

Enzymes

SYNOPSIS

This chapter will cover the following topics

1. Catalysis
2. Enzyme catalyzed reactions
3. Mechanism of enzyme catalysis reaction
4. Enzyme classification
5. Mechanism of enzyme action
6. Enzyme kinetics
7. RNA catalysis

CATALYSIS

Humans have known about catalysis for many centuries, even though they knew nothing about the chemical process that was involved. The making of soap, the fermentation of wine to vinegar, and the leavening of bread are all processes involving catalysis. In 1812 Russian chemist Gottlieb Sigismund Constantin Kirchhof studied the behavior of starch in boiling water. Under most circumstances, Kirchhof found, no change occurred when starch was simply boiled in water. But adding just a few drops of concentrated sulfuric acid to the boiling water had a profound effect on the starch. In very little time, the starch broke down to form the simple sugar known as glucose. When Kirchhof found that the sulfuric acid remained unchanged at the completion of the experiment, he concluded that it had simply played a helping role in the conversion of starch to sugar.

94 Syllabus
Catalysis reactions are usually categorized as either homogeneous or heterogeneous reactions. A homogeneous catalysis reaction is one in which both the catalyst and the substances are in the same phase, i.e either solid, liquid or gas. A heterogeneous catalysis reaction is one in which the catalyst is in a different phase from the substances on which it acts. One of the most interesting and important catalytic reactions is the Haber-Bosch process.

Some of the most interesting and important catalysts are those that occur in living systems: The enzymes. All of the reactions that take place within living bodies occur naturally, whether or not a catalyst is present. But they take place so slowly on their own that they are of no value to the survival of an organism. For example, when a sugar cube is placed in a glass of water, it eventually breaks down into simpler molecules with the release of energy. But that process might take years. A person who ate a sugar cube and had to wait that long for the energy to be released in the body would die. Our body also contains catalysts (enzymes) that speed up such reactions. They make it possible for the energy stored in sugar molecules to be released in a matter of minutes.

INTRODUCTION OF ENZYMES

An enzyme is a specialized protein produced with an organism which is capable of catalyzing a specific chemical reaction. Since the enzyme acts as a catalyst, it is also called a biocatalyst. A catalyst influences the rate of a chemical reaction, usually without undergoing any change itself so in this respect an enzyme differs from a normal catalyst.

STRUCTURE OF ENZYMES

All enzymes are proteins which are high molecular weight macromolecules. An enzyme may consist of a single polypeptide chain or a cluster of polypeptide chains. A polypeptide chain is made up of number of amino acid units linked by peptide bonds.

The sequence and number of the 20 amino acids which make up enzyme varies in different enzymes. This arrangement of enzymes is specific for a particular enzyme and determines the

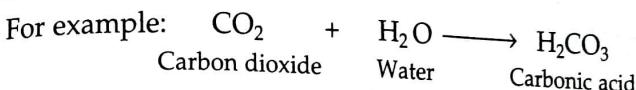
Enzymes

properties of the enzyme. The polypeptide chain has an amino (-NH₂) terminal and a carboxyl (-COOH) terminal biosynthesis of the enzyme begins at amino terminal.

The different parts of the polypeptide chain are linked by disulphide (-S-S-) bridges. These bridges may be within a polypeptide chain or may connect two polypeptide chains.

ACTIVE SITE

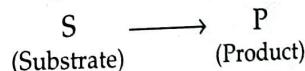
An enzyme has a distinct cavity or cleft in which the substrate is bound. A substrate is a specific compound acted upon by an enzyme. The part of the enzyme where the substrate binds is called the **active site** (since that is where the catalytic "action" happens).



In the absence of the enzyme carbonic anhydrase this reaction is very slow, producing two hundred molecules of carbonic anhydrase in an hour but in the presence of carbonic anhydrase present in the cytoplasm, this reaction speeds up dramatically with roughly six lakhs molecules formed every second.

| NATURE OF ENZYME ACTION

As we already know that an enzyme has a distinct cavity in which substrate bounds. The cleft contains an active centre in which amino acids are grouped together in such a way as to enable them to combine with substrate, it is the chemical which converts into a product. Thus enzymes include active sites which are capable of converting substrate (S) into a product (P).



