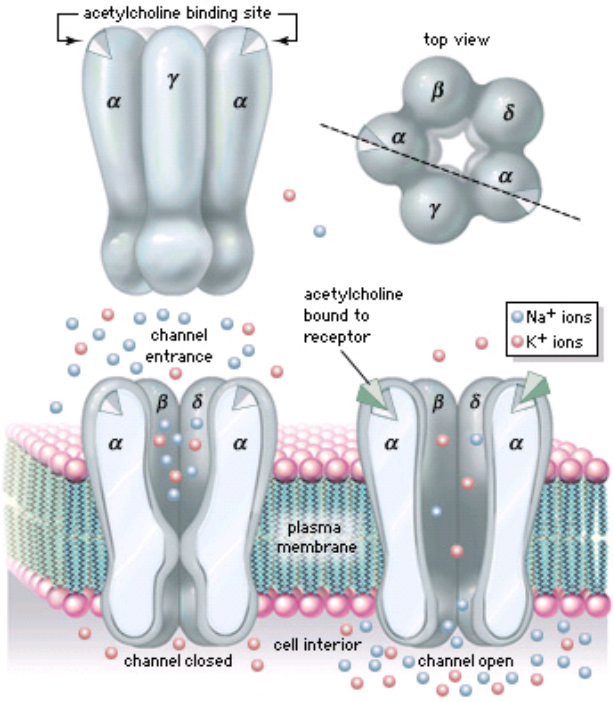
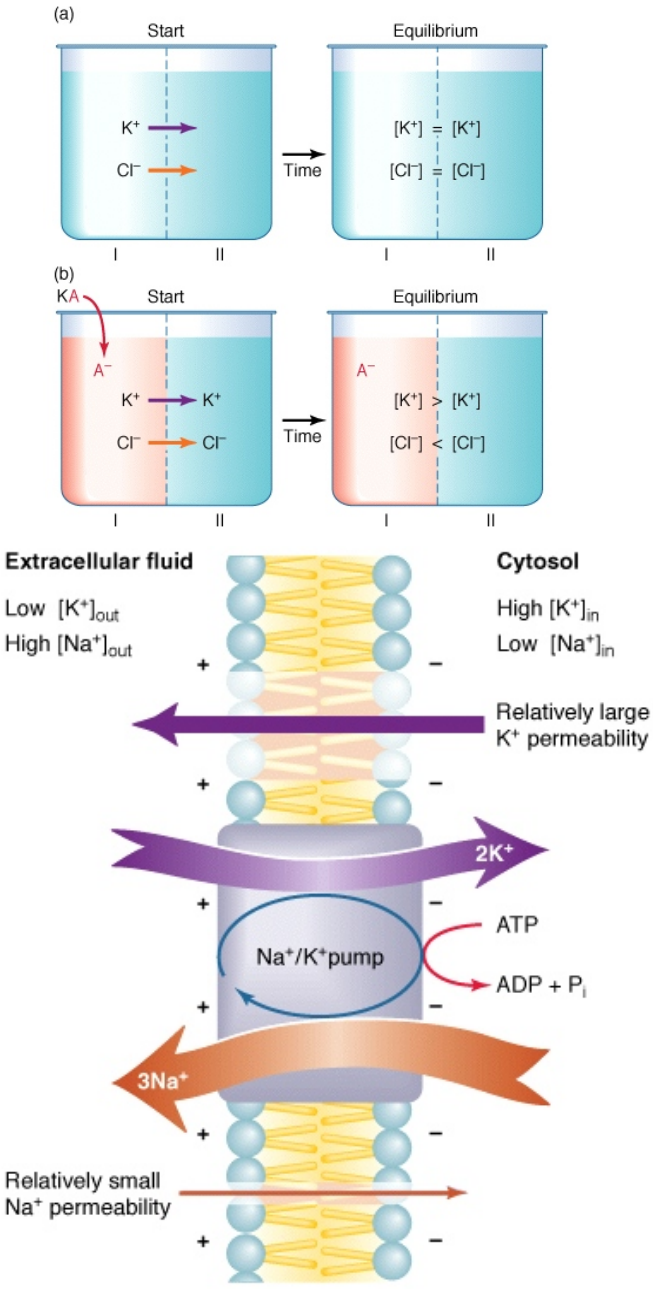
KARNATAKA STATE OPEN UNIVERSITY

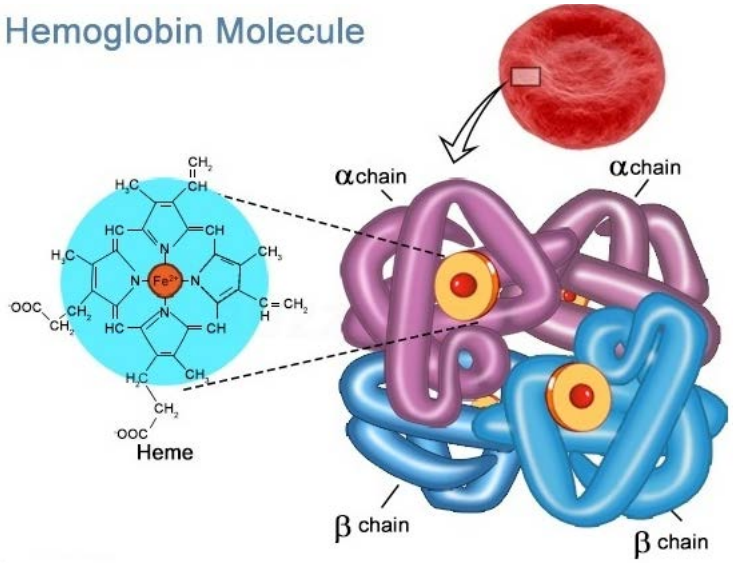
Mukthagangotri, Mysore – 570 006

M.Sc. CHEMISTRY

(THIRD SEMESTER)







Course: MCH T 3.4 **Block 1, 2,3 and 4**

BIO-INORGANIC & BIO-PHYSICAL CHEMISTRY

M.Sc. CHEMISTRY

THIRD SEMESTER

Course: MCHT 3.4

BIO-INORGANIC

AND

BIO-PHYSICAL

CHEMISTRY

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COURSE INTRODUCTION

Biophysical chemistry studies the properties of biological molecules along with the principles and methods of physics and physical chemistry. Experimentation and computation combine at Cornell to probe the structure, dynamics, interactions, and functions of individual biological

macromolecules and supramolecular complexes. Primary biological conformational processes such as protein folding, protein dynamics, and binding are addressed by theoretical methods and experiments. X-ray crystallography determines structures of biologically active molecules such as anti-tumor agents, immunosuppressents, and protein complexes.

“Bioinorganic Chemistry“ is at the gate-way of inorganic chemistry and biochemistry, i.e. it describes the mutual relationship between these two sub-disciplines, with focus upon the function of inorganic “substances“ in living systems, including the transport, speciation and, eventually, mineralisation of inorganic materials, and including the use of inorganics in medicinal therapy and diagnosis. These “substances” can be metal ions (such as K+, ferrous and ferric), composite ions (e.g. molybdate), coordination compounds like cisplatin and carbonyltechnetium), or inorganic molecules such as CO, NO, O_3. Medicinal inorganic chemistry on the one hand, and biomineralisation on the other hand, are important integral parts.

UNIT-1

Structure

1.0 Objectives of the unit

After studying this unit you are able to

- List the different essential elements and non-essential elements
- Differentiate the essential elements and non-essential elements
- Discuss the availability of biometals and bionon-metals in cells
- Identify the different metallo biomolecules in cells
- Explain the biochemistry of Sodium, Potassium and Chlorine

1.1Introduction

Bioinorganic chemistry is one of the most recently explored fields in inorganic chemistry. The major goal of this branch is as follows

i) To identify and study the inorganic compounds involved in various biological processes.

ii) The mathematical and chemical modeling of biological systems with such compounds.

iii) The control and optimize these systems.

Along with systematic analysis of the elements of the periodic system, inorganic chemistry also delves into the problems of 'bioinorganic chemistry with emphasis on the biological role of particular inorganic compounds.

Bioinorganic chemistry deals not only with the elements and their compounds that are present in a normally functioning living organism but also with those which, being extrinsic to a healthy organism, may exert a particular effect on the latter as they find their way into it.

The task of inorganic biochemistry includes investigation of the structure and biological role of inorganic compounds. Such investigations are carried out using a variety of physicochemical methods. Bioinorganic studies are of paramount importance in medicine, environmental control, and inorganic technology.

1.2 Essential Elements and Non-Essential Elements

The living beings are two types of the constituents according to chemical view point, they are

a) **Organic constituent**.

It is the main constituent and it is derived from inorganic elements (C, H, O and N). This organic constituent comprises of about 90% of the solid matter in living system. Examples of organic constituent are proteins, carbohydrates, fats, etc.

b) **Inorganic constituent**.

The 10% of the living system is inorganic constituent. Although it constitutes a relatively small amount of the total body, yet it is more important than organic constituent for maintaining the vital activities of a living being.

When the analysis of ash of animal tissues is carried out, it is having at least 30 elements. It ' that the body is having at least 30 elements.

It is possible to divide the 30 elements into two main groups such as essential and non-essential elements.

1.3 Essential elements

These are the elements which are indispensable for maintaining normal living state of a tissue or the whole of the body. Depending upon their absolute amounts in the body, these elements are further divided into two sub-groups. They are

i) **Macro elements**. *These are the elements which are required to be present in the diet in amounts more than 1 mg. These elements form nearly 60-80% of all the inorganic minerals in the body.* Macro elements include twelve elements such as carbon, hydrogen, oxygen, nitrogen, sodium, potassium, calcium, magnesium, iron, phosphorous, sulphur and chlorine. Among these the first four elements occur in substantial amounts in every body tissue and get derived from dietary carbohydrates, liquids and proteins. The body gets oxygen directly from atmosphere. Nearly 85% of the total oxygen and nearly 70% of the total hydrogen occur together in the form of water which makes nearly 3/5th of the total body weight. The remaining amount of oxygen and hydrogen, nitrogen, most of the carbon and some of sulphur are derived from carbohydrates, liquids and proteins which fulfill the basic requirement of tissue structure and the synthesis of various biochemical substances within these structures. Among the twelve elements, only Na, K and Cl have been described in this unit.

ii) **Micro elements**. *These are the elements which are needed by-the body in very small amounts, almost in micrograms or monograms*.

The examples of micro elements are copper, zinc, cobalt, manganese, molybdenum, iodine and fluorine. In this unit, only zinc and cobalt have been described.

Molybdenum and iodine also are essential, and tungsten-containing enzymes have also been found.

It is important to note that the elements essential at low concentrations; such as F, Se, As, and even Fe are toxic at higher concentrations. Even NaCI is toxic at high concentrations because it upsets the essential, electrolyte balance.

1.4 Non-essential elements.

These include the remaining ten elements which are not actually non-essential but their function in the body is not yet known. Examples of non-essential elements are bromine, boron, silicon, arsenic nickel, aluminum, lead, tin, vanadium and titanium.

Composition of human body		Composition of seawater		Composition of earth's crust	
H	63%	H	66%	O	46.6%
O	25.5%	O	33%	Si	27.7%
C	9.5%	Cl	0.33%	AL	8.1%
N	1.4%	Na	0.28%	Fe	5.0%
Ca	0.31%	Mg	0.033%	Ca	3.6%
P	0.22%	S	0.016%	Na	2.8%
Cl	0.03%	Ca	0.006%	K	2.6%
K	0.06%	C	0.0014%	Mg	2.1%
S	0.05%	Br	0.0005%	Ti	0.44%
Na	0.03%			H	0.14%
Mg	0.01%			C	0.20%

One of the major roles played by metallic elements in biochemistry is in metalloenzymes. In this enzymes metal ion participate at the active site to fiction.

Metalloenzymes are considered as a subclass of the metalloproteins, that is, proteins that incorporate one or more metal atoms as part of their structures. This includes respiratory proteins like hemoglobin and myoglobin, electron transport proteins such as cytochromes and ferredoxins, and metal storage proteins. In many cases it is possible to remove the metal atoms and then restore them (or replace them by other without collapse of the overall protein structure.

It is possible to classify the elements on the basis their function in the complex dynamic system of a living cell. The main classification of elements according to their occurrence in three different biological environments is as follows

a. In the extracellular fluids and on the outer wall of the cell membrane.
b. Inside the cell, starting from the inner wall of the membrane in the aqueous phase known as the cytoplasmic fluid.
c. In particles called organelles inside tile cell, including nuclei and mitochondria (the localized structures in a cell commonly identified with its source of power).

The location and the host environment of the elements gives the first clue of their role in biological systems. Some examples are given in below table.

The distribution of elements in the biological cell

Extracellular	Organelles	Cytoplasm
Na,Ca	K,Mg	K,Mg
Cu(Mo)	Fe,Co	Co
Cl,Si	P(S)	Zn
AI	Se	P(S)
		Se

1.5 Availability of biometals and bionon-metals in cells

An organism adapts to its surrounding environment by using the raw materials available to it and coping with unwanted or even toxic substances. Metal ions are available to organisms by the formation of metal complexes.

In basic soils, iron, as $Fe(OH)_3$, is quite insoluble. The Fe is much less abundant in seawater than the earth's crust. Plants can synthesize chelating agents to form soluble Fe complexes. Other chelate complexes are formed for the transport of Fe through cell membranes. Where the soil is deficient in Fe, a synthetic soluble Fe complex of ethylene-diaminetetraacetate ion (edta) is added. If the concentration of a metal ion is too high, the ions can be tied up as metal complexes or sequestered. The treatment for toxic metals, such as lead, is to inject a chelating agent (such as edta) to form a soluble complex that can be excreted. Although phosphorus is not abundant in the earth's crust or in seawater (Table 30.1), it serves essential functions in plant and animal life. In the normal balance of nutrients, P is likely to be the limiting nutrient. The mean life of inorganic phosphate added to lake water is on 1) a few minutes-it is used rapidly. Phosphate detergents thus upset the delicate natural balance,

The primary means for energy storage and release in cells involves the formation and hydrolysis of polyphosphates. The low abundance of phosphorus makes its compounds good candidates for energy carriers. The molecules storing energy are less likely to get lost in a multitude. The nucleic acids **deoxyribonucleic acid (DNA**) and **ribonucleic acid (RNA**) consist of long chains of cyclic five-carbon sugars joined by phosphate linkages. Attached to the sugars along the chains of cyclic five- carbon sugars joined by phosphate linkages. Attached to the sugar long chain are heterocyclic nitrogenous bases, the sequence of which constitutes the genetic code. The intertwined strands of DNA joined by hydrogen ponds to form the double helix permits the replication of the code. A portion of the double-helix structure of DNA shows the four bases occurring in DNA

Interestingly, the ratio of the chemical elements in living organisms is not in a simple relationship with their abundance on the Earth. Indeed, although the most abundant element, oxygen, is a major component of the compounds constituting animal and plant organisms, whereas the relatively scarce elements, including cobalt, copper, and molybdenum, perform important biological functions. It should also be pointed out that among the bioelements ie, elements essential for the structure of a living organism and its vital processes, there are metals and nonmetals of widely varying chemical properties, particle sizes, and electron structures. For example, the bimetals (often called "metals of life") include elements forming ions with a noble gas electron subshell, typically unable to have variable valence (Na^+, K^+, Mg^{2+}, Ca^{2+}). At the same time they include elements with an l8-electron (Zn^{2+}) or incomplete 18-electron (Cu^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+},Mo(V), Mo(VI) subshell. The latter may 'Change their oxidation state in the course of metabolic processes.

Some of the above bimetals form primarily ionic (Na, K) and covalent (Mo, Zn) bonds there are also such strong complexing agents among them as Fe^{3+}, Co^{2+}, Cu^{2+}, and Zn^{2+}. However, even the weaker complexes formed, for example, by the' a2+, Mg^{2+}, and Mn^{2+} ions play an important biological role and even ions of alkali metals (Na^+ K^+) become involved in complexing with macrocyclic ligands during metabolism.

The insignificant difference in the ionic radii of Na^+ (0.98 A^0) and K^+ (1.33 A^0) is responsible for widely different radii of the hydrated ions. This results in dissimilar functions of the Na^+ and K^+ ions in metabolism, Na^+ being an extracellular ion and K^+, an intracellular one. It is the size and the type of bonding characteristic of a given ion that determines which ions can be substituted for others in metabolic processes. *It has been established that the K^+ ions may be substituted in living tissues by large single-charged ions of alkalimetals (Rb^+, Cs^+) as well as by the NH_4^+, and Tl^+ of comparable sizes. On the other hand, the relatively small Na^+ ion can be substituted only by Li^+. Interestingly, the substitution by Cu^+ ions does not take place because Cu^+ tends to form covalent bonds, yet Cu^+ and Na^+ are similar size.*

It is extremely important that the Mg^{2+} and Ca^{2+} ions do not substitute for each other in bio systems. This is believed to be due to the pronounced covalence of the bond between Mg^{2+}, as opposed to Ca^{2+} and ligands. Even more covalent bonds with ligands are formed by Zn^{2+} which is not substituted by Mg^{2+} in spite of the closely similar ionic radii of the two.

According to Yatsimirsky, the ionic and covalent natures of the bonds between biometal ions and ligands should be estimated as follows. The ionicity of a bond is proportional to the ratio between the square of the ionic charge and the ionic radius. This ratio varies from one to five

for most ions. Only in the case of beryllium is this ratio abnormally high and equals 11.7, which is precisely what accounts for the high toxicity of the Be^{2+} ion.

The covalence of the metal-ligand bond can be determined, according to Yatsimirsky, as the ratio

$$\frac{I_M^2 S_{ML}^2}{I_M - I_L}$$

where I_M and I_L are the ionization (valence state potentials of the metal and ligand, respectively, and S_{ML} is the integral of overlap of the orbitals interacting during covalent bonding. The covalence of biometals, determined in this manner, usually varies from 20 to 135. When the covalence of a bond is low, the most stable are compounds of metal ions with oxygen. As the covalence increases, compounds with a metal-nitrogen and especially metal-sulphur bond grow in stability. The same correlation is observed in Pearson's classification according to which a "hard acid combines with a "hard" base and a "soft" acid combines with a "soft" base.

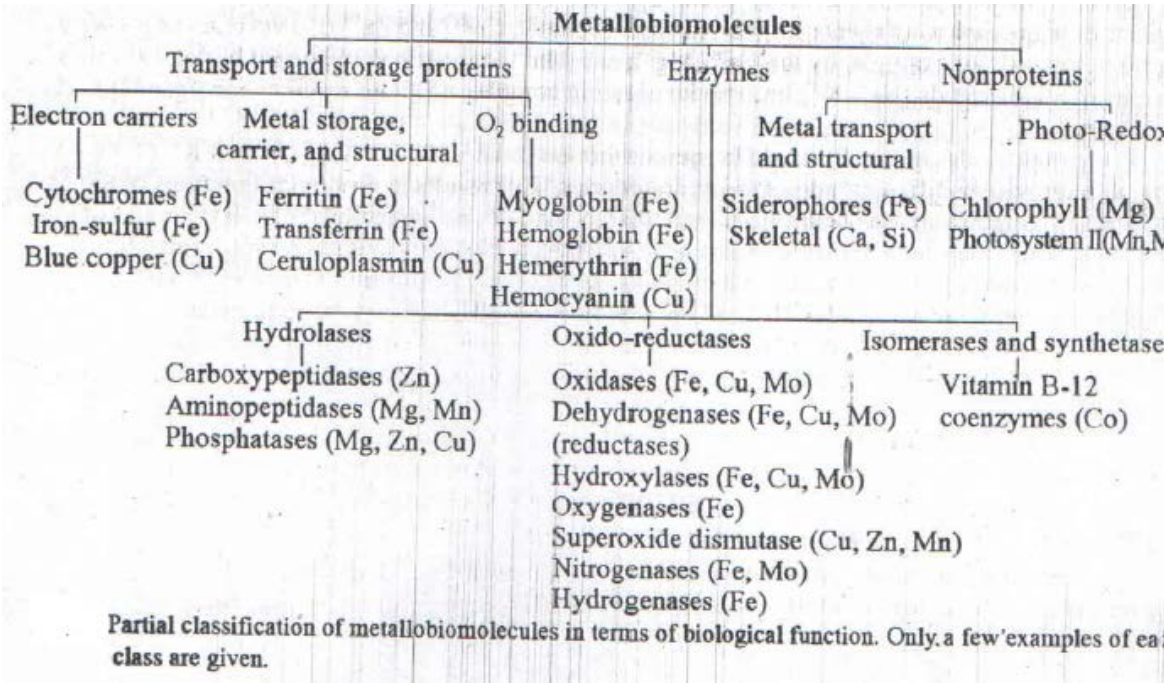
The concentrations of various elements and their compounds in the living organism are very important. It has been demonstrate that one and the same element may produce a positive effect on the organism as a whole, yet be a strong poison if present in excess amounts. For examplezinc belongs to biometals of major importance. The Zn^{2+} ions form part of many enzymes catalyzing vitally important processes. At the same time, it has been found that excessively high concentrations of Zn^{2+} in tissues are carcinogenic. :.

Similarly, selenium which is generally not classified as biometals, it has been established that decrease in the selenium content in man's daily food intake from 0.3-0.5 mg leads to an increase in the incidence of mammal cancer in women (more than five-fold). It has been speculated that the low content of selenium in the foodstuffs produced in countries with highly developed chemical industry is associated with a high content of sulphur in the atmosphere, which displaces selenium from natural substrates.

1.6 Metallo biomolecules

Metallobiomolecules are natural products which contain one or more metallic elements. A classification scheme for some of the more extensively studied metallobloc molecules is given below. These molecules are complex coordination compounds whose metal-containing sites (called "active sites") are usually involved in electron transfer, the binding of exogenous molecules, and catalysis. The comparative study of these sites and analogously structured

synthetic coordination compounds is the main part of theinterdisciplinary field of bioinorganic chemistry.



Partial classification of metallobiomolecules in terms of biological function. Only a few examples of ea class are given.

1.7 Biochemistry of Sodium, Potassium and Chlorine

The pairs of elements Na^+ - K^+ and Ca^{2+} - Mg^{2+} are chemically very much similar but surprising they differ so greatly in their biological functions. The Ca^{2+} and Na^+ ions are concentrated in body fluids outside of cells. Calcium forms solid skeleton materials such as bones, stabilizes conformations of proteins and triggers muscle contraction and the release of hormones. The ions Mg^{2+} and K^+ are concentrated in side the cells. Magnesium ion forms a complex with ATP and is required for most enzymatic reactions involving ATP within the cell.

As there exists intimate relations along sodium, potassium and chlorine, these should be discussed together rather than separately.

1.7.1 Sources

The main source of sodium and chlorine in the diet is the common table salt (sodium chloride) which finds use in cooking. In general, food from plant source is generally low in sodium, Potassium occurs in almost all foods, both plant and animal. The good sources in potassium include coffee, tea, cocoa, dried beans, molasses, most vegetables (especially green, leafy vegetables), milk, fish, chicken, beef liver, beef, pork; dried apricots, dried peaches, bananas, the juices or oranges tangerines f and pineapples, yams, winter squash, broccoli, potatoes, and Brussels sprouts.

Healthy individual, under normal dietary conditions, seldom suffers from a deficiency of sodium, chlorine, or potassium. However, extreme perspiration over a prolonged period, diarrhoea or vomiting may give rise to a sodium-chloride deficiency. On the other hand, under *certain health condition, like congestive heart disease, hypertensive high blood pressure, and kidney disease, a sodium-restricted diet is preferred. As sodium in the blood and interstitial fluids is a major factor in determining the osmotic pressure of these fluids, a reduction in sodium causes the transfer of water into the cell and subsequent loss of water and sodium chloride in the urine*.

Generally, sodium, potassium and chlorine are almost completely absorbed from the gastrointestinal tract, less than 2% of ingested sodium and less than 10% of the potassium being eliminated in the faces.

Sodium and potassium occur in plants as well as in animals as the salts (chlorides, phosphates and carbonates) of inorganic acid and salts of proteins and organic acids. Sodium has been the main extracellular cation, whereas potassium has been the main intracellular cation.

1.7.2 Functions

The metabolism of sodium, potassium and chlorine has been intimately related to certain fundamental physiological mechanisms.

1. *Maintenance of normal hydration and osmotic pressure*

The main function of these three ions in the body is to maintain the normal osmotic pressure of the different body fluids throughout the body and thereby to protect the body against excessive loss of fluids. Otherwise it will disturb the normal hydration. The osmotic pressure of a fluid is dependent upon the total cations of that fluid. Therefore sodium, which is the chief citation of extracellular fluid, plays a dominant role in this connection.

2. *Maintenance of normal acid-base equilibrium*

The sodium salts and potassium salts with the corresponding weak acids are known to form the chief buffer systems respectively of extracellular and intracellular fluids. These buffer systems play vital role in the regulation of pH of various fluids under various physiological conditions.

3. *Transport of* Co_2

All the three ions mainly chloride ions are known to play role in the gaseous transport of CO_2,

4. *Neuromuscular irritability*

Both sodium and potassium play an important role in the maintenance of normal neuromuscular irritability and excitability. They have been antagonistic to calcium and magnesium ions which are expressed by the following equation

$$\text{Irritability} \propto \frac{Na^+ + K^+}{Ca^{2+} + Mg^{2+} + H^+}$$

Thus, the ratios of various ions as well as their absolute amounts in the body fluids and cells have been of vital significance.

5. *Maintenance of proper viscosity of blood*

In blood plasma, sodium and potassium chlorides are having the outstanding function not only of keeping the globulins in physical solution but also of regulating the degree of hydration of the plasma proteins, so important in the maintenance of the proper viscosity of blood.

6. In secretion of digestive fluids

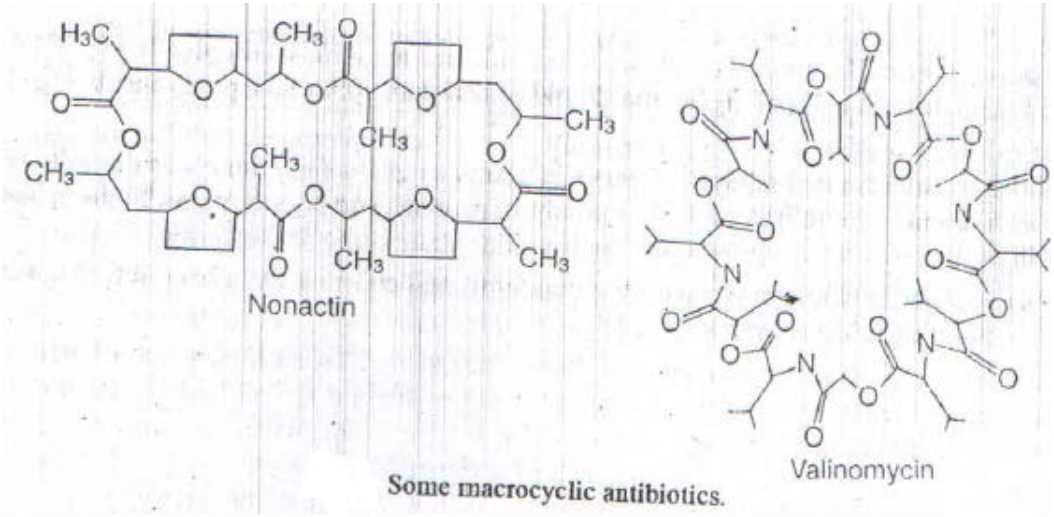
Gastric HCl gets derived from NaCl of blood and the base in alkaline digestive juices, like the pancreatic juice and bile gets derived from blood sodium and potassium salts.

7. In the storage of protein a d glycogen

Potassium enters the intracellular fluid during the period of cell growth and hormone ie, increased protein anabolism taking place spontaneously or induced such agents as growth hormone or androgen. Deposition of 1 g of cell protein needs retention of about 0.4 mEq of potassium. Similarly storage of 1g of glycogen in the liver or muscles causes the passage of about 0.15 mEq potassium in the intracellular fluid. Because of this reason, insulin and glucose, administered into a patient of diabetic comma to enhance the glycogenesis, may bring about hypokalemia.

8. *In antibiotics*

In alkaline metal ions are weak Lewis acids and form weak complexes. The encapsulation of a cation by crown ethers is selective, depending on the cation size. Some natural ionophores (ion-bearers) for alkali metal ions have O donor atoms from carboxylates, cyclic peptides, and so on. Some of these such as nonaction and valinomycin shown in below figure are antibiotics. The formation constants for nonacid in acetone containing some H_2O for Na^+, K^+ and Cs^+ are 210, $2X10^4$ and 400 respectively. Thus the nonaction is elective K^+.



Some macrocyclic antibiotics.

9. Excretion

Both sodium and chloride get excreted mainly by kidneys in urine, to a lesser extent, by skin in the form of perspiration and appreciably small in the digestive fluids by the gastrointestinal tract. Potassium on the other hand gets normally excreted almost entirely by kidneys in the urine.

Under normal physiological conditions the daily excretion of these electrolytes is almost equal to the intake. But when the conditions are not normal, the excretion of these electrolytes may get decreased or increased giving rise to hyper or hypo level in the plasma.

1.8 Summary of the unit

Of the approximately 115 elements known, only the 19 elements are the essential and are absolutely required in the human diet. These elements are restricted to the first four rows of the periodic table with only two or three exceptions (molybdenum, iodine, and possibly tin in the fifth row). Some other elements are essential for specific organisms. For example, boron is required for the growth of certain plants, bromine is widely distributed in marine organisms, and tungsten is necessary for some microorganisms.

An essential element is one that is required for life and whose absence results in death. Most living matter consists of *bulk elements*: oxygen, carbon, hydrogen, nitrogen, and sulfur. These are the building blocks of the compounds that constitute our organs and muscles. These five elements also constitute the bulk of our diet. Six other elements—sodium, magnesium, potassium, calcium, chlorine, and phosphorus—are often referred to as *macrominerals* because they provide essential ions in body fluids and form the major structural components of the body. In addition, phosphorus is a key constituent of both DNA and RNA, the genetic building blocks of living organisms. The six macrominerals are present in the body in somewhat smaller amounts than the bulk elements, so correspondingly lower levels are required in the diet. The remaining essential elements called *trace elements* are present in

very small amounts, ranging from a few grams to a few milligrams in an adult human. Measurable levels of some elements are found in humans but are not required for growth or good health. Examples are rubidium and strontium, whose chemistry is similar to that of the elements immediately above them in the periodic table (potassium and calcium, respectively, which are essential elements). Because the body's mechanisms for extracting potassium and calcium from foods are not 100% selective, small amounts of rubidium and strontium, which have no known biological function, are absorbed.

1.9 Key Words

Essential Elements; Non-Essential Elements; Metallo biomolecules

1.10 References for further studies

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1.11 Questions for self understanding

1) What are essential elements and non-essential elements? Give examples for each
2) Write a note on
    i) Macro elements.
    ii) Micro elements.
3) Discuss the availability of biometals and bionon-metals in cells
4) What are metallo biomolecules? Give example and discus their role
5) Briefly explain the biochemistry of Sodium, Potassium and Chlorine

UNIT-2

Structure

2.0 Objectives of the unit

After studying this unit you are able to

- List the general mechanisms by which molecules cross membranes
- List the two properties that determine how well a substance can diffuse across a membrane.
- Compare and contrast transport mediated by channel proteins versus carrier proteins.
- List the forms of energy used by carrier proteins to mediate active transport.
- Describe the differences in the concentrations of Na^+, K^+, Ca^{2+} and Cl^- on the outside of typical plasma membranes of eukaryotic cells versus the inside.
- Explain how the gradients of Na^+, K^+ and Ca^{2+} are maintained.
- Explain the influence of the membrane potential on the electrochemical gradients of Na^+, K^+ and Ca^{2+} and Cl^- across membranes.
- Explain how the Na^+/K^+-ATPase operates. Describe the consequences of inhibition of the Na^+/K^+-ATPase.
- Compare and contrast symport and antiport. Name a symporter and an antiporter and explain how each of them functions.
- Explain the contribution of P-glycoprotein to the blood-brain barrier.
- Name three ways in which an ion channel can be gated.

2.1 Introductions

An important function of a biological membrane is to serve as a barrier to the outside world. However, membranes are not impenetrable walls. Obviously, nutrients must enter the cell and waste products have to leave in order for the cell to survive. For this and many other reasons, it is crucial that membranes be selectively permeable. For example, the movement of ions across membranes is important in regulating vital cell characteristics such as cellular pH and osmotic pressure. Membrane permeability is also a key determinant in the effectiveness of drug absorption, distribution, and elimination. For example, a drug taken orally that targets cells in the central nervous system must cross several membranes: first the barrier presented by the intestinal epithelium, then the walls of the capillaries that perfuse the gut, then the blood-brain barrier. Some endogenous substances and many drugs easily diffuse across the lipid bilayer. However, the lipid bilayer presents a formidable barrier to larger and more hydrophilic molecules (such as ions). These substances must be transported across the membrane by special proteins. We will first look briefly at the three major ways that both endogenous substances and drugs cross the barriers presented by cell membranes. We will

then discuss in more detail two of these mechanisms, which are the primary ways that drugs cross membranes.

2.1 Mechanisms for ions to getting in and out of cells

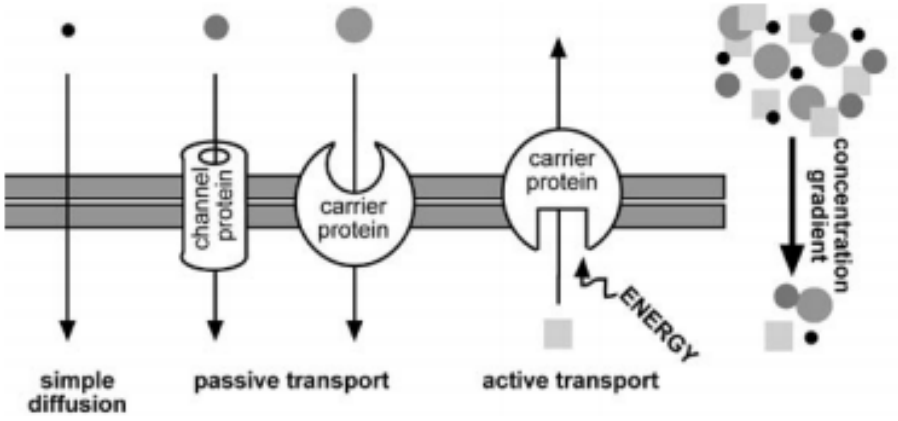


Figure 1: Types of movement across membranes

A) Diffusion across the lipid bilayer.

Since membranes are held together by weak forces, certain molecules can slip between the lipids in the bilayer and cross from one side to the other. This spontaneous process is termed Diffusion. This process allows molecules that are small and lipophilic (lipid-soluble), including most drugs, to easily enter and exit cells.

The difference in the concentration of a molecule on one side of a membrane versus the other is called a gradient. A molecule's concentration gradient (also called the chemical gradient) drives movement across the membrane until the molecule is at equilibrium. Movement from a high concentration to a low concentration is also referred to as movement "with" or "in the direction of" the concentration gradient or "downhill." Movement from a low concentration to a high concentration is also referred to as "against" the concentration gradient or "uphill."

B) Protein-mediated transport

In order to cross the hydrophobic interior of the bilayer, water-soluble molecules (those that are either charged or have polar groups) and large molecules require the action of membrane transport proteins. These integral membrane proteins provide a continuous protein-lined pathway through the bilayer. There are two classes of membrane transport proteinsare present they are

Carrier proteins, which literally carry specific molecules across, and

Channel proteins, which form a narrow pore through which ions can pass.

Channel proteins carry out passive transport, in which ions travel spontaneously down their gradients. Some carrier proteins mediate passive transport (also called facilitated diffusion),

while others can be coupled to a source of energy to carry out active transport, in which a molecule is transported against its concentration gradient. Membrane transport proteins are important pharmacologically for two reasons. First, some drugs exploit endogenous membrane transport processes to enter or exit cells. Second, membrane transport proteins are major drug targets.

C) Endocytosis/exocytosis

Large macromolecules (e.g., proteins, viruses, lipoprotein particles) require more complex mechanisms to traverse membranes, and are transported into and out of cells selectively via endocytosis and exocytosis (secretion). Interestingly, endocytosis and exocytosis are not only important for the import/export of large molecules. Often, essential small molecules that are hydrophobic or toxic (e.g., iron) travel through the bloodstream bound to proteins, which enter and exit cells via these mechanisms. *Lipid diffusion and protein-mediated transport mechanisms are the key means by which drugs travel into and out of cells. These processes are critical for movement of endogenous molecules (ions, sugars, amino acids, etc.) across membranes as well.*

2.2 Lipid diffusion (the major means of drug absorption and permeation)

The rate at which a molecule diffuses across a membrane depends on its size and its degree of hydrophobicity. Hydrophobic substances such as gases and steroid hormones diffuse across membranes easily.

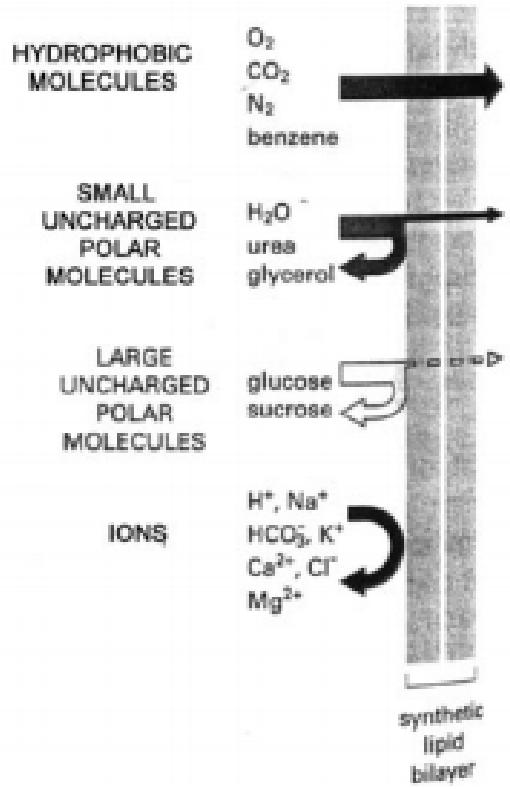


Figure 2: The relative permeability of a lipid bilayer to different classes of molecules

Due to the fact that they are repelled by the hydrophobic interior of the bilayer, polar molecules do not diffuse across the bilayer as easily, unless they are very small and uncharged (e.g., water and EtOH). Lipid bilayers are much less permeable to larger polar molecules and are virtually impermeable to ions, which are surrounded by a cage of water. However, most drugs are small amphipathic compounds, and therefore rely heavily on lipid diffusion to move through different compartments in the body. The ability of drugs to diffuse across membranes is heavily influenced by the ionizability of the drug in the surrounding medium. The pH of body compartments influences lipid diffusion of drugs. A large fraction of drugs are weak acids or weak bases. Drugs that are weak acids or weak bases exist in either charged (ionized) or uncharged (nonionized) forms as (Figure 3).

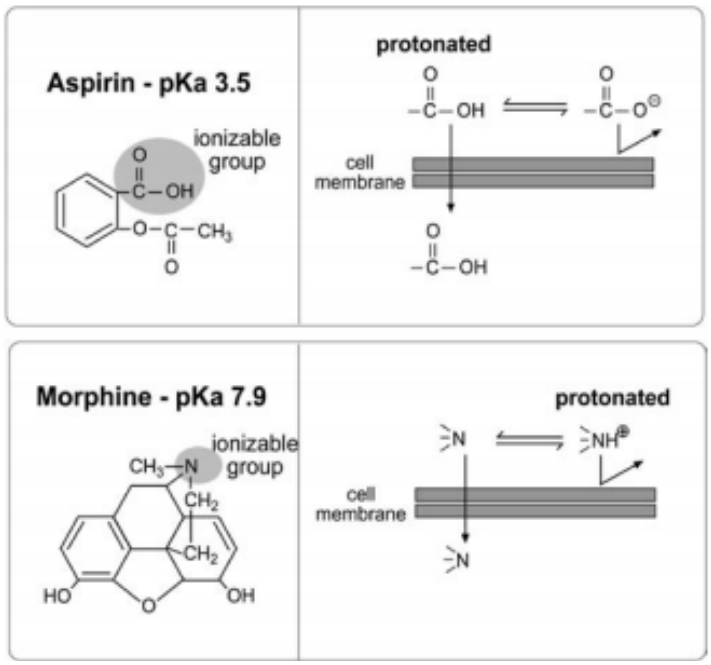


Figure 3: Ionization of weak acids and bases. Weak acids and bases have groups that can donate or accept protons.

The ratio of the charged to uncharged form depends on the drug's pKa, and the pH of the environment. Weak acids are neutral molecules that can dissociate from a proton to form an anion (e.g., aspirin). Weak bases are defined as neutral molecules that can form a cation when protonated (e.g., morphine). Since diffusion across a lipid bilayer requires that a drug be lipidsoluble, the ionized form of a drug cannot cross membranes. Thus, weak acids that are nonprotonated and weak bases that are protonated cannot diffuse across membranes. At a pH that is equal to a drug's pKa, equal amounts of the protonated and nonprotonated forms are present. Assuming the pH is the same on both sides of a given membrane, the drug will be at equilibrium across the membrane. If the pH is less than the pKa (such that there are excess protons available), the protonated form of a drug predominates. Thus weak acids exposed to a

low pH environment are favored to diffuse across membranes, while weak bases are not. The opposite is true at a higher pH.

2.3 Membrane transport proteins (carriers and channels)

There are two major classes of membrane transport proteins they are carrier proteins and channels. Membrane transport proteins can be classified further by whether they mediate active or passive transport.

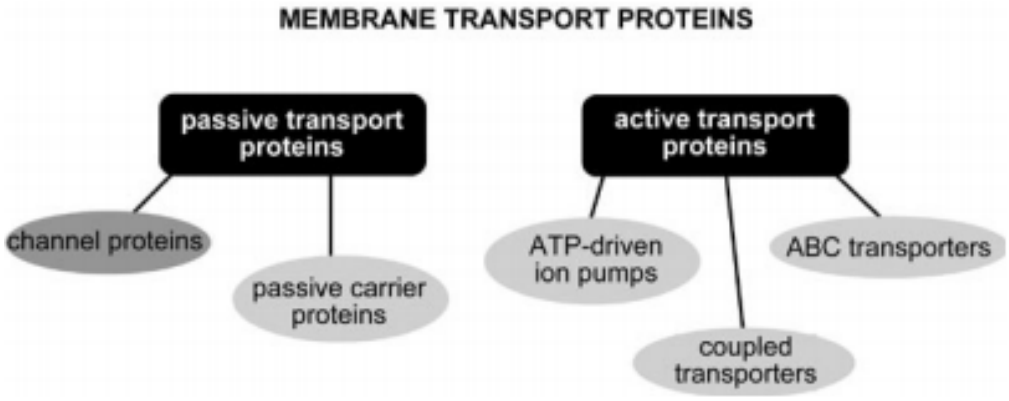


Figure 4: Classification of the types of membrane transport proteins

Channel proteins most of which transport ions, open to make a hole in the membrane through which ions can diffuse down their gradients. Channels serve simply as a gateway through a membrane. There are no highly specific interactions that take place between the protein and ions passing through it. In contrast, each carrier protein (also called permeases or simply carriers) actually binds to a specific molecule and physically carries it across the membrane via a conformational change. Consequently carriers are considerably slower than channels.

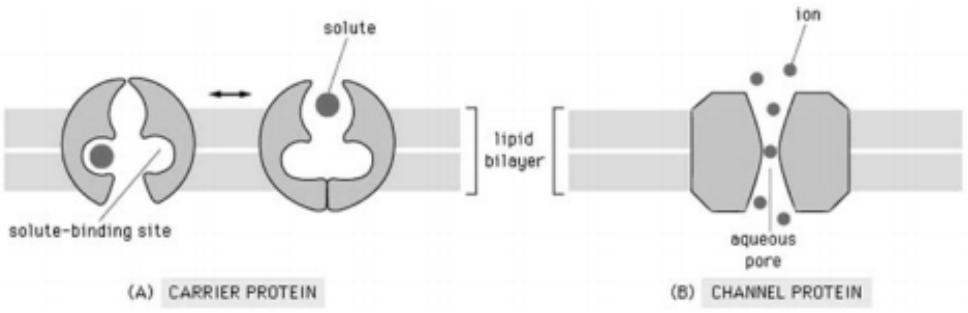


Figure 5: Carrier proteins and channel proteins

Carriers bind their substrates on one side of a membrane, undergo a conformational change and then release the substrate on the opposite side of the membrane. There are two major types of carrier proteins are present they are active carrier and passive carrier.

i) Active carriers

Active transport of solutes against their gradients is important for maintaining the balance of ions across membranes concentrating metabolites in certain organs or cellular compartments, and exporting foreign substances from cells.

There are three types of active transporters and they are

1) ATP-driven ion pumps

2) Coupled transporters, and

3) ABC transporters.

Transport of a molecule against its gradient is energetically unfavorable, and therefore requires that the transporter harness an energy source. ATP-driven ion pumps and ABC transporters utilize ATP hydrolysis to power uphill transport. Coupled transporters are driven by the energy stored in ion gradients.

2.3.1 ATP-driven ion pumps

ATP-driven ion pumps utilize the energy liberated by ATP hydrolysis to move ions across membranes, against their gradients. These proteins maintain ion gradients across both the plasma membrane and intracellular membranes.

Before we examine how ion pumps work, it is useful to understand the special nature of ion gradients, and why their maintenance is critically important to the cell. The internal ion composition of a cell is very different from that of the extracellular fluid, and the maintenance of these gradients is crucial for the viability of a cell.

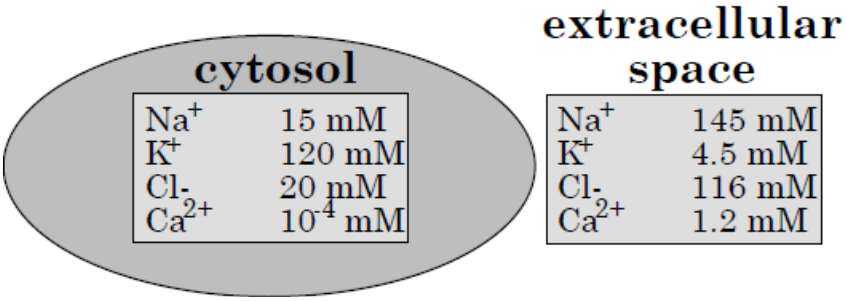


Figure 6: Typical concentration gradients of cellular ions across the plasma membrane

There are four major ion gradients that are necessary for cell functions, those are

Sodium ion (Na^+)

Calcium ion (Ca^{2+})

Potassium ion (K^+) and

Chloride ion (Cl^-).

The concentration of Na^+ is higher outside of a cell than on the inside. The constant active transport of Na^+ out of the cell maintains this concentration gradient, which helps to balance

osmotic pressure on either side of the membrane. If Na^+ were not actively transported out of the cell, water would rush in to dilute the intracellular contents, and the cell would eventually burst. Ca^{2+} and Cl^- concentrations are also higher outside the cell than inside. The K^+ gradient is the opposite ie, the intracellular concentration of K^+ is higher than the extracellular concentration.

Ion gradients are influenced by membrane potential: In the case of charged molecules, movement across a membrane is influenced by the molecule's concentration gradient, and by the fact that *the interior of the plasma membrane is negatively charged relative to the extracellular side of the membrane. This difference in charge across the membrane is referred to as membrane potential*. The inside-negative membrane potential exerts a force on any molecule carrying an electrical charge, which determines an electrical gradient (or electrical energy difference) for that molecule. The effects of the electrical and concentration gradients of a molecule are combined in what is called the electrochemical gradient (Figure 7).

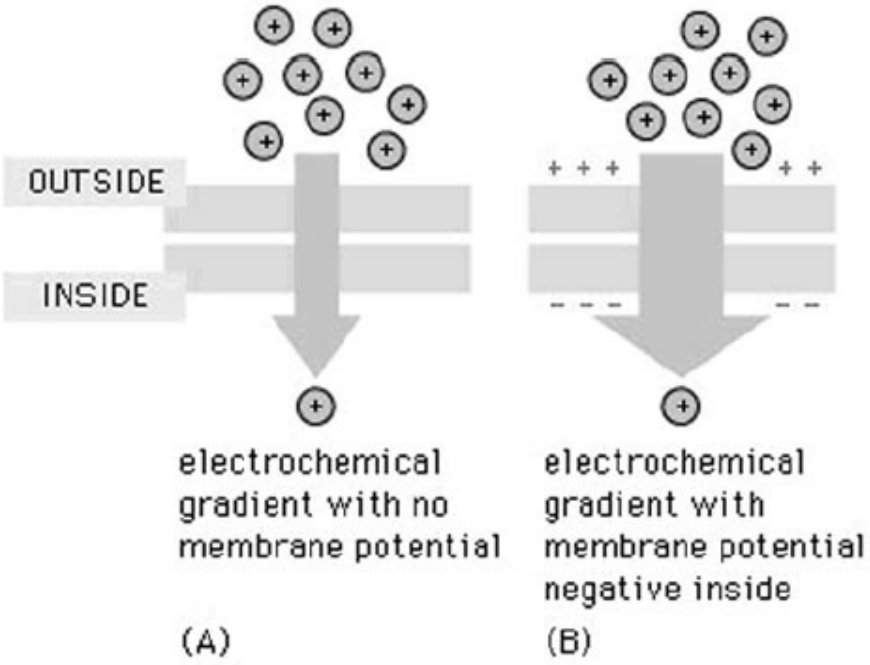


Figure 7: Two forces at work in an electrochemical gradient

In the case of K^+ and Cl^-, the electrical and concentration gradients of molecules work against each other. K^+ is attracted toward the inside of a cell because of the inside-negative membrane potential, but the molecule's concentration gradient works in the other direction. Thus, K^+ comes almost to equilibrium across the plasma membrane though it tends to move out of cells when given the opportunity. Cl^- also tends move out of cells, despite its higher extracellular concentration, because of the inside-negative membrane potential.

With Na^+ (and to a lesser degree Ca^{2+}), the electrical and chemical gradients work in the same direction. Molecules are driven into the cell down the chemical gradient and the membrane potential also pulls Na^+ into the cell. Simply stated, Na^+ enters cells whenever it can, because there is a large electrochemical force driving it to do so. This driving force can be harnessed by coupled transporters to power active transport of other molecules. The best-understood and perhaps the most important example of an ion pump is the Na^+/ K^+-ATPase, which is also called the Na^+/ K^+ pump (Figure. 8).

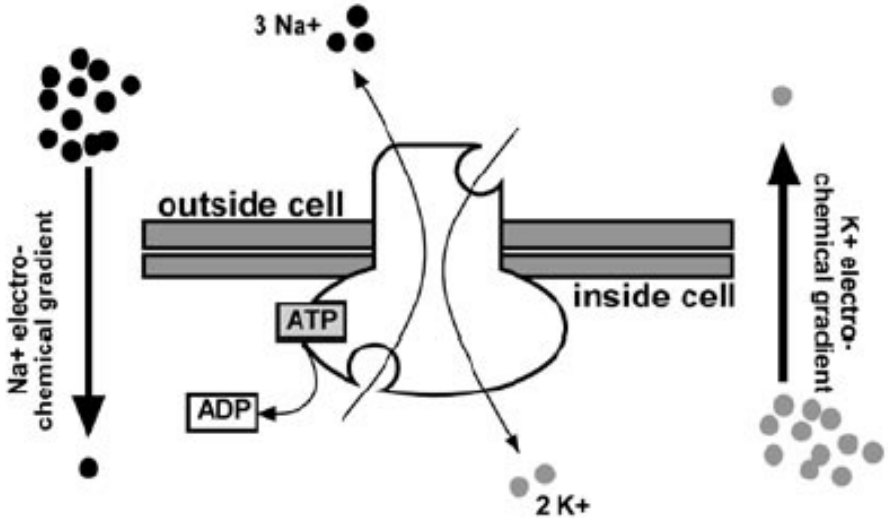


Figure 8: The Na^+/K^+-ATPase

This pump is an enzyme embedded in the plasma membrane that hydrolyzes ATP so that Na^+ and K^+ can be transported against their concentration gradients. As stated above, if Na^+ were not continually pumped out, the gradient would rapidly be lost, and cells would swell and burst. For this reason, the Na^+/ K^+ -ATPase is in continual operation. The Na^+/K^+ pump brings two K^+ ions in and three Na^+ ions out for every molecule of ATP hydrolyzed. An important feature of this mechanism is that ATP hydrolysis and ion transport are tightly coupled. ATP is not hydrolyzed unless the ions are transported.

The action of the Na^+/K^+ pump is thought to follow a series of steps explained in figure 9.

1) Na^+ and ATP bind the carrier protein. There are three binding sites for Na^+ on the intracellular side of the carrier.
2) ATP is hydrolyzed by the ATPase part of the carrier. As a result, ADP is released and the internal side of the carrier is phosphorylated. Subsequently, a conformational change causes the carrier to transfer Na^+ across the membrane and release it.
3) Two molecules of K^+ then bind to the extracellular surface.

4) The carrier is dephosphorylated and returns to its original conformation, which transfers the K^+ across the membrane. When K^+ is released into the cytosol, the carrier is ready to start the cycle again.

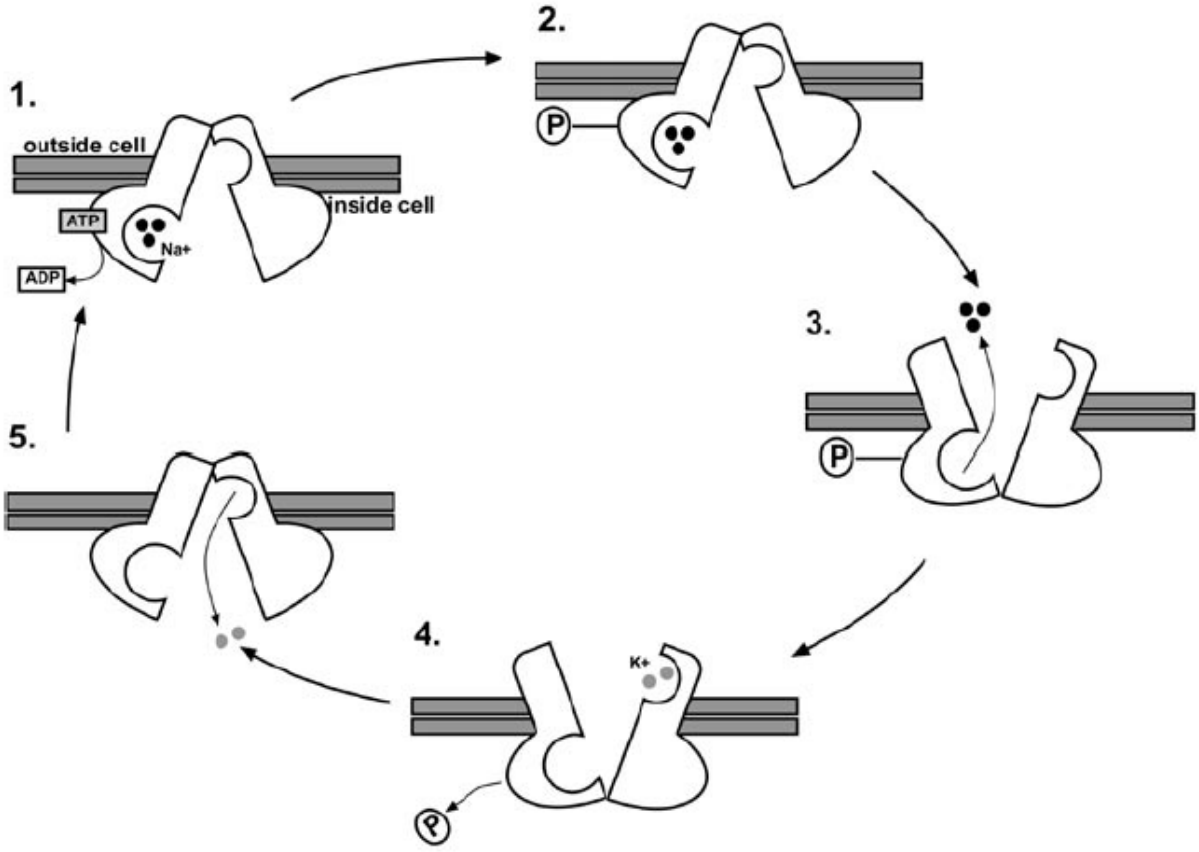


Figure 9: A schematic model of the Na^+/K^+ ATPase pumping cycle

There are other examples of ion pumps that operate in much the same way as the Na^+/K^+-ATPase. A Ca^{2+}-ATPase is located in the membrane of the sarcoplasmic reticulum (SR), a specialized version of the ER located in muscle cells. In this case, for every ATP hydrolyzed, two Ca^{2+} ions are pumped out of the cytosol and into the SR. This sequesters Ca^{2+} in the SR and maintains the Ca^{2+} gradient, which is important for muscle contraction and other cellular functions. Proton pumps are another important example of ATP-driven carrier proteins. These are important for many cellular events, including acidification of intracellular compartments.

