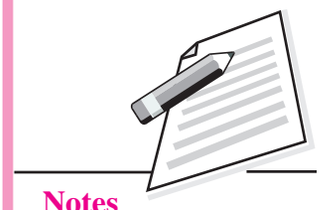


8

ENZYMES

8.1 INTRODUCTION

The global life depends on a series of chemical reactions. Most of the chemical reactions proceed too slowly on their own to sustain life. Hence catalysts are required to greatly accelerate the rates of these chemical reactions. In nature enzymes posses the catalytic power to facilitate life processes in essentially all life-forms from viruses to man. Most of the enzymes retain their catalytic potential even after extraction from the living organism. The above catalytic power of enzyme leads to commercial usage of enzymes.

In ancient days enzymes are used in manufacture of cheeses, breads, and alcoholic beverages, and for the tenderizing of meats. Today enzymes are also of fundamental interest in the health sciences. Human disease processes can be linked to the aberrant activities of one or a few enzymes. Because of their role in maintaining life processes, the assay and pharmacological regulation of enzymes have become key elements in clinical diagnosis and therapeutics.

The macromolecular components of almost all enzymes are composed of protein, except for a class of RNA modifying catalysts known as ribozymes. Ribozymes are molecules of ribonucleic acid that catalyze reactions on the phosphodiester bond of other RNAs. Enzymes are found in all tissues and fluids of the body. Intracellular enzymes catalyze the reactions of metabolic pathways. Plasma membrane enzymes regulate catalysis within cells in response to extracellular signals, and enzymes of the circulatory system are responsible for regulating the clotting of blood.

Almost every significant life process is dependent on enzyme activity. So the modern pharmaceutical research is based on the search for potent and specific inhibitors of these enzymes. This chapter briefs you about basic nature, classification and clinical significance of enzymes.

Notes

OBJECTIVES

After reading this lesson, you will be able to:

- define enzymes
- classify enzymes
- explain co-enzymes
- explain the factors affecting enzyme activity
- describe isoenzymes
- explain the Clinical significance of enzymes

8.2 DEFINITION AND CHARACTERISTICS OF ENZYMES

Enzymes are protein catalyst produced by a cell and responsible 'for the high rate' and specificity of one or more intracellular or extracellular biochemical reactions. Enzymes are biological catalysts responsible for supporting almost all of the chemical reactions that maintain animal homeostasis. Enzyme reactions are always reversible. The substance, upon which an enzyme acts, is called as substrate. Enzymes are involved in conversion of substrate into product. Almost all enzymes are globular proteins consisting either of a single polypeptide or of two or more polypeptides held together (in quaternary structure) by non-covalent bonds. Enzymes do nothing but speed up the rates at which the equilibrium positions of reversible reactions are attained. In terms of thermodynamics, enzymes reduce the activation energies of reactions, enabling them to occur much more readily at low temperatures - essential for biological systems. The basic characteristics of enzymes includes

(i) Almost all the enzymes are proteins and they follow the physical and chemical reactions of proteins

(ii) Enzymes are sensitive and labile to heat

(iii) Enzymes are water soluble

(iv) Enzymes could be precipitated by protein precipitating agents such as ammonium sulfate and trichloroacetic acid.

INTEXT QUESTIONS 8.1

1. Enzymes are ..... biocatalyst produced by cells.
2. Enzymes follow physical and chemical properties of .....

3. Enzymes are ..... soluble.
   (a) Water (b) acid (c) organic acids
4. Precipitation of enzymes could be achieved by using ..... and .....

8.3 CLASSIFICATION OF ENZYMES

Since earlier days to still date, fanciful names such as pepsin, chymotrypsin, etc were used to name enzymes. Later the suffix "ase" to the substrate was used to name enzymes. For example the enzymes lactase acts upon the lactate and produces glucose and galactose. The above method is known as "trivial naming" of enzymes. Currently enzymes are grouped into six functional classes by the International Union of Biochemists and Molecular Biology (IUBMB). As per the IUBMB system, each enzyme name starts with EC (enzyme class) followed by 4 digits.

