Chapter - 9
Enzymes

Introduction

The existence of the living system depends upon co-ordination of numerous specific and complex but systematic biochemical processes. Inside the living cell several chemical reactions going on efficiently at normal temperature. Out of these reactions, only a few can be conducted outside the cell in very high and extreme conditions of temperature, pressure, acidity and salinity. Apart from this their efficiency is also very less than the cellular reactions. Inside the cell various biochemical reactions take place in the presence of active organic compounds present in very less quantity. These substrates behave like biocatalysts are called enzyme or biocatalyst. Enzyme increases the rate of chemical reactions by reducing the activation energy of the reactants, some reaction in the cells are of synthesis type such reactions need energy these are called anabolic processes. Whereas some reactions are of dissociation type that release energy. These are called catabolic processes. These two processes combine to form metabolic paths in the cells. The most accepted definition of an enzyme is : organic substances having proteinous nature which act as biocatalyst in living cell, are called as enzymes.

History of Enzymes

Enzyme were identified as biocatalysts by Bergelius, 1835. Edward Buchner discovered an enzyme complex named zymase in yeast cells. Willy Kuhne (1878) for the first time used the term enzyme in fermentation of yeast solution. J.B. Summer (1926) was first to crystalise the pure form of urease enzyme. Summer and Northrope, (1930) established with evidence that chemically enzymes are protein. Summer, Northrope and Stanley were awarded with nobel prize in 1947 for protein related work. Non proteinacious enzymes (catalytic molecule of RNA) was first discovered by T. Cech and Altman, and called ribozyme.

Structure of Enzyme

All enzyme are protein but all proteins are not enzymes. Ribozyme is an enzyme made up of RNA helps in RNA which splicing. Some enzymes such as : Pepsin, Urease etc are completely made up of protein but in most of the enzyme along with the main protein some non protein substances are also found which are essential for the activily of the protein. Such type of an enzyme is called Holoenzyme or The protein component of holo enzyme is called apoenzyme and non protein component is called cofactor. Cofactor can be

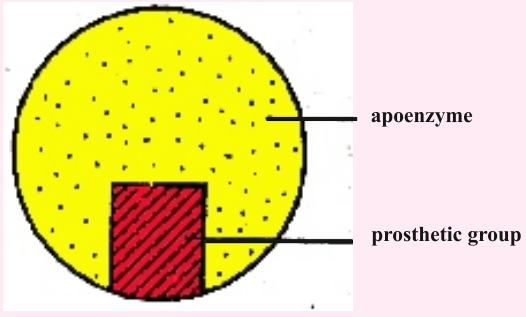

Fig. 9.1 Holoenzyme

divided in to three types:

(i) Prosthetic group : When non protein part of an organic nature is in tightly associated with apoenzyme it is called prosthetic group e.g. cytochrome, flavo protein.

(ii) Co-enzyme : When the non protein part is loosely associated with apoenzyme or it can be easily scparated and capable is attach again then this is called co enzyme such as NAD, NADP, FAD, Co-A etc.

(iii) Activator : When the non protein part is a metal ion of inorganic nature. It is called activator e.g. Fe.

Holoenzyme = Apoenzyme + Non protein part
(Cofactor)
(i) prosthetic group
(ii) Co-enzyme
(iii) Activator

Co-enzyme can be easily isolated from the holoenzyme by the dialysis process.

Apoenzyme : It is the main part of an enzyme. The length of protein molecules in different enzyme is different and the esequence of amino acids is specific. Protein are colloidal because of which more surface area is provided per unit volume. Protein part of an enzyme (apoenzyme) have one or more specific sites that are called active sites. The basic substrates bind to these active sites.

Specific properties of enzymes

All enzymes are found in cells but they are not evenly distributed. Such as enzymes needed for respiration are found in mitochondria, whereas enzymes required for photosynthesis are found in chloroplast. Enzyme can be isolated in pure form without destroying their catalytic capacity. After detailed study of enzymes following different properties are mentioned :

(I) **Protein Nature** :- All the enzymes are chemically proteins. Sometimes organic or inorganic molecules, ion or group may be present in them other than protein.

