

# Enzymes

- Enzymes are **biological catalyst** produced by living tissues.
- They are proteins with the exception of few classes of RNA molecules called **ribozyme**.
- They accelerate specific chemical reactions without being consumed in the process.
- They function in aqueous solutions under very mild conditions of temperature and pH.

- The catalytic activity of enzyme depends on their native protein conformation. If an enzyme is denatured or dissociated into its subunits, catalytic activity is usually lost.
- Thus, the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity

# Enzyme Classification

- Enzymes are classified according to the type of reaction they catalyse.
- All enzymes have formal 'EC' (Enzyme Commission) number and names, and most have trivial names.

- According to the International Union of Biochemistry and Molecular Biology (**IUBMB**) **system**, enzymes are classified into **seven** major classes
- Enzymes are classified according to the type of reaction they catalyse.

The seven classes as per **IUBMB** are as follows:

1. EC-1: Oxidoreductase

2. EC-2: Transferase

3. EC-3: Hydrolase

4. EC-4: Lyase

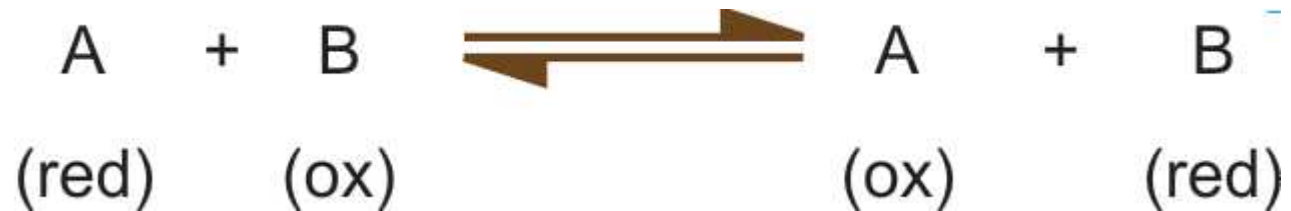
5. EC-5: Isomerase

6. EC-6: Ligase

7. EC-7: Translocases

## EC-1 Oxidoreductases

Catalyzes oxidation-reduction reactions.



Enzymes in this category include :

- Dehydrogenases
- Reductases
- Oxidases
- Peroxidases.



## EC-2 Transferases

Catalyses the transfer of a group such as, *amino*, *carboxyl*, *methyl* or *phosphate*, etc. from one molecule to another



Enzymes in this category include :

- **Amino transferase** or transaminase
- **Kinase**: catalyzes the transfer of phosphate groups
- **Transcarboxylase**.

## EC-3 Hydrolases

Catalyze the cleavage of C-O, C-N, C-C and some other bonds with the addition of water.

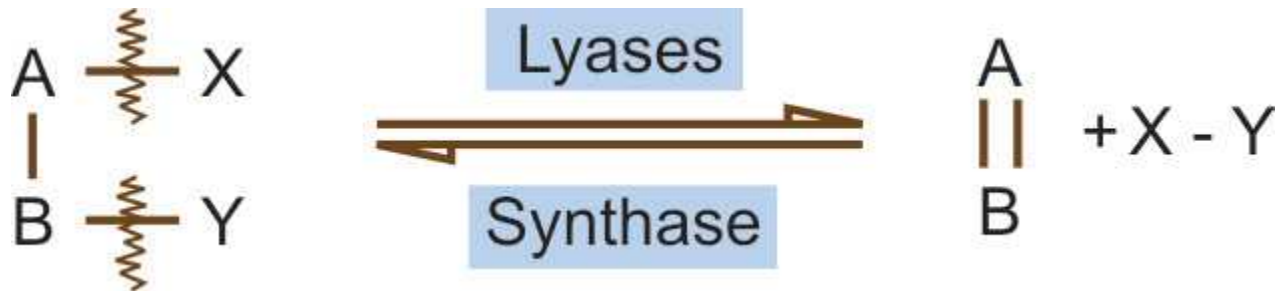


## Enzymes in this category are:

- All digestive enzymes like:
  - $\alpha$ -amylase,
  - pepsin,
  - trypsin,
  - chymotrypsin, etc.
- Acid phosphatase

## EC-4 Lyases

Catalyze the cleavage of **C-O**, **C-C** and **C-N** bonds by means other than hydrolysis or oxidation, giving rise to compound with **double bonds** or catalyze the reverse reaction, by the addition of group to a double bond.



In cases where reverse reaction is important, then **synthase**, (not **synthetase** of group EC-6) is used in the name.

## EC-5 Isomerases

Catalyze **intramolecular structural rearrangement** in a molecule. They are called **epimerases**, **isomerases** or **mutases**, depending on the type of isomerism involved.



## EC-6 Ligases (Synthetases)

Catalyze the **joining of two molecules** coupled with the hydrolysis of ATP.





## EC-7: Translocases (A new EC Class)

Translocases catalyze the movement of ions or molecules across membranes or their separation within membranes.

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Examples are:

- **Enzymes catalyzing the translocation of:**

Hydrons ( $H^+$ ), inorganic cations, inorganic anions, amino acids and peptides, and carbohydrates and their derivatives.

- **Enzymes of the reaction that provided the driving force for the translocation linked to:**

Oxidoreductase reactions, hydrolysis of a nucleoside triphosphate, hydrolysis of a diphosphate, and decarboxylation reaction.

## International IUBMB classification of enzymes.

| <i>Class</i>            | <i>Types of reaction catalyzed</i>                                                                                                   | <i>Examples</i>                                                                                                                                      |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| EC-1<br>Oxidoreductases | Oxidation-reduction reactions (transfer of electrons, hydride ions, or H atoms)                                                      | Lactate dehydrogenase (LDH)<br>Glucose-6-phosphate dehydrogenase (G6PD)<br>Ferroxidase (ceruloplasmin)<br>Cytochrome oxidase<br>Malate dehydrogenase |
| EC-2 Transferases       | Groups (like amino, carboxyl, methyl, or phosphoryl, etc.) transfer reactions                                                        | Aspartate transaminase (AST)<br>Alanine transaminase (ALT)<br>Ornithine carbamoyltransferase<br>Hexokinase<br>Creatine kinase                        |
| EC-3 Hydrolases         | Hydrolysis reactions (enzymes of this class catalyze the cleavage of C-O, C-N, C-C, and some other bonds with the addition of water) | Lipase<br>$\alpha$ -amylase<br>Trypsin<br>Chymotrypsin<br>Lactase<br>Sucrase<br>Alkaline phosphatase<br>Pepsin                                       |

|                 |                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                         |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
| EC-4 Lyases     | <p>Cleavage of C-O, C-C, and C-N or other bonds by means other than hydrolysis or oxidation, giving rise to compound with double bonds or catalyze the reverse reaction by the addition of group to a double bond. In cases where addition of groups to double bonds occurs, then synthase (not synthetase of group EC-6) is used in the name</p> | <p>Aldolase<br/> Porphobilinogen synthase<br/> Fumarase<br/> Argininosuccinase<br/> Carbonic anhydrase<br/> Cysteine desulfurase<br/> Decarboxylase</p> |
| EC-5 Isomerases | <p>Transfer of groups within molecules to yield isomeric forms</p>                                                                                                                                                                                                                                                                                | <p>Phosphoglucomutase<br/> Triphosphate isomerase or mutase<br/> Phosphohexose isomerase<br/> Glucose 4-epimerase<br/> Retinal isomerase</p>            |

|                   |                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                            |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| EC-6 Ligases      | Joining of two molecules by condensation reactions at the expense of ATP hydrolysis. They may form C-O, C-S, C-N, C-C, or other bonds | Glutamine synthetase<br>Pyruvate carboxylase<br>DNA ligases                                                                                                                                                                                                                                                                                                                                |
| EC-7 Translocases | Catalyze the movement of ions or molecules across membranes or their separation within membranes                                      | Enzymes catalyzing the translocation of hydrons ( $H^+$ ), inorganic cations, inorganic anions, amino acids and peptides, carbohydrates, and their derivatives<br><br>Enzymes of the reaction that provided the driving force for the translocation linked to oxidoreductase reactions, hydrolysis of a nucleoside triphosphate, hydrolysis of a diphosphate, and decarboxylation reaction |

## Zymogen OR Proenzyme

- Enzymes found in an inactive (precursor) form, called *zymogen* or Proenzyme.
- Zymogen have the prefix “**pro**” or suffix “**ogen**”.

- For example,
  - prothrombin,
  - proelastase
  - chymotrypsinogen,
  - trypsinogen,
  - pepsino- gen

## Cofactors (Coenzyme And Activator)

- Some enzymes require an additional non-protein component for its activity. This additional component is called *cofactor*
  - Inorganic ions, called *activators*.
  - Organic compounds, called *coenzymes*



- Enzymes without its cofactor is referred to as an *apoenzyme*
- The complete catalytically active enzyme is called *holoenzyme*.
- Apoenzyme + Cofactor = Holoenzyme.