The first digit represents the class, the second digit strands for the subclass, the third digit represents the sub-subclass or subgroup and the fourth digit provides the particular enzyme (Table 8.1)

Table 8.1 Classification of enzymes

Sl.No.	Classification	Biochemical Properties
1.	Oxidoreductases	Act on many chemical groupings to add or remove hydrogen atoms. e.g. Lactate dehydrogenase
2.	Transferases	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules. e.g. Aminotransferase.
3.	Hydrolases	Add water across a bond, hydrolyzing it. E.g. Acetyl choline esterase
4.	Lyases	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds. e.g. Aldolase.
5.	Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others. e.g. Triose phosphate isomerase
6.	Ligases	Catalyze reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP. e.g. Acetyl CoA carboxylase

Notes

These rules give each enzyme a unique number and specifies a textual name for each enzyme. Enzymes are also classified on the basis of their composition. Enzymes composed wholly of protein are known as simple enzymes in contrast to complex enzymes, which are composed of protein plus a relatively small organic molecule. Complex enzymes are also known as holo-enzymes. The non-protein component of an enzyme may be as simple as a metal ion or as complex as a small non-protein organic molecule. Enzymes that require a metal in their composition are known as metalloenzymes. Metalloenzymes bind and retain their metal atom(s) under all conditions with very high affinity. Enzymes with lower affinity for metal ion, but still require the metal ion for activity, are known as metal-activated enzymes. Based on requirement of ATP, enzymes are further classified into two types namely synthetases and synthase. Synthetases are ATP-dependent enzymes catalyzing biosynthetic reactions. Synthetases are enzyme belong to the class 6 (Ligases). Enzymes such as Carbamoyl phosphate synthetase, Arginino succinate synthetase and Glutamine synthetase are examples of the synthetases group of enzymes. The enzyme class other than ligases includes synthases. Synthases group of enzymes involves in catalyzing biosynthetic reactions that do not require ATP directly. Enzymes such as glycogen synthase and Alanine synthase are examples of synthase group.

INTEXT QUESTIONS 8.2

1. There are ..... main groups of enzymes.
2. Lactate dehydrogenase is example for ..... group of enzyme.
3. Enzymes that require a metal in their composition are known as .....
4. ..... are ATP dependent enzymes.
5. ..... and ..... are examples for synthase group of enzymes.

8.4 COENZYMES

Enzymes may be simple proteins, or complex enzymes. A complex enzyme contains a non-protein part, called as prosthetic group (co-enzymes). Co-enzymes are heat stable low molecular weight organic compound. The combined form of protein and the co-enzyme are called as holo-enzyme. The heat labile or unstable part of the holo-enzyme is called as apo-enzyme. The apo-enzyme gives necessary three dimensional structures required for the enzymatic chemical reaction. Co-enzymes are very essential for the biological activities of the enzyme. Co-enzymes combine loosely with apo-enzyme and are released easily by dialysis. Most of the co-enzymes are derivatives of vitamin B complex

group of substance. One molecule of the co-enzyme with its enzyme is sufficient to convert a large group of substrate.

Co-enzymes are further divided into two groups. The first groups of co-enzymes are a part of reaction catalyzed by oxidoreductase by donating or accepting hydrogen atoms or electrons. The first group of co-enzymes are also called as co-substrates or secondary substrates. Because they are involved in counter-balance in change occurring in the substrate. The second group of co-enzymes involves in reactions transferring groups other than hydrogen.

8.4.1 Role of Coenzymes

The functional role of coenzymes is to act as transporters of chemical groups from one reactant to another. The chemical groups carried can be as simple as the hydride ion ($H^+ + 2e^-$) carried by NAD or the mole of hydrogen carried by FAD; or they can be even more complex than the amine ($-NH_2$) carried by pyridoxal phosphate. Since coenzymes are chemically changed as a consequence of enzyme action, it is often useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different holoenzymes. In all cases, the coenzymes donate the carried chemical grouping to an acceptor molecule and are thus regenerated to their original form. This regeneration of coenzyme and holoenzyme fulfills the definition of an enzyme as a chemical catalyst, since (unlike the usual substrates, which are used up during the course of a reaction) coenzymes are generally regenerated.

INTEXT QUESTIONS 8.3

1. Non protein part of complex enzymes is called as .....
2. Combination of ..... and ..... are called as holo enzymes.
3. Co-enzymes are heat stable low molecular weight ..... compound.
4. There are ..... groups of co-enzymes.

