Indira Gandhi National
Open University
School of Sciences

BBCCT-107
ENZYMES

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ENZYMES

The branch of biochemistry that deals with property, activity and significance of enzymes is called as enzymology. It is one of the core branches of Biochemistry. It is study of enzymes, their kinetics, structure and function. Lately, there have been several major developments in the field of enzymology. This course is mainly for the students taking degree courses with substantial biochemistry component. However, it is of immense value to students from other science backgrounds–chemistry and life sciences. This course is written with the requirements of distance learners firmly in mind. It is intended to provide an introduction to enzymology. A balanced approach has been adopted to include theoretical, analytical and applied concepts of the subject.

We have attempted to define most of the scientific terms. They have been placed in context when they first appear. We have tried to deal comprehensively with the kinetics, structures and mechanism of enzymes. Enzymology also involves a basic level of mathematics and some of the equations which are derived may seem complicated at first sight. The derivation of the equations on enzyme kinetics are based on the biochemical assumptions and then followed on logical reasoning and analysis.

In the first block, (Block 1) the concept of enzymes has been introduced to the learners (Unit 1). Sections on nomenclature of enzymes are further subdivided into subclasses of enzymes. The principle and theories underlying enzyme catalysis are described in the following unit (Unit 2). Several factors affecting the enzyme activity are also discussed in the Unit 3.

The second block, (Block 2) deals specifically with the enzyme catalysis. The kinetics of mono substrate and bisubstrate reactions has been described in Unit 4 and Unit 5 respectively. The derivation of Michaelis-Menten equation has been described in the Unit 4. Unit 6 is mainly concerned with the inhibition of single substrate reactions obeying Michaelis Menten kinetics.

The mechanism and regulation of enzyme activity has been addressed in the Block 3. Mechanisms important in controlling enzyme activity have been explained with relevant examples (Unit 7). Several different mechanisms and their classification enable the learner to understand the regulation of metabolic pathways at several levels of organization of life forms (Units 8 and 9).

Since there has been a remarkable increase in the past few decades in the enzyme industry, in the last block, (Block 4) we have allowed the distance learners to keep up with the latest developments and applications of enzymes (Unit 11). The role of coenzymes (Unit 10) has also been described. The last unit (Unit 12) reflects the latest advances in enzyme technology.

Objectives

- explain the concept of enzymes, their nomenclature and their components;
- describe the enzymes kinetics;
- discuss the mechanism and regulation of enzyme; and
- explain the role of multienzyme complexes.

Indira Gandhi National
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BBCCT-107
ENZYMES

Block

1

ENZYMES AS BIOCATALYSTS

BLOCK 1 : ENZYMES AS BIOCATALYSTS

Cells function largely because of the action of enzymes. Life is a vibrant process that engrosses continuous changes in the chemical composition. These changes are regulated by catalytic reactions, which are regulated by enzymes. Enzymes are large molecules of proteins that are made from many amino acids. There are thousands of different enzymes. The name of enzymes indicates their functional nature. You must have read about the enzymes in your school textbooks.

In the first block we have given an introduction of enzymes, their components and their classification based on IUPAC. How enzymes speed up the rate of biochemical reactions has been discussed in the second unit of the block. Enzymes help to overcome the energy barrier so that more of the substrate molecules can be converted into product. Several theories such as famous Fischer theory of Lock and Key hypothesis and Induced Fit Model in this regard are described in Unit 2 of the block. The last unit focuses on the role of several factors affecting the enzyme activity.

Objectives

After studying this block, you should be able to:

- explain the fundamental concept of enzymes and their classification;
- illustrate enzyme-substrate interaction;
- discuss the features of enzyme catalysis; and
- describe about the factors affecting enzyme activity.

We hope that after studying this block you will acquire understanding of the fundamentals of enzymes.

Wishing you success in this endeavour!

UNIT 1

INTRODUCTION TO ENZYMES

Structure

1.1 INTRODUCTION

In this unit you will learn about the basic concept of enzymes and their role as biological catalysts. Enzymes are remarkable **highly specialized proteins** catalyzing biochemical transformations effectively and efficiently with very high specificity. The reactants of enzyme catalyzed reactions are called as substrates which are converted to products. Enzymes are large biological molecules which are colloidal and thermolabile in nature. They play a vital role in biochemical processes. Enzymes catalyze reactions in an organized manner to sustain life which could not have been possible without them in a given time scale. They are highly selective molecules and their catalytic activity depends on the integrity of their native protein conformation. Enzymes employ additional non-protein chemical component known as cofactor or complex organic molecules called as co enzymes to perform their catalytic activity. This additional component may be bound tightly or loosely. The distinguishing feature of an enzyme catalyzed reaction is the catalytic site, a small portion of the enzyme molecule termed as substrate binding site or active site. In this unit you will learn how enzymes are classified by the nature of reactions they catalyze. General or trivial names lead to confusion. In order to remove inconsistency and ever increasing number of enzymes, an international system of naming the enzymes has

been adopted. Each enzyme is assigned a four part classification number. Enzymes also have immense commercial and industrial value.

Enzyme is a Greek word meaning in yeast (*en*: in, *zyme*: leaven), the term coined in 1878 by Friedrich Wilhelm Kühne to designate biological catalysts which were earlier known as *ferments*. Interestingly, alcoholic fermentation is one of the most popular reactions wherein the production of ethyl alcohol and carbon dioxide occurs. The reaction is catalyzed by enzyme zymase which is present in yeast. This reaction led to the discovery of enzymes.

Most enzymes are proteins but all proteins are not enzymes. Exception is ribozymes where RNA acts as enzyme.

Objectives

After studying this unit, you should be able to:

- define enzyme;
- explain the characteristics of enzymes;
- distinguish between holoenzyme and apoenzyme;
- differentiate between cofactors and coenzymes; and
- classify enzymes.

1.2 BIOLOGICAL SIGNIFICANCE

Tight control of enzymes is essential for homeostasis. They play a crucial role in the maintenance and progression of life. A number of enzymes are involved in the development of an ovum and sperm, during fertilization and development of gametes, and during growth and development of an individual. Energy exchange processes such as photosynthesis and respiration are carried out with the help of enzymes in the living organisms. Enzymes also assist in metabolizing food and nutrients which we consume in our diet into simpler moieties and provide us with the building blocks needed for growth and repair. Apart from maintaining and sustaining life, enzymes also have several medical and commercial applications. Any malfunction (mutation, overproduction, deletion or underproduction) of enzyme can lead to serious disorders and severe illnesses. Now a day's many enzyme levels are monitored to check for the particular disease for e.g. lactate dehydrogenase (LDH) and creatine kinase (CK) in myocardial infarction. The imbalances in the enzyme activity are addressed by pharmacological agents using several strategies (enzyme inhibition, gene therapy etc).

1.3 NATURE OF ENZYMES

Nearly all enzymes are proteins with the exception of ribozymes (RNA acting as enzymes). However, many enzymes will lack their catalytic activity in the absence of non-protein component called as co-factor/coenzyme. Enzymes can be denatured and precipitated with salts, solvents and other reagents. They have molecular weights ranging from 10,000 to 2,000,000. Denaturation of enzymes (subjecting enzymes to unfavorable pH and temperature), like other proteins leads to loss in enzymatic activity. So, enzymes are thermolabile in nature i.e. they lose their characteristics when subjected to an increase in the temperature.

Until 1980s it was strongly believed that all enzymes are proteins. Then Tom Cech and Sidney Altman independently reported that some RNA molecules can be effective catalysts. These RNA molecules exhibiting catalytic activity are now known as ribozymes. Probably the first biocatalysts on earth were RNA instead of proteins. Ribozymes may have played a very crucial role during the evolutionary phase.

Recall about nucleic acids you have studied in your school text books. RNA exhibits various forms. This shows the ability of RNA to adopt complex structures (recall the structure of t-RNA). These specific yet complex structures might be related to their catalytic ability. For example, the first ribozyme identified from a plant virus termed as “hammerhead” ribozyme possesses a specific structure needed for catalysis. Another example is ribonuclease P (RNase P) which catalyses the maturation of tRNA by removing nucleotides from the 5' end of the precursor molecule and so on.

SAQ 1

Tick [✓] mark the correct option:

a) Enzymes are made up of proteins and lipids. [True/False]

b) Deletion or underproduction of enzyme can lead to serious disorders and severe illnesses. [True/False]

c) Change in pH and temperature does not affect enzyme activity. [True/False]

d) Colloidal nature of enzymes is due to its large size. [True/False]

1.4 CHARACTERISTICS OF ENZYMES

Let us focus on the characteristic properties of enzymes. As you know enzymes are the biological catalysts. The enzyme acts on a molecule or substances known as **substrate** to convert it into a particular product or products.

$$\text{Substrate(S)} \xrightarrow{\text{Enzyme}} \text{Product(P)}$$

Enzymes catalysis differ from chemical catalysis in several different aspects. Some of the general characteristics of enzymes are:

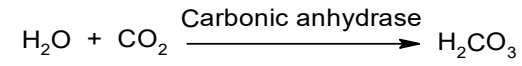
- The rate of reaction of an enzyme catalyzed reaction is 10^6 to 10^{12} times more than the uncatalyzed one.
- Another key feature of enzyme catalyzed reaction is specificity towards the substrate as well as their products.
- The physical conditions like temperature and pH at which enzymatic catalysis occurs is very mild as compared to the chemical catalysis. Almost all the enzyme catalysed reactions occur at temperature below $100^{O}C$ and nearly neutral pH.
- In addition to this, control mechanism of enzymes is remarkable. Catalytic activities of enzymes can be controlled by covalently modifying the enzyme, allosteric regulation and many others which you will study in later units.

