Unit 4 ENZYMES

Anselme Payen

Anselme Payen was a French chemist who first isolated the enzyme diastase in 1833 and showed that it can catalyze the conversion of starch to maltose. However only in 1926 enzymes were shown to be purely made of proteins by James B Sumner, who crystallized the enzyme urease to confirm its purity.

F6WT1

Learning Objectives

After studying this unit the students will be able to:

- Classify enzymes based on their function and assign EC numbers.
- Describe the factors influencing enzyme activity.
- Explain the different types of enzyme inhibitors.
- Explain isoenzymes.
- List the uses of enzymes in various fields such as medicine, industries, scientific research etc...

Introduction

Enzymes are a type of protein present in all living things. Enzymes are produced in animal, bacteria, fungi, yeast and plants. Enzymes act as biological catalysts, increasing the rate of chemical reactions without undergoing any permanent change to themselves. Enzymes are specific in their catalytic activity. They are colloidal in nature and thermo-labile in character.

Enzymes are used to speed up biochemical reactions. The reactions can be the transformation of a chemical compound or a protein. For example, in our stomach, enzymes break down food

molecules into simpler molecules. For example, amylase coverts starch into maltose and pepsin breaks proteins into smaller peptides.

4.1. Nature and Properties of Enzymes:

1. Enzymes are proteins.
2. Enzymes are complex macromolecules with high molecular weight.
3. Enzymes act as catalysts for biochemical reactions and are called biocatalysts.
4. They help in the breakdown of larger molecules into smaller molecules (catabolism) and in the synthesis of larger molecules (anabolism).
5. Enzymes possess high degree of specificity.
6. Most of the enzymes have high turnover number.
7. Some enzymes possess only one polypeptide chain. They are known as monomeric enzymes. (Ribonuclease, Trypsin etc.)
8. Some enzymes possess more than one polypeptide chain. They are known as oligomeric enzymes. (Lactate dehydrogenase (LDH)).
9. The activity of an enzyme increases with increase in substrate concentration and ultimately reaches a steady maximum velocity.
10. Some enzymes can possess multiple functions and when there are more number of polypeptide chains are known as multi-enzyme complexes. Example: Fatty acid synthase.
11. Each enzyme shows high activity at a particular pH and temperature called its optimum pH and temperature, respectively.

Note

Turnover number of an enzyme is defined as the maximum number of substrate molecules which can be converted to products per molecule of enzyme per unit time.

4.2 Nomenclature and Classification of Enzymes:

In early days the suffix – *ase* was added to the substrate for naming the enzymes.

Example : *Lipase* acts on lipids.

These names are known as trivial names. They do not convey complete information about the enzymatic reaction.

The International Union of Biochemistry and Molecular Biology (IUBMB) has assigned a systematic nomenclature for enzymes. The systematic name has two parts.

- The first part represents the substrate. In enzyme catalyzed reactions the reactants are known as substrates.

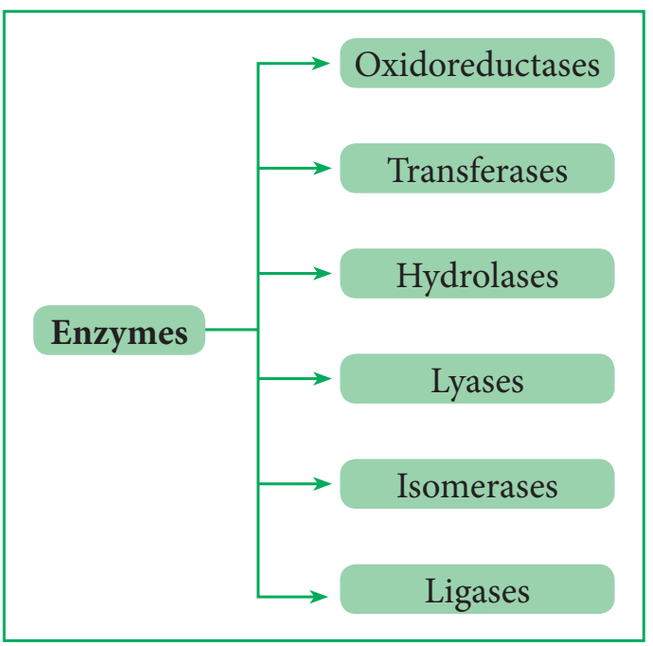
- The second part, ending in –*ase*, indicates the type of reaction catalysed.

Each enzyme is assigned a four-digit code number called Enzyme Commission (EC) number.

- The first digit represents the major class to which the enzyme belongs.
- The second digit denotes the subclass.
- The third digit denotes the sub-sub class of the enzyme within the major class.
- The fourth digit represents the serial number of the enzyme within the sub-sub class.

Example: Hexokinase (EC 2.7.1.1) and Glutamine synthetase (EC 6.3.1.2)

According to International Union of Biochemistry and Molecular Biology, the enzymes are classified into six major classes on the basis of the reaction they catalyse. The six major classes of enzymes are:



1) Oxidoreductases:

These are enzymes which catalyze the oxidation – reduction reactions between two substrates.

