6 Enzymes

Enzymes are biocatalysts – the catalysts of life. A ***catalyst*** is defined as a ***substance that increases the velocity*** or rate of a chemical reaction without itself undergoing any change in the overall process.

The student-teacher relationship may be a good example to understand how a catalyst works. The students often find it difficult to learn from a text-book on their own. The teacher explains the subject to the students and increases their understanding capability. It is no wonder that certain difficult things which the students take days together to understand, and sometimes do not understand at all – are easily learnt under the guidance of the teacher. Here, the teacher acts like a catalyst in enhancing the understanding ability of students. A good teacher is always a good catalyst in students' life!

Enzymes may be defined as biocatalysts synthesized by living cells. They are protein in nature (exception – RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action.

In the laboratory, hydrolysis of proteins by a strong acid at 100°C takes at least a couple of days. The same protein is fully digested by the enzymes in gastrointestinal tract at body temperature (37°C) within a couple of hours. This remarkable difference in the chemical reactions taking place in the living system is exclusively due to enzymes. The very existence of life is unimaginable without the presence of enzymes.

HISTORICAL BACKGROUND

Berzelius in 1836 coined the term ***catalysis*** (*Greek* : to dissolve). In 1878, Kuhne used the word ***enzyme*** (*Greek* : in yeast) to indicate the catalysis taking place in the biological systems. Isolation of enzyme system from cell-free extract of yeast was achieved in 1883 by Buchner. He named the active principle as zymase (later found to contain a mixture of enzymes), which could convert sugar to alcohol. In 1926, James

Sumner ***first*** achieved the ***isolation*** and crystallization of the enzyme ***urease*** from jack bean and identified it as a protein.

NOMENCLATURE AND CLASSIFICATION

In the early days, the enzymes were given names by their discoverers in an arbitrary manner. For example, the names pepsin, trypsin and chymotrypsin convey no information about the function of the enzyme or the nature of the substrate on which they act. Sometimes, the suffix-***ase*** was added to the substrate for naming the enzymes e.g. lipase acts on lipids; nuclease on nucleic acids; lactase on lactose. These are known as ***trivial names*** of the enzymes which, however, fail to give complete information of enzyme reaction (type of reaction, cofactor requirement etc.)

Enzymes are sometimes considered under two broad categories : (a) **Intracellular enzymes** – They are functional within cells where they are synthesized. (b) **Extracellular enzymes** – These enzymes are active outside the cell; all the digestive enzymes belong to this group.

The International Union of Biochemistry (IUB) appointed an Enzyme Commission in 1961. This committee made a thorough study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes. Since 1964, the ***IUB system of enzyme classification*** has been in force. Enzymes are divided into ***six major classes*** (in that order). Each class on its own represents the general type of reaction brought about by the enzymes of that class (***Table 6.1***).

Table 6.1 Classification of enzymes

*Enzyme class with examples**	*Reaction catalysed*
1. Oxidoreductases	
Alcohol dehydrogenase (alcohol : NAD^+ oxidoreductase E.C. 1.1.1.1.), cytochrome oxidase, L- and D-amino acid oxidases	Oxidation ⟶ Reduction $AH_2 + B \longrightarrow A + BH_2$
2. Transferases	
Hexokinase (ATP : D-hexose 6-phosphotransferase, E.C. 2.7.1.1.), transaminases, transmethylases, phosphorylase	Group transfer $A - X + B \longrightarrow A + B - X$
3. Hydrolases	
Lipase (triacylglycerol acyl hydrolase E.C. 3.1.1.3), choline esterase, acid and alkaline phosphatases, pepsin, urease	Hydrolysis $A - B + H_2O \longrightarrow AH + BOH$
4. Lyases	
Aldolase (ketose 1-phosphate aldehyde lyase, E.C. 4.1.2.7), fumarase, histidase	Addition ⟶ Elimination $A - B + X - Y \longrightarrow AX - BY$
5. Isomerases	
Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, E.C. 5.3.1.1), retinol isomerase, phosphohexose isomerase	Interconversion of isomers $A \longrightarrow A'$
6. Ligases	
Glutamine synthetase (L-glutamate ammonia ligase, E.C. 6.3.1.2), acetyl CoA carboxylase, succinate thiokinase	Condensation (usually dependent on ATP) $A + B \xrightarrow[ATP \quad ADP + Pi]{} A - B$

*For one enzyme in each class, systematic name along with E.C. number is given in the brackets.