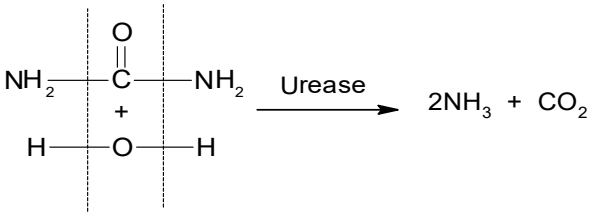
A numbers of enzymes are involved in physiological processes related to the growth and development of an individual. Energy exchange processes such as photosynthesis and respiration are carried out with the help of enzymes in the living organisms.

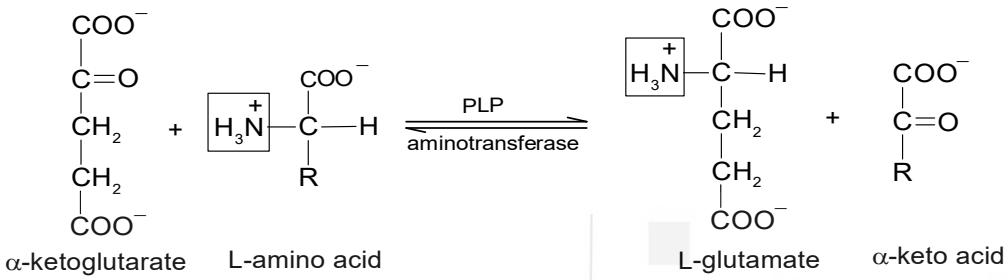
Have you ever questioned yourself how these diverse functions are performed by enzymes with great level of efficiency? What are the key characteristic properties which makes enzymes an efficient molecule? The answer is **catalytic efficiency** and **substrate specificity**.

Let us focus on these two characteristic properties, catalytic efficiency and substrate specificity of enzymes. Like any other catalyst catalyzing a reaction, enzymes do not alter the point of chemical equilibrium in a reversible reaction. They only increase the speed of the reaction or in other words they increase the rate of a reaction in both forward and backward direction and is also not consumed or used up in the process. So, enzymes increase the rate of a reaction which would otherwise take much longer time to be completed. For example, enzyme **carbonic anhydrase** catalyzes the conversion of CO_2 and H_2O into carbonic acid (H_2CO_3). This reaction is vital for our survival as the CO_2 generated in the tissues must be excluded but uncatalyzed reaction occurs at a very slow pace. Carbonic anhydrase speeds up the formation of carbonic acid. The catalyzed reaction is 10^7 times faster than the uncatalyzed one. Each enzyme molecule can hydrate 10^6 molecules of CO_2 *per second*. This is termed as the **catalytic efficiency** of an enzyme.

They may act on one specific substrate molecule or on a group of structurally-related compound. For example carbonic anhydrase and urease enzymes are substrate specific (reactions as shown) while dehydrogenases and transaminases work on structurally related compounds.







The specificity of an enzyme is due to the precise interactions of the substrate with the enzyme. This precision is a result of substrate interaction with the intricate three-dimensional structure of the enzyme protein.

Four types of enzyme specificity have been recognized. Absolute specificity (catalyzing only one substrate), group specificity (catalyzing structurally related group of compounds), optical specificity (catalyzing only one of the two optical isomers either D or L form) and geometrical specificity (catalyzing only a *cis* or *trans* bond).

You will study more about enzyme specificity and binding energy in the next unit. Binding energy is the energy arising due to weak interactions between enzyme and substrate molecule. This binding energy drives the catalysis as well as is the basis of enzyme specificity.

SAQ 2

State whether following statements are True or False:

a) Enzymes are effective biocatalysts due to substrate specificity. [True/False]

b) Enzymes do not alter the point of chemical equilibrium in a reversible reaction. [True/False]

c) Carbonic anhydrase catalyzed reaction is 10^7 times faster than uncatalyzed one. [True/False]

d) Urease enzyme catalyze reactions of structurally related compounds. [True/False]

1.5 CO-FACTOR, CO-ENZYME AND PROSTHETIC GROUP

When coenzyme is loosely associated, it is more like co-substrate because it binds and is released from the enzyme just as substrates and products are released after the catalysis.

We know that enzymes are highly specialized proteins catalyzing biochemical transformations effectively and efficiently with very high specificity. Like other proteins, molecular weight of enzymes ranges from about 12000 to more than 1 million. In some enzymes the three dimensional structure composed of amino acids is sufficient enough to carry out the catalysis and no additional chemical groups are needed. But many enzymes need an additional chemical component called cofactor or coenzyme or both for their catalytic activity. This additional moiety may be the integral part of enzyme (prosthetic group) or only needed during enzyme activity. Let us study in detail.

A complete, catalytically active enzyme together with its bound coenzyme and/ or metal ions is called a **holoenzyme or conjugated enzyme**. The protein part of such an enzyme is called the **apoenzyme** or **apoprotein** (Figure 1.1).

Apoenzyme/Apoprotein + cofactor/ co-enzyme or prosthetic group = Holoenzyme

Fig. 1.1: Schematic representation of a Holoenzyme.

Cofactor

Cofactors bind in a temporary dissociable manner to the enzyme or to the substrate. They can be subdivided into two groups: metal ions and small organic molecules. Many enzymes require either one or more inorganic ions, such as Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+} for their activity. Enzymes that require these metal ions for their catalytic activity are called as metal activated enzymes. Metal ions such as Mg^{2+}, Mn^{2+}, or Zn^{2+} bind at the enzymes active site as well as with the substrate simultaneously. A list of important enzymes and their associated metal ions are given in the Table 1.1.

Co-enzyme

Cofactors that are **small organic molecules** are called coenzymes. Coenzymes are often derived from vitamins and can be either tightly or loosely bound to the enzyme. If coenzyme is tightly bound to the enzyme it is termed as **prosthetic** group. Same coenzyme can be used by a variety of enzymes for catalysis. However, enzymes that use the same coenzyme are usually mechanistically similar i.e. their mechanism of catalysis remains same.

Table 1.1: Enzymes and their associated metals ions (co-factors)

Sl. No.	Enzyme	Associated Metal ion
1	Cytochrome oxidase	Fe^{2+}/ Fe^{3+}
2	Xanthine oxidase	Fe^{2+}/ Fe^{3+}
3	Peroxidase	Fe^{2+}/ Fe^{3+}
4	Catalase	Fe^{2+}/ Fe^{3+}
5	Phosphofructokinase	Mg^{2+}
6	Hexokinase	Mg^{2+}
7	Creatine kinase	Mg^{2+}
8	*Eco*RV	Mg^{2+}
9	Alcohol dehydrogenase	Zn^{2+}
10	Carbonic anhydrase	Zn^{2+}
11	DNA polymerase	Zn^{2+}
12	Carboxypeptidase	Zn^{2+}
13	Arginase	Mn^{2+}
14	Phosphoglucomutase	Mn^{2+}
15	Glycosyl transferase	Mn^{2+}
16	Superoxide dismutase	Mn^{2+}/ Fe^{2+}/ Ni^{2+}/ Cu^{+}
17	Cytochrome oxidase	Cu^{2+}/ Cu^{+}
18	Tyrosinase	Cu^{2+}/ Cu^{+}
19	Lipase	Ca^{2+}
20	Glutathione peroxidase	Se
21	Nitrate reductase	Mo
22	Pyruvate kinase	K^{+}
23	Propionyl CoA carboxylase	K^{+}
24	Urease	Ni^{2+}

Examples of well known coenzymes involved in the transfer of specific atoms or functional groups are given in Table 1.2.

Table 1.2: Enzymes and their associated coenzymes with functional groups involved in catalysis

Enzyme	Coenzyme	Group transferred
Pyruvate carboxylase	Biotin	Carbon dioxide
Acetyl CoA carboxylase	Coenzyme A (CoA)	Acyl group
Methylmalonyl mutase	Coenzyme B_{12} (5'-Deoxyadenosyl cobalamin)	Alkyl groups or hydrogen atoms
Monoamine oxidase	Flavin adenine dinucleotide (FAD)	Hydrogen atoms
Lactate dehydrogenase	Nicotinamide adenine dinucleotide (NAD^+)	Hydride ions (H-)
Glycogen phosphorylase	Pyridoxal phosphate	Amino groups
Thymidylate synthase	Tetrahydrofolate (THF)	One carbon groups other than CO_2

Prosthetic Group

A coenzyme or metal ion that is tightly or even covalently bound to the enzyme protein is called a prosthetic group. Binding of prosthetic group with the enzyme is permanent. Dissociation of the prosthetic group results in an irreversible loss of the catalytic activity of the enzyme. Some of the examples include pyridoxal phosphate, flavin mononucleotide (FMN), flavin dinucleotide (FAD), thiamin pyrophosphate, biotin, and the metal ions (Co, Cu, Mg, Mn, Se, and Zn). Metal ions are the most common prosthetic groups. One third of enzymes contain tightly bound metal ions (metallo-enzymes). They have metal ions as their integral part and are different from the enzymes that need metal ions as cofactor (metal-activated enzyme).

SAQ 3

Tick [✓] mark the correct option:

a) The term apoenzyme is applicable to

i) Simple enzyme (ii) Organic cofactor of a conjugate enzyme

iii) Protein part of conjugate enzyme (iv) Inorganic cofactor of a conjugate enzyme

b) An organic substance bound to an enzyme and essential for its activity is called

i) isoenzyme (ii) coenzyme (iii) apoenzyme (iv) holoenzyme

c) Prosthetic group is a part of the holoenzyme. It is

i) Loosely attached organic part (ii) Loosely attached inorganic part
iii) Accessory non protein substance attached firmly (iv) None of these

SAQ 4

Fill in the blanks:

a) Enzymes that require inorganic metal ions only during catalysis are called ..... enzymes while enzymes in which metal ion is the integral part is termed as ..... .

b) Acetyl CoA Carboxylare need coenzyme ..... for activity.

c) Ni^{2+} is essential for the activity of ..... enzyme.