2.3.2 Coupled Transporters

Coupled transporters transport two molecules across membranes simultaneously. Coupled transporters use the energy stored in ion gradients to actively transport molecules across membranes. *Because they rely on the gradients generated by ion pumps, coupled transporters are also known as secondary active transporters*. Most often, the source of energy for

coupled transporters is the flow of Na^+ down its electrochemical gradient. There are also some coupled transporters that use proton gradients to drive uphill transport;

There are many well-understood and physiologically important coupled transporters. A few are discussed below.

Symport

If the carrier protein transports two solutes in the same direction, this is called symport.

Examples:

The Na^+-driven glucose pump on the apical side of intestinal epithelial cells is an example of a symporter.

Antiport

If carrier protein traverses the ion in to membrane and transported the metabolite in the other direction is called Antiport.

Example: The Na^+/Ca^{2+} exchanger uses the electrochemical gradient of Na^+ to drive transport of another molecule.

In this case, Na^+ flows down its gradient into the cell, which allows Ca^{2+} to be exported from the cell against its gradient. Three Na^+ molecules enter the cell for every Ca^{2+} that exits.

The below figure demonstrate the function of symport and antiport

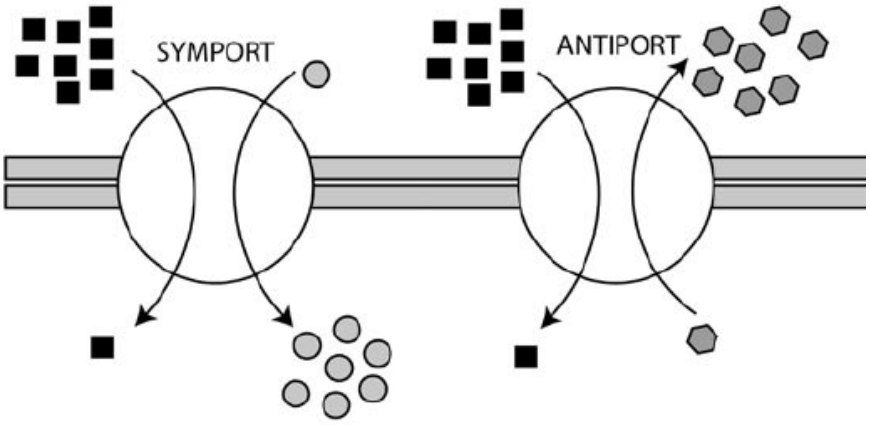


Figure 10: The action of coupled transporters

2.3.3 ABC transporters

The third type of active carrier is the ABC transporter (ATP-binding cassette) superfamily. ABC transporters all have a similar structure, consisting of two ATP binding domains facing the cytosol and two transmembrane domains (Figure 13). Similar to the situation seen with ATP-driven ion pumps, the binding and hydrolysis of ATP by ABC transporters is thought to drive conformational changes that transport molecules across the membrane. But while ion pumps transport ions in or out of cells, most ABC transporters in eukaryotes are specialized

for pumping small compounds out of cells. In general, ABC transporters seem to be crucial for getting foreign substances (drugs and other toxins) out of cells, making them extremely important clinically.

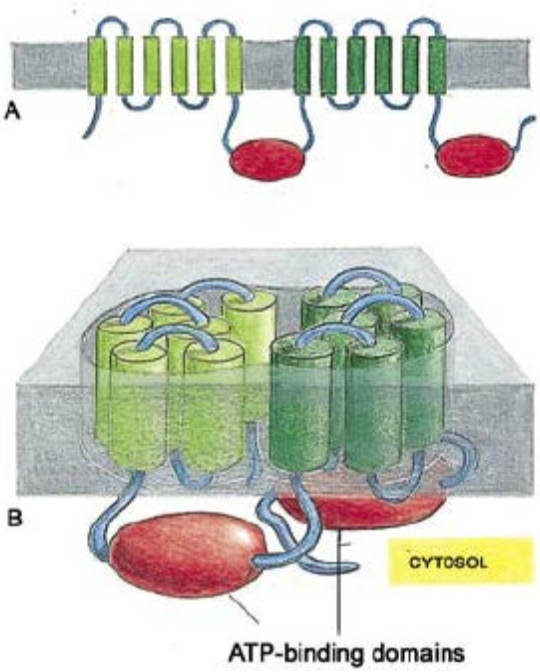


Figure 11: A typical ABC transporter. (A) A diagram of the topology of the protein. (B) A hypothetical arrangement of the polypeptide chain in the membrane.

2.4 Passive carriers

Passive carrier proteins facilitate the downhill transport of substances across membranes. An example of a carrier protein that carries out passive transport is the glucose transporter, which is located in the plasma membrane of all mammalian cell types. On liver cells the carrier can be open toward the outside of a cell or toward the inside (Figure 11). The glucose transporter can carry glucose in either direction, depending on the direction of the concentration gradient.

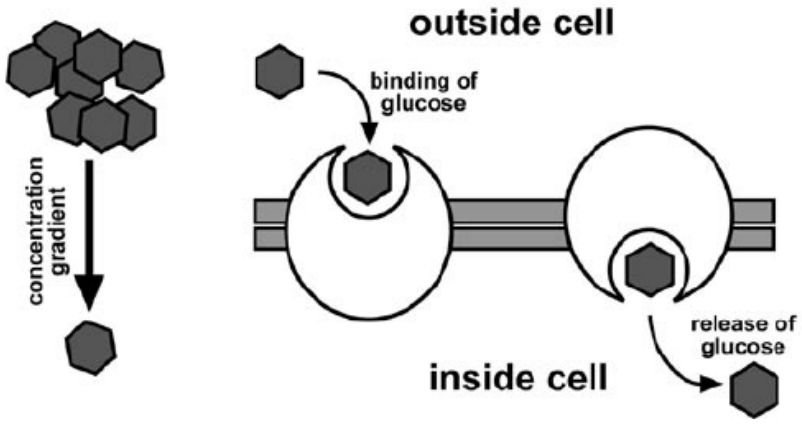


Figure 11: Passive transport by the glucose carrier

For example, just after a meal, glucose levels are high in the blood. The glucose transporter opens toward the outside of the cell and shuttles glucose across the membrane by undergoing a conformational change that opens the carrier toward the inside of the cell. Glucose is then released into the cells, where the concentration is lower. During fasting, blood sugar is low, and the transporter moves glucose from the liver out to the blood. This transporter is also found on the basolateral side of mammalian epithelial cells, which contributes to transcellular transport of absorbed solutes.

2.5 Ion channels

Ion channels are ion-permeable pores in the lipid membranes of all cells. They open and close the pores in response to stimuli and gating the flow of specific small ions. The ions flow downhill thermodynamically. Lipid bilayers on their own are virtually impermeable to charged molecules. However, membranes contain channel proteins, which form protein-lined passageways through the membrane and facilitate the trafficking of hydrophilic and charged molecules. Transport through channels does not require an additional input of energy. *Channels have an advantage over carrier proteins in terms of the speed of transport*. For example, up to a hundred million ions can pass through an ion channel per second which is 100,000 times greater than any measured rate of transport via a carrier protein.

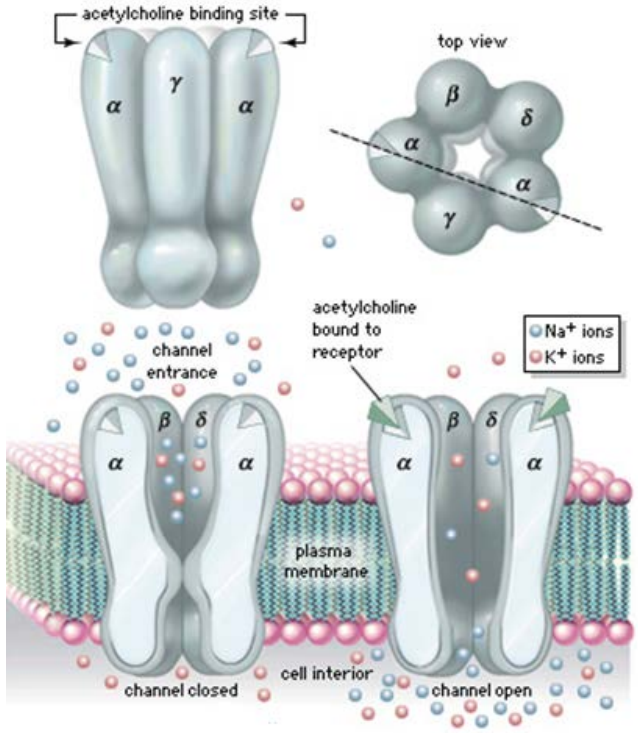


Figure 12: A model for the structure of the ion channel

Ion channels are membrane proteins. Typically they are oligomeric complexes of several subunits. The majority of channels have three, four, or five homologous or identical subunits,

arranged in circular symmetry, forming a single aqueous pore at the axial intersection. Regulation of ion transport is key for all cells. Specifically, ion channels contribute to the electrical excitability of muscle cells and signaling in the nervous system.

2.5.1 Function

Ion channels serve three principal physiological roles, they are

i) Ion channels set up the resting membrane potentials of all cells. Since the flow of ions moves charge and constitute an electric current, channel opening and closing underlie all electrical signaling of electrically excitable cells such as nerve and muscle. Thus, when open, potassium ion-selective channels and anion channels hyperpolarize the cells (cause the membrane potential to become more negative), whereas sodium- or calcium-selective channels and non-selective cation channels depolarize the cells (cause the membrane potential to become more positive).

ii) Flux of ions through ion channels contributes to the electrolyte movements required for volume regulation of single cells and for the net polarized transport of salt across epithelia like gut, kidney, or the choroid plexus.

iii) A few ions, notably Ca^{2+}, make regulatory signals inside cells. Cytoplasmic calcium signals are generated by the opening of Ca^{2+}-permeable ion channels that let Ca^{2+} ions flow into the cytoplasm. The Ca^{2+} may come from the extracellular medium or from intracellular organelles.

The ability of ion channels to accomplish these three physiological functions also requires the housekeeping operation of another class of membrane proteins, the transporters and pumps, to set up standing ion concentration gradients across cell membranes. Ion concentration gradients and electrical forces drive the flow of ions through channel pores.

Conceptually three significant functional domains of all ion channels are:

Ion conducting pore

An aqueous pathway for ions with a narrow selectivity filter that distinguishes among the ions that do go through and the ions that does not

Gates

A part of the channel that can open and close the conducting pore

Sensors

Detectors of stimuli that respond to electrical potential changes chemical signals. The sensors couple to the channel gates to control the probability that they open or close.

2.6 Selectivity of ion channels

Ion channels must allow the passage of ions but exclude other hydrophilic molecules. Clearly, the size of the central pore plays a role in selectivity. Ion channel pores are extremely narrow for example; Na^+ and K^+ channels have the smallest pores, from 3-5 Å.

Ion channels also discriminate between cations and anions. The distribution of charged amino acids along the outside of a channel's contributes to charge selectivity. When the channel is open, any cation with a diameter of less than 65 nm (which includes Ca^{2+}, K^+ and Na^+) has the potential to pass through it.

Some channels are exquisitely selective for particular ions. For example, K^+ leak channels discriminate between K^+ and Na^+ by a factor of 10,000, despite both cations being of similar size (Na^+ diameter = 0.95 Å, K^+ diameter = 1.33 Å).

The channel contains what is termed a selectivity filter (Figure 17). The filter is an area of the channel that is lined by carbonyl oxygen atoms from the polypeptide backbone. In order to pass through the selectivity filter, an ion must shed its coat of water (all ions are surrounded by a shell of water molecules) and interact with the carbonyl atoms. The filter is large enough to accommodate a dehydrated K^+ ion or a dehydrated Na^+ ion. However, only K^+ ions are large enough to be able to contact the carbonyl oxygens. This energetically favorable interaction balances the energy lost in removal of the waters from the ion. In contrast, when Na^+ loses its surrounding waters, it is too small to intimately contact the wall of the channel. It is energetically more favorable for Na^+ to retain its shell of water and not pass through the filter.

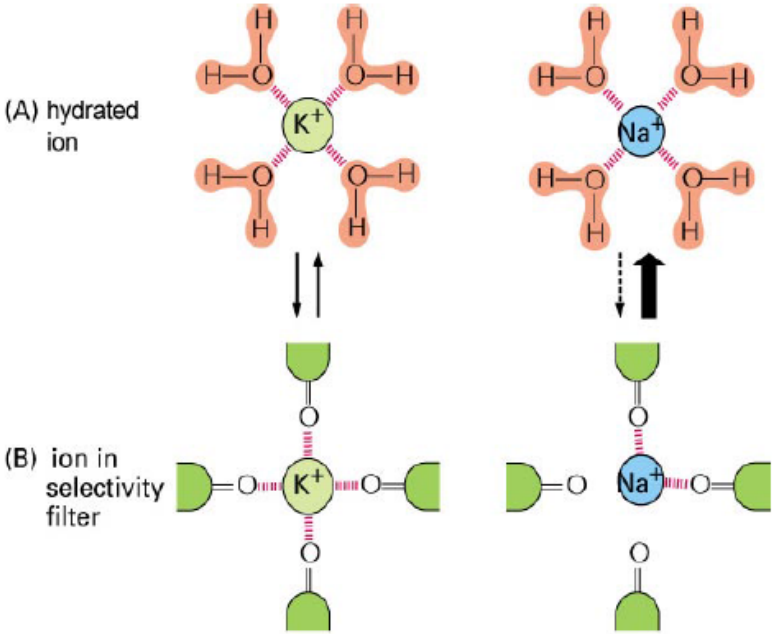


Figure 13: K^+ specificity of the selectivity filter of a K^+ channel. (A) Na^+ and K^+ ions hydrated with an outer shell of water. (B) The selectivity filter of the pore, viewed in cross section.

2.7 Gating of ion channels

In most cases, the central pore of an ion channel is not continuously open, and the regulation of the open versus closed state is crucial to maintaining the balance of ions in a cell. Thus, ion channels are gated ie., they can switch between an open and closed state by a change in conformation. The open state has an extremely short lifetime, typically a millisecond. The conformational change can occur in response to several types of stimuli:

Voltage-gated channels respond to changes in voltage across the membrane,

ligand-gated channels respond to binding of another molecule to the channel itself, and mechanically-gated channels respond to physical stimuli (Fig. 18).

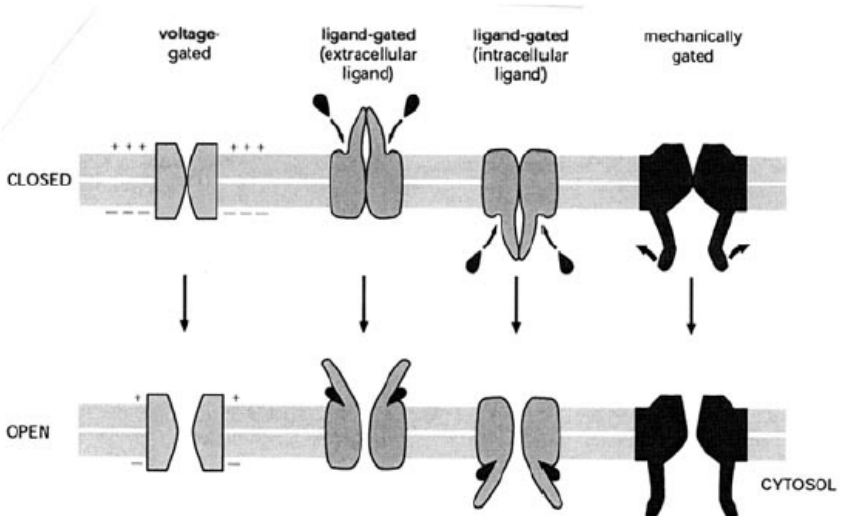


Figure 18: The gating of ion channels

2.8 Sodium and Potassium pump

Cells are enclosed by a membrane about 7000 pm ($70A^0$) thick and composed of double layers of protein separated by lipids. Cations cannot pass through the lipid layer without encapsulation and thus the enclosed cation presents an organic, lipid-soluble surface to the membrane.

In most animal cells the concentration of K^+ is about 0.15 M and that of Na^+ is about 0.0 1 M. In the fluids outside the cells the concentration of Na^+ is about 0.15 M and that of K^+ is less than 0.004 M. Maintenance of these large-concentration gradients requires a "sodium pump".

The sodium-potassium pump, also known as the Na,K-ATPase, a member of the P-type class of ATPases, is a critical protein found in the membranes of all animal cells. It functions in the active transport of sodium and potassium ions across the cell membrane against their concentration gradients.

The energy of transport of the ions is provided by the hydrolysis of ATP. Kidney and brain cells use about 70% of the energy from ATP for this transport. In some cells, each ATP

molecule hydrolyzed transports $3Na^+$ out of the cell and $2K^+$, (+ H^+) into the cell. The K^+ is required in the cell for glucose metabolism, protein synthesis, and activation of some enzymes. The transport of glucose and amino acids into the cell is coupled with Na^+ transport, which is favored by the great concentration gradient. The Na^+ entering the cell in this way must be pumped out again.

The membrane enzyme Na/K-ATPase serves as an Na^+ /K^+ pump. It is thought that the large protein spans the membrane with internal and external sites for both Na^+ and K^+. When the outer sites are filled with K^+ and the inner ones with Na^+, a confrontational change occurs rotating the sites and alternative g the site preferences, This releases K^+ inside the cell and Na^+ outside the cell. This is a "revolving door" for transport of Na^+ and K^+ in opposite directions. The pump is reversible if there is a large enough concentration gradients for Na^+ /K^+.

The sodium-potassium pump creates an electrochemical gradient across cell membranes. The electrical gradient, created by the outflow of more positive sodium ions than the inflow of positive potassium ions, resulting in a relatively negatively charged cytoplasm,is used in neurons and muscles to create the action potentials responsible for nervous system function and muscular contraction. The chemical gradient, which is created by the higher concentration of sodium ions in the extracellular space as opposed to the cytoplasm, results in the tendency of sodium ions to flow down their concentration gradient and back into the cytoplasm through other transmembrane proteins. Cells use the chemical gradient to transport essential nutrients such as glucose and amino acids into the cell in a process called secondary active transport

The operation of the ion pump was demonstrated by Jens Skou by an enzyme of molar mass about 110 kg mol^{-1} that hydrolyzes ATP only if Na^+ and K^+ are present in addition to the Mg^{2+} required by all ATPaes. The activity of this enzyme correlates quantitatively with the extent of ion transport. Another clue came from the observation that this ATPase is phosphorylated at an aspartate site only in the presence of Na^+ and Mg^{2+} ions. Moreover, the phosphorylated product is hydrolyzed if K^+ ions are present. It was also observed that the enzyme undergo conformational change when it is phosphorylated.

Using these the mechanism outlined in below figure was proposed.

1. In conformation E_1, three sodium ions bind to the pump from the cytoplasmic side of the phospholipid bilayer membrane.

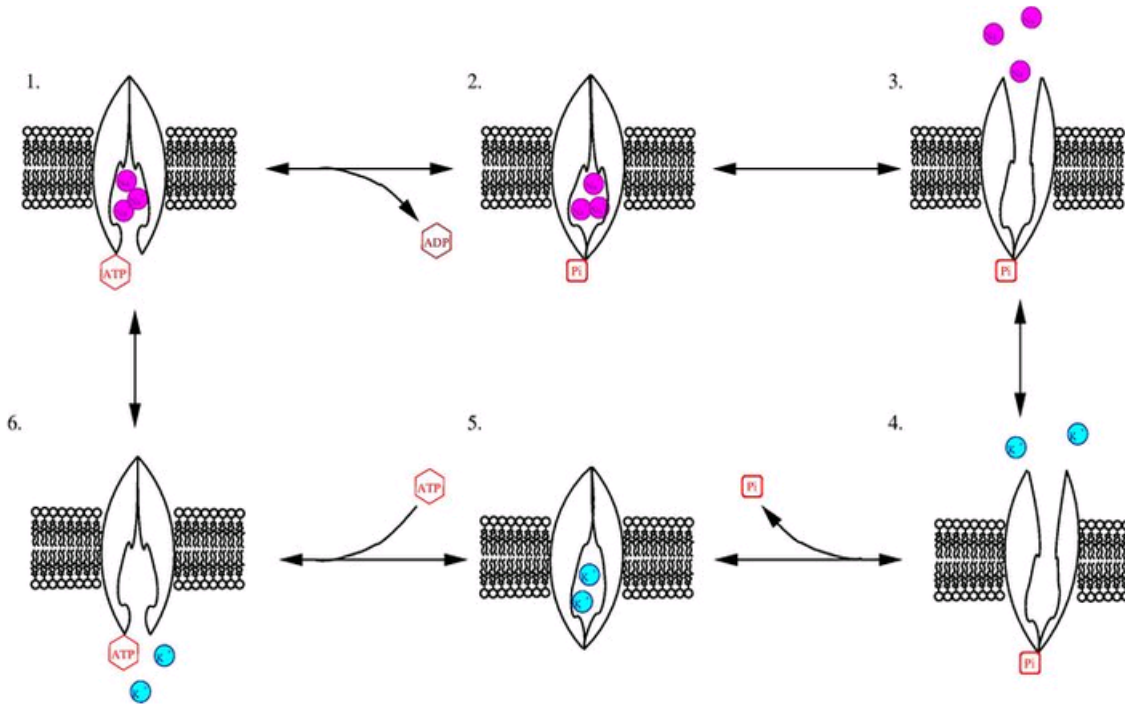
2. ATP phosphorylates the enzyme, as indicated by the P attached to the cytoplasmic side of the pump, and the remaining ADP is released.

3. The conformation then changes to E_2, and the three sodium ions are released into the extracellular space.

4. Two potassium ions bind to the pump from the extracellular side.

5. The pump is dephosphorylated and returns to conformation E_1.

6. ATP binding occurs and the potassium is released into the cytoplasm.



First, ATP and three Na^+ ions bind to the inside of the membrane and enzyme is phosphorylated. The product of the reaction undergoes a conformational change called **eversion,** which is like motion through a revolving door, that brings the bound Na^+ ions to the outside the cell membrane. There the three Na^+ ions are replaced by two K^+ ions. The attachment of the K^+ ions triggers dephosphorylation and the loss of the ATP induces a conformational change that carries the two K^+ ions into the interior of the cell, where they are released. The process builds up a charge gradient across the, membrane because three Na^+ ions are expelled for two K^+ ions incorporated, and the outer surface becomes relatively positively charged.

This mechanism is supported by the fact that the minute concentrations (10^{-9} M) of vanadate ions (VO_4^{3-}) can inhibit the operation ATPase. The difference between phosphate and vanadate ions is that the five-coordinate phosphate intermediate formed when water attacks is unstable and readily breaks down to completing hydrolysis. However there coordinate species formed by vanadate does not break down thereby the conformational change does not endure, therefore the bound K^+ ions remain on the outside of the cell membrane.

2.9 The selectivity of the process

The selectivity of complication between K^+ and Na^+ is uncommon in simple systems. But the enzyme can be selective first to Na^+ and then to K^+ in its different conformations. The Group 1 captions are usually not strongly complexed, so selectivity in simple systems is mainly dependent upon columbic forces and differences in ionic radii. Thus, basses that are stronger than H_2O will bind preferentially to the smaller, hard acid Na^+, and bases that are weaker than H_2O will bind preferentially to K^+ because displacement of H_2O from this soft cation is easier. However, complexes formed with ligands that are weaker bases than H_2O will be very weakly bound so it is difficult to see how to make a practically and selective for K+ ions. One possible solution to the problem is revealed by another biochemically important ligand. It is known that crown ethers that are antibiotically active increase the permeability of cell membranes to cations. The chelating ligand binds both Na^+ and K^+ ions strongly but favors K^+ by a factor of nearly 100. The origin of the selectively is stereo chemical, for a K^+ ion fits nearly into the cavity in the hollow crown. Thus selectivity between Na^+ and K^+ can be achieved by a ligand that is able to adopt conformations providing a chelating site of exactly the right size.

2.10 Summary of the unit

Cell membranes have special mechanisms for transporting molecules and ions from outside the cell into its interior. In particular, the distributions of the ions Na^+, K^+, and Mg^{2+} are controlled by pumps and channels in the membrane of cells. Since these three species of ion are major components of the cell, their location establishes the pattern of charge distribution and columbic fields. That iron is located in specific regions of membranes suggests that it has a highly specific role there, and one of its principal roles is to control the distribution of protons and electrons.

Electrochemical potential gradients across membranes that stem from the presence of O_2 as oxidizing agent are controlled by systems that exploit both manganese and iron. The electric fields produced by the ion gradients are of great biological significance, because as well as controlling ATP synthesis, they influence cell volume, render nerve and muscle cells excitable, and drive the transport of sugars and amino acids across the membrane. The control of physical motion and mechanical stress (e.g. in muscle) are associated with the distribution of zinc, calcium, and magnesium. Zinc is found in the nucleus and is a characteristic metal ion of the genetic apparatus. The relationship between several transport mechanisms can be observed at the neuromuscular junction when a nerve impulse stimulates a muscle cell to

contract. The response requires the action of at least five sets of ion channels, sequentially activated as follows.

1. The nerve impulse reaches the nerve terminal and depolarizes the plasma membrane of the terminal. This opens voltage-gated Ca^{2+} channels in this membrane. Ca^{2+} flows into the nerve terminal, which triggers the localized release of acetylcholine into the synaptic cleft.

2. The local depolarization of the muscle cell plasma membrane opens voltagegated Na^{+} channels in this membrane, allowing more Na^{+} to enter. This opens neighboring voltage-gated Na^{+} channels, creating a large depolarization (or action potential) that spreads to involve the entire plasma membrane.

3. The depolarization of the entire plasma membrane results in the opening of Ca^{2+} channels in the sarcoplasmic reticulum. Ca^{2+} then rushes into the cytosol, and its sudden increase in concentration triggers a series of events that cause the muscle cell to contract. To end the process of contraction, Ca^{2+} must be removed from the cytosol of both the nerve terminal and the muscle cell. In both cells, the Na^{+}/Ca^{2+} exchanger pumps Ca^{2+} out of the cell using the energy of the Na^{+} gradient. The Na^{+}/K^{+}-ATPase pumps. Na^{+} back out of the cell, maintaining the Na^{+} gradient. In the muscle cells, the Ca^{2+}-ATPase, located in the membrane of the SR, pumps Ca^{2+} from the cytosol back into the SR using the energy of ATP hydrolysis.

2.11 Key words

ABC transporter; Electrochemical gradient; Active transport; Membrane transport protein; ATP-driven pump Na+/ K+ -ATPase; Carrier proteins; Passive transport; Channel proteins; Concentration gradient; Coupled transporter;

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10) Bioinorganic Chemistry -- Inorganic Elements in the Chemistry of Life: An Introduction and Guide; Wolfgang Kaim, Brigitte Schwederski, Axel Klein; *John Wiley & Sons*, **2013**.

2.13 Questions for self understanding

1) Explain the various mechanisms for ions to getting in and out of cells

2) Write a note on

i) Lipid diffusion (the major means of drug absorption and permeation)

ii) Membrane transport proteins (carriers and channels)

3) Discuss ATP-driven ion pumps

4) What are coupled transporters? Explain their role in ion transporting

5) Discuss the following with example

i) Symport

ii) Antiport

6) Write a note on ABC transporters

7) What are passive carriers? Give example and explain how they transport ion

8) What are ion channels? Explain their function

9) Explain the selectivity of ion in ion channels

10) Discuss the gating of ion channels

11) Explain Sodium and Potassium pump and the selectivity of the process

UNIT-3

Structure

3.0 Objectives of the unit

After studying this unit you are able to

- List the metal deficiency and related disease
- Explain the detoxification therapy
- Explain the Chelation therapy
- Explain the mechanism of blood clotting
- List the different metals used in medicine
- Identify the metal complexes as anticancer agents
- Identify the metal complexes used in antiarithritic drug

3.1 Introduction

Metal ions are required for many critical functions in humans. Scarcity of some metal ions can lead to disease. Well-known examples include pernicious anemia resulting from iron deficiency, growth retardation arising from insufficient dietary zinc, and heart disease in infants owing to copper deficiency. The ability to recognize, to understand at the molecular level, and to treat diseases caused by inadequate metal-ion function constitutes an important aspect of medicinal bioinorganic chemistry.

Metal ions can also induce toxicity in humans, classic examples being heavy metal poisons such as mercury and lead. Even essential metal ions can be toxic when present in excess. Understanding the biochemistry and molecular biology of natural detoxification mechanisms, and designing and applying ion-specific chelating agents to treat metal overloads, are two components of a second major aspect of the new science that is evolving at the interface of bioinorganic chemistry and medicine. Metal ions are required in biology is their role as pharmaceuticals. Two major drugs based on metals that have no known natural biological function, Pt (cisplatin) and Au (auranofin), are widely used for the treatment of genitourinary and head and neck tumors and of rheumatoid arthritis, respectively. This unit introduces three broad aspects of metals in medicine: nutritional requirements and diseases related thereto.

3.2 Metal deficiency and disease

We know that our living body is made up of fifty many compounds such as proteins, aminoacids and they are possesting various elements. Aboutfifty to sixty occurring elements has been detected in living system. Four main group (Na, K, Mg, and Ca) and ten transition (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, and Cd) metals are currently known or thought to be required for normal biological functions in humans. Below table lists these elements, their relative abundances, and the medical consequences of insufficient quantities where known.

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Essential metals and medical consequences resulting from their deficiency.

Metal	Abundance: Sea Water mg/1 (ppm)	Abundance: Earth's Crust mg/1 (ppm)	Diseases resulting from metal deficiency
Na	1.05×10^4	2.83×10^4	
K	380	2.59×10^4	
M-	1.35×10^3	2.09×10^4	
Ca	400	3.63×10^4	bone deterioration
V	2×10^{-3}	135	
Cr	5×10^{-5}	100	glucose tolerance (?)
Mn	2×10^{-3}	950	
Fe	1×10^{-2}	5.00×10^4	anemia
Co	1×10^{-4}	25	anemia
Ni	2×10^{-3}	75	
Cu	3×10^{-3}	55	brain disease, anemia, heart disease
Zn	1×10^{-2}	70	growth retardation, skin changes
Mo	1×10^{-2}	1.5	
Cd	1.1×10^{-4}	0.2	

3.2.1 Sodium Ion

Salted foods and NaCl salt in cooking are the main source of sodium. The content of sodium is high in bread cheese, clams carrots, cauliflower, eggs, milk, nuts, spinach etc… 5 to 10g of NaCl is required daily for adults. Na+ is the major contains of blood .

Functions of sodium

It maintain O.P (Osmotic Pressure) of Body. It play important role to adsorption of glucose, aminoacids and galectose. It also associated with chloride and bicarbonate in regulator of acid base balance.

Sodium deficiency

Due to high environmental temperature extreme sweating may causes loss of sodium ion. This regard is muscular cramps of the abdomen, headaches. Thigh intake of sodium salt causes high blood pressure.

3.2.2 Potassium Ion

High content of K is present in chicken, beef liver, bananas, orange juice, pine apples and potatoes. About 4g of K per day is required for adults.

Functions of potassium ion

It is a principle cat ion of intracellular fluid ie, cytoplasma of cell. It increases the acitivity of muscles especially cardiac muscles. It maintains Osmotic pressure and acid base balance of the body. It increases the activity of glycolic enzymes, pyruvate kinase. It also regulates the he heart beat. It play important role to synthesis of ribosomes.

Deficiency of potassium ion

Potassium ion deficiency in the body causes depression and low activeness of cardiac and nervous system. It deficiency also causes muscular weakness (cramping of muscles)

3.2.3 Calcium ion

It present in milk, eggs, beans, nuts, figs cabbage, cauliflower and asparagus etc… it needs about 800mg darty of the age of 18^{th} for men and women and below 18^{th} age it require 1-1.2 gram per day.

Functions of calcium ion

It is a major constituent of bones and teeth. About 99% of the body calcium is in the skelton, where it is maintained as deposits of calcium phosphate is a soft fibrous matrix. Ionized calcium is of great importance in blood coagulation. It maintains the normal excitability of heart also.

Deficiency of calcium

The low concentration of calcium causes irritation, weakness of bones in children and causes Osteoporosis in adults.

3.2.4 Copper ion

The main sources of copper are nuts, fish, cow milk etc…. The adult required 2.5mg per day of copper. Infant and children required 0.5mg of copper perday.

Function of cooper

It is essential constituents of many proteins metallo enzyme and some naturally occurring pigments. It is also essential for formation of hemoglobin and normal bone formation.

Deficiency of copper

Deficiency effect of copper has never been positively demonstrated but it has been suspected in case of spours or in nephrosis.

3.2.5 Magnesium ion

It is present in cocoa various nuts, soyabean and sea foods. It also presents in beans peas in small quantities. It need about 300mg/day for adult womwn and 350 mg/day for adult men.

Mg^{2+} ion is one of essential cation of soft tissue. The body contains about 21mg 300mg of magnesium, 70% combined with calcium and phosphorus is complex salt of bones. In muscles and other tissue intra cellular Mg^{2+} ions probably functions as activator of phosphates group transfer enzyme.

3.2.6 Iron ion

The best dietary sources of iron are meats, liver, heart, egg York, wheat, molasses, apples, bananas etc….. For children of 1-8yrs 15mg per day of iron is required, for adult 10mg and

for female 18mg per day is required. After 51 years of age 10mg per day of iron is required for all.

Function of iron

Iron is required for tissue growth and blood hemoglobin. The role of iron is almost confined to the pressure of cellular respiration. Iron porphysin groups are essential component of hemoglobin and myoglobin which is responsible for intake of oxygen and circulation of oxygen, then also removal of CO_2.

Deficiency of iron

Iron deficiency especially in women after the 45 age causes black dot spot on skin and it is due to low concentration of H.B. Iron deficiency leads anemia type of hypo chronic and microcytics type. Low concentration of Fe causes slower rate of formation of RBC. It develops the disease with pale skin. Fe deficiency causes low energy palpitation and shortness of breath.

3.2.6.1 Anemia and Iron

Anemia results from insufficient oxygen supply, often because of a decrease of hemoglobin (Hb) levels in blood. Approximately 65 to 70 percent of total body iron resides in Hb. In the body metals are adequately absorbed by cells, appropriately stored, and ultimately inserted into the proper environment to carry out the requisite biological function. For iron, these tasks are performed by specific iron-chelating agents, the storage protein ferritin and the transport protein transferring.

3.2.6.2 Iron toxicity

Acute iron poisoning is resulting from accidental ingestion of $FeSO_4$ tablets. This results in corrosion of the gastrointestinal tract. Chronic iron poisoning, or hemochromatosis, arises from digestion of excess iron usually supplied by vessels used for cooking. Chelation therapy is used to treat iron overload. The chelating agent of choice for iron toxicity is the siderophore desferrioxamine, a polypeptide having a very high affinity for Fe(III) but not for other metals. Ferrioxamine chelates occur naturally in bacteria as iron-transport agents.

3.2.7 Zinc ion

The main sources of zinc metal are liver, egg, sea foods, milk, whole grains etc…. about 15mg of zinc is required per day for adult.

Zinc is essential for normal growth. It is essential for tissue repairing and wound healing. It is essential components of enzymes present in human body such as alcohol dehydrogenace, alkaline, phosphate carbonic anhydrase. It maintains normal concentration of vitamin A in plasma and the function of insulin is also regulated with zinc.

3.2.7.1 Causes and Consequences of Zinc deficiency

The average adult contains ~ 2 g of zinc and requires a daily intake of 15 to 20 mg, only half of which is absorbed, to maintain this level. Although food in many technologically advanced societies contains sufficient zinc to afford this balance, zinc deficiencies occur in certain populations where there is either an unbalanced diet or food that inhibits zinc absorption. Zinc deficiency produces growth retardation, testicular atrophy, skin lesions, poor appetite, and loss of body hair. Little is known about the biochemical events that give rise to these varied consequences, although the three most affected enzymes are alkaline phosphatase, carboxypeptidase, and thymidine kinase. About 30 percent of zinc in adults occurs in skin and bones, which are also likely to be affected by an insufficient supply of the element. Zinc deficiency is readily reversed by dietary supplements such as $ZnSO_4$, but high doses (>200 mg) cannot be given without inducing secondary effects of copper, iron, and calcium deficiency.

3.3 Copper deficiency

More copper is found in the brain and heart than in any other tissue except for liver, where it is stored as copper thionein and released as ceruloplasmin or in the form of a complex with serum albumin. The high metabolic rate of the heart and brain requires relatively large amounts of copper metalloenzymes including tyrosinase, cytochrome c oxidase, dopamine-{3-hydroxylase, pyridoxal-requiring monamine oxidases, and Cu-Zn superoxide dismutase. Copper deficiency, which can occur for reasons analogous to those discussed above for Fe and Zn, leads to brain disease in infants, anemia (since cytochrome oxidase is required for blood formation), and heart disease. Few details are known about the molecular basis for copper uptake from foods.

3.4 Toxic effects of metals

The presence of excess quantities of an essential metal can be as deleterious as insufficient amounts. This situation can arise from accidental ingestion of the element or from metabolic disorders leading to the incapacitation of normal biochemical mechanisms that control uptake and distribution phenomena. These possibilities constitute one major class of metal toxicity. The other broad classes results from entry of nonessential metals into the cell through food, skin absorption, or respiration. The toxicities associated with latter class have cause much risks for public health, for example; chemical and radioisotopic environmental pollutants. *The study of bioinorganic chemistry can contribute to the removal of these toxic metals and restoration of normal function. One way involves chelation therapy, in which metal-specific chelating agents are administered as drugs to complex and facilitate excretion of the*

unwanted excess element. For example; the use of desferrioxamine to treat iron poisoning is one example of this approach.

A second way is to identify fundamental biological mechanisms that regulate metal detoxification, and to apply the principles that emerge to help control the toxic effects of metal ions in the environment. The study of mercury resistance and detoxification in bacteria provide an elegant example of the way in which biochemistry and molecular biology can be used to elucidate events at the molecular level.

3.5 Copper overload and Wilson's disease

Wilson's disease is a rare inherited disorder that causes too much copper to accumulate in your liver, brain and other vital organs. Wilson's disease results from a genetically inherited metabolic defect in which copper can no longer be tolerated at normal levels. The clinical manifestations are liver disease, neurological damage, and brown or green (Kayser-Fleischer) rings in the cornea of the eyes.

Copper plays a key role in the development of healthy nerves, bones, collagen and the skin pigment melanin. Normally, copper is absorbed from your food, and any excess is excreted through bile, a substance produced in our liver.

But in people with Wilson's disease, copper isn't eliminated properly and instead accumulates, possibly to a life-threatening level. This is because patients suffering from Wilson's disease have low levels of the copper-storage protein ceruloplasmin. The gene and gene products responsible for the altered metabolism have not yet been identified. Chelation therapy, using $K_2Ca(EDTA)$, the Ca^{2+} ion being added to replenish body calcium stores depleted by EDTA coordination, 2,3-dimercaptopropan-I-ol (BAL), or d-penicillamine to remove excess copper, causes the symptoms to disappear. The sulfhydryl groups of the latter two compounds presumably effect removal of copper as Cu(I) thiolate complexes.

3.6 Toxic effects of other essential metals

When present in concentrations above their normal cellular levels, most of the other metals listed in above table are toxic. Calcium levels in the body are controlled by vitamin D and parathyroid hormones. Failure to regulate Ca^{2+} leads to calcification of tissue, the formation of stones and cataracts. Chronic manganese poisoning which can occur following ingestion of metal-oxide dust, e.g., miners suffers neurological symptoms similar to Parkinson's disease. The Zn toxicity is rare, but it can lead to deficiencies in other essential metals, notably calcium, iron, and copper. Cobalt poisoning leads to gastrointestinal distress and heart failure. Metal poisoning by those elements has been treated by chelating agents, most frequently

$CaNa_2$(EDTA), but the selectivity offered by the ferrioxamine class of ligands available for iron has not even been approached. Fortunately, there are few cases involving these metals.

3.6.1 Mercury toxicity and bacterial resistance

Mercury is released into the environment as Hg(II) ions through weathering of its most common ore HgS and red cinnabar. Organomercurials of general formula RHgX used in agriculture have also entered the environment as toxic waste. Both RHgX and HgX_2 compounds bind avidly to sulfhydryl groups in proteins, which can lead to neurological disease and kidney failure. Metallothionein is a favored protein target, which may help to limit mercury toxicity. The volatile, elemental form of mercury, Hg(0) is reportedly nontoxic, but its conversion to alkylmercury compounds by anaerobic microorganisms utilizing a vitamin B-12 biosynthetic pathway constitutes a serious health hazard.

3.6.2 Cadmium and Lead toxicity

Gastrointestinal, neurological, and kidney toxicity are among the symptoms experienced by acute or chronic exposure to these heavy metals. The use of unleaded gasoline and the removal of lead-containing pigments from paint have substantially diminished the quantity of this element released to the environment each year. Cadmium sources include alkaline batteries, pigments, and plating. Lead poisoning can be treated by chelation therapy using $CaNa_2$(EDTA) (acute) or penicillamine (chronic). Although both Cd(II) and Pb(II) bind to sulfhydryl groups in thionein.

3.7 Detoxification therapy

Detoxification is the removal of toxic materials that have accumulated in the body. These materials are called toxic because they interfere with the activity of the cells. Toxins come from two sources. First of all, humans are by their very metabolisms producers of toxins. These toxins are byproducts of normal human metabolism. The other source of toxins is the environment. They can come in the form of pesticides, artificial chemicals in our foods, chemicals that we place on our bodies or inhale. They also include many drugs. The worst of all of the environmental toxins are the heavy metals.

Heavy metals refer to certain toxic minerals that attach so firmly to cells that they cannot easily be removed. Because of this they will accumulate over time. Up on prolonged years body accumulated a substantial amount of heavy metals. As soon as the levels get high enough they start to interfere with healing and cause disease. The main heavy metals are lead, mercury, arsenic, cadmium, and uranium.

Detoxification refers to stimulating the liver, kidneys, lungs, skin, and lymphatic system to remove the toxins. This can be done by using Hydrozone colon therap, Ozonated sauna

therapy, Major autotherapy with ozone, bowel detoxification herbs, exercise, ionic foot baths, Chelation therapy, and Rife therapy.

3.8 Chelation therapy

Chelation therapy is a treatment used in conventional medicine for removing heavy metals from the blood. It involves intravenous injections of a chelating agent, for example; EDTA (ethylene diamine tetra-acetic acid). EDTA binds to heavy metals and minerals in the blood so that they can be excreted in the urine. Another intravenous agent used by some physicians for mercury detoxification is called DMPS (2,3-Dimercapto-1-propanesulfonic acid).

An oral chelating agent called Succimer (Dimercaptosuccinic acid, also known as DMSA) is FDA-approved for treatment of lead poisoning and is used to remove mercury from the body. The drug combines with metals in the bloodstream and then both the metals and the drug are removed from the body by the kidneys and then excreted. Chelation therapy as following advantages

i) EDTA chelation therapy might directly remove calcium found in fatty plaques that block arteries, thus breaking up the plaques.
ii) Chelation therapy may stimulate release of a hormone that in turn causes calcium to be removed from plaques or causes a lowering of cholesterol levels.
iii) Chelation therapy may reduce the damaging effects of oxygen ions (oxidative stress) on the walls of the blood vessels, which could reduce inflammation in the arteries and improve blood vessel function.

Despite it has a common side effect include diarrhea, loose stools, nausea and vomiting, poor appetite and skin rash.

3.9 Biochemistry of Calcium

Calcium is the major cation in the structural materials, bone and shell, where its role in these hard tissues stems from the insolubility of its carbonates and phosphates, such as calcite, $CaCO_3$, and apatite $CalO(PO_4MOH)_2$.

However, Ca^{2+} ions also have following diverse non-structural role

1) They function as messengers for hormonal action
2) In the triggering of muscle contraction and nerve signals
3) In the initiation of blood clotting, and
4) In the stabilization of protein structures.

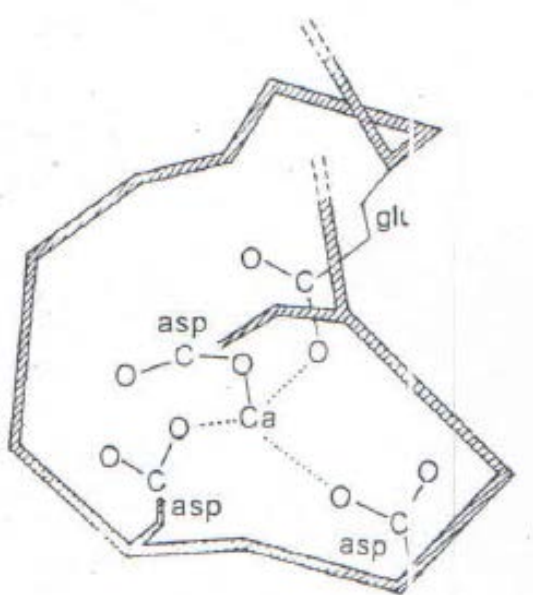
This diversity is despite the fact that the Ca^{2+} ion is not known for forming robust complexes with simple ligands in solution. It mean that, as in the case of Na^+ and K^+, ligands are involved that differ significantly from the familiar simple ones

One muscle protein has a formation constant of approximately 5 x 10^4 for Ca^{2+} binding. The same site can also bind other metal ions, and the order of formation constants is

$$Ca^{2+} > Cd^{2+} > Sr^{2+} > Mg^{2+}$$

Since the site favors Ca^{2+} over Cd^{2+}, it must contain fairly hard ligands. On the other hand, simple electrostatic considerations would lead us to expect that Mg^{2+} should be bound most strongly since it is the smallest of the ions. It is most plausible that a hard site precisely tailored in size to chelate the Ca^{2+} ion exists.

Calcium binding proteins are typically rich in aspartate (asp) and glutamate (glu), both of which have carboxylate groups as side chains and hence can act as hard anionic ligands. As an illustration of the kind of structure that occurs, one Ca^{2+} ion binding site that has been characterized by X-ray diffraction consists of four carboxylate O atoms in a distorted tetrahedral array as shown in below figure.



Initiation of muscle contraction results from the arrival of a nerve impulse at a motor ending in a fiber. This causes Ca^{2+} to be released from the sarcoplasmic reticulum (SR) of the muscle. The concentration of Ca^{2+} is increased by 100-fold in milliseconds by release of the SR. On relaxation of the muscle the Ca^{2+} is reabsorbed by SR. This process requires many cycles of a Ca pump identified as a Ca^{2+} /Mg^{2+}-ATPase similar to the sodium pump.

Among the most vital functions ofCa^{2+} is its involvement in enzymatic systems, including its being a regulator of muscle contraction, a transmitter of nervous pulses, and an agent of blood coagulation.

Calcium finds its way into the body with food in the form of neutral. The acidic medium in the digestive tract transforms this phosphate to 'the readily soluble acid phosphates $CaHPO_4$

and $Ca(H_2PO_4)_2$. It is precisely the acid phosphates that are absorbed in the intestine and penetrate the blood plasma.

The concentration of Ca^{2+} ions in human blood usually ranges from 0.0022 to 0.0028 mole/liter. Roughly one half of this calcium is in the form of aquo-ions capable of permeating membranes. The rest is bound with protein (albumin) and does not pass across membranes.

Along with K^+ and Mg^{2+}, the Ca^{2+} ions affect the rate of muscular contraction including the cardiac muscle, and also the action of cardiac glycosides (of the digitalis type). An overdose of glysosides leads to cardiac arrest. Injection of K^+ and Mg^{2+} ions into the cardiac muscle mitigates the action of glycosides, while that of' Ca^{2+} enhances it. However, Ca^{2+} ions may be bound into a stable complex, for example with EDTA. If EDTA is injected into the muscle of the arrested heart when the time is right, the heart resumes its beat.

When blood vessels are cut or damaged, the loss of blood from the system must be stopped before shock and possible death occur. This is accomplished by solidification of the blood and the process is called coagulation or clotting. Whenever our blood vessel broken, the sticky platelets present in the blood form clots and stop the blood flow. Calcium works together with vitamin K and a protein called fibrinogen in the clotting cascade. Without adequate levels of calcium and vitamin K, blood will take longer to clot, and if both nutrients are missing you might bleed to death. A blood clot consists of

1) A plug of platelets enmeshed in a

2) Network of insoluble fibrin molecules.

Platelet aggregation and fibrin formation both require the proteolytic enzyme thrombin. Clotting also requires calcium ions (Ca^{2+}) and about dozen other protein clotting factors. Most of these circulate in the blood as inactive precursors.

3.10 Mechanism of blood clotting

Clotting is cascade of complex enzymatic reaction. Each factor activates the many molecules of the next reaction. Clotting involves three major steps.

1) Step – I: Fromation of prothrombinase. It is formed in two pathways. One is the intrinsic pathway from platelets, and the other is the extrinsic pathway from damaged tissues. Intrinsic pathway is initiated by Hageman factor.

2) Step – II: Prothrombinase converts prothrombin into thrombin.

3) Step – III: Thrombin converts soluble fibrinogen into soluble fibrin. These fibres are stabilised by factor XIII as insoluble fibres.

Once a clot is formed, it plugs the ruptured area of the blood vessel and stops the bleeding of the blood. Clot reaction occurs later. It tightens the fibrin clot. The fibrin thread attached to

the damaged surface of blood vessels contract, pulling the damaged edges closer together. Platelets release factor-XIII, which stabilises and strengthens the clot.

Role of calcium ions in blood clotting

Calcium ions are required in both extrinsic and intrinsic pathways. Hence calcium ion removers are used in storing the blood in blood banks. EDTA or citrates and oxalates are used to remove calcium from the blood

Role of vitamin K in blood clotting

Vitamin K is required of the synthesis of factors II, VII, IX and X. These factors are synthesised by hepatic cells of liver.

Excess calcium adversely affects the body, giving rise to formation of stones (lithogenesis), deposition of salts, and so on. Since Ca^{2+} and Mg^{2+} ions are found in the tissues of bacterial cell walls, changes in the Ca^{2+} content in the system may kill microorganisms is effect is observed in-particular, if EDTA or another complexone of high dentation is introduced into system. As EDT A binds Mg^{2+} and Ca^{2+} into a stable complex, it destroys the walls of bacterial cells, killing microorganisms. The Ca^{2+} and other valuable ions may be washed out of the body when complex ones and chelating agents are used to remove ions of toxic metals such as Hg^{2+}, Pb^{2+}, and others. To minimize the washout the Ca^{2+} ions during treatment of certain diseases, EDT A is injected in the form of the calcium complex Ca $EDTA^{2-.}$

3.11 Metal interactions with DNA

Chemical interactions between ions and specific binding sites on DNA is important, since the presence of metal ions strongly affects the function of DNA in vivo as well as its stability and structure in vitro. Many metal ions control essential biological processes of living cells and without their catalytic presence many biological reactions would not take place. While Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} ions are important neurotransmitters, Zn^{2+} ions through the zinc finger play an important role in the regulation of DNA transcription and replication. On the other hand, the binding of metal ions to different sites on DNA stabilize or destabilize the secondary structure, but it can also cause a change in conformation. It is believed that many non-essential ions are even carcinogenic and mutagenic (e.g., Cd^{2+}, Hg^{2+} ions) which is a rather different consequence of metal-DNA interaction

3.12 Ligand interaction with DNA

DNA as carrier of genetic information is a major target for drug interaction because of the ability to interfere with transcription (gene expression and protein synthesis) and DNA replication, a major step in cell growth and division.

There are three principally different ways of drug-binding.

i) Through control of transcription factors and polymerases. Here, the drugs interact with the proteins that bind to DNA.

ii) Through RNA binding to DNA double helices to form nucleic acid triple helical structures or RNA hybridization (sequence specific binding) to exposed DNA single strand regions forming DNA-RNA hybrids that may interfere with transcriptional activity.

iii) Small aromatic ligand molecules that bind to DNA double helical structures by

a) Intercalating between stacked base pairs thereby distorting the DNA backbone conformation and interfering with DNA-protein interaction. These ligands are called intercalators

b) The minor groove binders. These cause little distortion of the DNA backbone. Both work through non covalent interaction.

DNA has a strong affinity for many heterocyclic aromatic ligands, such as acridine and its derivatives. Organic intercalators can inhibit nucleic acid synthesis in vivo, and they are now common anticancer drugs in clinical therapy.

The covalent attachment of organic intercalators to transition metal coordination complexes, yielding metallointercalators, can lead to novel DNA interactions that influence biological activity. Metal complexes with σ-bonded aromatic side arms can act as dual-function complexes, they bind to DNA both by metal coordination and through intercalation of the attached aromatic ligand. These aromatic side arms introduce new modes of DNA binding, involving mutual interactions of functional groups held in close proximity. The biological activity of both cis- and trans-diamine PtII complexes is dramatically enhanced by the addition of σ-bonded intercalators.

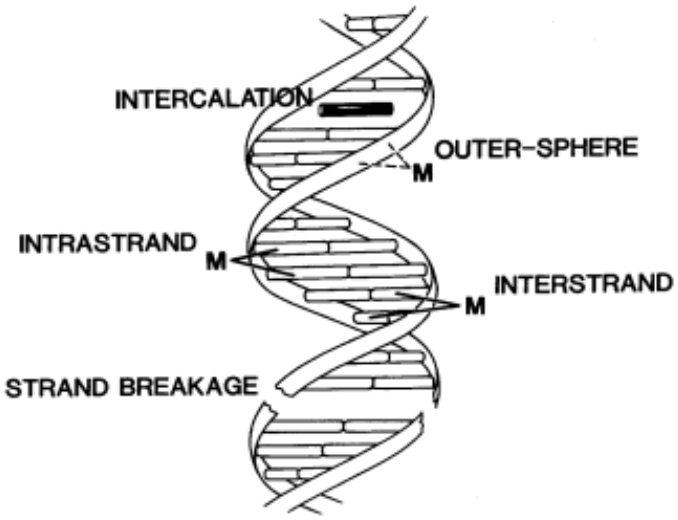
3.13 Metal Ligand interactions with DNA

The sequence and structure of our DNA is intrinsically linked to many biological processes those required for us to function. Certain diseases are associated with specific base sequences, or base-pair mismatches that sometimes occur during DNA replication. In particular, DNA mismatches have emerged as promising targets for biomedical diagnostics and therapeutics with carefully designed small molecules as the active agents.

Metal lignad can interact with DNA in two ways, they are

1) Covalently binding to inner-sphere
2) Non-covalently binding to outer sphere

Below figure illustrate the different modes of metal –ligand interactions with DNA

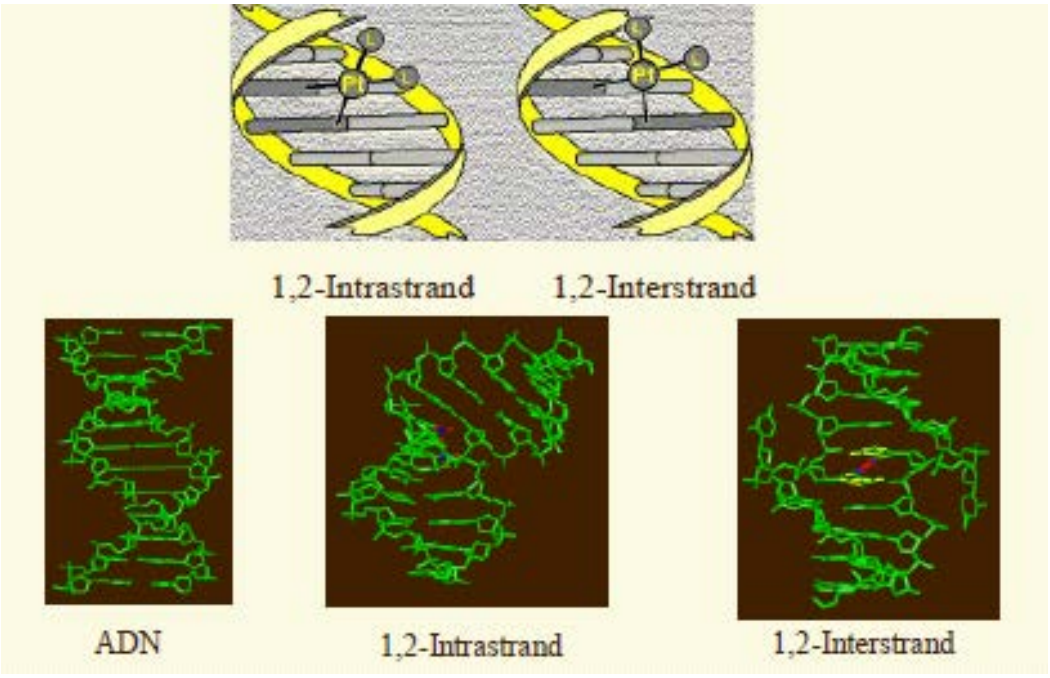


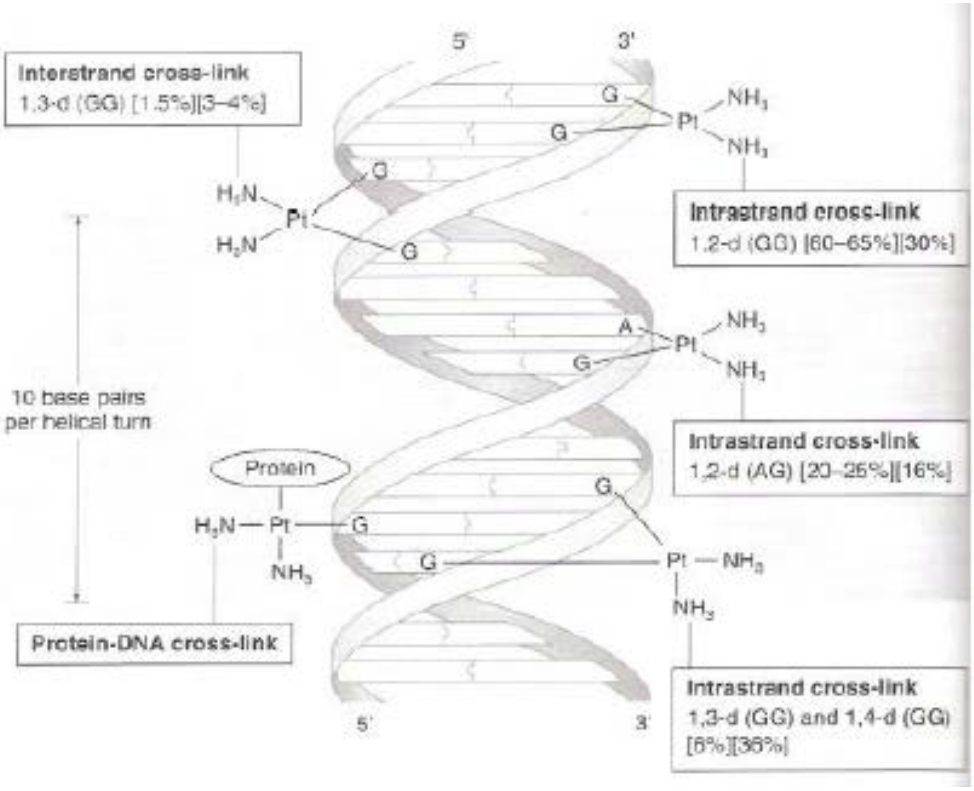
Schematic presentation of the modes of interaction of metal complexes with DNA.