1. The substrate 'S' binds to the active site of the enzyme for which it has to diffuse towards the active site. There is the formation of 'ES' (enzyme substrate) complex. This complex formation lasts for a short time and is called transient phenomenon.

2. When substrate binds to the active site of enzyme a new structure of the substrate called transition state structure is formed.
3. The molecules of the substrate group undergo chemical changes, breaking or making of bonds and finally the product is formed and is released from the active site.
4. The pathways of this transformation of substrate into product must go through the so called transition state structure.
5. The molecules of the substrate group undergo chemical changes, breaking or making of bonds and finally the product is formed and is released from the active state.
6. The pathways of this transformation of substrate into product must go through the so called transition structure. There can be many altered structural states between the stable substrate and the product. In this change of substrate to product, all intermediate structural states are unstable.

How do Enzymes Catalyze Reaction

Each enzyme has an active site to which substrate binds and forms a short-lived highly reactive enzyme substrate complex. This is followed by enzyme product complex (EP). Finally the enzyme product complex dissociates into product (P) and the enzyme freed, remains unchanged and is able to bind more substrate molecules (Fig. 5.1).

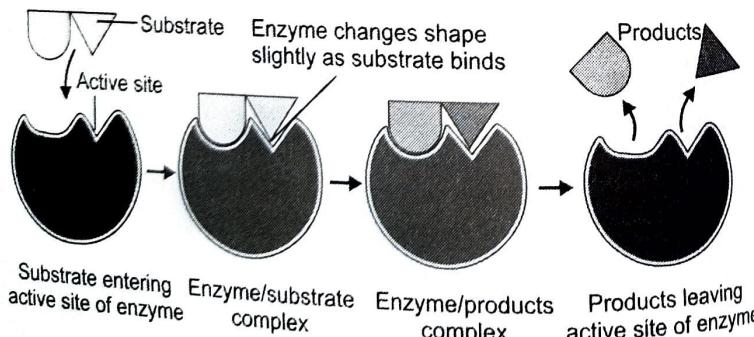


Fig. 5.1: Substrate and active site binding mechanism

The formation of the ES complex is essential for catalysis
 $E + S \rightarrow ES \rightarrow EP \rightarrow E + P$

The catalytic cycle of an enzyme can be described as:

1. The substrate binds to the active site of enzyme
2. The binding of the substrate induces the enzyme to alter its shape and fit tightly around the substrate.
3. The active site of the enzyme which is in close proximity of the substrate break the chemical bonds of the substrate and an enzyme product complex is formed.
4. The enzyme releases the products of the reaction and the free enzyme is ready to take up another molecule of substrate.

FACTORS AFFECTING ENZYME ACTIVITY

Enzymes are proteins with tertiary structure. Any change in tertiary structure would affect the action of enzymes. Factors which affect enzyme action are as follows:

1. **Temperature:** Enzyme action is greatly affected by temperature. The temperature at which enzymes show their highest activity is called optimum temperature. Enzyme activity declines both above and below the optimum temperature. At low temperature, enzymes become temporarily inactive and increasing the temperature to normal, they regain their lost activity. At high temperature there is a loss of enzyme activity due to protein denaturation. At higher temperature kinetic energy of molecules in an enzyme becomes strong to break the weak hydrogen bond present in tertiary structure of enzyme resulting in loss of catalytic activity. This change in structure is called denaturation of enzyme. Once an enzyme denatures, it remains inactive as temperature is lowered down (Fig. 5.2).

The optimum temperature for human enzymes is 35–40°C. The enzyme activity decreases with decrease as well as increase in temperature and stops at 0°C and above 80°C.

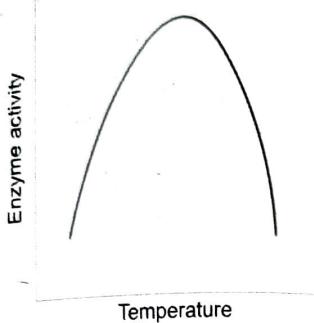


Fig. 5.2: Effect of change in temperature on enzyme activity

2. **pH:** At optimum pH the activity of enzymes is maximum for most enzymes, the effective pH range is 4–9. Beyond these limits denaturation of enzymes takes places. For example, the optimum pH for pepsin is 2 and for trypsin is 8 (Fig. 5.3).