1.6 CLASSIFICATION OF ENZYMES

Classification of enzymes can be done in number of ways depending on various factors like the composition of enzyme, type of substrate on which the enzyme acts, type of reaction catalyzed and so on. We will discuss about this general system of enzyme classification briefly with suitable examples along with the enzyme classification given by Enzyme Commission (EC) which is uniform in nature and followed universally.

1.6.1 General System of Enzyme Classification

Composition of Enzyme

All enzymes are proteins, except ribozymes. On the basis of composition of enzymes we can classify enzymes into three groups.

- Enzyme consisting of only protein for example trypsin, amylase and urease.
- enzyme consisting of **protein and metal ion** (metalloenzymes): approximately one-third of the enzymes need metal ion for their activity. This metal ion may be a co-factor or covalently linked to the enzyme.
- enzyme consisting of **protein and organic moiety** (commonly known as cofactor or prosthetic group), for example: **Iron porphyrin enzymes** (catalase, cytochrome c peroxidase I and II), **Flavoprotein enzymes** (glycine oxidase, pyruvate oxidase, histamine), **Enzymes requiring other coenzymes** (phosphorylase, amino acid decarboxylase), etc.

Type of substrate on which the enzyme acts: Enzyme derives its name simply by adding the suffix -***ase*** in the name of the substrate catalyzed. For example proteinases act on proteins, lipases act on lipids, maltase act on maltose, urease act on urea and so on.
Nature of end product, Enzyme derives its name from the nature of end product for example enzyme fumarase that catalyse the formation of fumarate (end product) irreversibly from L-malate.
Type of reaction catalyzed: Depending on the type of reaction and simply by adding –*ases* to it, enzymes can also be classified. For example, **hydrolases**

(catalyzing hydrolysis), **isomerases** (isomerization), **oxidases** (oxidation), **dehydrogenases** (dehydrogenation), **transaminases** (transamination), **transaldolases** (transaldolation), **transketolases** (transketolation), **phosphorylases** (phosphorylation) etc.

So far you have studied four different ways of classifying enzymes. It can be done in more ways but these systems of nomenclature are inconsistent. They tells us about only one aspect of the reaction catalyzed i.e. either only the nature of substrate or type of reaction catalyzed or end product and so on but not over-all chemical reaction. Whenever a new enzyme is characterized a great care has to be taken to give it a unique name. In order to bring consistency to the classification of enzymes and to remove ambiguities associated with the use of trivial nomenclature, in 1964, the International Union of Biochemistry and Molecular Biology (IUBMB) established an Enzyme Commission (EC) to develop a nomenclature for enzymes which is uniform and universally acceptable.

1.6.2 EC Numbers

The Enzyme Commission divided enzymes into six main classes, on the basis of the reaction catalyzed. Each class is assigned a code number. Each major class is divided into subclass and each subclass has numerous sub-subclasses. Every class, subclass and each sub-subclass is assigned a code number. Thus, each enzyme is given a four digit EC number (X1.X2.X3.X4) and a systematic name, which identifies the reaction it catalyzes. First digit (X1) shows the main class to which enzyme belongs. Six main classes of enzymes are given below in Table 1.3.

Second (X2) and third digit (X3) in the code corresponds to the subclass and sub-subclass, respectively and further describes the kind of reaction being catalyzed. Fourth number (X4) indicate the actual substrate. As an example, the formal systematic name of the enzyme catalyzing the reaction:

ATP + D-glucose ⟶ ADP + D-glucose 6-phosphate

is ATP: glucose phosphotransferase, which indicates that it catalyzes the transfer of a phosphoryl group from ATP to glucose. Its Enzyme Commission number (E.C. number) is 2.7.1.1. The first number (2) denotes the class name (transferase); the second number (7), the subclass (phosphotransferase); the third number (1), a phosphotransferase with a hydroxyl group as acceptor; and the fourth number (1), D- glucose as the phosphoryl group acceptor. EC classification system of enzyme brings out clarity and complexities of the reaction catalyzed by the enzyme but it makes the names lengthy and relatively cumbersome. So common name of the enzymes is still being used. For instance hexokinase for ATP:glucose phosphotransferase. Some examples of enzymes with their EC number, systematic and common names are given in the Table 1.4.

Table 1.3: IUBMB System of Enzyme Classification

First digit	Class	Type of reaction/ catalyzed	Some examples of subclass with enzyme names
1.	Oxidoreductases	Oxidation/ reduction reactions	**Dehydrogenases** (Succinate dehydrogenase, Malate dehydrogenase) **Peroxidases** (Catalase)
2.	Transferases	Transfer of an atom or group between two molecules	**Transaldolases Transketolases Kinases** (Hexokinase)
3.	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)	**Esterases** (Acetylcholinesterase) **Peptidases** (Trypsin, Chymotrypsin) **Glycosidases** (Lactase,amylase, chitinase, neuraminidase, lysozyme)
4.	Lyases	Addition or removal of a group from substrate (not by hydrolysis) to form double bonds	**Decarboxylase (**Phosphoenolpyruvate carboxylase,pyruvate decarboxylase, RuBisCO) **Dehydratase** (Serine dehydratase or L-serine ammonia lyase) **Synthase** (ATP synthase, Citrate synthase, Tryptophan synthase, Fatty acid synthase)
5.	Isomerases	Isomerization reactions (intramolecular group transfer)	**Isomerases** (Triose phosphate isomerase) **Mutases** (Bisphosphoglycerate mutase, Phosphoglucomutase)
6.	Ligases	Ligation of two substrates at the expense of ATP hydrolysis	**Synthetases** (Aminoacyl tRNA synthetase)

Table 1.4: IUBMB System of Enzyme Classification

EC Number	Systematic name	Common or trivial name
1.1.1.1	Alcohol : NAD oxidoreductase	Alcohol dehydrogenase
1.11.1.6	H_2O_2: H_2O_2 oxidoreductase	Catalase
2.7.1.1	ATP : D-hexose-6-phosphotransferase	Hexokinase
3.1.1.3	Glycerol ester hydrolase	Lipase
4.1.2.7	Ketose-1-phosphate aldehyde-lyase	Aldolase
4.2.1.2	L-malate hydro-lyase	Fumarase
5.3.1.9	D-glucose-6-phosphate keto-isomerase	Glucosephosphate isomerase
6.3.1.2	L-glutamate : ammonia ligase (ADP)	Glutamine synthetase

SAQ 5

Match the following:

Sl.No.	Enzyme	Sl.No.	Class
a	Malate dehydrogenase	i	Isomerase
b	Acetylcholinesterase	ii	Transferase
c	Phosphoglucomutase	iii	Oxidoreductase
d	RuBisCO	iv	Hydrolase
e	Hexokinase	v	Lyases

1.7 SUMMARY

1) Enzymes are biological *catalysts.* They are highly specialized proteins catalyzing biochemical transformations effectively and efficiently with very high specificity.
2) They possess a three dimensional structure and may require organic (e.g. biotin) and/or inorganic (e.g. ferric/ferrous ion) cofactors to assist in catalysis.
3) A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a holoenzyme or conjugated enzyme. The protein part of such an enzyme is called the apoenzyme or apoprotein.
4) Enzymes can increase reaction rates a billion fold, which enables biochemical reactions to occur under physiological conditions.
5) In 1964, the International Union of Biochemistry and Molecular Biology (IUBMB) established an Enzyme Commission to develop a nomenclature

for enzymes which is uniform and universally accepted. The Enzyme Commission divided enzymes into six main classes *Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases,* and *Ligases.*

1.8 TERMINAL QUESTIONS

1) What are the important characteristic properties of enzyme?

2) Explain co-enzyme, cofactor and prosthetic group giving examples.

3) Differentiate between metal activated enzyme and metalloenzyme.

4) Give a brief account of the EC classification of enzymes.

1.9 ANSWERS

Self-Assessment Questions

1) a) False, b) True, c) False, d) True

2) a) True, b) True, c) True, d) False

3) a) iii; b) ii; c) iii

4) a) metal activated, metalloenzyme; b) CoA; c) urease;

5) a) iii; b) iv; c) i; d) v; e) ii

Terminal Questions

1) The characteristic property of an enzyme is its high specificity. Refer to section 1.4

2) Refer to section 1.5

3) Metal activated enzymes require metal ions for their catalytic activity while metal ion is the integral part of metalloenzymes. Refer to section 1.5

4) The Enzyme Commission (EC) grouped enzymes into six main classes, on the basis of their functional specificity. Each class is assigned a number. Each major class is divided into sub-class and subclass has many sub-subclasses. Thus, each enzyme has a unique four digit EC number (X1.X2.X3.X4) and a systematic name, which identifies the enzyme on the basis of reaction it catalyzes. Refer to section 1.6

1.10 SUGGESTED READINGS

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3) Donald J Voet Principles of Biochemistry, John Wiley and Sons, Inc, USA.

4) Eric E Conn, Paul K Stumpf: Outlines of Biochemistry, John Wiley and Sons, Inc, USA.

5) S. Shanmugan and T. Sathishkumar: Enzyme Technology, I K International Publishing House Pvt Ltd, New Delhi.