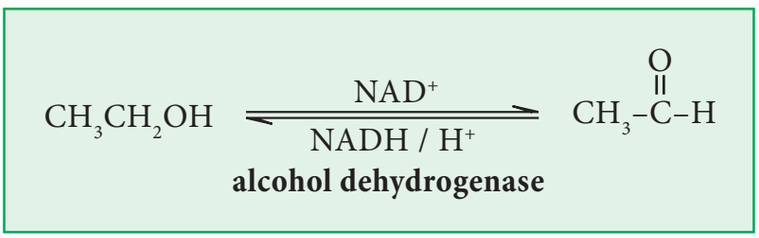
Examples: a) Dehydrogenase (Alcohol dehydrogenase)

b) Oxidase (Cytochrome oxidase)

c) Peroxidase (Glutathione peroxidase)

Alcohol dehydrogenase (EC 1.1.1.1) :

This enzyme oxidizes ethanol into acetaldehyde. It requires the coenzyme NAD^+ (Niacinamide Adenine Dinucleotide) which gets reduced to NADH.



2) Transferases:

These are enzymes which catalyze the transfer of certain groups such as phosphate, amino or acetyl groups from one substrate to another.

Examples:

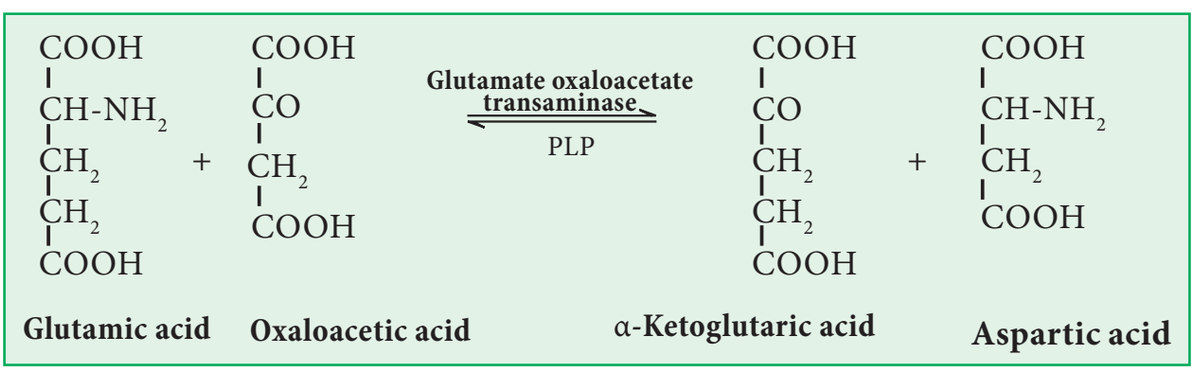
a) Transaminase (Transfers an amino group Example Aspartate amino transferase)

b) Transacylase (Transfers an acyle group Example Malonyl transacylase)

c) Phosphorylase (Transfers a phosphate group Example Glycogen phosphorylase)

Transaminases:

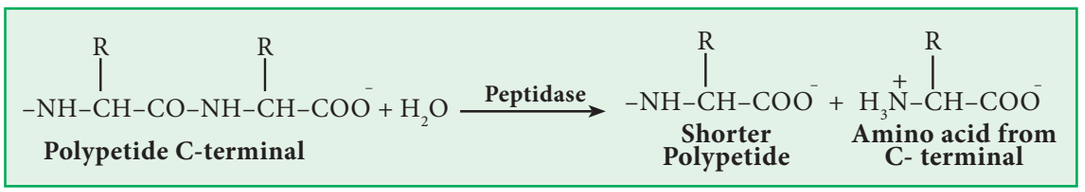
They catalyse the transfer of amino group from an amino acid to a keto acid. Example: Glutamate oxaloacetate transaminase (GOT) or Aspartate transaminase (AST; EC 2.6.1.1). This enzyme catalyses the transfer of an amino group from glutamic acid to oxaloacetic acid. It requires pyridoxal phosphate (PLP) as coenzyme for its activity.



3) Hydrolases:

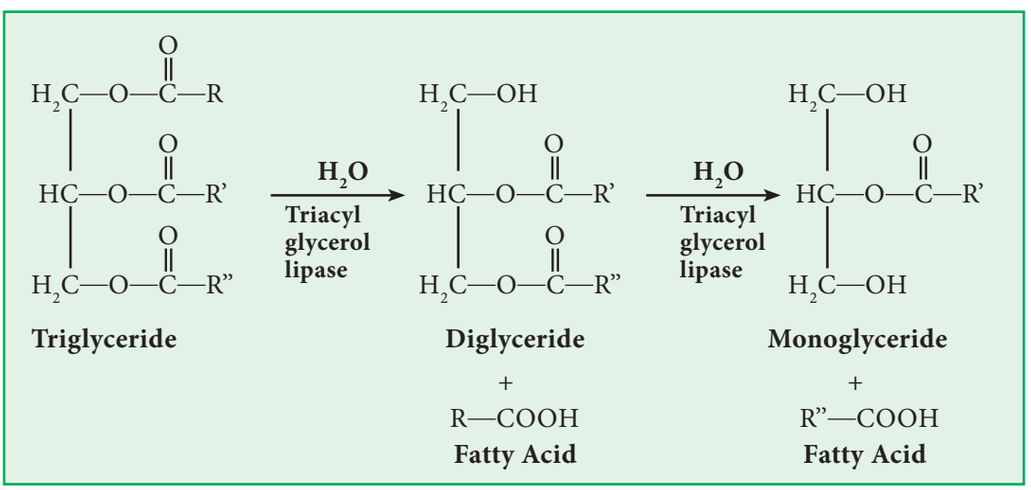
These are enzymes which catalyze the hydrolysis of substrates. They bring about the hydrolysis by adding water.

Example : a) Lipase b) Urease c) Glycosidase.



