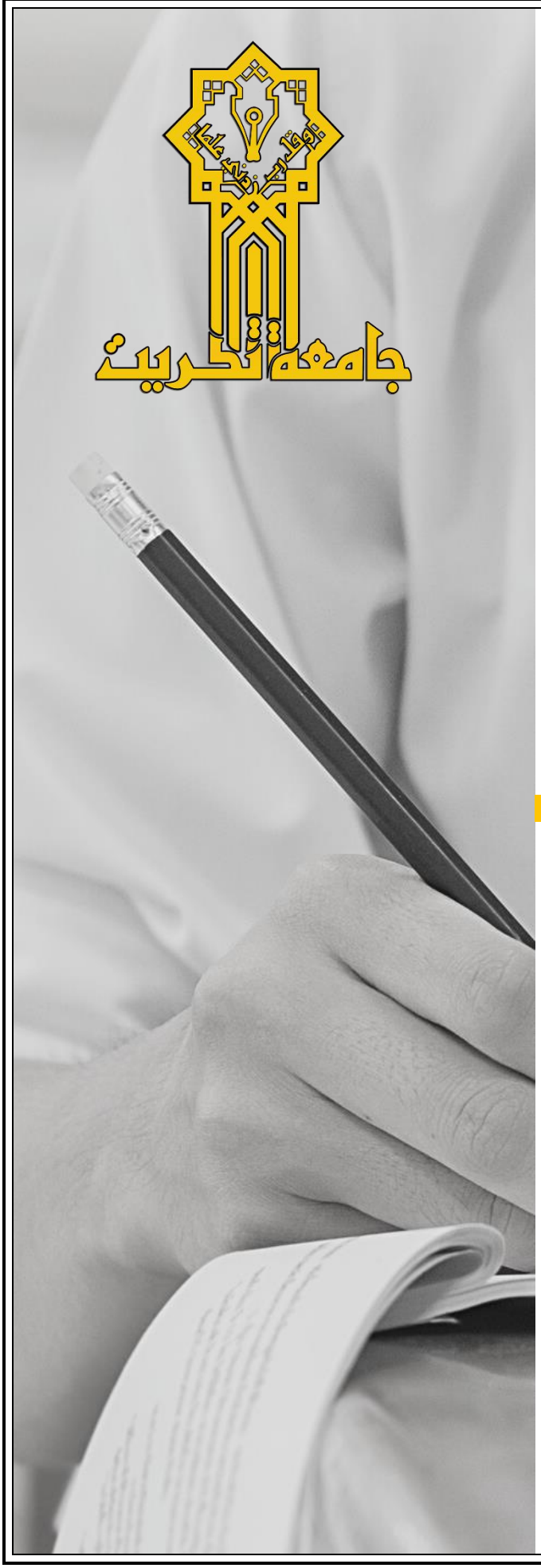

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Enzymes

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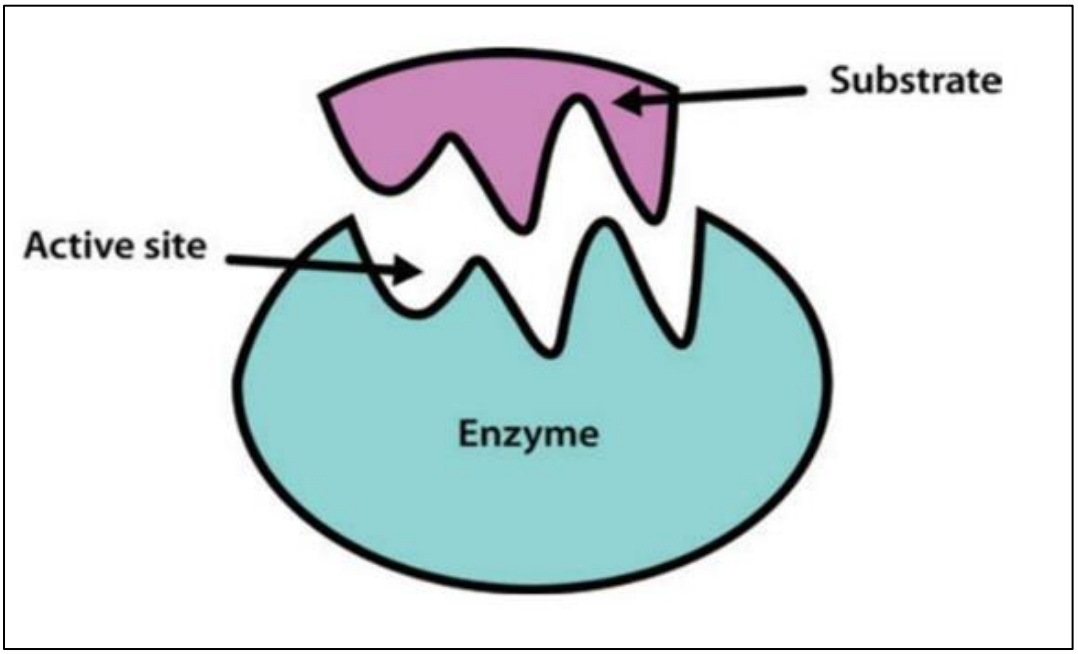


Are macromolecular biological catalysts. Enzymes accelerate, or catalyze, chemical reactions. The molecules at the beginning of the process upon which enzymes may act are called substrates and the enzyme converts these into different molecules, called products. Almost all metabolic processes in the cell need enzymes in order to occur at rates fast enough to sustain life. The set of enzymes made in a cell determines which metabolic pathways occur in that cell. The study of enzymes is called enzymology.

Enzymes are known to catalyze more than 5,000 biochemical reaction types.

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.)

- 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**).

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, CO2 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.