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UNIT 2

FEATURES OF ENZYME CATALYSIS

Structure

2.1 INTRODUCTION

In the previous unit you have learnt that enzymes are biocatalysts that are highly specialized proteins. Enzymes increase the rate of chemical reaction many times higher than the uncatalyzed reactions. In the present unit you will learn how enzymes work? How enzymes increase the rate of reactions by lowering free energy barrier that splits reactants and products? How do they lower the activation energy? However, they do not alter the equilibrium of the reaction.

In the first section of this unit you will learn about various theories of chemical kinetics. Purpose of studying these theories is to gain an understanding of reaction mechanism which will later help us in understanding enzyme kinetics. The concept of reaction rates, thermodynamics of reaction, transition state, activation energy, and collision theory will facilitate our understanding of enzyme kinetics and thus enzyme catalysis.

You have read about the two characteristic properties of enzymes in the previous unit, catalytic efficiency and substrate specificity. What accounts for the specificity of enzymes? The specificity of enzymes seems to be contributed by the arrangement of functional groups in the enzyme active site.

In the later sections we will also discuss lock and key hypothesis and induced fit hypothesis, the two models that explains the enzyme substrate interaction.

Objectives

After studying this unit, you should be able to:

- define rate of reaction;
- define Gibbs free energy;
- explain how enzymes lower the activation energy;
- describe active site; and
- differentiate between lock & key and induced fit hypothesis

2.2 THERMODYNAMICS OF CHEMICAL REACTION

Do you know the three laws of thermodynamics? These laws of thermodynamics are equally applicable to biological processes. Let us see how these laws of thermodynamics are applicable in an enzyme catalyzed reactions. The first law states that energy can neither be created nor destroyed, but can be converted into other forms of energy. The second law of thermodynamics is the "law of increased entropy". Entropy (denoted by S) is a measure of randomness or chaos within a closed system. This law states that total entropy of the system and surroundings always increase for a spontaneous process.

Now, for all the chemical or biochemical reactions, there are two driving forces, enthalpy and entropy. Enthalpy is defined as the heat content of a system (denoted by H). In biological systems temperature is assumed to be constant (denoted by T). In the year 1878, J W Gibbs gave a relation as a function of T and S, which is popularly referred to as free energy or Gibbs free energy.

$\Delta G = \Delta H - T\Delta S$ where ΔG represents free energy change.

The free energy change during the reaction process under standard set of conditions (Temperature, 298 K; partial pressure, 1 atmosphere; concentration of solutes, 1M) is called as standard free energy change $\Delta G°$. **Free energy change must be negative for a reaction to be spontaneous.** A reaction is said to be spontaneous if it occurs without being driven by some external force.

Thus, for a reaction, if ΔG is negative, reaction is spontaneous and if ΔG is positive, reaction will not take place spontaneously. For a biological reaction, ΔG is the energy available for work (osmotic work, muscular work or biosynthetic work).

SAQ 1

Do as Directed:

a) What amount of enzyme is consumed in the enzyme catalyzed reaction?

b) Each enzyme generally catalyzes ..... reaction.
(Specific/Any) (Fill in Blank)

c) The ΔG of a reaction depends only on the free energy of the products (the final state) minus the free energy of the reactants (the initial state). (True/False)

d) If the free energy change in a reaction is negative, it indicate that the reaction releases/absorbs energy. (Choose one option)

e) Free energy change must be positive for a reaction to be spontaneous. (True/False)

2.3 FACTORS AFFECTING RATE OF CHEMICAL REACTIONS

Molecules react when they come in contact with each other. Therefore any factor that increases the rate of collisions i.e increased concentration of reactants or an increase in the temperature of a reaction will increase the rate of reaction. There are several factors that influence the rate of chemical reaction. As you know the uncatalysed reactions in a biological system tend to be slow as most of the biomolecules are stable at neutral pH and mild temperature. Simple reactions such as digestion of food or any muscle movement will not occur or occur at a very slow rate without catalysis. Enzymes catalysts enhance the rate of reaction. Let us discuss factors affecting the rate of chemical reaction.

2.3.1 Collisions, Activation Energy

You would like to recollect from your school chemistry class that for chemical reactions to occur, molecules must collide with one another. However, not all colliding molecules react. Not all the colliding molecules of similar types will have equivalent amount of energy. The reactions will take place only when molecules collide in specific orientation and sufficient energy. The molecules must possess energy to overcome the energy barrier to the reaction. This is also known as **activation energy**. The activation energy is an energy barrier between substrates and products. For conversion of substrates to products, energy barrier is the energy required to align reacting molecules as well as formation of transient unstable intermediates before the formation of products.

Look at Fig 2.1, in the coordinate diagram, free energy of the system is plotted against the reaction process. In the Fig. you will notice that there is an **energy barrier** between S (substrate) and P (product). This energy barrier is known as **activation energy** or **energy of activation (E_A).** E_A is defined as the

energy required to overcome the energy barrier so that substrate/s can be converted into product.

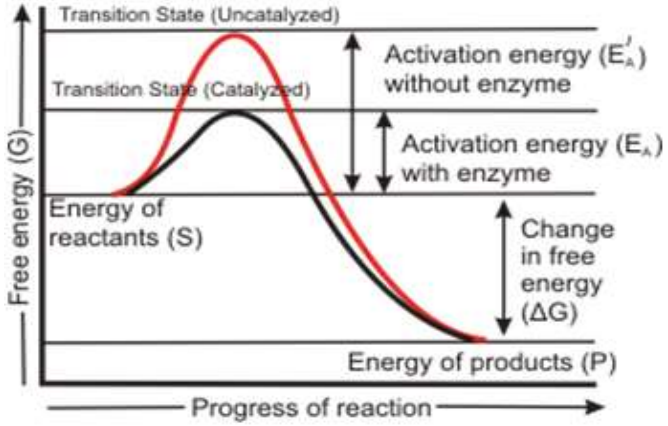


Fig. 2.1: Effect of enzyme on the activation energy. S and P denote the ground state of substrate and product respectively.

Once again look at Fig. 2.1 and you will notice that the activation energy of an uncatalysed reaction is very high than the catalyzed one. Here comes the role of a catalyst. It lowers the activation energy and thus facilitates the achievement of **transition state**, a phase which eventually leads to the formation of product and vice versa. Enzymes help to overcome the energy barrier so that more of the substrate molecule can be converted into product. How does it help the reaction process? The enzyme and the substrate form a reaction intermediate which helps to lower the activation energy than the reaction between reactant molecules without a catalyst. The enzyme substrate interaction creates a new reaction pathway whose **transition state** energy is lower than the reaction taking place without the enzyme. Breaking of older bonds and formation of new bonds require energy for the alignment of reacting groups, formation of transient unstable charges, and this energy comes from the binding energy from enzyme – substrate interaction. The free energy (binding energy) released by the interactions of enzyme and substrate offsets the energy required to reach the top of energy hill (Fig. 2.1) and results in lower activation energy.

2.3.2 Transition State Theory

The binding energy is a major supply of free energy used by enzymes to lower the activation energies of reactions. It adds to the specificity as well as to the catalytic power of the enzyme. In other words, binding energy acts as a driving force for the enzyme catalysis. There are several other factors that play a significant role related to the free energy -physical and thermodynamic factors. These include- entropy, solvation etc. The weak bonds between substrate and enzyme in transition state during reaction will reduce the hydrogen bonds between substrate and water (desolvation). The binding energy holds the molecules in precise alignment so that they can undergo the reaction, thereby reducing the entropy. The reduction in the motion of reactants along with weak interactions between enzyme and the substrate molecules will increase rate of reaction in an enzyme catalyzed reaction. Look at Fig. 2.1 & 2.2 it is evident from the reaction coordinate, the substrate has to overcome a free energy

barrier before the products can be formed. This energy barrier is known as the **activation energy (E)** of the reaction. The molecular structure and conformation corresponding to the peak position in this profile is called the **transition state** of the reaction. For an enzyme catalyzed reaction we call it enzyme-substrate complex or ES-complex. In the transition state the chemical bonds are in the process of being formed or broken. It is, therefore, the most activated and hence highly unstable entity in the reaction pathway.

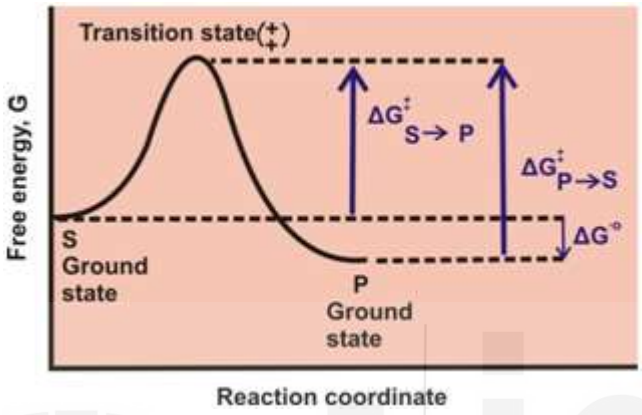
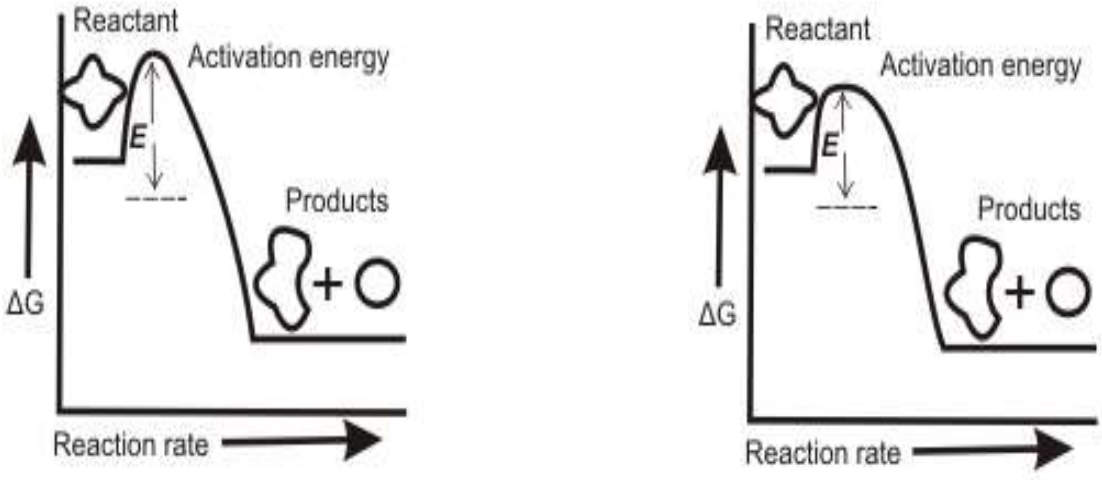


Fig. 2.2: Reaction coordinate showing free energy gap between substrate and product and transition state.

