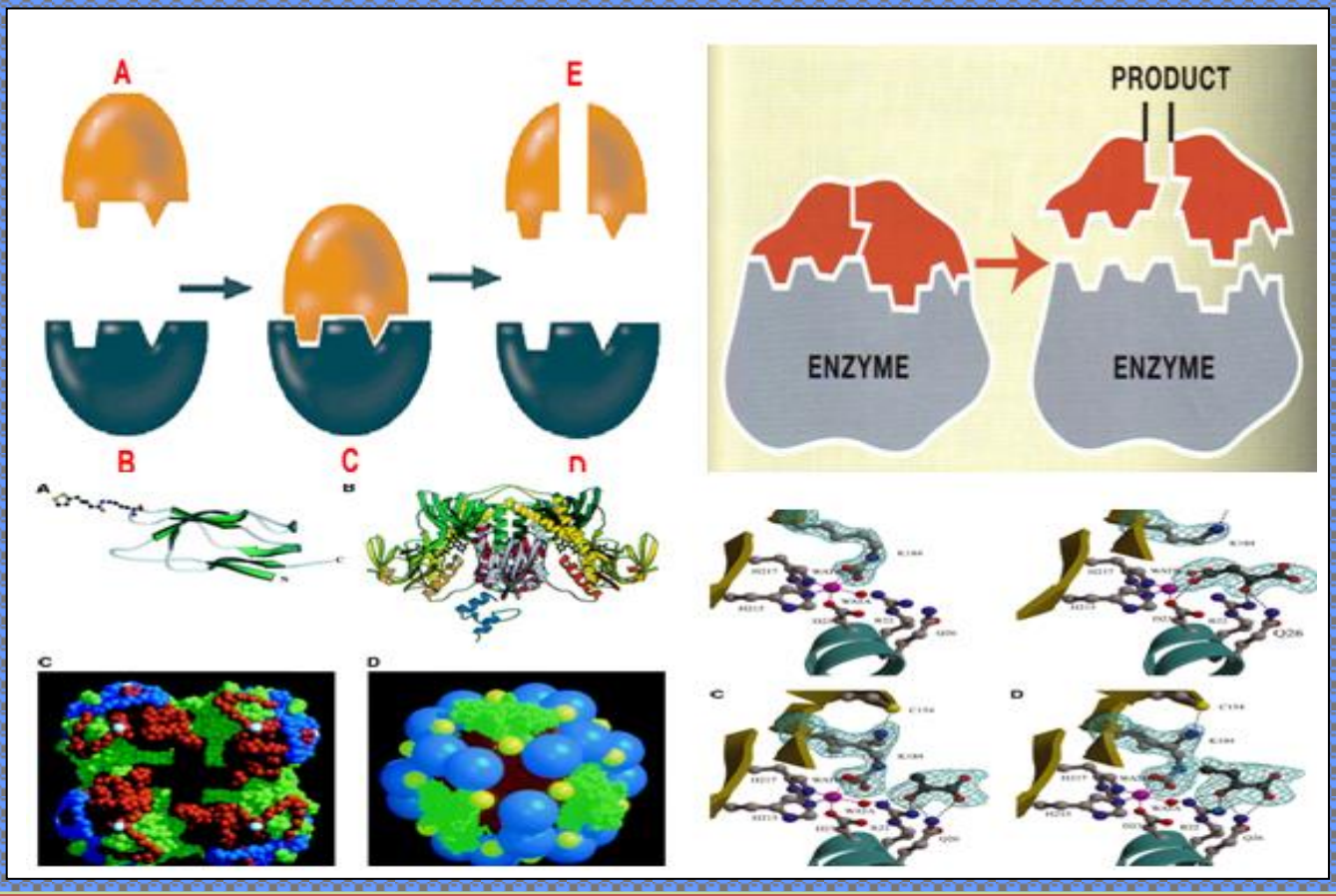
Karnataka State Open University

Mukthagangothri, Mysore-570006

M.Sc. Biotechnology

First Semester



ENZYMOLOGY

BTP- 1.3 **BLOCKS- I,II,III and IV** **UNITS - 1 To 16**

Karnataka State Open University

M.Sc. in Biotechnology

FIRST SEMESTER

BT 1.3 - ENZYMOLOGY

(Blocks -I, II, III and IV; Units - 1 TO 16)