: Some common coenzymes and their functions.

| <b><i>Vitamins</i></b>                    | <b><i>Coenzymes</i></b>                                                                       | <b><i>Coenzyme for</i></b>                                                |
|-------------------------------------------|-----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Thiamine (vitamin B <sub>1</sub> )        | Thiamine pyrophosphate (TPP)                                                                  | Oxidative decarboxylation and transketolase reaction                      |
| Riboflavin (vitamin B <sub>2</sub> )      | Flavin adenine dinucleotide and flavin mononucleotide (FAD and FMN)                           | Oxidation and reduction reactions                                         |
| Niacin                                    | Nicotinamide adenine dinucleotide (NAD)<br>Nicotinamide adenine dinucleotide phosphate (NADP) | Oxidation and reduction reactions                                         |
| Pyridoxine (vitamin B <sub>6</sub> )      | Pyridoxal phosphate (PLP)                                                                     | Transamination, deamination, and decarboxylation reactions of amino acids |
| Biotin                                    | Biocytin                                                                                      | Carboxylation reactions                                                   |
| Folic acid                                | Tetrahydrofolate (THF)                                                                        | Carrier of one carbon group                                               |
| Pantothenic acid                          | Coenzyme A                                                                                    | Acyl carrier                                                              |
| Cyanocobalamin (vitamin B <sub>12</sub> ) | Methylcobalamin and deoxyadenosylcobalamin                                                    | Transfer of CH <sub>3</sub> group and isomerization                       |

## Inorganic ions that serve as cofactors for enzymes.

| <b><i>Ions</i></b>                   | <b><i>Enzymes</i></b>                                               |
|--------------------------------------|---------------------------------------------------------------------|
| $\text{Cu}^{2+}$                     | Cytochrome oxidase                                                  |
| $\text{Fe}^{2+}$ or $\text{Fe}^{3+}$ | Cytochrome oxidase, catalase, and peroxidase                        |
| $\text{K}^{+}$                       | Pyruvate kinase, propionyl-CoA carboxylase, and acetyl-CoA thiolase |
| $\text{Mg}^{2+}$                     | Hexokinase, glucose-6-phosphatase, and pyruvate kinase              |
| $\text{Mn}^{2+}$                     | Arginase, ribonucleotide reductase                                  |
| $\text{Mo}^{+}$                      | Dinitrogenase, nitrate reductase                                    |
| $\text{Ni}^{2+}$                     | Urease                                                              |
| Se                                   | Glutathione peroxidase                                              |
| $\text{Zn}^{2+}$                     | Carbonic anhydrase, alcohol dehydrogenase, and carboxypeptidase     |

# MECHANISM OF ENZYME ACTION

Formation of an **enzyme-substrate (ES)** complex is the first step in enzymatic catalysis which is subsequently converted to product and free enzyme.



- Substrate is bound through **non-covalent** interactions at the **active site** of the enzyme.
- The **active site** of an enzyme is the region that binds the substrate and which contains the **specific amino acid residues**.

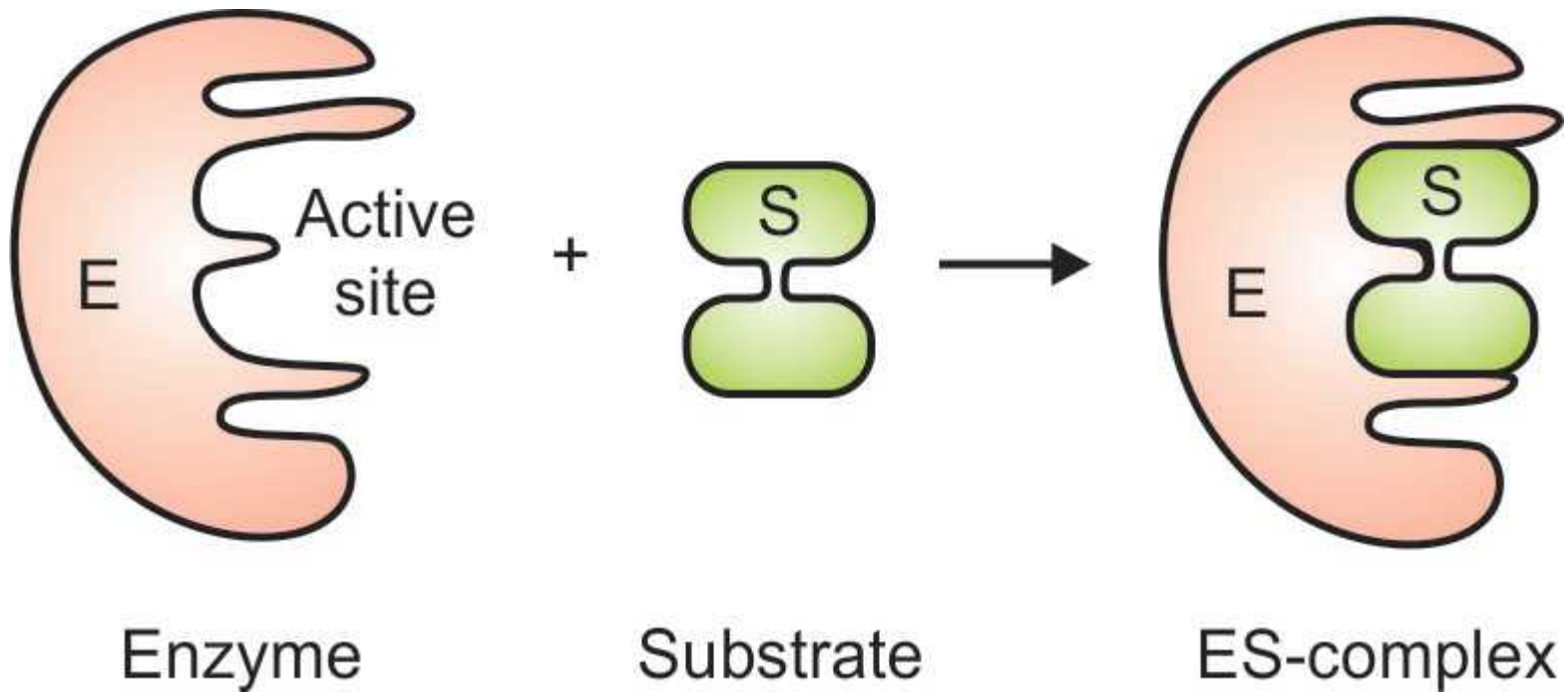
# Models for binding of substrate to enzyme

1. **Lock and key** model or rigid template model of Emil Fisher.
2. **Induced fit model** or **hand-in-glove** model of Daniel E Koshland

# Lock and Key Model or Rigid Template Model of Emil Fisher

- Enzyme is **pre-shaped** and the active site has a **rigid** structure, **complementary** to that of the substrate.
- Substrate fits into the active site in much the same way that a key fits into a lock.

Representation of Fisher's lock and key model.





This model explains all mechanisms but do not explain the changes in the enzyme activity in the presence of **modulator**.

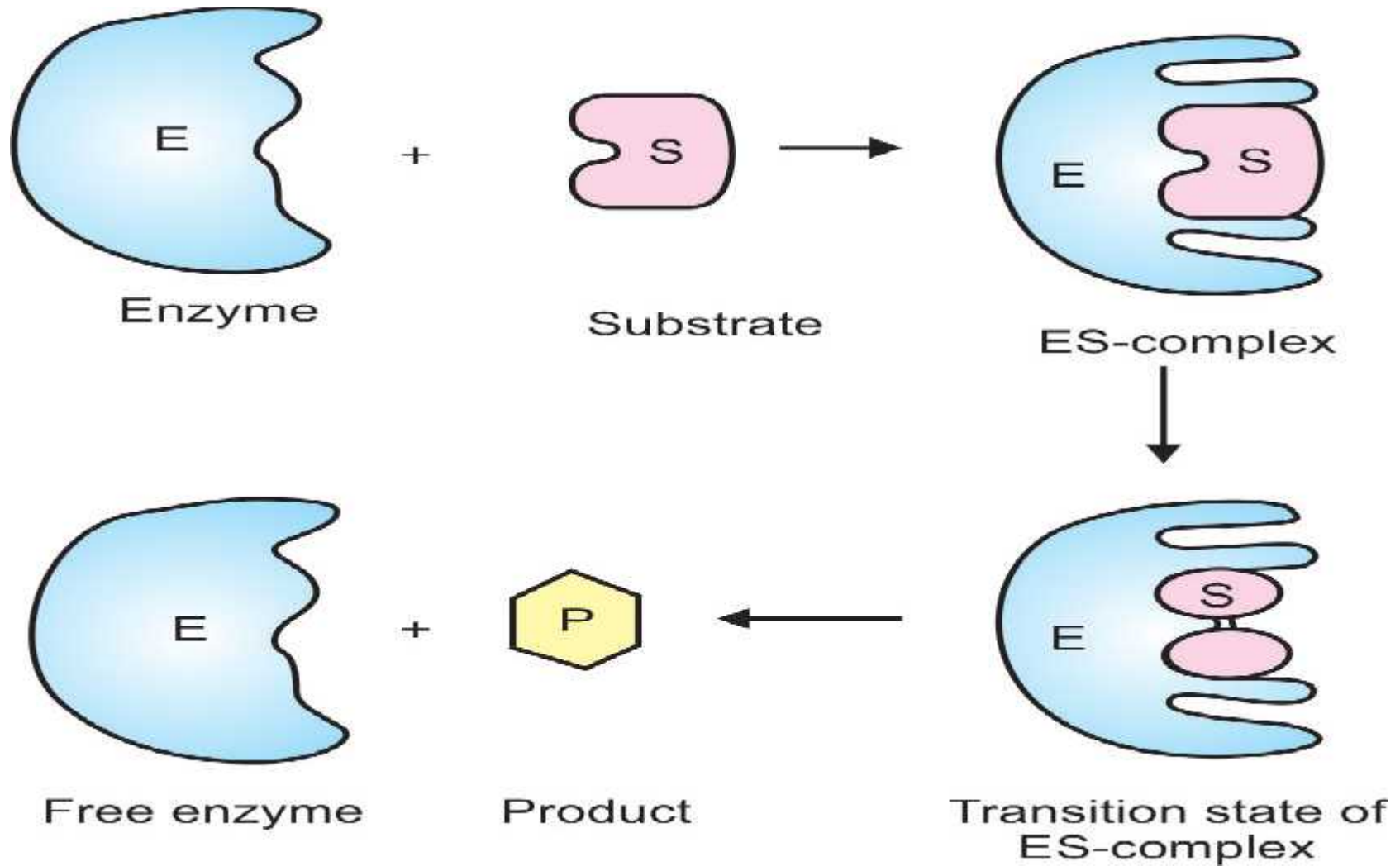
# Induced Fit Model or Hand-in-glove Model of

Daniel Koshland

- Enzymes are **flexible**
- Shapes of active site can be modified by the binding of the substrate.
- Substrate induces a **conformational** change in enzyme.