The concept of the transition state helps to express the rate of reaction, in terms of the free energy of activation. Since the molecules in the transition state are the ones which lead to the formation of products, the rate of reaction will depend on the concentration of molecules in the transition state.

Enzymes help in achievement of transition state easily and thus bring about a remarkable increase in the reaction rate. It should be clear from Fig. 2.3(a) and Fig. 2.3(b), that enzyme only decreases the free energy of activation, so that more and more of reactant molecules pass the energy barrier to form the products. Here you must note that, the enzymes do not change the equilibrium point of the reaction which is related to the free energy of the reaction (ΔG;) but merely speed up the rate at which the equilibrium point is reached.



(a) Uncatalyzed Reaction **(b) Enzyme Catalyzed Reaction**

Fig. 2.3 a & b: Effect of enzymes on the activation energy.

2.3.3 Reaction Rate

You would have read about rate of reaction and order of a reaction in your school curriculum. Let us go through it briefly. For any reaction, the rate is proportional to the product of the activities of reactant, each activity being raised to the power of the number of that reactant taking part as given in the following equation:

For example for a reaction $aA + bB \rightarrow P$

where A and B are two moieties reacting together to form product P, for practical purposes activity is usually replaced with concentrations

rate is expressed as $Rate = k[A]^a[B]^b$

where k is proportionality constant known as rate constant.

In simpler terms reaction rate is the measure of velocity, whether reaction is happening fast or slow. Rate of reaction at time t (V_0) is the measure of the amount of substrate transformed per unit time or product formed per unit time. Normally V_0 is expressed in terms of µmoles per minute or µmol min^{-1} [1 µ (micro) = 10^{-6}]

Whereas order of a reaction corresponds to the molecularity of the reaction, i.e the number of molecules which must simultaneously collide during the reaction so that product can be formed.

For example, $A \rightarrow P$, is unimolecular or first order reaction

$2A \rightarrow P$, is bimolecular or second order reaction, also

$A + B \rightarrow P$, is bimolecular or second order reaction.

So, molecularity or order of a reaction is measure of the number of molecules simultaneously colliding for reaction to take place. Generally, reactions are of the first or second order i.e. unimolecular or bimolecular. Trimolecular reactions also occur unusually. There is zero order reaction as well. In zero order reactions, reaction rate is independent of the substrate concentration.

SAQ 2

State whether the following statements are True or False:

a) Catalysis alter the state of energy of reactants and products (True/False)

b) Collision theory states that effective collisions lead to chemical reactions. (True/False)

c) Enzymes increase the energy of activation so that more activated substrate molecules can be converted into product. (True/False)

d) The transition state of a catalyzed reaction is lower in energy than that of an uncatalyzed reaction. (True/False)

e) The rate of formation of the transition state intermediate determines the overall reaction rate. (True/False)

2.4 SPECIFICITY OF ENZYMES

In this section you will study about the key characteristic property of enzyme; specificity. Enzymes are highly selective molecules and their catalytic activity depends on the integrity of their native protein conformation.

The catalytic power of enzymes is due to the precise molecular interactions between enzyme-substrate that occur at the active site, which lower the energy barrier and enable the formation of transition state. These interactions are the same which are responsible for the three dimensional structure of a protein i.e. non-covalent interactions like hydrogen bonding, van der Waal's attraction, hydrophobic and ionic interactions. The energy is released from these interactions and is termed as **binding energy**. Binding energy serves two purposes: it is the driving force for catalysis as well as responsible for enzyme specificity.

What makes the enzyme extraordinary? There are two explanations. First lies in rearrangement of covalent bonds in an enzyme catalyzed reaction. Covalent interactions between substrate and the enzyme lower the activation energy. The second explanation lies in non-covalent interactions between substrate and enzyme. These weak binding interactions between enzyme and the substrate also provide ample driving force for enzymatic catalysis.

Thus, specificity of enzymes seems to be contributed by the arrangement of functional groups in the enzyme active site. A well defined arrangement of atoms in the active site of enzyme molecule enables the enzyme to discriminate between substrate and a competing molecule. It gives enzymes its specificity. Specificity means the complementarity between substrate and active site of the enzyme i.e. enzyme-substrate interaction subsequently leading to formation of ES-complex. Lock and key hypothesis beautifully explain the concept of complementarity. Every lock has its own key, every key fit very well in the space given in the lock and is capable of opening it. Similarly, substrates show specificity/complementarity towards specific enzyme. It may be **geometric** (shape) or **electronic** (electrostatic attraction). Geometric specificity can be understood with the help of lock and key hypothesis whereas electronic specificity can be explained with the help of induced fit hypothesis. You will study about these two hypotheses in the next section.

Let us focus on different types of specificity with their relevant examples. **Absolute specificity** refers to enzyme catalysis of reaction with only one substrate for eg.*carbonic anhydrase* which acts only on carbonic acid, *lactase* which acts on lactose etc, while **group specificity** means enzyme catalysis of reactions involving structurally related group of compounds for eg. *Endopeptidases* like pepsin, trypsin etc. **Optical specificity** is about catalyzing only one of the two optical isomers either D or L form, eg. *L-amino acid oxidase* acting on L-amino acids while **geometrical specificity** is catalyzing only a *cis* or *trans* bond for eg. *fumarase* catalyzes the interconversion of fumaric and malic acids, it does not react with maleic acid which is the *cis* isomer of fumaric acid or with D-malic acid.

Substrate molecules are comparatively much smaller than the enzyme molecules, we have mentioned about ES interaction and formation of ES complex many a times. So where do these interactions occur? Have you ever thought in which part of the enzyme they bind? Is entire enzyme molecule or only few of its constituent amino acids are involved in the catalysis? Answer to this lies in the **active site** which you will study now.

2.4.1 Active Site

You would like to know whether entire enzyme molecule is involved in the catalysis or only few of its constituent amino acids? The answer to the above questions lies in the relocation of covalent bonds during catalysis. The functional groups of enzymes and substrate/substrates interact with each other and may form a transient covalent bond. These interactions take place at the **active site** of enzymes and help to lower the activation energy, thereby increasing the rate of reaction.

The active site is also termed as 'catalytic site'. Any enzyme-catalyzed reaction takes place within the confines of active site (Fig. 2.4). The molecule that is bound in the active site and acted upon by the enzyme is called the 'substrate'. Although the enzymes differ widely in their properties, the active site of any enzyme molecule possesses some common features.

- Active site is very small as compared to entire enzyme structure.
- It is three dimensional cleft or crevice.
- Amino acid sequences from different parts of linear protein are present in the active site. For example, chymotrypsin (His 57, Asp 102, Ser 195) [Fig. 2.5a], carbonic anhydrase II (His 94, His 96, His 119) [Fig. 2.5b]
- Binding of substrate with the amino acid functional group in the active site is by relatively weak molecular interactions.
- Generally, water is largely excluded from the active sites in the enzyme molecule. The active site contains charged or hydroxy amino acids such as aspartic acid, glutamic acid, lysine serine etc. The side chain groups like -COOH, $-NH_2$, $-CH_2OH$ etc., serve as catalytic groups in the active site. Besides, the crevice creates a micro-environment in which certain polar residues acquire special properties which are essential for catalysis.

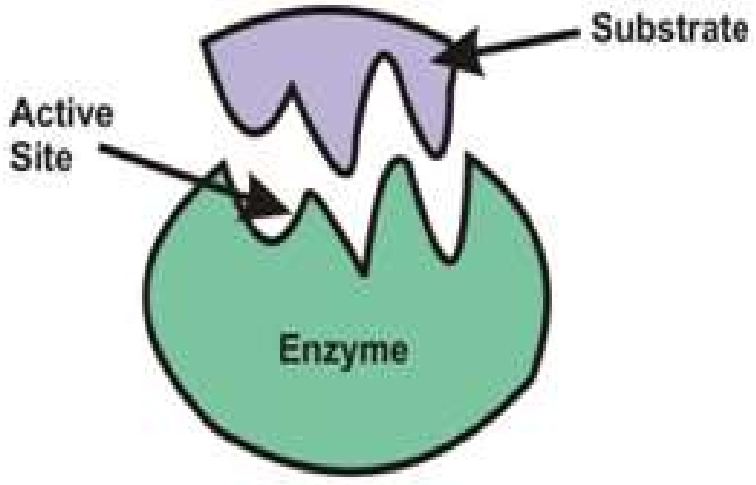


Fig. 2.4: Schematic representation of the active site of an enzyme.