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) $A + B \xrightarrow[ATP \quad ADP + Pi]{} A - B$

Each enzyme is given a specific name indicating the substrate, coenzyme (if any) and the type of the reaction catalysed by the enzyme .

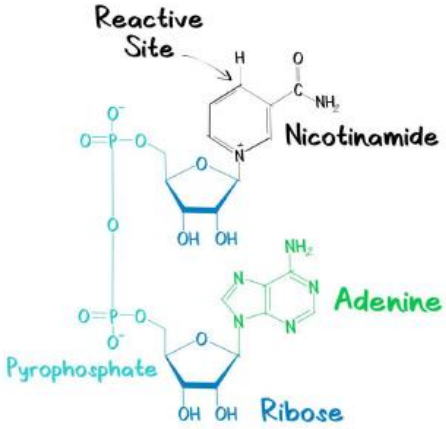
CHEMICAL NATURE AND PROPERTIES OF ENZYMES

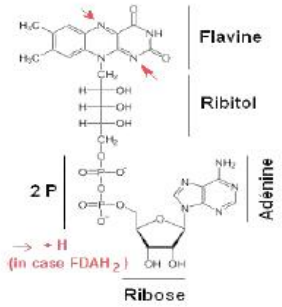
- All the enzymes are invariably proteins.
- The functional unit of the enzyme is known as **holoenzyme** which is often made up of **apoenzyme** (the protein part) and a **coenzyme** (non-protein organic part).

$$\underset{\text{(active enzyme)}}{\text{Holoenzyme}} \longrightarrow \underset{\text{(protein part)}}{\text{Apoenzyme}} + \underset{\text{(non-protein part)}}{\text{Coenzyme}}$$

- if it is an in organic groups it is called activator. (Fe 2+, Mn 2+ , or Zn 2+ ions). If the cofactor is bound so tightly to the apoenzyme and is difficult to remove without damaging the enzyme it is sometimes called a prosthetic group.