1. **Oxidoreductases** : Enzymes involved in oxidation-reduction reactions.

2. **Transferases** : Enzymes that catalyse the transfer of functional groups.

3. **Hydrolases** : Enzymes that bring about hydrolysis of various compounds.

4. **Lyases** : Enzymes specialised in the addition or removal of water, ammonia, CO_2 etc.

5. **Isomerases** : Enzymes involved in all the isomerization reactions.

6. **Ligases** : Enzymes catalysing the synthetic reactions (*Greek* : ligate—to bind) where two molecules are joined together and ATP is used.

[The word ***OTHLIL*** (first letter in each class) may be memorised to remember the six classes of enzymes in the correct order].

Each class in turn is subdivided into many sub-classes which are further divided. A four digit *E*nzyme *C*ommission (***E.C.***) number is assigned to each enzyme representing the class (first digit), sub-class (second digit), sub-sub class (third digit) and the individual enzyme (fourth digit). Each enzyme is given a specific name indicating the substrate, coenzyme (if any) and the type of the reaction catalysed by the enzyme. Although the IUB names for the enzymes are specific and unambiguous, they have not been accepted for general use as they are complex and cumbersome to remember. Therefore, the trivial names, along with the E.C. numbers as and when needed, are commonly used and widely accepted.

CHEMICAL NATURE AND PROPERTIES OF ENZYMES

All the enzymes are invariably proteins. In recent years, however, a few RNA molecules have been shown to function as enzymes. Each enzyme has its own tertiary structure and specific conformation which is very essential for its catalytic activity. The functional unit of the enzyme is known as ***holoenzyme*** which is often made up of ***apoenzyme*** (the protein part) and a ***coenzyme*** (non-protein organic part).

Holoenzyme ⟶ Apoenzyme + Coenzyme
(active enzyme) (protein part) (non-protein part)

The term ***prosthetic group*** is used when the non-protein moiety tightly (covalently) binds with the apoenzyme. The coenzyme can be separated by dialysis from the enzyme while the prosthetic group cannot be.

The word ***monomeric enzyme*** is used if it is made up of a single polypeptide e.g. ribonuclease, trypsin. Some of the enzymes which possess more than one polypeptide (subunit) chain are known as ***oligomeric enzymes*** e.g. lactate dehydrogenase, aspartate transcarbamoylase etc. There are certain ***multienzyme complexes*** possessing specific sites to catalyse different reactions in a sequence. Only the native intact multienzyme complex is functionally active and not the individual units, if they are separated e.g. pyruvate dehydrogenase, fatty acid synthase, prostaglandin synthase etc. The enzymes exhibit all the general properties of proteins (***Chapter 4***).

Genetic engineering and modified enzymes

Recent advances in biotechnology have made it possible to modify the enzymes with desisable characters-improved catalytic abilities, activities under unsual conditions. This approach is required since enzymes possess enormous potential for their use in medicine and industry.

Hybrid enzymes : It is possible to rearrange genes and produce ***fusion proteins***. e.g. a hybrid enzyme (of glucanase and cellulase) that can more efficiently hydrolyse barley β-glucans in beer manufacture.

Site-directed mutagenesis : This is a technique used to produce a specified mutation at a predermined position in a DNA molecule. The result is incorporation of a desired amino acid (of one's choice) in place of the specified amino acid in the enzyme. By this approach, it is possible to produce an enzyme with desirable characteristics. e.g. tissue plasminogen activator (used to lyse blood clots in myocardial

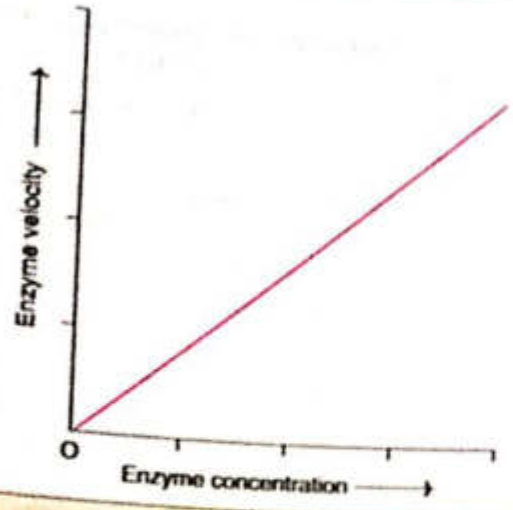


Fig. 6.1 : *Effect of enzyme concentration on enzyme velocity.*

infarction) with increased half-life. This is achieved by replacing asparagine (at position 120) by glutamine.