- Conformational change in enzyme induces reciprocal **changes in its bound substrate** that alters their **orientation** and **configuration** and strains the structure of the bound substrate.
- **intrinsic binding energy** is liberated.
- Intrinsic binding energy converts substrate into product.

## Schematic representation of induced fit model of Koshland



## SPECIFICITY OF ENZYME ACTION

- Ability of enzyme to discriminate between two substrates.
- Enzymes are highly specific both in the **reaction** catalyzed and in their choice of **substrates**.
- Specificity makes it possible for number of enzymes to co-exist in cell without interfering in each other's actions.

# Types of Specificity

1. Substrate specificity
2. Reaction specificity
3. Stereo specificity

# Substrate Specificity

- i. Absolute substrate specificity
- ii. Relative substrate specificity
- iii. Broad substrate specificity.

## Absolute substrate specificity

Certain enzymes will act on only one substrate and catalyze one reaction, e.g. Glucokinase, lactase, urease, etc.



Glucose  $\xrightarrow{\text{Glucokinase}}$  Glucose-6-phosphate

Lactose  $\xrightarrow{\text{Lactase}}$  Glucose + Galactose

Urea  $\xrightarrow{\text{Urease}}$  Ammonia + CO<sub>2</sub>

## Relative substrate specificity

Enzyme acts on more than one substrate.

- Group specificity
- Bond specificity.

- Chymotrypsin acts on several **proteins** by hydrolyzing **peptide bonds** attached to **aromatic amino acids**.
- Trypsin hydrolyzes peptide linkages involving **arginine** or **lysine**.

➤  $\alpha$ -amylase, cleaves  $\alpha$ -(1  $\rightarrow$  4) glycosidic bonds of carbohydrates.

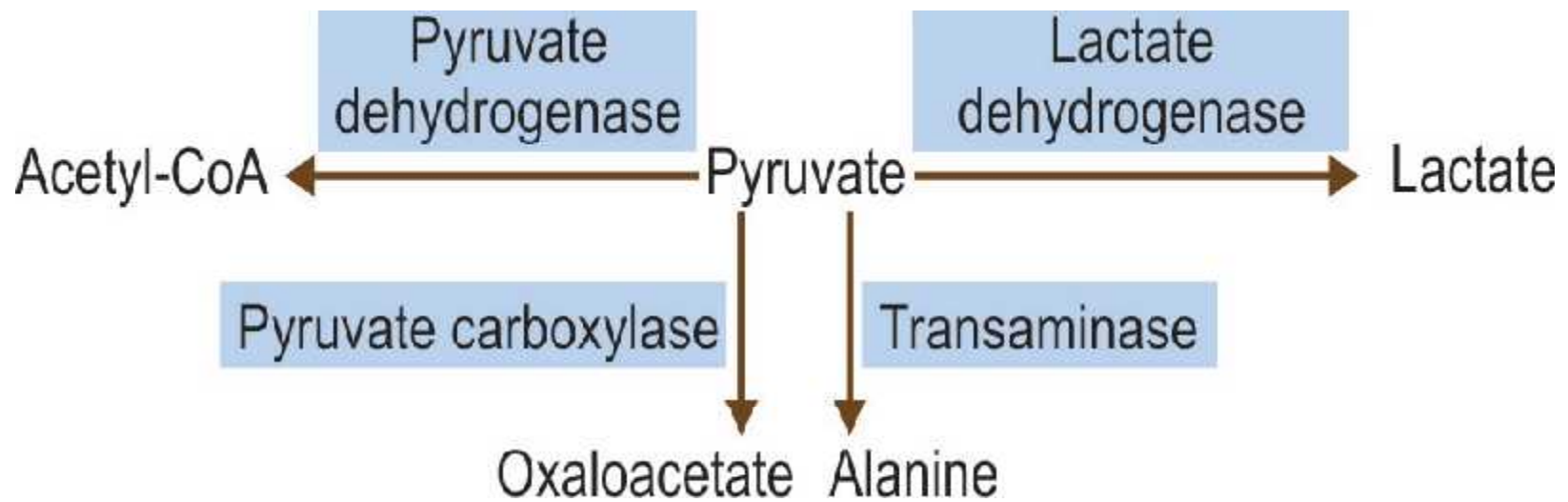
➤ Lipase hydrolyzes ester bonds of lipids.

## Broad substrate specificity

- Enzyme acts on more than one **structurally related** substrates.
- hexokinase catalyzes the phosphorylation of more than one kind of hexoses such as glucose, fructose and mannose.

# Reaction Specificity

Enzyme is specific to a particular reaction but not to substrate (s) and catalyzes only one type of reaction.



Example of reaction specificity.

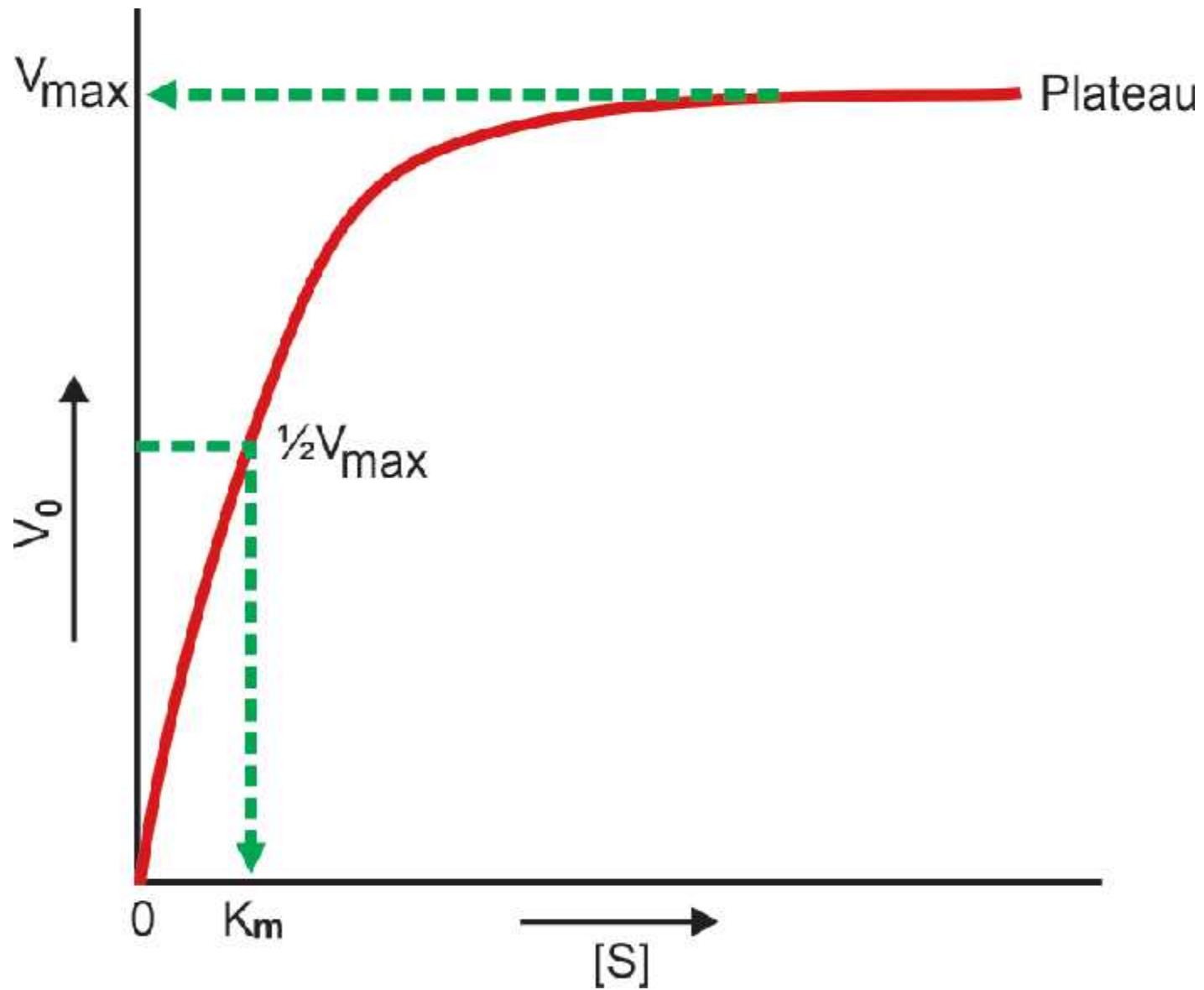
## Stereo Specificity

- L-lactate dehydrogenase will act only on L-lactic acid and not D-lactic acid.
- L-amino acid oxidase and D-amino acid oxidase act only on L and D-amino acids.
- Salivary  $\alpha$ -amylase acts on the  $\alpha$ -1,4 glycoside linkage and is inactive on  $\beta$ -1,4 glycoside bond



# Factors Affecting The Velocity Of Enzyme Reaction

- Substrate concentration
- Enzyme concentration
- pH i.e.  $H^+$  ion concentration
- Temperature
- Product concentration
- Activators and coenzymes
- Time
- Physical agents