Lipase (EC 3.1.1.3):

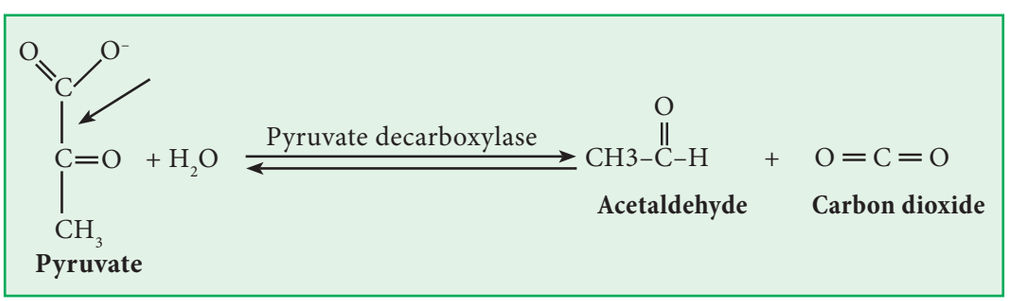
These are enzymes which hydrolyze the ester linkage. For example triacyl glycerol lipase (EC 3.1.1.3) splits the ester linkage between glycerol and fatty acid.



4) Lyases:

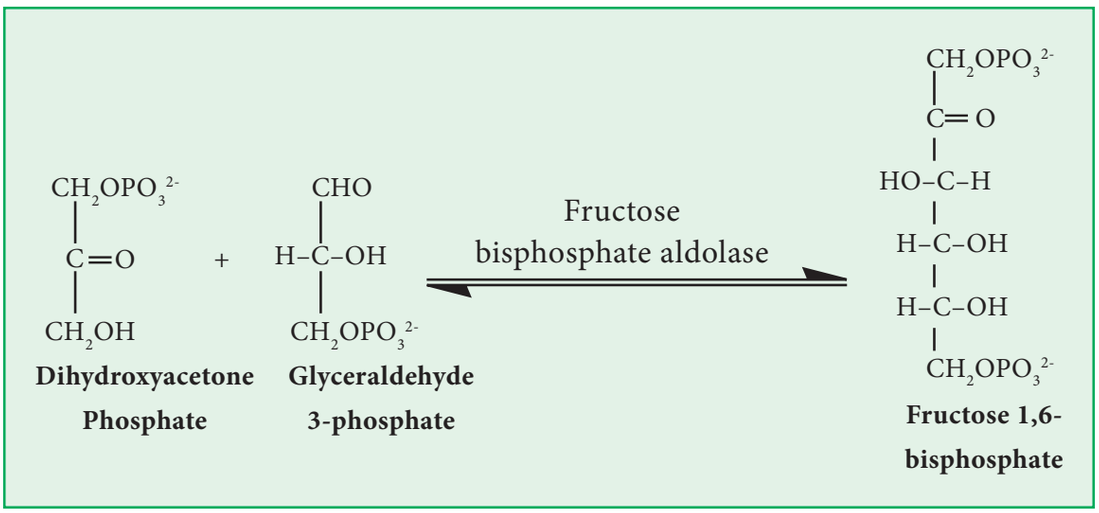
These enzymes catalyze the addition or elimination of groups like H_2O, CO_2, and NH_3 etc. Example: Aldolase, decarboxylase

a) Pyruvate carboxylase (EC 4.1.1.1):



b) Fructose bisphosphate aldolase (EC 4.1.2.13):

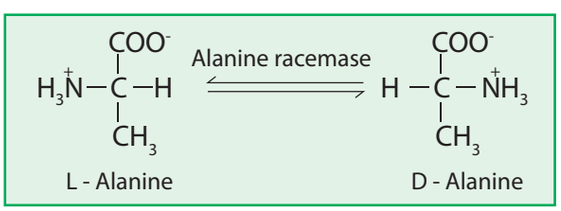
It catalyzes the reversible conversion of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate by aldol cleavage of the C3–C4 bond.



5) Isomerases:

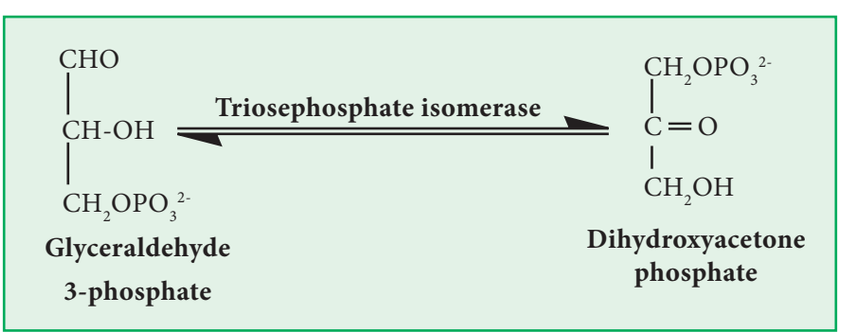
These enzymes catalyze the inter-conversion of isomers such as optical, geometrical or positional isomers.

Example : a) Alanine racemase (EC 5.1.1.1)



b) Triosephosphate isomerase (EC 5.3.1.1):

This enzyme catalyzes the isomerization of glyceraldehyde-3-phosphate into dihydroxy acetone phosphate.