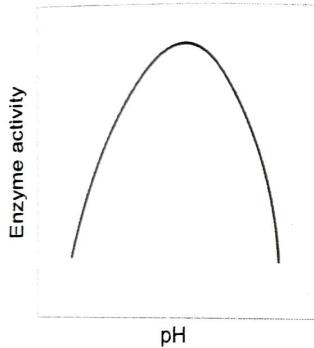


Fig. 5.3: Effect of change in pH on enzyme activity

3. **Concentration of substrate:** Increase in substrate concentration, increases the velocity of enzymatic reaction. The reaction soon reaches a maximum velocity (V_{max}) which is not exceeded by further rise in concentration of substrate. This is because, at this stage the enzyme molecules become fully saturated and no active site is left free to bind to additional substrate molecules.
4. **Product concentration:** Accumulation of the product of enzyme reaction lowers the enzyme activity. Enzyme molecules must be freed to combine with more substrate

Enzymes

molecules. Normally the products are quickly removed from the site of formation and reaction does not suffer.

NOMENCLATURE AND CLASSIFICATION OF ENZYMES

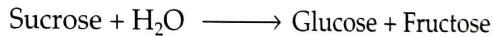
Enzymes are generally named by adding 'ase' to the root indicating the substrate on which the enzyme acts. The International Union of Biochemistry report of 1962 contains a scheme for enzyme classification. Enzymes have been divided into 6 groups.

1. **Oxireductases dehydrogenases:** Enzymes which catalyze oxidation reduction reactions involving transfer of electrons/ H^+ from one molecule to another, in these reactions one compound is oxidized and the other is reduced.
Example: Dehydrogenase, oxidase, reductase



2. **Transferases:** These enzymes catalyse the transfer of specific group other than hydrogen from one substrate to another.
Example: Kinase catalyse the phosphorylation of substrate by transferring phosphate group from ATP.

3. **Hydrolysis:** These enzymes catalyze the breakdown of larger molecules into smaller molecules with the addition of water. These bring hydrolysis of ether, peptide and ester.
Examples: Amylase, lipase, maltase



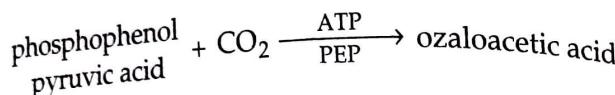
4. **Lyases:** These enzymes catalyze the cleavage of substrate into two parts, without the use of water or removal of group without hydrolysis. A double bond is formed at the place of removal of group.

Examples: Decarboxylase, carbonic anhydrase, etc.

5. **Isomerase:** These enzymes catalyse the rearrangement of molecular structure to form isomers. Isomers are the molecular compounds that are similar in having the same molecular formula but have different arrangement of atoms. Example: Isomerase



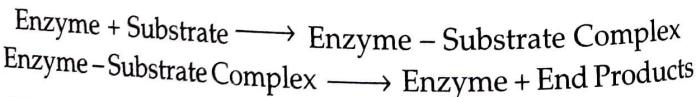
6. Ligases: These enzymes catalyse covalent bonding of two substrates to form a large molecule. They catalyze joining of C-O, C-S, P-O bonds by using ATP as energy source.
Examples: RUBP carboxylase, phosphoenol pyruvate (PEP)



MECHANISM OF ENZYME ACTION

Two hypotheses have been put forward to explain the mode of enzyme action.

1. Lock and key hypothesis: This hypothesis was given by Emil Fischer in 1984. According to this hypothesis, both enzyme and substrate molecules have specific geometrical shapes. It is similar to the system of lock and key, which have special geometrical shapes in the region of their activity. The active site contains special groups having $-\text{NH}_2$, $-\text{COOH}$, $-\text{SH}$ for establishing contact with the substrate molecules. Just as a lock can be opened by its specific key, a substrate molecule can be acted upon by a particular enzyme. This explains the specificity of enzyme action. After coming in contact with the active site of enzyme, the substrate molecule forms a complex called enzyme substrate complex. In this enzyme substrate complex, the molecules of the substrate undergo chemical change and form products. The product no longer fits into the active site and escapes in surrounding medium, leaving the active site free to receive more substrates molecules (Fig. 5.4).