Effect of Substrate Concentration

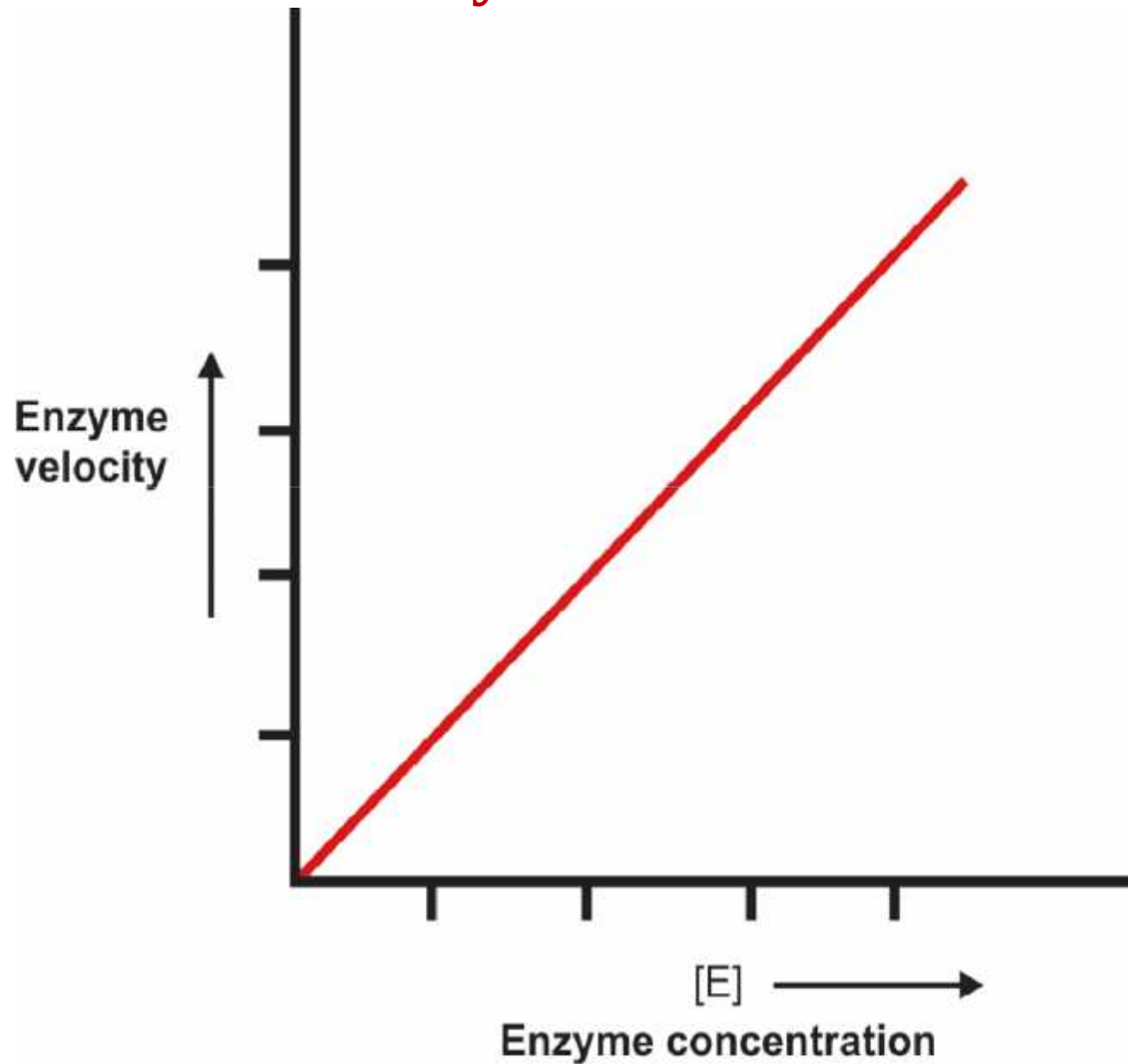
$V_0$  : initial velocity

$V_{\max}$  : maximum velocity

$K_m$  :  $1/2 V_{\max}$  = Michaelis Menten constant

$[S]$  : substrate concentration

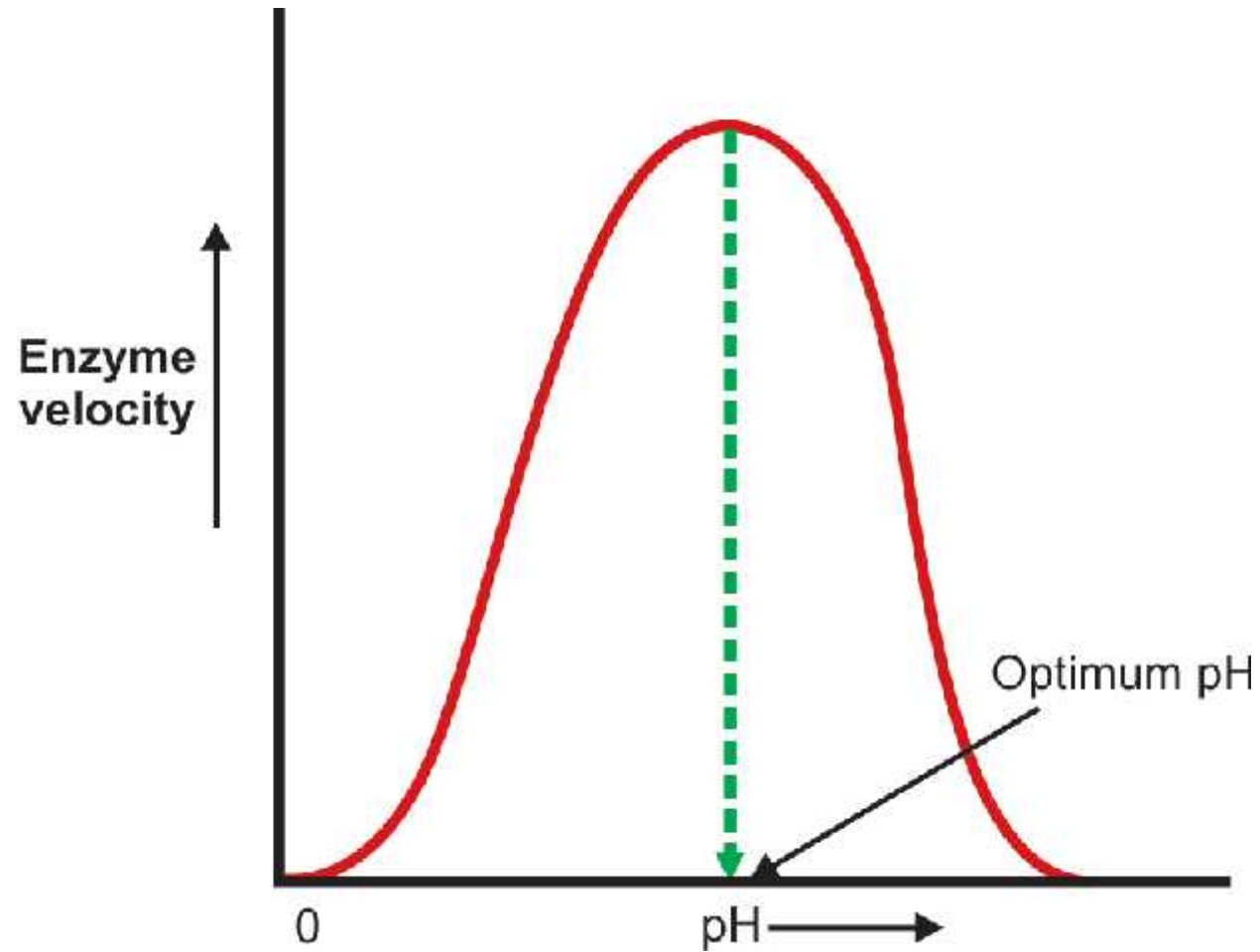
# Effect of Enzyme Concentration



## Effect of Hydrogen Ion Concentration pH

- Each enzyme has an *optimum pH*, i.e. a pH at which the enzyme activity is maximum.
- Below or above this pH, enzyme activity is decreased.
- The optimum pH differs from enzyme to enzyme.
  - Pepsin = 1.2
  - Trypsin = 8.0