1.3 ENZYMOLOGY

COURSE DESIGN

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COURSE INTRODUCTION

BT 1.3 Enzymology

Enzymology is one of the branches of Biochemistry in which numerous advances are made. Enzymes are proteins produced in cells that catalyse and accelerate the metabolism in living organsisms. The Enzymes have been used indirectly since the origin of human. Curdling of milk by lactose fermentation and preparation of wine, beer, vinegar etc are some of the processes that are in use since thousands of years. The protease rennin is perhaps the first cell free enzyme used in making of cheese which is in practice since hundreds of years.

Probably the protease Trypsin which improves the efficiency of detergent isolated from animal source was the first commercial enzyme to be reported in Germany in 1914. Subsequently in 1959 microbial protease from Bacillus sp was used in washing power. Later on use of enzymes in food industry gained momentum.

Commercial Enzymes can be extracted from different sources such as Microorganisms, Plants and Animals but Microorganisms form major source because they are economical and so on. With the advancement in Genetic Engineering and r-DNA technology it is possible to not only synthesize and obtain desired enzymes but to produce tailor made enzymes as per customers requirement.

The prospectus of Enzyme Industry is definitely bright due to its increased applications in different Industries such as Food Industry, Brewing Industry, Agricultural Industry, Laundry Industry , Pharmaceutical Industry , Textile Industry , Animal feed Industry, Medical, clinical Diagnostics fields and many more.

The information in this book is condensed but maximum attempt has been made to provide complete information required for understanding the basics of Enzymology and its applications. All the units have been brought up to date by collecting information from different sources and modified in keeping pace with the learning interest and potential of Open University Students.

Each Unit begins with clearly stated learner-oriented objectives followed by terms important for thorough understanding of the text. Every unit at the end includes key words to easily remember the subject and questions to help the readers to self evaluate their grasp of the concepts.The complete format of self learning material should act as necessary tool in creating interest and learning the Enzymology as a part of M.Sc. Biotechnology programme.

The content of this book is organized into 4 blocks, each block with 4 units.

The Block I consist of four Units: Unit-1 covers history, nomenclature, classification of enzymes, and their relationship to recent developments in the field of biotechnology. The Unit-2 explains Nomenclature of enzymes and different key factors that affect enzyme activity. The Unit-3 defines Enzyme Kinetics, explains the reaction mechanism and

Michaelis - Menten equation. Unit-4 explains behavior of many enzymes by Michaelis-Menten equation and VMax and KM.

The Block II consists of four Units: Unit-5 explains different types of enzyme inhibitions. Unit-6 discusses molecular mechanism of action of chymotrypsin and ribonuclease enzymes. In Unit-7 topics related to Allosterism namely positive and negative co-operativity, Sigmoidal kinetics, MWC and KNF models and ATCase & Haemoglobin are dealt. Unit-8 explains Bi substrate enzyme catalyzed reactions, Cleland notation with, ping pong and random ordered reactions and general rate equilibrium.

The Block III consists of four Units; Unit-9 discusses Multienzyme complexes-PDC and fatty acid synthetase complex and Multifunctional enzymes –DNA polymerase. Unit-10 describes the mechanism of endoprotein degradation- the Ubiquitin pathway and Lysosomal pathway. Unit-11 explains degradation of nucleic acids by endo- exo- nucleases and Restriction endonucleases. Unit-12 describes the Immobilization of enzymes and properties of immobilized enzymes.

The Block IV consists of four Units; Unit-13 discusses Isoenzymes Lactate dehydrogenase and its Clinical application. Unit-14 explains Applications of enzymes and leather Industry. Unit-15 discusses various applications of immobilized enzymes as biosensors, in medicine, antibiotic production, food industry, biodiesel production, bioremediation. Unit-16 explains Co-enzymes-NAD and NADP, Coenzyme A, FMN, FAD and cyclic nucleotides.