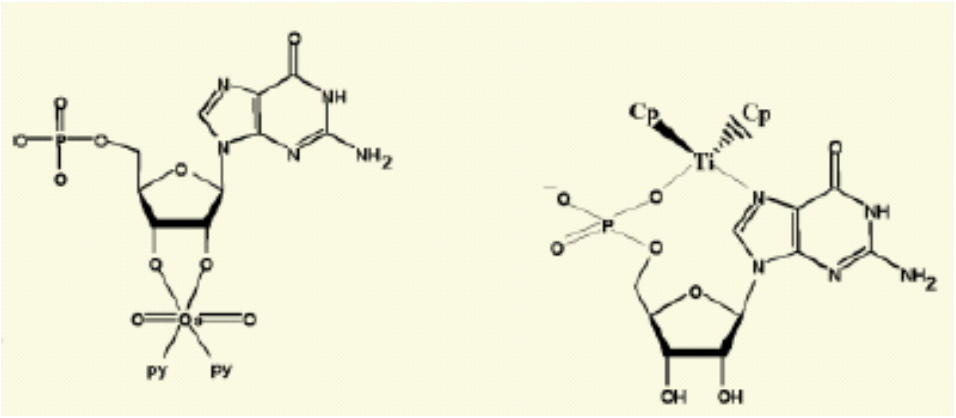
Covalently bindingthrough inner-sphere

This kind of interaction involves covalent bond formation of the metal complex to either the phosphate or the nucleic acid bases. In these case more than one metal-DNA bond is formed by cross linking, which can be either *intra-* or *inter*-strand.

Best-known molecule that binds to DNA is cis-diammine-dichloro-platinum(II) a simple square-planar complex that gained international recognition under the name *cisplatin*. It is worth noting that whereas the cis isomer has significant anticancer activity, its trans counterpart does not offering a glimpse into just how specific interactions with DNA, and effects on its biological role, are.





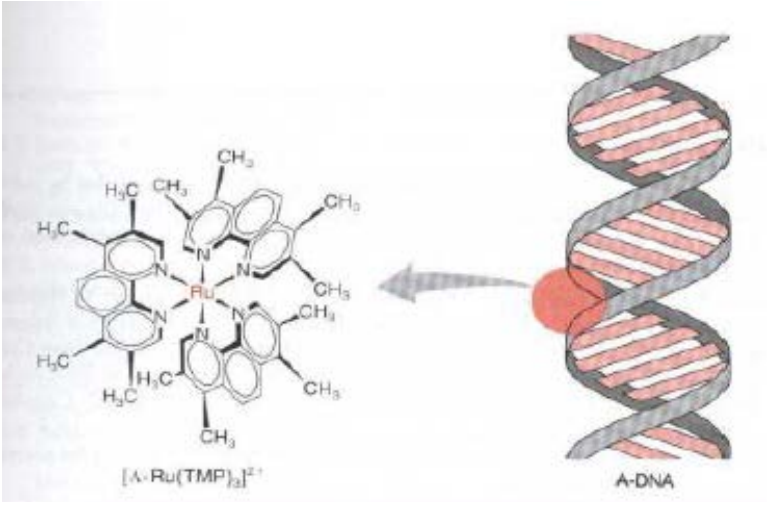


Covalently bindingthrough inner-sphere

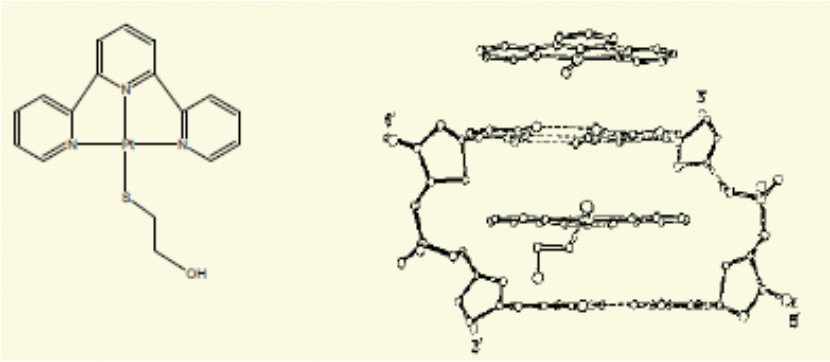
This kind of interaction involves hydrogen bonding, electrostatic interaction or minor and major groove interactions.

Negatively charged backbone of DNA interacts with positively charged molecules through electrostatic interactions or phosphate – oxygen binding. Exocyclic groups on the purines/pirimidines can be involved trough hydrogen bonding to suitable ligand atoms. This interaction depends on the nature and concentration of the metal.

Molecules that bind to DNA do not necessarily need similar shapes or composition. This is highlighted by another class of metal-containing compounds 'light-switch' polyazaaromatic ruthenium complexes which bind to DNA in a very noticeable manner.



The luminescence of these complexes is DNA dependent and their emission in aqueous solutions is often (albeit not always) switched on in the presence of DNA. Conveniently, it can even be enhanced if the DNA contains base-pair mismatches. This characteristic offers a very practical route to sensing, and holds the promise of being able to switch on a therapeutic effect through irradiation.



3.14 Metals in medicine

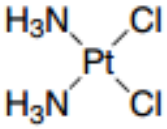
Radiodiagnostic agents

Metal complexes having radioactive nuclei find many applications in medicine, such as in organ, and tissue imaging. Early detection of cancer, for example, by selective and imaging of the tumor using a radioactive metal compound can facilitate surgical removal or chemotherapeutic treatment before the disease reaches an advanced stage. Radioisotopes used for diagnostic purposes be emit low-energy γ and no α or β particles.

Table 9.2 lists the radionuclides most commonly employed for purpose in nuclear medicine. Among these, 99mTc is perhaps the most desirable for it gives off a 140-keV γ ray that is detected scintillation cameras and produces clear images.

3.14.1 Metal complexes as anticancer agents

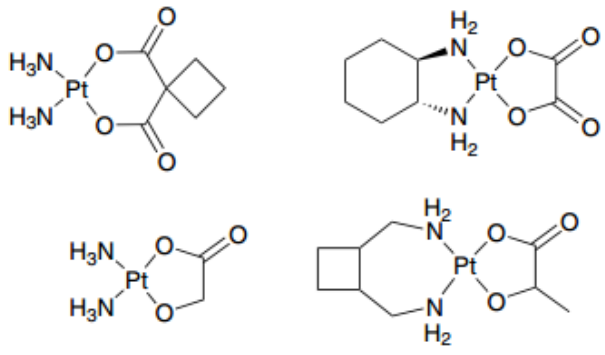
Platinum (II) complexes has been used as anti cancer drugs since long, among them cisplatin has proven to be a highly effective chemotherapeutic agent for treating various types of cancers. Ci splatin moves into the cell through diffusion and active transport. Inside the cell it causes platination of DNA, which involves interstrand and intrastrand cross-linking as well as formation of adducts, usually through guanine, as it is the most electron rich site and hence, easily oxidized.



Formation of cis platin DNA adducts causes distortion and results in inhibition of DNA replication. Cis platin DNA adducts also serve as binding site for cellular proteins such as repair enzymes, histones, transcription factors and HMG-domain proteins. The binding of HMGprotein to cisplatin-DNA adduct has been suggested to enhance anticancer effect of the drug. Beside the effectiveness of cisplatin against cancer, it has encountered side effects.

Drugs like cis platin does not specifically affect cancer cells but it also effect the rapidly dividing cells of certain normal tissues, such as those found in hair follicles, bone marrow, and the lining of the gastrointestinal tract. Inside the cell it interacts with a number of other negatively charged bio molecules besides DNA such as proteins, sulphur-containing compounds like metallothioneins and glutathiones that sequester heavy metals like Pt and remove it from the cell.

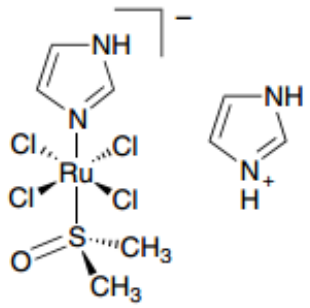
Pt (II) and Pt (IV) complexes are photo reactive. Irradiation of cis-platin modified DNA with UV light can induce cross-links with the protein HMG, which can inhibits RNA transcription. The clinically used platinum complexes are shown in below figure.



Structures of the clinically used platinum anticancer agents.

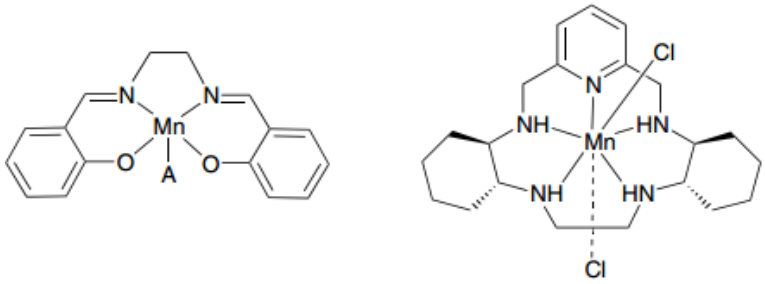
Nonplatinum anticancer agents

In the early development of analogs of the platinum compounds, complexes with similar reactivity to the platinum complexes were extensively examined. Ni and Pd analogs of Pt complexes are kinetically too reactive to be of use as drugs whereas Ir and Os ammine compounds are too inert. Ruthenium and rhodium compounds have produced greatest promise. In ruthenium ammine complexes of the general series $[RuCl_n(NH_3)_{6-n}]^{z+}$ (where n = 3, 4, or 5), *fac*$[RuCl_3(NH_3)_3]$ showed good activity but was not sufficiently water soluble for extensive testing. Ruthenium-ammine compounds have a rich DNA chemistry. the DMSO complexes cis/trans-$[RuCl_4(DMSO)Im]$ with imidazole ligands has led to an interesting clinical candidate(NAMI-A). The mechanism of action of this compound is not related to DNA binding; rather, it is an antimetastatic agent.

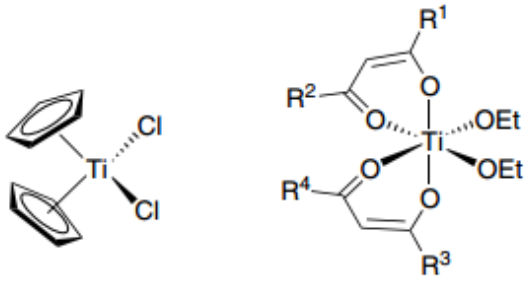


Structure of a potential ruthenium-based antimetastatic agent, NAMI-A.

Manganese chelate compounds especially those based on cyclam (1,4,8,11-tetraazacyclotetradecane) and N,N-bis(salicylaldehydo) ethylenediamine (salen), have shown promise anticancer agents their structure are shown below



The titanocene compound $[TiCl_2Cp_2]$ known as MTK4 and budotitane, a -diketonate derivative, shown below are structurally quite different compounds. But they show promising anticancer agents.



a: $R^1 = R^3 = Me$; $R^2 = R^4 = Ph$
b: $R^1 = R^3 = Ph$; $R^2 = R^4 = Me$
c: $R^1 = R^4 = Me$; $R^2 = R^3 = Ph$

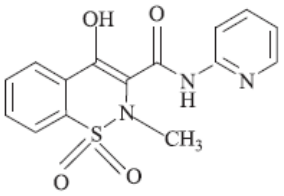
Titanium anticancer agents that have undergone clinical trials.

3.14.2 Metal complexes used in antiarithritic drug

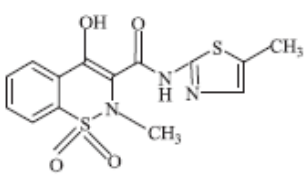
Arthritis is a typically, pain, aching, stiffness and swelling in and around one or more joints characterize rheumatic conditions. The symptoms can develop gradually or suddenly. Certain rheumatic conditions can also involve the immune system and various internal organs of the body. *The term "arthritis" is used to describe numerous rheumatic diseases and conditions that affect joints.* Some forms of arthritis, such as rheumatoid arthritis and lupus, can affect multiple organs and cause widespread symptoms.

Arthritis is more common among adults aged 65 years or older, but people of all ages (including children) can be affected. Transition metals have been used as anti-inflammatory and anti-arthritic agents. Due to the physiological importance of copper (Cu) and its unique redox activity, many different Cu complexes and Cu chelators have been synthesized and investigated for their therapeutic and diagnostic potential in human disease. The metal complexes of Fe(II), Co(II), Ni(II), Cu((II), Zn(II) and Cd(II) with piroxicam (1) ,

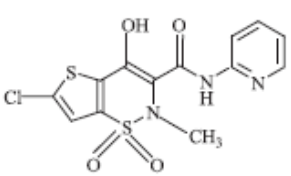
Meloxicam (2), Lornoxicam (3), Tenoxicam (4) and Isoxicam (5) ligands shows very good anitarthritis activity.



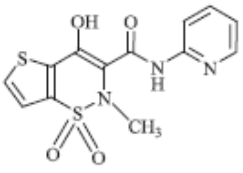
[1]



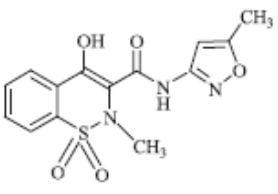
[2]



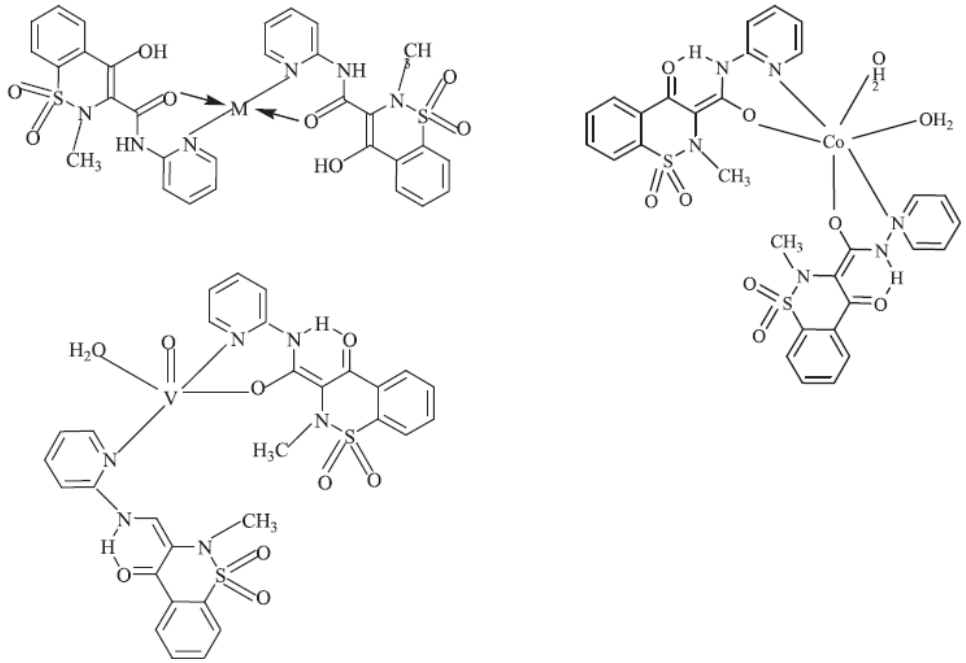
[3]



[4]



[5]



3.15 Summary of the unit

Ionophores are special carrier molecules that wrap around metal ions so they can pass through the membrane by diffusion. Ion Channels are large, membrane-spanning molecule that forms a hydrophilic path for diffusion. Ion Pumps are molecules using energy to transport ions in one direction through a membrane Passive Transport involves movement of ions down the concentration gradient, requiring no energy source. Ionophores and Ion Channels are Passive. Active transport involves movement of ions against the concentration gradient, requiring energy from ATP hydrolysis. Ion pumps are active.

Na^+/K^+-ATPase is a Membrane-Spanning Protein Ion Pump. Conformational changes pump the ions. One conformation binds Na^+ best, the other binds K^+ best. Hydrolysis of ATP provides the energy for conformational changes (30% of a mammal's ATP is used in this reaction). In antiport transport like charged ions are transported in opposite directions. Reversing the normal reaction can generate ATP and reaction can occur 100 time per second

3.16 Key word

Metal deficiency and disease; Anemia and Iron; Toxic effects of metals; Copper overload and Wilson's disease; Detoxification therapy; Chelation therapy; Blood clotting; Metal interactions with DNA; Anticancer agents; Antiarithritic drug.

3.17References for further studies

1) Bioinorganic Chemistry: A Short Course; 2nd ed. Rosette M. Roat-Malone; *John Wiley & Sons*, **2007**.

2) Biological Inorganic Chemistry: Structure and Reactivity; Ivano Bertini; *University Science Books*, **2007**.
3) Bioinorganic Chemistry; K. Hussain Reddy; *New Age International*, **2007**.
4) Principles of Bioinorganic Chemistry; Stephen J. Lippard, Jeremy Mark Berg; *University Science Books*, **1994**.
5) Bioinorganic Chemistry -- Inorganic Elements in the Chemistry of Life: An Introduction and Guide; Wolfgang Kaim, Brigitte Schwederski, Axel Klein; *John Wiley & Sons*, **2013**.

3.18 Questions for self understanding

1) Discuss the cause of deficiency and disease related with sodium ion
2) Discuss the cause of deficiency and disease related with potassium ion
3) Discuss the cause of deficiency and disease related with calcium ion
4) Discuss the cause of deficiency and disease related with copper ion
5) Discuss the cause of deficiency and disease related with magnesium ion
6) Discuss the cause of deficiency and disease related with iron ion
7) Discuss the cause of deficiency and disease related with zinc ion
8) Write a note on toxic effects of metals
9) Discuss copper ion overload and Wilson's disease
10) Discuss the mercury toxicity and bacterial resistance
11) Explain the consequences of cadmium and lead toxicity
12) Write a note on detoxification therapy
13) Write a note on chelation therapy
14) Write a note on biochemistry of calcium
15) Explain the Mechanism of blood clotting
16) Write short notes on followind
i) Metal interactions with DNA
ii) Ligand interaction with DNA
iii) Metal Ligand interactions with DNA
iv) Metal Ligand Covalently bindingthrough inner-sphere
v) Metal Ligand Covalently bindingthrough inner-sphere
17) Write a note on metal complexes as anticancer agents
18) Write a note on metal complexes used in antiarithritic drug

UNIT-4

Structure

4.0 Objectives of the unit

After studying this unit you are able to

- List the different proteins used for Iron storage in cell
- Explain the structure and iron storage method in Ferritin
- Explain the structure and iron storage method in Transferrin
- Explain the structure and iron storage method in Sidrophores (Bacterial iron transmit)

4.1 Introduction

We know that charged ions must pass through a hydrophobic membrane in order to enter inside the cell. Neutral gases like O_2, CO_2 and low charge density ions like anions can move directly through the membrane. But high charge density cations require help. Once metal ions enter inside the cell, they must be transported to the location of their use and then released or stored for later release. Release from ligand is often not trivial and storage requires additional molecules.

Iron is truly ubiquitous in living systems. It is at the active centre of molecules responsible for oxygen transport and electron transport and it is found in metalloenzymes such as nitrogenases, various oxidases, hydrogenases, reeducates, dehydrogenases, deoxygenases, and dehydrates. Iron is extremely abundant in the earth's crust and it has two readily interconverted oxidation states.

4.2 Iron storage

Three properties of iron can account for its extensive use in terrestrial biological reactions

a) Facile redox reactions of iron ions

b) An extensive repertoire of redox potentials available by ligand substitution or modification

c) Abundance and availability under conditions apparently extant when terrestrial life began

Ferrous ion appears to have been the environmentally stable form during prebiotic times. The combination of the reactivity of ferrous ion and the relatively large amounts of iron used by cells may have necessitated the storage of ferrous ion; recent results suggest that ferrous ion may be stabilized inside ferritin long enough to be used in some types of cells.

There are two principal iron-storage compounds in the human body. They are ferritin and hemosideri. Hemosiderin is poorly understood compared to ferritin.

4.2.1 Ferritin

Ferritins are family of proteins composed of a protein coat and an iron core of hydrous ferric oxide $[Fe_2O_3(H_2O)_n]$ with various amounts of phosphate. Iron is stored mainly in ferritins and as many as 4,500 iron atoms can be reversibly stored inside the protein coat in a complex that is soluble in aqueous media.

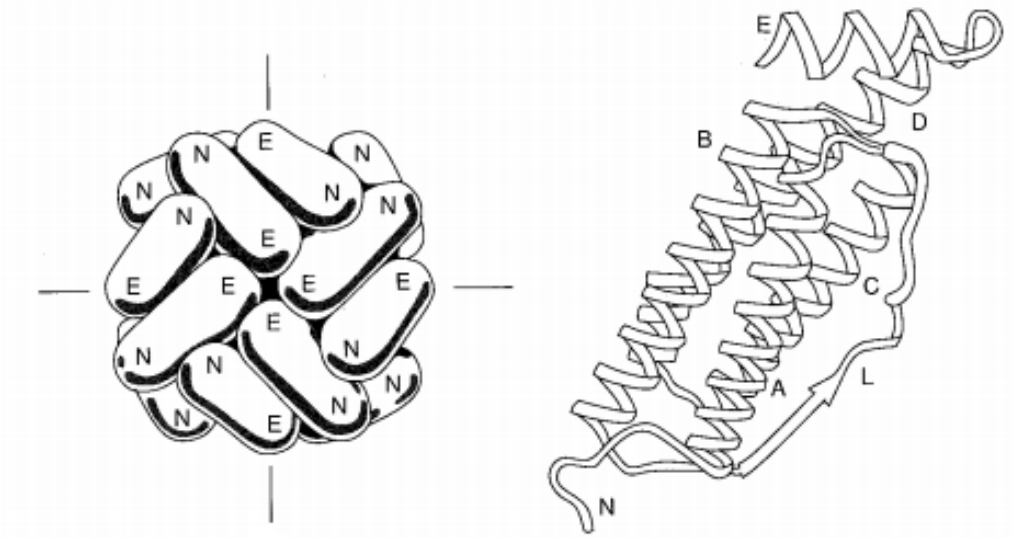


Figure 1: Sub-unit assembly of ferritin. Each sub-unit (shaped like a sausage) is made up of four parallel, α-helical polypeptide chains. Sub-unit assembly of ferritin. Each sub-unit (shaped like a sausage) is made up of four parallel, α-helical polypeptide chains.

Ferritin serves as a depot in which surplus iron can be stored within cells in nontoxic form and it can be released in usable forms whenever required, either within that cell itself or in other cells of the organism. It is widely' distributed throughout the various organs of all mammals, especially high concentrations being found in liver, spleen, and bone marrow. It is also found in plants and bacteria.

Ferritin consists of a shell of protein surrounding a core that contains the iron. The diameter of the essentially spherical core varies from 40 to 88 A^0 and may contain up to 4500 iron atoms. The core is quasicrystalline and has a composition approximating closely to $(FeOOH)_8FeO.H_2PO_4$. The phosphate is not a part of the bulk structure, but appears instead to play some role in covering the $(FeOOH)_x$ particles and attaching them to each other and to the protein sheath. The X-rays crystal structure of the protein sheath -called the apoferritin from horse spleen. It is made up of 24 subunits, each of which is a polypeptide chain coiled so as to form a slightly prolate spheroid or lozenge. The arrangement of the lozenges, shown in below figure is very symmetrical.It has full cubic symmetry with fourfold, threefold, and twofold axes and forms face centered cubic crystals. ,

The manner in which iron enters and leaves ferritin is still poorly understood. The core can be formed only from aqueous iron (II), so that, oxidation accompanies incorporation. Iron release is controlled by the protein shell and can occur very rapidly when necessary.

Protein Coat

Twenty-four peptide chains (with about 175 amino acids each), folded into ellipsoids, pack to form the protein coat, which is a hollow sphere about 100 Å in diameter. The organic surface

is about 10 Å thick. Channels which occur in the protein coat at the trimer interfaces may be involved in the movement of iron in and out of the protein.

Iron-Protein Interface

Formation of the iron core appears to be initiated at an Fe-protein interface where Fe(II)-O-Fe(Ill) dimers and small clusters of Fe(Ill) atoms have been detected attached to the protein and bridged to each other by oxo/hydroxo bridges. Evidence for multiple nucleation sites has been obtained from electron microscopy of individual ferritin molecules.

Iron Core

Only a small fraction of the iron atoms in ferritin bind directly to the protein. The core contains the bulk of the iron in a polynuclear aggregate with properties similar to ferrihydrite, a mineral found in nature and formed experimentally by heating neutral aqueous solutions of $Fe(III)(NO_3)_3$.

4.2.2 Transferrin

*Transferrin are proteins with a molecular weight of ~ 80,000 and they contain two similar but not identical sites that bind iron tightly but reversibly in the presence of certain anions, mainly CO_3^{2-} or HCO_3^-.*This molecule carries ingested iron from the stomach and introduces it into the iron metabolic processes of the body. As iron passes from tile stomach (which is acidic) into the blood (pH ~7.4) it is oxidized to Fe^{III} in a process catalyzed by the copper metalloenzymeceruloplasmin, then picked up by transferrin molecules. The details of the nature of binding sites are still unknown. The binding constant is ~ 10^{26}; therefore transferrin is an extremely efficient scavenger of iron. It remove iron from stable complexes with phosphate or citrate ions.

Transferrin bound to the cell wall of an immature red cell, which requires iron, and the transfer is made under control of a complex mechanism for transporting the iron through the cell membrane.

Transferrin also carries iron to ferritin in vitro and the process of transfer is requiring ATP and ascorbic acid and proceeding via Fe^{II}. The nature of the binding sites of transferrin has not been established, but tyrosine, histidine, and the carbonate ion have been suggested as ligands. .

4.3 Sidrophores (Bacterial iron transmit)

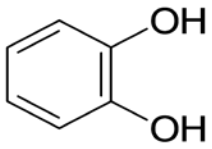
The iron is not spontaneously available to aerobic organisms in an aqueous environment because of the very low solubility of ferric hydroxide. Thus Fe^{3+} ions at pH of 7 have a molar concentration of only 10^{-18} and simple diffusion into cells could never sufficient to supply their needs. Indeed, simple inward diffusion could not occur, since iron is already more

concentrated than this in living cell. Therefore special chelating agents called *sidrophores* are produced by bacteria and ejected in to their environment to gather iron and transfer it through the cell wall into the cell.

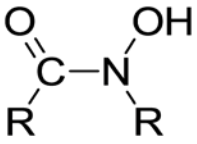
In some cases it appears that the iron is released by the chelator at the wall and it passes through alone, whereas in others the entire complex enters the cell.

The siderophores are diverse chemically but have in common the use of chelating, oxygen-donor-type ligands. A very large fraction of the siderophores has hydroxamate moieties as the ligating units.

For example, a type of siderophore called a ferrichrome consists of a cyclic hexapeptide in which three successive amino acid residues have side chains ending in hydroxamate groups. These three hydroxamate groups bind iron by forming a trischelate octahedral complex as shown in below figure.



Catechol



Hydroxamic Acid

Another type of siderophore common in prokaryotes such as enteric bacteria is called *enterobactin*. The ligating units are catecholate anions that also chelate very effectively. The siderophores form Fe^{3+} complexes that are thermodynamically very stable (i.e., they have very high formation constants).

It has been estimated that the enterobactin complexes Fe^{3+} with a formation constant of $\geqq 10^{45}$. However, these complexes are kinetically labile, and such lability is essential so that iron can be easily taken up before transport and released afterward.

4.4 Summary of the unit

In humans, iron is stored mainly in the bone marrow, spleen and liver. About 10 per cent of all the iron in the body is in storage. Two proteins are involved in iron storage; these are called ferritin and haemosiderinIn humans, iron is stored mainly in the bone marrow, spleen and liver. About 10 per cent of all the iron in the body is in storage. Two proteins are involved in iron storage; these are called ferritin and haemosiderin.

ach ferritin molecule can store iron up to about 20 per cent of its total mass. This is a very high percentage, considering that less than 0.2 per cent of the total mass of proteins like transferrin and myoglobin is iron. Ferritin is a large protein with a relative molecular mass of

440 000.ach ferritin molecule can store iron up to about 20 per cent of its total mass. This is a very high percentage, considering that less than 0.2 per cent of the total mass of proteins like transferrin and myoglobin is iron. Ferritin is a large protein with a relative molecular mass of 440 000.

The overall structure shows that ferritin is a huge, hollow protein, with a wall mostly made up of α-helical peptide chains. The structure is quite symmetrical, being roughly dodecahedral, and is one of the outstanding examples of symmetry in chemistry. The wall contains channels, which lead from the inside to the outside of the hollow 'sphere'. The channels are rich in amino acids with carboxylate side-chains, which are capable of chelating iron.The overall structure shows that ferritin is a huge, hollow protein, with a wall mostly made up of α-helical peptide chains. The structure is quite symmetrical, being roughly dodecahedral, and is one of the outstanding examples of symmetry in chemistry. The wall contains channels, which lead from the inside to the outside of the hollow 'sphere'. The channels are rich in amino acids with carboxylate side-chains, which are capable of chelating iron.

4.5 Key words

4.6 References for further studies

1) Bioinorganic Chemistry: A Short Course; 2nd ed. Rosette M. Roat-Malone; *John Wiley & Sons*, **2007**.
2) Biological Inorganic Chemistry: Structure and Reactivity; Ivano Bertini; *University Science Books*, **2007**.
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4) Principles of Bioinorganic Chemistry; Stephen J. Lippard, Jeremy Mark Berg; *University Science Books*, **1994**.
5) Bioinorganic Chemistry -- Inorganic Elements in the Chemistry of Life: An Introduction and Guide; Wolfgang Kaim, Brigitte Schwederski, Axel Klein; *John Wiley & Sons*, **2013**.

4.7 Questions for self understanding

1) List the different proteins used for Iron storage in cell
2) Explain the structure and iron storage method in Ferritin
3) Explain the structure and iron storage method in Transferrin
4) Explain the structure and iron storage method in Sidrophores (Bacterial iron transmit)

UNIT-5

Structure

5.0 Objectives of the unit

After studying this unit you are able to

- Describe the metalloproteins
- Explain the functions of metalloproteins
- Classify the metalloproteins based on their functions
- Explain the role of Heme proteins
- Point out the similarities and differences between hemoglobin and myoglobin
- Explain the mechanism of di oxygen binding to myoglobin and hemoglobin

5.1 Introduction

Many transition metals have been found serving as prosthetic groups in the active sites of proteins or as the co-factor in metal activated proteins, or metalloproteins. These metalloproteins exploit the redox and electronic properties of the transition metals to perform a variety of complex biological functions, being the metal ions critical to the protein's function, structure, or stability. One third of all proteins are metalloproteins, using metallic ions such as Ca, Mg, Mn, Fe, Co, Ni, Cu, Zn, Mo, W, V.

The term metalloprotein is used to designate those types of metal-protein complexes in which the strength of binding is so great that a metal atom can be considered an integral part of the structure of the protein. Thus, at "physiological" conditions of pH and temperature, and in the absence of strong competing metal-binding agents, there is no appreciable dissociation of the metal ion, which, consequently, is not in reversible equilibrium with "free" metal ions in solution.

In many cases, for example with heme proteins (6), the metal interaction is not primarily with the protein, but the metal ion is instead bound to a prosthetic group.

5.2 Metalloproteins

In biological systems, metals are found as constituents of proteins. Metal ions in metalloproteins are critical to the proteins function, structure, and stability. Because numerous essential biological functions require metal ions and most of these functions involve metalloproteins, understanding metalloproteins and how to manipulate them is of great importance in the biological and medical fields. *Many proteins bind metal ions, permanently as prosthetic groups or more transiently as ligands and these proteins are generally called metalloproteins.*

Metalloproteins represent one of the most diverse classes of proteins, containing intrinsic metal ions which provide catalytic, regulatory, or structural roles critical to protein function.

The metal ion is usually coordinated by nitrogen, oxygen, or sulfur atoms belonging to amino acids in a polypeptide chain and/or in a macrocyclic ligand incorporated into the protein.

The presence of a metal ion rich in electrons allows metalloproteins to perform functions such as redox reactions, phosphorylation, electron transfer, and acid-base catalytic reactions that cannot easily be performed by the limited set of functional groups found in amino acids. Approximately onethird of all proteins possess a bound metal1, and almost half of all enzymes require the presence of a metal atom to function2. The most abundant metal ions in vivo are Mg and Zn, while Fe, Ca, Co, Mn, and Ni are also frequently observed. Metalloproteins play important roles in key biological processes such as photosynthesis, signaling, metabolism, proliferation, and immune response.

5.3 Functions of metalloproteins

The use of metals in proteins can be broadly separated into four classes:

i) Transport:

Metalloproteins in this category are responsible for molecular transport. The most obvious examples of this class of metalloproteins are the oxygen transport systems used in respiration including myoglobin-hemoglobin family, hemocyanins and hemerythrins.

ii) Electron Transport

Unlike carbon, many metals have other stable oxidation states that are energetically accessible at physiological conditions. These metals are ideal for the transport of electrons in processes that require oxidations or reductions to a substrate. Some examples of common electron transport metalloproteins include iron-sulfur clusters and cytochromes.

iii) Structural Support

Metal ions have the ability to access a wide variety of coordination states, many of which are different than the traditional sp^3 tetrahedral carbon center found in organic chemistry. In some cases, the metal simply defines the overall tertiary shape of the protein while in other cases the presence or the absence of metal ions and subsequent changes in protein configuration may be involved in protein/enzyme activity (metalloregulation). The well-known “zinc fingers” are an example of the first type of structural support that involve a Zn^{2+} ion which forms the central core of a wide variety transcription factor proteins. Iron-sensitive metalloproteins control the regulation and production of ferritin and other iron transport systems in mammals are examples of the second type of structural support.

iv) Metalloenzymes

Metalloenzymes are an important subclass of metalloproteins wherein the metal is active in some catalytic cycle in a biologoical system. Many of the transformations accomplished by metalloproteins involve small molecule substrates.

5.4 Functional classification of metalloproteins

Metal centers in proteins can be classified according to their function as follows

i) permanent centers ii) catalytic centres iii) transient centers iv) electron transfer v) exciton transfer vi) gas sensing vii) gas storage viii) metal sensing ix) metal storage and transport.

Some metal centers can switch their roles. For example the iron-responsive element binding protein (IRE-BP) functions either as an active aconitase (aconitate hydratase) when cells are iron-replete, or as an active mRNA-binding protein, when cells are iron. Aslo many metalloproteins have more than one functional metal center. Table given below provide the different mentalloproprins, the metal present in them and their functions.

Function of protein	Role of compound	Compound binding mode		Example	
		Permanent	Transient	Protein	Compound
Electron transfer	Electron transfer	√		Cytochrome b_5 Adrenodoxin Plastocyanin	haem *b* Fe_2S_2 Cu
Light harvesting	Excitation energy transfer	√		Light-harvesting complex LH-II	BChl-*a*
Catalysis	Substrate activation	√		Nitrile hydratase DMSO reductase Nitrogenase MoFe protein Manganese superoxide dismutase	Fe Moco FeMoco Mn
	Electron transfer	√		Hydroxylamine oxidoreductase	haem P460 haems *c*
	Reactant		√	Manganese peroxidase Ferrochelatase	Mn Fe, haem
Catalysis and regulation	Switch of function	√		Holoenzyme: aconitate hydrolase	Fe_4S_4
			–	Apoenzyme: IRE-BP	
Translocation	To be translocated		√	Copper-transporting ATPase	Cu^+
Catalysis or transport	Inhibitor		√	Ca^{2+}-ATPase D-xylose isomerase	La^{3+} Ca^{2+}
Storage (uptake, binding and release)	Gas coordination	√		Nitrophorin Haemocyanin Haemophore HasA	haem (coordinates NO) 2 Cu^{2+} (coordinates O_2) haem
	To be transported or stored		√	Metallothioneins Lactoferrin Bacterioferritin	Cd^{2+}, Hg^{2+}, Pb^{2+}, Tl^+ Fe Fe (in form of hydrated ferric phosphate)
Various	Structural	√		Lignin peroxidase Zinc finger Endonuclease III	Ca^{2+} Zn^{2+} Fe_4S_4

5.5 Heme proteins

Heme proteins are a large subclass of metalloproteins which have a metallomacrocyle at their active site. The common motif is a metal ion captured within an N-heterocycle corresponding to a porphyrin-derived system, e.g. Myoglobin and Hemoglobin

Hem protein describes an iron-containing protein where the Fe/porphyrin complex is finely tuned to provide a variety of functions. The heme moiety is a useful way of capturing reactive Fe ions. Macrocyclic effect is important in giving system great stability. Whole complex unit shows properties different from those of the free metal ion including solubility.

5.6 Hemoglobin

It is made up of four protein subunits. Normal human adult hemoglobin A is a tetramer comprised of a pair of alpha-globin chains and a pair of beta-globin chains. Nestled deep in each of these protein's folds, is a planar structure called a porphyrin, which binds in its center a single atom of iron, most commonly in the 2+ valency state. The iron-porphyrin group is called heme. Hemoglobin is contained in red blood cells, serves as the oxygen carrier in blood. The name hemoglobin comes from heme and globin, since each subunit of hemoglobin is a globular protein with an embedded heme group. Each heme group contains an iron atom, and this is responsible for the binding of oxygen. The presence of hemoglobin in blood increases the oxygen carrying ability of a litre of blood from 5 to 250 ml. Hemoglobin also plays a major role in the transport of carbon dioxide from the tissues back to the lungs. Myoglobin, on the other hand, is located in muscle, and serves as a reserve supply of oxygen and also facilitates the movement of O_2 within muscle

5.7 Myoglobin

Myoglobin is a protein found in the muscle cells of animals. It functions as an oxygen-storage unit, providing oxygen to the working muscles. Diving mammals such as seals and whales are able to remain submerged for long periods because they have greater amounts of myoglobin in their muscles than other animals do.

Myoglobin consists of a single polypeptide chain of about 153 amino acids. Approximately 70% of the main chain is folded into eight major, right-handed α -helices (identified as segments A-H with the first residue of segment A being A1, etc.) The rest of the chain forms turns between helices devoid of symmetry. The interior consists almost entirely of nonpolar residues, two histidines are the only polar residues which play an integral role in the binding of heme oxygen. The outside of the protein has both polar and nonpolar residues.

5.8 Similarities and differences between hemoglobin and myoglobin

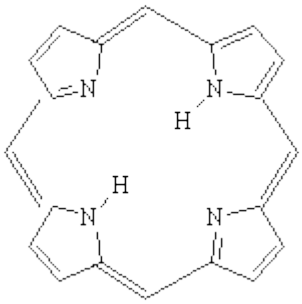
There is a close chemical similarity between myoglobin and hemoglobin, the oxygen-binding protein of red blood cells. Both proteins contain a molecular constituent called heme, which enables them to combine reversibly with oxygen. The heme group, which contains iron, imparts a red-brown colour to the proteins. The bond between oxygen and hemoglobin is

more complex than that between oxygen and myoglobin and accounts for the dual ability hemoglobin has to transport oxygen as well as to store it.

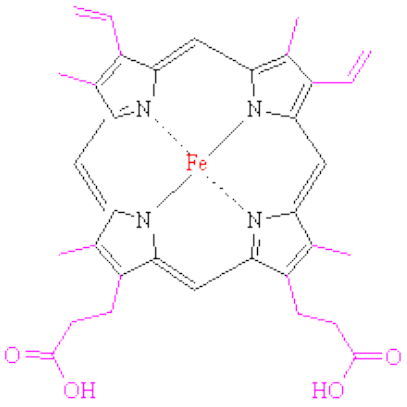
In contact with venous blood, oxygen combines more readily with myoglobin than it does with hemoglobin, favouring the transfer of oxygen from blood to muscle cells. Thus, the oxygen that the working muscle requires for the energy-producing biochemical reactions is provided.

5.9 The Heme Porphyrin

Although the hemoglobin and myoglobin molecules are very large, complex proteins, the active site is actually a non-protein group called heme. The heme consists of a flat organic ring surrounding an iron atom. The organic part is a porphyrin ring based on porphin (a tetrapyrrole ring), and is the basis of a number of other important biological molecules, such as chlorophyll and cytochrome.



Porphin: the building block of heme

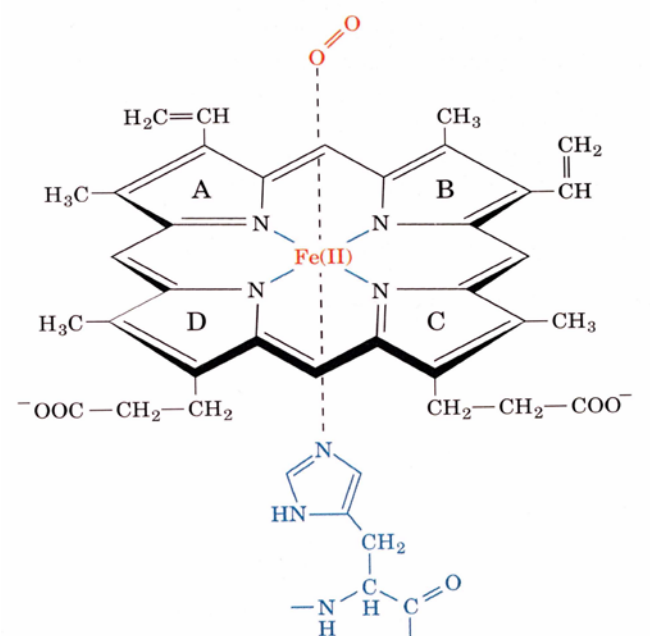


Heme: the non-protein active site within myoglobin and hemoglobin.

The ring contains a large number of conjugated double bonds, which allows the molecule to absorb light in the visible part of the spectrum. The iron atom and the attached protein chain modify the wavelength of the absorption and gives hemoglobin its characteristic colour. Oxygenated hemoglobin (found in blood from arteries) is bright red, but without oxygen (as in blood from veins), hemoglobin turns a darker red.

5.10 Oxygen uptake

The oxygen that we need for survival is transported from the lungs to peripheral tissues by the hemoglobin. In the lung, oxygen diffuses across the alveolar membrane, and then the red cell membrane in lung capillaries. When it encounters a molecule of hemoglobin, it wedges itself between the iron atom and nitrogen attached to the globin chain, which helps to hold the heme group in the protein. One molecule of hemoglobin with its four heme groups is capable of binding four molecules of diatomic oxygen, O_2.



The loaded pigment is called oxyhemoglobin, and it is a brilliant red color as in arterial blood. Pressure from dissolved oxygen in plasma and in the surroundings in the red cell helps to keep the oxygen on its binding site.

When molecular oxygen encounters an isolated heme molecule, it rapidly converts the Fe(II) to Fe(III). The oxidized heme binds oxygen very poorly. Oxidation of the heme iron is prevented by the presence of the distal histidine side chain, which prevents the O_2 from forming a linear Fe–O–O bond. The bond between Fe and O_2 is bent, therefore this bond is not as strong as it might be. Weaker oxygen binding helps for easier oxygen release. This is an important principle in understanding not only heme chemistry but also the regulation of hemoglobin's affinity for oxygen.

As the blood circulates to the periphery, the small amount of dissolved oxygen is consumed first by cells in organs and tissues. This release in pressure makes available the much larger reservoir of heme-bound oxygen, which begins a sequential unloading of its four oxygen molecules. At the most, under normal circumstances only 3 molecules of oxygen are unloaded. *Partially or fully unloaded hemoglobin is called deoxyhemoglobin*. It is a dark blue to purplish color as in venous blood.

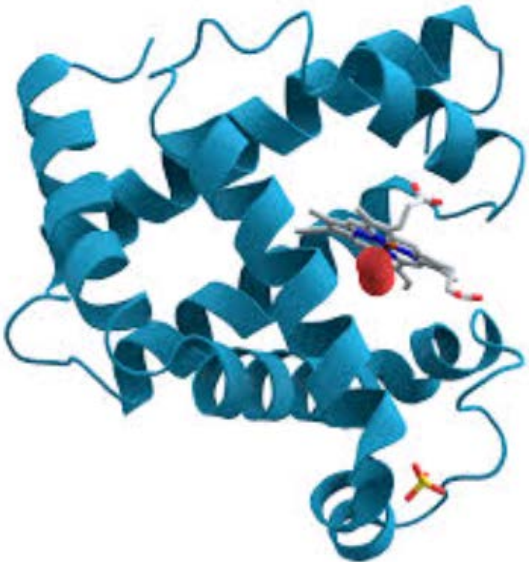
During oxygen unloading, the hemoglobin tetramer undergoes subtle intramolecular conformational changes called cooperativity. As a result of cooperativity, once the first oxygen has been unloaded, the unloading of the second oxygen is facilitated. The second oxygen can dissociate after a much smaller change in oxygen pressure than was needed to unload the first. Another conformational change facilitates dissociation of the third oxygen. *Cooperativity is an important phenomenon that permits the loading and unloading of large*

amounts of oxygen at physiologically relevant oxygen pressures. Oxygen lack is known as hypoxia, the complete absence of oxygen is called anoxia.

5.11 Structure and functions of Hemoglobin and Myoglobin

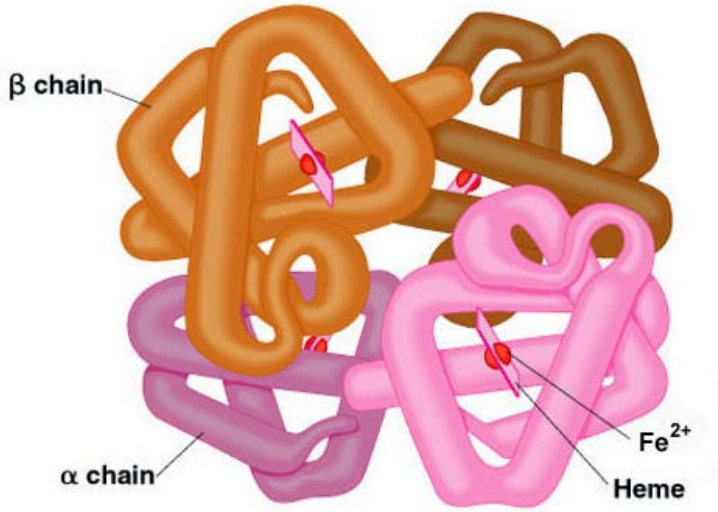
Myoglobin and hemoglobin are hemeproteins whose physiological importance is principally related to their ability to bind molecular oxygen. Myoglobin is a monomeric heme protein found mainly in muscle tissue where it serves as an intracellular storage site for oxygen. During periods of oxygen deprivation oxymyoglobin releases its bound oxygen which is then used for metabolic purposes.

Myoglobin contains a very high proportion (75%) of α-helical secondary structure. A myoglobin polypeptide is comprised of 8 separate right handed α-helices, designated A through H, that are connected by short non helical regions. Amino acid R-groups packed into the interior of the molecule are predominantly hydrophobic in character while those exposed on the surface of the molecule are generally hydrophilic, thus making the molecule relatively water soluble. The tertiary structure of myoglobin is a typical water soluble globular protein.



Each myoglobin molecule contains one heme prosthetic group inserted into a hydrophobic cleft in the protein. Each heme residue contains one central coordinately bounded iron atom normally in the Fe^{2+}(ferrous) oxidation state. The oxygen carried by hemeproteins is bound directly to the ferrous atom. Oxidation of the iron to the Fe^{3+} (ferric) oxidation state renders the molecule incapable of normal oxygen binding. Carbon monoxide also binds coordinately to heme iron atoms in a manner similar to that of oxygen, but the binding of carbon monoxide to heme is much stronger than that of oxygen.

Adult hemoglobin is a [α(2):β(2)] tetrameric hemeprotein found in erythrocytes where it is responsible for binding oxygen in the lung and transporting the bound oxygen throughout the body where it is used in aerobic metabolic pathways



Each subunit of a hemoglobin tetramer has a heme prosthetic group identical to that described for myoglobin.

The differences between hemoglobin and myoglobin are most important at the level of quaternary structure. Hemoglobin is a tetramer composed of two each of two types of closely related subunits, alpha and beta. Myoglobin is a monomer (so it doesn't have a quaternary structure at all). Myoglobin binds oxygen more tightly than does hemoglobin. This difference in binding energy reflects the movement of oxygen from the bloodstream to the cells, from hemoglobin to myoglobin

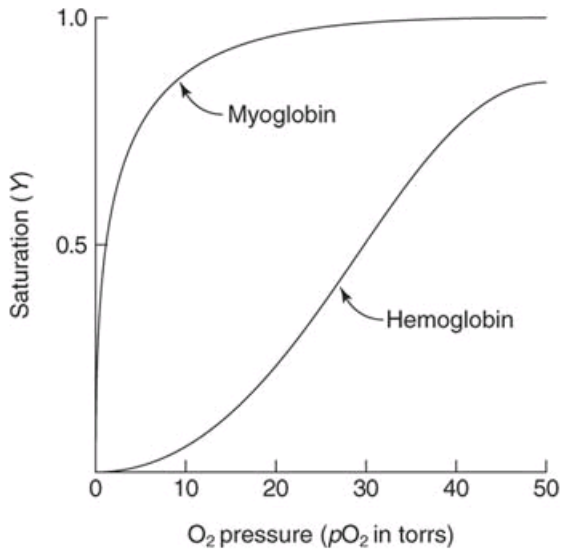
5.12 Di oxygen binding to myoglobin

The binding of O_2 to myoglobin is a simple equilibrium reaction

$$Mb + O_2 \leftrightarrow MbO_2$$

Each myoglobin molecule is capable of binding one oxygen, because myoglobin contains one heme per molecule. Even though the reaction of myoglobin and oxygen takes place in solution, it is convenient to measure the concentration of oxygen in terms of its **partial pressure,** the amount of gas in the atmosphere that is in equilibrium with the oxygen in solution.

The titration curve of myoglobin with oxygen is a hyperbola, as shown in figure



where Y is the fraction of oxygenated myoglobin, pO_2 is the partial pressure of O_2, expressed in torr (mm Hg; 760 torr = 1 atmosphere)

5.13 Di oxygen binding to hemoglobin

Because hemoglobin has four subunits, its binding of oxygen can reflect multiple equilibria

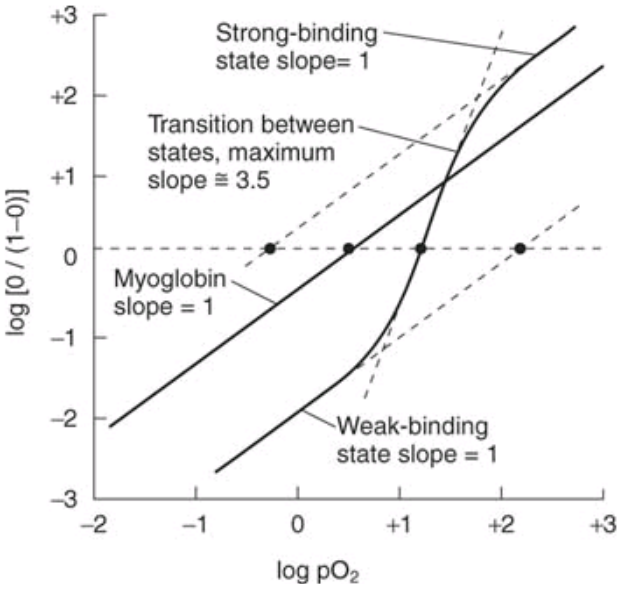
$$Hb + O_2 \rightarrow Hb\text{-}O_2$$

$$Hb\text{-}O_2 + O_2 \rightarrow Hb\text{-}(O_2)_2$$

$$Hb\text{-}(O_2)_2 \rightarrow Hb\text{-}(O_2)_3$$

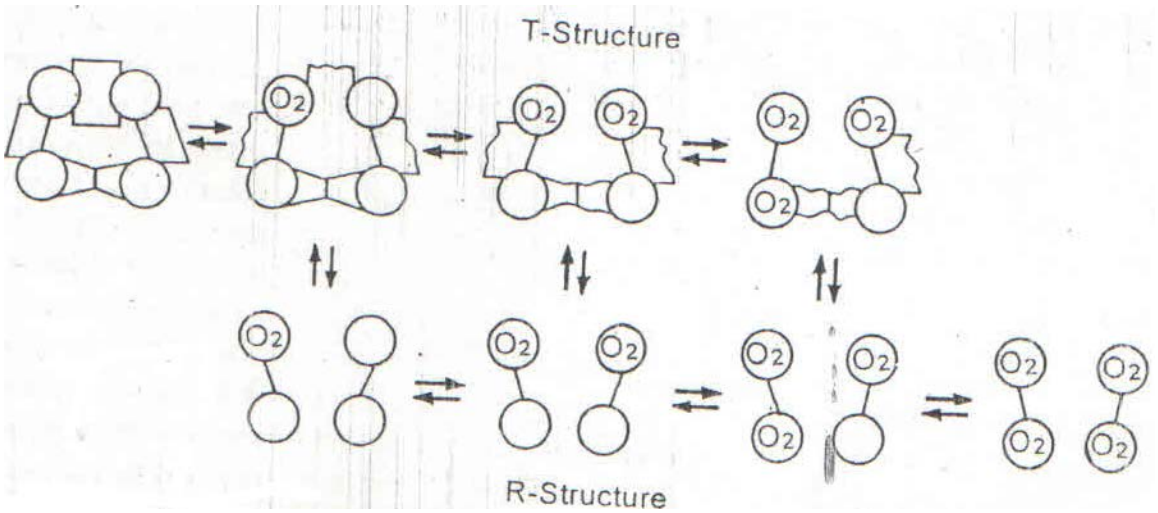
$$Hb\text{-}(O_2)_3 \rightarrow Hb\text{-}(O_2)_4$$

The equilibrium constants for these four O_2 binding events are dependent on each other and on the solution conditions. The influence of one oxygen's binding on the binding of another oxygen is called a homotropic effect. Overall, this cooperative equilibrium binding makes the binding curve sigmoidal rather than hyperbolic, as shown in below figure. The P_{50} of hemoglobin in red blood cells is about 26 torr under normal physiological conditions. In the alveoli of the lungs, p_{O2} is about 100 torr, and close to 20 torr in the tissues. Hence hemoglobin is about 80% loaded in the lungs and a little over 40% loaded with oxygen in the tissue capillaries. ie, hemoglobin is more O_2saturated in the lungs and less saturated in the capillaries.