- Coenzymes are derivatives of vitamins without which the enzyme cannot exhibit any reaction. One molecule of coenzyme is able to convert a large number of substrate molecules with the help of enzyme.
- Coenzymes are called co - substrate because the changes that take place in substrates are complimentary to the changes in coenzymes.
- Example: NAD, FAD, Coenzyme A .





Flavine Adénine Dinucléotide
(FAD)

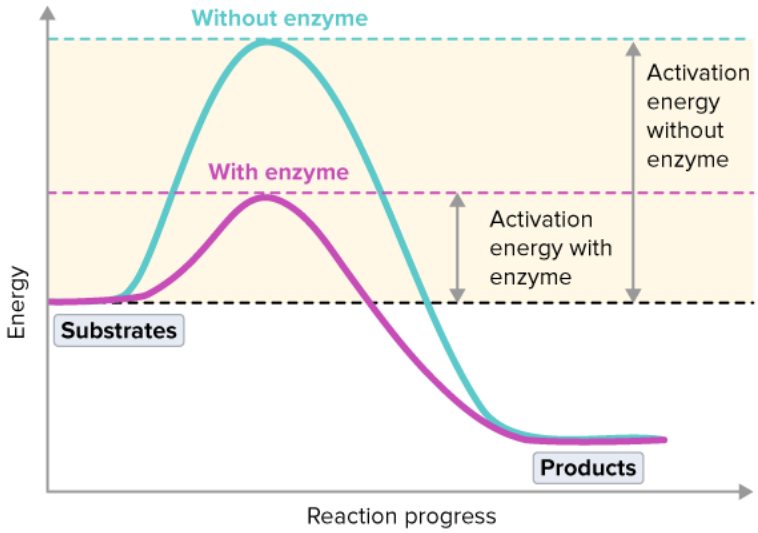
- Enzyme molecules contain a special pocket or cleft called the active site. The active site contains amino acid chains that create a three-dimensional surface complementary to the substrate. The active site binds the substrate, forming an enzyme-substrate (ES) complex.
- ES is converted to enzyme-product (EP); which subsequently dissociates to enzyme and product.
- Some enzymes are produced in nature in an inactive form which can be activated when they are required. Such type of enzymes are called

Zymogens (Proenzymes). Many of the digestive enzymes and enzymes concerned with blood coagulation are in this group Examples: Pepsinogen - This zymogen is from gastric juice.When required Pepsinogen converts to Pepsin Trypsinogen - This zymogen is found in the pancreatic juice, and when it is required gets converted to trypsin.

- **Isoenzymes (Isozymes) :-**These are enzymes having similar catalytic activity, act on the same substrate and produces the same product but originated at different site and exhibiting different physical and chemical characteristics
- Examples LDH (Lactate dehydrogenase) . H= Heart and M=Muscle. and CPK.

MECHANISM OF ENZYME ACTION

- Enzymes lower activation energy : The energy required by the reactants to undergo the reaction is known as activation energy



- The reactants when heated attain the activation energy. The catalyst (or the enzyme in the biological system) reduces the activation energy and this causes the reaction to proceed at a lower temperature.
- Thereby making the reaction go faster. The enzymes reduce the activation energy of the reactants in such a way that all the biological systems occur at body temperature (below 40°C).

Enzyme-substrate complex formation

- Enzyme catalysis is that the substrate (S) must combine with the enzyme (E) at the active site to form enzyme substrate complex (ES) which ultimately results in the product formation (P).
- A few theories have been put forth to explain mechanism of enzyme-substrate complex formation.

Lock and key model or Fischer's template theory

- **Lock: Key model** of enzyme action implies that the active site of the enzyme is complementary in shape to that of its substrate, i.e. the shape of the enzyme molecule and the substrate molecule should fit each other like a lock and Key.
- **Induced fit theory or Koshland's model**

In 1958, Daniel Koshland, postulated another model; which implies that the shapes & the active sites of enzymes are complementary to that of the substrate only after the substrate is bound.