(ii) **Colloidal nature :-** All enzymes are colloidal in nature. Due to the large surface area they provide an extensive surface for biochemical processes because of which the reactions complete at faster rate.

(iii) **Specificity :-** All enzymes are specific for their reaction usually one enzyme catalyses only one reaction or remains functional for few special chemical bonds. Where as, few enzymes are there which catalyse two or more reactions. Usually enzyme specificity is of four types :

(a) **Absolute specificity :-** Enzyme having this type of specificity catalyse only one type of reaction. Such as urease which hydrolyses urea only.

(b) **Group specificity :-** Some enzymes catalyse related substrates of specific group example- Hexokinase enzyme acts on different types of hexose sugars.

(c) **Bond Specificity :** Some enzyme identify some specific chemical bond and act on it example- Ribonuclease enzyme hydrolysis phosphodiester bond of pyrimidine base 3' (3 prime) ends.

(d) **Stereo Specificity :** Some enzymes act only on special type of stereoisomer of substrate and not on other forms. Such as : amino acid oxidase catalyses reaction of L (Levo) amino acid and not of D-amino acid.

(iv) **Large size :-** The size of Enzyme being large in size it provides large surface to many substrate which helps in increase the reaction rate even when enzyme is present in small amount.

(v) **Capacity to react with acidic and basic substrates :** Major part of an enzyme is made up of protein and protein being amphoteric reacts with both basic and acidic substrates.

(vi) **Enzyme Optima :** Most of the enzymes show maxium activity at specific condition, this is called as enzyme optima. Enzyme activity depends on temperature, pH, substrate

concentration. At high temperature they lose their catalytic capacity because of there proteinacious nature.

(vii) **Enzyme inhibition :** Enzyme activity can be reduced or inhibited by other chemicals. These chemicals are called enzyme inhibitors Enzyme inhibition can be direct or indirect.

Factors affecting enzymatic action

Following factors affect the enzyme action :

1. **Temperature -** Enzymes due to proteinacious nature are extremely sensitive to temperature changes. Optimum temperature for an enzyme action is 20^0 C - 35^0 C. 10^0C rise of temperature in this range, increases the reactivity by 2 to 2½ times. At temperature above 35^0C, the reactivity slows down due to thermal denaturation and at very high temperature, it stops.

2. **pH value :** All enzymes are sensitive to pH of the medium. Each enzyme can act on a specific optimum pH value. Most of the enzymes work efficiently at pH 5.0 to 7.5. Increase or decrease in the pH values affects the action of an enzyme.

3. **Concentration of substrate :** Concentration of the substrate can be low. optimum or excess. At low concentration enzyme activity is less as substrates are not attached on all the active sites present on an enzyme surface. On increase of substrate concentration the enzyme activity also increases. On concentration, beyond a definite concentration the enzyme activity becomes constant. At adequate concentration the maximum activity of enzyme is called its maximum rate.

4. **Enzyme concentration :** On adequate concentration of substrate, the enzyme activity is proportional to the enzyme concentration. On the increase of enzyme concentration the activity gradually increases but due to limitation of substrate concentration at some point it stops and becomes constant. Thus on increase of enzyme concentration along with substrate reaction rate of the enzyme activity increases.

5. **Concentration of end product :** Due to the increase in concentration of end product enzyme catalytic activity decreases. This decrease gradually makes the catalytic activity zero at equilibrium state and after equilibrium state the activity completly stops and after normal condition the activity starts to reverse.

6. **Enzyme inhibitors and prisons :** Those substances which reduce the catalytic activity of enzymes are called enzyme inhibitors. These inhibitors usually associate with active sites of enzymes and inactivate (block) them. Inhibition processes are of two types.