# Effect of pH on enzyme activity



## Changes in pH can alter the following:

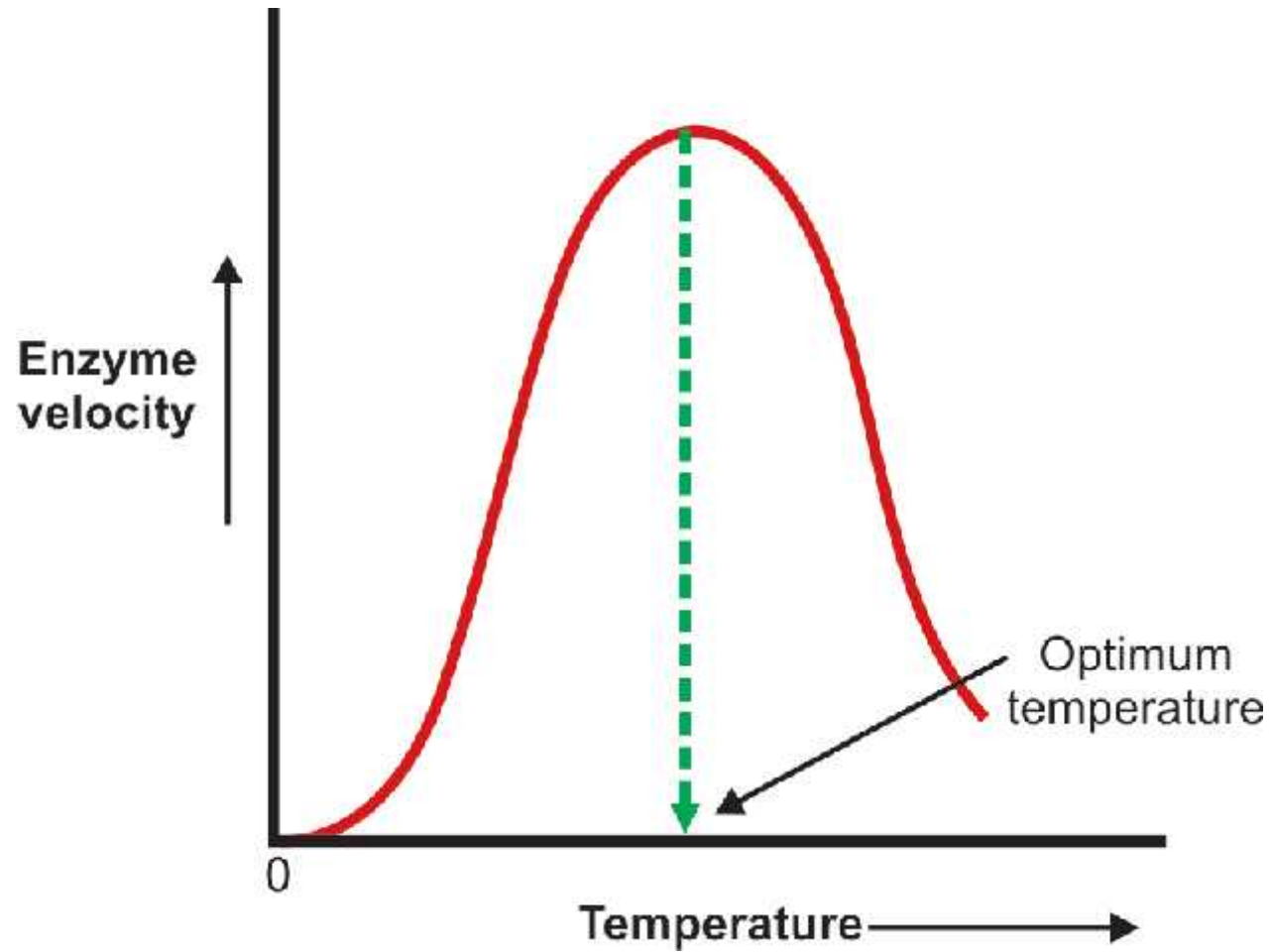
- Ionization state of the amino acids  
present in the active site of the enzyme.
- The ionization state of the substrate.
- Drastic change in pH denatures enzyme



## Effect of Temperature

- Enzyme catalyzed reactions show an increase in rate with increasing temperature only within a relatively small and low temperature range.
- Each enzyme shows the highest activity at a particular temperature called *optimum temperature*.
- The activity progressively declines both above and below this temperature.

## Effect of temperature on enzyme activity



- Increase in velocity is due to the increase in the kinetic energy.
- Further elevation of the temperature results in a decrease in reaction velocity due to denaturation of the enzyme protein.

- Low temperature also decreases enzyme activity and enzymes may be completely inactive at temperature of 0°C and below.
- The inactivity at low temperature is reversible.

- Most of the body enzymes have the optimum temperature close to  $37^{\circ}\text{C}$  to  $38^{\circ}\text{C}$  and have progressively less activity as the temperature rises.

## Effect of Product

Accumulation of products of the reaction causes the inhibition of enzyme activity for some enzymatic reactions.

## Effect of Activators and Co-enzymes

In absence of activators and coenzymes, enzymes become functionally inactive.

## Effect of Time

- Under optimum conditions of pH and temp, time required for an enzyme reaction is less.
- The time required for the completion of an enzyme reaction increases with changes in temperature and pH from its optimum.



# ENZYME KINETICS

- The study of enzyme reaction rates and how they change in response to changes in experimental parameters is known as *kinetics*.
- One of the key factors affecting the enzyme reaction rates is the concentration of *substrate* [S].

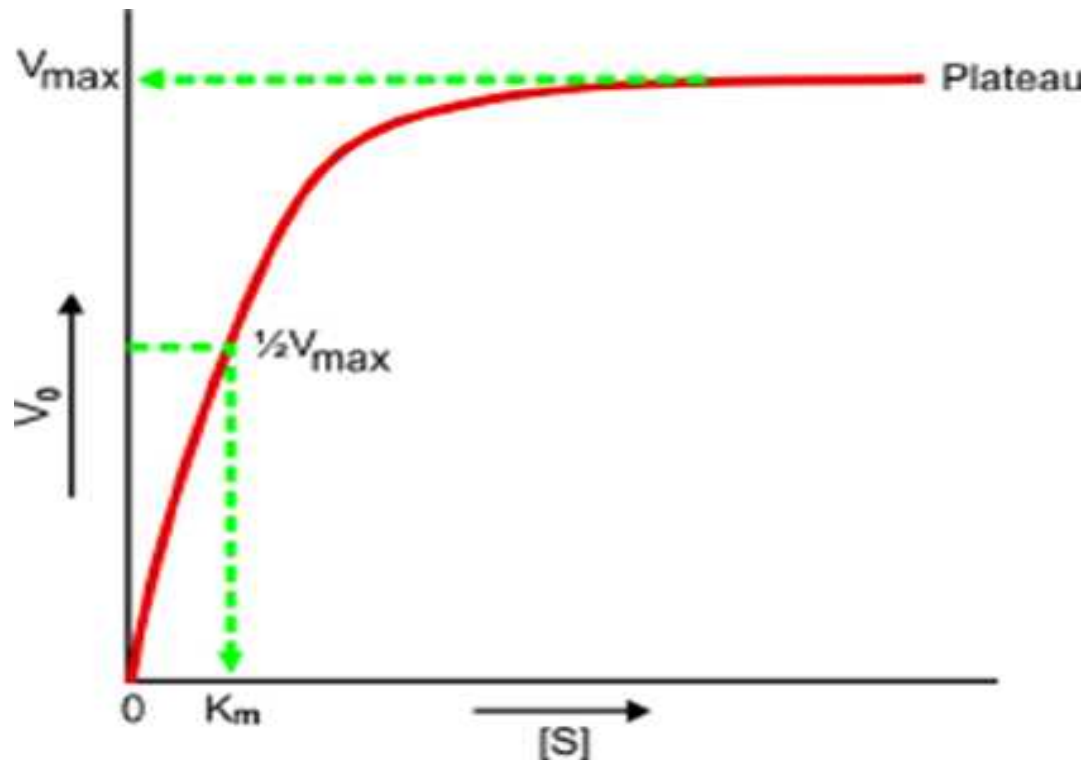
## Effect of Substrate Concentration

$V_0$ : initial velocity

$V_{\max}$ : maximum velocity

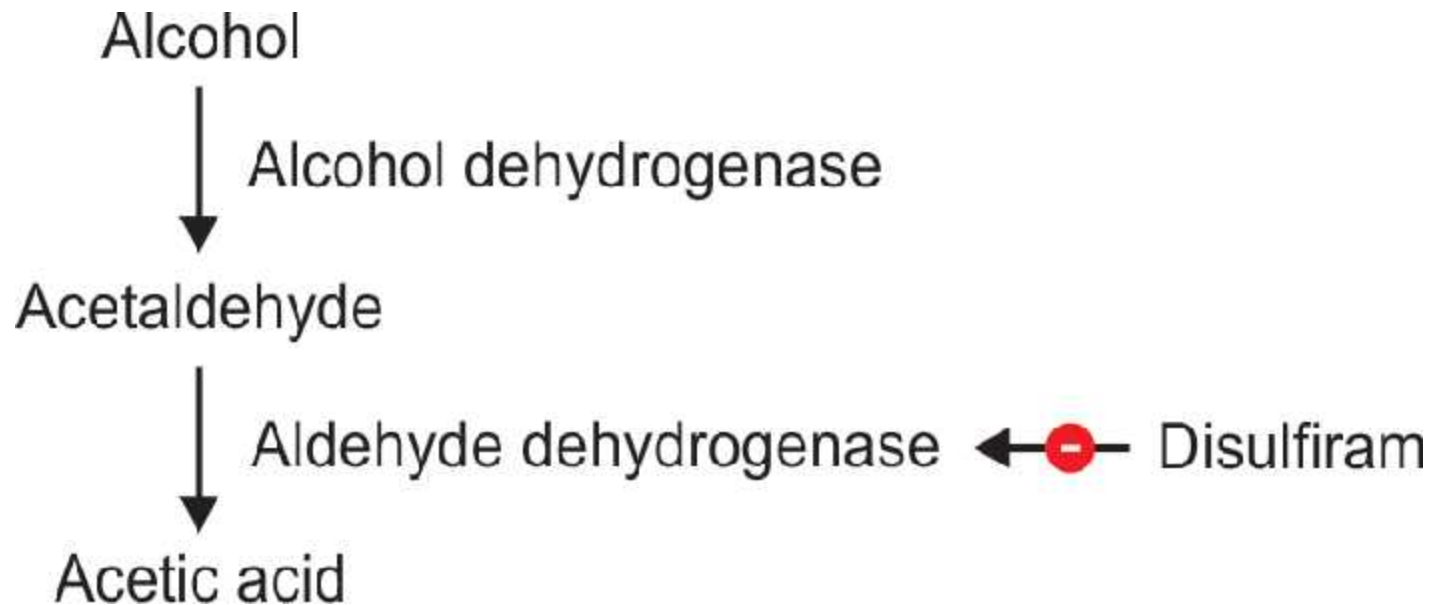
$K_m$ :  $1/2 V_{\max}$  = Michaelis Menten constant

$[S]$ : substrate concentration



## Significance of $K_m$ (Michaelis Constant)

1.  $K_m$  provides a amount of the substrate required for significant catalysis to occur.
2. It is a measure of the affinity of the enzyme for its substrate, a high  $K_m$  indicates weak binding and a low  $K_m$  indicates strong binding with its substrate.