This theory explains how a small concentration of enzyme can act upon a large amount of the substrate. It also explains how the enzyme remains unaffected at the end of chemical reaction. The theory explains how a substance

having a structure similar to the substrate can work as a competitive inhibitor.

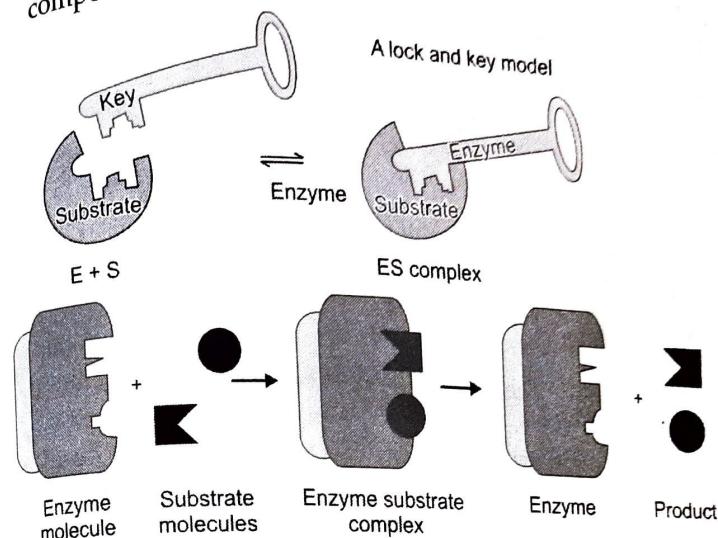


Fig. 5.4: Lock and key hypothesis to show the specificity of enzymes

2. Induced fit hypothesis: This hypothesis was proposed by Koshland in 1960. According to this hypothesis, the active site of the enzyme does not initially exist in a shape that is complementary to the substrate but is induced to assume the complementary shape as the substrate becomes bound to the enzyme. According to Koshland, the active site reaches a complementary shape in a similar way as a hand induces a change in the shape of glove. An active site of an enzyme is a crevice or a pocket into which the substrate fits. Thus, enzymes through their active site, catalyze reactions at a higher rate. Hence according to this model, the enzyme and its active site is flexible and the active site of the enzyme contains two groups:

- Buttressing group meant for supporting substrate.
- Catalytic group meant for catalyzing the reaction when substrate comes in contact with the buttressing group, the active site changes to bring the catalytic group opposite the substrate bonds to be broken (Fig. 5.5).

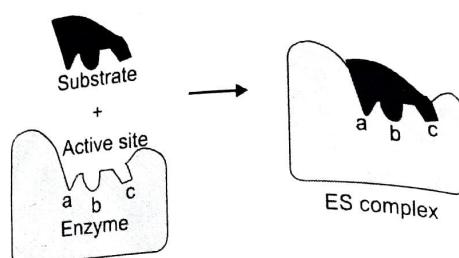


Fig. 5.5: Induce fit hypothesis

ENZYME KINETICS

In an enzymatic reaction $S \rightarrow P$, the rate of the reaction is dependent on the substrate concentration. At low substrate concentration, the rate of reaction 'v' is proportional to substrate concentration. As the substrate concentration is increased, the velocity of reaction falls and is no longer proportional to the substrate concentration. With the further increase in substrate concentration, the rate of reaction becomes constant and independent of substrate concentration. The enzyme at this stage shows the saturation effect, i.e. it has become saturated with the substrate and levels off to a flat plateau at high substrate concentration.

The plateau occurs because the enzyme is saturated, meaning all available enzyme molecules are tied up with substrates. Any additional substrate molecule will have to wait around till another enzyme is available, so we can say rate of reaction is limited by enzyme concentration. This maximum rate of reaction is characteristic of a particular enzyme at a particular concentration and is known as the maximum velocity or V_{max} .

The substrate concentration that gives a rate that is halfway to V_{max} is called K_m and is a useful measure of how quickly reaction rate increases with substrate concentration. K_m is also a measure of an enzyme affinity (tendency to bind) to its substrate. A low K_m corresponds to a higher affinity for substrate, while a higher K_m corresponds to a lower affinity for the substrate.

This saturation effect led Michaelis and Menten to a general theory of enzyme action and kinetics in the year 1913 (Fig. 5.6).