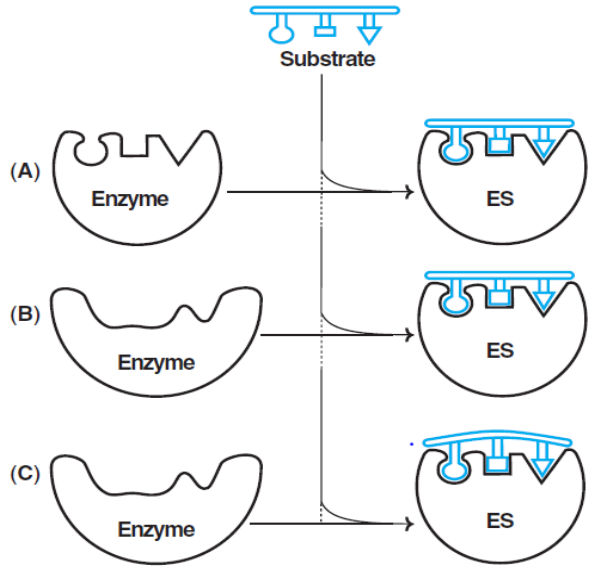


Fig. 6.12 : Mechanism of enzyme-substrate (ES) complex formation (A) Lock and key model (B) Induced fit theory (C) Substrate strain theory.

FACTORS AFFECTING ENZYME ACTIVITY

1. **Concentration of enzyme** As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases 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,

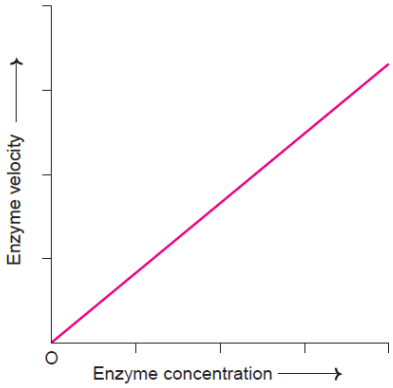


Fig. 6.1 : Effect of enzyme concentration on enzyme velociy.

2. **Concentration of substrate** Increase in the substrate concentration gradually increases the velocity of enzyme reaction within the limited range of substrate levels.

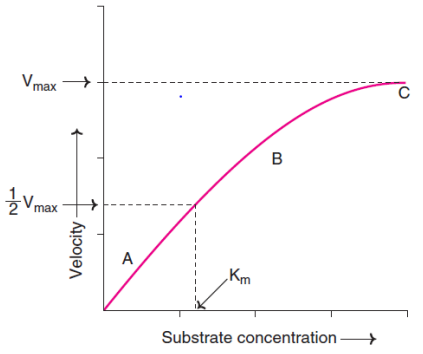


Fig. 6.2 : Effect of substrate concentration on enzyme velocity (A-linear; B-curve; C-almost unchanged).

Enzyme kinetics and Km 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). Km or the Michaelis-Menten constant is defined as the substrate concentration (expressed in moles/l) to produce half-maximum velocity in an enzymes reaction.

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

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

- ✓ A low Km value indicates a strong affinity between enzyme and substrate, whereas a high Km value reflects a weak affinity between them. Lineweaver-Burk double reciprocal plot : For the determination of Km value.