(a) **Competitive inhibition :** The structure of such inhibitors resemble the substrate structure. Thus such inhibitors complete with substrate to combine at active sites of enzyme molecule because of which the enzyme activity decreases such as : Malonic acid is a competitive inhibitor of succinic acid. This types of inhibition can be checked by increasing the substrate concentration.

(b) **Non-competitive inhibition :** This type of inhibitors permanently attach with enzyme molecules and bring structural change in it. The permanent association denatures the enzyme molecule. Such substances are called cell poisons. Such as : Pb^{++}, Hg^{++}, Ag^{++} ions etc. Cynide destroys cytochrome oxidase in respiration process, this type of destruction can be stopped by increasing the enzyme concentration.

Mode of enzyme action

The working mode of all enzymes depends on the nature of enzyme and participating substrates in the process. Initiation of enzyme activity and its rate is related to formation of enzyme substrate complex. And decrease in require for the initiation of the

process of activation energy. Thus the enzyme action can be understood by the following manner.

1. Formation of enzyme substrate complex (ESC)
2. Lowering of activation energy.

1 Formation of enzyme substrate complex (ESC) :

In all the enzyme catalysed actions, first of all enzyme combines with substrate to form a unstable complex called enzyme substrate complex (ESC). This complex dissociates to form end product and enzyme is released.

Substrate + Enzyme ----------> enzyme substrate complex (unstables)

Enzyme substrate complex ----------> Enzyme + Product

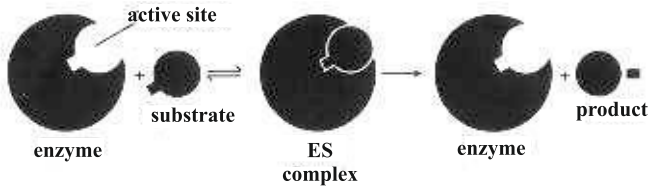

Fig. 9.2 Mechanism of enzyme

On the surface of each enzyme some specific site are present to which the substrate attaches. Those sites are called active sites. Active sites on enzyme are so close that substrate attaches and reacts easily. When the substrate attaches to the enzyme surface, the enzyme changes their shapes and forms a bond with substrate. The end products formed gets a separted from the enzyme due to the bond. Enzyme becomes free again to bind with other substrate molecules and forms new enzyme substrate complex.

The formation of enzyme substrate complex can be understood by following two theories:

(I) Lock and key theory : This theory was postulated by Emil Fischer (1984). According to this theory as one lock can be opened by its key only. Similarly, a specific active substrate only can form bond with the active site of enzyme. Thus a specific type of substrate can only bind active site on enzyme, in such enzyme catalysed reactions.

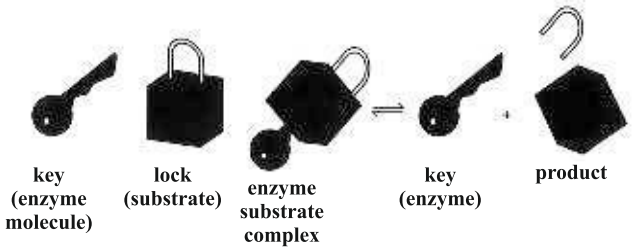

Fig. 9.3 Lock and Key Theory of Enzymatic Action

(ii) Induced fit theory : This theory was postulated by Koshland, (1966). According to this theory the active sites found in enzyme are not rigid i.e. these active sites are not complementary to substrate, but as soon as substrate comes in contact with enzyme, active sites changes and becomes according to it. According to this theory active site present in enzyme changes under the influence of substrate. Because of this it is called induced fit theory. It can be said that unlike lock and key theory, the active site of the enzyme is not definite or rigid but changeable. Thus on the contact of substrate the enzyme under goes some minute structural change to accomodate it, theory there by enzyme substrate complex is formed.