Michaelis-Menten plot

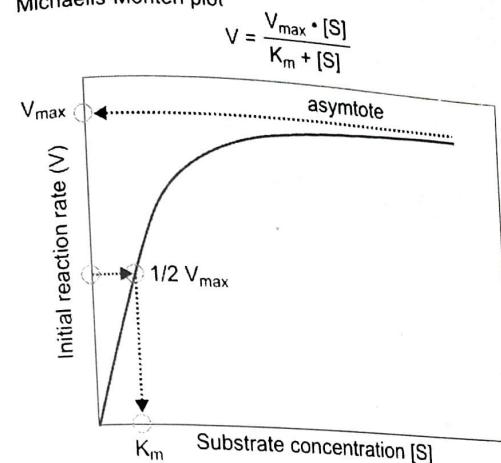


Fig 5.6: Effect of change in concentration of substrate on enzyme activity

S = Substrate

V = Reaction velocity

V_{max} = Maximum velocity

K_m = Michaelis-Menten constant

INHIBITION OF ENZYME ACTION

Any substance which can diminish the velocity of an enzyme catalysed reaction is called inhibitor. They act in three different ways.

1. Competitive inhibition: This type of inhibition occurs when the inhibitor binds reversibly at the same site where substrate would normally bind and therefore competes with the substrate for that site.

Effect on V_{max} : The effect of a competitive inhibitor can be reversed by increasing substrate. At a high substrate concentration, the reaction velocity reaches V_{max} as observed in the absence of inhibitor.

Effect on K_m : A competitive inhibitor increases K_m for the given substrate. This means that in the presence of a competitive inhibitor more substrate is needed to achieve $\frac{1}{2} V_{max}$ (Fig. 5.7).

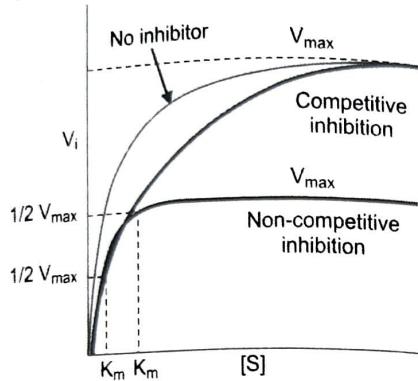


Fig. 5.7: Effect of a competitive inhibitor on the reaction velocity (V_0) versus substrate $[S]$ plot

2. Non-competitive inhibition: This inhibition is brought about by a substance which does not resemble the substrate in structure. This non-competitive inhibitor binds to the enzyme at some site other than the substrate binding site, thus no product is formed.

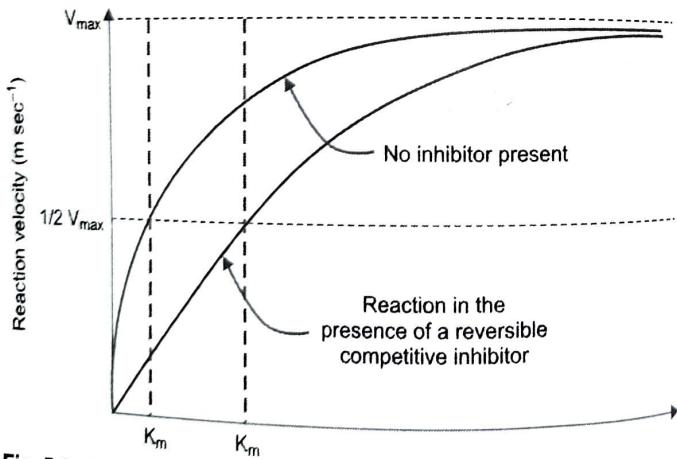


Fig. 5.8: Effect of a non-competitive inhibitor on the reaction velocity (V_0) versus substrate $[S]$ plot

Effect on V_{max} : Non-competitive inhibitor cannot be overcome by increasing the concentration of substrate, so non-competitive inhibitors decrease the V_{max} of the reaction.

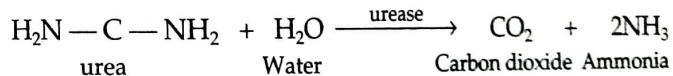
Effect on K_m : Non-competitive inhibitors do not interfere with binding of substrate to enzyme. Thus the enzyme shows the same K_m in presence or absence of non-competitive inhibitor.