5.14 Cooperativity in hemoglobin

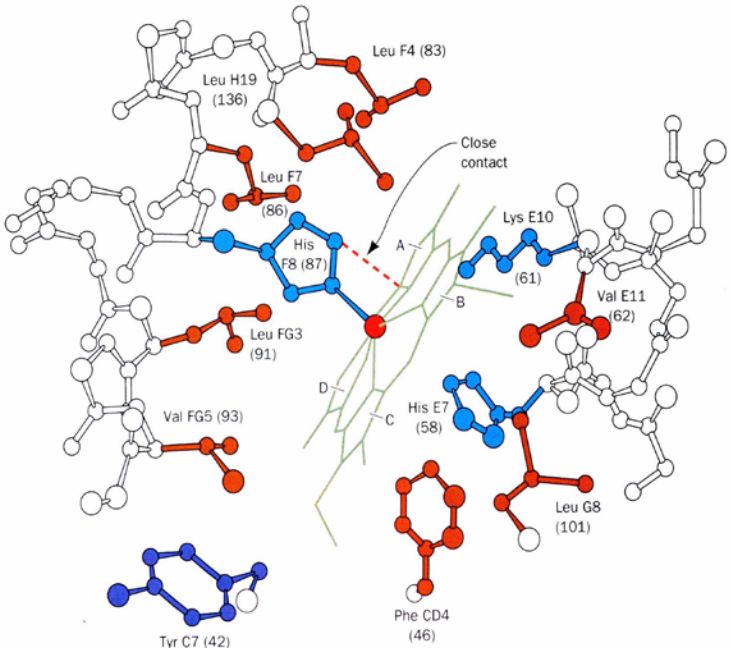
Cooperativity is a complex subject, one model is the interconversion of the hemoglobin between two states ie, the T (tense) and the R (relaxed) conformationsof the molecule. The R state has higher affinity for oxygen. Under conditions where pO2 is high (such as in the lungs), the R state is favored; in conditions where p_{O2} is low (as in exercising muscle), the T state is favored. The conformation of the deoxy state is called the T state. The conformation of the oxy state is called the R state. Individual subunits have a T or R if in the deoxy or oxy state and it is associated with the binding of oxygen.



The schematic representation of the equilibrium between the T and R structure if hemoglobin

The positive cooperativity of O_2 binding to Hb arises from the effect of the ligand-binding state of one heme on the ligand-binding affinity of another. The Fe iron is about 0.6 Å out of the heme plane in the deoxy state. When oxygen binds it pulls the iron back into the heme plane. Since the proximal His F_8 is attached to the Fe this pulls the complete F helix like a

lever on a fulcrum. Hence binding of the oxygen on one heme is more difficult but its binding causes a shift in the α1-β2 contacts and moves the distal His E_7 and Val E_{11} out of the oxygen's path to the Fe on the other subunit as shown in below figure. This process increases the affinity of the heme toward oxygen.



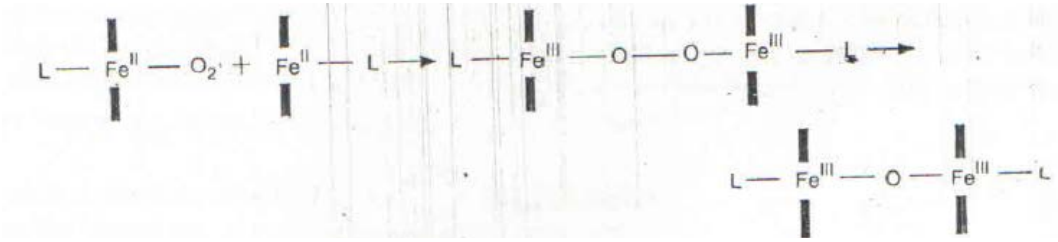
Thus the T state with reduced oxygen affinity will be changed to the R state without binding oxygen because the other subunits switch upon oxygen binding. Now unbound R state has a much higher affinity for oxygen, and this is the rational for co-operativity.

5.15 Di oxygen binding model complexes

Naturally occurring oxygen carriers and storage proteins contain a transition metal ion to which O_2 can reversibly bind, typically iron (in the form of ferrous heme in proteins such as myoglobin and hemoglobin) or copper (hemocyanin).

Factors relevant to the biological functioning of hemoglobin can be understood by studying changes in chemical behavior of model compounds. Inorganic chemist made an attempt to understand the functioning of hemoglobin by studying model compounds. Model compounds are simple Fe-containing species that function like the hemoglobin prototype.

It has been discussed for a long time that if only a simple iron-prophyrin complex is used, oxygen reacts in an irreversible manner with two such homelike units, as represented below



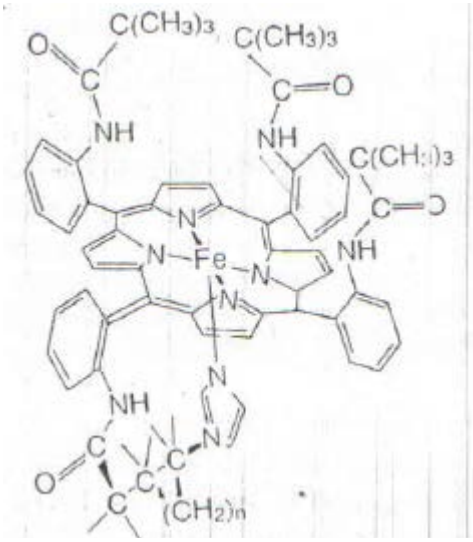
In nature this is prevented because individual heme units are attached to the bulky globin(protein) molecules and the close approach required for oxygen binding is impossible. In the model systems the biomolecular reaction is usually precluded by some kind of steric hindrance, deliberately introduced in several way

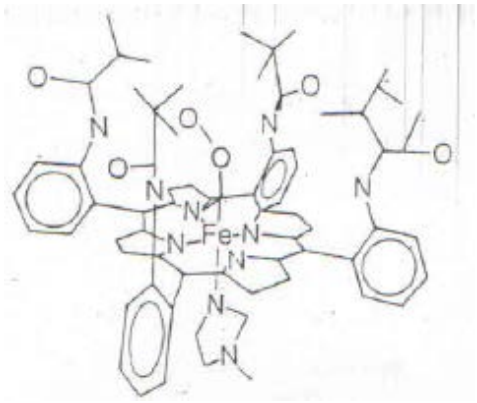
1. The *picket fence* (I) approach in which three or four large groups stand around the iron atom on the side where oxygen is to be bound
2. *Strapped models* (II) in which one chain extends over the iron atom but leaves room for the O_2.
3. *Roofed models* (III) in which there is more complete enclosure of the binding site.

Model studies have shown that irreversible oxidation can occur through dimerization. Two approaches are developed to preventing the formation of dimers. They are

a) Attaching a Fe porphyrin to a surface so that the units cannot come together to form a dimers
b) Picket fence porphyrins in which bulky substituents on tetraphenylprophine form a fence around the O_2 bonding site

Metals usually react with O_2 irreversibly to give metal oxides or other oxo compounds. Knowing which metals in what oxidation states and with what ligands can add O_2 reversibly is important for production of synthetic blood and understanding the functioning of hemoglobin.

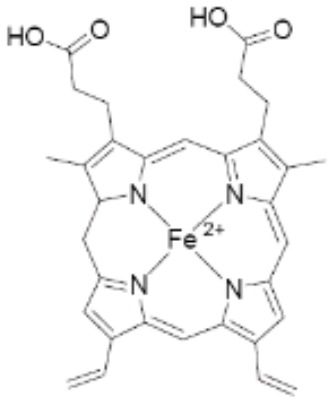




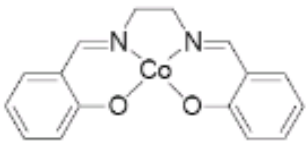
Dioxygen binding to a Picket fence porphyrin

One of the Picket fence models for a heme-protein oxygen

The most successful models for O_2 carriers have been those of cobalt(II) and iron(II) compounds.A simple synthetic cobalt complex reversibly binds dioxygen. Many complexes of this type have been used as models to aid in the understanding of how the proteins function. The complex Co(salen) [wheresalen = N,N-bis(salicylaldehyde)ethylenediimine] reversibly binds O_2, thereby acting as afunctional model for myoglobin. As one might imagine, most spectroscopic and measurement techniques are easier to perform and interpret on a molecule which has two orders of magnitude fewer atoms than a protein

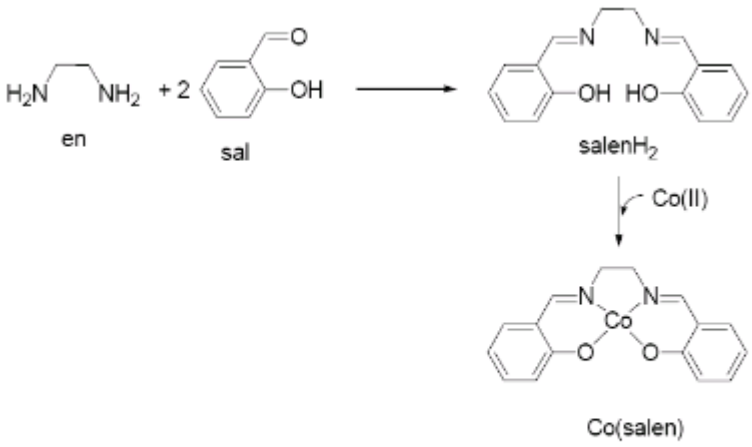


Ferrous Heme



Co(salen)

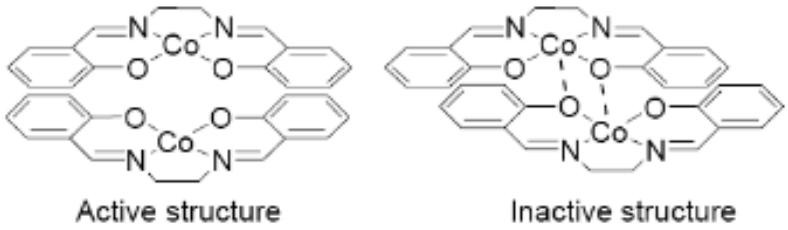
When Co(salen) was first prepared, it was observed that the red-brown crystals darkened on exposure to air. Later that it was established that the color change was due to reversible uptake of O_2. SalenH_2 is a Schiff-base ligand formed by the condensation of two molecules of salicylaldehyde (sal) with ethylenediamine (en).



Synthesis of Co(salen).

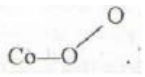
Later, it was found that different crystalline forms existed depending on the solvent used in the preparation, and that these crystalline forms had varying capacity for oxygenation in the solid state. This variation in oxygenation has been related to the presence of voids in the crystal lattice, sufficient to allow the passage of molecular oxygen. This suggestion is

supported by the x-raycrystal structure determination of the so-called "inactive" form which shows that the structure consists of dimeric units $[Co(salen)]_2$. The active forms of Co(salen) are presumed to contain dimeric units with a more open lattice packing relative to the inactive form.

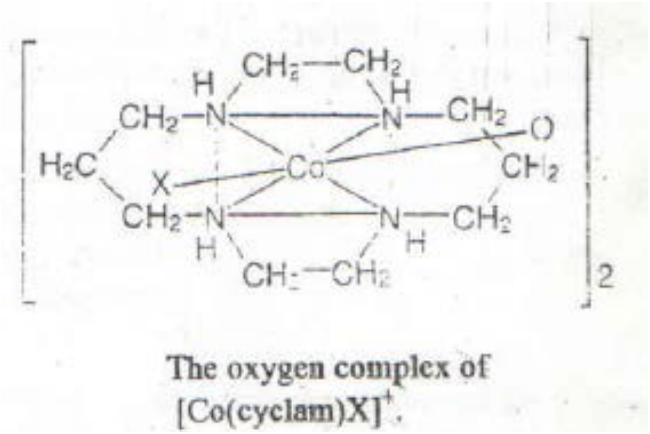


. Structures of active and inactive Co(salen) in the solid state.

The cobalt(II) complexe formed by *N*,*N*'-ehylene-bis(acetylacetonimide) adds O_2 reversibly below 0^OC to give a 1:1 complex. Oxidation of the ligand occurs at higher temperature. An X-ry crystallographic study reveals that the $Co^{III}O_2$- binding is bent end-on



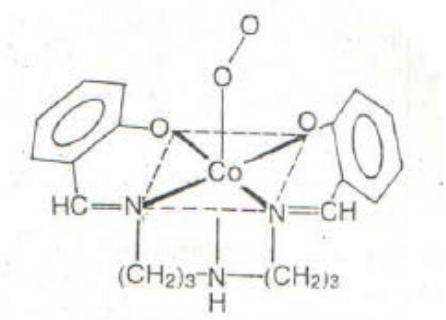
The five coordinate complex formed by the ligand derived from $NH_2C_3H_6NHC_3H_6NH_2$ and salicyladehyde adds O_2 to give a 1:1 complex as shown in figure



The oxygen complex of $[Co(cyclam)X]^+$.

The similar cation complex of cobalt(II) with $(NH_2C_2H_4NHC_2H_4)_2$ gives a dimeric (2: 1) complex with O_2.

The 1:1 complex formed initially would contain Co^{III} and superoxide ion. The superoxide ion easily could oxidize another Co^{II} coordinated to a strong field ligand, to produce the dimer. Synthetic **coboglobins,** prepared from cobalt(II) protoporphyrin IX and globin from hemoglobin or myoglobin, function as O_2 carriers. The coordination of the Co^{II} and the orientation of the porphyrin in deoxy cobalt hemoglobin or myoglobin are the same as in hemoglobin or myoglobin, respectively.



The 1 : 1 salicylaldimine-cobalt(II)-dioxygen complex

5.16 Summary of the unit

Myoglobin and hemoglobin are hemeproteins whose physiological importance is principally related to their ability to bind molecular oxygen.

Hemoglobin is an [α_2:β_2] tetrameric hemeprotein found in erythrocytes where it is responsible for binding oxygen in the lung and transporting the bound oxygen throughout the body where it is used in aerobic metabolic pathways. Each subunit of a hemoglobin tetramer has a heme prosthetic group. Although the secondary and tertiary structure of various hemoglobin subunits are similar, reflecting extensive homology in amino acid composition, the variations in amino acid composition that do exist impart marked differences in hemoglobin's oxygen carrying properties In addition, the quaternary structure of hemoglobin leads to physiologically important allosteric interactions between the subunits, a property lacking in monomeric myoglobin which is otherwise very similar to the α-subunit of hemoglobin.

Myoglobin is a monomeric protein that has 153 amino acids residues. It consists of eight α-helicies connected through the turns with an Oxygen binding site. It has a globular structure. Myoglobin contains a heme (prosthetic) group which is responsible for its main function (carrying of oxygen molecules to muscle tissues). Myoglobin can exist in the oxygen free form, deoxymyoglobin, or in a form in which the oxygen molecule is bound, called oxymyoglobin. Myoglobin is a protein found in muscles that binds oxygen with its heme group. Oxidation of the iron atom ($Fe^{2+} \rightarrow Fe^{3+}$) is mainly responsible for the color of muscle and blood. At the center of protporphyrin, the iron atom is bonded to nitrogen atoms from four pyrrole rings. The iron atom can form two additional bonds, one on each side of the heme plane. These binding sites are called the fifth and sixth coordination sites.

The binding affinities for oxygen between myoglobin and hemoglobin are important factors for their function. Both myoglobin and hemoglobin binds oxygen well when the concentration of oxygen is really high, however, hemoglobin is more likely to release oxygen in areas of low concentration. Since hemoglobin binds oxygen less tightly than myoglobin in

muscle tissues, it can effectively transport oxygen throughout the body and deliver it to the cells. Myoglobin, on the other hand, would not be as efficient in transferring oxygen. It does not show the cooperative binding of oxygen because it would take up oxygen and only release in extreme conditions. Myoglobin has a strong affinity for oxygen that allows it to store oxygen in muscle effectively. This is important when the body is starving for oxygen, such as during anaerobic exercise. During that time, carbon dioxide level in blood streams is extremely high and lactic acid concentration builds up in muscles. Both of these factors cause myoglobin (and hemoglobins) to release oxygen, for protecting the body tissues from getting damaged under harsh conditions. If the concentration of myoglobin is high within the muscle cells, the organism is able to hold the breath for a much longer period of time.

5.17 Key words

Metalloproteins; Heme proteins; Hemoglobin; Myoglobin; Heme (Porphyrin)

5.18 References for further studies

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5.19 Questions for self understanding

1) What are metalloproteins? Give few examples.

2) Discuss the functions of metalloproteins in biological system.

3) What are heme proteins? Give examples.

4) Write a shoer notes on

a) Hemoglobin

b) Myoglobin

5) List the similarities and differences between hemoglobin and myoglobin

6) Write a note on heme geoup (Porphyrin)

7) Discuss the oxygen uptake in biological system

8) Explain the structure and functions of Hemoglobin and Myoglobin

9) Discuss the followins

a) Di oxygen binding to myoglobin

b) Di oxygen binding to hemoglobin

10) What is cooperativity in hemoglobin means? Explain briefly

11) Briefly explain di oxygen binding model complexes

UNIT -6

Structure

6.0 Objectives of the unit

After studying this unit you are able to

- Explain the significance of electron transfer forces in biological system
- Identity the difference between various types of Cytochromes
- Explain the role of Cytochrome C in electron transfer process
- Explain the structure and function of Cytochrome c oxidase
- Explain the structure and function of Ferredoxin
- Explain the structure and function of Rubredoxins

6.1 Introduction

Electron-transfer reactions play key roles in many biological processes, including collagen synthesis, steroid metabolism, the immune response, drug activation, neurotransmitter metabolism, nitrogen fixation, respiration, and photosynthesis. The latter two processes are of fundamental significance-they provide most of the energy that is required for the maintenance of life. In biological system this electron-transfer reaction is taking place by oxidation-readuction (redox) reaction between certain molecules present in the living system.

Mainly three types of oxidation-reduction (redox) centers are found in biology and they are

i) Protein side chains

ii) Small molecules and

iii) redox cofactors.

The first class is frequently overlooked by mechanistic enzymologists. The sulfhydryl group of cysteine is easily oxidized to produce a dimer, known as cystine

$$2R\text{-}SH \xrightarrow[-2H^+]{-2e^-} R\text{-}S\text{-}S\text{-}R$$

This type of interconversion is known to occur in several redox proteins, including xanthine oxidase, mercuric ion reductase, and thioredoxin.

A variety of small molecules, both organic and inorganic, can function as redox reagents in biological systems. Of these, only the nicotinamide and qui- none coenzymes are found throughout the biosphere. Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) participate in a wide variety of biological redox reactions. The 4-position of the pyridine ring is the reactive portion of both molecules and both typically function as 2-electron redox reagents.

Metalloproteins containing a single type of redox cofactor can be divided into two general classes: electron carriers and proteins involved in the transport or activation of small

molecules. Some of the factors that seem to be characteristic of electron-transfer proteins (these proteins are sometimes called "electron transferases") are

a) Possession of a suitable cofactor to act as an electron sink

b) Placement of the cofactor close enough to the protein surface to allow electrons to move in and out

c) Existence of a hydrophobic shell adjacent to, but not always entirely surrounding, the cofactor

d) Small structural changes accompanying electron transfer and

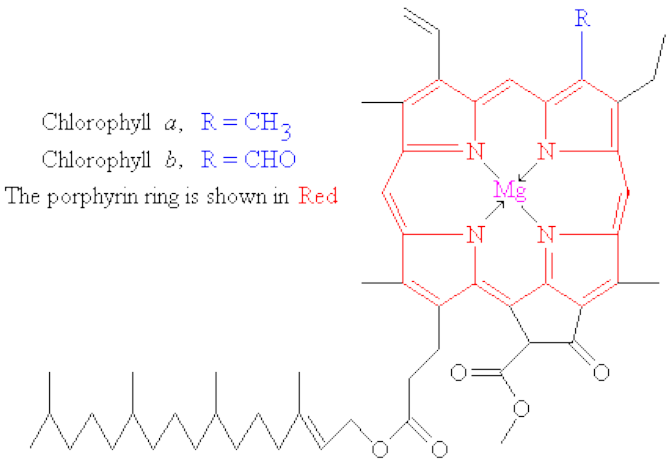
e) An architecture that permits slight expansion or contraction in preferred directions upon electron transfer.

In this unit we will discuss the structure and functions of some metalloproteins involved in electron transfer process.

6.2 Electron transfer forces

One of the most important chemical reactions is electron transfer from one atomic/molecular unit to another. This reaction accompanied by proton and hydrogen atom transfers, occurs in a cascade in many biological processes, including photosynthesis.

Electron transfer reactions are fast, can occur across relatively well separated units, can be triggered by light and can induce secondary reactions. All these features are fully exploited by nature. Biological processes involve complex reactions dominated by electron and hydrogen movement. There are proteins that facilitate electron transfer from one part to another. These proteins have metal ions capable of existing in different oxidation states. The ions are embedded in an environment that responds to change in the oxidation states to transfer an electron from one part to another. The important ligand systems are tetrapyrrole macrocycles,viz., porphyrins.



Molecular structure of the plant pigments, tetrapyrroleunits.

Proteins that function as electron transferases typically place their prosthetic groups in a hydrophobic environment and may provide hydrogen bonds (in addition to ligands) to assist in stabilizing both the oxidized and the reduced forms of the cofactor. Metal-ligand bonds remain intact upon electron transfer to minimize inner-sphere reorganization. Many of the complex multisite metalloenzymes (e.g., cytochrome C oxidase, xanthine oxidase, the nitrogenase FeMo protein) contain redox centers that function as intramolecular electron transferases, shuttling electrons to/from other metal centers that bind exogenous ligands during enzymatic turnover.

There are four classes of electron transferases, they are

1) Flavodoxins

2) Blue copper proteins

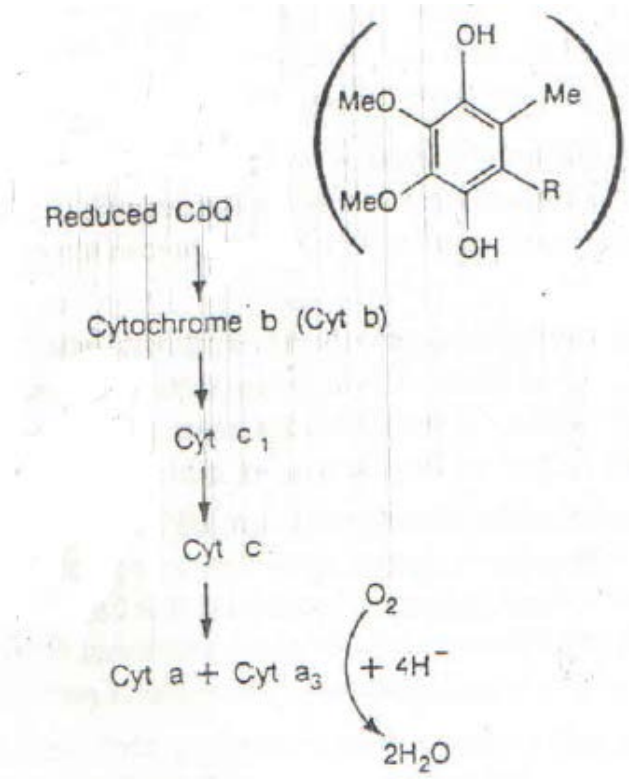
3) Iron-sulfur proteins and

4) Cytochromes

Each of which contains many members that exhibit important structural differences.

6.3 Cytochromes

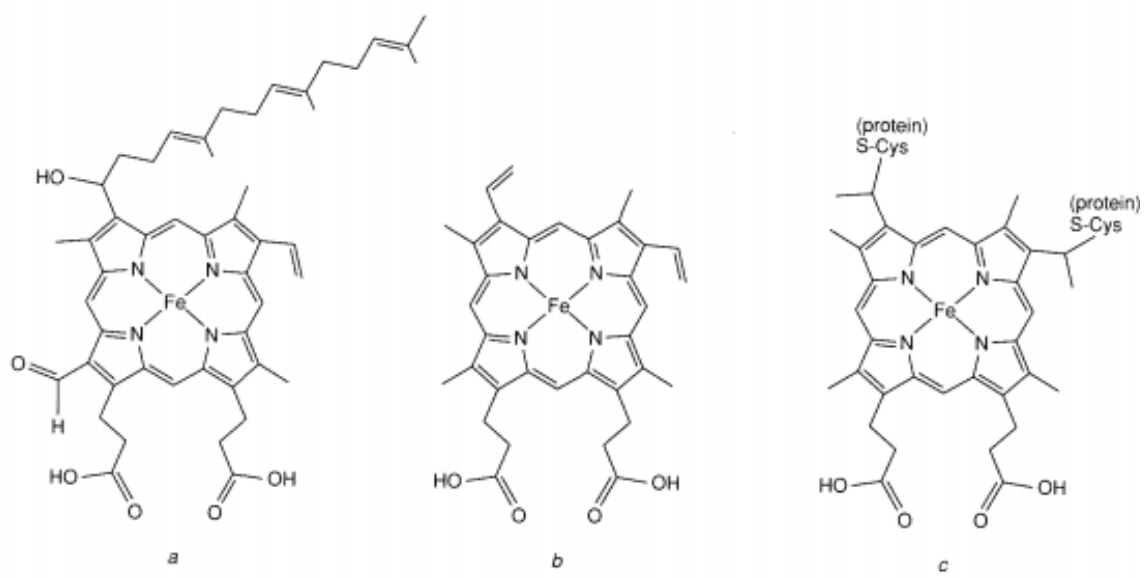
Cytochromes are heme proteins that act as electron carriers, linking the oxidation of substrates to the reduction of O_2, as shown schematically in figure.



A scheme showing the sequence of cytochrome (Cyt) thast intervene between coenzume Q (CoQ) and the reduction of dioxygen to water

Cytochromes are found in all aerobic forms of life. They are small (MW~12,000) molecules, and they operate by shuttling of the iron atom between Fe(II) and Fe(III). Cytochromes contain one or more heme cofactors. These proteins were among the first to be identified in cellular extracts because of their distinctive optical properties, particularly an intense absorption in the 410-430 nm region (called the Soret band)

Cytochromes are broadly divided into types a, b, and c according to the type of prophyrin they contain. Blow figure displays the three most commonly encountered types of heme.



Structures of hemes *a*, *b*, and *c*.

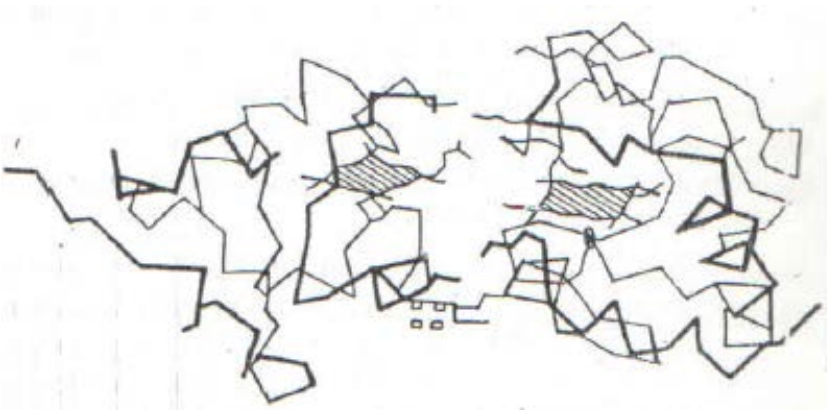
Heme a possesses a long phytyl "tail" and is found in cytochrome c oxidase

Heme b is found in b-type cytochromes and globins

Heme c is covalently bound to c-type cytochromes via two thioether linkages.

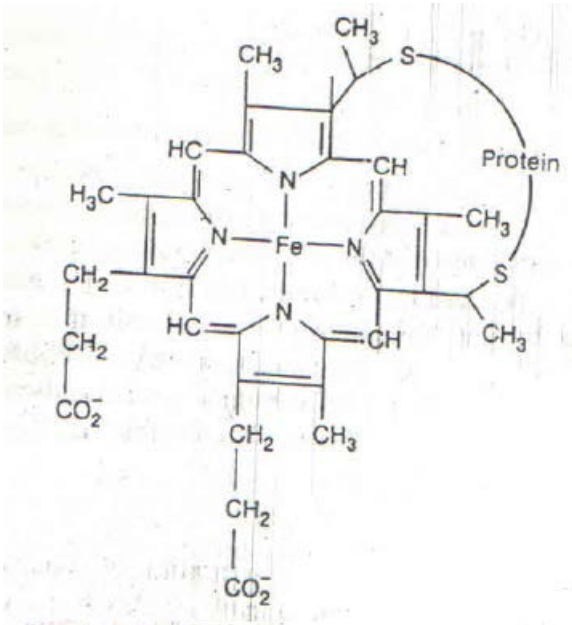
Cytochromes function by shuttling iron between Fe(II) and F e(III) at the active site and hence are one-electron transfer reagents. The Fe atoms are in porphyrin ring coordination environments buried in the middle of the protein. Despite the complexity of the complexes it is possible to model the encounter complex that is formed when two of these proteins meet to exchange an electron. Computer model of the encounter between two cytochromes is shown in below figure. It is easy to believe from that, because the Fe atoms of the two encountering complexes always remains far apart the reaction cannot be a case of inner-sphere electron transfer and reaction must take place by-long-distance outer-sphere electron transfer.

The iron atom is ligated on both sides of the porphyrin plane by the $CH_2CH_2SCH_3$ side chain of a methionine and a nitrogen atom of a histidine. The rest of the pocket is lined by hydrophobic side chains and only a small part of the rim of the porphyrin ring is at or near the exposed surface of the molecule.



One stage in a computer simulation of the encounter between two electron transfer proteins. The shaded areas are the iron porphyrins.

The electrons originating with reduced coenzyme Q (CoQ) are accepted by a cytochrome b and proceed through a series of' intermediate cytochromes. Overall four electrons are delivered at an appropriate potential to carry out the reduction of O_2 'to water. The redox potentials of the intervening cytochromes gradually increase to cover the gap between the oxidation of reduced CoQ and reduction of dioxygen. To do this the cytochromes have distinctive structures and properties, In Cyt_b, Cyt_{c1}, and Cyt_c the heme group is the same as that in hemoglobin and myoglobin. In Cyt_b the heme is not covalently attached to the protein, but is held only by ligation to the iron atom. In Cyt_{c1} and Cyt_c the heme is covalently connected by thioether linkages as shown in below figure and the iron atom is coordinated by protein side chains



The iron porphyrin unit in cytochromes *c*.

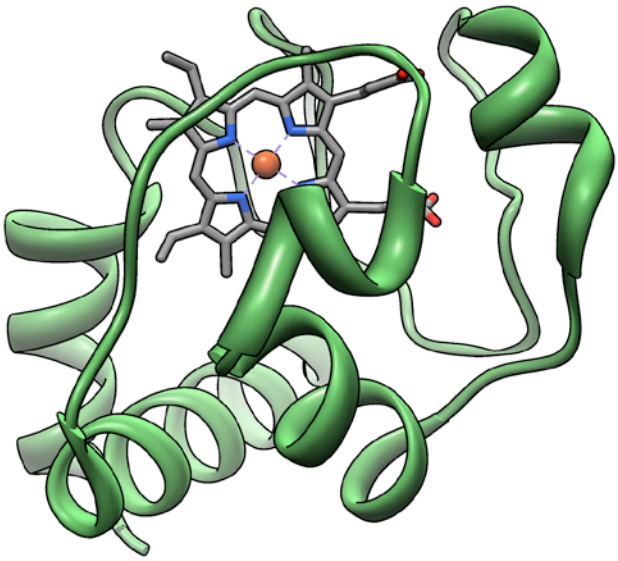
Cytochrome a and Cyt_{a3} exist together as a complex, sometimes led cytochrome oxidase, and they contain a heme with several different substituents on the periphery of the porphyrin ring.

Cytochrom a_3 also contains copper, which go s from Cu^{III} to Cu^{I} and thus transfers an electron from the heme of Cyt a_3 to the dioxygen molecule.

6.4 Cytochrome C

Cytochrome C occupies a prominent place in the mitochondrial electron-transport chain. Its water solubility, low molecular weight (12.4 kDa), stability, and ease of purification have allowed many experiments, which, when taken together, present a detailed picture of the structure and biological function of this electron carries.

Cytochrome c consists of a single polypeptide chain of 104 amino acid residues and covalently attached to a heme group. They are roughly spherical with a diameter of 34Å. The heme is surrounded by many tightly packed hydrophobic side chains. Only one edge of the planar heme ring is accessible to the surface. Small channels on either side of the ring permit only small hydrophobic molecules to the heme iron. The mechanism of electron transfer is unclear, however, it is believed that features on the surface of the molecule near the heme may be involved in electron-transfer activities. The cationic side chains of several lysine and arginine residues are clustered at the surface on one face of the molecule and are thought to provide a binding site for anionic groups on cytochrome oxidase. Likewise, on the opposite face of the molecule, a cluster of anionic residues including glutamic and aspartic acids may provide a binding site for a reductase or other components of the electron transport systems. Cytochrome c has 19 positively charged lysine residues, plus two arginines also positively charged, but only 12 acidic residues (aspartic or glutamic acids). Cytochrome c is very basic with an isoelectric point near pH 10



Cytochrome c plays important roles in two processes, they are oxidative phosphorylation and apoptosis.

As an electron carrier in oxidative phosphorylation, cytochrome c shuttles four electrons, one at time, via its heme group from cytochrome c reductase to cytochrome c oxidase.

As a crucial player in apoptosis (programmed cell death), cytochrome c is released from the mitochondria to the cytosol where it binds to an adaptor subunit, APAF-1 in the presence of dATP, leading to activation of caspase 9. Caspase 9 triggers activation of other caspases which cleave and destroy other proteins. This results in cell death.

Control of the reduction potential of the iron center in cytochrome C is depends on the following factors, they are

i) The nature of the axial ligands

ii) The stability and solvent accessibility of the heme crevice and

iii) The hydrophobicities of the amino acids that line the heme crevice.

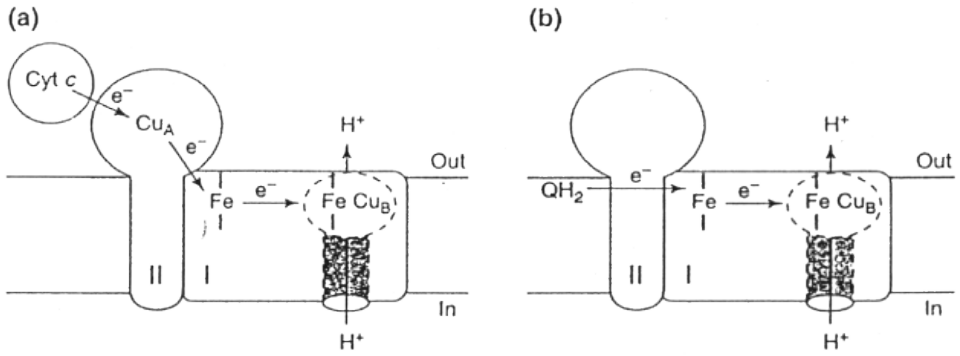
6.5 Cytochrome c oxidase

Cytochrome c oxidase is the terminal enzyme in the respiratory chain, is located in the inner membrane of mitochondria and bacteria. It catalyses the reduction of dioxygen to water and pumps an additional proton across the membrane for each proton consumed in the reaction. The resulting electro-chemical gradient is used elsewhere, for ex, in the synthesis of ATP.

The protein contains two heme and two copper sites. The reaction center for the reduction of oxygen is a binuclear center formed by the iron of a heme and copper (Cu). In the course of the reaction cycle four protons have to be transferred from the matrix side of the membrane to the oxygen, and an additional four protons are pumped across the membrane using the free energy released in the reaction.

There are two catalytic subunits, I and II are present in cytochrome c oxidase. Subunit I contains two heme centers. The first of these (heme a in cytochrome oxidaes) acts as an electron input device to the second. The second heme (heme a_3 in cytochrome oxidase) is part of a binuclear center, with a Cu (CuB in cytochrome oxidase) as the other metal. The binuclear center is the site of oxygen reduction.

Subunit II processes the electron donation. In cytochrome c oxidases, the subunit contains a Cu center (CuA) with 2Cu atoms, which is thought to be the immediate electron acceptor from cytochrome c. A possible electron transfer pathway from this center to heme a has been identified in the structure (see below). In quinol oxidases, this subunit processes the quinol substrate. In addition to the H^+ uptake in the N-phase associated with reduction of O_2, the enzymes also transfer additional protons across the membrane, from N- to P-phase.



aa_3-type (cow, *P. denitrificans*, yeast, *Rb. sphaeroides*)
ba_3-type (*Thermus thermophilus*)

bo_3-type (*E. coli*)
ba_3-type (*Acetobacter aceti*)
aa_3-type (*Bacillus subtilis*)

6.6 Ferredoxin

There are non heme iron proteins that participate in electron-transfer processes. They contain Fe bonded to Sulphur.

Ferredoxins are iron-sulfur proteins that mediate electron transfer in a range of metabolic reactions. Or

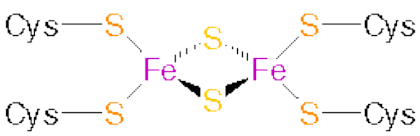
Ferredoxins are acidic, low molecular weight, soluble iron-sulfur proteins found in various organisms, and act as multifuncitonal electron carriers in diverse redox systems.

Iron-sulfur proteins are defined as proteins carrying iron-sulfur cluster(s) in which the iron is at least partially coordinated by sulfur. Iron-sulfur clusters are prosthetic groups commonly found in various proteins that participate in oxidation-reduction reactions and catalysis. In most instances, the iron is bound to sulfur(s) from cysteine residues in polypeptide backbone and also to inorganic sulfurs in the iron-sulfur cluster. The chief role of the iron-sulfur cluster is to facillitate electron transfer, while in others, the clusters contribute to catalytic function.

The major forms of iron-sulfur cluster are as follows

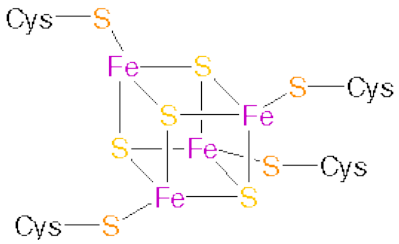
The [2Fe-2S] cluster

The two iron atoms are bridged to one another by two inorganic sulfur atoms and ligated to four cysteines from peptide backbone.



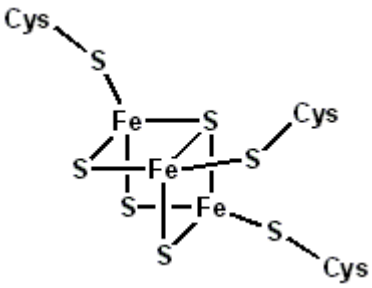
The [4Fe-4S] cluster

The four iron atoms are ligated to four cysteines and form a cubic structure with the four inorganic sulfur atoms.



The [3Fe-4S] cluster

A single iron of the [4Fe-4S] cluster is absent.

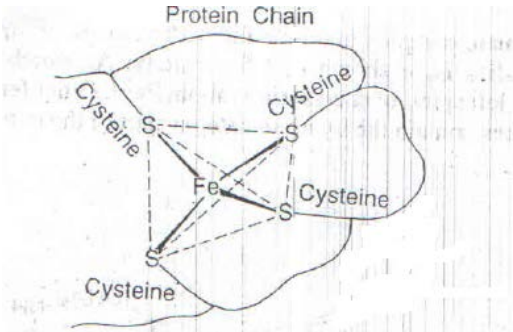


Ferredoxin is also called "red enzyme". This protein plays a vital role in electron transfer processes in clostridial and plant cells. The ferredoxins display variable molecular weights and contain different amounts of iron, depending upon their source.

One group, originally found in the bacterium Clostridium pasteurianum, has been termed “bacterial-type”, in which the active center is an Fe_4S_4 cluster. Bacterial-type ferredoxins may in turn be subdivided into further groups, on the basis of their sequence properties. Most contain at least one conserved domain, including four cysteine residues that bind an Fe_4S_4 cluster. In some bacterial-type ferredoxins, one of the four conserved cysteine residues is lost. These ferredoxins bind an Fe_3S_4 cluster instead of an Fe_4S_4 cluster

6.7 Rubredoxins

Rubredoxin is a small iron-sulfur protein found in various sulfur-metabolizing bacteria. Rubredoxin was firs isolated from the bacterium *Clostridiu pasteurianum,* but it also occurs in other anaerobic bacteria.



The FeS_4 flattened tetrahedron (smallest angle 101°) in rubredoxin. There is one short Fe–S bond (205 pm), with others at 224-235 pm.

The single Fe is bonded to four S atoms of cysteine [$HSCH_2CH(NH)_2CO_2H$] as part of a protein of about 54 amino acid residues (molar mass 6000). The S atoms form a distorted tetrahedron around iron with one very short Fe-S bond.

The reduction potential of rubredoxin in neutral solution is close to 0 V, The oxidized protein is red in color, and the reduced form is colorless.

Rubredoxins are found in strictly anaerobic bacteria and archea and have oxidation/reduction potentials (+30 to 60 mV, relative to the hydrogen electrode) that appear to be too high to be relevant for bacterial metabolism. Although assumed to function as electron carriers, the redox partners of rubredoxins in anaerobes have not been identified. Recently, a rubredoxin-oxygen oxidoreductase was found to function in vitro at the end of a soluble electron transfer chain that couples NADH oxidation to oxygen consumption in Desulfovibrio gigas. There is another protein in D. gigas containing a single $Fe(Cys)_4$ unit in each of its two small subunits, named desulforedoxin. Rubredoxin-like proteins that contain two $Fe(Cys)_4$ clusters are also found in Pseudomonacee. These aerobic rubredoxins transfer electrons to enzymes that catalyze the oxidation of alkanes.

6.8 Summary of the unit

Electron transfer, or the moving an electron from one place to another, is amongst the simplest of chemical processes, yet certainly one of the most critical. The process of efficiently and controllably moving electrons around is one of the primary regulation mechanisms in biology. Without stringent control of electrons in living organisms, life could simply not exist. For example, photosynthesis and nitrogen fixation (to name but two of the most well-known biochemical activities) are driven by electron transfer processes. It is unsurprising, therefore, that much effort has been placed on understanding the fundamental principles that control and define the simple act of adding and/or removing electrons from chemical species.

Transition metals such as copper and iron play leading roles in electron transport as one electron redox-active centers within proteins that are used to effectively move electrons around. Well-known examples are the blue copper proteins ($Cu^{I}\leftrightarrow Cu^{II}$), cytochromes ($Fe^{II}\leftrightarrow Fe^{III}$ porphyrins), and iron-sulfur proteins ($Fe^{II}\leftrightarrow Fe^{III}$ with sulfur ligands). Significant efforts have been placed on developing our understanding of how biological systems control which electron transfer processes are feasible (i.e., reduction potentials) and how fast they will occur (i.e., rate constants). The factors that affect the properties of these important biological electron transfer sites are generally considered as either intrinsic (i.e., an inherent behavior and/or property of the site itself) or extrinsic (i.e., modulation of the basic properties

by external factors) to the active site. Our recent efforts have served to provide insights into the intrinsic properties of mononuclear iron centers using both experimental and theoretical methods to evaluate their inherent electronic structure and to correlate it to their observed redox properties.

6.9 key words

Electron transfer forces; Cytochromes; Cytochrome C; Cytochrome c oxidase ; Ferredoxin; Rubredoxins

6.10 References for further studies

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6.11 Questions for self understanding

1) What are electron transfer forces?

2) Give few examples of proteins which are participated in electron transfer

3) Mention any few biological processes which required electron transfer reaction

4) What are cytochromes?

5) Discuss the structure and function of cytochrome c

6) Discuss the structure and function of Cytochrome c oxidase

7) Discuss the structure and function of Ferredoxin

8) Discuss the structure and function of Rubredoxins

UNIT-7

Structure

7.0 Objectives of the unit

After studying this unit you are able to

- Expalin the role of metalloenzymes in biological system
- Identify the different zinc enzymes present in biological system
- Explain the structure and function of carboxypeptidase
- Identify the different copper enzymes present in biological system
- Discuss the structure and role of superoxide dismutases (SOD)
- Identify the different molybdenum enzymes present in biological system
- Identify the different iron enzymes present in the biological system
- Explain the structure and role of cytochrome P-4S0 enzymes.

7.1 Introduction

Enzymes are very efficient catalysts for biochemical reactions. They speed up reactions by providing an alternative reaction pathway of lower activation energy. Enzymes are usually highly selective, catalysing specific reactions only. This specificity is due to the shapes of the enzyme molecules. Many enzymes consist of a protein and a non-protein (called the cofactor). The proteins in enzymes are usually globular. The intra- and intermolecular bonds that hold proteins in their secondary and tertiary structures are disrupted by changes in temperature and pH. This affects shapes and so the catalytic activity of an enzyme is pH and temperature sensitive.

The pH of a solution can have several effects of the structure and activity of enzymes. For example, pH can have an effect of the state of ionization of acidic or basic amino acids. Acidic amino acids have carboxyl functional groups in their side chains. Basic amino acids have amine functional groups in their side chains. If the state of ionization of amino acids in a protein is altered then the ionic bonds that help to determine the 3-D shape of the protein can be altered. This can lead to altered protein recognition or an enzyme might become inactive.

Changes in pH may not only affect the shape of an enzyme but it may also change the shape or charge properties of the substrate so that either the substrate con not bind to the active site or it cannot undergo catalysis. In geneal enzyme have a pH optimum. However the optimum is not the same for each enzyme.

Cofactors may be i) organic groups that are permanently bound to the enzyme (prosthetic groups), ii) cations - positively charged metal ions (activators), which temporarily bind to the active site of the enzyme, giving an intense positive charge to the enzyme's protein

7.2 Metalloenzymes

Enzymes are proteins synthesized from amino acids by cells. The names of the enzymes are obtained by adding the suffix ase to the name of the process they catalyzed or to the name of the molecule on which they acts (the **substrate**). Some enzymes function alone, whereas others require the cooperation of a ***cofactor*** usually a metal ion or an organic molecule. *The enzymes requiring metal ions as cofactors are called metalloenzymes*. Some name of the metalloenzymes, the metal ions they associated and their functions are given in Table.

Mg^{II}	Phosphohydrolases Phosphotransferases	Mn^{II}	Arinase Oxaloaceteate decarboxylase Phosphotransferrases
Fe^{III} or Fe^{III}	Cytopchromes Peroxidase Catalase Ferredoxin	Zn^{II}	Alcohol dehydrogenase Alkaline phosphatease Carbonic anhydrase Carboxypeptidase
Cu^{II} or Cu^{I}	Tyrosinase Amine Oxidases Cytochrome oxidase Ascorbate oxidase Galactose oxidase Dopamine β-hydroxylase	Fe and Mo	Nitrogenase

Coenzymes that are tightly bound to the enzyme are, called prosthetic groups. The active centers of metalloenzymes often involve distortion (the entatic state) of the normal stereochemistry of the metal ion with changes in stereochemistry occurring during the reaction cycle. Enzyme reactions yields are often 100%, and occur under the mildest conditions. High Yields are achieved in enzyme catalyzed reactions because the rate of the reaction might be increased by a factor of as much as 10^{12}, making side reactions unimportant.

7.3 Examples of metalloenzymes

The rhizobium organisms which are living in the root nodules of various legume species (clover, alfalfa, beans, peas, etc.) share with several bacteria and blue-green algae and convert the atmospheric N_2 to NH_3. The enzyme nitrogenase carries out this redox reaction under anaerobic conditions.

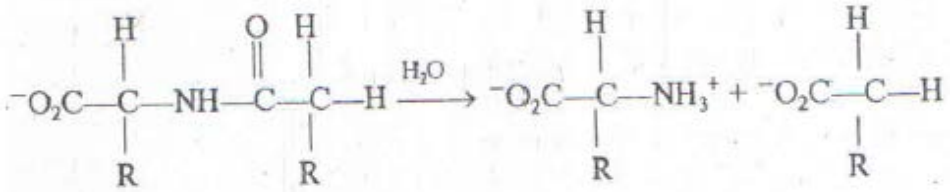
7.4 Zinc enzymes

Zinc is required for the activities of more than 300 enzymes, which are divided in to six classes. Zinc binding sites in proteins are often distorted tetrahedral or trigonal bipyramidal geometry, made up of the sulfur of cysteine, nitrogen of histidine or the oxygen of aspartate

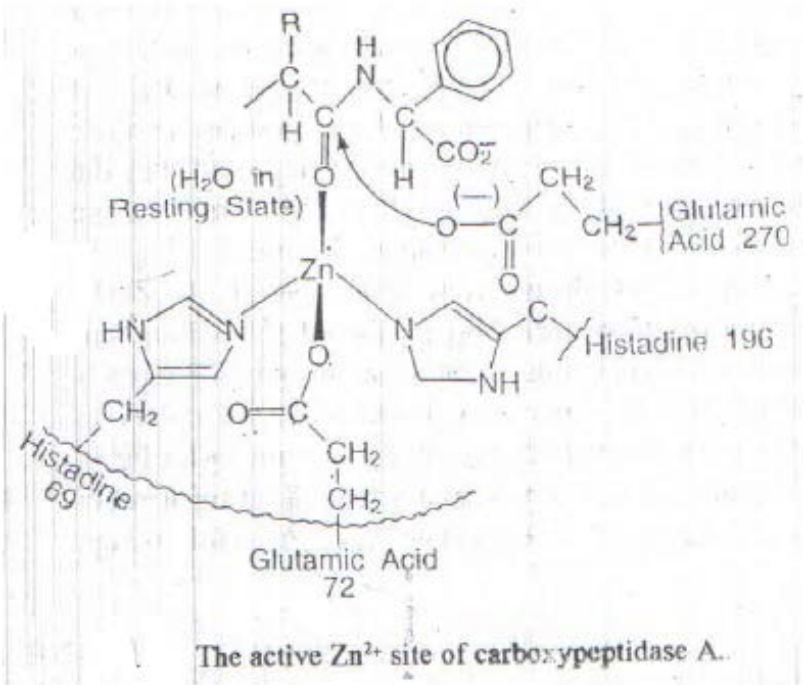
and glutamate, or a combination. Zinc in proteins can either participate directly in chemical catalysis or be important for maintaining protein structure and stability. In all catalytic sites, the zinc ion functions as a Lewis acid.

7.5 Carboxypeptidase

The carboxypeptidases are enzymes containing Zn(II) that catalyze the hydrolysis of the peptide bonds in peptides and proteins.



Bovine carboxypeptidase-A is an enzyme composed of 307 amino acids in a single polypeptide chain and binds one Zn^{2+} ion per molecule. The structure of carboxypeptidase-A has been studied extensively by X-ray crystallography. The one Zn^{2+} per molecule is bonded to two imidazoles of histidine side chain, one glutamic acid group, and an H_2O molecule that can be replaced by the substrate. The Zn^{2+} sits in a depression in the surface of the molecule. This is the active site, from which extends a pocket into the interior of the molecule for accommodating the substrate. The enzyme protects its own terminal end against self-digestion by tucking it inside. The Zn^{2+} site shows coordination of histidine residues 69 and 196 and glutamic acid residue 72. Arginine-l45 and tyrosine-248 are seemed to be involved in hydrogen bonding to the substrate, causing dramatic conformational changes of the protein. The glutamic acid residue 270 serves as a nucleophilc aiding cleavage of the peptide linkage.



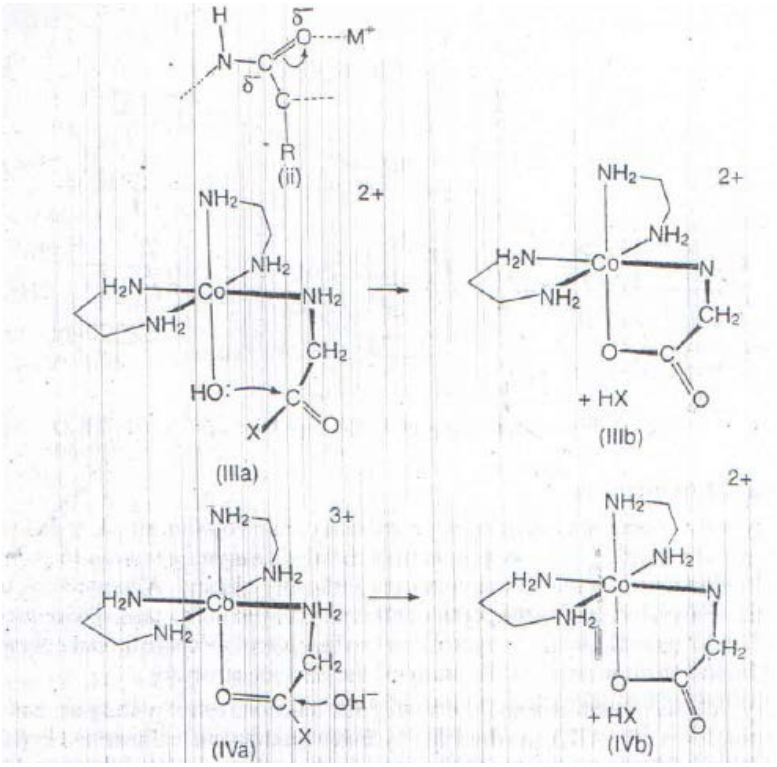
The active Zn^{2+} site of carboxypeptidase A.

Zinc ion can be replaced by cobalt(II) in carboxypeptidase and in several other zinc enzymes with retention of activity of the enzyme. In some cases there is even enhanced activity is observed. Because Co was not available in greater extent therefore the nature had to settle for Zn. Replacement of Zn^{2+} by Co^{2+} has been particularly useful because Zn^{2+} (d^{10}) forms colorless complexes whereas Co^{2+}(d^7) complexes absorb in the visible region. Thus spectral studies of the Co-substituted enzyme provide valuable information about the metal ion environment at the active site. Cobalt serves as a spectral probe for the study of the active side. Other divalent metal ions that can be substituted for Zn with retention of some peptidase or esterase activity are Ni, Cd, Hg, and Pb.

7.8 Mechanisms of hydrolysis

The hydrolysis reaction requires the promotion of carbonyl group O atom of the peptide bond. The metal ion promotes proton loss from the H_2O molecule to make it into the more nucleophilic (OH^-) ligand. Alternatively, the metal ion act as a Lewis acid catalyst by binding the peptide carbonyl group so reduce the electron density at its C atom.

Recent X-ray studies of a model substrate evidences of hydroxo mechanism. The carbonyl group to be attacked is first hydrogen bonded to arginine residue 127.An H_2O molecule remains in the coordination sphere of the Zn^{2+} ion and attacks the substrate by the hydroxo mechanism. The primary intermediate in the reaction is a idol structure shown in Figure. This proposal is consistent with the kinetics or the reaction and with observations on binding of analogs of the substrate. The mechanism implies the existence of transient five-coordination at the Zn^{2+} ion.



7.9 Copper enzymes

Copper proteins/enzymes occur widely in nature and are responsible for performing a diverse array of functions. The classification of copper centers found in enzymes is based on the spectroscopic properties of the center. There are presently four types of copper centers:

Type 1 (TI) copper centers (blue copper) are normally involved in electron transport

Type 2 (T2) centers are termed "normal" copper centers because of their typical EPR spectra that resemble inorganic Cu(I1) complexes

Type 3 (T3) centers are EPR silent, owing to the magnetic coupling of their binuclear copper ions in the oxidized state; as in hemocyanin, tyrosinase, and catechol oxidase.

The fourth class is the multicopper containing enzymes that usually contain all three types of copper centers.

T1 Copper Centers

Blue copper proteins are important electron transfer proteins with an intense absorption near 600 nm resulting in their blue color.

T2 Copper Proteins

With the exception of galactose oxidase, the T2 copper proteins usually do not have intense absorbance in the visible region. Superoxide dismutase, galactose oxidase, amine oxidase along with peptidylglycine α-amidating monooxygenase and dopamine β-monooxygenase all belong to this class.

T3 Copper Proteins

Hernocyanin (Hc), tyrosinase, and catechol oxidase contain a coupled binuclear (T3) copper center. All three proteins contain two copper atoms that are antiferromagnetically coupled in the Cu(I1) state. The ground state of oxygenated Hc is EPR silent due to a strong antiferromagnetic coupling of the two Cu(I1) ions by an endogenous ligand that bridges the two metal centers. This Cu-Cu interaction results in strong exchange coupling of the unpaired electron. T3 proteins exhibit a relativity intense LMCT band centered near 350 nm derived from peroxide to Cu(I1) charge transfer of the bound O_2.