Constructive suggestions, comments and criticism for the improvement of this book are most welcomed.

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BLOCK - I

UNIT- 1

Introduction, Nomenclature and Classification of Enzymes

STRUCTURE

1.0 OBJECTIVES

After studying this Unit you will be able to understand

- Some aspects of the history of enzymes
- Nomenclature of enzymes
- Classification of enzymes
- Their relationship to recent developments in the field of biotechnology

1.1 INTRODUCTION

Enzymes are biocatalysts. Like any other catalyst, an enzyme brings the reaction catalyzed to its equilibrium position more quickly than would occur otherwise; an enzyme cannot bring about a reaction with an unfavorable change in free energy unless that reaction can be coupled to one whose free energy change is more favorable..

The activities of enzymes have been recognized for thousands of years; for example the fermentation of sugar to alcohol by yeast.

However, in recent years the properties of enzymes been understood properly. At present the research on enzymes has now entered a new phase with the fusion of ideas from protein chemistry, molecular biophysics, and molecular biology.

Early Concepts of Enzymes:

The term ‘‘enzyme’’ (literally ‘‘in yeast’’) was coined by **KUHNE** in 1876. Yeast, because of the acknowledged importance of fermentation, was a popular subject of research. A major controversy at that time, associated most memorably with **LIEBIG** and **PASTEUR**, was whether or not the process of fermentation was separable from the living cell. No belief in the necessity of vital forces, however, survived the demonstration by **BUCHNER** (1897) that alcoholic fermentation could by carried out by a cell-free yeast extract. The existence of extracellular enzymes had, for reasons of experimental accessibility, already been recognized. For example, as early as 1783, **SPALLANZANI** had demonstrated that gastric juice could digest meat in vitro, and SCHWANN (1836) called the active substance pepsin. KUHNE himself appears to have given trypsin its present name, although its existence in the intestine had been suspected since the early 1800s.

Enzymes as Proteins By the early 1800s, the proteinaceous nature of enzymes had been recognized. Knowledge of the chemistry of proteins drew heavily on the improving techniques and concepts of organic chemistry in the second half of the 1800s; it culminated in the peptide theory of protein structure, usually credited to **FISCHER** und **HOFMEISTER**. However, methods that had permitted the separation and synthesis of small

peptides were unequal to the task of purifying enzymes. Indeed, there was no consensus that enzymes were proteins. Then, in 1926, **SUMNER** crystallized urease from jack bean meal and announced it to be a simple protein. However, **WILLSTATTER** argued that enzymes were not proteins but ‘‘colloidal carriers’’ with ‘‘active prosthetic groups.’’ However, with the conclusive work by **NORTHROP** et al., who isolated a series of crystalline proteolytic enzymes, beginning with pepsin in 1930, the
proteinaceous nature of enzymes was established.

The isolation and characterization of intracellular enzymes was naturally more complicated and, once again, significant improvements were necessary in the separation techniques applicable to proteins before, in the late 1940s, any such enzyme became available in reasonable quantities. Because of the large amounts of accessible starting material and the historical importance of fermentation experiments, most of the first pure intracellular enzymes came from yeast and skeletal muscle. However, as purification methods were improved, the number of enzymes obtained in pure form increased tremendously and still continues to grow. Methods of protein purification are so sophisticated today that, with sufficient effort, any desired enzyme can probably be purified completely, even though very small amounts will be obtained if the source is poor.

Primary Structure

After the protein nature of enzymes had been accepted, the way was clear for more precise analysis of their composition and structure. Most amino acids had been identified by the early 20th century. The methods of amino acid analysis then available, such as gravimetric analysis or microbiological assay were quite accurate but very slow and required large amounts of material. The breakthrough came with the work of **MOORE** and **STEIN** on ion-exchange chromatography of amino acids, which culminated in 1958 in the introduction of the first automated amino acid analyzer.