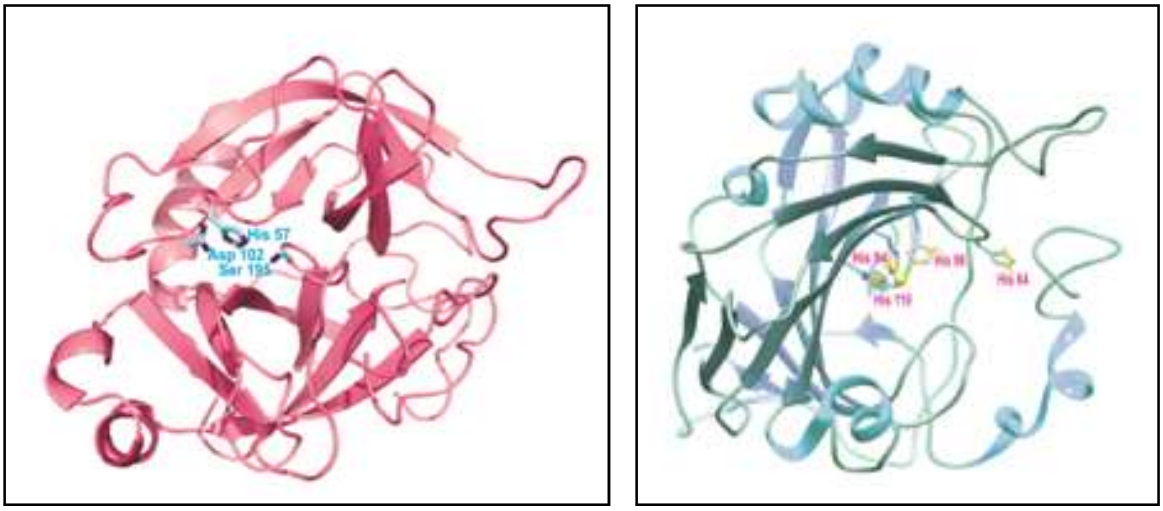


Fig. 2.5: Folded structure of a) Chymotrypsin and b) Carbon anhydrase revealing amino acids from different positions in the active site.

SAQ 3

Do as Directed:

a) The part of enzyme where substrate binds is called as ..... . (Fill in the Blank)

b) The decrease of activation energy in an enzyme catalyzed reaction comes from the ..... interactions between substrate and enzyme in the ES complex. (Fill in the Blank)

c) Active site of enzyme is big/small as compared to entire enzyme structure (Choose one option).

d) An active site is normally hydrophilic in nature. (True/False)

To understand the enzyme substrate binding, studies on enzyme specificity has led to the postulation of two important models as discussed in the following sections.

2.5 FISHER LOCK AND KEY HYPOTHESIS

In 1894, Emil Fisher proposed that 'enzymes are structurally complementary to their substrates, so that they fit together like a lock and key' (Fig. 2.6). This model is also known as template model. According to this model, enzyme-substrate interaction is like a key fitting into its lock. As we have different set of keys for different locks, similarly different enzymes possess unique active sites for variable substrates. This also accounts for enzyme-substrate specificity. This analogy has proved to be very useful and has greatly influenced the development of biochemistry, since such interactions lie at the heart of many biochemical processes.

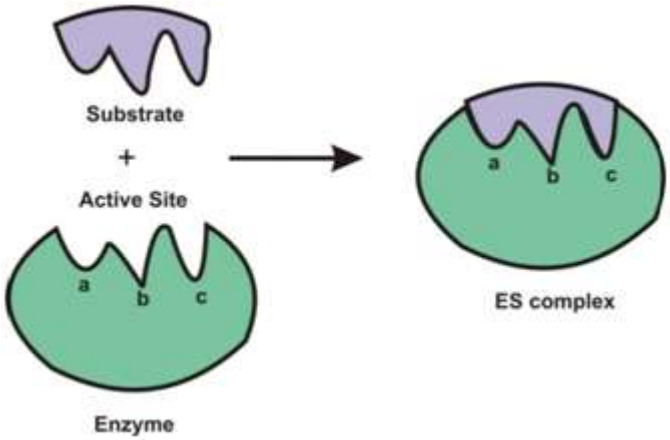


Fig. 2.6: Representation of the enzyme–substrate interaction in Lock and Key model.

The lock and key hypothesis is focused on the active site of enzyme. It fails to explain the stabilization of enzyme. The active site of enzyme as per Lock and Key mechanism seems rigid (lock) and matches with the structure of substrate (key). It does not visualize the weakening of enzyme substrate bonds proposed in transition state theory. Active site seems to be more static and a single entity with no separate catalytic group. The observations from X-ray diffraction studies reveal that active site of enzyme is a much more flexible structure than postulated by Lock and Key model of enzyme catalysis.

2.6 INDUCED FIT MODEL

Koshland Jr., in 1958 postulated that "Enzymes are flexible and that the shapes of the active sites can be markedly modified by the binding of substrate'. In the Fisher's lock and key model, the active sites are rigid structures. But this is not the case always. It has been observed that the active sites of some enzymes assume a shape that is complementary to that of the transition state only *after* the substrate is bound. That is, the proximity of the substrate induces a conformational change in the enzyme molecule aligning the groups for both substrate binding and catalysis. This process of dynamic recognition is called *induced fit* (Fig. 2.7). It has also been postulated that enzyme induced reciprocal changes in substrate and harness the binding energy to facilitate the transformation of substrates to products.

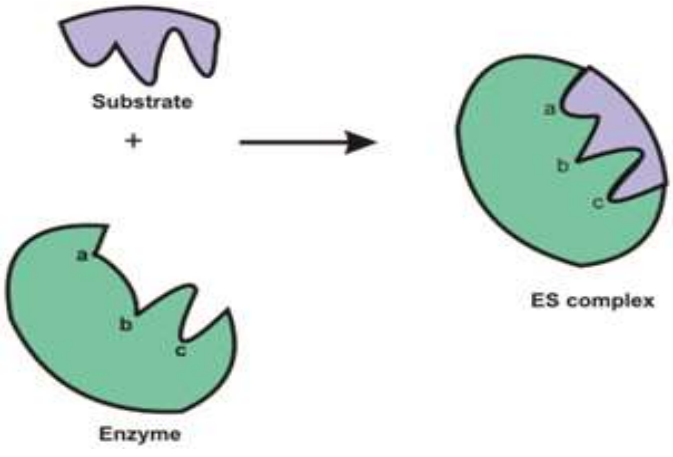


Fig. 2.7: Pictorial representation of enzyme–substrate interaction in the induced fit model.

This model envisages the presence of catalytic group in the active site. The interactions between substrate and the enzyme and transition state formation as well as weakening of interactive bonds by nucleophilic or electrophilic attack are well explained in the induced fit mechanism.

Generally, an enzyme catalyzed reaction is 10^6 to 10^{12} times faster than any non-catalyzed reaction. The enzyme-substrate interactions occur at the molecular level. So it will be difficult to find the exact and accurate nature of these processes. Even enzymes differ in their specificity, some of the enzymes are compatible with several substrates- similar side chains or functional groups. There are several other factors that affect enzyme activity such as temperature and pH. An increase or decrease in the temperature alters the shape of active site of enzyme. There are six types of enzymes with different characteristics and functions. The definitive answer to enzyme substrate catalysis remains unclear.

SAQ 4

Do as Directed:

a) The Lock and Key hypothesis explain the mechanism of .....
(Fill in the Blank)

b) In the Fisher's lock and key model, the active sites are rigid structures.
(True/False)

c) The active site of the enzyme perfectly matches the shape of the substrate just as a lock perfectly matches the shape of its respective key.
(True/False)

d) From the two models (Induced Fit and Lock and Key) of enzyme catalysis, which one suggests that enzyme is changed by the reaction?

2.7 SUMMARY

1) Enzymes can increase reaction rates many fold, which enables biochemical reactions to occur under physiological conditions.

2) The role of catalyst is to lower the energy barrier to reach the transition state so that more and more substrate molecules can be converted to product.

3) The rate of reaction of an enzyme catalysed reaction is 10^6 to 10^{12} times more than the uncatalysed one.

4) Free energy is the energy available to do work. Free energy change during the reaction process under standard set of conditions (Temperature, 298K; partial pressure, 1 atmosphere; concentration of solutes, 1M) is called as standard free energy change $\Delta G°$.

5) Free energy change must be negative for a reaction to be spontaneous. A reaction is said to be spontaneous if it occurs without being driven by some external force.

6) Collision theory explains that for a reaction to happen, reaction species must collide with each other in right orientation so that product formation can take place. There is an **energy barrier** between S (substrate) and P (product). This energy barrier is known as **activation energy** or **energy of activation (E_A).** E_A is defined as the energy required to overcome the energy barrier so that substrate/s can be converted to product.

7) A catalyst lowers the activation energy and facilitates the achievement of **transition state**. The enzyme substrate interaction creates a new reaction pathway whose **transition state** energy is much lower than the reaction taking place without the enzyme. Lowering of activation energy is achieved by bringing the reactants in close proximity and in correct orientation for reaction to occur.

8) At the active site, substrate is subjected to stress and strain, which facilitates bond breaking and making. The energy for this is derived from enzyme-substrate interaction in the active site. Enzyme substrate interaction leads to release of energy. The energy released from these interactions is termed as **binding energy**. Binding energy serves two purposes: it is the driving force for catalysis as well as responsible for enzyme specificity.

9) Enzymes are characterized by an active site, where the substrate (reactant) binds, undergoes transformation and then the product is subsequently released. The active site of an enzyme consists of amino acid side chains, some of which act as binding groups while others act as catalytic groups. The amino acid side chains of catalytic groups are of polar type only.