In recent years, it has also become possible to produce ***hybrid enzymes*** by rearrangement of genes. Another innovative approach is the production ***abzymes*** of catalytic antibodies, the (antibody enzymes).

FACTORS AFFECTING ENZYME ACTIVITY

The contact between the enzyme and substrate is the most essential pre-requisite for enzyme activity. The important factors that influence the velocity of the enzyme reaction are discussed hereunder

1. Concentration of enzyme

As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases (***Fig.6.1***). In fact, this property of enzyme is made use in determining the serum enzymes for the diagnosis of diseases. By using a known volume of serum, and keeping all the other factors (substrate, pH, temperature etc.) at the optimum level, the enzyme could be assayed in the laboratory.

2. Concentration of substrate

Increase in the substrate concentration gradually ***increases the velocity of enzyme reaction*** within the limited range of substrate levels. A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration (***Fig.6.2***). Three distinct phases of the reaction are observed in the graph (A-linear; B-curve; C-almost unchanged).

Order of reaction : When the velocity of the reaction is almost proportional to the substrate concentration (i.e. [S] is less than K_m), the rate of the reaction is said to be ***first order*** with respect to substrate. When the [S] is much greater than K_m, the rate of reaction is independent of substrate concentration, and the reaction is said to be ***zero order***.

Enzyme kinetics and K_m value : The enzyme (E) and substrate (S) combine with each other to form an unstable enzyme-substrate complex (ES) for the formation of product (P).

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

Here k_1, k_2 and k_3 represent the velocity constants for the respective reactions, as indicated by arrows.

K_m, the Michaelis-Menten constant (or ***Brig's and Haldane's constant***), is given by the formula

$$K_m = \frac{k_2 + k_3}{k_1}$$

The following equation is obtained after suitable algebraic manipulation.

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad \text{equation (1)}$$

where v = Measured velocity,
V_{max} = Maximum velocity,
S = Substrate concentration,
K_m = Michaelis – Menten constant.

Let us assume that the measured velocity (v) is equal to $\frac{1}{2}V_{max}$. Then the equation (1) may be substituted as follows

$$\frac{1}{2}V_{max} = \frac{V_{max}[S]}{K_m + [S]}$$

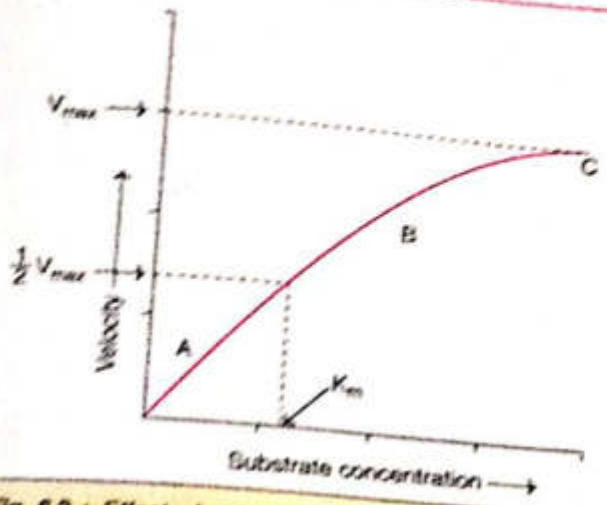


Fig. 6.2 : Effect of substrate concentration on enzyme velocity (A-linear; B-curve; C-almost unchanged)

$$K_m + [S] = \frac{2V_{max}[S]}{V_{max}}$$

$$K_m + [S] = 2[S]$$

$$K_m = [S]$$

K stands for a constant and ***m*** stands for *Michaelis* (in K_m).

K_m or the ***Michaelis-Menten constant*** is defined as the ***substrate concentration*** (expressed in moles/l) ***to produce half-maximum velocity*** in an enzyme catalysed reaction. It indicates that half of the enzyme molecules (i.e. 50%) are bound with the substrate molecules when the substrate concentration equals the K_m value.