3. **Allosteric inhibition:** Some inhibitors join an enzyme at a specific site and change the form of the active site meant for the substrate. These inhibitors are also known as modifiers.

IMPORTANCE OF ENZYMES IN BIOLOGY

Enzymes play a significant role in variety of processes:

1. **Biological uses:** A large number of chemical reactions take place in a living cell. These reactions made to occur outside a living cell would require a very high temperature or would occur very slowly. These biological enzymes make the biochemical reactions occur at ordinary temperature and also at quick pace. For example, one molecule of enzyme urease can break down 30000 molecules of urea into carbon dioxide and ammonia in one second. In the absence of enzyme, this reaction would take years.



2. **Physiology:** Enzymes present in stomach quickly and efficiently carry out the process of digestion. Enzymes are also important for respiration, nerve impulse transmission, blood clotting, etc; these enzymes are essential for carrying out biochemical processes.

3. **Medical diagnosis:** ELISA (enzyme linked immunosorbent assay) is used for detecting diseases like AIDS, Lyme disease.

4. **Medical treatment:** Enzyme streptokinase is used for dissolving blood clot formed inside blood vessels.

5. **Genetic engineering:** Enzyme like ligases and endonucleases are used in genetic engineering.

Enzymes work efficiently in association with various factors which enhance its activity, these factors may be:

Cofactors which are small non-protein inorganic molecule that carries out chemical reactions that cannot be performed by the standard 20 amino acids. Examples of cofactors include metal ions like iron and zinc.

Coenzymes which are organic molecules that are non-proteins and mostly derivatives of vitamins soluble in water by phosphorylation. Examples of coenzyme include thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), biotin

Apoenzyme is an inactive form of enzyme lacking the association of coenzyme and/or cofactors. Activation of the enzyme occurs upon binding of an organic or inorganic cofactor.

Holoenzyme is a complete and catalytically active form of enzyme. An apoenzyme together with its cofactor is holoenzyme. Examples of holoenzymes include DNA polymerase and RNA polymerase which contain multiple protein subunits (Fig. 5.9).

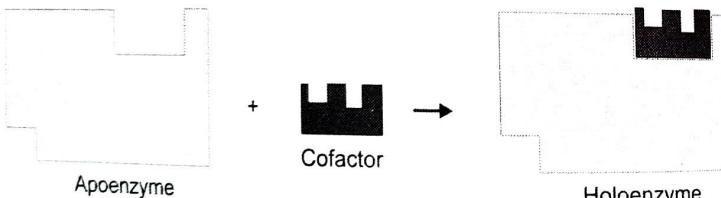


Fig. 5.9: Apoenzyme + Cofactor = Holoenzyme

RNA Catalysis

Ribozymes are (RNA molecules that accelerate chemical reactions) the enzymes that happen to be made up of RNA rather than protein. Two most important reactions of the cells catalysed by RNA are:

1. Splicing
2. Viral replication

Investigators studying origin of life have produced ribozymes in the lab which are capable of catalyzing their own synthesis from monomers.

Application

1. Ribozymes have been proposed and developed for the treatment of disease through gene therapy.
2. Synthetic ribozyme has been developed and entered clinical testing for HIV infection.
3. Ribozyme have been designed to target the hepatitis C virus.

KEY POINTS

- A catalyst influences the rate of a chemical reaction, usually without undergoing any change itself.
- Homogeneous catalysis reaction is one in which both the catalyst and the substances are in the same phase, i.e. either solid, liquid or gas.
- Heterogeneous catalysis reaction is one in which the catalyst is in a different phase from the substances on which it acts.
- An enzyme is a specialized protein produced with an organism which is capable of catalyzing a specific chemical reaction.
- All enzymes are proteins which are high molecular weight macromolecules.
- Active site: An enzyme has a distinct cavity or cleft in which the substrate is bound.
- Factors affecting enzyme action are temperature, pH, concentration of substrate and product concentration
- Mechanism of enzyme action: Two hypothesis 1. Lock and key hypothesis, 2. Induced fit hypothesis:
- Inhibition of enzyme action: Act in three different ways
 1. Competitive inhibition, 2. Non-competitive inhibition
 3. Allosteric inhibition