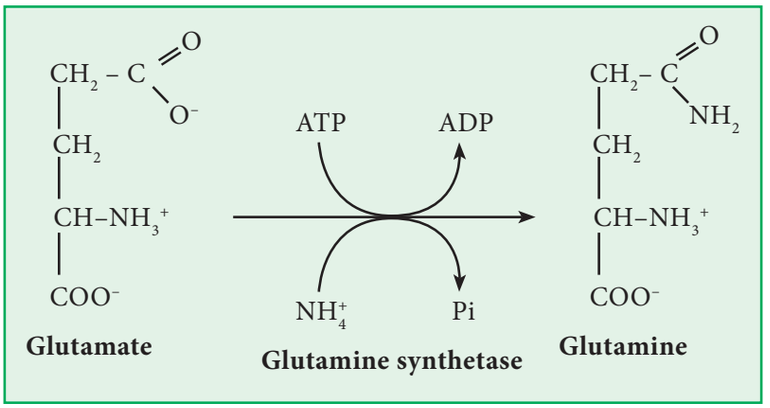
6) Ligases:

These enzymes catalyze the synthetic reactions. They link two substrates together with the utilization of ATP or GTP.

Example : Glutamine synthetase.

Glutamine synthetase (EC 6.3.1.2):

This is a ligase which catalyzes the synthesis of glutamine from glutamate and NH_3.



4.3 Coenzyme

1. Some of the enzymes are simple proteins. Examples: amylase, trypsin. Many enzymes require one or more non-protein components called cofactors. If the cofactor is an organic molecule, it is known as coenzyme. The cofactor may also be a metal ion.

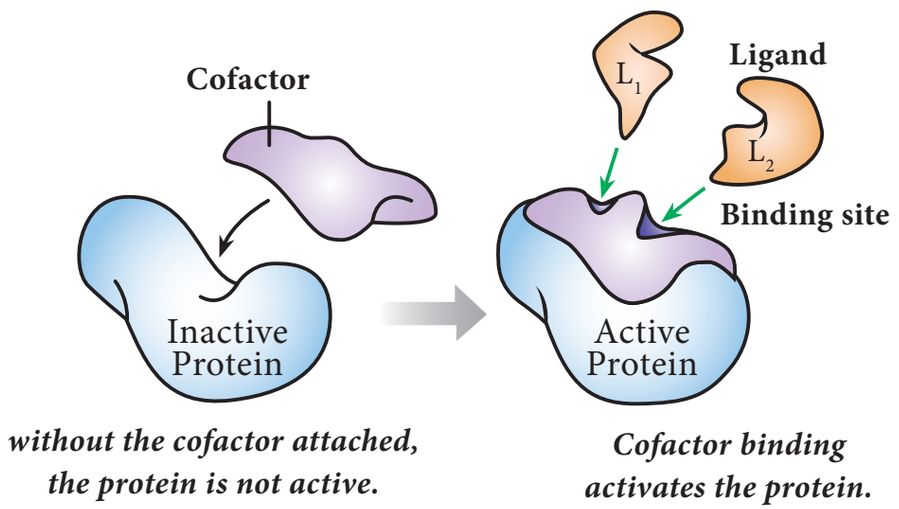


Figure 4.1 Cofactor binding

2. Coenzymes may be defined as non-protein, low molecular weight organic compounds required for the activity of enzymes. Example: Thiamine pyrophosphate (TPP).

3. The protein part of the enzyme is known as 'apoenzyme'. The entire enzyme system consisting of the apoenzyme and coenzyme (or) prosthetic group is known as **holoenzyme**.

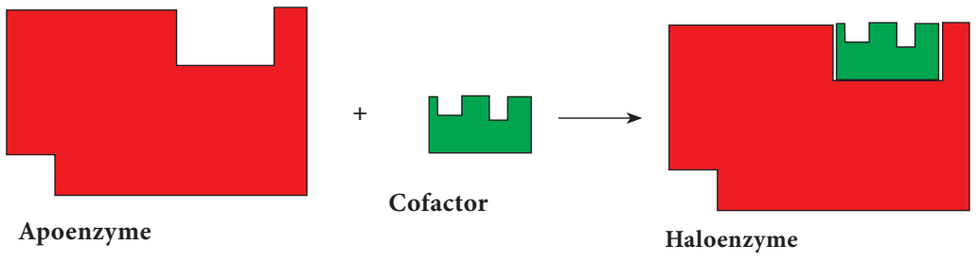


Figure 4.2 Apoenzymes and haloenzyme

4. Most of the coenzymes are linked to their apoenzymes by noncovalent forces. Example**:** The coenzyme ATP is attached to its apoenzyme hexokinase through weak non-bonding interactions.

5. Some of the coenzymes are tightly bound to their apoenzymes through covalent bonds. These are termed as prosthetic groups. Example: The prosthetic group biotin is attached to its apoenzyme carboxylase through a covalent bond.

6. The coenzymes undergo alterations during enzymatic reaction. Hence the coenzymes are regarded as second substrates (or) cosubstrates.

7. Many coenzymes are the derivatives of water soluble B-complex vitamins. **Examples:** Niacin.

8. The Coenzymes can also be organic substances other than vitamins. **Example :** ATP (adenosine triphosphate), CDP (Cytidine diphosphate)

9. Nucleotides and their derivatives can act as coenzymes.

   Example**:** NAD, FMN, FAD, coenzyme-A etc.

10. The specificity of an enzyme is mostly dependent on the apoenzyme and not on the coenzyme. For example, NAD^+ functions as coenzyme for several enzymes like alcohol dehydrogenase and lactate dehydrogenase.