K_m value is a constant and a characteristic feature of a given enzyme (comparable to a thumb impression or signature). It is a representative for measuring the strength of ES complex. A ***low K_m value indicates a strong affinity between enzyme and substrate***, whereas a high K_m value reflects a weak affinity between them. For majority of enzymes, the K_m values are in the range of 10^{-5} to 10^{-2} moles. It may however, be noted that K_m is not dependent on the concentration of enzyme.

Lineweaver-Burk double reciprocal plot : For the determination of K_m value, the substrate saturation curve (***Fig.6.2***) is not very accurate since V_{max} is approached asymptotically. By taking the reciprocals of the equation (1), a straight line graphic representation is obtained.

$$\frac{1}{v} = \frac{K_m + [S]}{V_{max}[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

The above equation is similar to y = ax + b. Therefore, a plot of the reciprocal of the velocity $\left(\frac{1}{v}\right)$ vs. the reciprocal of the substrate concentration $\left(\frac{1}{[S]}\right)$ gives a straight line. Here the slope is K_m/V_{max} and whose y intercept is $1/V_{max}$.

The Lineweaver-Burk plot is shown in ***Fig.6.3***. It is much easier to calculate the K_m from the intercept on x-axis which is $-(1/K_m)$. Further, the double reciprocal plot is useful in understanding the effect of various inhibitions (discussed later).

Enzyme reactions with two or more substrates : The above discussion is based on the presumption of a single substrate-enzyme reaction. In fact, a majority of the enzyme-catalysed reactions involve two or more substrates. Even in case of ***multisubstrate***

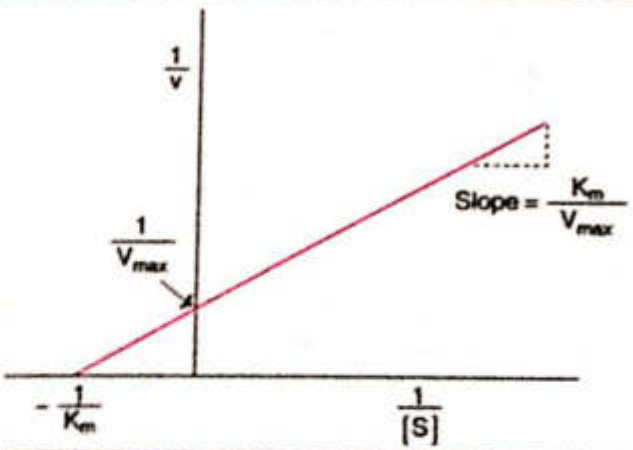


Fig. 6.3 : Lineweaver-Burk double reciprocal plot

enzymes, despite the complex mathematical expressions, the fundamental principles conform to Michaelis-Menten Kinetics.

3. Effect of temperature

Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A ***bell-shaped curve*** is usually observed (***Fig.6.4***).

Temperature coefficient or Q_{10} is defined as increase in enzyme velocity when the temperature is increased by 10°C. For a majority of enzymes, Q_{10} is 2 between 0°C and 40°C. Increase in temperature results in higher activation energy of the molecules and more molecular (enzyme and substrate) collision and interaction for the reaction to proceed faster.

The optimum temperature for most of the enzymes is between 40°C–45°C. However, a few enzymes (e.g. venom phosphokinases, muscle adenylate kinase) are active even at 100°C. Some plant enzymes like urease have optimum activity around 60°C. This may be due to very stable structure and conformation of these enzymes.

In general, when the enzymes are exposed to a temperature above 50°C, ***denaturation*** leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above 70°C).

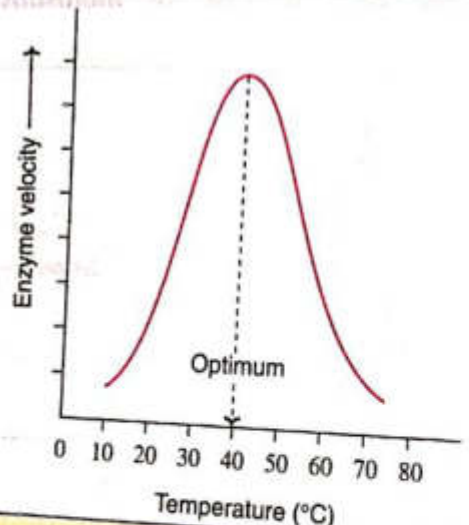


Fig. 6.4 : Effect of temperature on enzyme velocity.

