Chapter 3

ENZYMES

Life would not be possible with out metabolic activities of the cell. This in turn is depends upon the Catalytic molecules called the enzymes. With-out enzymes, the dynamic, steady state of the cell would cease to exist.

Life to a complex mesh work involving a perfect co-ordination of a vast majority of chemical reactions. Some of these reactions result in synthesizing large molecules, others in cleaving large molecules and still others either utilize energy or liberate energy. All these reactions would occur very slowly at low temperature and atmospheric pressures, the conditions under which living cells carry on their life proteases. But in the living system these reactions proceed at extremely high rates. This is due to the presence of some specialised substances or Biocatalysts which are synthesized inside the living cells. These biocatalysts are called **enzymes** (Gr: En = in; zyme = yeast). The term 'enzyme' was coined by Friedrich Wilhelm Kuhne (1878). Enzymes may be defined as organic substances capable of catalysing specific chemical reactions in the living system.

Just a few years ago, it was considered that all enzymes were proteins. During the 1980s, however, Thomas Cech and Sidney Altman discovered that certain molecules of ribonucleic acid also function as enzymes. These molecules are called **ribozymes;** which catalyze reactions involved in processing genetic information to be used by a cell. But generally enzymes are proteinaceous in nature.

3.1 ENERGY OF ACTIVATION

The questions arise here, how enzymes are able to accomplish such effective catalysis and why thermodynamically favourable reactions do not proceed on their own at relatively rapid rates in the absence of enzyme?

Chemical transformation requires that certain covalent bonds be broken within the reactants. To do so the reactants must contain sufficient kinetic energy (energy of motion) to overcome a barrier called Energy of activation or Activation energy. The important role played by the enzymes during reactions is that they lower the activation energy of the reaction. The enzyme reacts with the energy rich and energy poor molecules to form an intermediate complex. This complex again breaks into product and enzyme. If activation energy of this complex is low, many molecules can participate in reaction. In this way activation energy is lowered by the enzyme but in this action equilibrium (ratio of concentration of reactant and product) is never altered, it remains the same.

3.2 CHARACTERISTICS OF ENZYME

Enzymes are biocatalysts produced in the protoplasm. They are synthesized in the cell. The basic properties of enzymes are:

i) Most of the enzymes are proteinaceous in nature. They are macromolecules of globular proteins with higher molecular weight. They may entirely consist of protein e.g **amylase** or **pepsin** or may contain, along with protein, a non-protein part. e.g. holoenzyme.

ii) They react with both acidic and alkaline substances due to the presence of protein as their major part.

iii) Enzymes generally act within the living cell where they have been produced but sometimes they diffuse out of the cell and perform catalytic function out side the cell or in other cells. An enzyme which acts within the same cell is called intracellular enzymes or **endoenzyme** and the enzyme which acts outside the cell is called **exoenzyme**.

iv) They are specific in their nature and their action.

v) Their molecules are much greater in size than the substrate.

vi) They have particular sites to react with the substrates called **active site**.

vii) They are biocatalyst, which speed up the rate of reaction. They are required in very small quantities which are capable of bringing about a change in large amounts of substrates.

viii) Enzyme activities can be accelerated by certain ions or salts called **activators** e.g. Mn, Ni, Mg, Cl, etc.

ix) Enzymes activities can be inhibited by certain factors called inhibitors e.g. substrate concentration, enzyme concentration, pH.

x) They are heat sensitive i.e. they are thermolabile and pH sensitive i.e they work on specific pH.

xi) They remain chemically unchanged during and after the chemical reactions.

3.2.1 Mode of action:

Action of enzyme is related to it's structure which is complex and three dimensional. Each enzyme has a dimple or groove of a specific shape called the **active site**, into which substrate can fit

In order to explain the mode of action of enzyme, Fischer (1898) proposed a 'Key-Lock' theory which was later improved by Paul Filder and D. D Woods. They proposed that a particular enzyme acts on a particular substrate like particular lock can be unlocked by a particular key. This theory depends upon physical contact between substrate and enzyme molecules.

The active site of each enzyme has a distinct shape and distribution of charge which is complementary to its substrate, like lock and key, where a lock allows very few keys to fit in. Similarly enzymes allow a few complementary molecules to fit in and react while rejecting even fairly similar molecules.

On the other hand, some molecules may be able to fit in the active site of an enzyme but do not have chemical bond upon which the enzyme can act, so no reaction occurs.