The more complex question–the arrangement of the constituent amino acids in a given protein, generally referred to as its primary structure–was solved in the late 1940s. The determination in 1951 of the amino acid sequence of the b-chain of insulin by **SANGER** and **TUPPY** demonstrated for the first time that a given protein does indeed have a unique primary structure. The genetic implications of this were enormous. The introduction by **EDMAN** of the phenyl isothiocyanate degradation of proteins stepwise from the N-terminus, in manual form in 1950 and subsequently automated in 1967 , provided the principal chemical method for determining the amino acid sequences of proteins.

The primary structures of pancreatic ribonuclease and egg-white lysosomes were published in 1963. Both of these enzymes, simple extracellular proteins, contain about 120 amino acids. The first intracellular enzyme to have its primary structure determined was glyceraldehyde 3-phosphate dehydrogenase, which has an amino acid sequence of 330 residues and represents a size (250– 400 residues) typical of many enzymes. Protein sequencing is increasingly performed by liquid chromatography/mass spectrometry (LC/MS) techniques, and several tools and software packages are now available for protein identification and characterization.

The methods of protein sequence analysis are now so well developed that no real practical deterrent exists, other than time or expense, to determination of the amino acid sequence of any polypeptide chain.

Active Site:

The fact that enzymes are highly substrate specific and are generally much larger than the substrates on which they act quickly became apparent. The earliest kinetic analyses of enzymatic reactions indicated the formation of transient enzyme– substrate complexes. These observations could be explained easily if the conversion of substrate to product was assumed to occur at a restricted site on an enzyme molecule. This site soon became known as the active center or, as is more common today, the active site.

Particular compounds were found to react with specific amino acid side chains and thus inhibit particular enzymes. This suggested that such side chains might take part in the catalytic mechanisms of these enzymes. An early example was the inhibition of glycolysis or fermentation by iodoacetic acid, which was later recognized as resulting from reaction with a unique cysteine residue of glyceraldehyde 3-phosphate dehydrogenase, which normally carries the substrate in a thioester linkage.

Many such group-specific reagents have now been identified as inhibitors of individual enzymes; often they are effective because of the hyper-reactivity of a functionally important side chain in the enzyme's active site. However, a more sophisticated approach to the design of enzyme inhibitors became possible when the reactive group was attached to a substrate; in this way, the specificity of the target enzyme was utilized to achieve selective inhibition of the enzyme. Such active-site directed inhibitors have acquired major importance not only academically in the study of enzyme mechanisms but also commercially in the search for a rational approach to selective toxicity or chemotherapy.

Three-Dimensional Structure:

Chemical studies showed that the active site of an enzyme consists of a constellation of amino acid side chains brought together spatially from different parts of the polypeptide chain. If this three-dimensional structure was disrupted by denaturation, that is, without breaking any covalent bonds, the biological activity of the enzyme was destroyed. In addition, it was found that all the information required for a protein to fold up spontaneously in solution and reproduce its native shape was contained in its primary structure.

The X-ray crystallography of proteins demonstrated unequivocally that a given protein has a unique three-dimensional structure. Among the basic design principles was the tendency of hydrophobic amino acid side chains to be associated with the hydrophobic interior of the folded molecule, whereas charged side chains were almost exclusively situated on the hydrophilic exterior or surface. The first high-resolution crystallographic analysis of an enzyme, egg-white lysozyme, confirmed these principles and led to the proposal of a detailed mechanism. The active site was located in a cleft in the structure, which has subsequently proved to be a common feature of active sites. According to this, the enzymatic reaction takes place in a hydrophobic environment, and the successive chemical events involving substrate and protein side chains are not constrained by the ambient conditions of aqueous solution and neutral pH.

1.2. NOMENCLATURE AND OF ENZYMES

Strict specificity is a distinguishing feature of enzymes, as opposed to other known catalysts. Enzymes occur in myriad forms and catalyze an enormous range of reactions. By the late 1950 s the number of known enzymes had increased so rapidly that their nomenclature was becoming confused or, worse still, misleading because the same enzyme was often known to different workers by different names; in addition, the name frequently conveyed little or nothing about the nature of the reaction catalyzed.