11. Coenzymes function as group transfer agents (Table 4.1).

Table 4.1 : Vitamin related Coenzymes

Coenzyme	Derived from Vitamin	Atom (or) group transferred (function)	Dependent enzyme
Thiamine pyrophosphate (TPP)	Thiamine (B_1)	Aldehyde	Transketolase
Flavin mono nucleotide (FMN)	Riboflavin (B_2)	Hydrogen and electrons	L-amino acid oxidase
Flavin adenine dinucleotide (FAD)	Riboflavin (B_2)	Hydrogen and electrons	D-amino acid oxidase
Nicotinamide adenine dinucleotide (NAD^+) (or) Diphospho pyridine nucleotide (DPN)	Niacin (B_3)	Hydrogen and electrons	Lactate dehydrogenase
Pyridoxal phosphate (PLP)	Pyridoxine (B_6)	Amino	Alanine transminase
Biotin	Biotin (B_7) or (H)	CO_2	Pyruvate carboxylase
Coenzyme A	Pantothenic acid (B_5)	Acyl	Thiokinase
Tetrahydrofolate	Folic acid	one carbon unit	formyl transferase

4.4 Factors influencing Enzyme activity

The important factors that influence an enzyme catalyzed reaction are; pH, Temperature, Substrate concentration, Enzyme concentration, Activators and inhibitors.

4.4 .1 Effect of pH :

1. Change in hydrogen ion concentration influences the enzyme activity. When the velocity is plotted against pH, a bell shaped curve is obtained.

2. The pH at which the enzymatic reaction has maximum velocity is known as **optimum pH.** Most of the enzymes possess optimum pH between 5 and 9. However there are exceptions like pepsin, alkaline phosphatase etc.

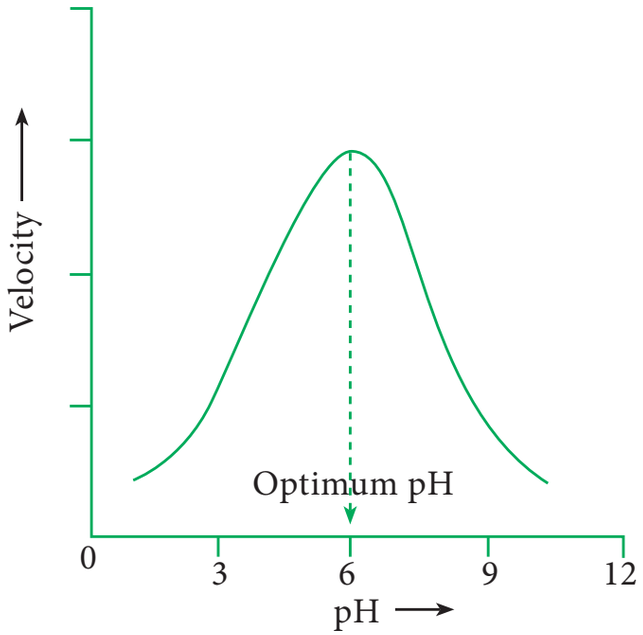


Figure 4.3 Effect of pH

Note

Denaturation: Nonproteolytic change in structure of a native enzyme that causes it to lose some or all of its unique or specific characteristics.

3. The optimum pH of some of the common enzymes are as follows:

Enzyme	Optimum pH
Pepsin	1-2
Alkaline phosphatase	10-11
Acid phosphatase	4-5

4. Enzymes possess only low activity or even become inactive at extreme pH values. This is due to the following reasons;

a) The hydrogen ion concentration affects the ionic charges on the active site of the enzyme.

b) Thus the extreme pH values lower the effective concentration of active form of enzyme and substrate. Therefore, the reaction velocity will be lowered.

c) Denaturation of enzyme occurs at extreme pH values.

4.4.2. Effect of temperature on enzyme acitivity:

1. Velocity of an enzyme reaction increases with increase in temperature upto a maximum and then declines.

2. When the velocity is plotted against temperature we obtain a plot as shown in figure 3.4.

3. The temperature at which the enzymatic reaction has maximum velocity is known as **optimum temperature.**

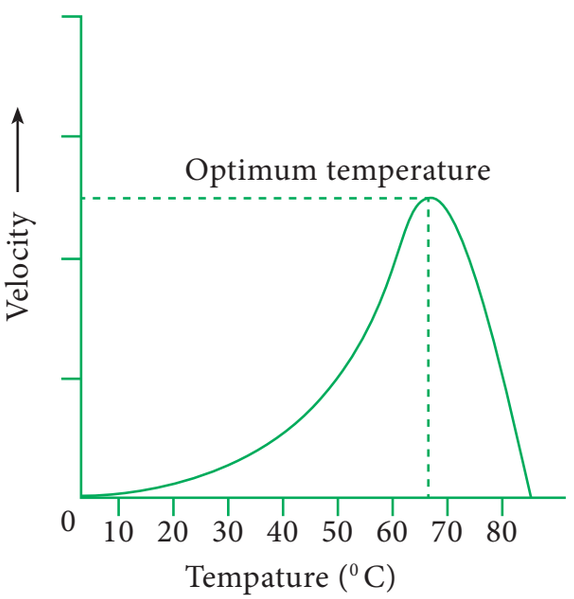


Figure 4.4 Effect of temperature

4. The optimum temperatures of some of the common enzymes are as follows:

Enzyme	Optimum temperature (^{0}C)
Plant urease	60
Human enzymes	37

But enzymes like venom phosphokinase, muscle adenylate kinase are active even at 100^{0}C.