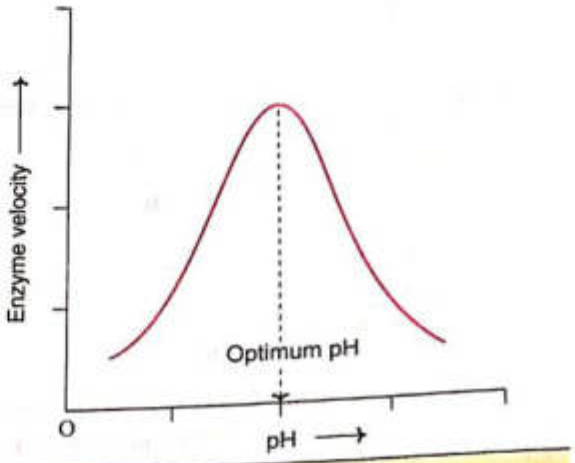


Fig. 6.5 : Effect of pH on enzyme velocity.

It is worth noting here that the enzymes have been assigned optimal temperatures based on the laboratory work. These temperatures, however, may have less relevance and biological significance in the living system.

4. Effect of pH

Increase in the hydrogen ion concentration (pH) considerably influences the enzyme activity and a ***bell-shaped curve*** is normally obtained (***Fig.6.5***). Each enzyme has an optimum pH at which the velocity is maximum. Below and above this pH, the enzyme activity is much lower and at extreme pH, the enzyme becomes totally inactive.

Most of the enzymes of higher organisms show optimum activity around neutral pH (6-8). There are, however, many exceptions like pepsin (1-2), acid phosphatase (4-5) and alkaline phosphatase (10-11). Enzymes from fungi and plants are most active in acidic pH (4-6).

Hydrogen ions influence the enzyme activity by altering the ionic charges on the amino acids (particularly at the active site), substrate, ES complex etc.

5. Effect of product concentration

The accumulation of reaction products generally decreases the enzyme velocity.

For certain enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity. In the living system, this type of inhibition is generally prevented by a quick removal of products formed. The end product inhibition by feedback mechanism is discussed later.

6. Effect of activators

Some of the enzymes require certain inorganic ***metallic cations*** like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^{+}, K^{+} etc. for their optimum activity. Rarely, anions are also needed for enzyme activity e.g. chloride ion (Cl^{-}) for amylase. Metals function as activators of enzyme velocity through various mechanisms—combining with the substrate, formation of ES-metal complex, direct participation in the reaction and bringing a conformational change in the enzyme.

Two categories of enzymes requiring metals for their activity are distinguished

- **Metal-activated enzymes :** The metal is not tightly held by the enzyme and can be exchanged easily with other ions

  e.g. ATPase (Mg^{2+} and Ca^{2+})

  Enolase (Mg^{2+})

- **Metalloenzymes :** These enzymes hold the metals rather tightly which are not readily exchanged. e.g. alcohol dehydrogenase, carbonic anhydrase, alkaline phosphatase, carboxypeptidase and aldolase contain zinc.

  Phenol oxidase (copper);

  Pyruvate oxidase (manganese);

  Xanthine oxidase (molybdenum);

  Cytochrome oxidase (iron and copper).

7. Effect of time

Under ideal and optimal conditions (like pH, temperature etc.), the time required for an enzyme reaction is less. Variations in the time of the reaction are generally related to the alterations in pH and temperature.

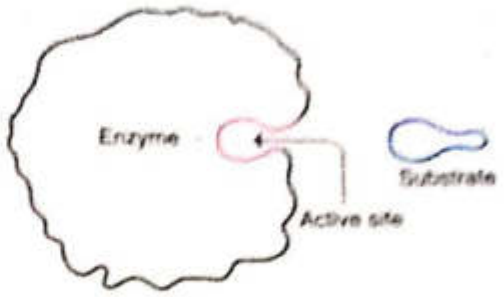


Fig. 6.6 : A diagrammatic representation of an enzyme with active site.

8. Effect of light and radiation

Exposure of enzymes to ultraviolet, beta, gamma and X-rays inactivates certain enzymes due to the formation of peroxides. e.g. UV rays inhibit salivary amylase activity.

ACTIVE SITE

Enzymes are big in size compared to substrates which are relatively smaller. Evidently, a small portion of the huge enzyme molecule is directly involved in the substrate binding and catalysis (***Fig.6.6***).