8.5 FACTORS AFFECTING ENZYME ACTIVITY

Velocity or rate of enzymatic reaction is assessed by the rate of change in concentration of substrate or product at a given time duration. Various factors which affect the activity of enzymes include:

1. Substrate concentration
2. Enzyme concentration
3. Product concentration

4. Temperature
5. Hydrogen ion concentration (pH)
6. Presence of activators
7. Presence of inhibitor

8.5.1 Effect of substrate Concentration

Reaction velocity of an enzymatic process increases with constant enzyme concentration and increase in substrate concentration. The velocity (V) is expressed in micromoles of substrate converted per minute. As the concentration of substrate increases, the velocity of the reaction increases. Continued increase in substrate concentration may lead to a reduction in rate of the reaction and leads to flattened curve. The maximum velocity obtained from a enzymatic reaction is called as Vmax. Vmax represents the maximum reaction rate possible in the presence of excess substrate.

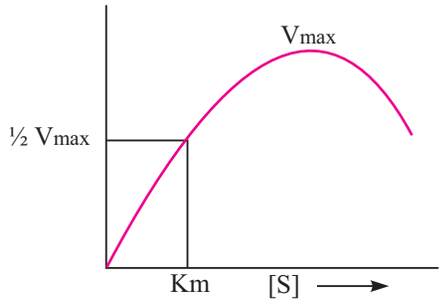


Fig. 8. 1: Effect of substrate concentration

8.5.2 Effect of enzyme Concentration

As there is optimal substrate concentration, rate of an enzymatic reaction or velocity (V) is directly proportional to the enzyme concentration. Presence of excess substrate and an increase in the enzyme concentration may result in some limitations. It is worthy of note that the enzymes are rarely saturated with substrates under physiological conditions.

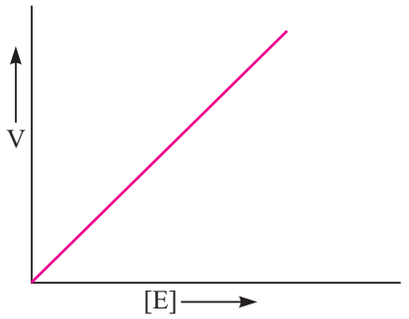


Fig. 8.2: Effect of enzyme concentration

8.5.3 Effect of product concentration

In case of a reversible reaction catalyzed by a enzyme, as per the law of mass action the rate of reaction is slowed down with equilibrium. So, rate of reaction is slowed, stopped or even reversed with increase in product concentration. This phenomena can be better explained by the equation

$$A \xrightarrow{E1} B \xrightarrow{E2} C \xrightarrow{E3} D$$

In the above equation, in case of absence of the enzyme E3, the product C will accumulate. Enzymatic activity of E2 will be inhibited with accumulation of the product C. In such inborn error of one enzyme will block the whole pathway.

8.5.4 Effect of temperature

Velocity of enzymatic reaction increases with temperature of the medium which they are most efficient and the same is termed as optimum temperature. As temperatures increases it leads to denaturation; a molecular arrangement which causes a loss of the active sites of the enzyme surfaces and results in a loss of efficiency.

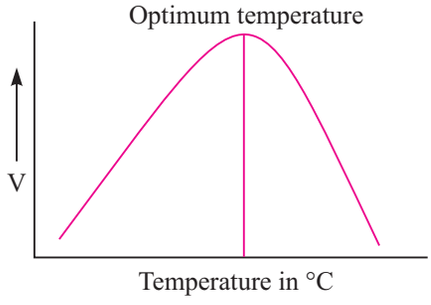


Fig. 8.3: Effect of temperature

8.5.5 Effect of pH

Like temperature, all enzymes have a optimum pH, at which the enzymatic activity will be at maximum. Many enzymes are most efficient in the region of pH 6-7, which is the pH of the cell. Outside this range, enzyme activity drops off very rapidly. Reduction in efficiency caused by changes in the pH is due to changes in the degree of ionization of the substrate and enzyme. Highly acidic or alkaline conditions bring about a denaturation and subsequent loss of enzymatic activity. Some exceptions such as pepsin (with optimum pH 1-2), alkaline phosphatase (with optimum pH 9-10) and acid phosphatase (with optimum pH 4-5) are even noticed.

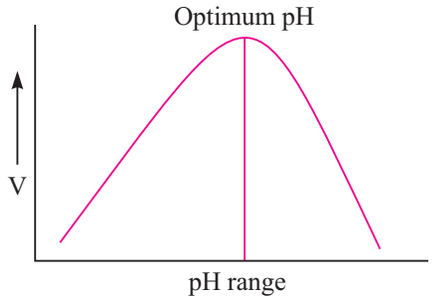


Fig. 8.4: Effect of pH

8.5.6 Presence of activators

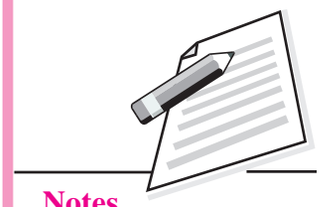
Presence of certain inorganic ions increases the activity of enzymes. The best examples are chloride ions activated salivary amylase and calcium activated lipases.