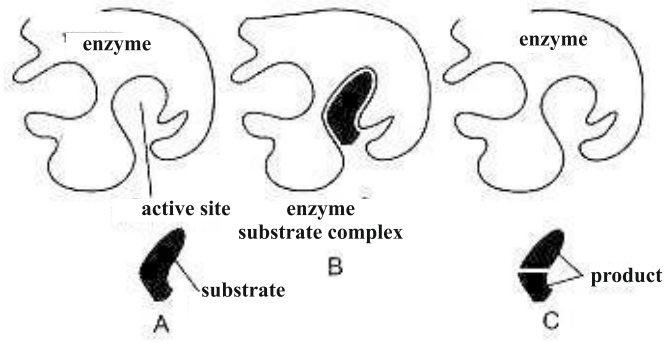

Fig. 9.4 Induced fit diagram of Enzymatic Action

2. Lowering of activation energy : In chemical reactions energy is released but some energy is also required to prevent the initiation of these reaction. The energy required for intiating the reaction is called activation energy. Enzymes

have this speciality that they can reduce the energy required for activation of substrate molecule. Thus in the presence of enzyme substrate gets converted into end products at lower activation energy.

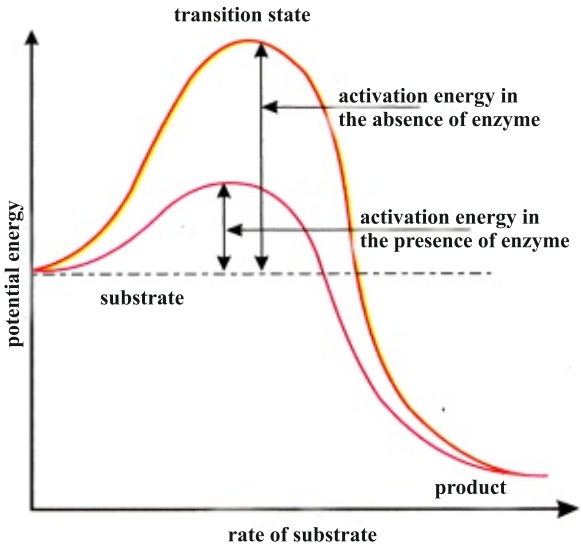

Fig. 9.5 Activation energy

Nomenclature of enzymes

Nomenclature of enzymes is usually done on the basis of the substrate they acte upon or the catalytic activity. According to Duclaux (1883) the suffix 'ase' is used to name the enzyme. The two systems for nomenclature of enzymes are :

1. **On the basis of substrate :** According to this system the suffix 'ase' when attached to the name of substrate it becomes the name of enzyme that acts on the substrate. Such as : sucrase, maltase, lipase that acts on sucrose, maltose and lipids respectively.
2. **One the basis of chemical reaction :_**This nomenclature system is very popular and in use. According to this system, enzymes are named according to the reaction catalysed by them i.e. Hydrolase, oxydase, Carborylase, Delydrogenase Many enzymes are named according to both, the basis of substrate they catalyse and the nature of the reaction.

Eg.L. Glutamic dehydrogenase, in which L-glutamic acid is substrate and dehydrogenation is the name of reaction being catalysed by it.

International beiochemical organisation in 1955 postulated the standards and systematic nomen clature of the enzymes. In the enzymes standard and systematic naming their substrate, reaction they catalyse, should be included, so that the information regarding substrate and reactions they catalyse etc. can be obtained. In this way the standard name of Hexokinase is ATP : D-Hexose - 6 Phospho transferase. Standard name of uricase is urate O_2 oxidoreductase. Some standard names of enzyme too complex so, it is difficult to recall. Due to this reason, small names are postulated which are called recommended names.

Classification of enzymes

International union of biochemistry (IUB) had recommended the use of overall formal reaction equation for the classification of enzymes. Enzyme commission has classified all the enzymes into six classes and each enzyme has been allotted an enzyme code (EC) these 6 classes are as following :

1. **Oxidoreductases :** All enzymes that catalyse oxidation and reduction reaction are included in this class. These enzymes remove/add electron or hydrogen to their substrate and oxidise or reduce it. Such as : Cytochrome oxidase, Alcohol dehydrogenase, Reductase
2. **Transferases :** These enzymes transfer any ion or group except hydrogen from the substrate to any other substrate. Main groups transferred by them are : amino, phosphate, methyl, thiole, Ketone etc. e.g. Transaminase Transphosphatase.
3. **Hydrolases :** The enzymes of this class catalyse. The reactions involing addition or removal of water molecules. In any reaction, addition of water molecules is done in the presence of hydrolase enzyme. These enzymes break large molecules into smaller molecules by addition of water molecules such as : esterase, carbohydrase, amylase, nuclease.