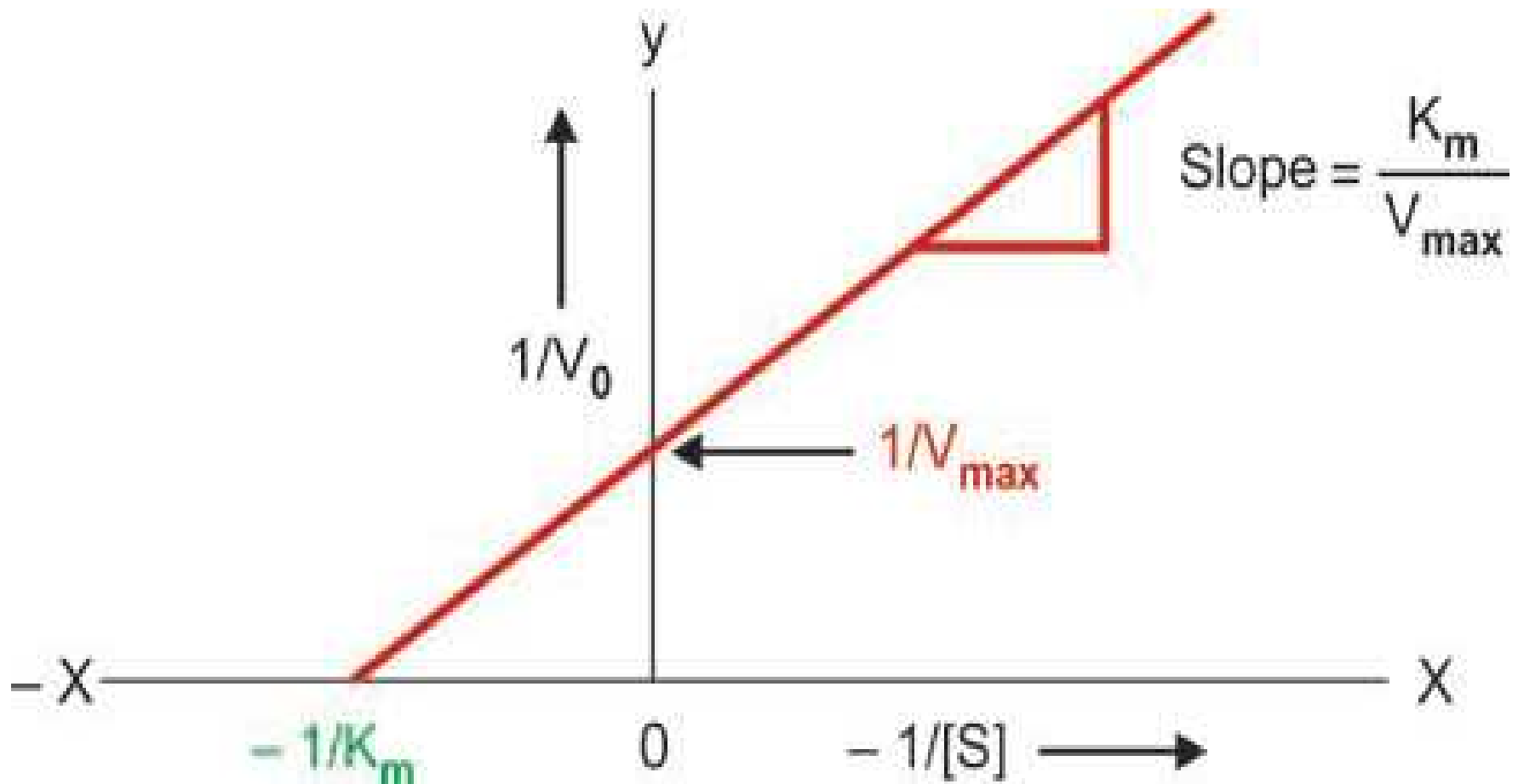


- A low  $K_m$  mitochondrial form
- A high  $K_m$  cytosolic form.

## Significance of $V_{\max}$

- The  $V_{\max}$  of a reaction is an index of the catalytic efficiency of an enzyme.
- The  $V_{\max}$  is useful in comparing the activity of one enzyme with that of another.

# Lineweaver-Burk plot (Double reciprocal plot)



# ENZYME INHIBITION

- Any substance that can diminish the velocity of an enzyme reaction is called inhibitor.
- Two general classes of inhibitors are:
  1. Reversible inhibitor
  2. Irreversible inhibitor.

# Enzyme inhibitors

```
graph TD; A[Enzyme inhibitors] --> B[Reversible]; A --> C[Irreversible]; B --> D[Competitive or substrate analog]; B --> E[Noncompetitive]; B --> F[Un-competitive]; C --> G[Reactive substrate analog or affinity labels]; C --> H[Group specific]; C --> I[Suicide or mechanism based inactivation];
```

Reversible

Irreversible

Competitive  
or substrate  
analog

Noncompetitive

Un-  
competitive

Reactive  
substrate  
analog or  
affinity labels

Group  
specific

Suicide or  
mechanism  
based  
inactivation



# REVERSIBLE INHIBITOR

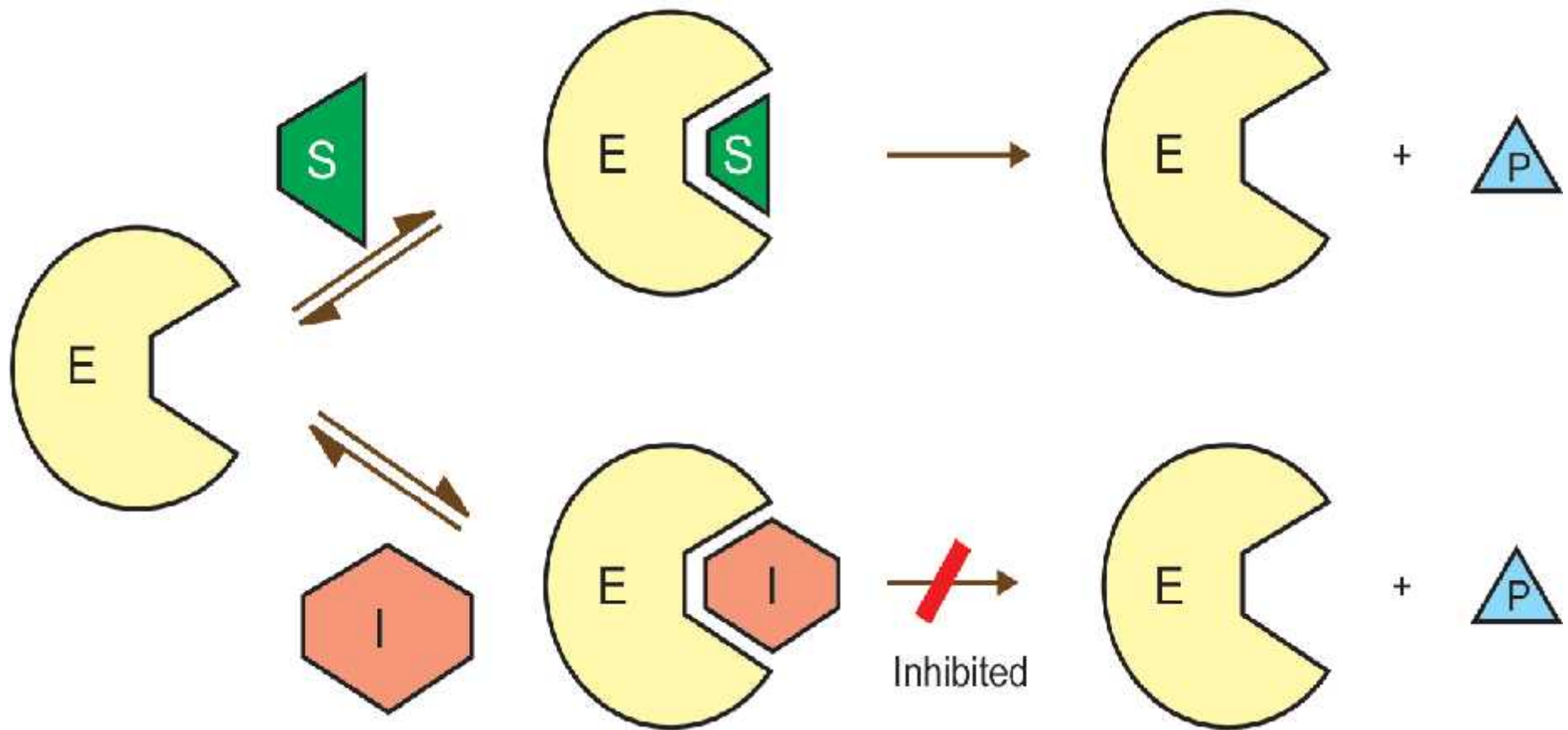
Reversible inhibitors bind to enzymes through non-covalent bonds and the activity of the enzyme is restored fully when the inhibitor is removed from the system.

Different types of reversible inhibitors are:

- i. Competitive or substrate analogue inhibitor
- ii. Non-competitive inhibitor
- iii. Uncompetitive inhibitor.