2.8 TERMINAL QUESTIONS

1) Define and explain the 'transition state' of a reaction.

2) How does an enzyme contribute to the lowering of energy of activation of an enzyme catalyzed reaction?

3) Describe enzyme specificity.

4) What are the characteristic features of an active site?

5) Explain the difference between lock and key hypothesis and induced fit model.

2.9 ANSWERS

Self-Assessment Questions

1) a) Nil, b) Specific, c) True, d) releases, e) False

2) a) False, b) True, c) False, d) True, e) True

3) a) Active site, b) Non-Covalent interactions, c) Small, d) False

4) a) Enzyme specificity, b) True, c) True, d) None

Terminal Questions

1) Transition state is a phase which eventually leads to the formation of product. The enzyme substrate interaction creates a reaction pathway whose transition state energy is lower than the reaction taking place without the enzyme. During the transition state breakage of older bonds and formation of new bonds takes place.

2) Energy barrier between substrate and product known as activation energy or energy of activation (E_A) is lowered by enzyme substrate interaction. In enzyme catalysed reaction, transition state energy or energy required to overcome the energy barrier is much lower than the reaction taking place without the enzyme. Lowering of activation energy is achieved by bringing the reactants in close proximity and in correct orientation for the reaction to occur. Also, at the active site, substrate is subjected to stress and strain, which facilitates bond breakage and formation. The energy for this is derived from enzyme-substrate interaction at the active site.

3) Specificity of enzymes seems to be contributed by the arrangement of functional groups in the enzymes active site. A well defined arrangement of atoms in the active site of enzyme molecule enables the enzyme to discriminate between substrate and a competing molecule. It gives enzymes its specificity. By specificity, we mean the complementarity between substrate and active site of the enzyme i.e. enzyme-substrate interactions subsequently leading to the formation of ES-complex. It may be geometric (shape) or electronic (electrostatic attraction).

4) Refer to section 2.4.1

5) Refer to section 2.4 and 2.5

2.10 SUGGESTED READINGS

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UNIT 3

FACTORS AFFECTING ENZYME ACTIVITY

Structure

3.1 INTRODUCTION

In the first two units of this Block you have learnt about what are enzymes and how do they work. There are a number of factors that have an effect on the enzyme's activity, such as the temperature, hydrogen ion concentration (pH), concentration of the enzyme, concentration of the substrate and inhibitors. In this unit you will study more about these factors. As you know that enzymes are proteins and three- dimensional structure of a protein is crucial for its catalytic activity. The change in physical factors such as temperature and pH profoundly affect the speed of an enzymatic reaction by altering the enzyme activity. Similarly, the concentration of the reactant (substrate) and product also affects the enzyme activity. We will also discuss the effect of metal ions on the enzyme activity in this unit.

Objectives

After studying this unit, you should be able to:

- enlist factors affecting enzyme catalysis;
- explain the effect of pH and temperature on enzyme; and
- discuss how substrate concentration affects enzyme activity.

3.2 FACTORS AFFECTING ENZYME ACTIVITY

While studying proteins in Course BBCCT-101, you have learnt that the three dimensional structure of proteins (enzymes) is important for their catalytic activity. Apart from the peptide bond which links amino acids together in a chain, a protein is stabilized by various intermolecular forces like hydrogen bonding, vander Waals attraction, electrostatic interaction and so on. Changes in the physical or chemical environment of an enzyme affect its structure, which in turn affects its activity. In this section you will study about various factors like temperature, pH, concentration of substrate, concentration of enzyme, concentration of product and how these factors affects the enzyme activity.

Effect of Temperature

Enzymes are biological catalysts. So how does change in temperature affects the enzyme activity? Every enzyme has an optimum range of temperature at which enzyme shows its maximum activity. Generally, most of the enzymes have optimal temperature of 40^{o}C, which is close to the physiological body temperature (37.5^{o}C). At high temperature typically between 42-70^{o}C enzymes get irreversibly denatured.

At temperatures above and below the optimum range, activity of enzyme declines. This is because, at very low temperature enzyme activity gets arrested while at very high temperatures the three dimensional structure of the enzyme is lost. High temperature destroys the intermolecular attractions like hydrogen bonding which lead to loss of functional structure of enzyme, hence the affinity of enzyme for substrate diminishes and then its activity declines. For details, refer to tertiary structure of proteins in Course BBCCT-101, Unit 4.

If you look at the temperature versus activity graph (Fig. 3.1), it's a bell shaped curve, depicting maximum enzyme activity at the optimum temperature. In this context, one important fact which you must note is that increase in rate of reaction with increase in temperature is due to increase in the number of collisions between the enzyme and substrate molecules as well as also due to increase in the energy of these collisions.

The change in rate of reaction for any 10^{o} rise in temperature is known as its **Q_{10}, or temperature coefficient value**. This value is close to 2 for most of the chemical reactions. However, for enzymatic reactions, Q_{10} values are lower.

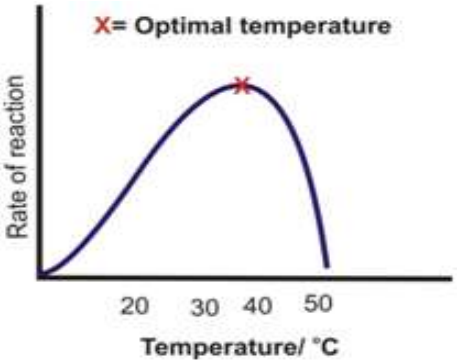

Fig. 3.1: Plot of temperature versus rate of reaction.

The activity of most of the enzymes is usually destroyed by heat. However, some exceptions are the enzymes of bacteria, living in hot springs at temperature of 60-80^{o}C.

Effect of pH

pH i.e. change in hydrogen ion concentration has similar effect on the activity of enzyme as temperature. Enzymes in general work within a small range and most of them have a particular pH at which they exhibit optimal activity. The particular pH of enzymes is the pH value at which the bonds within enzyme are influenced by the H^+ and OH^- ions in such a way that the shape of the active site of enzyme is most complementary to the shape of its substrate. Any change in pH can alter the state of ionization (e.g amino acids Asp, Lys) that plays a crucial role in the substrate binding and/ or the catalytic action. Therefore, alteration in pH leads to loss of enzyme structure and its activity. Each enzyme, thus, acts best in a certain pH range which is specific to it and its activity slows down with any appreciable change i.e. increase or decrease in the H^+ ion concentration. At optimum pH, enzymes show maximum activity (Fig. 3.2). Generally most of the enzymes have optimal pH of 7.4 which is also the physiological pH. The activity generally falls off on either side of this value. Deviations above 0.5 pH unit from the normal blood pH of 7.4 leads to protein denaturation. Although the covalent bonds are not broken but even disruption of some hydrogen bonding leads to loss of enzymatic activity.

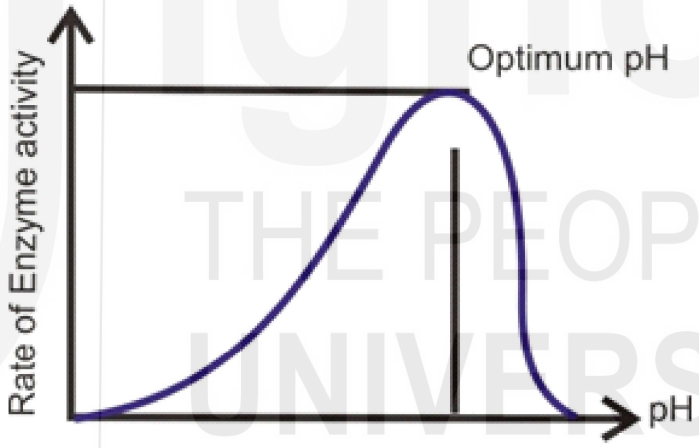

Fig. 3.2: Plot of pH versus enzyme activity.

Question: Have you ever thought why metabolic rate increases during fever?

Answer: The metabolic rate increases partially due to increased temperature and also because of the effect of temperature on enzyme activity.

Usually maximum enzyme activities are obtained at or near the isoelectric point (pI) of the enzymes, pH optimum of some enzymes is given in the Table 3.1. For example, trypsin, whose pI value is 10.1, shows maximum activity at pH between 7 and 9.

Table 3.1: pH optimum of some common enzymes

Sl. No.	Enzyme	pH optimum
1	Pepsin	1.5-1.6
2	Lipase (Stomach)	4.0-5.0
3	Lipase (Pancreas)	8.0
4	Catalase	7.0
5	Trypsin	7.8-8.7

The little changes in the pH above or below the optimum do not lead to a lasting change to the enzyme, since the bonds can be renewed. However, acute changes in pH may cause denaturation of enzymes and they will lose their function permanently. Enzymes in dissimilar locations may have diverse optimum pH values since their environmental settings may be different.

Concentration of Substrate

The changes in the substrate concentration will affect the rate of an enzyme catalyzed reaction if it is a limiting factor or in other words substrate concentration becomes a factor if it stops the reaction from taking place at a higher rate. Therefore, in such condition, an increase in substrate concentration will lead to an increase in the rate of reaction.

Let us consider a single substrate-single enzyme reaction to understand the effect of substrate concentration on the rate of reaction. As you have studied in the previous section, in an enzymatic reaction the effective collision between the enzyme [E] and substrate [S] leads to the formation of an enzyme-substrate complex [ES], in which the substrate is firmly bound to the active site of the enzyme. This complex then breaks down to give the product [P] and release the original enzyme.