Koshland (1959) proposed **Induce Fit Model**. He stated that when a substrate combines with an enzyme, it Induces changes in the enzyme structure, this change enables the enzyme to perform its catalytic activity more effectively.

3.3 TYPES OF ENZYMES

Enzymes are generally proteinaceous in nature. They may entirely consist of protein e.g amylase or may contain a non-protein part with protein. If an enzyme consists only of protein it is called simple enzyme (**Proteozyme**) and if it contains another group with protein it is called conjugated enzymes. Euler (1932) proposed that conjugated enzyme showing complete activity be called **holoenzyme**. It contains two parts, the protein part of enzyme is called **apoenzyme** and the non protein part is called **prosthetic** group.

On the basis of the nature of prosthetic group, conjugated enzymes or holoenzymes are of two types:

i) The holoenzymes in which prosthetic group is an inorganic ions are known as **co-factor**. Role of magnesium, manganese, calcium and potassium on enzymes like phosphatases, phosphorylase, amidase, peptidase, carboxylase are well known.

ii) The holoenzymes in which prosthetic group is an organic compound, although inorganic ions may also be present in it are called **co-enzymes**. A co-enzyme constitutes about 1% portion of the entire enzyme molecule. This part of enzyme is more or less easily separable, usually heat resistant. Some co-enzymes of oxidation and reduction processes are NAD (Nicotinamide adenine dinucleotide), NADP (Nicotinamide adenine dinucleotide phosphate), FMN (Flavin mononucleotide), ATP (Adenosine triphosphate) etc.

3.4 FACTORS AFFECTING ENZYME ACTIVITY

Following are the factors which affect the enzyme activity:

(1) Concentration of substrate (2) Temperature (3) pH

(4) Co-enzymes, activators and inhibitors (5) Water (6) Radiation.

1) Concentration of Substrate:

The rate of reaction increases with an increase in the concentration of substrate until the available enzyme becomes saturated with substrate. There is no increase in the enzymatic activity to a certain higher level of substrate concentration. At a very high concentration the substrate exerts a retarding effect upon enzyme action.

This may be due to two reasons:

(a) Higher quantity of substrate than enzyme.

(b) Accumulation of end product in high quantity.

Hence, substrate and enzyme concentration are directly proportional upto a certain maximum velocity after which further increase in substrate concentration has no effect on the rate of reaction.

2) Effect of temperature:

Enzymes are sensitive to temperature. Each enzyme has its optimum temperature for its maximum activity, above and below this temperature its rate of reaction decreases. Most of the enzymes are highly active at about 37°C and all are completely destroyed at 100°C, whereas at minimum i.e. 0°C, activity is reduced to minimum but enzymes are not destroyed.

3) Effect of pH:

The activity of enzyme varies considerably with pH and there is generally a marked optimum pH for each enzyme e.g. pepsin of stomach has an optimum pH of 1.4. It is inactive in neutral or alkaline solution.

4) Co-enzymes, activators and inhibitors:

Enzyme action is frequently accelerated or inhibited by the presence of other substances called **co-factors**. Co-factors have been divided into three categories.

i) Co-enzymes: If the co-factor is an organic molecule, its is called co-enzyme. Without co-enzyme certain enzymes are unable to function e.g. CoA,

NAD, FAD etc. Most vitamins are co-enzymes or raw materials from which co-enzymes are made.

ii) Activators: Inorganic substances which increase the activity of an enzyme are called activators. Magnesium (Mg^{+2}) is an inorganic activators for the enzyme phosphatase and Zinc ion (Zn^{+2}) is an activator for enzyme carbonic anhydrase.

iii) Inhibitors: Substances which decrease the activity of an enzyme are called inhibitors. The inhibitors may act by combining directly with the enzyme or they may react with the activator therefore, activator does not remain available to enzyme for activation.

Some inhibitors resemble the normal substrate molecule and compete for admission into the active site. These mimics, called **competitive inhibitors**, reduce the productivity of enzyme by blocking the substrate from entering into the active site. If the inhibition is reversible, it can be overcome by increasing the concentration of substrate so that as active site become available, more substrate molecule than inhibitor molecules are around to gain entery to these site.

Non-competitive inhibitors obstruct enzymatic reactions by binding to a part of the enzyme away from the active site. This interaction causes the enzyme molecule to change its shape, rendering the active site unreceptive to the substrate, or leaving the enzyme less effective at catalyzing, for the conversion of substrate to product. In non-competitive inhibition, a molecule binds to an enzyme other than its active site. This other binding site is called **allosteric site** (allo = other, steric = space or structure) and the inhibitor which acts, at this site is called allosteric inhibitor.