5. In general, at high temperatures the enzymes undergo denaturation and this leads to the rapid loss of catalytic activity.

6. Temperature co-efficient or Q_{10} is defined as the increase in velocity of the enzymatic reaction when the temperature is increased by 10^0 C. For most of the enzymes the value of Q_{10} is "2" in the temperature range 0^0 C to 40^0 C.

4.4.3 Concentration of substrate:

Formation of an enzyme-substrate complex (ES complex) is the first step in enzyme catalysis. Increase in the substrate concentration gradually increases the velocity of the enzymatic reaction up to a particular value.

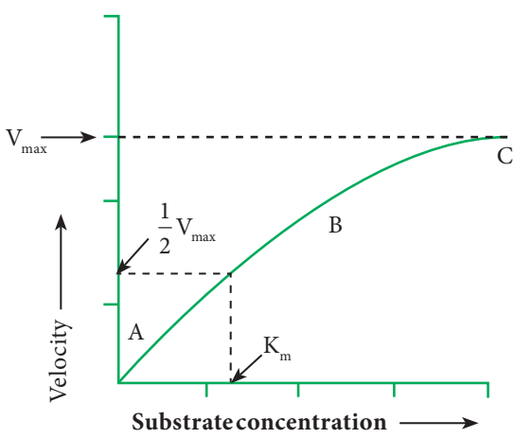


Figure 4.5 Effect of substrate concentration

A hyperbolic curve is obtained when velocity is plotted against the substrate concentration. This graph shows three distinct phases.

i) In the first phase (A), the velocity of the reaction is directly proportional to the substrate concentration.

ii) In the second phase (B), the substrate concentration is not directly proportional to the enzyme activity.

iii) In the third phase (C), the velocity remains constant and does not change with increase in the substrate concentration. (plateau)

4.4.4 Concentration of enzyme

At a constant substrate concentration, the velocity of an enzyme catalyzed reaction increases proportionately with the increase in the concentration of the enzyme. This property is utilized in determining the level of serum enzymes for the diagnosis of diseases. On plotting the velocity of the enzymatic reaction with the enzyme concentration, a straight line is obtained.

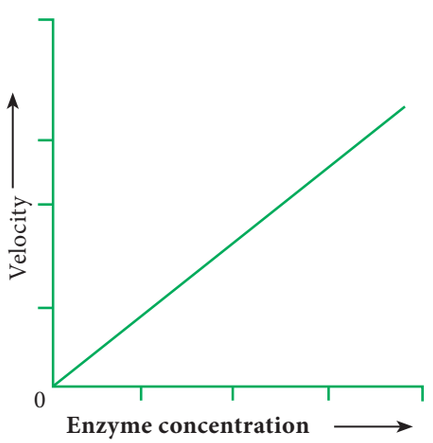


Figure 4.6 Effect of enzyme concentration

4.4.5 Activators

Activators are the inorganic ions (cations or anions) or compounds that enhances the activity of the enzymes.

Enzyme	Activator
Phenol oxidase	Cu^{2+}
Amylase	Cl^{-}

Metal ions can be bound to the enzyme permanently or can be used just for activation. When metal ions used only for the activation of enzyme, they are called metal activated enzymes.

Examples: ATPase (Mg^{2+}, Ca^{2+}) and Enolase (Mg^{2+}).

If metal ions are bound with enzymes using chemical bond, they are called metalloenzymes.

Examples: Alcohol dehydrogenase-Zn^{2+} and Carbonic anhydrase-Zn^{2+},

Time, radiations and co-enzymes are also the other factors which affect the velocity of an enzyme reaction.

4.5 Inhibitors

An inhibitor is defined as a substance which binds with the enzyme and brings about a decrease in catalytic activity of that enzyme. For example, anti-oxidants are added as inhibitors to food to retard its spoilage on exposure to air (oxygen) and inhibition could be either reversible or irreversible.

Allosteric activators and inhibitors:

This type of inhibition takes place due to the presence of allosteric site (Greek allo = 'other'; stereos = 'space' or 'site') on the surface of the enzyme, away from the active site. The final end-product fits in the allosteric site and in some way brings about a change in shape of the enzyme so that the active site of the enzyme becomes unfit for making a complex with its substrate. Allosteric inhibition may be reversible. In many metabolic reactions, when the concentration of the final end product (usually acts as an allosteric inhibitor) in the cell falls, the activity of the enzyme is restored. Similary an enzyme can also be activated by an activator that binds to an allosteric site. This activator is called as an allosteric activator.

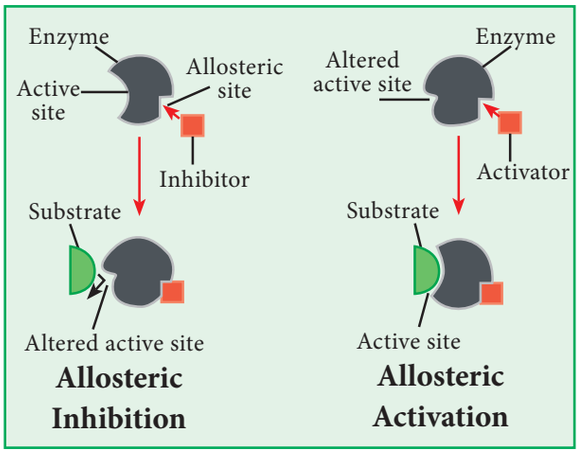


Figure 4.7 Allosteric inhibition

4.5.1 Types of Inhibition

i) Competitive Inhibition

Competitive inhibition is usually reversible. A competitive inhibitor usually closely resembles the substrate and is regarded as a substrate analogue. The inhibitor competes with substrate and binds at the active site of the enzyme but does not undergo any catalysis. As long as the competitive inhibitor is bound to the active site, the enzyme will not be available for the substrate to bind. This type of inhibition can be reversed by increasing the concentration of substrate.