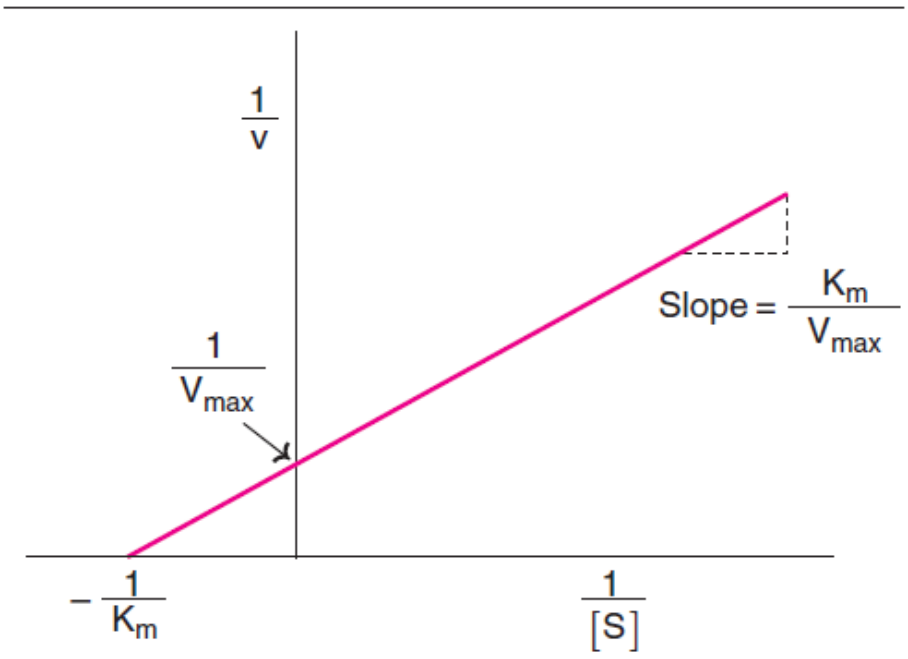
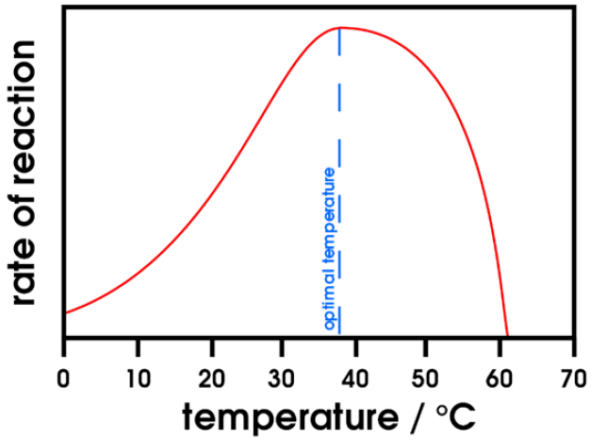


Fig. 6.3 : Lineweaver-Burk double reciprocal plot.

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

3. Effect of temperature

Starting from low temperature as the temperature increases to certain degree the activity of the enzyme increases because the temperature increase the total energy of the chemical system . There is an optimal temperature at which the reaction is most rapid (maximum). Above this the reaction rate decreases sharply, mainly due to denaturation of the enzyme by heat. , The temperature at which an enzyme shows maximum activity is known as the optimum temperature for the enzyme. For most body enzymes the optimum temperature is around 370c, which is body temperature.



4. Effect of pH

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.

5. Effect of product concentration

The accumulation of reaction products generally decreases the enzyme velocity. the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity.

Enzyme Inhibition

Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor and the process is known as inhibition. There are two major types of enzyme inhibition, Irreversible and Reversible.

Irreversible Inhibition

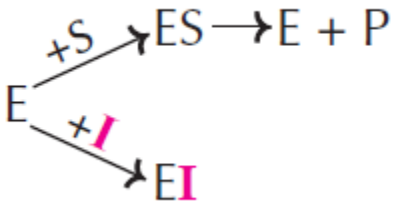
The type of inhibition that can not be reversed by increasing substrate concentration or removing the remaining free inhibitor is called Irreversible inhibition.example Acetyl cholinesterase catalyzes the hydrolysis of Acetylcholin (to acetic acid and choline) a neurotransmitter substance functioning in certain portions of the nervous system.