## Competitive or Substrate Analogue Inhibitor

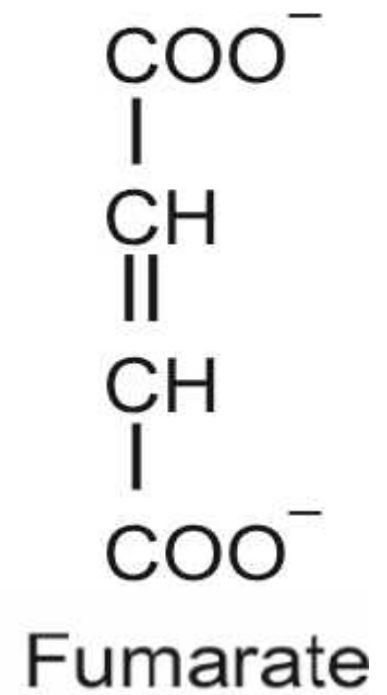
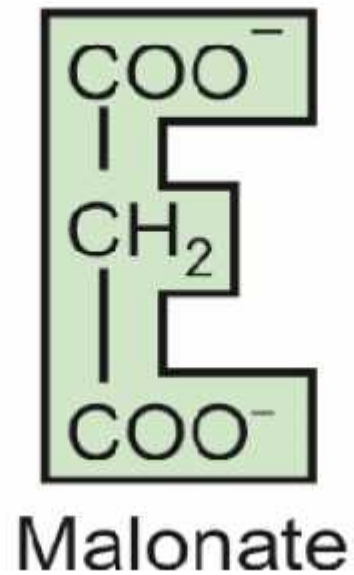
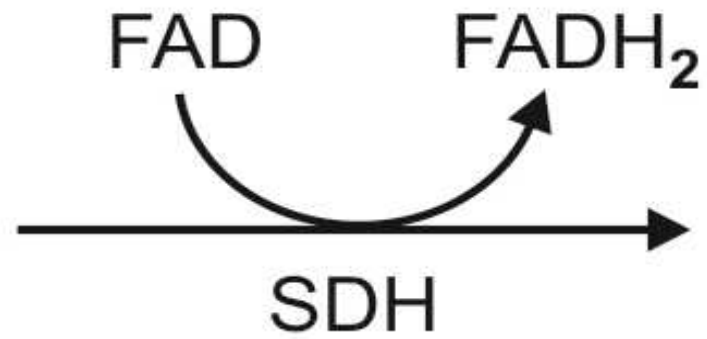
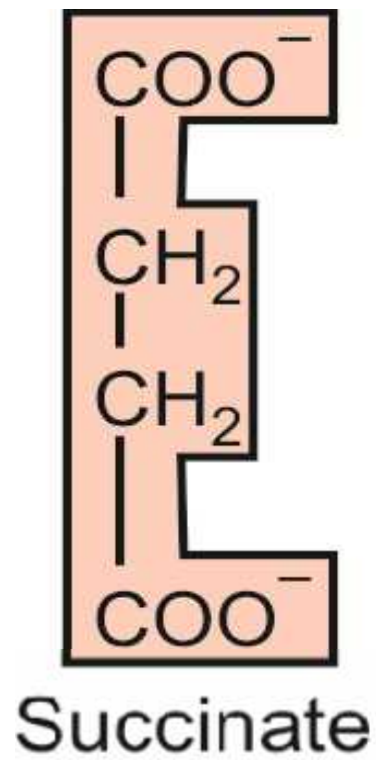
- A competitive inhibitor is a structural analogue of the substrate.
- Chemical structure of inhibitor (I) resembles that of substrate (S) and binds to enzyme at active site, forming EI complex rather than ES-complex.



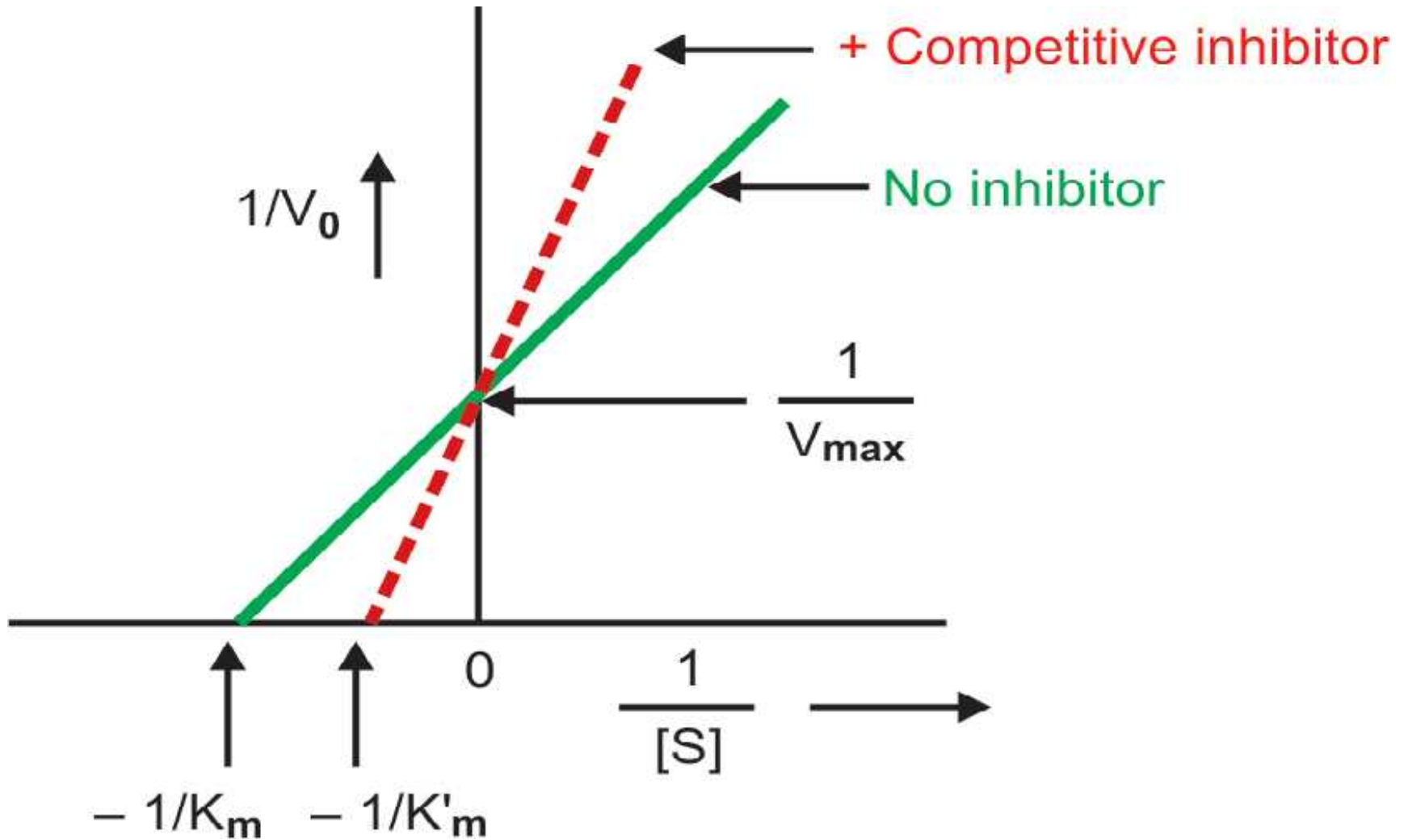
Diagrammatic representation of competitive inhibition

E: Enzyme; S: Substrate; I: Competitive inhibitor; P: Product

The inhibition could be overcome by increasing  
substrate concentration



# Enzyme kinetics of competitive inhibitor



## Enzyme kinetics of competitive inhibitor

$V_{\max}$  is unaltered

$K_m$  is increased



Drugs act as competitive inhibitors

## ➤ Sulphonamide

Analogue of P- aminobenzoic acid (PABA) and inhibits the synthesis of folic acid in microorganisms.

## ➤ Isoniazide [Isonicotinic acid hydrazine (INH)]

It is an anti-tuberculosis drug, inhibits the biosynthesis of NAD and restrict the growth of the organisms that cause tuberculosis.

### ➤ Dicumarol

It is an **anticoagulant drug** structurally similar to **vitamin K**. It inhibits the vitamin K activity and inhibits the formation of **prothrombin**.