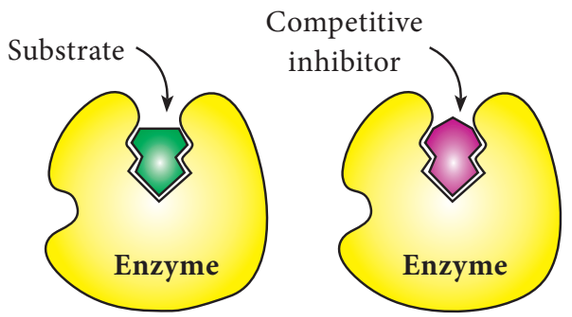


Figure 4.8 Competitive inhibition

Example: 1) Enzyme - Xanthine oxidase; Substrate - Hypoxanthine; Inhibitor – Allopurinol.

Significance of the inhibitor: Used in the control of Gout to reduce excess production of uric acid from hypoxanthine.

Example: 2) Enzyme - Succinate dehydrogenase; Substrate - Succinate; Inhibitor - Malonate

ii) Noncompetitive inhibition:

Usually a noncompetitive inhibitor binds either to free enzyme or to ES complex at a site other than the active site on the enzyme surface. This results in the change of conformation of the enzyme as well as its active site, which makes the substrate unable to bind to the enzyme effectively. This type of inhibitor has no structural resemblance with the substrate like competitive inhibitors.

Non-competitive inhibitors do not interfere with the enzyme-substrate binding. But catalysis is prevented, possibly due to the distortion of enzyme conformation.

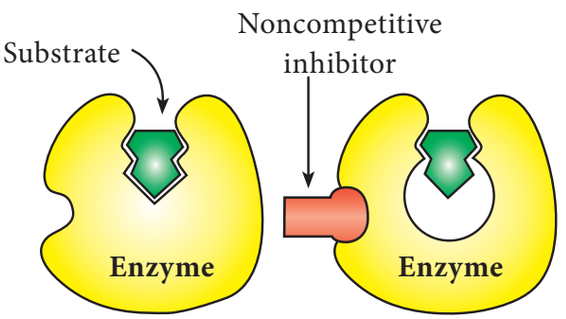


Figure 4.9 Noncompetitive inhibition

iii) Uncompetitive inhibition:

Uncompetitive inhibitors binds only to the ES complex. However, the binding of the inhibitor affects the binding of the substrate. By adding the inhibitor usually follows an allosteric effect where it binds to a different site on the enzyme than the substrate. This binding to an allosteric site changes the conformation of the enzyme so that the

affinity of the substrate for the active site is reduced.

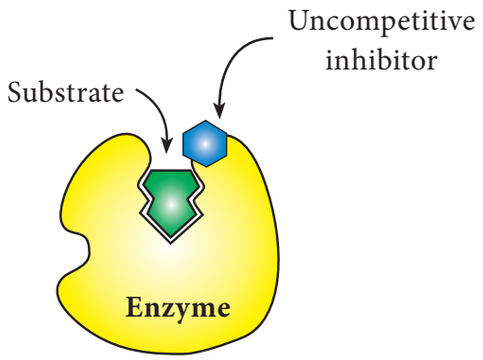


Figure 4.10 Uncompetitive inhibition

Table 4.2: Comparison between Competitive and Noncompetitive inhibition

Sl. No.	Competitive inhibition	Noncompetitive inhibition
1.	Inhibitor resembles the substrate	Inhibitor has no structural resemblance with the substrate.
2.	Inhibitor binds at the active site.	Inhibitor binds at a site other than active site
3.	Enzyme binds either with substrate or inhibitor	Enzyme binds with both substrate and inhibitor
4.	Reversible	Reversible
5.	Can be overcome by increasing substrate concentration.	Cannot be overcome by increasing substrate concentration

4.6 Industrial applications of enzymes

Enzymes are widely used in food, pharmaceutical and chemical industries. Bacterial enzymes are used for the fermentation of food items. For example,

i. Making of curd from milk by *Lactobacillus acidophilus*.

ii. Producing yoghurt and cheese from milk by *Streptococcus thermophilus*.

iii. Fermenting rice and blackgram by *Leucanostoc mesenteroides* for preparing delicious idlies.

iv. In washing powders enzymes are incorporated to remove stains from clothes.

v. Fructose syrup is produced by the isomerization of glucose by glucose isomerase.

vi. Penicillin acylase is used to convert penicillin to semisynthetic penicillins.

vii. Glucose and galactose are produced from the discarded portion during cheese making by lactase enzyme

viii. Enzymes are used for desizing fabrics. This kind of enzymatic desizing does not weaken the fabrics.

ix. In the manufacture of leather, the hide is made free from hair. This is done by employing pancreatic enzymes.

x. Pepsin is used to digest gelatin in the process of recovering silver from photographic films.

4.7 Medical applications of enzymes

i. Streptokinase or Urokinase is sometimes used to lyse intravascular blood clots.

ii. Gastrointestinal tract enzymes (pepsin, trypsin and lipase) are given to patients suffering from indigestion.

iii. Enzyme asparaginase is used as an anticancer drug.

iv. Enzymes are used to diagnose various diseases such as AIDS.

v. Immobilized enzymes like glucose oxidase (GOD) and peroxidase (POD) are used for the estimation of blood glucose.