Reversible Inhibition

This type of inhibition can be Competitive, Non-competitive and uncompetitive

Competitive Inhibition: This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy, therefore, competes with the substrate for that site.

in competitive inhibition the inhibitor and substrate compete for the same active site on the enzyme as a result of similarity in structure. The enzyme substrate complex will be broken dawn to products where as enzyme inhibitor complex; (EI) will not be broken down to products.



Uncompetitive Inhibition

Uncompetitive Inhibitor binds only to ES complex at locations other than the catalytic site. Substrate binding modifies enzyme structure, making inhibitor-binding site available. Inhibition cannot be reversed by substrate.

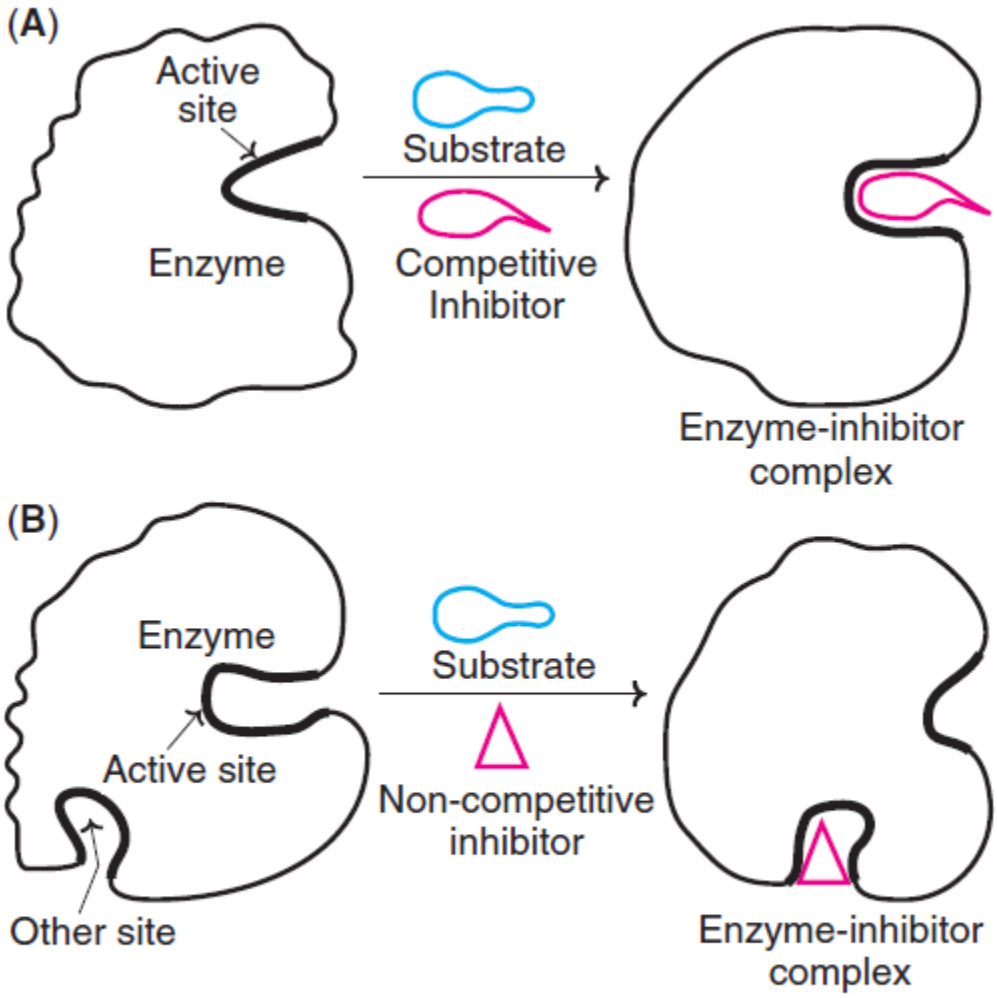


Fig. 6.7 : A diagrammatic representation of (A) Competitive and (B) Non-competitive inhibition.