8.5.7 Effect of Inhibitors

The catalytic enzymatic reaction may be inhibited by substances which prevent the formation of a normal enzyme-substrate complex. The level of inhibition then depends entirely upon the relative concentrations of the true substrate and the inhibitor. Such inhibition, which depends on competition with the substrate for the active sites of the enzyme, is termed competitive inhibition. In other cases, the inhibitor combines with the enzyme-substrate complex to give an inactive enzyme-substrate-inhibitor complex, which cannot undergo further reaction to give the usual product. This is termed uncompetitive inhibition. Non competitive inhibition involves combination of the inhibitor with the enzyme or the enzyme substrate complex, to give inactive complexes. In this case, the inhibitor binds to sites, on the enzyme other than enzyme sites, resulting in deformation of the enzyme molecule so that the formation of the enzyme-substrate complex is slower than normal. Some enzymes undergo irreversible inactivation; reaction of the inhibitor with a functional group of the enzyme, resulting in a loss of its catalytic activity. Enzyme inhibitor plays a vital role in clinical utility and is listed in table 8.2.

Table 8.2: Effect of Enzyme Inhibitors

Sl.No	Enzymatic inhibitor/drug	Enzyme inhibited	Clinical use
1.	Allopurinol	Xanthine oxidase	gout
2.	Dicoumarol	Vitamin-K-epoxide-reductase	Anti-coagulant
3.	Penicillin	Transpeptidase	Anti-bacterial
4.	Sulphonamide	Pteroid synthetase	Anti-bacterial
5.	Pyrimethamine	FH2-reductase	Anti-malarial
6.	5-fluorouracil	Thymidylate synthetase	Anti-cancer



INTEXT QUESTIONS 8.4

1. At optimal substrate concentration the rate of an enzymatic reaction or velocity (V) is directly proportional to the .....
2. Velocity of ..... increases with temperature of the medium.
3. The optimum pH of alkaline phosphatase are .....
4. Allopurinol are ..... inhibitor and used for treatment of .....

8.6 ISO-ENZYMES

Iso-enzymes are physically distinct forms of the same enzyme activity. Higher organisms have several physically distinct versions of a given enzyme, each of which catalyzes the same reaction. Isozymes arise through gene duplication and exhibit differences in properties such as sensitivity to particular regulatory factors or substrate affinity that adapts them to specific tissues or circumstances. Some isozymes enhance survival by providing a "backup" copy of an essential enzyme. Isozymes are expressed only in specific cell types. The expression of isozymes in specific cells occurs during certain periods of development, or in response to specific physiologic or pathophysiologic changes. Thus analysis of the presence and distribution of enzymes and isozymes are often useful in diagnosis. Isoforms of Lactate dehydrogenase is useful in diagnosis of myocardial infarction. While study of alkaline phosphatase isoforms are helpful in diagnosis of various bone disorder and obstructive liver diseases.

INTEXT QUESTIONS 8.5

1. Physically distinct forms of the same enzyme are called as .....
2. Isozymes arise through ..... duplication.
3. Isoforms of ..... is useful in diagnosis of myocardial infarction.
4. Alkaline phosphatase isoforms are helpful in diagnosis of various ..... disorder.

8.7 CLINICAL SIGNIFICANCE OF ENZYMES

The measurement of enzymes level in serum is applied in diagnostic application. Detection of certain enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood. Hence, when a physician indicates that he/she is going to assay for liver enzymes, the purpose is to ascertain the potential for liver cell damage.

Notes

Commonly assayed enzymes are the amino transferases: alanine transaminase, ALT (sometimes still referred to as serum glutamate-pyruvate aminotransferase, SGPT) and aspartate aminotransferase, AST (also referred to as serum glutamate-oxaloacetate aminotransferase, SGOT); lactate dehydrogenase, LDH; creatine kinase, CK (also called creatine phosphokinase, CPK); gamma-glutamyl transpeptidase, GGT. Other enzymes are assayed under a variety of different clinical situations but they will not be covered here.