7.10 Superoxide dismutases (SOD)

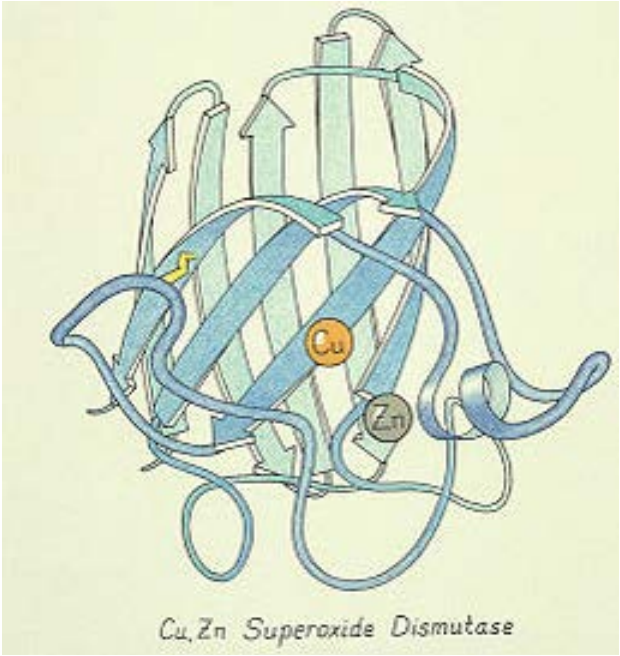
Superoxide dismutases are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide.

$$Cu^{2+}\text{-SOD} + O_2^- \rightarrow Cu^{+}\text{-SOD} + O_2$$
$$Cu^{+}\text{-SOD} + O_2^- + 2H^+ \rightarrow Cu^{2+}\text{-SOD} + H_2O_2$$

Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen. One of the exceedingly rare exceptions is Lactobacillus plantarum and related lactobacilli, which use a different mechanism**.**

Studies have shown that SOD acts as both an antioxidant and anti-inflammatory in the body, neutralizing the free radicals that can lead to wrinkles and precancerous cell changes. Researchers are currently studying the potential of superoxide dismutase as an anti-aging treatment, since it is now known that SOD levels drop while free radical levels increase as we age. Superoxide Dismutase helps the body use zinc, copper, and manganese.

There are two types of SOD: copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD. Each type of SOD plays a different role in keeping cells healthy. Cu/Zn SOD protects the cells' cytoplasm, and Mn SOD protects their mitochondria from free radical damage.



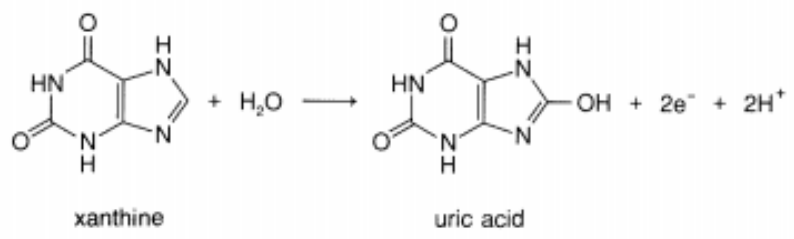
Cu/Zn SOD is a dimeric enzyme with each monomeric unit containing an active site of one copper and one zinc bridged by a histidine imidazole. The copper is bound by 3 additional histidines with an overall distorted square planar structure and an additional water molecule. The zinc is bound by two histidines and an aspartate in addition to the bridging imidazole. Copper is the redox-active metal, changing between the $2^{+}/3^{+}$ oxidation states during catalysis, and zinc appears to play a role in overall enzyme stability and in facilitating a large pH independence in activity. Upon reduction of the Cu^{2+}/Zn^{2+} SOD to Cu^{+}/Zn^{2+} SOD, the bridging imidazole-copper coordination is lost, as is the bound water and the Cu^{+} shifts position and is three coordinate; otherwise both oxidized and reduced enzymes are generally structurally similar.

7.11 Molybdenum enzymes

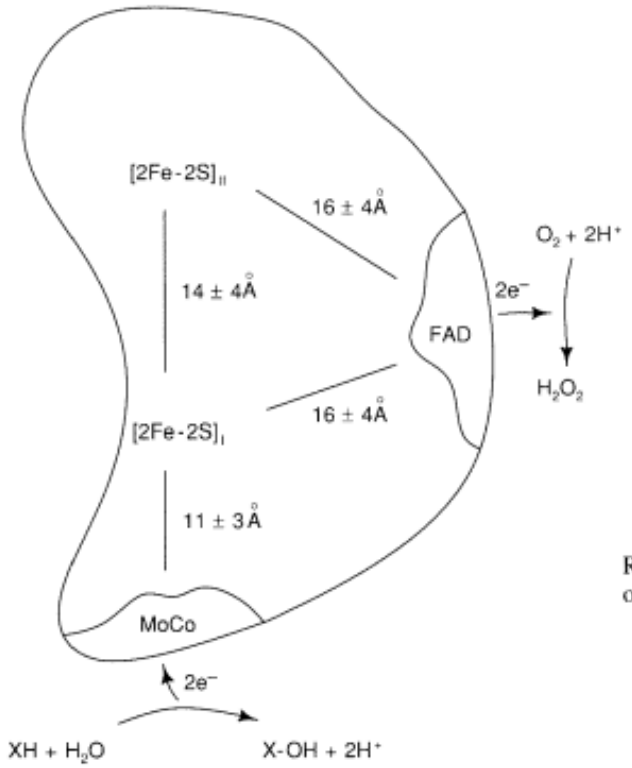
There are many molybdenum containing enzymes distributed throughout the biosphere. The availability of molybdenum to biological systems is due to the high water solubility of oxidized forms of the metal. Molybdenum enzymes can be grouped on the basis of the structure of the metal centre. Three principal families of enzyme exist, with active sites consisting of (ppt)MoOS(OH) (the molybdenum hydroxylases), (ppt)MoO_2(S-Cys) (the eukaryotic oxotransferases) and $(ppt)_2$MoOX (the bacterial oxotransferases). Here, ppt represents a unique ppt cofactor (pyranopterin) that co-ordinates to the metal, and X is a metalliganded serine, cysteine or selenocysteine. The molybdenum hydroxylases catalyse their reactions differently to other hydroxylase enzymes, with water rather than molecular oxygen as the ultimate source of the oxygen atom incorporated into product, and with the generation rather than consumption of reducing equivalents. The active sites possess a catalytically labile Mo-OH (or possibly Mo-OH_2) group that is transferred to substrate in the course of the hydroxylation reaction. These enzymes invariably have other redoxactive centres. The eukaryotic oxotransferases consist of the sulphite oxidases and plant nitrate reductases. They catalyse the transfer of an oxygen atom to or from their substrate (to and from nitrate) in a manner that involves formal oxidation-state changes of the molybdenum. As with the molybdenum hydroxylases, the ultimate source of oxygen is water rather than molecular oxygen. The bacterial oxotransferases and related enzymes differ from the other two groups of molybdenum enzymes in having two equivalents of the ppt cofactor co-ordinated to the metal. This family is quite diverse, as reflected in the fact that serine, cysteine or selenocysteine may be found co-ordinated to the molybdenum, depending on the enzyme. As in the case of the molybdenum hydroxylases, both eukaryotic and bacterial oxotransferases utilize water (rather than molecular oxygen) as the source of the oxygen atom incorporated into product, although for these enzymes, the catalytically labile oxygen in the active site is an Mo = O group rather than an Mo-OH.

7.12 Xanthine oxidase

Xanthine oxidase catalyzes the two-electron oxidation of xanthine to uric acid



This enzyme plays a prominent role in the biodegradation of purines and is the target of drugs administered to patients suffering from gout (joint inflammation, due to precipitation of sodium urate). Below figure displays the cofactors in a subunit which containsa Mo-pterin, termed MoCo, two [2Fe-2S] centers; and one FAD.



Representation of the cofactors in one subunit of xanthine oxidase.

The binuclear iron-sulfur sites serve to shuttle electrons between the reduced substrate (XH) and O_2.

7.13 Iron enzymes

There are many mononuclear iron containing enzymes in nature that utilize molecular oxygen and transfer one or both oxygen atoms of O_2 to substrates. These enzymes catalyze many processes including the biosynthesis of hormones, the metabolism of drugs, DNA and RNA base repair and, the biosynthesis of antibiotics. Therefore, mononuclear iron containing enzymes are important intermediates in bioprocesses and have great potential in the commercial biosynthesis of specific products since they often catalyze reactions regioselectively or stereospecifically. Understanding their mechanism and function is important and will assist in searches for commercial exploitation.

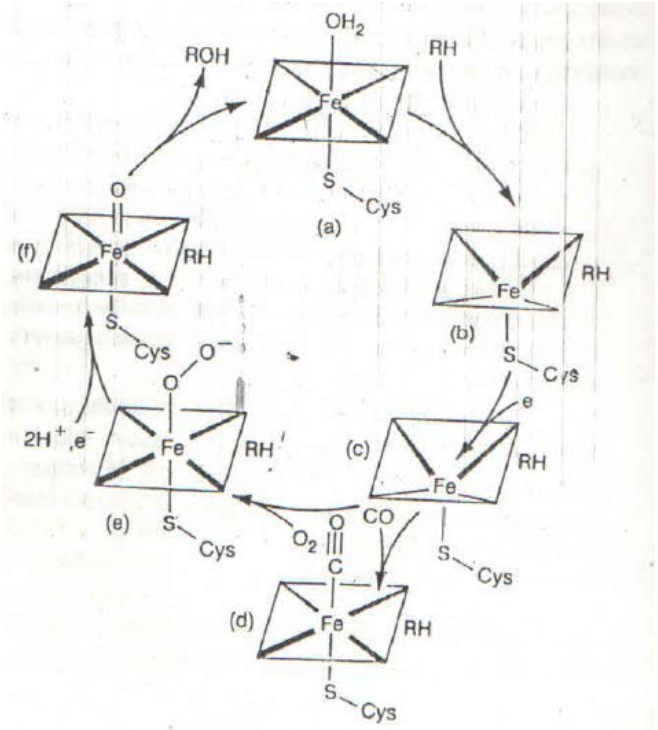
7.14 Cytochrome P-4S0 enzymes.

Cytochrome P-450 is the designation of a family of enzymes with iron porphyrin active sites that catalyze the addition of oxygen to a substrate. The mo. t important representative of this class of reactions is

$$R - H + \frac{1}{2} O_2 - -> R - OH$$

The insertion of O into a R-H bond which is just the sort of redox reaction likely to occur by an atom transfer mechanism. It is a part of the body's defense against hydrophobic compounds such as drugs, steroid precursors, and pesticides. The hydroxylation of RH to ROH renders the target compounds more water soluble and thereby aids their elimination. The name 'P-450' is taken from the position of the characteristic blue to near-ultraviolet absorption band of the porphyrin called the 'Soretband' which is red-shifted to 450 nm in carbonyl complexes of these molecules.

The proposed catalytic cycle for P- 450 is shown in below figure. The sequence begins at (a) in the figure with the enzyme in a resting state with iron present as Fe(III). The hydrocarbon substrate then binds (b) and one electron is transferred (c). The resulting Fe(II) complex with, bound substrate proceeds to bind O_2 (e). (At this point in the cycle, a competing reaction with CO to give (d) leads to a species which is easily identified and is responsible for the absorption at 450 nm which gives the family its name). A key reaction is the reduction of the porphyrin ring of the oxygen complex (e) by a second electron, which produces the ring radical anion. Uptake of two H^+ ions then leads to the formation of the Fe(IV) complex (1) which attacks the substrate to insert oxygen. Loss ofROH and uptake of an H_20 molecule at the vacated coordination position brings the cycle back to the resting state.



The cycle of reactions of P-450. The resulting state of the enzyme is (a) and the important Fe(IV) oxo species is (f).

7.15 Summary of the unit

Metals play roles in approximately one-third of the known enzymes. Metals may be a co-factor or they may be incorporated into the molecule, and these are known as metalloenzymes. Side chains of aminoacids in protein posses groups that can form coordinate-covalent bonds with the metal atom. The free amino and carboxy group bind to the metal affecting the enzymes structure resulting in its active conformation. Metals main function is to serve in electron transfer. Many enzymes can serve as electrophiles and some can serve as nucleophilic groups. This versatility explains metals frequent occurrence in enzymes. Some metalloenzymes include hemoglobins, cytochromes, phosphotransferases, alcohol dehydrogenase, arginase, ferredoxin, and cytochrome oxidase. Ferredoxin is an electron transferring proteins involved in one-electron transfer processes. Cytochrome oxidase contains copper ions easily accommodate electron removed from a substrate and can just as easily transfer them to a molecule of oxygen.

7.16 Key words

Metalloenzymes; Zinc enzymes; Carboxypeptidascs; Copper enzymes; Superoxide dismutases (SOD); Molybdenum enzymes; Iron enzymes; Cytochrome P-4S0 enzymes.

7.17 references for further studies

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7.18 Questions for self understanding

1) What are metalloenzymes?

2) Give few examples of metalloenzymes present in biological system and mention their role

3) Write a various biological processes carried out by zinc enzymes

4) Discuss the structure and function of Carboxypeptidase

5) What are the different biological processes carried out by copper enzymes

6) Discuss the structure and function of Superoxide dismutases (SOD)

7) Write a note on molybdenum enzymes

8) Discuss the structure and function of Xanthine oxidase

9) Discuss the structure and function of cytochrome P-4S0 enzymes.

UNIT-8

Structure

8.0 Objectives of the unit

After studying this unit you are able to

- Identify the different species involved in biological nitrogen fixation
- Explain the structure of the nitrogenase complex
- Discuss the role of dinitrogenase reductase in biological nitrogen fixation
- Discuss the role of dinitrogenase in biological nitrogen fixation
- Write the structure of metal clusters present in dinitrogenase
- Identify the different electron transfer species involved in Photosynthesis
- Discuss the role and structure of chlorophyll
- Discuss the role and structure of Photosystem II

8.2 Introduction

The primary source of energy for nearly all life is the Sun. The energy in sunlight is introduced into the biosphere by a process known as photosynthesis, which occurs in plants, algae and some types of bacteria. Photosynthesis can be defined as the physico-chemical process by which photosynthetic organisms use light energy to drive the synthesis of organic compounds. The photosynthetic process depends on a set of complex protein molecules that are located in and around a highly organized membrane. Through a series of energy transducing reactions, the photosynthetic machinery transforms light energy into a stable form that can last for hundreds of millions of years. This introductory chapter focuses on the structure of the photosynthetic machinery and the reactions essential for transforming light energy into chemical energy.

Nitrogen is a critical limiting element for plant growth and production. It is a major component of chlorophyll, the most important pigment needed for photosynthesis, as well as amino acids, the key building blocks of proteins. It is also found in other important biomolecules, such as ATP and nucleic acids. Even though it is one of the most abundant elements (predominately in the form of nitrogen gas (N_2) in the Earth's atmosphere), plants can only utilize reduced forms of this element. Plants acquire these forms of "combined" nitrogen by: 1) the addition of ammonia and/or nitrate fertilizer (from the Haber-Bosch process) or manure to soil, 2) the release of these compounds during organic matter decomposition, 3) the conversion of atmospheric nitrogen into the compounds by natural processes, such as lightning, and 4) biological nitrogen fixation.

Biological nitrogen fixation (BNF), discovered by Beijerinck in 1901 is carried out by a specialized group of prokaryotes. These organisms utilize the enzyme nitrogenase to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Plants can readily assimilate

NH_3 to produce the aforementioned nitrogenous biomolecules. These prokaryotes include aquatic organisms, such as cyanobacteria, free-living soil bacteria, such as Azotobacter, bacteria that form associative relationships with plants, such as Azospirillum, and most importantly, bacteria, such as Rhizobium and Bradyrhizobium, that form symbioses with legumes and other plants.

8.3 Nitrogen fixation

Nitrogen fixation is a natural process where nitrogen gas is converted into ammonium either by microbes or by lightning. This process is vital to life on the planet, as well as food production, as nitrogen is needed for biomass production. Or

Nitrogen fixation is a process by which nitrogen (N_2) in the atmosphere is converted into ammonia (NH_3). Atmospheric nitrogen or elemental nitrogen (N_2) is relatively inert, it does not easily react with other chemicals to form new compounds. Dinitrogen is quite inert because of the strength of its N≡N triple bond, breaking all three chemical bonds between nitrogens required huge energy. Therefore, nitrogen fixation is essential for agriculture and the manufacture of fertilizer. Microorganisms that fix nitrogen are bacteria called diazotrophs.

Nitrogen fixation would not occur without a special enzyme called nitrogenase, an enzyme found in some types of microbes that allows for the reduction of nitrogen gas to ammonium.

There are two main forms of these nitrifying microbes

1. Symbiotic nitrogen fixing bacteria

These microbes live on plants and are found around the world. They are most commonly associated with Rhizobia bacteria that live on a class of plants called legumes. These bacteria live in nodules on the roots, which look like little pearls, for protection from the outside world. Nitrogenase is incapable of functioning when oxygen is present. The plant provides simple sugars to the microbe, and in return, the plant receives the excess nitrogen fixed by the microbe.

There are other species of bacteria called Frankia that can also fix nitrogen through symbiotic relationships with some trees and shrub. These bacteria also live in root nodules. Some cyanobacteria also have symbiotic relationships with some hornworts, liverworts, mosses, horsetails, ferns, cycads, lichens, and other plants. These bacteria have special cells called heterocysts, which do not carry out oxygen reactions in photosynthesis, allowing nitrogen fixation to occur.

2. Free-living nitrogen fixing bacteria.

These bacteria do not require associations with other organisms to survive. These include some cyanobacteria, green sulphur bacteria, and purple sulphur and non-sulphur bacteria.

8.4 Nitrogenase

Nitrogenase is the enzyme responsible for fixing atmospheric nitrogen, and it's found in various microbes that may live alone in the nature or in symbiotic relationships with plants.

Nitrogenases carry out the reduction of molecular dinitrogen (N_2) to ammonium (NH_4) according to the reaction shown in eqn [I].

$$N_2 + n\text{MgATP} + 8e^- + 10H^+ \rightarrow 2NH_4^+ + n\text{MgADP} + H_2 \qquad \text{[I]}$$

$(n \geq 2 \text{ per electron})$

The nitrogenase enzyme system is widely distributed among Bacteria and the Archaea, but no eukaryotic system has been shown to contain a nitrogenase. (Some plants, most notably the legumes, form ymbioses with nitrogen-fixing bacteria and thus nitrogen fixation is often considered to be a 'plant process'. However, the actual nitrogen fixation in these symbioses is performed by the nitrogenase enzyme complex encoded, synthesized and localized in the bacterial endophyte of these symbioses.) The nitrogenases from all studied systems have very similar properties. The nitrogenase molecule looks similar to a sandwich. On both ends of the molecule are two iron containing proteins that are bonded to a molybdenum/iron protein.

8.5 Structure of the nitrogenase complex

The nitrogenase enzyme complex consists of two component proteins they are

i) Dinitrogenase and

ii) Dinitrogenase reductase.

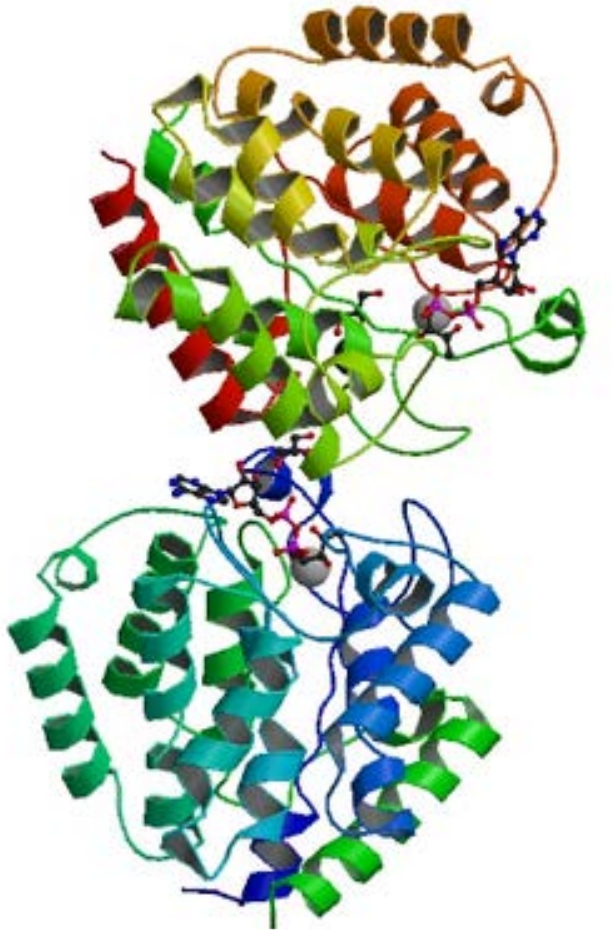
Dinitrogenase reductase is the unique and specific electron donor to dinitrogenase. Dinitrogenase accumulates electrons and catalyses the reduction of N_2.

The properties of the nitrogenase proteins are remarkably conserved in nature. The primary sequence of the dinitrogenase reductase protein is at least 70% identical mong species and across kingdoms, and the primary sequences of the dinitrogenase subunit proteins are also highly conserved.

8.6 Dinitrogenase reductase

Dinitrogenase reductase (also referred as the iron protein) is an α_2 dimer and it contains a single Fe_4S_4 cluster that bridges the two subunits of the protein at cysteines 98 and 132 of each subunit. The protein contains about 290 amino acids. The protein has the shape shown

in below figure, with the Fe_4S_4 cluster sitting near the surface at the c2 axis of protein symmetry.



α_2 dimer structure of dinitrogenase reductase

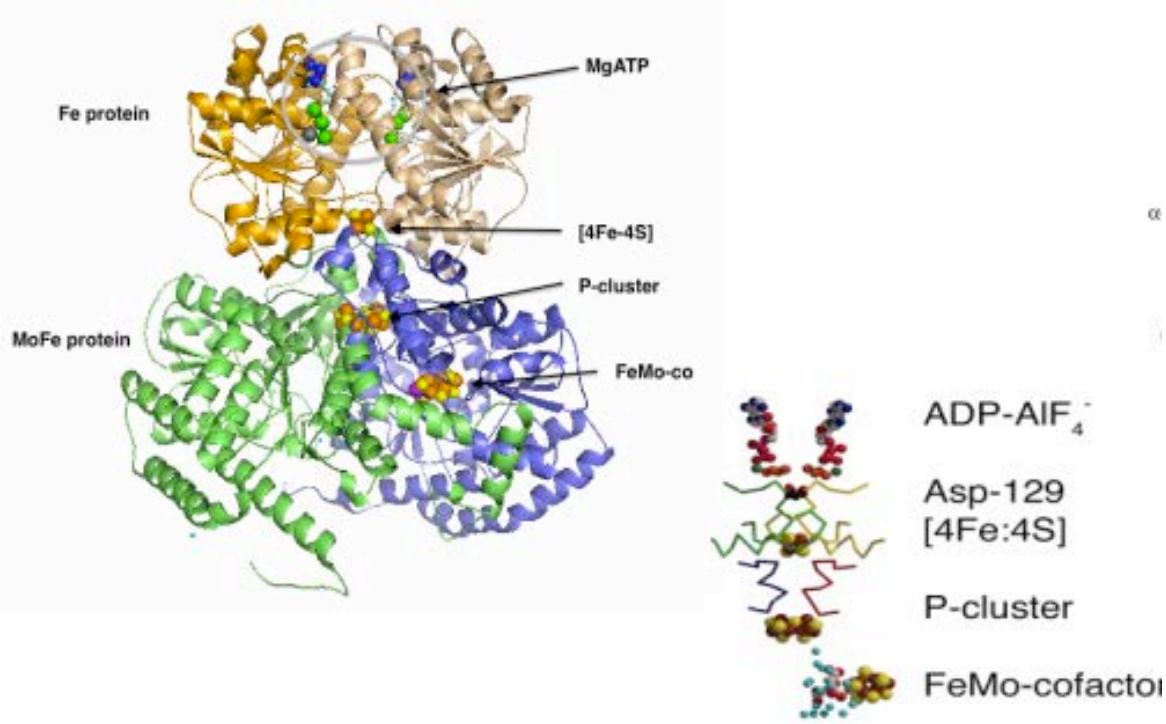
Dinitrogenase reductase exists in the oxidized ($[Fe_4S_4]^{2+}$), one-electron reduced ($[Fe_4S_4]^{+}$) and twoelectron reduced ($[Fe_4S_4]^{0}$) forms. Of these, the oxidized and one-electron reduced forms are considered to bephysiologically and catalytically relevant. Dinitrogenase reductase binds two molecules of MgATP at a pair of sites distal from the site of the Fe_4S_4 cluster. Each subunit binds one molecule of MgATP. Upon binding MgATP, dinitrogenase reductase undergoes a conformational change.

Dinitrogenase reductase performs other roles in addition to the transfer of electrons to dinitrogenase. In the absence of dinitrogenase reductase, the cells fail to complete the synthesis of the iron–molybdenum cofactor of the dinitrogenase protein. Furthermore, dinitrogenase reductase is required for the proper insertion of the iron–molybdenum cofactor into the dinitrogenase protein. Thus, the protein has at least three roles in the nitrogen fixation process. Dinitrogenase reductase that lacks its Fe_4S_4 cluster is unable to transfer electrons, but can still perform its roles in the biosynthesis and insertion of the iron–molybdenum cofactor.

8.7 Dinitrogenase

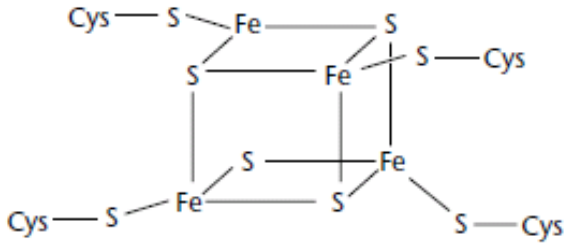
Dinitrogenase is an $\alpha_2\beta_2$ tetramer of a molecular weight of approximately 240kDa. Dinitrogenase is the site of substrate reduction, and this protein contains two each of two unique metal clusters, the P cluster and the iron–molybdenum cofactor (FeMo-co). The dinitrogenase tetramer is essentially a pair of dimers, each of which functions independently in the reduction of substrates.

The MgATP molecules that bind to dinitrogenase reductase and are hydrolysed during electron transfer from dinitrogenase reductase to dinitrogenase. Electrons are thought to enter the protein from the Fe_4S4 cluster of dinitrogenase reductase via the P cluster, finally to FeMo-co, which serves as the site of substrate reduction on the enzyme.



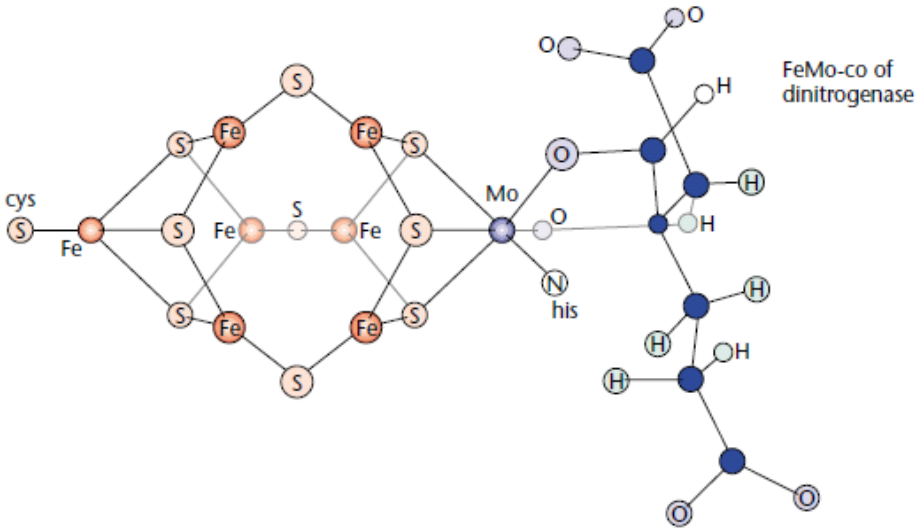
8.8 Metal clusters of dinitrogenase

. The Fe_4S_4 cluster of dinitrogenase reductase

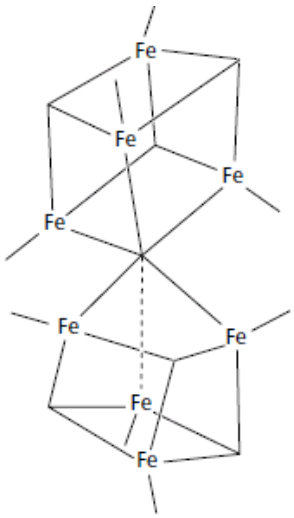


The Fe_4S_4 cluster of dinitrogenase reductase

The other unique cofactor of dinitrogenase is the iron– molybdenum cofactor (FeMo-co). FeMo-co consists of MoFe7S9 and the seven-carbon organic acid, homocitrate.



Dinitrogenase contains two unique metal clusters. The first of these, the P cluster, All iron atoms of the P cluster are in the ferrous state and the cofactor is thought to undergo oxidation and reduction by the reversible cleavage of one iron bond to the central sulfur atom.



The P cluster
of dinitrogenase

The basic mechanism of nitrogenase involves

a) Complex formation between the reduced Fe-protein with two bound ATP and the MoFe protein

b) Electron transfer between the two proteins coupled to the hydrolysis of ATP

c) Dissociation of the Fe-protein accompanied by re-reduction (via ferredoxins or flavodoxins) and exchange of ATP for ADP and

d) Repetition of this cycle until sufficient numbers of electrons (and protons) has been accumulated so that available substrates can be reduced on the FeMo-cofactor.

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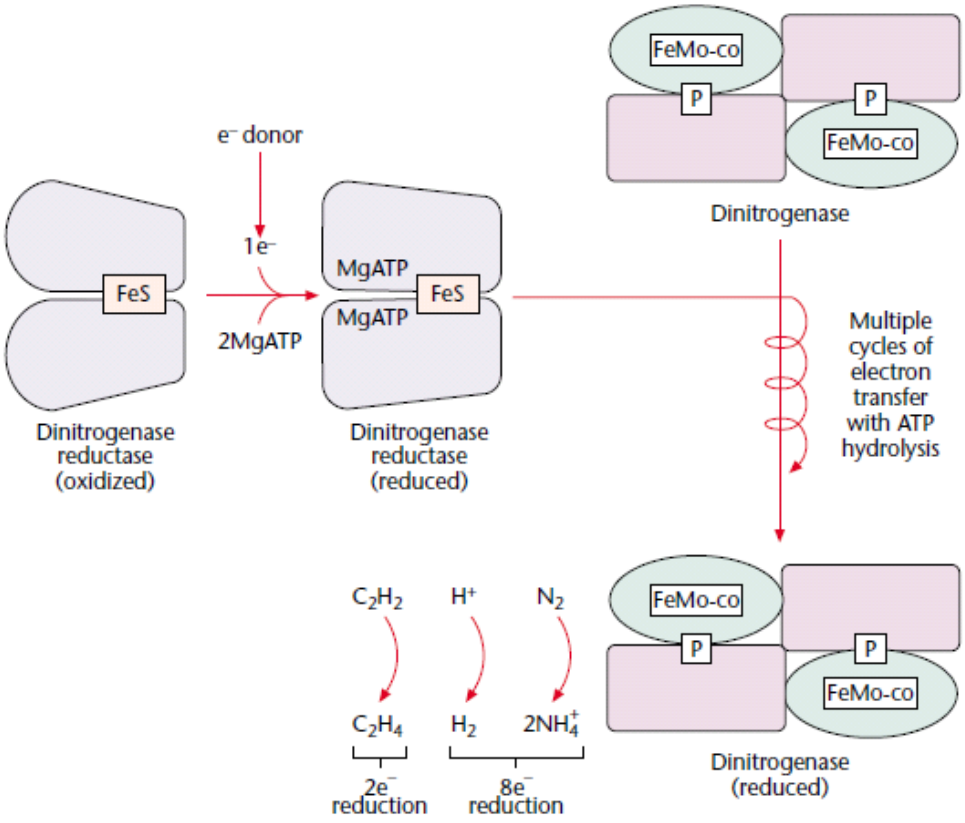


The path of electron flow in nitrogenase.

A key intermediate in the nitrogenase mechanism is consequently the formation of the Fe-protein-MoFe-protein complex where ATP hydrolysis is coupled to interprotein electron transfer

8.9 Photosynthesis

Photosynthesis is the process of converting light energy to chemical energy and storing it in the bonds of sugar. This process occurs in plants and some algae (Kingdom Protista). Plants need only light energy, CO_2, and H_2O to make sugar. The process of photosynthesis takes place in the chloroplasts, specifically using chlorophyll, the green pigment involved in photosynthesis. Photosynthesis takes place primarily in plant leaves. The mesophyll cells have chloroplasts and this is where photosynthesis occurs.

The overall chemical reaction involved in photosynthesis is

$$6CO_2 + 6H_2O \text{ (+ light energy)} \rightarrow C_6H_{12}O_6 + 6O^2$$

Photosynthesis is taking place in two parts. They are called the light reaction and the dark reaction.

The light reaction happens in the thylakoid membrane and converts light energy to chemical energy. This chemical reaction must, therefore, take place in the light. Chlorophyll and several other pigments such as beta-carotene are organized in clusters in the thylakoid membrane and are involved in the light reaction. Each of these differently-colored pigments

can absorb a slightly different color of light and pass its energy to the central chlorphyll molecule to do photosynthesis. The central part of the chemical structure of a chlorophyll molecule is a porphyrin ring, which consists of several fused rings of carbon and nitrogen with a magnesium ion in the center.

The energy harvested via the light reaction is stored by forming a chemical called ATP (adenosine triphosphate), a compound used by cells for energy storage.

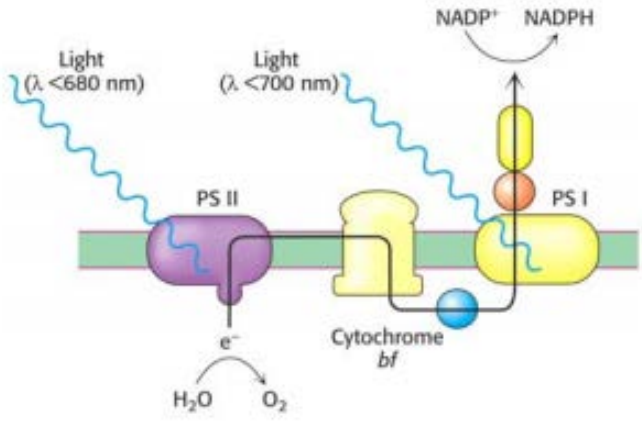
The dark reaction takes place in the stroma within the chloroplast, and converts CO_2 to sugar. This reaction doesn't directly need light in order to occur, but it does need the products of the light reaction (ATP and another chemical called NADPH). The dark reaction involves a cycle called the Calvin cycle in which CO_2 and energy from ATP are used to form sugar. The first product of photosynthesis is a three-carbon compound called glyceraldehyde 3-phosphate. Almost immediately, two of these join to form a glucose molecule.

Within the thylakoid membranes of the chloroplast, are two photosystems they are Photosystem I (PSI) and Photosystem II (PSII).

(PSI) optimally absorbs photons of a wavelength of 700 nm and Photosystem II optimally absorbs photons of a wavelength of 680 nm.

[It is important to note that I and II numbers indicate the order in which the photosystems were discovered, not the order of electron transfer]. Under normal conditions, electrons flow from PSII through cytochrome *bf* (a membrane bound protein) to PSI.

PS II uses light energy to oxidize two molecules of water into one molecule of molecular oxygen. The 4 electrons removed from the water molecules are transferred by an electron transport chain to reduce $2NADP^+$ to 2NADPH. During the electron transport process a proton gradient is generated across the thylakoid membrane. This proton motive force is then used to drive the synthesis of ATP. This process requires PSI, PSII, cytochrome *bf*, ferredoxin-$NADP^+$ reductase and chloroplast ATP synthase. The sequence of reaction occurs is represented by following cartoon.



8.10 structure of chlorophyll

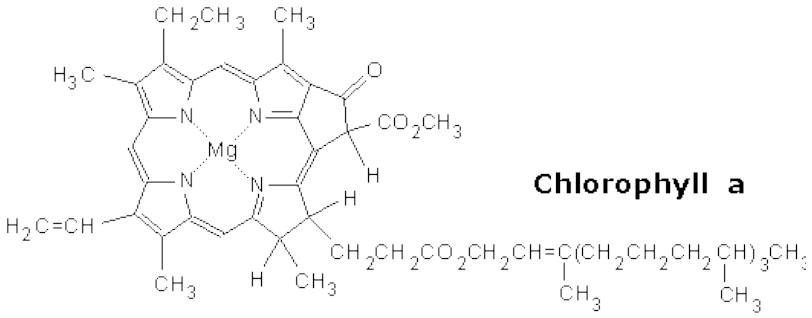
Chlorophyll is a green compound found in leaves and green stems of plants. A chlorophyll molecule has a hydrophobic "tail" that embeds the molecule into the thylakoid membrane. The "head" of a chlorophyll molecule is a ring called a porphyrin. The porphyrin ring of chlorophyll, which has a magnesium atom at its center, is the part of a chlorophyll molecule that absorbs light energy. Two types of chlorophyll are found in plants and the green algae they are

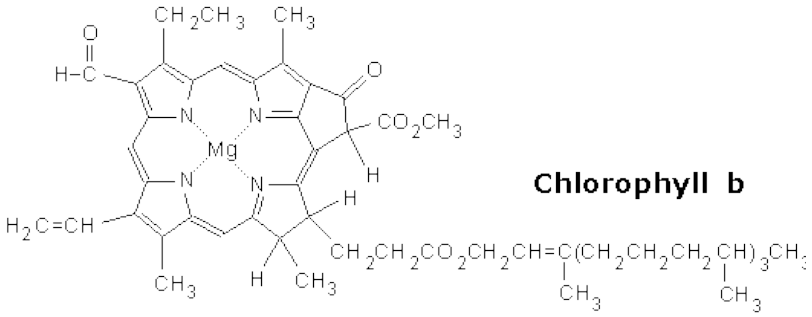
i) Chlorophyll a and

ii) Chlorophyll b

The difference in their structures is shown in the figure.

In the chloroplast, both types are associated with integral membrane proteins in the thylakoid membrane.

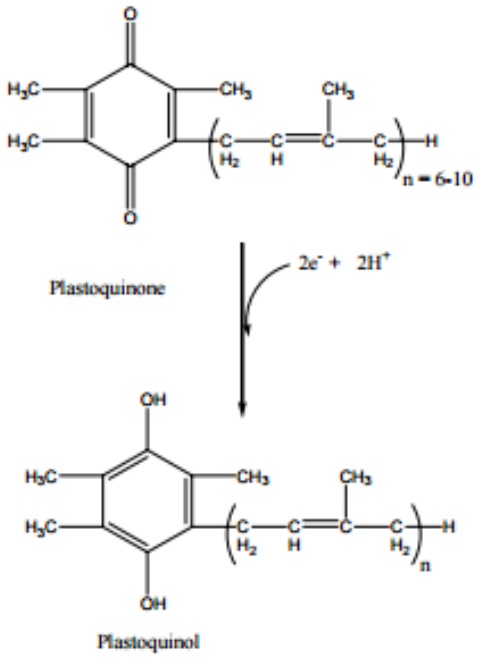




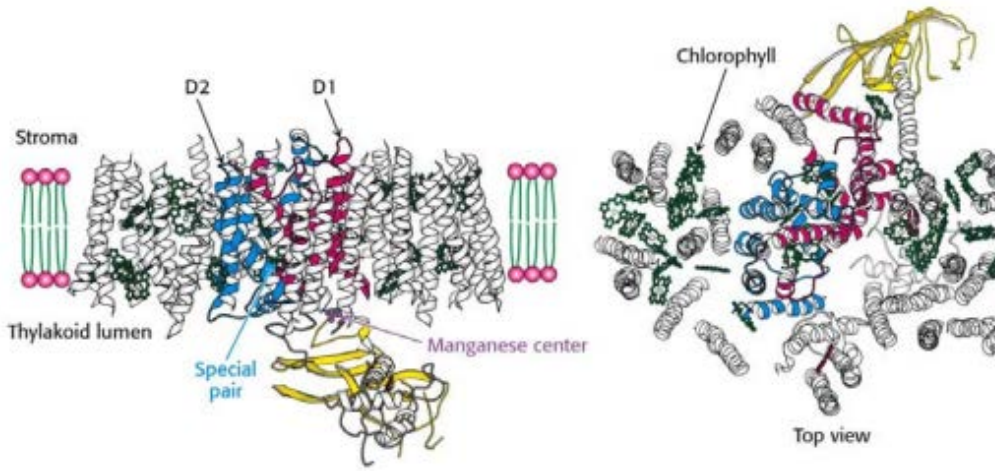
.Both chlorophylls absorb light most strongly in the red and violet parts of the spectrum. Green light is absorbed poorly. Thus when white light shines on chlorophyll-containing structures like leaves, green light is transmitted and reflected therefore the structures appear green.

8.11 Photosystem II

PSII transfers electrons from water to plastoquinone and in the process generates a pH gradient. Plastoquinone (PQ) carries the electrons from PSII to the cytochrome *bf* complex. Plastoquinone can functions as a one or two electron acceptor and donor. When it is fully reduced to PQH_2 it is called plastoquinol.

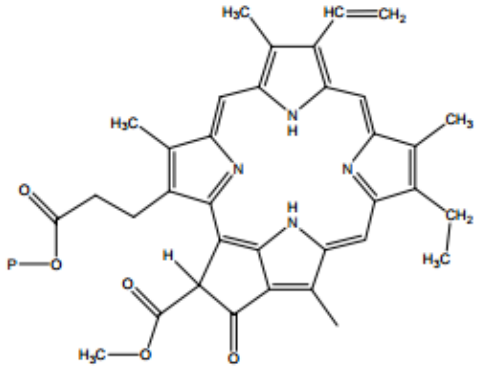


PSII is an i The overall reaction of PSII is shown below. $2PQ + 2H_2O \rightarrow O_2 + 2PQH_2$ mbrane protein is formed by two subunits D1 and D2. These two subunits span the membrane and are homologous to subunits L and M of the bacterial photosystem.

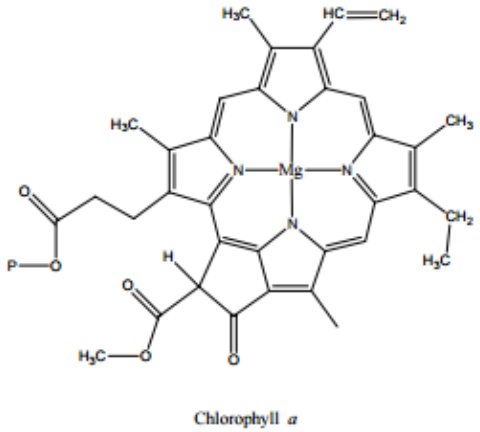


PSII contains a lot more subunits and additional chlorophylls to achieve a lot higher efficiency than bacterial systems. The ribbon diagram of the crystal structure of PSII is shown abow.

There is a special pair of chlorophylls in PSII bound by D1 and D2 that are in close proximity of each other. This special pair is called P680, absorb light at an optimal wavelength of 680 nm. On excitation by the absorption of a photon P680* rapidly transfers an electron to a nearby pheophytin a. Pheophytin a is a chlorophyll a molecule with the Magnesium replaced by two protons.



Pheophytin *a*

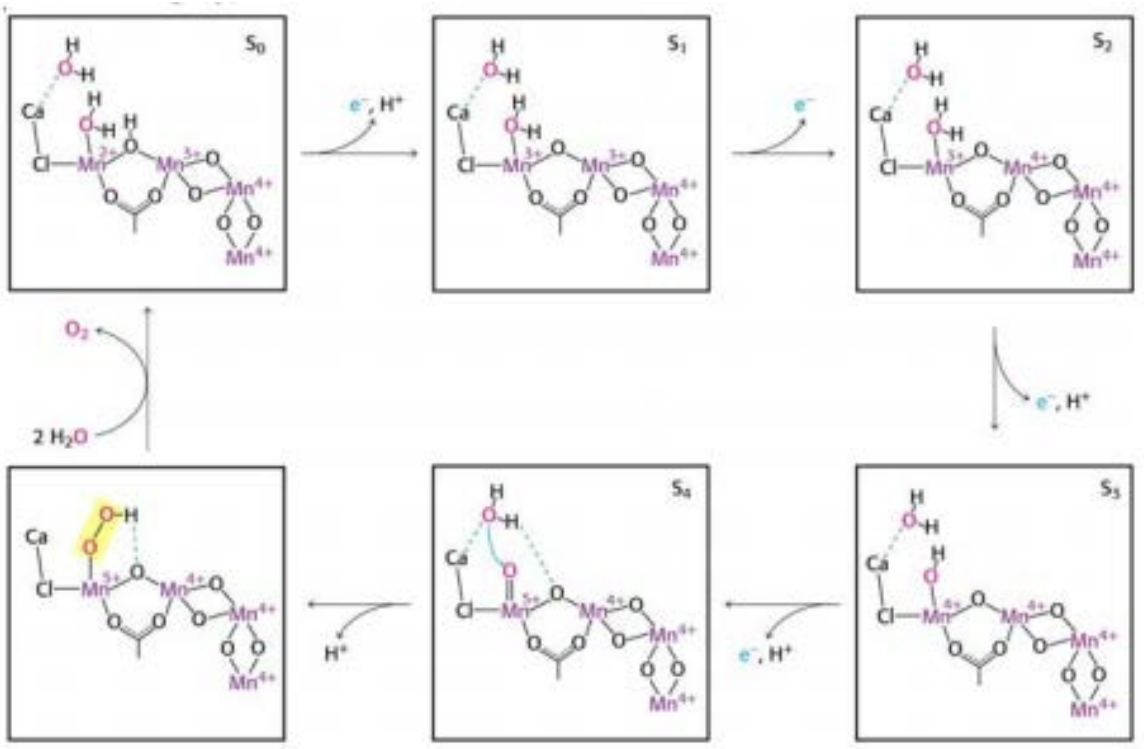


Chlorophyll *a*

The electron is then transferred to a tightly bound plastoquinone at the Q_A site. The electron is then transferred to an exchangeable plastoquinone located at the Q_B site of the D2 subunit and plastoquinol, PQH_2 is produced.

When the electron is rapidly transferred from P680* to pheophytin a, a positive charge is formed on the special pair, $P680^+$. $P680^+$ is an incredibly strong oxidant which extracts electrons from water molecules bound at the manganese center. Manganese is the core of this redox center because it has four stable oxidation states (Mn^{2+}, Mn^{3+},Mn^{4+} and Mn^{5+}) and coordinates tightly to oxygen containing species. Each time the P680 is excited and an electron is kicked out, the positively charged special pair extracts an electron from the manganese center.

$$2H_2O \rightarrow O_2 + 4e^-$$



4 electrons must be transferred to 2 molecules of plastoquinone in order to oxidize H_2O to molecular oxygen. This requires 4 photochemical steps. The Manganese center is oxidized one electron at a time, until two molecules of H_2O are linked to form O_2 which is then released from the center. The manganese center exists in five different oxidation states numbered for S_0 to S_4 as shown above. One electron and a proton are removed during each photochemical step. When S_4 is attained, an O_2 molecule is released and two new molecules of water bind. The reason the third pulse of light produces O_2 is because the resting state of the PSII in the chloroplast is S_1 not S_0. Over all for every four electrons harvested from H_2O, 2 molecules of PQH_2 are formed extracting four protons from the stroma. The four protons formed during the oxidation of water are released into the thylakoid lumen. This distribution of protons across the thylakoid membrane generate a pH gradient

18.12 Cytochrome *bf*

The plastoquinol formed by PSII contributes its electrons through an electron transport chain that terminates at PSI. The intermediary electron transfer complex between PSII and PSI is cytochrome bf also known as cytochrome b_6f. In this electron transfer complex electrons are passed one at a time from plastoquinol to plastocyanin (Pc) a copper protein of the thylakoid lumen. The reaction is shown below

$$PQH_2 + 2Pc(Cu^{2+}) \rightarrow 2Pc(Cu^{+}) + 2H^{+}$$

The protons are released into the thylakoid lumen.

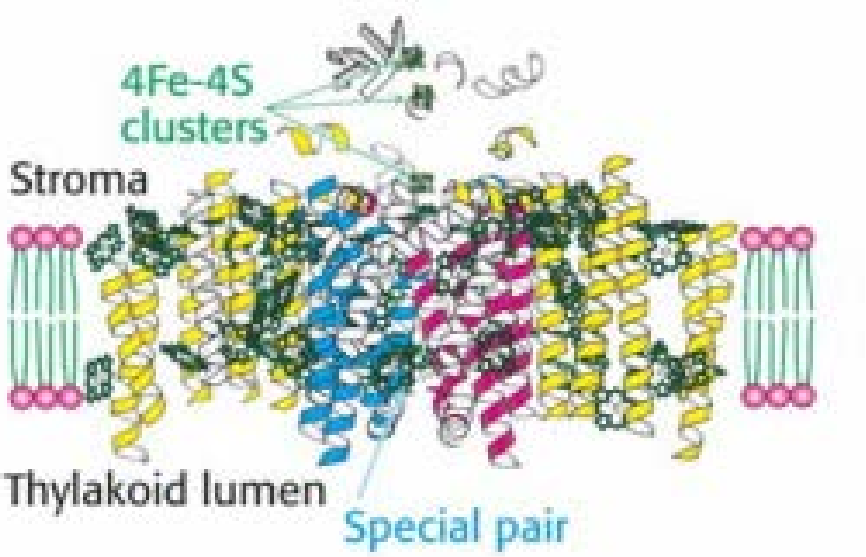
Plastocyanin is a water soluble electron carrier found in the thylakoid lumen of chloroplasts.

It contains a single Copper atom coordinated to two histidine residues and a cysteine residue in a distorted tetrahedron. The molecule is intensely blue in the cupric form. This mobile electron carrier carries electrons from cytochrome bf to PSI.

The cytochrome *bf* contains two b-type heme cytochromes. This enzyme transfers electrons from plastoquinol. The net result is two protons are picked up from the stroma side of the thylakoid membrane and 4 protons are released into the lumen contributing to the pH gradient.

8.13 Photosystem I

The final stage of the light reactions is catalyzed by PSI. This protein has two main components forming its core, psaA and psaB. A special pair of chlorophyll a molecules lies at the center of the structure which absorbs light maximally at 700 nm. This special pair is denoted P700.



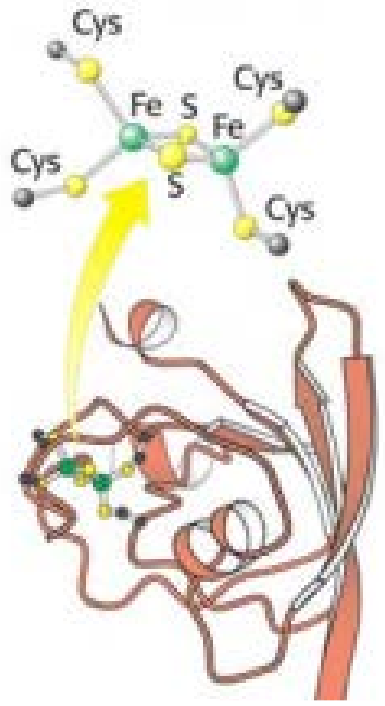
Upon excitation, P700* transfers an electron through a chlorophyll and a bound quinone (QA) to a set of 4Fe-4S clusters. From these clusters the electron is transferred to ferredoxin (Fd) a water soluble mobile electron carrier located in the stroma which contains a 2Fe-2S cluster coordinated to 4 cysteine residues.

The electron transfer produces a positive charge on the special pair which is neutralized by the transfer of an electron from a reduced plastocyanin.

The overall reaction is shown below

$$Pc(Cu^{+}) + Fd_{ox} \rightarrow Pc(Cu^{2+}) + Fd_{red}$$

The structure of ferredoxin is shown below. It contains a 2Fe-2S cluster which accepts electrons from PSI and carries them to ferredoxin-$NADP^{+}$ reductase.



$Pc(Cu^{2+}) + e^- \rightarrow Pc(Cu^+)$ $E^{o'} = +0.37$ V

$Fd_{ox} + e^- \rightarrow Fd_{red}$ $E^{o'} = -0.45$ V

The electron acceptor in the overall reaction shown above is the oxidized ferredoxin, the electron donor is the reduced plastocyanin. From the reduction potentials listed above, the change in reduction potential is:

$\Delta E^o = -0.45 - 0.37 = -0.82$ V which corresponds to a $\Delta G^o = 79.1$ kJ/mol, very endergonic.

This uphill electron transfer is driven the by absorption of a 700-nm photon of light which has an energy of 171 kJ/mol. The electron transport pathway between PSII and PSI is called the Z-scheme because the redox diagram looks like a sideways letter Z.

8.14 Summary of the unit

Biological nitrogen fixation (BNF) is the process whereby atmospheric nitrogen (N=N) is reduced to ammonia in the presence of nitrogenase. Nitrogenase is a biological catalyst found naturally only in certain microorganisms such as the symbiotic Rhizobium and Frankia, or the free-living Azospirillum and Azotobacter.

Biological nitrogen fixation can be represented by the following equation, in which two moles of ammonia are produced from one mole of nitrogen gas, at the expense of 16 moles of ATP and a supply of electrons and protons (hydrogen ions)

$$N_2 + 8H^+ + 8e^- + 16\ ATP = 2NH_3 + H_2 + 16ADP + 16Pi$$

This reaction is performed exclusively by prokaryotes (the bacteria and related organisms), using an enzyme complex termed nitrogenase. This enzyme consists of two proteins - an iron

protein and a molybdenum-iron protein. The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to $H_2N\text{-}NH_2$, and this in turn is reduced to $2NH_3$. Depending on the type of microorganism, the reduced ferredoxin which supplies electrons for this process is generated by photosynthesis, respiration or fermentation.

The photosynthetic reactions that require light occur within the thylakoid membrane. These light-dependent photosynthetic reactions employ the thylakoid membrane-embedded antenna system to harness energy delivered by a photon. The light-dependent, photosystem reactions ultimately transduce the energy of light to generate molecules of ATP and NADPH, which act as energy-transfer molecules in the light-independent, "dark" reactions of the Calvin cycle.

There are two kinds of photosystems in eukaryotes PSI and PSII. The reaction center chlorophyll molecule within the antenna of photosystem I responds most strongly to 700 nm light, and is therefore termed P700. The reaction center within the antenna of photosystem II responds most to 680 nm light, and is accordingly called P680. Photosystem I evolved very early, and it is found in nonoxygenic phototrophs; photosystem II evolved later. Because the PSII photosystem is most sensitive to shorter wavelength 680 nm light, it absorbs slightly more energy than the P700-PSI system.

The electron transport system of each photosystem is embedded within the thylakoid membrane and functions in the production of ATP. The system comprises membrane-bound electron carriers that pass electrons from one molecule to the next. Water is split to generate an electron, hydrogen ions, and oxygen.

By receiving the energized electron (reduction), the first carrier of the P680 (PSII) electron transport system gains energy. It utilizes some of the energy to pump H^+ into the thylakoid lumen, then passes the less energetic electron to the second of four carrier molecules. Each successive carrier in the electron transport chain utilizes some of the energy of the received electron to pump H^+ from the stroma into the thylakoid lumen, and then passes the further depleted electron along to the next carrier. Thus, the electron transport system functions to generate a concentration gradient of H^+ inside the thylakoid. The chemical potential energy of the H^+ concentration gradient is employed to synthesize ATP.

The “light” reactions of noncyclic photophosphorylation produce both ATP and NADPH, which act as energy-transfer molecules in the light-independent (“dark”) reactions of the Calvin cycle. The “dark”, or light-independent Calvin anabolic reactions occur in the stroma of the chloroplast in either light or dark conditions. The light-independent reactions function to reduce CO_2 to glucose.

$$6CO_2 + 6H_2O \rightarrow \text{Energy} + C_6H_{12}O_6 + 6O_2.$$

8.15 Key words

Nitrogen fixation; Nitrogenase; Dinitrogenase reductase; Dinitrogenase; Photosynthesis; Chlorophyll; Photosystem II; Cytochrome *bf*; Photosystem I

8.16 References for further studies

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8.17 Questions for self understanding

1) What is biological nitrogen fixation?

2) Discuss the structure of the nitrogenase complex

3) Explain the role of dinitrogenase reductase

4) Explain the role of dinitrogenase

5) Write a short note on metal clusters present in dinitrogenase and explain their role

6) What is photosynthesis?

7) Discuss the structure of chlorophyll

8) Explain the reactions occurs in Photosystem II

9) Explain the role of Cytochrome *bf*

10) Explain the reactions occurs in Photosystem I

UNIT-9

Structure

9.0 Objectives of the unit

After studying this unit you are able to

- Derive the expression for effect of [substrate] on enzyme catalyzed reaction or Michalein-Menten equation
- Explain the significance of M.M equation
- Discuss the effect of pH enzyme catalyzed reaction
- Explain the effect of temperature enzyme catalyzed reaction

9.1 Introduction

When studying biochemical and physiological processes, it is often necessary to measure the rate at which a given reaction or process proceeds to completion. For example, if we examine the rate of biochemical reactions catalyzed by enzymes, or the rate of carrier-mediated transport of molecules across biological membranes, we commonly find that at low enzyme or transporter substrate concentrations, the reaction rate increases almost in a linear fashion with increasing substrate concentration. However, as the substrate concentration is increased to higher and higher levels, the reaction rate no longer increases in proportion to the increase in substrate concentration. Thus, at higher substrate concentrations, the reaction rate no longer increases in a linear manner. Rather, increases in the substrate concentration lead to progressively smaller and smaller increases in the reaction rate. In fact, at very high substrate concentrations, the rate begins to asymptote to a steady-state level, and additional increases in the substrate concentration do not lead to an increase in the reaction rate. This type of relationship is referred to as hyperbolic and demonstrates saturation of the enzyme or transporter at high substrate concentrations. Saturation is caused by the fact that there is a fixed number of enzyme or transporter molecules, each with a fixed number of substrate binding sites. At high substrate concentrations, all of the binding sites have substrate bound and each enzyme or transporter molecule is working as fast as its intrinsic rate to catalyze the reaction (for enzymes) or transport the substrate across the membrane (for transporters).