To bring order to this chaotic situation, an International Commission on Enzymes was established in 1956 under the auspices of the **International Union of Biochemistry** (IUB).

Its terms of reference were as follows:

‘‘To consider the classification and nomenclature of enzymes and coenzymes, their units of activity and standard methods of assay, together with the symbols used in the description of enzyme kinetics.’’

The Commission’s recommendations have formed the basis of enzyme nomenclature since its first report in 1961.

Responsibility for enzyme nomenclature passed to the Nomenclature Committee of IUB in 1977, which has subsequently published several reports, e.g. and supplements, e.g.,; it is expected that further supplements will be published from time to time in the European Journal of Biochemistry. The growth in scale can be appreciated from the fact that the 1961 Report of the Enzyme Commission listed 712 enzymes, whereas the 1992 version of Enzyme Nomenclature listed 3196.

The most recent information about changes or additions to enzyme nomenclature is available at **http://www.chem.qmw.ac.uk/iubmb/**, which offers also an up-to-date version of the Enzyme Nomenclature list.

General Principles of Nomenclature

The accepted system for classification and nomenclature of enzymes embodies three general principles.

1. The first is that enzyme names, especially those ending in -ase, should be used only for single enzymes, i.e., single catalytic entities. They should not be applied to systems containing more than one enzyme.
2. The second general principle is that an enzyme is named and classified according to the reaction it catalyzes. This refers only to the observed chemical change produced by the enzyme, as expressed in the chemical equation. The mechanism of action is ignored, and intermediate cofactors or prosthetic groups are not normally included in the name. Thus, an enzyme cannot be named systematically until the reaction it catalyzes has been identified properly.
3. The third general principle is that enzymes are named and classified according to the type of reaction catalyzed, which enables Enzyme Commission(E.C.) code numbers to be assigned to enzymes to facilitate subsequent unambiguous identification.

For the purpose of systematic nomenclature, all enzymes in a particular class are considered to catalyze reactions that take place in a given direction, although only the reverse direction may have been demonstrated experimentally. However, the recommended name for the enzyme may well be based on the presumed direction of the reaction in vivo.

Thus, a given enzyme often has two names, one systematic and the other recommended or trivial. The latter is generally the name in current usage, shorter and more readily applied. After its systematic name and E.C. code number have identified an enzyme, the recommended name can be used without fear of ambiguity. This practice is now generally followed in the literature.

1.3. CLASSIFICATION OF ENZYMES

According to the report of the first Enzyme Commission in 1961, enzymes are divided into six main classes according to the type of reaction catalyzed. They are assigned code numbers, prefixed by E.C., which contain four elements separated by points and have the following meaning:

1. The number first indicates to which of the six classes the enzyme belongs,
2. The second indicates the subclass,
3. The third number indicates the sub-subclass, and
4. The fourth is the serial number of the enzyme in its sub-subclass.

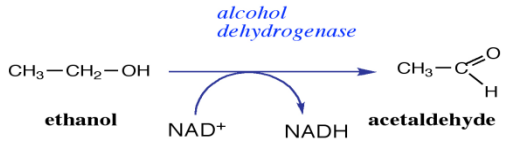
The six classes are distinguished in the following manner:

1. Oxidoreductases

This class encompasses all enzymes that catalyze redox reactions. The recommended name is dehydrogenase whenever possible, but reductase can also be used. Oxidase is used only whenO2 is the acceptor for reduction. The systematic name is formed according to donor: acceptor oxidoreductase.

Eg. Alcohol Dehydrogenase.