The active site (or active centre) of an enzyme represents as the small region at which the substrate(s) binds and participates in the catalysis.

Salient features of active site

1. The existence of active site is due to the tertiary structure of protein resulting in three-dimensional native conformation.

2. The active site is made up of amino acids (known as ***catalytic residues***) which are far from each other in the linear sequence of amino acids (primary structure of protein). For instance, the enzyme lysozyme has 129 amino acids. The active site is formed by the contribution of amino acid residues numbered 35, 52, 62, 63 and 101.

3. Active sites are regarded as ***clefts*** or ***crevices*** or pockets occupying a small region in a big enzyme molecule.

4. The active site is not rigid in structure and shape. It is rather ***flexible*** to promote the specific substrate binding.

5. Generally, the active site possesses a ***substrate binding site*** and a ***catalytic site.*** The latter is for the catalysis of the specific reaction.

6. The coenzymes or cofactors on which some enzymes depend are present as a part of the catalytic site.

7. The substrate(s) binds at the active site by weak noncovalent bonds.

8. Enzymes are specific in their function due to the existence of active sites.

9. The commonly found amino acids at the active sites are serine, aspartate, histidine, cysteine, lysine, arginine, glutamate, tyrosine etc. Among these amino acids, ***serine*** is the most frequently found.

10. The substrate[S] binds the enzyme (E) at the active site to form enzyme-substrate complex (ES). The product (P) is released after the catalysis and the enzyme is available for reuse.

$$E + S \rightleftharpoons ES \rightarrow E + P$$

ENZYME INHIBITION

Enzyme inhibitor is defined as a substance which binds with the enzyme and brings about a ***decrease in catalytic activity*** of that enzyme. The inhibitor may be organic or inorganic in nature. There are three broad categories of enzyme inhibition

1. Reversible inhibition.
2. Irreversible inhibition.
3. Allosteric inhibition.

1. Reversible inhibition

The inhibitor binds non-covalently with enzyme and the enzyme inhibition can be reversed if the inhibitor is removed. The reversible inhibition is further sub-divided into

I. Competitive inhibition (***Fig.6.7A***)

II. Non-competitive inhibition (***Fig.6.7B***)

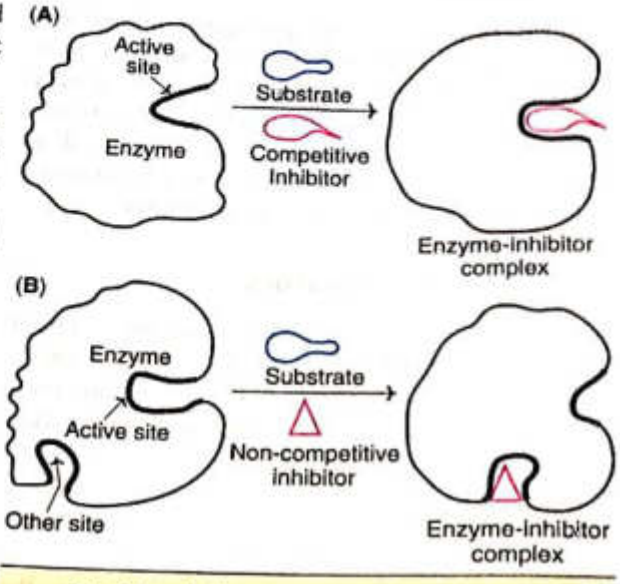
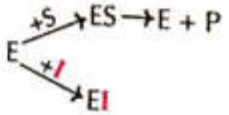


Fig. 6.7 : A diagrammatic representation of (A) Competitive and (B) Non-competitive inhibition.

I. **Competitive inhibition :** The inhibitor (I) which closely resembles the real substrate (S) 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 holds the active site, the enzyme is not available for the substrate to bind. During the reaction, ES and EI complexes are formed as shown below



The relative concentration of the substrate and inhibitor and their respective affinity with the enzyme determines the degree of competitive inhibition. The inhibition could be overcome by a high substrate concentration. In competitive inhibition, the ***K_m value increases*** whereas ***V_{max}*** remains ***unchanged*** (***Fig.6.8***).

The enzyme succinate dehydrogenase (SDH) is a classical example of competitive inhibition with succinic acid as its substrate. The compounds, namely, malonic acid, glutaric acid and oxalic acid, have structural similarity with succinic acid and compete with the substrate for binding at the active site of SDH.