Two scientists, Leonor Michaelis and Maud Leonora Menten, proposed the model known as Michaelis-Menten kinetics to account for enzymatic dynamics. The model serves to explain how an enzyme can cause kinetic rate enhancement of a reaction and explains how reaction rates depends on the concentration of enzyme and substrate.

The Michaelis-Menten model is the one of the simplest and best-known approaches to enzyme kinetics. It takes the form of an equation relating reaction velocity to substrate concentration for a system where a substrate S binds reversibly to an enzyme E to form an enzyme-substrate complex ES, which then reacts irreversibly to generate a product P and to regenerate the free enzyme E. This system can be represented schematically as follows

$$S \rightleftharpoons ES \rightarrow E + P$$

9.2 Introduction to Enzyme

Enzymes are complex nitrogeneous substance (proteins) but may be associated with non protein substances (known as co enzymes or prosthetic groups) that are essential to the activity of the enzyme. Enzymes catalyze most of the biochemical processes such as digestion and bio-synthesis etc….. in animals and human beings.

Enzymes are highly specific and each enzyme catalyses a particular reaction.

For example

Sucrose $\xrightarrow{Invertase}$ glucose + fructose

But Inverstase cannot be used for breaking of maltose because for reaction

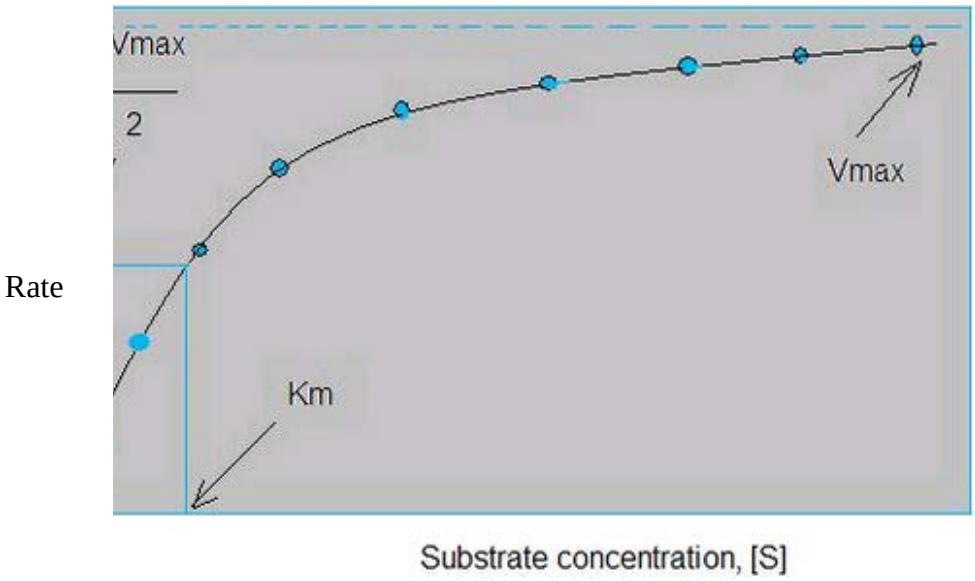
Maltose $\xrightarrow{enymes\ meltose}$ 2 glucose

Because maltose is used instead of inverse trace

Enzymes reactions are much sensitive to catalytic poisoning such as HCN, H_2S CS_2 etc. The activity of the enzyme depends up on the non-protein substance called co-enzyme. For each enzyme there is only one co-enzyme. Enzymes lose their activity in the presence of electrolytes or when expose to UV-radiation.

9.3 Effect of [substrate] on enzyme catalyzed reaction or Michalein-Menten equation

The rate of enzyme- catalyzed reaction is directly proportional to the concentration of enzymes [enzyme] when substrate concentration is very much greater than the concentration of enzyme. ie, [substrate] >> [enzyme]. But at constant enzyme concentration [enzyme], the rate increases at lower concentration of substrate[S] and become independent of substrate concentration [S] at higher substrate concentration [S]. This is explained by below graph



Michelis- menten reaction scheme is given by

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P + E \text{ ---------- } (1)$$

For the above reaction scheme

Rate = $k_2[ES]$----------(2)

Applying steady state concept

$$\frac{d}{dt} = k_1[E][S] - k_{-1}[ES] + k_2[ES] = 0 \text{ ------------ } (3)$$

Here the rate of formation of ES is equal to the disappearance of ES. So that the concentration of ES is constant. Hence $\frac{d}{dt}[ES] = 0$ this is called steady state concept.

$$\therefore k_1[E][S] = (k_2 + k_{-1})[ES]$$

$$[ES] = \frac{k_1}{(k_2 + k_{-1})}[E][S] \text{ ---------- } (4)$$

Substrate of equation (4) in (2) gives

$$rate(v) = \frac{k_2 k_1}{(k_2 + k_{-1})}[E][S] \text{ ---------- } (5)$$

This equation can be written as

$$rate(v) = k_M [E][S] \text{ ---------- } (6)$$

$$Here\ k_M = \frac{k_2 k_1}{k_2 + k_{-1}}$$

Equation (6) is called Michaleis Meutron equation and K_M is called Michaleis- Meutron constant

Equation (6) can be written as

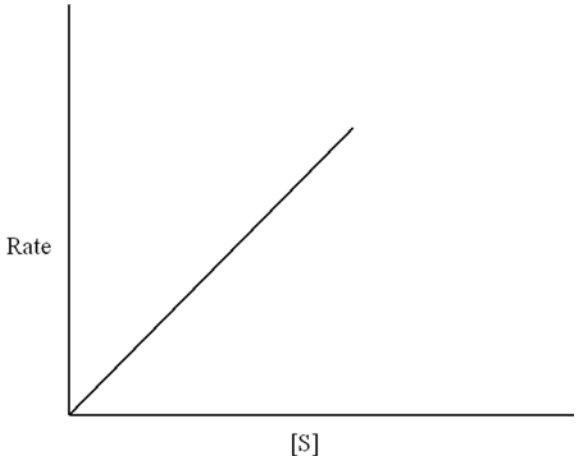
$$rate(v) = \frac{k_2 [E_0][S]_0}{K_M + [S]_0} \text{ ---------- } (7)$$

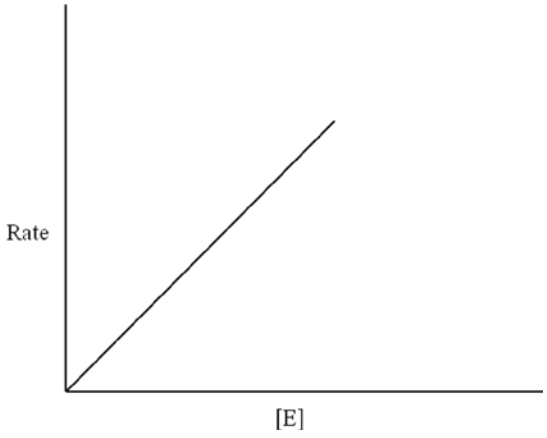
Here $[E_0]$ and $[S]_0$ are initial concentration of enzymes and substrate

Case1. At low $[S]_0$

$[S_0]$ in the denominator in equation 7 is negligible here

$rate = \frac{k_2}{K_M}[E]_0[S]_0$ ---------- (8). Indicates rate directly proportional to enzyme and substrate concentration.



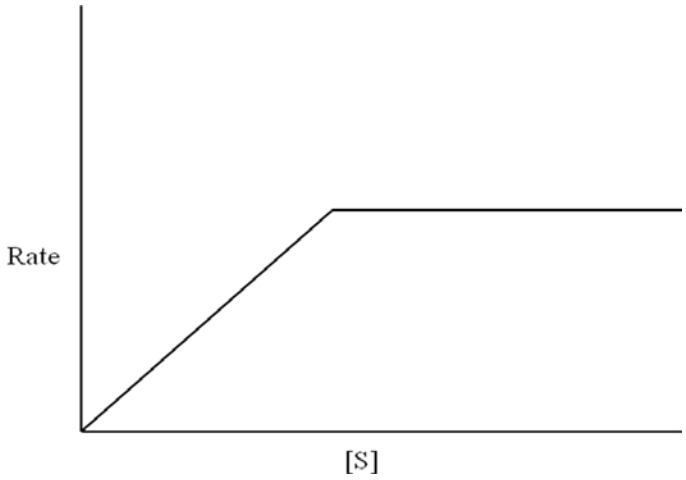


Case 2. At high $[S]_0$ K_M is neglected

$$v = \frac{k_2 [E_0][S_0]}{[S_0]}$$

$$v = k_2 [E_0] \text{ ---------- } (9)$$

Here rate is independent of substrate concentration



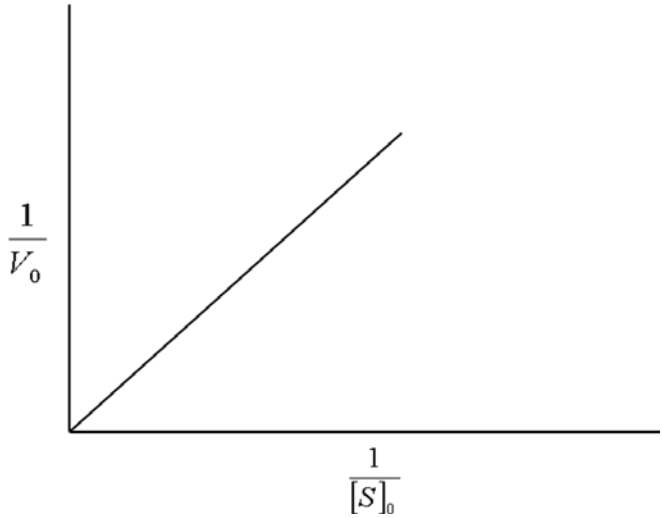
9.4 Significance of M.M equation

i) M.M equation can be written as

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]_0}$$

V_{max} = rate at high [S]

A plot of $\frac{1}{V_0} v/s \frac{1}{[S]_0}$ gives



Value of V_{max} from the intercept and K_M from the slope can be determined.

ii) M.M equation (6) is applicable only when one intermediate ES is formed if more than one intermediate involved then MM equation (6) is fails

For example

$$E + S \underset{}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_1^1} (ES)^1 \xrightarrow{k_2} E + P$$

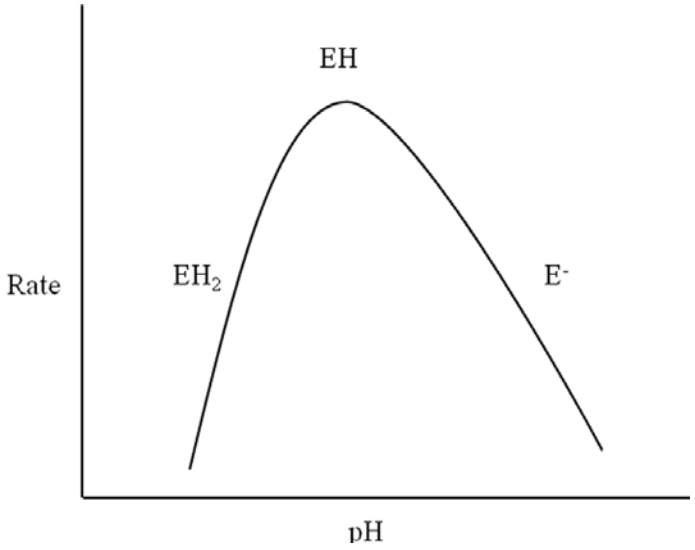
Then the rate is given by

$$v = \left[\frac{k_1 k_1^1}{(k_1 + k_1^1)}\right] \frac{[E_0][S_0]}{\left[[S_0] + K_M\left(\frac{k_1^1}{k_1 + k_1^1}\right)\right]}$$

Hence we can get the information whether only one intermediate is formed or more than one intermediate formed in the reaction.

9.5 Effect of pH

In general enzymes exhibit maximum catalytic activity at optimum pH ie, at pH 7 ± 1.0. Above or below this pH activity of enzyme decreases



This is explained as follows

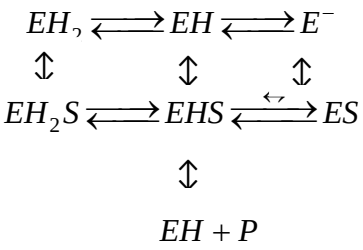
Enzymes can exist in three states of ionization i.e

$EH_2^+ \rightleftarrows EH \rightleftarrows E^-$ depending on pH

$\therefore$ Corresponding with substrate following complex are formed

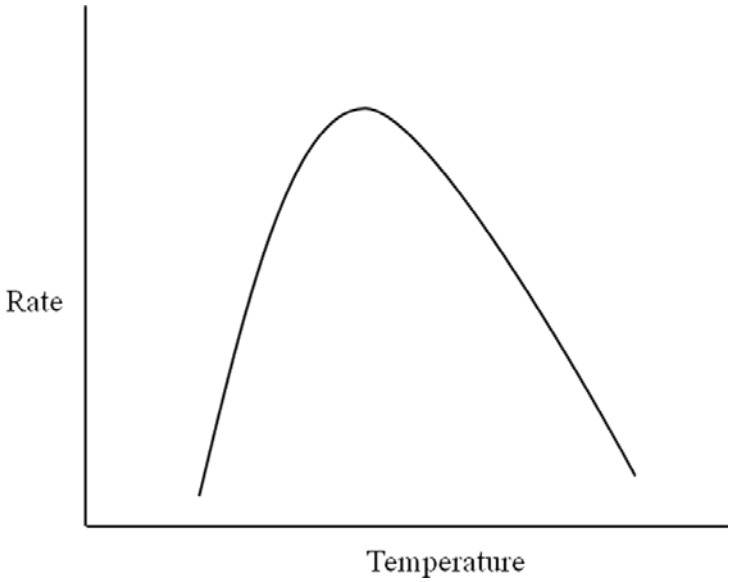
$EH_2^+ S \rightleftarrows EHS \rightleftarrows E^- S$

Among this EH hence EHS is more active than $EH_2^+ S$ or $E^- S$ and breaks up and give rise to products. The overall reaction scheme is given by



9.6 Effect of temperature

The rate of enzyme catalyzed reaction increases with temperature, reaches maximum and then decreases.



This is because in general enzymes are stable at lower temperature and catalyzed the reaction. But temperature greater than 35^0C the kinetic energy of the enzyme exceeds the energy barrier for breaking of the weak hydrogen and hydrophobic bands that maintain its secondary and tertiary structure so that at higher temperature the denaturation of enzyme occurs and activity decreases. Hence the rate of reaction is decreases.

9.7 Summary of the unit

The Michaelis-Menten equation is commonly used to study the kinetics of reaction catalysis by enzymes as well as the kinetics of transport by transporters. Typically, the rate of reaction (or reaction velocity) is experimentally measured at several substrate concentration values. The range of substrate concentrations is chosen such that very low reaction rates as well as saturating rates are measured. A plot of the reaction rate versus the substrate concentration reveals two important kinetic parameters: V_{max} and K_m. V_{max} is the maximum reaction rate that is observed

at saturating substrate concentrations. V_{max} is a function of the intrinsic rate of the enzyme or transporter as well as a function of the total number of enzyme/transporter molecules that give rise to the measured rate. *K_m is referred to as the Michaelis constant and is the substrate concentration at which the reaction rate is exactly half of V_{max}*. K_m is inversely related to the apparent affinity of the enzyme/transporter for its substrate. Therefore, *a low numerical value of K_m refers to a very high affinity of interaction between the protein and its substrate*. This is because it takes a very small amount (i.e., low concentration) of the substrate to reach 50% of the saturating concentration. Conversely, a high numerical value of K_m is indicative of a low affinity of the enzyme/transporter for its substrate. This is because it takes a large amount (i.e., high concentration) of the substrate to reach 50% of the saturating concentration. Thus, K_m is a very useful parameter by which the affinity of the protein for various substrates can be compared.

It is important to emphasize that the kinetics of transport for many transport proteins exhibit features that are very similar to those of enzymes. Similar to enzymes, transporters show specificity with respect to the substrate transported and, in addition, the rate of substrate transport across a biological membrane exhibits saturation at high substrate concentrations. Therefore, the kinetics of many transport processes can be studied by using Michaelis-Menten kinetics. The Michaelis-Menten equation can adequately describe the dependence of transport rate on the substrate concentration for facilitative transporters, secondary active transporters (cotransporters and exchangers), and primary active transporters (i.e., pumps).

If the protein under study has more than one (i.e., two or more) substrate binding sites, and if there is cooperativity with respect to substrate binding to the protein, a plot of the reaction rate as function of the substrate concentration is no longer hyperbolic and may assume a sigmoidal shape. In this case, the Michaelis-Menten equation is no longer the appropriate equation to use for studying the rate of reaction as a function of the substrate concentration. Instead, the Hill equation is the appropriate equation to use. Indeed, the Michaelis-Menten equation is a special case of the Hill equation where the protein under study has only one substrate binding site.

9.8 Keywords

Substrate; Enzyme; Michalein-Menten equation; Significance of M.M equation; Effect of pH enzyme catalyzed reaction; Effect of temperature enzyme catalyzed reaction

9.9 References for further studies

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9.10 Questions for self understanding

1) Derive the expression for effect of [substrate] on enzyme catalyzed reaction or Michalein-Menten equation
2) Explain the significance of M.M equation
3) What is Michalein-Menten constant? Explain its significance
4) Discuss the effect of pH enzyme catalyzed reaction
5) Explain the effect of temperature enzyme catalyzed reaction

UNIT-10

Structure

10.0 Objectives of the unit

After studying this unit you are able to

- Derive the expression for kinetic and mechanistic application of glucose oxidase in the oxidation of glucose
- Explain the effect of concentration of substance on glucose oxidase in the oxidation of glucose
- Explain the effect of concentration of enzymes [E]on glucose oxidase in the oxidation of glucose
- Explain the effect of temperature glucose oxidase in the oxidation of glucose
- Derive the expression for kinetic and mechanistic application of Transaminase enzyme in the oxidation of amino acids.

10.1 Introduction

The kinetics of oxidation of D-glucose catalysed by the enzyme glucose oxidase has been studied over a wide range of experimental conditions. The reaction velocities increased with increase in the concentrations of the glucose oxidase and glucose, as well as increase in temperature and ionic strength of the solution. The reaction velocity initially increased with increase in pH, reaching a maximum at pH 6.5 and then decreased with further increase in pH. The reaction exhibited saturation kinetics and experimental data were analysed using the Michaelis- Menten equation. Arrhenius activation energy and thermodynamic activation parameters were measured and are reported. The large negative value of entropy of activation $\Delta S\neq$, $-148.8 JK^{-1}mol^{-1}$, and positive value of the enthalpy of activation $\Delta H\neq$ $26.3 kJmol^{-1}$, give further support to the proposed mechanism. The results are interpreted in terms of a mechanism involving both an oxidative half reaction and a reductive half reaction.

10.2 Kinetic and mechanistic application of glucose oxidase in the oxidation of glucose

Glucose oxidase enzyme catalyzes the oxidation of β-D- glucose to glucoxoketone and then the glucolactone hydrolysed to gluconic acid. Because of considerable biological and industrial importance, glucose oxidase has found a wide range of applications. In diagnostics it is the basis of sensors for the determination of glucose concentration in blood serum or plasma.

The storichiometry reaction is given by

β-D-glucose + GOD(FDA) $\rightarrow$D-glucono-δ-lactone +$FDAGO_x(FDAH_2)$ ---------(1)

(enzyme)

$$GOD(FADH_2)+O_2 \rightarrow GOD\ (FAD)+H_2O_2 \text{----------2}$$

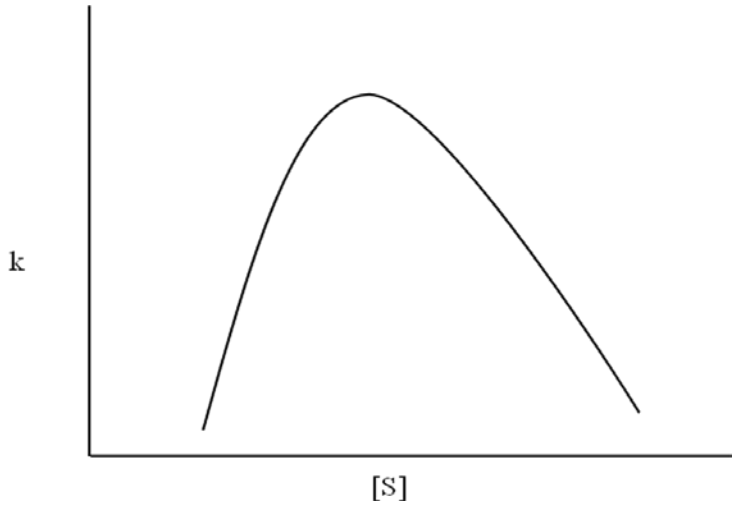
D-glucose-δ-lactone + $H_2O \rightarrow$ gluconic acid

Kinetics of reaction is followed by measuring disappearance of glucose concentration by Benedicts method.

The following kinetics results were observed for above reaction

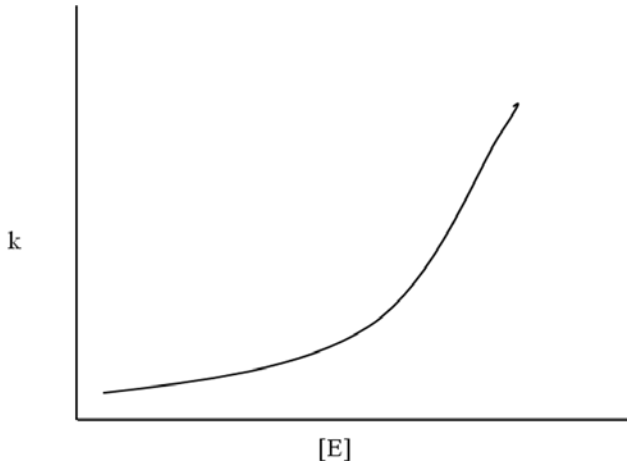
10.3 Effect of concentration of substance

Increase of [S] increases the rate, reaches a maximum and then decreases at constant concentration of enzyme



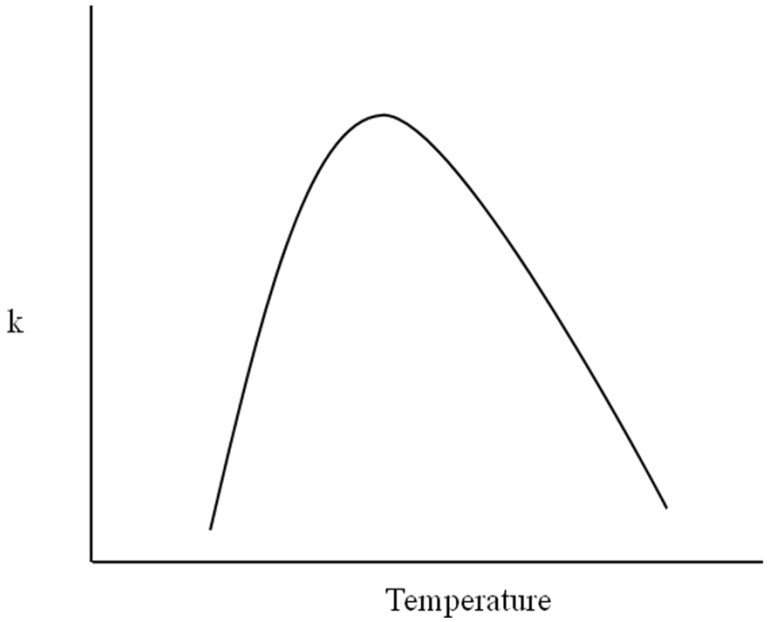
10.4 Effect of concentration of enzymes [E]

Increase of [E] increases the rate at constant [S]



10.5 Effect of temperature

Increase of temperature increase the rate, reaches a maximum at 35^0 C after that the rate decreases.



10.6 Reaction scheme

The following reaction scheme is proposed

$$E_{GOX} + G \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} (E_{GOX}G) \xrightarrow{k_2} Gluconic\ acid + E_{GOX}$$

(enzyme) (glucose)

$$\text{Rate} = k_2[E_{GOX}G] \quad \text{---------- (1)}$$

At steady state,

$$\frac{d}{dt}[(E_{GOX}G)] = k_1[E_{GOX}][G] - k_1[E_{GOX}G] + k_2(E_{GOX}G) = 0 \quad \text{------- (2)}$$

$$\therefore k_1[E_{GOX}](G) = (k_2 - k_{-1})(E_{GOX}G)$$

$$\text{Hence}[(E_{GOX}G)] = \frac{k_1}{k_2 - k_{-1}}[E_{GOX}][G] \quad \text{---------- (3)}$$

substituting (3) in (1) we get

$$\text{Rate}(\vartheta) = \frac{k_1 k_2}{(k_2 - k_{-1})}[E_{GOX}][G] \quad \text{---------- (4)}$$

$$\text{rate} = \vartheta = K_M[E_{GOX}][G] \quad \text{---------- (5)}$$

Equation (5) is called Michaelis menton equation and $K_m = \frac{k_1 k_2}{(k_2 - k_{-1})}$ is called Michaelis-Menten constant.

If initial enzyme and substrate consideration are expressed in terms of $(E_{GOX})_0$ and $(G)_o$ then

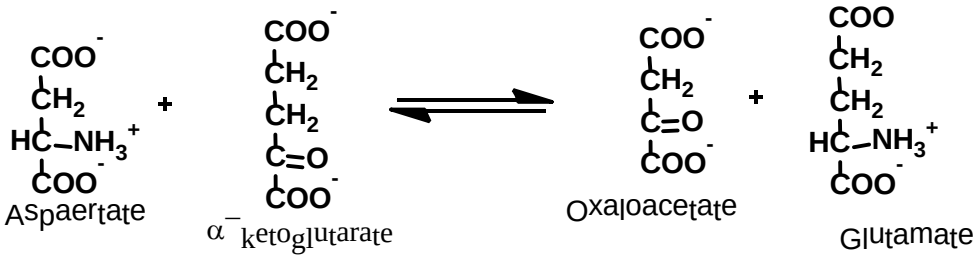
$$The\ Rate\ \vartheta = \frac{k_2 (E_{GOX})_o (G)_o}{K_m + (G)_0} \text{ ---------- } (6)$$

At low $[G]_0$ rate is directly proportional to $(E_{GOX})_0$ and at high concentration of glucose the rate decreases with [G] as it is observed in the experiment. The decrease of rate with increase of temperature is due to denaturation of enzyme at higher temperature.

10.7 Kinetic and mechanistic application of Transaminase enzyme in the oxidation of amino acids.

Transaminase (Aminotransferases) catalyzed the reaction of oxidation of aminoacids. There are multiple transaminase enzymes depends with substrate.

An example of a transminase reaction is shown below.

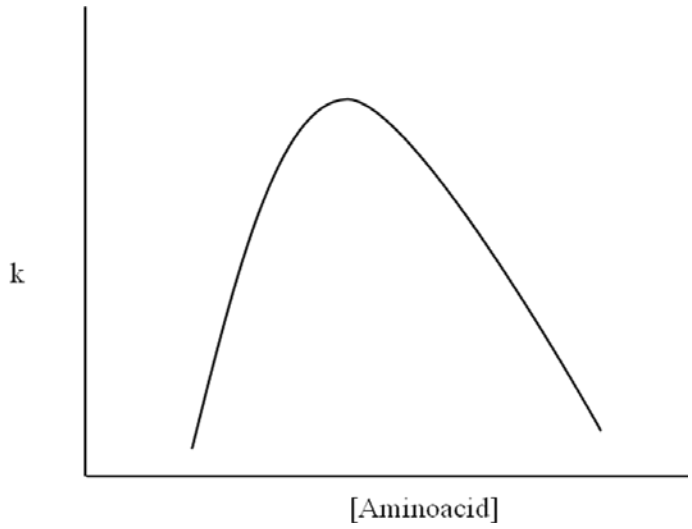


Here aspirate donates its amino group becoming the α-ketoacid oxaloacetate and α-ketoglutarate accepts the amino group becoming the amino acid gluctamate

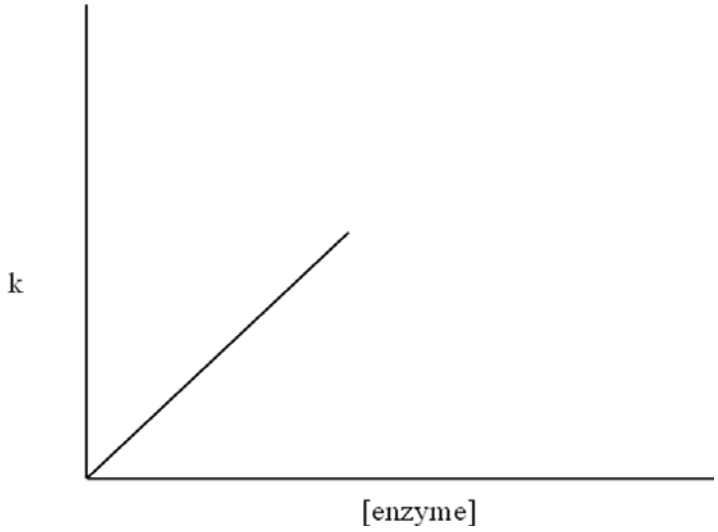
Kinetics is followed by studying rate of DPNH oxidation before and after the addition of transaminase

The following kinetic reactions were observed

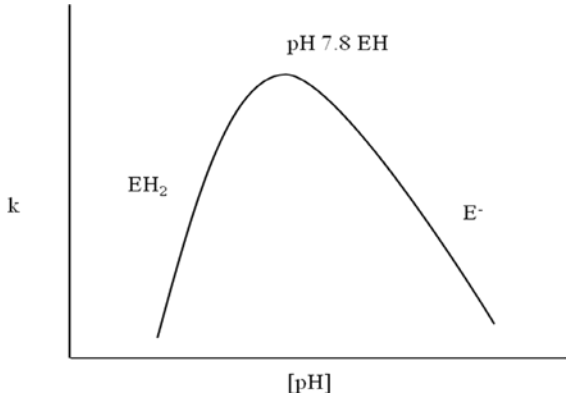
i) The rate increases with [Amino acid] and reaches a maximum and then decreases due to inhibition of excess concentration of substrate at high [amino acid]



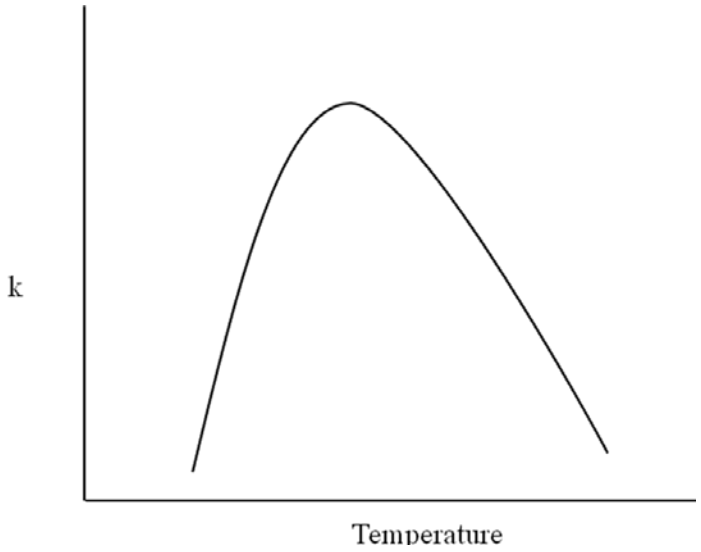
ii) At constant [amino acid] and pH the rate increases with [enzyme]



iii) The rate increases at low pH and reacts a maximum and then decreases due to existence of difference form of enzyme.



iv) Increases of temperature increases the rate of reaction and after 38^0C the rate of reaction decreases



The reaction scheme is assured to be $E_A + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (E_A A) \xrightarrow{k_2} P + E$

$$Rate = k_2[(E_A A)] - - - - - - - - - - - - - - - (1)$$

At steady state

$$\frac{d}{dt}[(E_A A)] = k_1[E_A][Aa] - k_1[(E_A Aa)] + k_2[(E_A Aa)] = 0 - - (2)$$

$$\therefore [(E_A . Aa)] = \frac{k_1}{k_2 - k_1}[E_A][Aa] - - - - - - - - - - - - - - - - - (3)$$

substituting equation (3) *in* (1) *we get*

$$Rate = \frac{k_1 k_2}{k_2 - k_{-1}}[E_A][Aa] - (4)$$

or

$$rate \ \vartheta = k_M .[E_A][Aa] - (5)$$

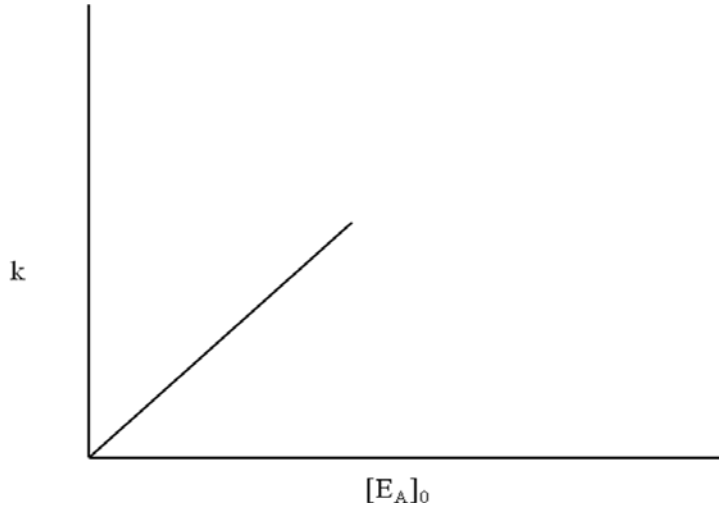
$$here \ k_M = \left(\frac{k_1 k_2}{k_2 - k_{-1}} \right)$$

Is called Michaelis-Menten constant and equation (5) is retunes as Michaelis-Marton equation

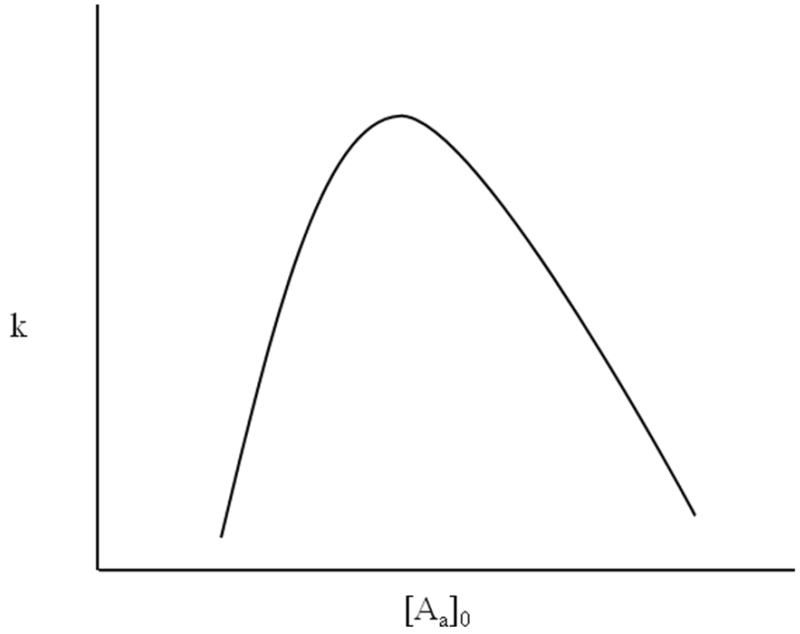
If the equation (5) is expressed in terms of (E_A) and $(Aa)_a$ the intial concentration of enzyme and amino acid then the equation is given by

$$rate\,\vartheta = \frac{k_2[E_A]_0[Aa]_0}{K_m + [Aa]_0} \text{----------(6)}$$

Case i) at low $[Aa]_0$ the rate linearly increases with $[E_A]_o$



Case ii) at high [Aa] the rate increases and reaches maximum then decreases



10.8 Summary of the unit

Glucose oxidase enzyme catalyzes the oxidation of β-D- glucose to glucoxoketone and then the glucolactone hydrolysed to gluconic acid. Increase of [S] increases the rate it reaches a

maximum and then decreases at constant concentration of enzyme. Similarly increase of [E] increases the rate at constant [S]. Increase of temperature increase the rate, reaches a maximum at 35^0 C after that the rate decreases. Transaminase (Aminotransferases) catalyzed the reaction of oxidation of aminoacids. There are multiple transaminase enzymes depends with substrate. The rate increases at low pH and reacts a maximum and then decreases due to existence of difference form of enzyme. Increases of temperature increases the rate of reaction and after 38^0C the rate of reaction decreases

10.9 Key words

Glucose oxidase; Oxidation of glucose

10.10 References for further studies

1) Textbook of Biophysical Chemistry; U N Dash; *Macmillan*, **2006**.

2) Biophysical Chemistry; Satake & Iqbal; *Discovery Publishing House*, **1997**.

3) Biophysical Chemistry; James P. Allen; *John Wiley & Sons*, **2009**.

4) Biophysical Chemistry; Alan Cooper; *Royal Society of Chemistry*, **2011**.

5) Biophysics & Biophysical Chemistry; D. Das; *Academic Publishers*, **1982**.

10.11 Questions for self understanding

1) Derive the expression for kinetic and mechanistic application of glucose oxidase in the oxidation of glucose
2) Explain the effect of concentration of substance on glucose oxidase in the oxidation of glucose
3) Explain the effect of concentration of enzymes [E]on glucose oxidase in the oxidation of glucose
4) Explain the effect of temperature glucose oxidase in the oxidation of glucose
5) Derive the expression for kinetic and mechanistic application of Transaminase enzyme in the oxidation of amino acids.

UNIT-11

Structure

11.0 Objectives of the unit

After studying this unit you are able to

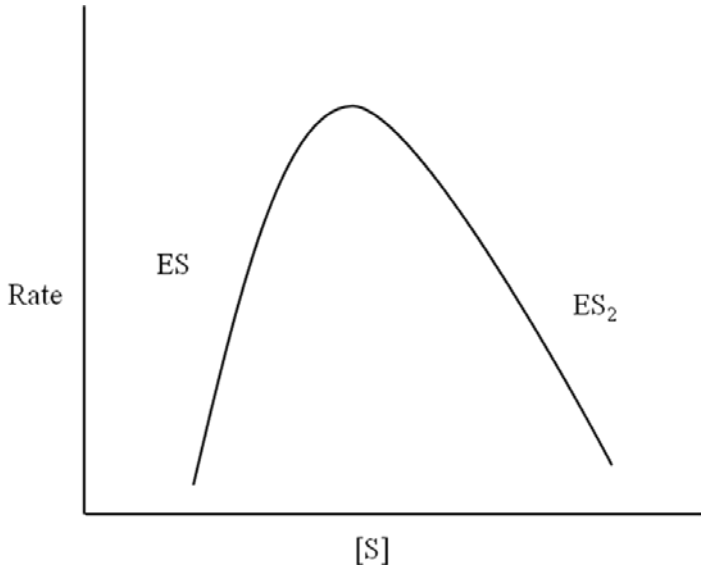
- Derive the expression of substrate inhibition of Enzyme catalyzed reaction at constant [E]
- Explain the effect of Cr^{3+} on oxidation of glucose by glucose oxidation
- Explain the effect of ZnO oxidation of glucose by glucose oxidation
- Explain the effect of Fe^{2+} on the oxidation of glucose
- Effect of Zeolite ($AlPO_4$-5) of D-glucose oxidation
- Effect of ZnO and UV light on the oxidation of glucose by glucose oxidation enzyme catalyzed reaction

11.1 Introduction

Enzyme inhibitors are substances which alter the catalytic action of the enzyme and consequently slow down, or in some cases, stop catalysis. There are three common types of enzyme inhibition - competitive, non-competitive and substrate inhibition. Most theories concerning inhibition mechanisms are based on the existence of the enzyme-substrate complex ES. Competitive inhibition occurs when the substrate and a substance resembling the substrate are both added to the enzyme. A theory called the "lock-key theory" of enzyme catalysts can be used to explain why inhibition occurs.

11.2 Substrate inhibition of Enzyme catalyzed reaction at constant [E]

In general at constant [E] increases of concentration of substrate increases the rate of reaction at low [S]. As the [S] increases the rate reaches a maximum and then it decreases



The reaction scheme in this is given by

$$E + S \rightleftharpoons ES \rightarrow E + P$$

$$+$$

$$S$$

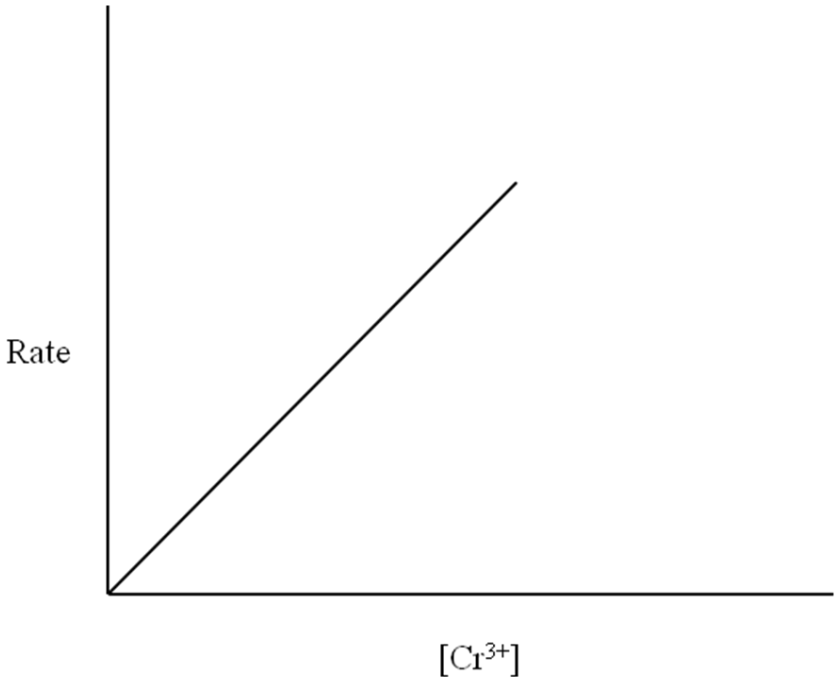
$$\downarrow$$

$$ES_2$$

Here ES_2 is less active than ES so that $[ES_2] >> [ES]$ hence the rate of reaction is decreased. As the[S] increases the substrate present in large molar excess over the enzyme and no free site of enzyme is available to react.

11.3 Effect of Cr^{3+}

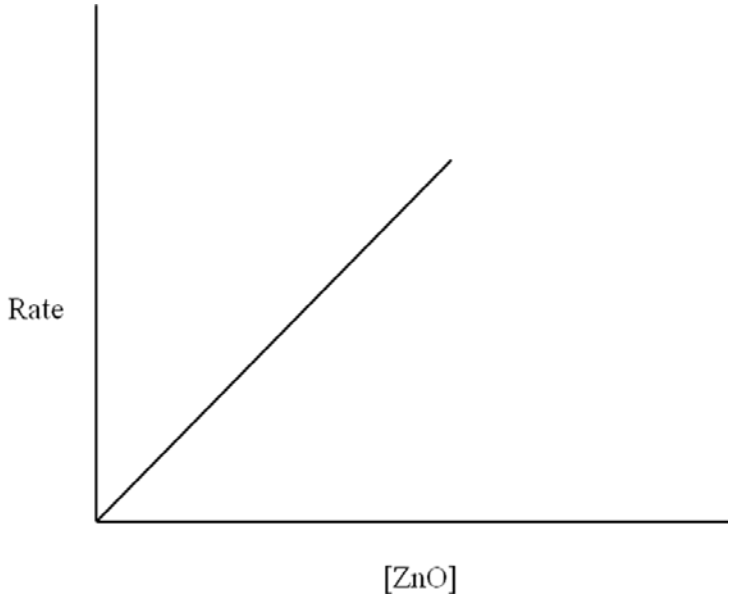
Addition of Cr^{3+} (trivalent chromium) in the oxidation of D-Glucose by enzyme glucose oxidize reaction in creaser the rate of reaction which increase of $[Cr^{3+}]$



Here Cr^{3+} binds to insulin activated receptors and results in stimulating its tyrosine kinase activity. Also Cr^{3+} improves glucose metabolism and resume lipid profile with or without diabetes.

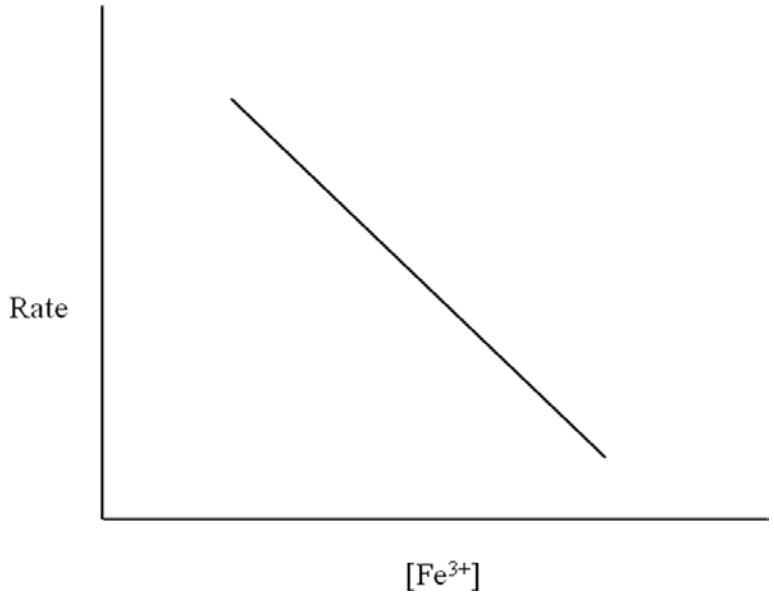
11.4 Effect of ZnO

Addition of ZnO in the oxidation of D-glucose by enzyme Glucose oxidase reaction increases the rate of reaction with increase of [ZnO]



11.5 Effect of Fe^{2+} on the oxidation of glucose

Addition of Fe^{2+} ions to the oxidation reaction of glucose by enzyme catalyst of glucose oxidation influence the rate of reaction. The increase of [Fe^{2+}] decrease the rate of reaction

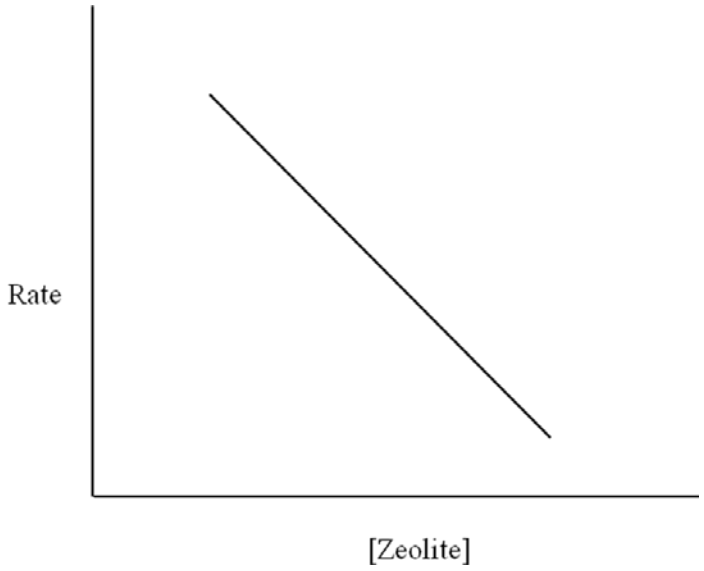


This indicates that the role of iron in the pathogenesis increase incidence of type 2 diabetes in diverse causes of iron overload, because of following 3 mechanism

i) Insulin deficiency.
ii) Insulin resistance
iii) Hepatic dysfunction

11.6 Effect of Zeolite ($AlPO_4$-5) of D-glucose oxidation

$AlPO_4$ -5 Q molecular zeolite used as a catalyst to inhibit the oxidation of D-glucose in NaOH medium. The increase of concentration of zeolite decreases the rate of oxidation of glucose.

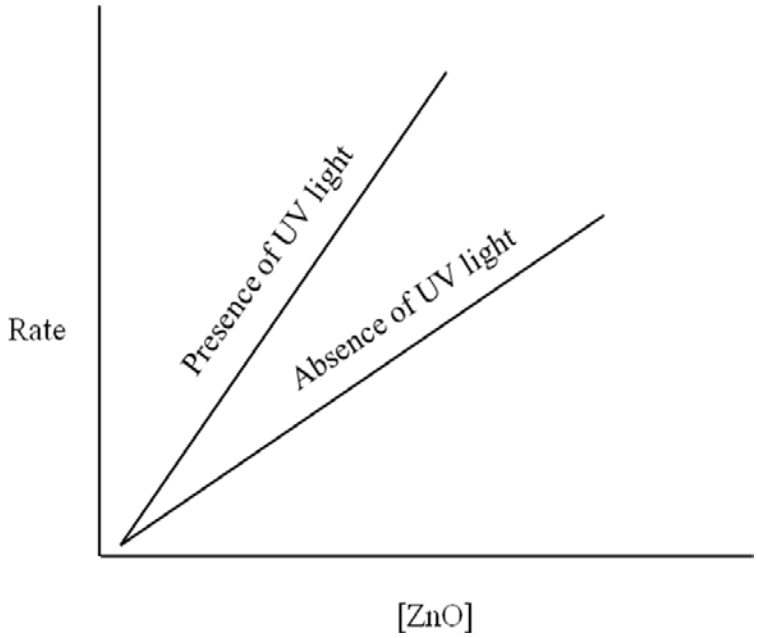


The inhibiting effect is explained as follows. The mesopores of the zeolite have a stronger influence on the OH^-ions adsorption in the inner part of the zeolite than the micropores. The transfer of OH^- ions from the solution to the zeolite pores decreases the concentration of OH^- in the solution. Hence the rate of reaction decreases. This may play very important role to slow down the oxidation of glucose as it is required for hypogluconic (low sugar) patients**.**

11.7 Effect of ZnO and UV light on the oxidation of glucose by glucose oxidation enzyme catalyzed reaction

ZnO acts as a photo catalyst. Irradiation of ZnO in presence of UV light lead to formation of species like e^-, holes and $^{\cdot}OH$ radicals. Hence the rate of reaction of oxidation of glucose by enzyme catalyzed reaction is influenced by ZnO and UV light. Addition of ZnO increases the

rate of reaction. However if the reaction is carried out in presence of U.V light the rate of oxidation is faster compare to absence of U.V irradiation



11.9 Summary of the unit

In general at constant [E] increases of concentration of substrate increases the rate of reaction at low [S]. As the [S] increases the rate reaches a maximum and then it decreases. Addition of Cr^{3+} (trivalent chromium) in the oxidation of D-Glucose by enzyme glucose oxidize reaction in creaser the rate of reaction which increase of [Cr^{3+}]. Cr^{3+} binds to insulin activated receptors and results in stimulating its tyrosine kinase activity. Also Cr^{3+} improves glucose metabolism and resume lipid profile with or without diabetes. Addition of Fe^{2+} ions to the oxidation reaction of glucose by enzyme catalyst of glucose oxidation influence the rate of reaction. The increase of [Fe^{2+}] decrease the rate of reaction. The mesopores of the zeolite have a stronger influence on the OH^-ions adsorption in the inner part of the zeolite than the micropores. The transfer of OH^- ions from the solution to the zeolite pores decreases the concentration of OH^- in the solution. Hence the rate of reaction decreases. This may play very important role to slow down the oxidation of glucose as it is required for hypogluconic (low sugar) patients. ZnO acts as a photo catalyst. Irradiation of ZnO in presence of UV light lead to formation of species like e^-, holes and $^{\cdot}OH$ radicals. Hence the rate of reaction of oxidation of glucose by enzyme catalyzed reaction is influenced by ZnO and UV light. Addition of ZnO increases the rate of reaction. However if the

reaction is carried out in presence of U.V light the rate of oxidation is faster compare to absence of U.V irradiation.

11.10 Key words

Effect of Cr^{3+}; Effect of ZnO; Effect of Fe^{2+}; Zeolite ($AlPO_4$-5); Effect UV light

11.11 References for further studies

1) Textbook of Biophysical Chemistry; U N Dash; *Macmillan*, **2006**.

2) Biophysical Chemistry; Satake & Iqbal; *Discovery Publishing House*, **1997**.

3) Biophysical Chemistry; James P. Allen; *John Wiley & Sons*, **2009**.

4) Biophysical Chemistry; Alan Cooper; *Royal Society of Chemistry*, **2011**.

5) Biophysics & Biophysical Chemistry; D. Das; *Academic Publishers*, **1982**.

11.12 Questions for self understanding

1) Derive the expression of substrate inhibition of Enzyme catalyzed reaction at constant [E]
2) Explain the effect of Cr^{3+} on oxidation of glucose by glucose oxidation
3) Explain the effect of ZnO oxidation of glucose by glucose oxidation
4) Explain the effect of Fe^{2+} on the oxidation of glucose
5) Explain the effect of Zeolite ($AlPO_4$-5) of D-glucose oxidation
6) Explain the effect of ZnO and UV light on the oxidation of glucose by glucose oxidation enzyme catalyzed reaction

UNIT-12

Structure

12.0 Objectives of the unit

After studying this unit you are able to

- Explain the Pharmacokinetics
- Explain the procedure of Measurement of drug concentrations
- Calculate the drug concentrations in blood, plasma or serum
- Draw the Plasma level-Time curve (Drug plasma level-Time curve)
- Explain the significance of plasma level-time curve

12.1 Introduction

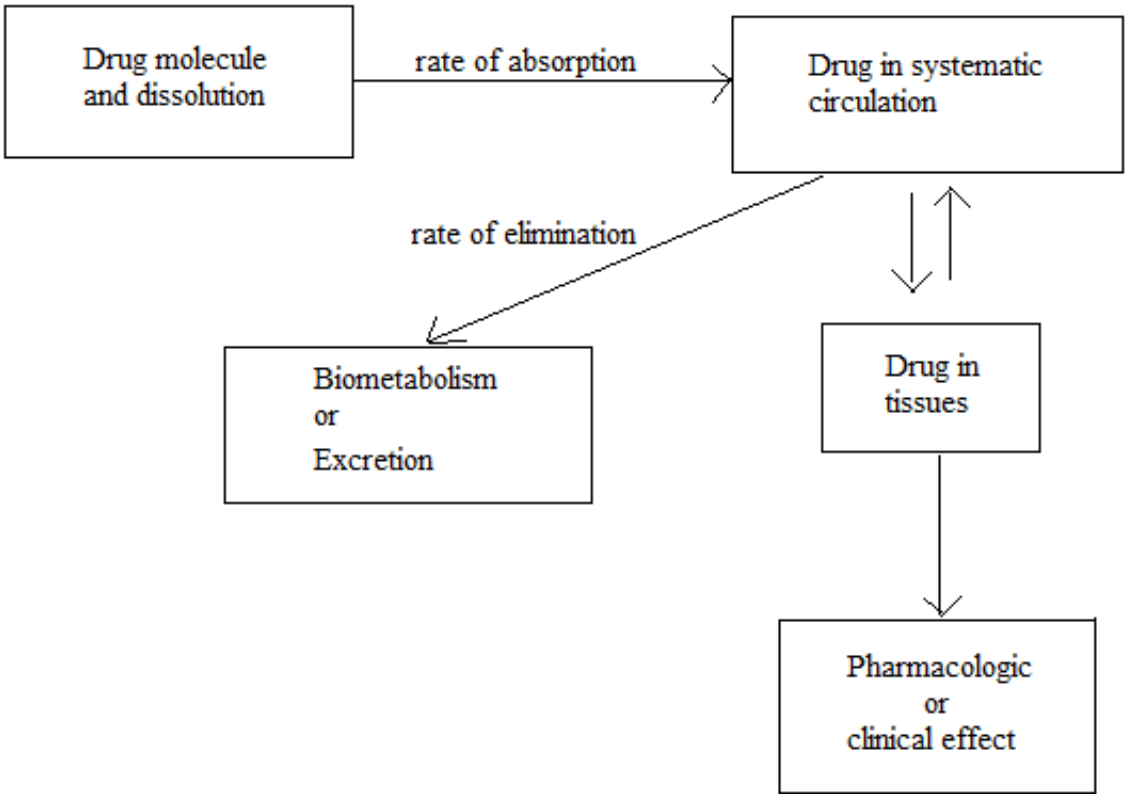
The percent of dose entering the systemic circulation after administration of a given dosage form. More explicitly, the ratio of the amount of drug "absorbed" from a test formulation to the amount "absorbed" after administration of a standard formulation. Frequently, the "standard formulation" used in assessing bioavailability is the aqueous solution of the drug, given intravenously. The amount of drug absorbed is taken as a measure of the ability of the formulation to deliver drug to the sites of drug action. Obviously depending on such factors as disintegration and dissolution properties of the dosage form, and the rate of biotransformation relative to rate of absorption. Dosage forms containing identical amounts of active drug may differ markedly in their abilities to make drug available, and therefore, in their abilities to permit the drug to manifest its expected pharmacodynamic and therapeutic properties.