Table 4.3 Some important therapeutic enzymes

Enzyme	Reaction	Therapeutic use
Asparaginase	L-Asparagine + H_2O → L-aspartate + NH_3	Leukaemia
Collagenase	Collagen hydrolysis	Skin ulcers
Glutaminase	L-Glutamine + H_2O → L-glutamate + NH_3	Leukaemia
Hyaluronidase	Hyaluronate hydrolysis	Heart attack
Lysozyme	Bacterial cell wall hydrolysis	Antibiotic
Ribonuclease	RNA hydrolysis	Antiviral
β-Lactamase	Penicillin → penicilloate	Penicillin allergy
Streptokinase	Plasminogen → plasmin	Blood clots
Trypsin	Protein hydrolysis	Inflammation
Uricase	Urate + O_2 → allantoin	Gout
Urokinase	Plasminogen → plasmin	Blood clots

Serum enzymes are used as markers to detec cellular damage which in turn helps in the diagnosis of diseases.

Table 4.4 Serum enzymes

Serum enzyme	Disease
Amylase	Acute pancreatitis
GPT or ALT	Liver diseases (hepatitis), jaundice, cirrhosis of liver
GOT or AST	Heart attack
Alkaline phosphatase	Rickets, obstructive jaundice, bone cancer, hyperparathyroidism.
Acid phosphatase	Cancer of prostate gland

Serum enzyme	Disease
Lactate dehydrogenase (LDH)	Heart attack, liver diseases, leukemia, pernicious anemia
Creatine Kinase (CK)	Myocardial infarction (early marker), hypothyroidism, alcoholism.
Aldolase	Muscular dystrophy, liver diseases
5´-Nucleotidase	Hepatitis, obstructive jaundice, tumour
γ-Glutamyltranspeptidase (γ-GT)	Alcoholism, infective hepatitis, obstructive jaundice.

EVALUATION

I. Choose the correct answer

1. The catalytic activity of an enzyme is restricted to its small portion called

   (a) active site (b) passive site

   (c) allosteric site (d) all Choices are correct

2. An active enzyme made of polypeptide chain and a co-factor is

   (a) coenzyme (b) substrate

   (c) apoenzyme (d) holoenzyme

3. In human body the optimum temperature for enzymatic activities is

   (a) 37^0C (b) 40^0C

   (c) 25^0C (d) 30^0C

4. Enzymes are sensitive to

   (a) changes in pH (b) changes in temperature

   (c) both a and b (d) none of these

5. Enzyme B requires Zn^{2+} in order to catalyze the conversion of substrate X. The zinc is best identified as a(n):

   (a) coenzyme (b) activator

   (c) substrate (d) product

II. Fill in the blanks

6. Glutamine synthetase is an example for the class of enzymes called ----------
7. The inhibitor that resembles the substrate is called ----------
8. ---------- is used to digest gelatin in the process of recovering silver from photographic films
9. ---------- and ---------- are the enzymes used therapeutically to treat blood clots
10. ---------- is the serum enzyme that acts as an early marker for myocardial infarction.

III. True or False.

11. Enzyme substrate complex is a permanent stable complex.
12. Malonate is the competitive inhibitor of succinate dehydrogenase.
13. An enzyme substrate complex is formed in all the enzymatic reactions.
14. The degree of competitive inhibition cannot be decreased by increasing the concentration of the substrate.
15. An uncompetitive inhibitor has affinity towards ES complex.

IV. Give short answers for the following:

16. What are enzymes? Why are enzymes important for living organisms?
17. Is there a difference between the initial and the final energy levels in catalyzed and non-catalyzed reactions?
18. Give any two examples for an enzymatic reaction.
19. Justify the need for a systematic nomenclature for enzymes.
20. Relate the reaction pH with enzyme activity.

V. Answer the following:

21. What are enzyme cofactors? Describe the relationship between vitamins and enzyme cofactors?
22. With appropriate examples describe the different classes of enzymes and their nomenclature system.

23. Describe the different types of enzyme inhibition mechanisms.

24. Compare competitive and non-competitive inhibition.

25. Write about the various industrial applications of enzymes.

26. Describe the different medical applications of enzymes.

27. Write in detail about the main factors that alter the speed of enzymatic reactions.

SUMMARY

Enzymes are proteins that work as biological catalysts. They catalyse the biochemical reactions that occur in living organisms. They are highly specific for a given reaction and accelerate the reaction rates to many folds. They help breakdown the food molecules to harvest energy from them and also to synthesise the necessary macromolecules for the development of living organisms. Enzymes have their maximum activity at a particular pH and temperature which are called the optimum pH and temperature for that enzyme. The concentration of the enzyme and the substrate concentration influence the activity of a particular enzyme. Some enzymes do require small organic molecules or metal ions in order to carry out their functions, these molecules are called co enzymes/cofactors.

Enzymes are classified into six classes based on their functions viz. oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. The International Union of Biochemistry and Molecular Biology (IUBMB) has assigned a 4 digit number called Enzyme Commission (EC) number.

The activity of an enzyme can be inhibited by small molecules which are known as inhibitors. Based on the type of inhibition they can be classified as competitive, uncompetitive and noncompetitive inhibitors.

Enzymes have many applications in industries as well as in medicine.

CONCEPT MAP