8.7.1 Pancreatic Enzymes

Acute pancreatitis is an inflammatory process where auto digestion of gland was noticed with activation of the certain pancreatic enzymes. Enzymes which involves in pancreatic destruction includes α-amylase, lipase etc.,

8.7.1.1 α-amylase

α-amylase (AMYs) are calcium dependent hydrolyase class of metaloenzyme that catalyzes the hydrolysis of 1, 4- α-glycosidic linkages in polysaccharides. Molecular weights of AMYs are human plasma ranges from 54 to 62 kDa. Due to its smaller size they could easily pass the glomeruli of the kidneys and AMY is the only plasma enzyme physiologically found in urine. The normal values of amylase is in range of 28-100 U/L. Marked increase of 5 to 10 times the upper reference limit (URL) in AMYs activity indicates acute pancreatitis and severe glomerular impairment. Pancreatic pseudocyst occurs if the plasma level of amylase activity fails to fall after an attack of acute pancreatitis.

8.7.1.2 Lipase

Lipase is single chain glycoprotein of molecular weight 48 kDa. Bile salts and a cofactor called colipase are required for full catalytic activity of lipase. Colipase is secreted by pancreas. Lipase is small molecule filtered through the glomerulus and totally reabsorbed by the renal tubules. Lipase is not normally detected in urine samples. The normal value of lipase ranges from 40-200 U/L. Increase in plasma lipase activity indicates acute pancreatitis and carcinoma of the pancreas. So determination of both amylase and lipase together helps in the diagnosis of acute pancreatitis.

8.7.2 Liver Enzymes

The assay of serum enzymes is very useful for the differential diagnosis and monitoring of various liver disorders. Liver enzymes acts as marker of hepatocellular damage, cholestasis and disturbances in the hepatocellular synthesis.

8.7.2.1 Markers of Hepatocellular Damage

In case of hepatocellular damage, the enzymes which are normally present inside the hepatocytes are released into the blood. Aminotransaminases such as aspartate transaminase (AST) and alanine transaminase (ALT) are routinely used in diagnosis of hepatocellular damages. Transaminases are enzymes involved in the transfer of an amino group from a 2-amino- to a 2-oxoacid. The 2-oxoglutarate acts as amino group acceptor and the L-glutamate serves as donor in all amino-transfer reactions. The specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group.

8.7.2.1.1 Aspartate transaminase (AST)

Aspartate transaminase is present in high concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels. The normal value of AST for male is <35 U/L and for female it is <31 U/L. Marked increase of AST activity in the range of 10 to 100 times the upper adult reference limit indicates myocardial infarction or acute viral or toxic hepatitis.

8.7.2.1.2 Alanine transaminase (ALT)

Alanine transaminase is present at high concentrations in liver and to a lesser extent, in skeletal muscle, kidney and heart. Thus in case of liver damage increase in both AST and ALT were noticed. While in myocardial infarction AST is increased with little or no increase in ALT. The normal value of ALT is <45 U/L and <34 U/L for male and female respectively. In acute viral hepatitis there is a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST.

8.7.2.2 Markers of cholestasis

Enzymatic markers of cholestasis are membrane bound enzymes. Markers of cholestasis includes alkaline phosphatases, gamma-glutamyltransferase and glutamate dehydrogenase.

8.7.2.2.1 Alkaline phosphatases

Alkaline phosphatases are a group of enzymes that hydrolyse organic phosphates at high pH. They are present in osteoblasts of bone, the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta.

8.7.2.2.2 Gamma-glutamyl-transferase (GGT)

Gamma-glutamyl-transferase catalyzes the transfere of the γ–glutamyl group from peptides. The activity of GGT is higher in men than in women. In male

Notes

the normal value of GGT activity is <55 U/L and for female it is <38 U/L. Rise in plasma GGT activity is due to infectious hepatitis and induction of enzyme synthesis, without cell damage, by drugs or alcohol.

8.7.2.2.3 Glutamate dehydrogenase (GLD)

Glutamate dehydrogenase is a mitochondrial enzyme found in liver, heart muscle and kidneys. Small amounts of GLD are even observed in brain, skeletal muscle tissue and leukocytes. GLD is increased in serum of patients with hepatocellular damage offering differential diagnostic potential in the investigation of liver disease. GLD is released from necrotic cells and is of value in estimation of the severity of liver cell damage. The GLD upper reference limits are 6U/L (women) and 8U/L (men).

8.7.3 Muscle Enzymes

Clinically important muscle enzymes include creatine kinase and lactate dehydrogenase.