"Amount absorbed" is conventionally measured by one of two criteria, either the area under the time-plasma concentration curve (AUC) or the total (cumulative) amount of drug excreted in the urine following drug administration. A linear relationship exists between "area under the curve" and dose when the fraction of drug absorbed is independent of dose, and elimination rate (half life) and volume of distribution are independent of dose and dosage form. Alinearity of the relationship between area under the curve and dose may occur if, for example, the absorption process is a saturable one, or if drug fails to reach the systemic circulation because of, e.g., binding of drug in the intestine or biotransformation in the liver during the drug's first transit through the portal system

12.2 Pharmacokinetics

Pharmacokinetics is the quantitative study of drug movement in, through and out of the body. Intensity of effect is related to concentration of drug at the site of action which depends on its pharmacokinetic properties.

Pharmacokinetic properties of particular drug is important to determine the route of administration, dose, onset of action, peak action time, duration of action and frequency of dosing. Below mentioned chart describe the actions involved in pharmacokinetics



12.3 Measurement of drug concentrations

Following methods are used for measurement of drug concentrations in biological samples

1. *Invasive method*

It involves any biological material that requires surgical intervention in the patient. Ex: blood, spinal fluid, tissue biopsy etc.

2. *Non-invasive method*

It involves any biological material that can be obtained without surgical intervention. Ex: urine, saliva, feces, expired air, etc..

Measurement of drug concentration in each of these cases gives different information. However the measurement of [drug] in the blood, serum or plasma is the most direct method to study the pharmacokinetics or pharmacological effect in the body.

12.4 Drug concentrations in blood, plasma or serum

Whole blood contains cellular elements, i.e., red blood cells, white blood cells, platelets and proteins such as albumin, globulins etc.

In general serum or plasma is used for drug measurement.

Serum: whole blood is allowed to clot and the serum is collected from the supernatant after centrifugation.

Plasma: to whole blood an anticoagulant ex: Hepasin is added then the plasma is obtained from the supernatant of centrifugation.

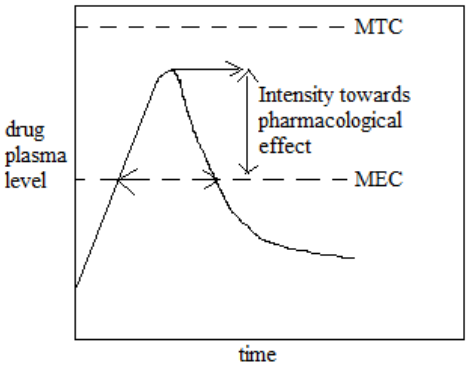
Therefore, the protein content of serum and plasma is different.

Plasma prefuses (reflects) all the tissues of the body including the cellular elements in the blood. Assuming that a drug in the plasma is in dynamic equilibrium with the tissues, then changes in the drug concentration in plasma will reflect changes in tissue drug concentration.

12.5 Plasma level-Time curve (Drug plasma level-Time curve)

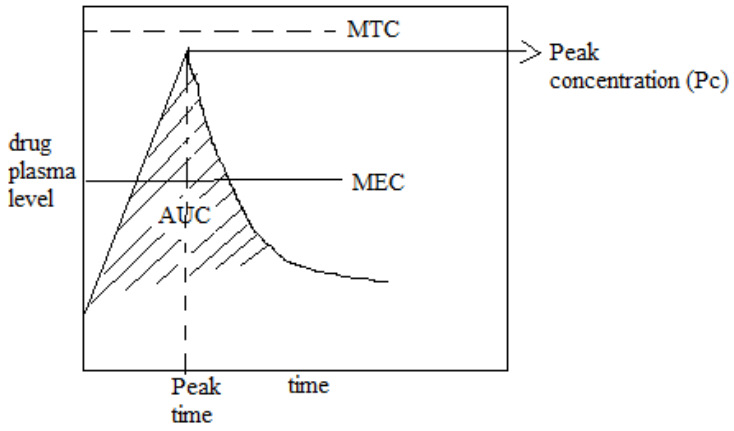
The plasma level-time curve is obtained by plotting [Drug] in plasma samples taken at various time intervals after a drug is administrated (given to patient). The [drug] in each plasma sample is plotted against time at which the plasma sample was removed. As the drug absorbed in the systematic circulation in the body, plasma [drug] increases, reaches a maximum, then decreases. Usually absorption is more rapid than elimination. The elimination of a drug can proceed by excretion or biotransformation or a combination of both.

The relationship between drug level- time curve is shown in figure.



Here MEC, *"minimum effective concentration"* i.e., assuming the drug concentration in the plasma is in equilibrium with the tissues, the MEC reflects the minimum concentration of drug required for a patient to produce the pharmacological effect. (Below this [drug] no pharmacological effect can be observed). The duration of drug action is the difference between the onset time and the time for the [drug] to come back MEC (After that time no pharmacological effect).

MTC: minimum toxic concentration. Above this toxic effect is dangerous.



The time for peak plasma level is the time of maximum drug concentration in the plasma (or indicates average rate of drug absorption). PC is related to the dose, the rate constant for absorption and elimination constant of the drug.

The AVC is related to the amount of drug absorbed systematically with time.

i) [drug] in one tissue is different compare to tissue of other part of the body. i.e., the blood flow rate is different to different tissues.

ii) [drug] in urine reflects the rate of absorption.

iii) [drug] in feces reflects the drug that is not absorbed after an oral dose.

iv) The saliva/plasma [drug] < 1 and depends with pKa of saliva and pH of the saliva (plasma pH= 7.4)

12.6 Significance of plasma level-time curve

i) Since tissue cells are related to plasma, the intensity of the pharmacological or toxic effect of a drug can be related to the [drug] and patient.

ii) Since physiological condition of patient to patient varies, it is very necessary to vary dose of [drug] which can be done using DPT curve.

iii) Also it is very necessary to change the amount of dose from time to time, which can be done using DPT curve.

iv) Also sometimes the change of drug itself required to observe pharmacological effects for which DPT curve is required.

v) By monitoring DPT curve allows the change of dose rate as according to patient disease condition.

However for some of the diseases the DPT curve is not used.

Ex: i) for drugs that acts irreversibly at the receptor site. Drugs used to cancer chemotherapy interact with nucleic acid or proteins to destroy tumor cells. In this case some other pathophysiologic parameters are used to study the patient conditions.

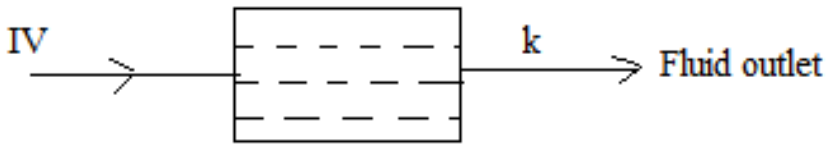
ii) for cardiac patients ECG is used etc..

12.7 One-compartment open model

To understand quantitative study of various kinetic process of drug absorption or desorption in the body various mathematical models is proposed (one-compartment model, two compartment model, three compartment model, etc..). These mathematical models make possible the development of equations to describe [drug] in the body as a function of time. [A model may predict how much of drug present in a given part of body ex: it may be possible to predict [drug] after I hour in liver is 20mg after an oral administration].

12.8 Compartment model

A compartment model is not a real physiological or anatomic region but it is tissue or group of tissues that have similar blood flow or drug affinity i.e., the drug distribute uniformly, rapidly and mixed, also the drug molecule has an equal probability of leaving the compartment conceptually, drugs move dynamically in and out of the compartments and the rate constants are used to explain this. The model is said to be open if the drug is eliminated from the system.



Assume a drug is given by intravenous injection (IV) so that drug rapidly dissolves (distributes) in the body fluids. Then the model is a tank containing a volume of fluid that is rapidly equilibrated with the drug. Then the concentration of the drug in the tank after a given dose is governed by two parameters:

i) The fluid volume of the tank which dilutes the drug

ii) The elimination in the rate of the drug/unit time.

If [drug] in the tank is determined at various time intervals then the volume of the fluid and the rate of drug elimination can be estimated.

(For more complex system computer programmes are used for parameters estimation). Then the rate expression is given by,

$$\frac{d\,DB}{dt} = -\,k.\,D_B \quad \text{.....(2)}$$

Equation 2 shows that rate of elimination in the body follows I order kinetics, which depends with k and D_B (amount of the drug in the body) remaining.

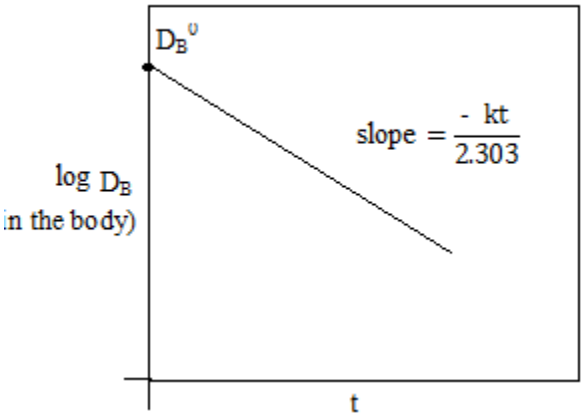
Integrating equation 2. We get,

$$\log DB = \frac{-kt}{2.303} \times \log D_B^{\,0} \quad \text{.....(3)}$$

D_B = [drug] in the body at anytime ‘t’

$D_B^{\,0}$ = [drug] in the body at time t = 0

Then a plot of log D_B versus‘t’ a straight line is obtained.



Hence the elimination rate constant k can be obtained.

The one component open model is the simpler one to describe the process of drug distribution and elimination in the body.

Apparent volume distribution (V_D) volume of drug uniformly distributed (dissolved) determined from pre injected amount of dose and plasma [drug] resulting from immediately after dose is injected. Another parameter is *elimination constant* which gives decrease of drug with time.

V_B = Apparent volume

D_B = Drug in the body

One component model not directly gives actual drug level in the tissues, but obtained by body fluids (such as blood) to determined the [drug].

12.9 Elimination rate constant

The rate of elimination for most of the drug follows I order kinetics. The total removal or elimination of the percent drug (active drug) from the compartment is effected by metabolism and excretion. Then elimination rate constant (k) is given by,

$$k = k_m + k_e \quad \text{…….. (1)}$$

k_m = I order rate constant for metabolism process.

k_e = I order rate constant for excretion process.

$$V_D = D_B/C_P$$

D_B = amount of drug in the body

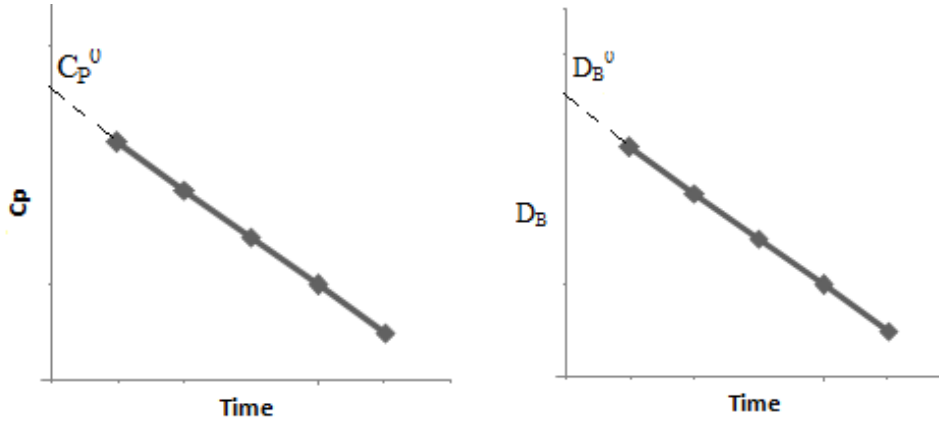
C_P = [drug] in the plasma

Therefore, $\log C_P = \frac{-kt}{2.303} + \log C_P^0$

C_P^0 = [drug] in plasma t = 0

Calculation of V_D

$V_D = D_B^0/C_P^0$



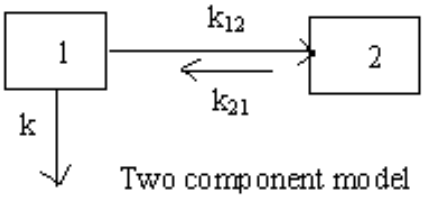
V_D can be

Significance of V_D

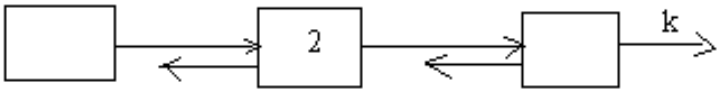
i) Parameter explains how drug distributes in vascular and extravascular tissues.

ii) Given the apparent V_D for a particular drug (usually content), the total amount of drug in the body at any time after administration can be measured.

iii) V_0 reflects the solubility of a given drug.



Two component model



Three component model

12.10 Bioavailability

Bioavailability refers to the extent and rate at which the active moiety (drug or metabolite) enters systemic circulation, thereby accessing the site of action.

Fraction of the dose of a drug contained in any dosage form that reaches the systemic circulation in unchanged or active form administered through any route is known as bioavailability.

Drugs injected using intravenous route of administration have 100% bioavailability, while others have much less bioavailability, because:

All of the drug may not be adsorbed

Metabolism of the drug might occur before reaching the site of action

Bioavailability of a drug is largely determined by the properties of the dosage form, which depend partly on its design and manufacture. Differences in bioavailability among formulations of a given drug can have clinical significance; thus, knowing whether drug formulations are equivalent is essential.

i) Physicochemical factors affecting bioavailability.

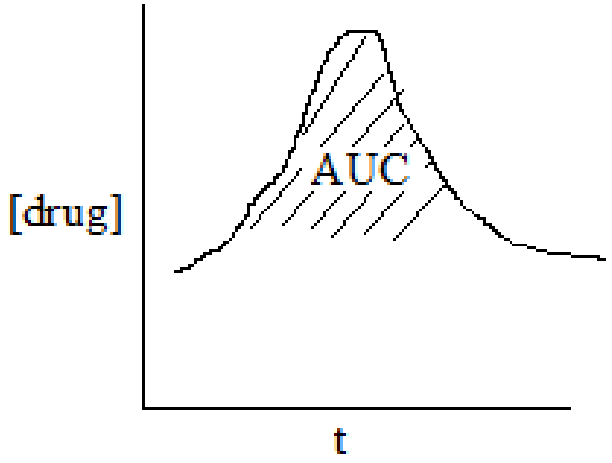
ii) Drug dissolution rate.

iii) Protein binding of drugs.

Bioavailability indicates "a measurement of the rate and extent (amount) of therapeutically active drug that reaches the systematic circulation and is available at the site of action". Bioequivalence requirement based on above how much of a given drug is required for in vitro/in vivo testing with respect to drug products before marketing is imposed by FDA (Food and drug administration). Therefore, in approving a drug product for marketing, FDA requires bioavailability stands for all drug products to know the strength, quality and purity. Bioavailability studies are used to know the effect of changes in the physicochemical properties of the drug substance and the effect of the drug product on the pharmacokinetic parameters.

12.11 Relative and absolute availability

AUC is a measure of total amount of drug that reaches the systemic circulation.



AUC α FD_0/k_1 (total amount of drug)

k_1 is a elimination constant.

F = 0 to 1 : for IV administration F = 1

But for oral administration F = 0 to 1

F = 0 no drug absorption

F = 1 complete drug absorption

The relative availability = $\frac{[AUC]_A / \text{dose A}}{[AUC]_B / \text{dose B}}$

Here A: new drug

B: reference recognized standard

Absolute availability = $\frac{[AUC]_{po} / \text{dose po}}{[AUC]_{IV} / \text{dose IV}} = F$

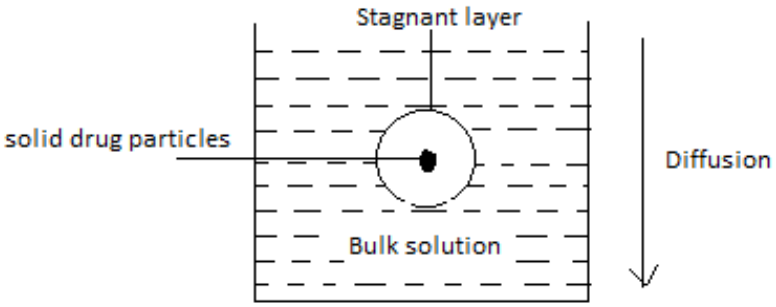
Here F indicates traction of the dose that is bioavailable.

F = 1 or 100% for IV injection i.e., drug is completely absorbed.

F < 1 all other extra vascular or oral routes of administration.

12.12 Drug dissolution rate

In biological systems drug dissolution in an aqueous medium is an important prior condition for systematic absorption. The rate of drug dissolution may affect the dug bioavailability.



Drug dissolution starts at the surface of the particle forming a standard solution of drug (called stagnant layer) then diffuses into the bulk of the solution.

The rate of dissolution dC/dt is given by Noyes –Whitney equation,

$$\frac{dC}{dt} = \frac{DA(Cs-C)}{h}$$

D = diffusion rate constant (stagnant layer to bulk of the solution)

Cs = concentration of the drug in the stagnant layer.

C = concentration of drug in the bulk of the sovent

h = thickness of the stagnant layer.

A = surface area of the particle

Drug in the body, particularly in the gastro intestinal tract, is considered to be dissolving in an aqueous environment. i.e., diffusion in GI = Drug dissolution in aqueous environment.

The rate of dissolution depends with;

i) Physicochemical nature of the drug
ii) The nature of the ingredients
iii) The method of manufacture

12.13 Physiological nature of the drug (pH)

The solubility of a drug is influenced by pH. Environment of the gastro intestinal tract varies from acidic in the stomach to slightly alkaline in the small intestine. The solubility –pH relationship gives information about dose rate of the drug in stomach or intestine. Solubility may improve y addition of an acidic or basic excipient. Ex: Erythromycin tablet

Its decomposition occurs rapidly in acidic medium (stomach), but stable in neutral or alkaline pH. Therefore, the tablet is coated (buffered) to protect against acid degradation.

Particle size

Greater the surface area, the more rapid is the rate of drug dissolution. Thus, smaller particle size increases rate of dissolution. Adding recipients, dissolution rate can be improved i. e., the recipients alter the medium or react with drug itself.

Ex: Lactose, Sucrose, Starch, Cellulose, etc..

12.14 Effect of food in gastrointestinal (GI) drug absorption

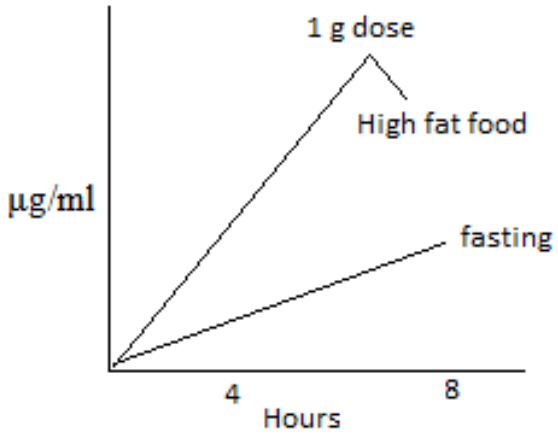
The presence of food in the GI tract affect the rate of dissolution, hence the bioavailability of the drug.

Absorption of antibiotics Ex: Penicillin, tetracycline is decrease with food.

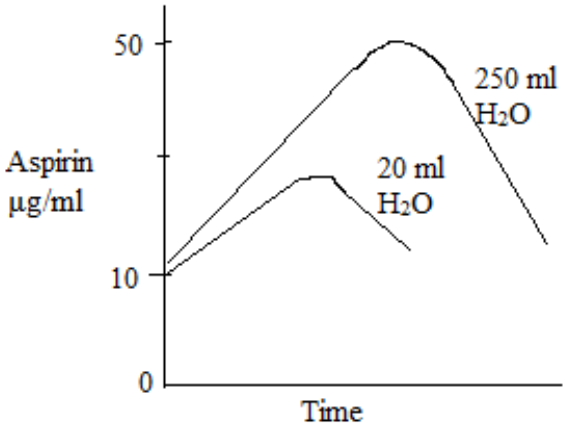
Some other drug ex: Griseofulvin is increase with food.

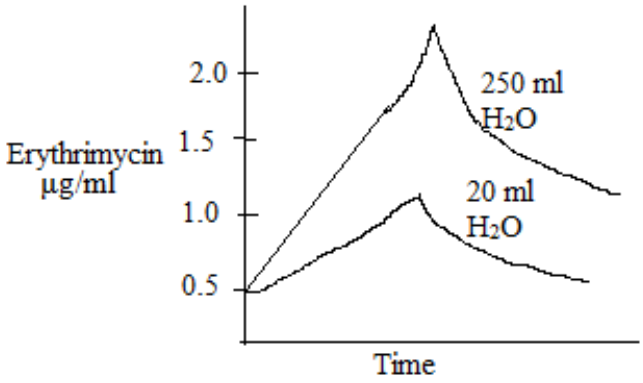
The presence of food in the GI lumen stimulates the flow of bile. The bile acid increases the solubility of the drugs through micelle formation. The food presence and absence changes the pH

in the GI tract. Hence depending upon whether the drug requires acidic or basic condition, the drug should be taken in presence of food or absence of food.



Griseofulvin with food and without food





Generally large volume of water dissolution is more.

12.15 Protein binding drugs

Once a drug is absorbed or injected into the blood stream, the drug molecules are carried by the blood to the target site for drug action. The [drug] in untargeted sites may produce the side effect.

The drug molecule distributed to liver, kidney, tissues such as brain, skin and muscle,etc..

Some drug molecule may be bound to proteins in the plasma or tissues. i.e., many drugs interact with plasma or tissue proteins or with other macromolecules like melanin and DNA to form a drug-macromolecule complex. The formation of a drug protein complex is named as drug-protein binding.

Drug protein binding → Irreversible

↘ Reversible

Irreversible binding is formed by covalent bonding which produces drug toxicity over a long time. Ex: chemical carcinogenesis or formation of chemical intermediates with very short time (high toxicity). Ex: high doses of “acetaminophen” which interact with river proteins.

Reversible binding is formed by weaker chemical bonds such as hydrogen bonds or vander waals forces. Ex: aminoacids have hydroxyl, carbonyl or amino sites available for reversible drug interactions.

12.16 Kinetics of protein binding

If P = protein concentration

D = [drug] [PD] = protein binding drug

$$P + D \underset{}{\overset{Ka}{\rightleftharpoons}} PD$$

Then $Ka = \frac{[PD]}{[P][D]}$ ……(1)

Value of Ka gives information whether binding is strong or weak.

Ka is high: drug is strongly bound

Ka is low: drug is weakly bound

Factors affecting; [protein], patient to patient, drug to drug, [drug], etc..

Drug	Renal boundary % bound
Chloramphenicol	53
Dessipramine	80
Oxazepam	95
Morphine	35

The kinetics of protein-drug binding play an important role in controlling the dose given to a patient.

Purified albumin is taken as standard protein source.

Experimentally at different intervals of time

[D] = Free drug

[PD] = protein bound drug can be obtained (i.e., initial [D]-[D] at anytime = [PD]). Therefore, total protein concentration = [P] + [PD]

To study binding behavior, a term "binding coefficient" (r) is used,

$$r = \frac{moles\ of\ drug\ bound}{total\ moles\ of\ protien}$$

$$r = \frac{[PD]}{[P]+[PD]} \quad \text{…….(2)}$$

$$r = \frac{Ka\ [P][D]}{[P]+Ka\ [P][D]}$$

$$r = \frac{Ka\ [D]}{1+Ka\ [D]} \quad \text{……..(3)}$$

This is for formation of 1:1 complex (single site)

If more than one site i.e., n identical sites then;

$$r = \frac{n\ Ka\ [D]}{1+Ka\ [D]} \quad \text{……(4)}$$

If different type of sites n_1, n_2 etc..

Then $r = \frac{n1\ Ka\ [D]}{1+K1\ [D]} + \frac{n2\ Ka\ [D]}{1+K2\ [D]}$ ……..(5)

Determination of binding sites and binding constants

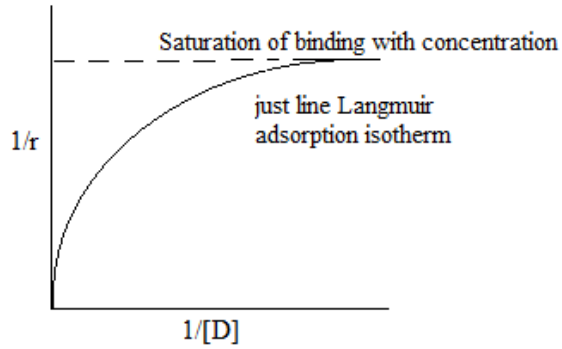
Using equation (4),

$$\frac{1}{r} = \frac{1 + Ka\,[D]}{n\,Ka\,[D]}$$

$$\frac{1}{r} = \frac{1}{n\,Ka\,[D]} + \frac{1}{n}$$

This is called Scatchard equation.

∴ A plot of 1/r versus 1/[D]



∴ Intercept = 1/n

Slope = 1/n.Ka

Hence 'n' the number of binding sites and Ka the binding constant can be calculated by following the kinetics.

∴ This plays very important role to control dose for particular patient.

12.17 Apparent volume of distribution (V_D): (V_D of drug)

V_D represents volume estimating the amount of drug in the body (sampling compartment) from the [drug].

The V_D cannot be obtained directly. It is obtained using C_P.

C_P = Drug concentration in plasma at different intervals of time.

D_B = amount of drug in the body.

i.e., $D_B = V_D C_P$ ……(1)

(since $D_B \alpha C_P$ and log DB = $\frac{-kt}{2.303}$ + log D_B^0)

We get, log Cp = $\frac{-kt}{2.303}$ + log C_P^0 ……..(2)

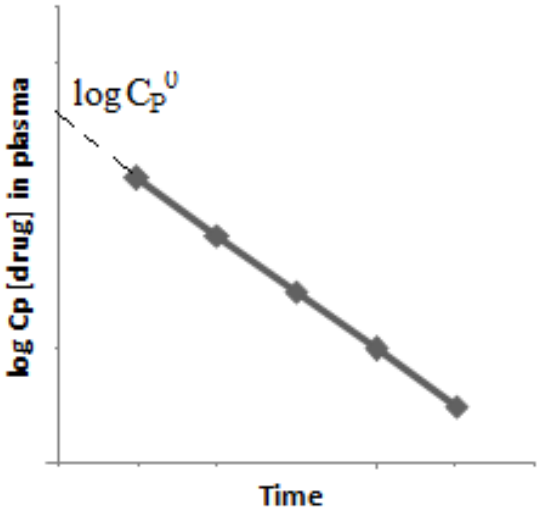
∴ V_D is calculated by equation (1)

i.e., $V_D = C_P/D_B$ or $V_D = C_P^0/D_B^0$ …….(3)

Here C_P^0 = [drug] in plasma at t = 0

D_B^0 = IV dose at t = 0

The C_P^0 is obtained by plotting and taking the intercept.



V_D is calculated from $V_D = C_P^0/D_B^0$

12.18 Calculation of V_D using AUC

We know, $dD_B/dt = - k. D_B$ ……(4)

But DB = $V_D C_P$

$dD_B/dt = -k. V_D C_P$

Here $\int dD_B = - k. V_D \int C_P$

If we consider the drug distribution in a given compartment from 0 to ∞ for AUC, then we can write

$-D_B^0 = -kV_D [AUC]_0^\infty$

$V_D = D_B / k. [AUC]_0^\infty$ …….(5)

Hence knowing area under the curve V_D can be obtained.

12.19 Significance of V_D

i) Parameter explains how the drug distributes in vascular and extravascular tissues.

ii) Given the apparent V_D for a particular drug (usually constant), the total amount of drug in the body at any time after administration can be measured.

iii)V_D reflects the solubility of a given drug.

(V_D α $1/C_P^0$: V_D is high: then drug distributed in the extravascular tissues. V_D is small, C_P^0 is high: then drug distributed in the intravascular i.e., binding of drug peripheral tissues.)

V_D is exposed in terms of body weight

If V_D = 1 lit = 1 kg. For a body weight 70 kg

Then, if V_D = 3500 mg = 3.5 kg.

Then V_D = 3.5 kg/70 kg 100 = 5% of body weight.

In general V_D is content for a given drug, but if body contains more water or less water, it varies.

12.20 Summary of the unit

Pharmacokinetics is the study of drug absorption, distribution, metabolism, and excretion. A fundamental concept in pharmacokinetics is drug clearance, that is, elimination of drugs from the body, analogous to the concept of creatinine clearance. In clinical practice, clearance of a drug is rarely measured directly but is calculated as either of the following

$$\text{clearance} = \text{dose/AUC (equation 1)}$$
$$\text{or}$$
$$\text{clearance} = \text{infusion rate}/C_{ss} \text{ (equation 2).}$$

AUC, the area under the curve, represents the total drug exposure integrated over time and is an important parameter for both pharmacokinetic and pharmacodynamic analyses. As indicated in equation 1, the clearance is simply the ratio of the dose to the AUC, so that the higher the AUC for a given dose, the lower the clearance. If a drug is administered by continuous infusion and a steady state is achieved, the clearance can be estimated from a single measurement of the plasma drug concentration (Css) as in equation 2. Clearance can conceptually be considered to be a function of both distribution and elimination. In the simplest pharmacokinetic model.

12.21 Key words

Pharmacokinetics; Measurement of drug concentrations; Drug concentrations in blood, plasma or serum; Plasma level-Time curve (Drug plasma level-Time curve); Significance of plasma level-time curve; Elimination rate constant; Bioavailability; Relative and absolute availability; Drug dissolution rate; Effect of food in gastrointestinal (GI) drug absorption; Protein binding drugs; Apparent volume of distribution (V_D): (V_D of drug).

12.22 References for further studies

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12.23 Questions for self understanding

1) What is Pharmacokinetics?

2) Explain the methods used for measurement of drug concentrations

3) Write a note on drug concentrations in blood, plasma or serum

4) What is Plasma level-Time curve (Drug plasma level-Time curve)?

5) Write the significance of plasma level-time curve

6) Write a note on

i) One-compartment open model

ii) Compartment model

7) What is elimination rate constant?

8) What is V_D? How it can be calculated?

9) What is Bioavailability?

10) Explain Relative and absolute availability

11) Discuss drug dissolution rate

12) Write a note on physiological nature of the drug (pH)

13) Explain the effect of food in gastrointestinal (GI) drug absorption

14) Write a note on Protein binding drugs

15) Derive the expression for kinetics of protein binding

16) Discuss determination of binding sites and binding constants

17) Write the expression for Calculation of V_D using AUC

18) Discuss the significance of V_D

UNIT-13

Structure

13.0 Objectives of the unit

After studying this unit you are able to

- Explain the Donnan membrane equilibrium
- Identify the biological significance of Donnan membrane equilibrium
- Explain the formation of micelles
- Discuss the effect of solute or substance on micellization
- Explain the formation of mixed micelles between bile salts and products of lipid digestion
- Discuss the diffusion of solution across biomembrane and mechanism of application in the respiratory exchange of O_2 and CO_2

13.1 Introduction

The presence of charged groups attached to the membrane structure plays a central role in many observed equilibrium and transport phenomena in biological and synthetic membranes. Charges can be due to the ionization of functional groups present in the membrane matrix, as well as to the adsorption of ions of external origin when the membrane is immersed in an electrolyte solution. Ion adsorption in biopolymers, membranes and conducting polymers constitutes a problem of considerable experimental interest. A number of theories describing the thermodynamics of such adsorption phenomena have been advanced. Most of these make use of the classical theory of the electrical double layer at charged interfaces. Others invoke the Donnan equilibrium theory. However, it seems that no theoretical study accounting simultaneously for the electrolyte concentration and pH dependence of reversibly adsorbed charged species on the one hand, and the electrostatic interaction between these species on the other hand, has been presented.

13.2 Donnan membrane equilibrium

When two solutions of electrolytes are separated by semipermeable membrane and if the movement of at least one of the ions through the semi permeable membrane is hindered then a potential is developed at the junction of semipermeable membrane at the equilibrium. This process is called Donnan membrane effect. an equilibrium is called Donnan equilibrium and potential is called Donnan membrane potential.

Let us consider a system divided into two compartments separated by semi permeable membrane. The semi permeable membrane allows small molecules and water so pass through but not large ions of colloidal dimensions

Na^+ (a mole)	Na^+(b moles)
R^- (a mole)	Cl^- (b moles)

(SPM)

Let a mole of Na are in compartment (A)

B mole of NaCl in compartment (B)

R^- is a large anion of polyelectrolyte in nature and R^- is not diffuse through SPM. NaCl is diffusing form (B) to (A) and also (A) to (B)

If X is the net concentration of NaCl diffuse from (B) to (A)

Na^+ (a+x) mole	Na^+(b-x) moles
R^- (a mole)	Cl^- (b-x) moles
Cl^- (x moles)	

Here $[Na^+]_B = [Cl^-]_B$

To maintain electrical neutrality in compartment 'A' it follows that the concentration 'n' of

$[Na^+]_n=[Cl^-]_A+[R^-]_A$

This indicates the concentration of diffusible anion is less in compartment which contains non-diffusable ion.

Also $[Na^+]_A > [Na^+]_B$ or $[Cl^-]_A < [Cl^-]_B$

Therefore the presence of non-diffusible ions causes unequal distribution of diffusible ions at equilibrium and hence the potential at compartments A with respect to compartment B is developed at the junction which is called Donnan potential.

Increasing concentration of R^- increases the unequal distribution hence the Donnan potential is increase at the junction. Or

Increasing concentration of NaCl decreases the unequal distribution hence the Donnan potential decreases.

Therefore Donnan effect is decreases at high concentration of electrolytes.

Osmotic pressure of Donnan effect is given by

$$P=\Delta C.RT$$

C = concentration of ions

ΔC = effective concentration change due to diffusion

The amount of NaCl (diffusible electrolytes) B to A can be calculated by the equation

$$\left\langle x=\frac{b^2}{a+2b}\right\rangle$$

Example, at 27^0C a = 8 moles, b = 4 moles, x =?

$$x=\frac{4^2}{3+8}=\frac{16}{11}=1.45\,moles$$

A	B
$Na^+(3+1.45)$	$Na^+(4-1.45)$
$R^-(3\,mole)$	$Cl^-(4-1.45)$
$Cl^-(1.95\,mol)$	
9.4	$5.1\,moles$

$$\Delta C=9.4-5.1=4.3ml$$

$$p=\Delta C\times R\times p$$

$$4.3\times 0.082\times 300$$

$$=105.78$$

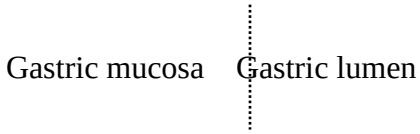
13.3 Biological Significance

Donnan effect plays a significant role in our body because

1. Most of our body membranes are dialyzing membranes i.e they allow the movement of H_2O, gases like O_2, CO_2 ions and organic molecules but not allowing colloidal particles
2. The non diffusible protein anions are present in large quantities in cells and in plasma but not in interstitial fluid

Because of above factors an unequal distribution of diffusible ions exits and a pH difference and osmotic pressure difference is established on either side of the biological membrane.

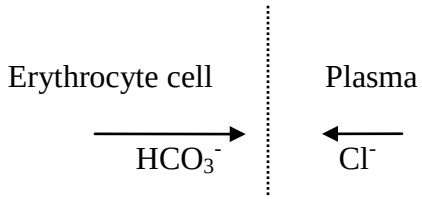
i) Maintenance of pH in stomach for digestion



$P.Cl + H.OH \rightarrow P.OH + HCl$

Minor change in pH in body is tolerable but drastic change in pH creates problem to the body. Gastric digestion takes place at low pH only. Here P is non diffusible protein ions. Because of hydrolysis the H^+ ions move form gastric mucosa to the gastric lumen so that the pH at gastric lumen become low. This helps for digestion

ii) Exchange of HCO_3 and Cl^- ion in red blood cells



Here erythrocyte membrane allows HCO_3^- and Cl^- anion but not protein haemoglobin (R^-). When blood flow through capillaries about 70% of HCO_3^- which formed in the red cells diffuse into the plasma. At the same time to maintain electrical neutrality only 70 % Cl^- it diffuses from plasma to erythrocyte (Cl^- concentration is more in plasma). Therefore Donnan effect maintains the distribution of HCO_3^- ions form red blood cells.

Example 1. Absorption of glucose by the intestine

2. Diffusible ions (Na^+ as K^+) between blood and cerebrospinal fluid

iii) Artificial kidney

Kidney is the main organ for acid base regulation. Also excrete many toxic substances through urine formation. If it is not working, problem causes for life. Then artificial kidney are used

Minute channels for blood | dialysing fluid

Dialysing membrane (cellulose membrane)

The cellulose membrane allows all constituents of blood to diffuse in the directions but not plasma proteins and cellular components. Therefore toxic substance accumulated in the blood is diffuse through cell membrane into the dialyzing fluid. The rate of diffusion can be controlled by controlling the non diffusible ions or diffusible ions calculated by Donnan effect.

Ex. Plasma albumin decreases then slat and H_2O retention takes place in the tissue then oedema of tissues occurs.

13.4 Micelles

Micelles are thermodynamically stable aggregates. During micellation the increase of entropy is observed. These micelles are made up of amphiphilic molecules ie, lyophpbic components in the core region and lyophilic groups on the surface.

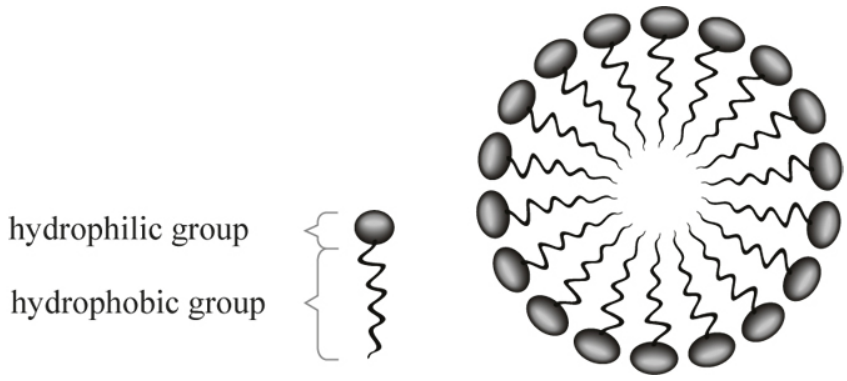


Figure 1 : Schematic representation of a micelle in aqueous solution

It is seen in the micelles the hydrocarbon groups or tails are loose and move freely with in the aggregate. The van der wall forces of attraction acting between the hydrocarbon groups keeps molecule within the frame use of the micelles

Micelles are widely used in industrial and biological fields for their ability to dissolve and move non polar substances through an aqueous medium, or to carry drugs which are, often, scarcely soluble in water. The carrying ability of micelles can be altered if parameters determining their size and shape are changed. Micelle aggregates form only when the concentration of the amphiphilic molecule reaches a given concentration called critical micelle concentration (cmc).

That condition is monitored by the sudden change in the chemical and physical properties of the solution.

Micellization depends on the balance of two main effects they are

1) The tendency of the non polar tails to avoid contacts with water and

2) The repulsion among the polar or charged heads, a destabilizing effect on the aggregation process.

Hydrocarbon tails avoid contacts with the solvent molecules pointing toward the aggregate interior, which lacks of water. Instead, the repulsion among the charged heads on the surface of the micelle is attenuated by the presence of oppositely charged ions (counter-ions). The favourable association among the non polar tails in the interior of the micelle occurs through the hydrophobic interaction, which is the prevailing effect in the formation process of these aggregates.

Amphiphilic molecules can form micelles not only in water, but also in non polar organic solvents. In such cases, micelle aggregates are called inverse micelles because the situation is inverted as respect to water. In fact, hydrocarbon tails are exposed to the solvent, while the polar heads point toward the interior of the aggregate to escape the contacts with the solvent.

Reverse micelles are able to hold relatively large amounts of water in their interior. In that way, a "pocket" is formed which is particularly suited for the dissolution and transportation of polar solutes through a non polar solvent.

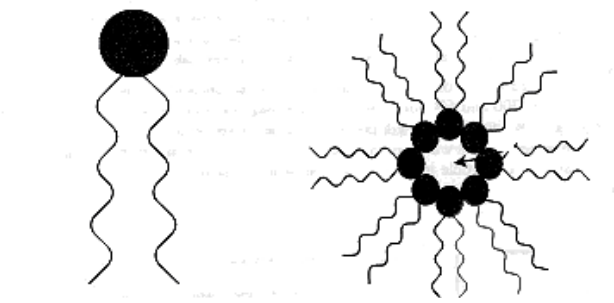


Figure 2: A reverse micelle Polar heads point toward the interior, while the non polar tails are exposed to the non polar solvent. The arrow indicates water "sequestered" in the interior.

It is seen that many molecules having segments in structure are not compatible but upon micelization these molecules become more compatible in a given solvent because of pack of these lyophobic and lyophillic groups

The association of many colloids form aggregates in solution. These aggregates of a particular diameter are called micelles. If we consider a substance, it may form micelles in one solvent bu may not form in another solvent ie, the formation of micelles depend on thermodynamic considerations.

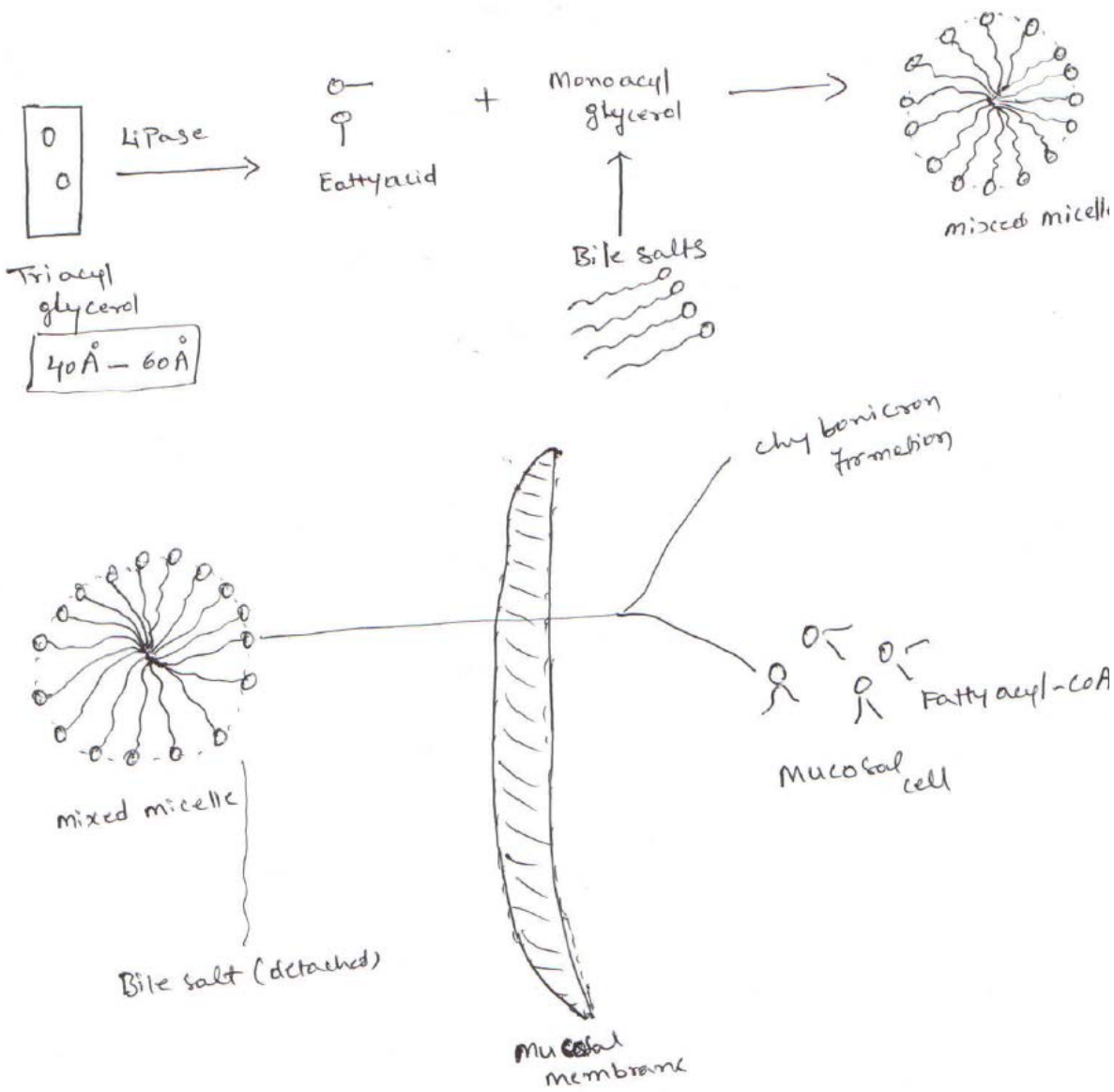
13.5 Effect of solute or substance on micellization

During micellization, as substance concentration increases, the number of temporary aggregates or premicelles increases. If we increase the concentration of substance or solute further, at a particular concentration the osmotic coefficient of the solute suddenly drops at and the premicelles form micelles through supra molecular aggregation and this concentration is called critical micelle concentration (cmc)

However practically it has been shown that the stable micelle formed not at a point of concentration but narrow range of concentration (for example cmc $\neq$ 0.3M, cmc = 0.3 to 0.4M)

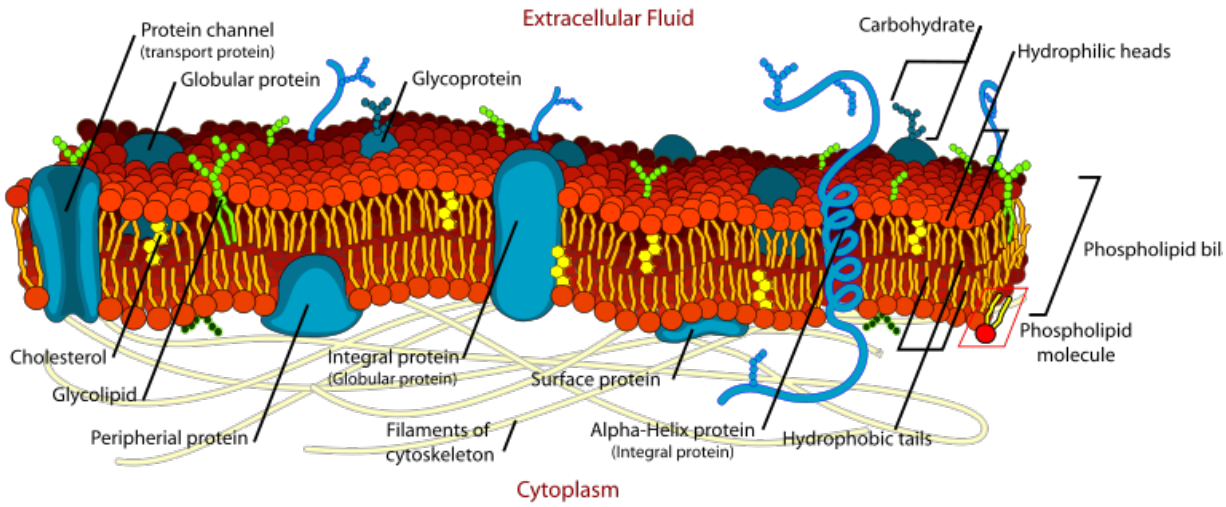
13.6 Formation of mixed micelles between bile salts and products of lipid digestion

Triacylglycerol with lipase action gives water- insoluble fatty acids and mono acylglycerols. Which then mix with bile acid salts form mixed micelles. The diameter of this is about 40Å-60Å units with polar head groups facing outword and hydrophobic hydrocarbon chains are located in the interior of the micelle. Fat-soluble steroid, cholesterol and phosphatidylcholine are also present. Thus the products of lipid digestions enter the aqueous phase. Hence increasing contact between the hydrophilic enzymes and their respective hydrophobic substrate. This increase the rate of hydrolysis process which in turn increases the rate of lipid breakdown. Then these are rapidly transformed to the intestinal muscular through muscular membrane. During this the bile salts are detached ans premicellar is not through membrane. Hence because of formation of micelles of this type the concentration of monoacylglycerol and concentration of fatty acid decreases Micelle formation also leads to unprcipitation of cholesterol and lecithin (insoluble in water). If there is any infection or super-saturation of cholesterol concentration, then disturbance caused to the micelle structure of formation. Then it results precipitation of cholesterol as crystals which can grown as gallstones. The disease called “cholelithiasis’ which is dangerous to life.



13.7 Diffusion of solution across biomembrane and mechanism of application in the respiratory exchange of O_2 and CO_2

Biological membranes have a lipid bilayer sandwiched between two layers of proteisn as shown in below figure. The lipid layer acts as an effective diffusion barriers.



1) A molecule has to break all its hydrogen bonds with water if it has to penetrate the lipid layer. In an aqueous environment this would be difficult and therefore the diffusion rate of this molecule is lowered when passing through a lipid medium.
2) The lipid medium structure has intermolecular hydrophobic bonds between adjacent side chains of the fatty acids. To create a single hole for movement of solute molecules a larger energy is needed to break the hydrophobic bonds. Hence the diffusion is reduced to a great extent.
3) For larger polyelectrolytes the diffusion is slowed down because larger holes to be created i.e larger the molecules lower the diffusion.
4) Greater the degree of hydration of a molecules lesser is the diffusion

Ex. Cations show slow diffusion than anions

The degree of hydration of molecule measures the shrinkage of the cell

Application

The respiratory exchange i.e oxygenation in the venous blood, removal of excess of CO_2 form the blood and distribution of oxygenated blood to the tissues is governed by this simple diffusion process.

$$the\ rate\ of\ diffusion\ of\ gas = \frac{dg}{dt} = \frac{k \propto \Delta p}{T_t}$$

$\propto$ = cross sectional area in which diffusion occurs(membrane area)

K= diffusion coefficient

T_t = thickness of the bio membrane

In man the value of k for oxygen or CO_2 can be determined form the measurement of diffusion of capacity of O_2 or CO_2, lung area and the distance through which diffusion occurs. The continuous cellular metabolism formed CO_2 in cells. The diffusion is swift as the O_2 tension in alveoli air is 100 mm Hg as compared to 45 mm Hg in venous blood. These pressure gradients from diffusion of O_2 into the blood and CO_2 out of the blood. Also CO_2 is absent 28 times more soluble than O_2 at 20^0 C so that its diffusion rate is about one 26 times faster than oxygen. Therefore the exchange of gases CO_2 and O_2 mainly control by the diffusion process

13.8 Summary of the unit

Almost all cells have voltage gradients across their plasma membranes. Membrane potential is depend on concentrations of ions, particularly K^+. One of the first mathematical models proposed to explain the resting membrane potential of cells was the Bernstein model, which argued that cells at rest are permeable to K^+ only. Thus, the cell's resting potential is simply the K^+ *Nernst potential*, V_K (also known as the K+ equilibrium potential). This model predicts that a resting cell should follow the V_K as we manipulate $C_K{}^o$ or $C_K{}^i$.

Another model that has been considered is a model in which several ions are in equilibrium simultaneously. This condition, known as Gibbs-Donnan equilibrium, requires a very specific relationship among ratios of internal and external concentrations and is not seen in practice. The Gibbs-Donnan equilibrium is also problematic in that it leads to osmotic imbalances.

Real cells exist in a multi-ion steady-state, non-equilibrium condition, implying that while all partial derivatives with respect to time = 0. his condition was first handled by Goldman, Hodgkin, and Katz (GHK). They derived the steady-state, non-equilibrium value of membrane potential V_m under two assumptions: (1) the assumption of a semi-permeable membrane and (2) a constant electrical field (implying a linear change in V_m) across the membrane. The GHK equation, also called the constant-field equation, looks like this for a system including Na^+, K^+, and Cl^-.

13.9 Key words

Donnan membrane equilibrium; Biological significance; Micelles; Mixed micelles; Bile salts; Lipid digestion; Diffusion of solution across biomembrane; Respiratory exchange of O_2 and CO_2

13.10 References for further studies

1) Textbook of Biophysical Chemistry; U N Dash; *Macmillan*, **2006**.

2) Biophysical Chemistry; Satake & Iqbal; *Discovery Publishing House*, **1997**.

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4) Biophysical Chemistry; Alan Cooper; *Royal Society of Chemistry*, **2011**.

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13.11Questions for self understanding

1) What is Donnan membrane equilibrium?

2) Discus the biological significance of Donnan membrane equilibrium

3) What are Micelles? How they are formed?

4) Explain the effect of solute or substance on micellization

5) Discuss the formation of mixed micelles between bile salts and products of lipid digestion

6) Discuss the diffusion of solution across biomembrane and mechanism of application in the respiratory exchange of O_2 and CO_2

UNIT-14

Structure

14.0 Objectives of the unit

After studying this unit you are able to

- Explain the procedure of precipitation of Colloids
- Discuss the salting in and salting out of proteins
- Explain the Effect of pH on salting in and salting out of proteins
- Write the Hofmeister series
- Explain the Osmotic behavior of cells and significance of somotic in biology osmoregulation
- Discuss the Significance of σ"
- Explain the mechanism of controlling osmo regulation

14.1 Introduction

Proteins are the most abundant biological macromolecules occurring in all cells and all parts of cells. Our understanding of protein structure and function has been derived from the study of many individual proteins. To study a protein in detail, the researcher must be able to separate it from other proteins and must have the techniques to determine its properties. The necessary method comes from protein chemistry, a discipline as old as biochemistry itself and one that retains a central position in biochemical research. A pure preparation is essential before a protein's properties and activities can be determined. Given that cells contain thousands of different kinds of proteins, how can one protein are purified? Methods for separating proteins take advantage of properties that vary from one protein to the next. The source of a protein is generally tissues or microbial cells. The first step in any protein purification procedure is to break open these cells, releasin their proteins into a solution called a crude extract. Once the extract is ready, various methods are available for purifying one or more of the protein it contains. Commonly, the extract is subjected to treatments that separate the proteins into different fractions based on a property such as size or charge, a process referred to as fractionation. Early fractionation steps in purification utilize differences in protein solubility.

Because a protein contains multiple charged groups, its solubility depends on the concentrations of dissolved salts, the polarity of the solvent, the ph, and the temperature. Some or all of these variables can be manipulated to selectively precipitate certain proteins while others remain soluble. The solubility of a protein at low ion concentrations increases as salt is added, a phenomenon called "salting in". The additional ions shield the protein's multiple ionic charges,

thereby weakening the attractive forces between individual protein molecules (such forces can lead to aggregation and precipitation). However, as more salt is added, particularly with sulfate salts, the solubility of protein again decreases.This "salting out" effect is primarily a result of the competition between the added salt ions and the other dissolved solutes (protein molecules) for molecules of solvent (water). At very high salt concentrations, so many of the added ions are solvated that there is significantly less bulk solvent available to dissolve other substances, including proteins.

14.2 Precipitation of Colloids

Precipitation of colloids depends on

1. Change on the colloidal particle
2. The degree of salvation.

and the stability of colloids depends with whether they are lyophobic or lyophilic

Addition of electrolyte to the system and mixed thoroughly after each addition it is observed that at a particular concentration of electrolytes rapid aggregation takes place and formation of co agulate (precipitate) or flocculate takes places. The volume or concentration of electrolyte to flocculate referred as "flocculation volume" of the colloidal system.

Hydrophobic collides easily flocculated (less stable) i.e Flocculated volume is less for lyophobic than lyophilic.

In hydrophobic it is due to Brownian and congregation moment, the particles come close to each other so that flocculation is fast. Higher valency of ion increases the flocculation. But in the lyophilic system particles have a very strong attraction for the solvent therefore as long as particle remains solvated neutralization of the charges by added electrolytes will not bring about precipitation. "dehydration will convert the lyophilic collides to lyophobic (suspended colloids) then if salt is added, flocculation become (emulsion) fast

This property has great biological significance as our physiological colloidal dispersions are of the lyophilic type. *Therefore living matters can tolerate slight changes of salt concentration i.e inorganic ions without dissociates as lyophilic colloids requires more salt for precipitation. This is called selting out process and depends with nature of the ions (cation, annion, valency etc). Depending on the dehydration of the particles, the ions are arranged in series called "Hofmeister series"* addition of large amounts of electrolytes causes dehydration by flocculation (irreversible colloids) and reversible reaction is not possible.