Feed-back inhibition:

The activity of almost every enzyme in a cell is regulated by feed-back inhibition. **Feed-back inhibition** is an example of a common biological control mechanism called **negative feedback**. When the product is in abundance, it binds competitively with it's enzyme's active site; once the product is used up, inhibition is reduced and more product can be produced. In this way concentration of the product is always kept within a certain range.

The pesticides DDT and parathion are inhibitors of key enzymes in the nervous system. Many antibiotic are inhibitors of specific enzyme in bacteria e.g. penicillin blocks the active site of an enzyme that many bacteria use to made cell-walls. These examples of enzyme inhibitors as metabolic poison may give the impression that enzyme inhibition generally abnormal and harmful

Most enzymatic pathways are also regulated by feed-back inhibition, but in these cases the end product of the pathway binds at an allosteric site on the first enzyme of the pathway. This binding shuts down the pathway and no more product is produced.

5) Effect of Water:

Water is necessary for enzyme activity as it influences the rate of enzymatic activity. In germinating seeds, with the increase in amount of water, to some extent, enzymes become active and germination proceeds.

6) Radiation:

Enzymes are generally inactivated rapidly by exposure to ultraviolet light and also to -.- and X-rays, because it alters the shape of protein i.e. enzymes.

KEY POINTS

- Enzymes are biocatalyst, speed up chemical reactions because they lower down the energy of activation. They can do this because they form a complex with their substrate(s) at the active site.
- Many enzymes have co-factors or co-enzymes that help them to carryout a reaction. Co-enzymes have non-protein organic molecules and are often derived, at least in part, from vitamins.
- Various factors affect the yield of enzymatic reactions, such as the concentration of the substrate(s), the temperature and the pH. A high temperature or a pH outside the preferred range for that enzyme can lead to denaturation, a change in structure that prevents the enzyme from functioning.

EXERCISE

1. Encircle the correct choice:

(i) Which molecule binds to the active site of an enzyme.
(a) Allosteric activator (b) Allosteric inhibitor
(c) Non-competitive inhibitor (d) Competitive inhibitor

(ii) Which metabolic process in bacteria is directly inhibited by the antibiotic pencillin.
(a) Cellular respiration (b) ATP hydrolysis
(c) Synthesis of fats (d) Synthesis of chemical components of the cell-wall

(iii) How does an enzyme increase the speed of a reaction?
(a) By adding activation energy
(b) By lowering activation energy requirements
(c) By decreasing concentration of reactants
(d) By increasing the concentration of products

(iv) An allosteric site on an enzyme is:
(a) The same as the active site
(b) Where ATP attaches and gives up its energy
(c) Often involved in feed-back inhibition
(d) At the opposite site of active site

(v) A temperature beyond optimum:
(a) Can affect the shape of an enzyme.
(b) Lowers the energy of activation.
(c) Makes cells less suspectible to disease.
(d) Both a and c.

(vi) Nucleic acid which also serve as enzymes are:
(a) Nucleoprotein (b) Ribozyme.
(c) Ribosome (d) Co-enzyme.

(vii) The activity of almost every enzyme in a cell is regulated by;
(a) Feed-back inhibition (b) Positive-feedback
(c) Negative-feedback (d) Feed-back control

(viii) The nonprotein part of an enzyme is:
(a) Prosthetic group (b) Co-enzyme
(c) Co-factor (d) All of them

(ix) The protein part of holo-enzyme is:
(a) Ribozoyme (b) Apoenzyme
(c) Acylglycerol (d) Co-enzyme.

(x) Magnessium (Mg^{+2}) is an inorganic activator for the enzyme:
(a) Manganase (b) Phosphatase
(c) Carbonic anhydrase (d) Hexokinase

2. Write detailed answers of the following questions:
(i) What are enzymes? Classify them and explain their role.
(ii) Write an essay on enzymes.
(iii) Write short notes on:

(a) Co-enzymes (b) Inhibitor
(c) Mode of action of enzyme

3. Give short answers of the following:

(i) Give three characters of enzyme.
(ii) Who proposed key and lock theory of enzyme action and how it works?
(iii) What is the effect of substrate concentration on enzyme activity?

4. Define the following terms:

(i) Enzyme
(ii) Activation Energy
(iii) Allosteric inhibitor
(iv) Active site
(v) Feedback inhibition