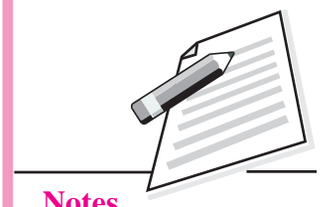
8.7.3.1 Creatine Kinase

Creatine kinase (CK) is most abundant in cells of brain, cardiac and skeletal. In addition to their abundance in above tissues, it also occurs in other tissues such as smooth muscle. In normal physiological condition the CK activity is 46-171 U/L (for male) and 34-145 U/L (for female). Serum CK level elevates in all types of muscular dystrophy. Quite high values of CK are noted in viral myositis, polymyositis and similar muscle disease. Under the circumstances of neurogenic muscle disease such as: myasthenia gravis, multiple sclerosis and Parkinsonism, the level of serum CK is normal. CK consist of two protein subunits, M (for muscle) and B (for brain) and exist as three different isoforms namely BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects. CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts.

8.7.3.2 Lactate Dehydrogenase

Lactate dehydrogenase (LD) catalyses the reversible interconversion of lactate and pyruvate. LD has a molecular weight of 134 kDa and is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes. Five isoforms (LD-1 to LD-5) of LD are existing. The normal physiological limit of LD is 180-360 U/L.

Other clinically important enzymes includes acid phosphatase, glucose -6-phosphate dehydrogenase, cystathionine α-synthase and sphingomyelinase.



INTEXT QUESTIONS 8.6

1. ..... and ..... are enzymes which involves in pancreatic destruction.
2. Molecular weights of amylases in human plasma ranges ..... to ..... kDa.
3. Plasma level of amylase activity fails to fall after an attack of .....
4. Alkaline phosphatases, gamma-glutamyltransferase and glutamate dehydrogenase are markers of .....
5. Clinically important muscle enzymes include ..... and .....

WHAT HAVE YOU LEARNT

- Enzymes are protein catalyst produced by a cell and responsible 'for the high rate' and specificity of one or more intracellular or extracellular biochemical reactions.
- Enzymes posses the catalytic power to facilitates life processes in essentially all life-forms from viruses to man.
- Enzymes are found in all tissues and fluids of the body.
- Intracellular enzymes catalyze the reactions of metabolic pathways.
- Plasma membrane enzymes regulate catalysis within cells in response to extracellular signals.
- Enzymes are broadly classified into 6 major groups namely Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases.
- Enzymes may be simple proteins, or complex enzymes (presence of non-protein part, called as prosthetic group).
- Coenzymes functions as transporters of chemical groups from one reactant to another.
- Activity of a particular enzyme may be affected by many external factors such as concentration of substrate, product, enzyme, hydrogen ion, temperature, activators and inhibitors.
- Enzyme inhibitors plays a vital role in clinical utility and are useful as anti-bacterial, anti-malarial, anti-cancer molecule and treatment of metabolic disorders such as gout. Thus measurement of enzymes level in serum is applied in diagnostic application.

TERMINAL QUESTIONS

1. Define enzyme?
2. Write about classification of enzymes?
3. Give a brief discussion about various factors affecting enzyme activity?
4. What are metallo enzymes?
5. Define isozymes?
6. What are co-enzymes?
7. Differentiate synthase and synthatase?
8. Write a note on clinically important enzymes?
9. Describe in detail about the liver enzymes used in clinical diagnosis?
10. Give some examples of enzyme inhibitory drugs?

ANSWERS TO INTEXT QUESTIONS

8.1

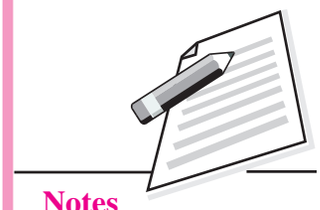
1. Protein
2. Proteins
3. Water
4. Ammonium sulfate and Trichloroacetic acid

8.2

1. Six
2. Oxidoreductases
3. Metallo enzymes
4. Synthetases
5. Glycogen synthase and Alanine synthase

8.3

1. Co-enzymes
2. Protein and co-enzyme
3. Organic
4. Two



8.4

1. Enzyme concentration
2. Enzyme reaction
3. 9-10
4. Xanthine oxidase and gout

8.5

1. Isozymes
2. Gene
3. Lactate dehydrogenase
4. Bone

8.6

1. α-amylase and lipase
2. 54 to 62 kDa
3. Acute pancreatitis
4. Cholestasis
5. Creatine kinase and lactate dehydrogenase