General reaction:



Enzyme Commission (EC) Number: 1.1.1.1

First digit: Main class

Second digit:

Second digit	hydrogen or electron donor
1	alcohol
2	aldehyde or the ketone
3	CH=CH

Third digit:

Third digit	hydrogen or electron acceptor
1	NAD+ OR NADP+
2	Fe3+
3	O2

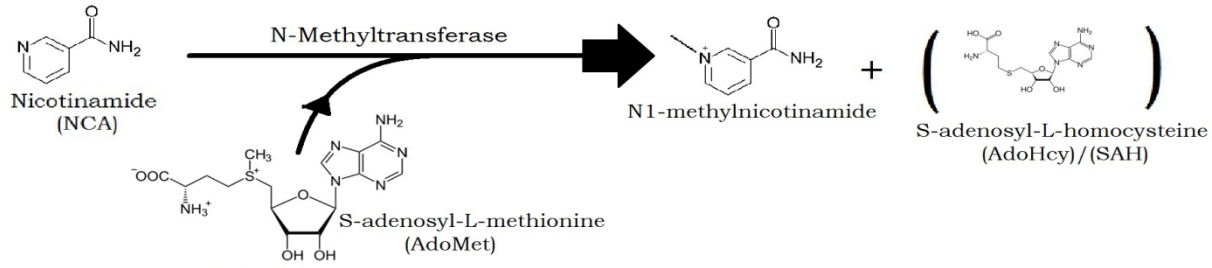
Fourth digit: Represents their sub-sub class

2. Transferases

Transferases catalyze the transfer of a specific group, such as methyl, acyl, amino, glycosyl, or phosphate, from one substance to another. The recommended name is normally acceptor group transferase or donor group transferase. The systematic name is formed according to donor: acceptor group transferase.

Eg. Nicotinamide N-methyltransferase as shown below.

General reaction:



Enzyme Commission (EC) Number: 2.1.1.1

First digit: Main class

Second digit:

Second digit	Group transferred
1	1 – Carbon transfer
2	Aldehyde or ketone
3	Acyl group
4	Glycosyl group
7	Phosphate group

Third digit:

Third digit	Group transferred
1	$-CH_3$
2	$-CH_2OH$
3	OH – C=O or H_2N – C = O

Fourth digit: Represents their sub-sub class

3. Hydrolases

Hydrolases catalyze the hydrolytic cleavage of C–O, C–N, C–C, and some other bonds. The recommended name often consists simply of the substrate name with the suffix -ase. The systematic name always includes hydrolase.

Eg. Triacylglycerol lipase

General reaction:

Enzyme Commission (EC) Number: 3.1.1.3

First digit: Main class

Second digit:

Second digit	bond hydrolysed
1	Ester
2	Glycosidic
4	Peptide

Third digit:

Third digit	type of bond bond hydrolysed
1	COO-
2	O=C-S
3	0-PO32-
4	PO4 2-

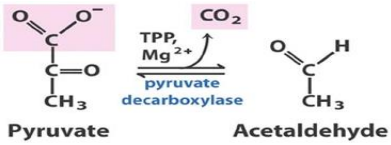
Fourth digit: Represents their sub-sub class

4. Lyases

Lyases catalyze the cleavage of C–C, C–O, C–N, and other bonds by elimination. The recommended name is, for example, decarboxylase, aldolase, dehydratase (elimination of CO_2, aldehyde, and water, respectively). The systematic name is formed according to substrate group-lyase.

Eg. Pyruvate decarboxylase

General reaction:



Enzyme Commission (EC) Number: 4.1.1.1

First digit: Main class

Second digit:

Second digit	bond broken
1	C-C
2	C-O
3	C-N
4	C-S

Third digit:

Third digit	Group transferred
1	$-CO_2$
2	-CH=O
3	HOOC – C=O

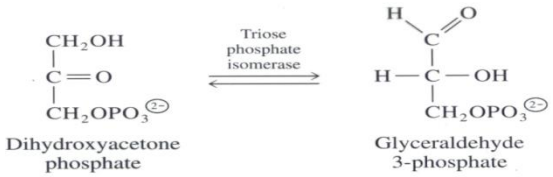
Fourth digit: Represents their sub-sub class

5. Isomerases

Isomerases catalyze geometric or structural rearrangements within a molecule. The different types of isomerism lead to the names racemase, epimerase, isomerase, tautomerase, mutase, or cycloisomerase.