Hydrolase enzymes join smaller molecules to form large molecular compounds where water molecules are released. Such as : fumarase

enolase etc.

4. **Lyases :** This type of enzyme catalyse reaction where substrate special covalent bond are dissociated to release co-group without hydrolysis, like- aldolase.
5. **Isomerases :** This type of enzyme catalyses reaction that rearranges the intra molecular arrangement in substrate and changes the light orientation i.e. any substrate is changed in to optical isomer, e.g. phosphohexo isomerase.
6. **Ligases :** This type of enzyme catalyses the reaction in which two compounds are associated by covalent bonds e.g. Pyruvate carboxylase. Citrate synthatase.

Important Points

1. Enzyme is a type of biocatalyst made up of protein
2. Urease enzyme was first of all crystalized by J.B. Sumner in 1926.
3. Protein part of enzyme is called apoenzyme and non protein part is called co-enzyme jointly both are called holoenzyme.
4. Co factor can be an organic compound or charged ion.
5. Due to amphoteric nature of enzymes they remain active in both acidic and basic medium.
6. Mode of action of enzyme can be understood by lock and key theory, induced fit theory and lowering of activation energy.
7. Naming of enzymes is done by adding sufix 'ase' to the name of substrate they act on and the reaction they catalyse.
8. Enzymes are classified into six classes based on the reactions they catalyse.
   (I) Oxidoreductases
   (ii) Transferases
   (iii) Hydrolases
   (iv) Lyases
   (v) Isomerases
   (vi) Ligases

Practice Question

Multiple choice questions-

1. Enzymes are different from inorganic catalyses :
   (a) In high diffusion rate
   (b) Functional at high temperature
   (c) Protein nature
   (d) Use themselves in the reaction
2. Non protein part of an enzyme is called :
   (a) Apoenzyme
   (b) Holoenzyme
   (c) Prosthetic group
   (d) All of these
3. Which statement is correct :-
   (a) All protein are enzymes
   (b) All enzymes are proteins
   (c) Most of enzymes are protein
   (d) Non of these
4. Which enzyme was first to be discovered :-
   (a) Zymase (b) Lipase
   (c) Pepsin (d) Isomerase
5. Enzyme activity is affected :-
   (a) by pH
   (b) by substrate concentration
   (c) by temperature
   (d) All of these
6. Non competitive inhibitors are those, which :
   (a) Associate with active sites of enzyme
   (b) Destroy the active sites of enzyme
   (c) Change the constructive combination of enzyme
   (d) Do not change the characteristics of enzyme

7. The enzyme to be first crystalised was :

(a) Urease (b) Catalase

(c) Amylase (d) Aldolase

Very short answer questions -

1. Lock and key theory was postulated by whom and when ?
2. What is the protein and non protein part of an enzyme called?
3. Name one non-protein enzyme.
4. Define Prosthetic group.

Short answer questions -

1. What is co-enzyme? Give one example.
2. What do you understand by competitive inhibition? How can it be stopped ?
3. Briefly explain the nomenclature of enzyme.
4. How can the activation rate be increased by an enzyme.

Essay type questions-

1. Describe the structure of an enzyme. Also explain the main features of it.
2. Describe the catalytic process of an enzyme.
3. Explain in detail the classification of enzymes.
4. What is an enzyme inhibition? How many types of inhibitors are there and how can its effects be controlled? Explain.

Answer Key-

1. (c) 2. (b) 3. (c) 4. (a)
5. (d) 6. (c) 7. (a)