If the amount of enzyme is kept constant and substrate concentration is gradually increased, the rate of reaction increases linearly until it reaches a **maximum known as "point of saturation"** (Fig. 3.3). The point of saturation is where maxima is attained and is denoted by V_{max}. V_{max} is the maximum possible rate of reaction. Once the maxima is achieved, addition of more substrate molecules will not lead to an increase in the reaction rate as free enzyme becomes unavailable. To put it into other words this can be also explained as, since the concentration of enzyme is fixed, as more and more amount of substrate is added enzyme undergoes saturation leading to formation of ES complex and then product. However, when all the available enzymes are occupied then further increase in substrate concentration has no effect on the rate of reaction.

Shape of the curve depicting the effect of substrate concentration on rate of reaction / enzyme activity is a hyperbola and well explained with a mathematical equation known as Michaelis- Menten equation which you will study in next unit.

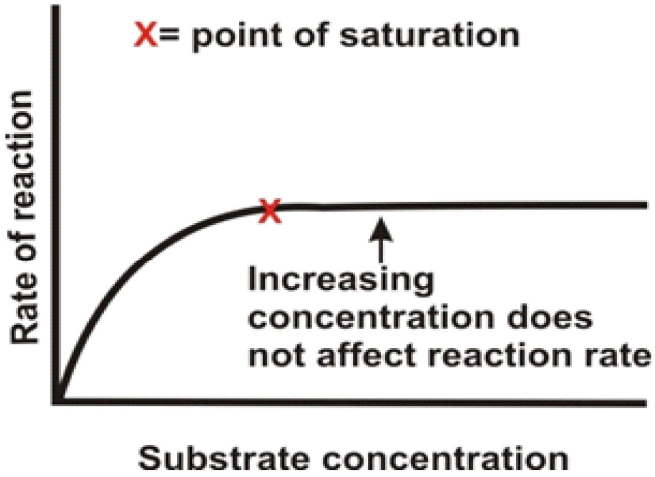

Fig. 3.3: Saturation curve for an enzyme obeying Michaelis-Menten equation, showing relation between substrate concentration and rate of reaction.

Concentration of Enzyme

Whether it is a chemical catalyst or an enzyme, catalysts are always needed in minute quantities. With other factors remaining constant during catalysis, if the concentration of enzyme is raised, rate of reaction will increase linearly upto a certain extent i.e. upto the “point of saturation” and then will become constant. This is because when all the available substrate has been bound to the active site of enzyme, then any further increase in the enzyme concentration has no effect on the rate of a reaction as substrate concentration becomes limiting.

But in the enzymatic reactions, the enzyme concentration is generally very low as compared to the substrate concentration. So, in order to study the effect of enzyme concentration on the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration. Any change in the amount of product formed over a specified period of time will be dependent upon the level of enzyme present. Generally under such conditions, rate of reaction is proportional to the concentration of the enzyme. If enzyme concentration is doubled, the rate of conversion of substrate to product shows a corresponding increase (Fig. 3.4).

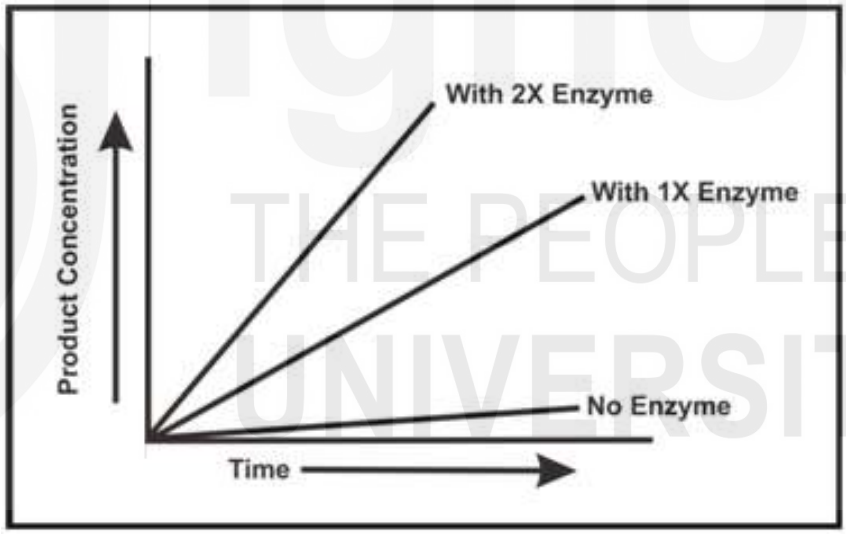

Fig. 3.4: Plot showing rate of product formation at different concentrations of enzyme.

Concentration of Product

Accumulation of reaction product decreases the enzyme activity. In case of certain enzymes, the product formed when present in large excess binds loosely at the active site of the enzyme and inhibits the enzyme activity. This is also known as feedback inhibition. This situation can be reversed by quick removal of the product. For example activity of aspartate transcarbamoylase. You will study this in detail in unit 8 (Block 3) of this course.

Inhibitor

Any substance which either slow down or stop the activity of enzyme is termed as inhibitor. The inhibition of enzyme activity can be reversible or irreversible. You will study all this in detail in unit 6 (Block 2) of this course.

3.3 EFFECT OF METAL IONS ON ENZYME ACTIVITY

Several metal ions play a significant role in performing the biological activity of enzymes. However, you may want to know how metal ions affect the enzyme activity. Let us discuss in the following section.

As you know, some of the well known metal ions include Sodium (Na^+), Potassium (K^+), Iron (Fe^{2+}), (Fe^{3+}), Calcium (Ca^{2+}), Zinc (Zn^{2+}), Magnesium (Mg^{2+}), Chloride (Cl^-) etc. Metal ions are an essential part of many enzymes and are indispensable in many catalytic reactions. So removal of metal ion often leads to loss of enzyme activity. The enzyme activity can also be restored by replacing the original metal ion. The various means of metal-protein (enzyme) interface include metal**-**, ligand**-**, and enzyme-bridge complexes. Metals can take up role as electron donors or acceptors, Lewis acids or structural regulators. Several mechanisms by which metal ions affect the rate of enzyme catalyzed biological reactions are:

a) Metallosubstrate: Metal ion joins the substrate to form metallosubstrate which further form enzyme metal substrate complex and then leads to the formation of product.

b) Metalloenzyme: Metal ion combines with the enzyme to form metalloenzyme and interact with substrate to form enzyme metal substrate complex. The complex then leads to the formation of product.

c) Change in enzyme conformation: The binding of metal ion to the enzyme brings a change in the conformation of enzyme, thereby transforming the enzyme into an active form.

d) Direct involvement in catalysis: Several metal ions participate in the biological electron transport reactions. These ions get involved directly in oxidation-reduction reactions by means of variation in their valency.

SAQ 1

Tick [✓] mark the correct option:

a) Following factors can affect enzyme activity

   i) temperature and pH

   ii) presence of certain metal ions

   iii) addition or removal of phosphate

   iv) all of the above

b) Which of these is not a regulator of enzymatic activity

   i) Concentration of enzyme/substrate

   ii) pH

   iii) Lipid

   iv) Temperature

c) Enzymes generally have

i) Same pH and temperature optima

ii) Same pH but different temperature optima

iii) Different pH but same temperature optima

iv) Different pH and different temperature optima

d) Beyond optimum temperature, enzyme

i) becomes hyperactive

ii) divides to form smaller enzymes

iii) becomes denatured

iv) all of the above

e) The initial rate of an enzyme catalyzed reaction depends on

i) the concentration of the enzyme

ii) the concentration of the substrate

iii) the affinity of the enzyme for its substrate

iv) all of the above

3.4 SUMMARY

1) Various factors affect or alter the activity of enzymes. These factors can be physical or chemical for example temperature, pH, concentration of substrate etc.

2) Every enzyme has an optimum temperature at which it shows its maximum activity. Generally, most of the enzymes have optimal temperature of 40^0 C. The change in rate of reaction for any 10^0 rise in temperature is known as its **Q_{10}, or temperature coefficient value**. This value is close to 2 for most of the chemical reactions. However, for enzymatic reactions, Q_{10} values are lower.

3) Generally most of the enzymes have optimal pH of 7.4 which is also the physiological pH. The activity generally falls off on either side of this value.

4) Enzyme activity is also affected by substrate concentration. Shape of the curve depicting the effect of substrate concentration on rate of reaction / enzyme activity is a hyperbola and well explained with a mathematical equation known as Michaelis- Menten equation.

5) Metal ions are an integral part of many enzymes and are essential in many catalytic reactions. The binding of some metal ions increases stability of proteins (enzymes) or protein domains.

3.5 TERMINAL QUESTIONS

1) What are the various factors affecting enzyme activity?

2) How does temperature and pH affect the activity of enzymes?

3) Enzyme activity depends on substrate concentration. Illustrate the statement

4) List some of the well-known metal ions affecting enzyme activity.

3.6 ANSWERS

Self-Assessment Questions

1) a) iv; b) iii; c) iv; d) iii: e) iv

Terminal Questions

1) pH, temperature, substrate concentration, enzyme concentration, product concentration, inhibitors.

2) Temperature and pH beyond optimum range denature the enzyme resulting in loss of enzyme activity.

3) To study the effect of substrate concentration on enzyme, the concentration of enzyme is kept constant, and as more and more amount of substrate is added to the reaction mixture, enzyme undergoes saturation and when all the available enzymes are occupied then further increase in substrate concentration has no effect on the rate of reaction. A hyperbolic curve is obtained.

4) Sodium (Na^+), Potassium (K^+), Iron (Fe^{2+}), (Fe^{3+}), Calcium (Ca^{2+}), Zinc (Zn^{2+}), Magnesium (Mg^{2+}), Chloride (Cl^-) etc (for details refer to section 3.3).