Eg. Triose-phosphate isomerase

General reaction:



Dihydroxyacetone phosphate — Glyceraldehyde 3-phosphate

Enzyme Commission (EC) Number: 5.3.1.1

First digit: Main class

Second digit:

Second digit	type of reaction
1	Racemisation /epimerization
2	Cis- trans isomerisation
3	Intra molecular oxidoreductase
4	Intra molecular transfer reaction

Third digit:

Third digit	substrate
1	amino acids
2	hydroxy acids
3	carbohydrate

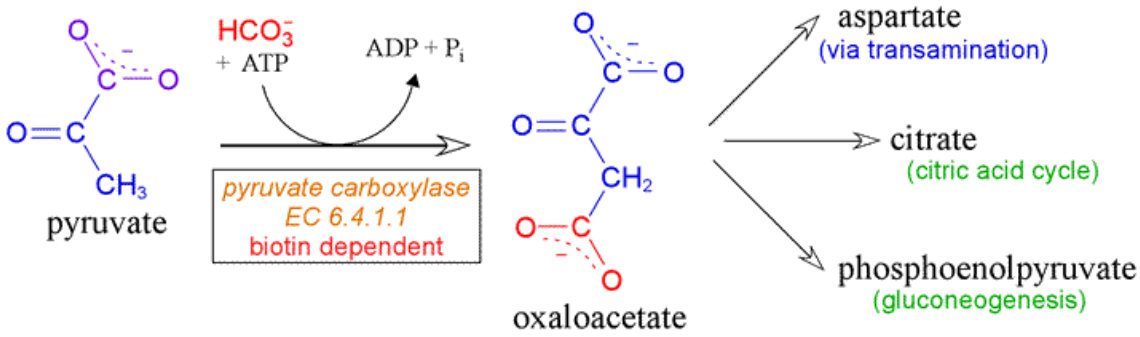
Fourth digit: Represents their sub-sub class

6. Ligases

Ligases catalyze the joining of two molecules, coupled with the hydrolysis of a pyrophosphate bond in ATP or another nucleoside triphosphate. Until 1983, the recommended name often included synthetase, but the current recommendation is that names of the type X–Y ligase be used instead, to avoid confusion with the name synthase (which is not confined to enzymes of class 6). The systematic name is formed according to X : Y ligase (ADP-forming).

Eg. Pyruvate Carboxylase as shown below

General reaction:



Enzyme Commission (EC) Number: 6.4.1.1

First digit: Main class

Second digit:

Second digit	Bond synthesized
1	C – O
2	C – S
3	C – N
4	C – C

Third digit:

Third digit	bond formed
1.	Amide
2.	Peptide

Fourth digit: Represents their place in the list (IUBMB).

1.4 SUMMARY

To date, over 2,000 different enzymes are known, of which the oxidoreductases, transferases and hydrolases predominate. Because official names are often lengthy, the trivial names of enzymes are generally used after initial identification. With the continuous increase in our

knowledge of enzymolog, various systems have evolved to name and classify the enzymes, using one or the other criterion as the basis. However, many of the enzymes were known before these systems of naming enzymes were adopted. The names of such enzymes were not changed under the new systems.

1.5 KEYWORDS

Catalyst: A catalyst is defined as a substance that increases the velocity or rate of a chemical reaction without itself undergoing any change in the overall process.

Enzymes: It may be defined as biocatalysts synthesized by living cells. They are protein in nature (exception - RNA acting as ribozyme), colloidal and thermophile in character, and specific in their action.

E C. Number:A four digit Enzyme Commission (E C.) number is assigned o each enzyme representing the class (first digit), sub-class(second digit), sub-sub class (third digit) and the individual enzyme (fourth digit).

1.6 QUESTIONS FOR SELF STUDY

1. Why do we classify enzymes?
2. What does EC.1.1.1.1 indicate?
3. Differentiate between hydrolases and lyases with suitable example.
4. How are enzymes classified? Explain with suitable examples.
5. Give an amount of IUB nomenclature and classification of enzymes.

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