14.3 Salting in and salting Out of proteins

Proteins molecules are changed species and when they placed in a solvent either attract or repeal with each other depending up on the number of changes on the molecules. In aqueous system (i.e in H_2O) the electrostatic forces of attraction between the charged groups of protein molecules is decreased because of high dielectric constant of H_2O and interaction between polar groups and water molecules increases and stability

Therefore it is seen that the solubility of protein increases by the addition of low salt concentration and increase in logarithm of solubility i.e

$\log s \propto \mu$ in the ionic system (given by Lewis and Randall)

Here $\mu = \left[\Sigma \frac{n_i Z_i^2}{2} \right]^{y_2} \text{----------}(1)$

μ = ionic strength

N_i= molal concentration

Z_i = valency of all the ions in solution

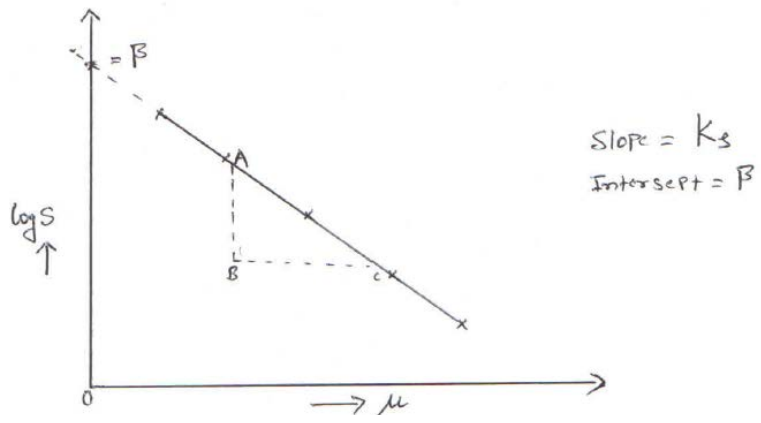
When concentration is low, because of solvent molecules strength and added salt ion acts as a shield (electrical double layer formation) it presents the attraction of protein molecules and increases the repulsion, so that the solubility increases and precipitation decreases.

But most of the proteins at higher salt concentration the logs decreases and is given by

$\log s = \beta - k_s \mu \text{----------}(2)$

Here k_s= salting out constant

β = characteristic constant, it depends with nature of proteins, varies with temperature and pressure of the system



Here the slat ions interact with enormous solvent molecules and decrease the interaction of polar groups of proteins with H_2O molecules. Hence the protein molecules becomes solvated and therefore flocculation becomes increased.

This process of formation of precipitation or aggregation of proteins molecules is called " salting out" process

This salting out process finding application in separation of proteins

This process depends with

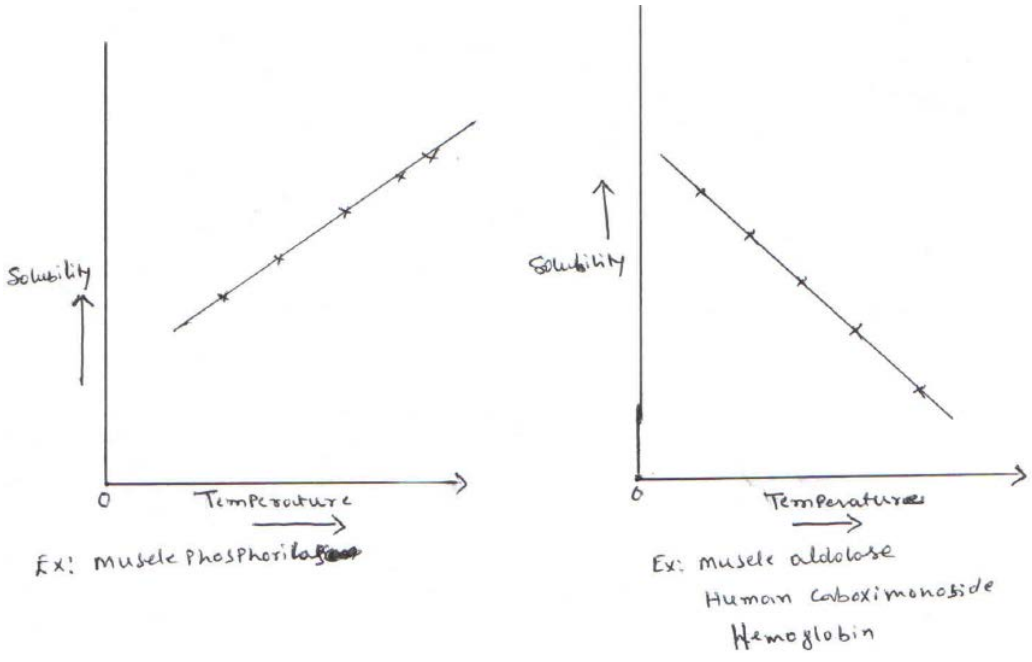
1. pH
2. Temperature
3. The nature of salt added.

14.4 Effect of pH

Proteins exist as cations or anions on either side of the isoelectric point. But at isoelectric point proteins is least soluble hence the pH of the minimum solubility is isoelectric point pH. Therefore it is necessary to use suitable buffers for setting required pH in the purification or separation of the proteins.

14.5 Effect of temperature

Temperature increases the solubility for some proteins and decreases the solubility for some proteins



Therefore sometimes –ve temperature coefficient finds application in getting proteins crystal by gradually increasing the temperature of the cold solution of proteins.

14.6 Types of slat

ks depends on which type of salt used for particular protein, ie, depends with extent of ion hydration i.e the interaction of ions and solvent molecules which depends with size and change of the ion (more hydration of ion increases the protein precipitation)

14.7 Degree of hydration (Hofmeister series)

Hofmeister discovered ion specific effects on precipitation of purified egg white. According to the efficiency of the different ions, they can be ordered reproducibly. This is known as the Hofmeister series. The effects of ions on biological and chemical processes in solution usually depend on the particular ions involved. These specific ion effects make up the Hofmeister phenomena. The Hofmeister series

Anions > cations

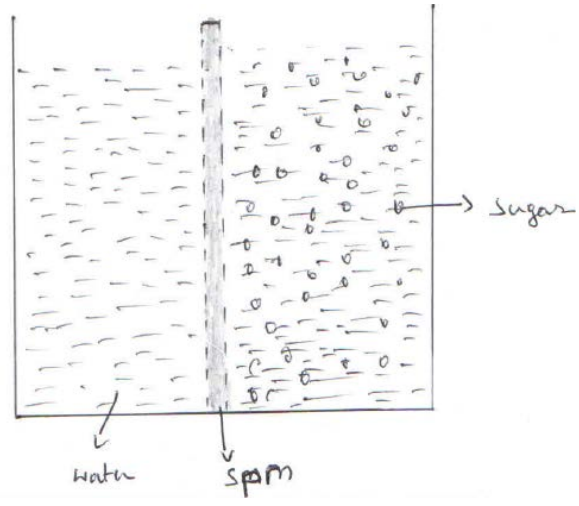
Anions : citrate> tartarate > SO_4> ClO_2> NO_3> I > Cl

Cations: Ti> Al> H> Ba> Sr> Ca> K> Na> Li

14.8 Osmotic behavior of cells and significance of osmotic in biology osmoregulation

We have observed "biological membrane" are perfect selective permeable membrane because they allow only the solvent and not solute molecules. However due to small pore size, even through the moment of the solute molecules are not diffused, some time it is observed very slow diffusion of low molecular weight solutes.

Let us consider a vessel filled with H_2O and divided in to two compartments A and B. A semi permeable membrane it a solute like sugar is added to B then H_2O will flow from A to B at the same time diffusion of sugar molecules from B to A at a very slow rate. Therefore the effective osmotic pressure obtained is less than the osmotic pressure calculated from the solute concentration.



i.e $\pi = RT.\Delta C$

ΔC= solvent concentration

As concentration of sugar decreases due to its moment from B to A

Then the effective osmotic pressure $\pi_{eff} = \sigma RT\Delta C ----------(1)$

σ = staverman's reflection co-efficients depends with nature of semi permeable and solute

If A_{solute}= effective fraction of area available on the membrane for diffraction of solute molecules.

$A_{Solvent}$= effective fraction of area available on the membrane for diffusion of solvent molecules.

$$Then\,\sigma = 1 - \frac{A_{solute}}{A_{solvnet}} ----------(2)$$

i.e semipermeable membrane not allow solute molecules then

$A_{solute} \rightarrow 0$

$\therefore \sigma > 1$

$\pi = RT.\Delta C$

The significance of σ is better understood when we study the osmotic behavior of living cells. Living cells have selectively permeable membranes i.e permeable to water and some low mol.wt solutes but not to other substances. Under these conditions the fraction o fthe total osmotic pressure due to solutes which are retained is termed as "tonicity" *[tonicity is also defined as a state of normal tension of the tissues by virtue of which the parts are kept in shape, alert, and ready to function in response to a suitable stimulus]*

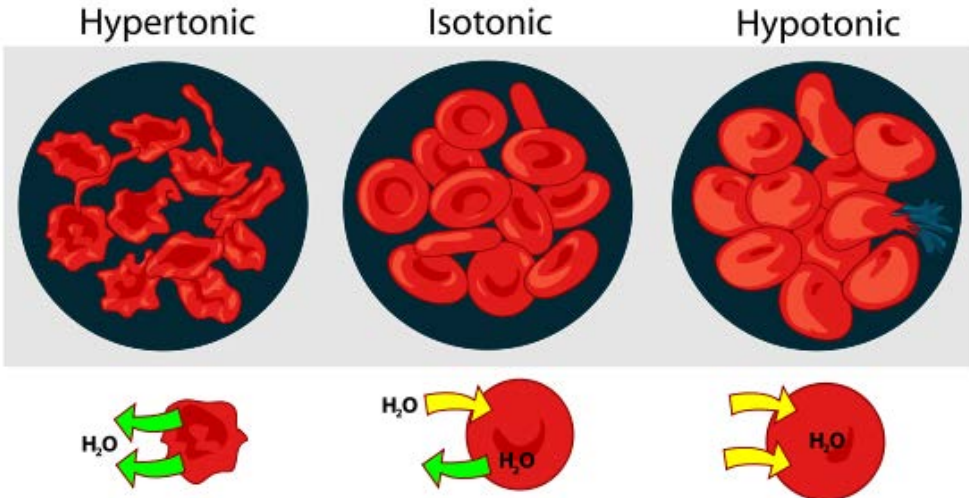
When the cells are placed in a medium

Osmotic pressure of medium = cell content inside

Then the cell size remains same and there is no unidirection flow of solvent. Then the medium is said to "isotonic" medium

If medium is more concentrated i.e osmotic pressure > cell interior flow from the cell resulting in the shrinkage of the cell then the solution is said to be "Hypertonic"

If the medium is diluted i.e osmotic pressure < cell interior then the cell absorbs water from outside and increase in size of the cell (swelling of the cell) then the medium is called Hypotonic ex- kidney become healing if more outside water is flow inside, the extent of swelling or decrease in size depends with the cell contents and surrounding fluid.



Toxicity of solution expresses the response or function of cells when immersed in solutions. In osmometry the concentration always expressed in molal (solute in definite weight of solvent) concentration which is independent of temperature while moles, concentration is dependents of temperature.

Therefore concentration of solutes in these solutions is expressed by "osmolality" and the unit is "osmoles". For the pressure of the body fluid since it is low it is convenient to use smaller unit i.e millisomol i.e mosm.

i.e $mosm = \frac{1}{10^3}.osm$

millisomol = solution of 1 millimole of non-electrolite/ liter of water

ex: one mol of glucose (non ionzable molecules) = 1 osmole

But one mole of NaCl = 2 osmole (because it ionize to Na^+ and Cl^- and produce 2 osmoler)

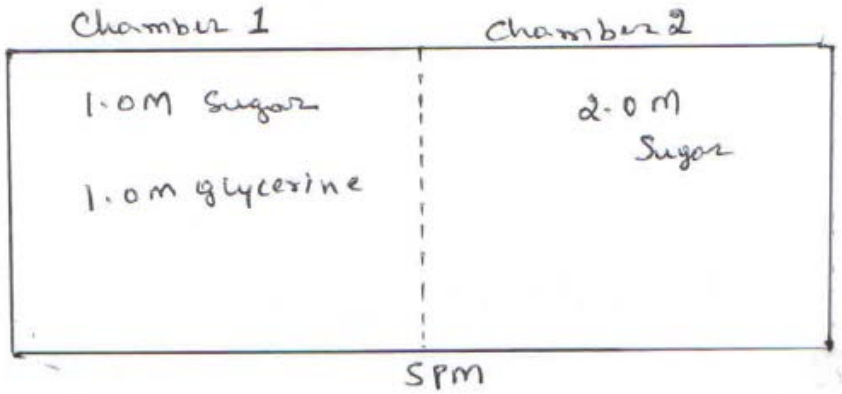
$CaCl_2$= 3 osmoler (ionizes to Ca^+, Cl^-, Cl^- produce 3 osmoles)

14.9 Significance of σ"

The osmolosity of the Erythrocytes is ≈280mm when erythrocytes (red blood cell) is suspended in 280mm manitol(medium) no change in cell size is observed because σ≈1 for manitol so that 280mm manitol concentration acts as isotonic for red blood cells.. But if it is suspended in

280mm glycerol the swelling of cells takes places i.e glycerol hypotonic to the same cells then to set isotonic condition. 318mm of glycerol is necessary because the σ value of glycerol is ≈ 0.88 similarly for malonamide solution σ≈0.83 then 337 mm required for isotonic condition for red blood cells.

The value of σ is essential or play an important role in preparation of isotonic solutions for living cells. Also it is observed the term isotonic, iso-osmotic and hyposmotic and hypotonic and hyper osmotic and hypertonic is not same.

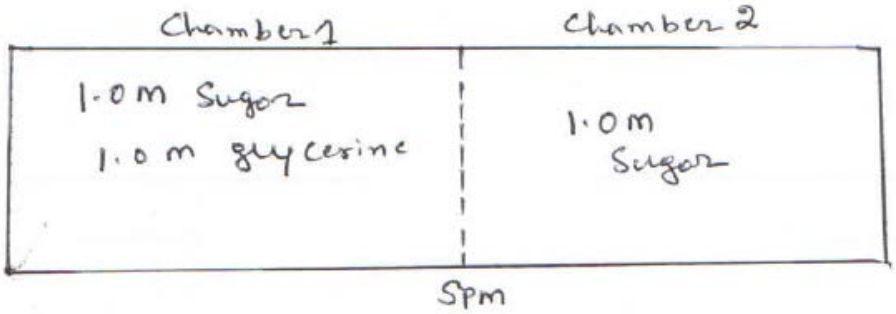


1.Chamber 1 is isosmotic with chamber 2

2.Chamber 1 is hypotonic to chamber 2

∴ direction of osmotic is from chamber 1 to chamber 2

Case (2)



1. Chamber 1 is hyper osmotic to chamber 2
2. Chamber 1 and chamber 2 are isotonic to each other ∴ no osmosis

Ex. Consider a two chambers as shows in figure. The semi permeable membrane allows solvent and Glycine but not sucrose. Then H_2O diffuse from chamber 1 to 2 in case (1) and osmosis take place.

But in case (2) no net transfer of H_2O molecules between the chambers therefore the selectivity of permeable membrane plays an important role in regulating osmosis.

Therefore in a normal body any stress to the homeostatic mechanism occurs as a result of injury, diseases, infection and electrolyte imbalance areise which the causes the problem in osmotic pressure which burns in the size of the cells (which is dangerous to life)

Hence "osmo regulation" is the ability of an organism to maintain both the electrolyte content and fluid content of its cells at a constant level.

"osmorgulatory capacity " is the capacity of species for its survival and express the degree of an organism to that extent it can tolerate charges in its external environment i.e it is ability of a species to maintain a content internal environment against a continuously changing external environment.

Osmo regulation can be controlled by following mechanism

1. Regulation of water entry into the living body by a charge in behavior or by a change in the permeability of external surface such as skin.
2. Regulation of water loss from the body by changes in kidney or by an alteration in the of the external surface.
3. Regulation of the rate of excretion of electrolytes by use systems
4. Regulation of the rate of excreation or entro electrolyte througes the external organ like skin and lungs.

By an alteration of the osmotic pressure of body fluids brought about by a change in the concentration of physiologically inactive solvents (water intake is controlled by " Thirst" mechanism i.e living being thirst whenever H_2O is needed

Examples

Organism living in the sea (sharks, skates, lay's) they maintain iso-smosity with their environment by retaining inert solutes like urea and trimethylamineoxide(TMAO). These compounds are relatively inert non-toxic and relatively diffusible, also retention of this is possible even up to 0.5m

Similarly in the desert, the insects living in deserts develop waxy substance on skin surface to prevent water loss. The toads have thick skin to prevent water loss.

The habits

"burrowing and nocturnal (night working) activity allow them to avoid the heat and aridity (dryness) of the desert.

The food habits

Desert animals use plant seeds and food materials which is high in "fat content" as oxidation of 1 gm fat gives 1.07g, H_2O while proteins and carbohydrates gives 0.4gm and 0.6gm of water after oxidation.

In human beings

The principle cation in extracellular fluid is Na^+. Sodium levels and fluid balance are always closely related. It affects the osmotic shifts and blood volume. The most important hormone secreted by the adrenal glands for salt balanced is "Aldosterone" this hormone facilitated the re-suspection of Na and Cl from sweat and saliva and also can increase in sodium reception in the gut. A sense of this hormone causes large extraction of Na by the kidney which triggers the secretion of aldosterone. Increase KCl decreased in cardiac output is occurs.

14.10 Summary of the unit

Osmoregulation is the process by which the body regulated the osmotic pressure of any organisms' fluids in order to keep the homeostasis of the organisms' water level constant. Therefore osmoregulation is used to keep the bodily fluid from being too diluted or too concentrated. An osmotic pressure is used to measure the ability of water to move from one solution to another solution through osmosis. *Osmotic pressure refers to tendency for a liquid solution to diffuse therefore moving from a lower to higher concentration across a membrane*. As it is critical for humans to maintain a regulated osmotic pressure they are able to gain an isotonic solution which would mean that there is no total loss or gain of fluids or salts into the cells of the body. Rather there is a steady and constant flow of substances in and out of the cell membrane.

There are a few types of osmoregulation; these are called osmoconformers and osmoregulators. In the body osmoconformers try to match the body osmolality to that of the environment the body is situated in. osmoregulators on the other hand regulate the osmolality of their body, therefore keeping the osmolality constant. In humans the kidney plays a huge part in the osmoregulation of the body's internal environment. Regulation of water in the human body is carried out through the excretion of waste urine from the body. Hormones which include: the

antidiuretic hormone, aldosterone and angiotensin II are used in the body to help to increase the permeability of thecollecting ducts found in the kidney. This further allows diffusing to occur easily, it also allows the kidney to be able to reabsorb water and prevent it from being excreted. Humans are also able to regulate by controlling the total amount of water that is passed out of the body through urine waste or sweat, this is carried out with the help of the excretory system.

To prevent the loss or the gain of water from cell in the body, the water potential of the blood is regulated to suit the individual; this is controlled by the hypothalamus. The hypothalamus is able to notice changes in the water potential off the blood that passes through the brain. This is process is carried out by the osmoreceptors cells in the hypothalamus. As a response to the change in water potential the hypothalamus controls the urge for thirds and further secrets a hormone called the antidiuretic hormone. The antidiuretic hormone which is stored in the pituitary gland mainly targets cells that are endothelial cells of the collecting ducts in the kidney nephrons. These cells are unlike normal cells because they only allow water molecules to pass through their cell membrane through the water aquaporins rather than through the lipid bilayer like normal cells would. This hormone makes the water channels to open and allow water molecules flow.

The body uses several processes to remove bodily waste from the system of an individual; one of these processes includes the metabolic process. This process is used to keep the level of salts and other substances that dissolve in the blood solution of the body at a constant; this is done by the removal of waste from the system of the organism. This process therefore encompasses the cells of an organism in an isotonic solution to allow maintenance of health in the cell.

There are two common ways in which the human body constantly shows osmoregulation in the body, this is through dehydration and waterlogging. In the process of dehydration the hypothalamus is sent a signal that the water level in the body are critically low, the hypothalamus then sends a signal to pituitary glands where the antidiuretic hormone is secreted for further use. This hormone is the sent to the kidney and causes it to reabsorb water from the body, where the net amount of antidiuretic hormone determines the amount of water absorbed by the kidneys. As the fluid in the body become concentrated it would be noticed in the colour of the urine passed by the individual, the kidneys however do not stop absorbing water until the pituitary glands send a signal to reduce the production of the hormone. When the reduction is sensed in the body,

another form of homeostasis called the negative feedback system occurs to take the body back to the desired set point.

Water logging on the other hand is the exact opposite of dehydration, in this case, the hypothalamus is signaled the level of water in the bodily fluids is too high, a signal is then sent to the pituary gland, which stops the production and secretion of antidiuretic hormones making the kidney cease the reabsorption of water from the blood. The cells in the body become waterlogged causing them to burst increasing the concentration of the blood. The hypothalamus signals the pituiry gland to end to secretion of the hormone, this is also carried out by the negative feedback mechanism.

14.11 Key words

Precipitation of Colloids; Salting in and salting Out of proteins; Degree of hydration (Hofmeister series); Osmotic behavior of cells; significance of Osomotic behavior in biology; Osmoregulation.

14.12 References for further studies

1) Textbook of Biophysical Chemistry; U N Dash; *Macmillan*, **2006**.

2) Biophysical Chemistry; Satake & Iqbal; *Discovery Publishing House*, **1997**.

3) Biophysical Chemistry; James P. Allen; *John Wiley & Sons*, **2009**.

4) Biophysical Chemistry; Alan Cooper; *Royal Society of Chemistry*, **2011**.

5) Biophysics & Biophysical Chemistry; D. Das; *Academic Publishers*, **1982**.

14.13 Questions for self understanding

1) Write a note on precipitation of Colloids

2) Discuss the process of salting in and salting out of proteins

3) Explain the effect of pH on salting in and salting out of proteins

4) Explain the effect of temperature on salting in and salting out of proteins

5) Discuss the influence of types of slats salting in and salting out of proteins

6) What is Hofmeister series?

7) Explain the osmotic behavior of cells

8) What is osmoregulation?

9) Discuss the significance of osmotic in biology osmoregulation

10) Explain the significance of σ"

11) How can Osmo regulation be controlled?

UNIT-15

Structure

15.0 Objectives of the unit

After studying this unit you are able to

Explain the effect of temperature on viscosity

Explain the effect of pH on viscosity of the liquid

Explain the significance of viscosity in biological systems (Mechanism of muscular contraction)

Explain the method of dDetermination of Intrastrand Disulfide bonds in proteins

Explain the polymerization of DNA

Explain the effect of viscosity on nature of blood flow through different vessel

15.1 Introduction

Viscosity is an internal property of a fluid that offers resistance to flow. For example, pushing a spoon with a small force moves it easily through a bowl of water, but the same force moves mashed potatoes very slowly. In fact, one of the major differences between styles of mashed potatoes is the viscosity of the starchy mass.

15.2 Effect of temperature on viscosity

The variation of the viscosity of a liquid with temperature is given by

$$n = A.e^{E/RT}$$

or

$$\log n = \frac{A}{T} + E$$

Here

n = viscosity the

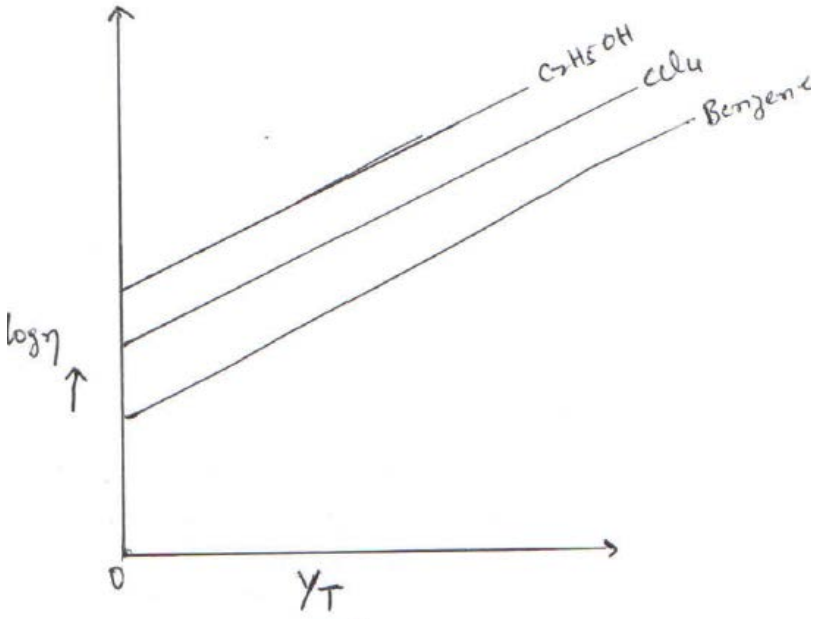
a = constant depends with molecular weight and nature of liquid.

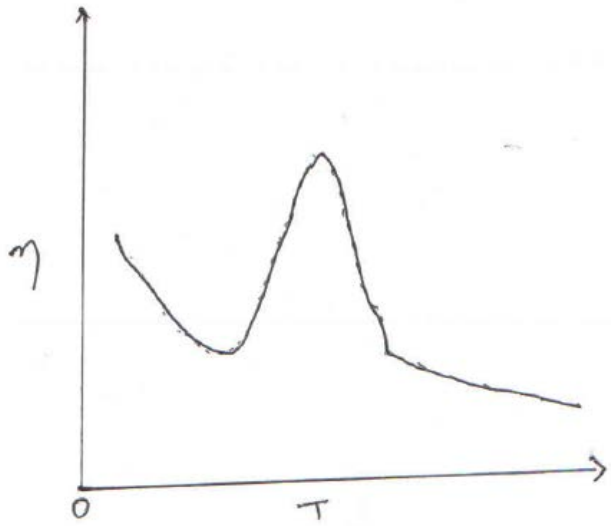
E = Energy of activation per mole

A plot of log n v/s 1/T gives a straight line

The increase of temperature increases the molecular activity in the liquid as a result the liquid now faces lesser resistance to its flow and hence liquid has less viscosity.

Ex: for albumins solution





15.2 Effect of pH on viscosity of the liquid

The protein molecules [macromolecules] are charged molecules includes opposite charges in the surrounding solvent. The electrical double layer formed moves along with the particles resulting in an increase its friction, hence increase in the viscosity. *This is called electro viscosity effect*, given by Krasny- Ergen equation.

$$n_r = 1 + 2.3\phi\left[1 + \frac{3}{2}\frac{1}{kn_o r^2}\frac{D[\xi]_\xi^2}{2\pi}\right]$$

n_r = viscosity of the solution

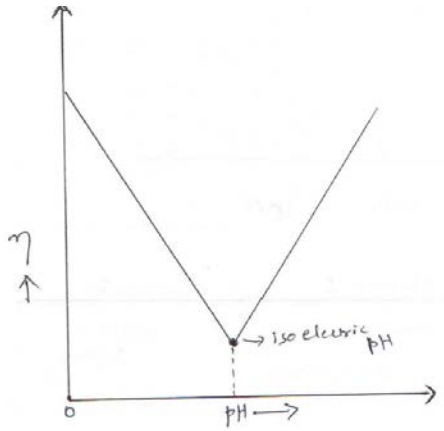
n_0 = viscosity of the solvent

D = dielectric constant

ξ = zeta potential

K = specific contance of the medium.

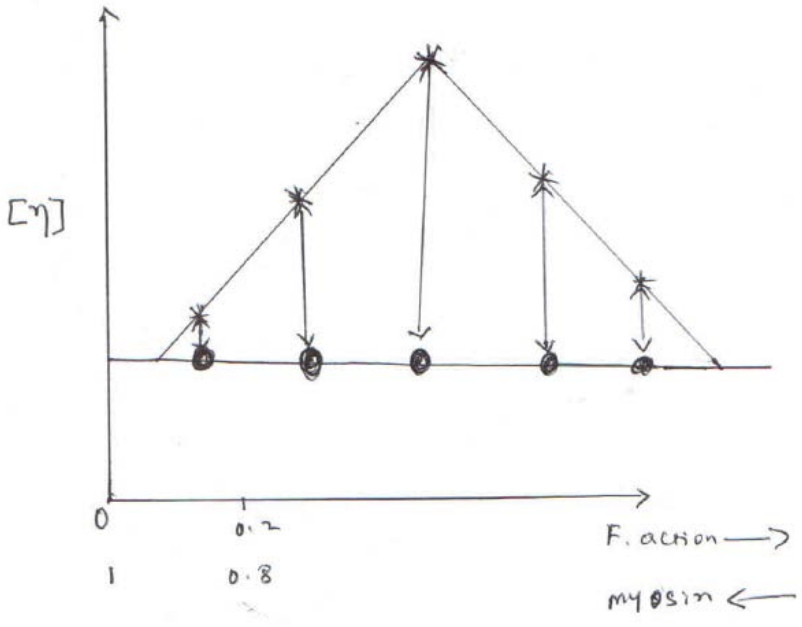
It is observed that at isoelectric pH (net charge is zero), the molecules contracts and decrease in viscosity. Above or below isoelectric pH there is excess of –Ve or +ve charges and the molecule expands so that he viscosity increases.



15.3 Significance of viscosity in biological systems (Mechanism of muscular contraction)

The interaction of actin and myosin controls the mechanism of muscle contraction. This relationship between actin and myosin and ATP was explain with the help of viscosity studies.

When actin and Myosin are mixed the viscosity of the solution rises. Addition of ATP makes the viscosity drop.



Actin+Myosin→Actomysin (X)

$$\text{Actomysin+ATP} \rightarrow \text{Actin+ Myosin (0)}$$

Therefore the interaction between Action myosin and ATP depends with the viscosity changes, which controls the muscle contraction.

15.4 Determination of Intrastrand Disulfide bonds in proteins

The existence of intrastrand disulphide bonds in proteins can be known from viscosity studies.

Polypeptides when disulphide bonds are present will not give much change in viscosity if treated with 6M of gnanidium chloride, but if such bonds are broken the compactness decreases and up on addition of 6M guanidinuim chloride protein will become random coil shape so that viscosity will increase.

Ex. Calf brainfubulin has [n]=36.0 ml/gm is presence of disulfide bonds.

Addition of β-mercaptorthanol can reduce the disulfide bridge into individual sulfhydryl groups then [n] increases to 44.o ml/gm .hence no change in viscosity indicates the presence of intrastand disulfide bonds in proteins.

15.5 Polymerization of DNA

DNA polymerization can be studied by viscosity study. When DNA polymerization occurs, the chain length of DNA increases and hence the viscosity increases.

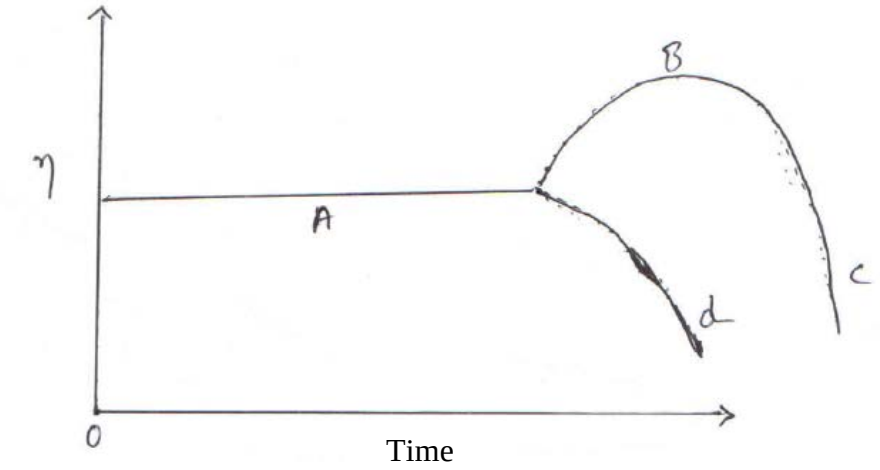
Ex. DNA, with all four 5`triphosphate nucleotides and Mg^{+}, undergo polumerization and increases the viscosity. But for absent of any one nucleotide or Mg^{+} polymerization is absent and viscosity not changes there no change in viscosity indicates absence of polymerization of DNA.

Circular DNA molecules

Circular DNA molecules + Nucleotides $\xrightarrow[break]{Single\ strand}$ no changes in configuration hence no change in viscosity

Circular DNA molecules + Nucleotides $\xrightarrow[break]{Doublestrand}$ Linear DNA For this increase in viscosity is observed

Circular DNA molecules + Nucleotides $\xrightarrow[break]{Multiplestrand}$ Multiple small fragments. Then viscosity once again decreases. Hence viscosity of circular DNA increases and then decreases shown ion figures.



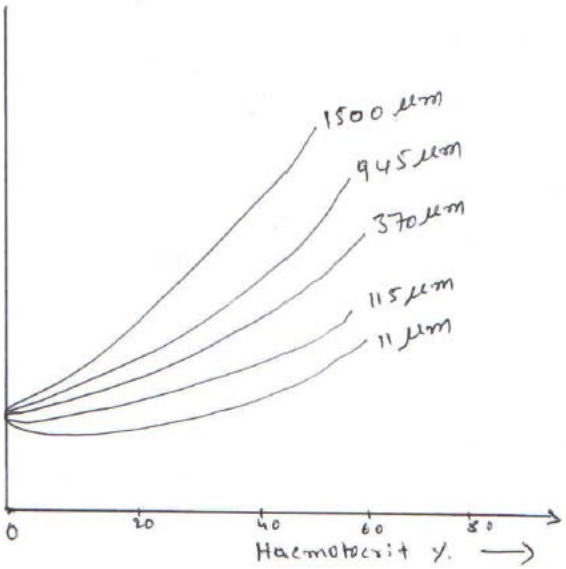
A: circular DNA.

B: Linear DNA

C and D: multiple fragments of Linear DNA.

Therefore viscosity studies explain the extent of polymerization in DNA molecules.

A plot of n of erythrocyte v/s Haemotoesit palys for different diameters of vessel is shown below.



For smaller diameter vessel the curve is flattened but for larger vessel the [n] is high. The red blood cells are biconcave with flexible membranes and become of this property the cells undergo extreme deformation (arrow head shape) with increasing of shearing stress which facilitate to flow in capillaries with smaller diameter. This process is known as axial migration as the shearing stress in these vessels increases the red cells themselves obliquely along the tube axis.

Therefore we observe viscosity of the blood decreases with smaller diameter vessel and increases for longer diameter vessel with change of shearing stress. The apparent viscosity for a man in terms of haematocnt value is about 45%. In this way variation of viscosity of blood influence the working of Heart

15.6 Effect of viscosity on nature of blood flow through different vessel

Viscosity plays an important role in circulation of blood in human being. The blood is highly viscous and is affected by even a small change in the viscosity of the medium. The blood flow varies inversely with the viscosity of the blood. This viscosity depends on the haematocrit value i.e the % of blood occupied by erythrocytes one of the major components of the blood.

For larger vessel like aorta and veins : haematocrit value is high

For capillaries and small veins: haematorit value is very low.

because rate of flow of blood is different in different vessels.

Blood due to the presence of blood cells behaves as a Newtorian fluid (viscosity independent of stress) then

$$\eta = \frac{\tau}{\gamma}$$

η = viscosity , γ = rate of shear. τ = shearing stress

15.7 Summary of the unit

The protein molecules [macromolecules] are charged molecules includes opposite charges in the surrounding solvent. The electrical double layer formed moves along with the particles resulting in an increase its friction, hence increase in the viscosity. *This is called electro viscosity effect*, given by Krasny- Ergen equation. The interaction of actin and myosin controls the mechanism of muscle contraction. This relationship between actin and myosin and ATP was explain with the help of viscosity studies. When actin and Myosin are mixed the viscosity of the solution rises. Addition of ATP makes the viscosity drop. The existence of intrastrand disulphide bonds in proteins can be known from viscosity studies.

Polypeptides when disulphide bonds are present will not give much change in viscosity if treated with 6M of gnanidium chloride, but if such bonds are broken the compactness decreases and up on addition of 6M guanidinuim chloride protein will become random coil shape so that viscosity will increase. DNA polymerization can be studied by viscosity study. When DNA polymerization occurs, the chain length of DNA increases and hence the viscosity increases.

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15.8 Key words

Effect of temperature on viscosity; Effect oF p^H on viscosity of the liquid; Significance of viscosity in biological systems; Intrastrand Disulfide bonds in proteins; Polymerization of DNA; Blood flow.

15.9 References for further studies

1) Textbook of Biophysical Chemistry; U N Dash; *Macmillan*, **2006**.

2) Biophysical Chemistry; Satake & Iqbal; *Discovery Publishing House*, **1997**.

3) Biophysical Chemistry; James P. Allen; *John Wiley & Sons*, **2009**.

4) Biophysical Chemistry; Alan Cooper; *Royal Society of Chemistry*, **2011**.

5) Biophysics & Biophysical Chemistry; D. Das; *Academic Publishers*, **1982**.

15.10 Questions for self under standing

1) What is viscosity?

2) Discuss the effect of temperature on viscosity

3) Explain the effect of pH on viscosity of the liquid

4) Discuss the significance of viscosity in biological systems (Mechanism of muscular contraction)

5) How Intrastrand disulfide bonds in proteins can be determined using viscosity measurement?

6) How polymerization of DNA can be identified using viscosity measurement?

7) Discuss effect of viscosity on nature of blood flow through different vessel.

UNIT-16

Structure

16.0 Objectives of the unit

After studying this unit you are able to

- Explain the effect of temperature on surface tension (γ)
- Explain the effect of solute on the surface tension
- Discuss the biological significance of surface tension (Stability of Alveoli in lungs)
- Explain the Interfacial Tension and (Danielli and Davson model)
- Discuss the Application of sedimentation velocity and sedimentation equilibrium method for molecular weight determination of proteins

16.1 Introduction

Surface tension is measured as the energy required increasing the surface area of a liquid by a unit of area. The surface tension of a liquid results from an imbalance of intermolecular attractive forces, the cohesive forces between molecules:

A molecule in the bulk liquid experiences cohesive forces with other molecules in all directions.

A molecule at the surface of a liquid experiences only net inward cohesive forces.

A microscopic view of water illustrates the difference between molecules at the surface of a liquid and water molecules within a liquid.

Forces of attraction between a liquid and a solid surface are called adhesive forces. The difference in strength between cohesive forces and adhesive forces determine the behavior of a liquid in contact with a solid surface.

Water does not wet waxed surfaces because the cohesive forces within the drops are stronger than the adhesive forces between the drops and the wax.

Water wets glass and spreads out on it because the adhesive forces between the liquid and the glass are stronger than the cohesive forces within the water.

Capillary action is the rise of a liquid that wets a tube up the inside of a small diameter tube (i.e., a capillary) immersed in the liquid.

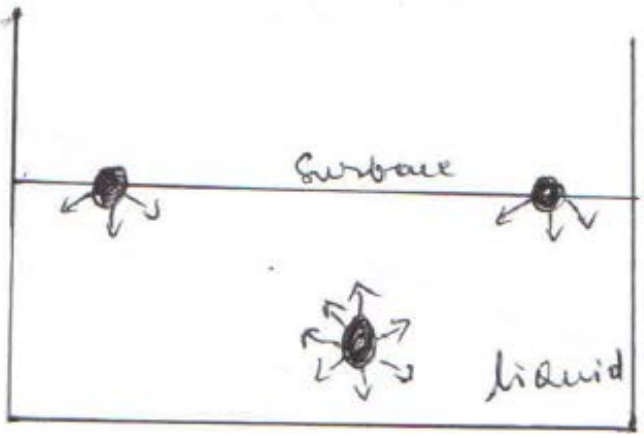
The liquid creeps up the inside of the tube (as a result of adhesive forces between the liquid and the inner walls of the tube) until the adhesive and cohesive forces of the liquid are balanced by the weight of the liquid. The smaller the diameter of the tube, the higher the liquid rises.

16.2 Surface tension

It is the force in dynes acting along the surface at right angle (90^0) to only line 1cm in length. Also it can be defined as *the special strain that the surface layer experience as a consequence of the unbalanced forces is called surface tension.*

The relationship between surface tension, surface area and the surface free energy is given by

Surface tension X Total surface Area = surface energy



At the surface the molecules are only partially surrounded by other molecules as a result the molecules experiences inward pull. But in the bulk of the liquid (solution) the molecules attract each other with a cohesive force which is uniform with all directions, because they are surrounded by other molecules. Therefore at the surface the concentration of the molecules lesser than bulk of the liquid and the surface contracts until its area is the smaller possible for a given volume of liquid. This results in surface tension

16.3 Effect of temperature on surface tension (γ)

As temperature raises surface tension of a liquid decreases i.e surface tension has a negative temperature coefficient.

The total energy at the surface/sq cm is given by

$$\mu = \gamma - \tau \frac{d\gamma}{d\tau} - - - - - - - (1)$$

Here $\frac{d\gamma}{dT}$ is the rate of change of surface tension with a change of temperature $\left(\frac{d\gamma}{dT}\right)$ is always -ve as γ decreases with increase of temperature

The equation which relates γ with M, ρ and temperature is given by

$$\gamma\left(\frac{M}{\rho-\rho'}\right)^{2/3}=k(t_c-t)----------(2)$$

Where

ρ= density of liquid at temperature t

ρ^1 = density of its vapour at temperature t

M = molecular weight of liquid

T_c = critical temperature

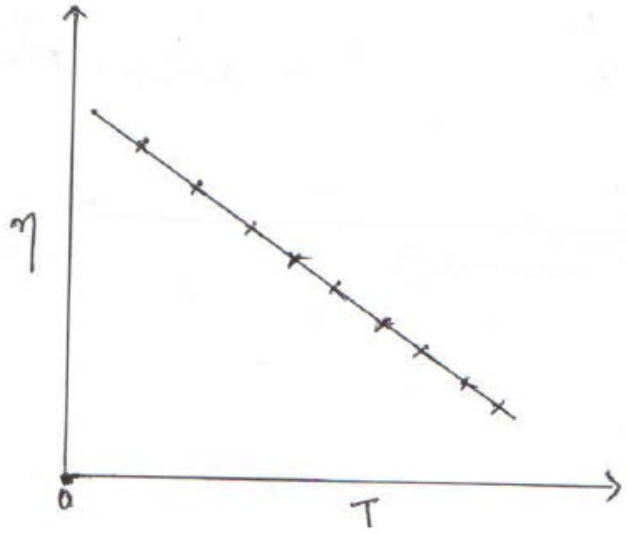
From (2) when t = t_c $\gamma\rightarrow 0$ but γ=0 when t is less than t_c by 6^0C and at a critical temperature (t_c) the γ of normal liquids will become negative

Another equation is given by

$$\frac{M\gamma^{1/4}}{\rho-\rho^1}=Mc=P$$

Here p is called *'parachor' a constant. It is defined as the molar volume of a liquid at a temperature at which the surface tension γ is equal to one.*

The increase of temperature increases the kinetic energy of the molecules. As a result the inward pull by the molecules decreases and hence γ is decreased with temperature.



16.4 Effect of solute on the surface tension

The decrease or increase of surface tension (γ) of a solvent on addition of a solute depends on chemical properties of the solute.

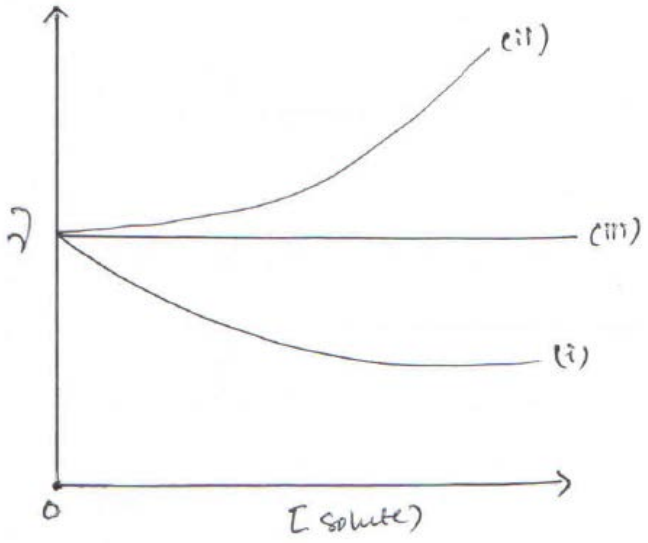
Solutes which decrease γ are called capillary active Ex. Soaps, proteins, alcohols, esters, arrines, ketones etc.

Solutes which increase γ are called capillary inactive Ex. Inorganic salts, salts of organic acids sugar, glucorine etc.

Case i) organic compounds neither typically polar nor completely non-polar, if added to the solvent surface concentrate preferentially at the surface and +vely adsorbed so that γ decreases and referred as capillary active.

Case ii) but inorganic electrolytes (polar) tend to pull water molecules into the interior of the solution i.e –vely adsorbed so that γ increases and referred as capillary inactive

Case iii) for addition of weak electrolytes or non electrolytes gradual and slow decrease of γ effect of solute concentration on γ is show in figure

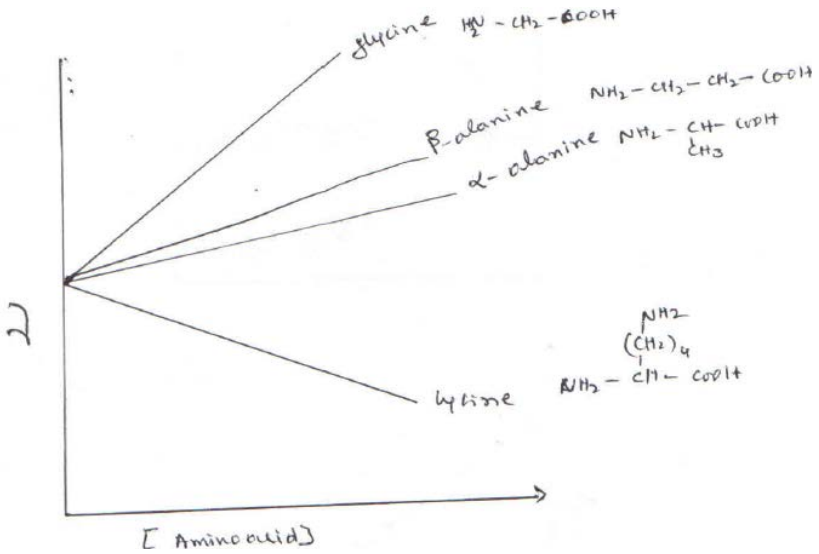


i) Surface active or capillary active solvents (soaps, proteins)

ii) Surface inactive or capillary inactive solutes (ionic salts)

iii)Weak electrolytes or non electrolytes.

Surface tension of H_2O by addition of different amino acids is shown in figure



The decrease or increase of γdepends with whether a given amino acids is capillary active or inactive

16.5 Biological significance of surface tension (Stability of Alveoli in lungs)

Lungs the respiratory organs communicate with the environment via a graduated series of tubes that terminates distally in sac-like structure called Alveoli. The gaseous exchange occurs because of contraction (expiration) and relaxation (inspiration) of these Alveoli.

The presence of surface active material lipids and proteins allows dense packing of the lipid film during respiration thereby reducing the γ generated by the aqueous film. But in the absence of surfactant γ is increased along the alveolar lining which results in the collapse of the lung at the end of each expiration. The collapsed alveoli like collapsed balloons would require greater pressure to inflate the balloon than partially collapsed alveoli and this is responsible for smooth gaseous exchange. Therefore presence of surface that intermediate play important role during inspiration and expiration for the human beings.

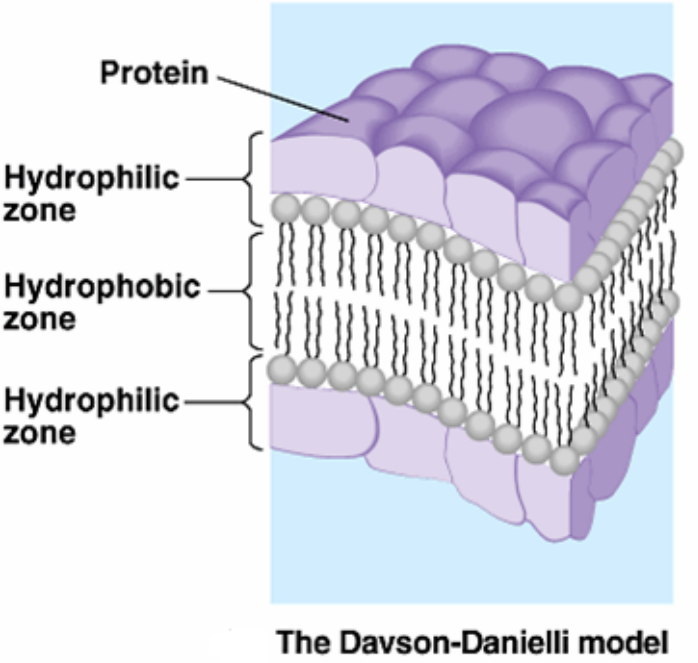
For premature infants the lung is interrupted by insufficient surfactant. Because of this respiratory distress syndrome (RDS) is observed in infants treatment of this compellation may be introduction of high [oxygen] through inspiration by level of surfactant proteins (mixture of phospholipid and surfactant proteins) in the lungs.

16.6 Interfacial Tension and (Danielli and Davson model)

Bio membranes mainly contain proteins and the lipid as among the protoplasmic constituents the proteins and lipids posses the high surface tension decreasing ability. The study of interfacial

tension leads to information about the arrangement of these two types of molecules in the membranes.

Gorther and Greandal proposed a model according to this model the lipids are arranged in two layers one above the other with the hydrophilic polar heads of the lipid molecules arranged externally but the hydrophobic tails face each other in the interior.



The Davson-Danielli model

Further, Danielli and Davson measure interfacial tension for different living cells (0.1 to 0.2 dynes/cm) but for lipid-water interfaces (10 to 15 dynes/cm) which is bigger than living cells. This observation lead Danielli and Davson to propose a model.

Danielli and Davson model comprising of a lipid bilayer with proteins attached to lipid- aqueous interfaces because very small amount of proteins were effective in reducing the surface tension of a model lipid-water system considerably

However present spectroscopy study shows dynamic nature of the cell membrane referred as fluid mosaic model.

16.7 Application of sedimentation velocity and sedimentation equilibrium method for molecular weight determination of proteins

Sedimentation velocity and sedimentation equilibrium method are useful to determine the molecular weight of proteins

1) *Sedimentation velocity method:* in this an object moving in a circle at a steady angular velocity with force experience directed outwards (called centrifugal force is the basis of

centrifugation). In this method the ultracentrifuge is operated at high speeds the randomly distributed particles migrate through the solvent radialy outwards from the centre of rotation. The experiment is carried out by using isoelectric proteins or high salt like 0.1 M KCl. The pH or ionic strength is adjusted to make the protein neutral. Then the molecular weight of the proteins molecule is given by Svedberg equation

$$M = \frac{RT.S}{D(1-ve)}$$

Here S=Sedimentation Coefficient of the molecule

D= Diffusion coefficient of the molecule

$\bar{v}$ =Partial specific volume of the proteins molecules

ρ = density of medium

M= molecular weight of protein molecules

R= gas constant

T= temperature

2) Sedimentaion equilibrium method

This method involves centrifugating a proteins solution at a speed which will exactly balance or equilibrium is set up between the sedimentation and diffusion of the protein molecules so that no net movement occurs any more, under this condition the molecular weight of protein molecule is given by

$$M = \frac{2RT\ln\left(C_2/C_1\right)}{w^2\left(1-\bar{v}e\right)\left(r_1^2-r_2^2\right)}$$

R=gas constant

T= absolute temperature

W= angular velocity

ρ = density of the solvent

$\bar{v}$ = partial specific volume

$C_1$and C_2= concentration of the protein at two distance r_1 and r_2

Significance

Determination of molecular weight by sedimentation velocity and equilibrium method gives information about the shape of the protein molecule. If the particle is spherical shape, the molecular weight would be same from both the methods but if the protein molecule is needle or

cylindrical shape (velocity for spherical molecules> needle shape molecules) then the molecular weight determined form sedimentation velocity method is less than sedimentation equilibrium method. Therefore determination of molecular weight by both the methods is useful to known whichever the protein molecule is spherical or needle shape molecules.

16.9 Problems

1) A protein has a sedimentation co-efficient value of $3.2x10^{-3}$ sec in water its difference co-efficient in water is formed to be $8.2x10^{-7}$ cm^2/sec. both the above values have been corrected for 20^0 C in water the particle specific volume of the protein is 0.735 and the density of water at 20^0 C is 0.9982 determine the weight of the proteins

$$M = \frac{RTs}{D(1-\bar{v}\rho)}$$

($S_{20\ ,w}$ becomes simply s because the values have been corrected for 20^0) R here will have a value of $8.31x10^7$ ergs per mole per degree

Substituting the date provided in the question in the above equation we get

$$M = \frac{8.31\times10^7 \times 293 \times 3.12\times10^{-13}}{8.2\times10^{-\bar{7}} \times (1-0.735\times0.9982)}$$

$$= 34{,}800$$

2) In an experiment carried out at 20^0 the following data was obtained when indigo red dye was subjected to sedimentation equilibrium

Distance from anis to rotation		Relation concentration of longo	
X_2	X_1	C_2	C_1
5.87	5.84	53.6	50.46
5.75	5.72	42.18	39.76
5.66	5.63	35.36	33.36

The intial concentration of dye 0.1g per liter dissolved in 0.1 M Nacl, the density of the sesulting solution being 1.0023. the speed of rotation of the centrifuge mass 17976 rpm. The partial specific volume of cango red is 0.6 from the above data calculate the weight of congo red for each set of distance from anis and relatine concentration.

$$M = \frac{2RT\ln(C_2/C_1)}{w^2(1-\bar{\gamma}\rho)(X_2^2 - X_1^2)}$$

$$R = 8.31X10^{-7}$$

$$(1-\bar{\gamma}\rho) = 0.39862$$

$$\pi = 3.142$$

$$w = 2X3.142X\frac{17966}{60}$$

$$w = 2\pi \times rev/see$$

$$w = 1882.7$$

$$M = \frac{2\times 8.31\times 10^{7}\times 293\times 0.0261\times 2.303\left(X_2^2 - X_1^2\right) = 0.3513}{(1882.7)^2 * (0.39862) * (0.3513)\ln\left(c_2 / c_1\right) = 0.0261\ for\ case 1}$$
$$= 5897$$

Thus for case (1) the molecular weight of cengo red comes to be 5897 ina similar neary for cases (ii) and (iii) the molecular weight was found to be approximately 5930 each.

16.9 Summary of the unit

Surface tension is most prevalent in earth's most important compound, water. "Normal" water at ambient temperature has a high surface tension, but the addition of different chemicals can alter this.

Without surface tension, even the smallest objects would sink underwater. The large particles of dust would not float on the water, but instead the particles would sink to the bottom and kill all of the marine life, causing the collapse of ecosystems.

Water particles at the atomic level help keep the cell membrane from collapsing on itself. Water is a critical component in cytoplasm (the jelly-like substance that fills the cell), this means that the surface tension of water allows the cytoplasm to hold-up the cell membrane. If the pressure is too extreme, then the water breaks down the cell membrane and the cell "drowns". The high surface tension of water is also essential for processes such as water and blood transport in plants and animals respectively. The concave motion of the surface of the water allows it to be "pulled up" by plants from the ground.

Overall, surface tension is a necessary property that contributes tremendously to our lives. I have not even started to explain the relevance that surface tension has with modern technologies (raincoats, car wax, etc.)

16.10 Key words

Effect of temperature on surface tension (γ); Effect of solute on the surface tension; Biological significance of surface tension (Stability of Alveoli in lungs); Interfacial Tension and (Danielli and Davson model)

16.11References for further studies

1) Textbook of Biophysical Chemistry; U N Dash; *Macmillan*, **2006**.

2) Biophysical Chemistry; Satake & Iqbal; *Discovery Publishing House*, **1997**.

3) Biophysical Chemistry; James P. Allen; *John Wiley & Sons*, **2009**.

4) Biophysical Chemistry; Alan Cooper; *Royal Society of Chemistry*, **2011**.

5) Biophysics & Biophysical Chemistry; D. Das; *Academic Publishers*, **1982**.

16.12 Questions for self understanding

1) What is surface tension?

2) Explain the effect of temperature on surface tension (γ)

3) Explain the effect effect of solute on the surface tension

4) Explain the effect Biological significance of surface tension (Stability of Alveoli in lungs)

5) What is Interfacial tension?

6) Explain the Danielli and Davson model

7) Discuss the application of sedimentation velocity and sedimentation equilibrium method for molecular weight determination of proteins

8) Explain the significance sedimentation velocity and sedimentation equilibrium method