### ➤ Physostigmine

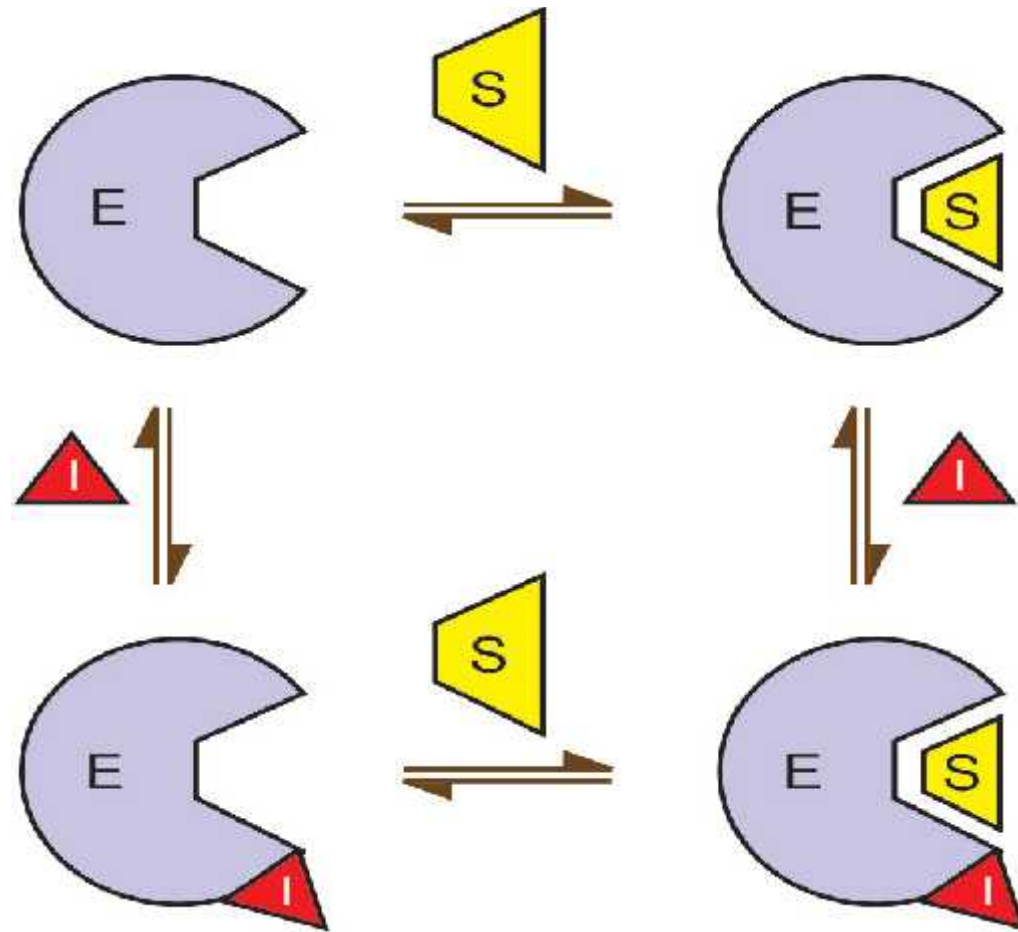
It inhibits **acetylcholinesterase** and use to treat glaucoma and myasthenia gravis.

Drugs such as **ibuprofen** (anti-inflammatory drug), **statin** (cholesterol lowering drug) are competitive inhibitors of enzymes, that involved in the prostaglandins and cholesterol synthesis respectively.

## Non-competitive Inhibitors

- No competition occurs between substrate and inhibitor.
- Inhibitor is usually structurally different from the substrate.
- It binds at a site on the enzyme molecule other than the substrate-binding site.

- Noncompetitive inhibitor can bind free enzyme (EI) or the enzyme substrate complex (EIS)
- However, EIS complex does not continue to form product.
- Noncompetitive inhibitor lowers the concentration of functional enzymes.
- Noncompetitive inhibition cannot be overcome by increasing the substrate concentration



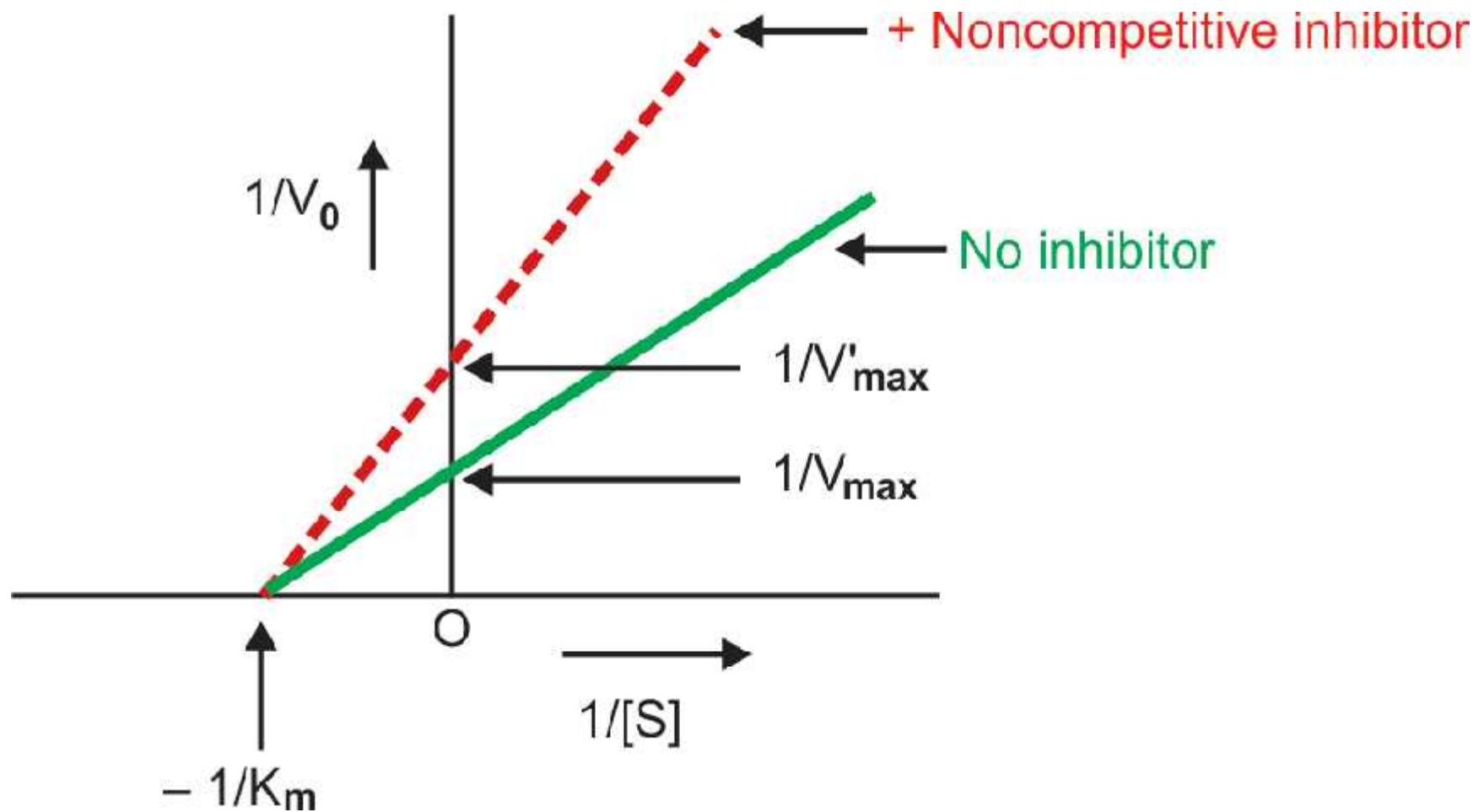
**Diagrammatic representation of noncompetitive inhibition.**

(E = Enzyme; S = Substrate; I = Non-competitive inhibitor; P = Product)

## Examples of non-competitive inhibitors are:

- Ethanol or certain narcotic drugs are non-competitive inhibitor of acid phosphatase.
- Trypsin inhibitors occur in soybean and raw egg white, inhibit activity of trypsin.
- *Ascaris* parasites (worm) contain pepsin and trypsin inhibitors, inhibit action of pepsin and trypsin.



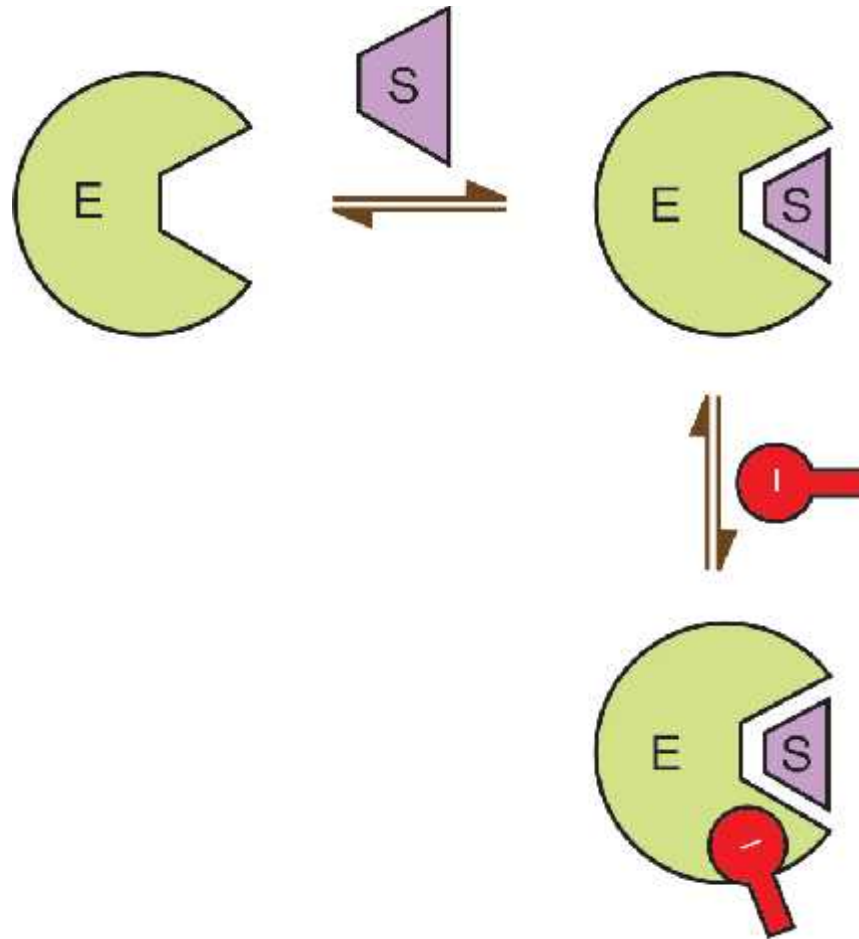


For non-competitive inhibition, the  $K_m$  value is unchanged while  $V_{\max}$  is lowered

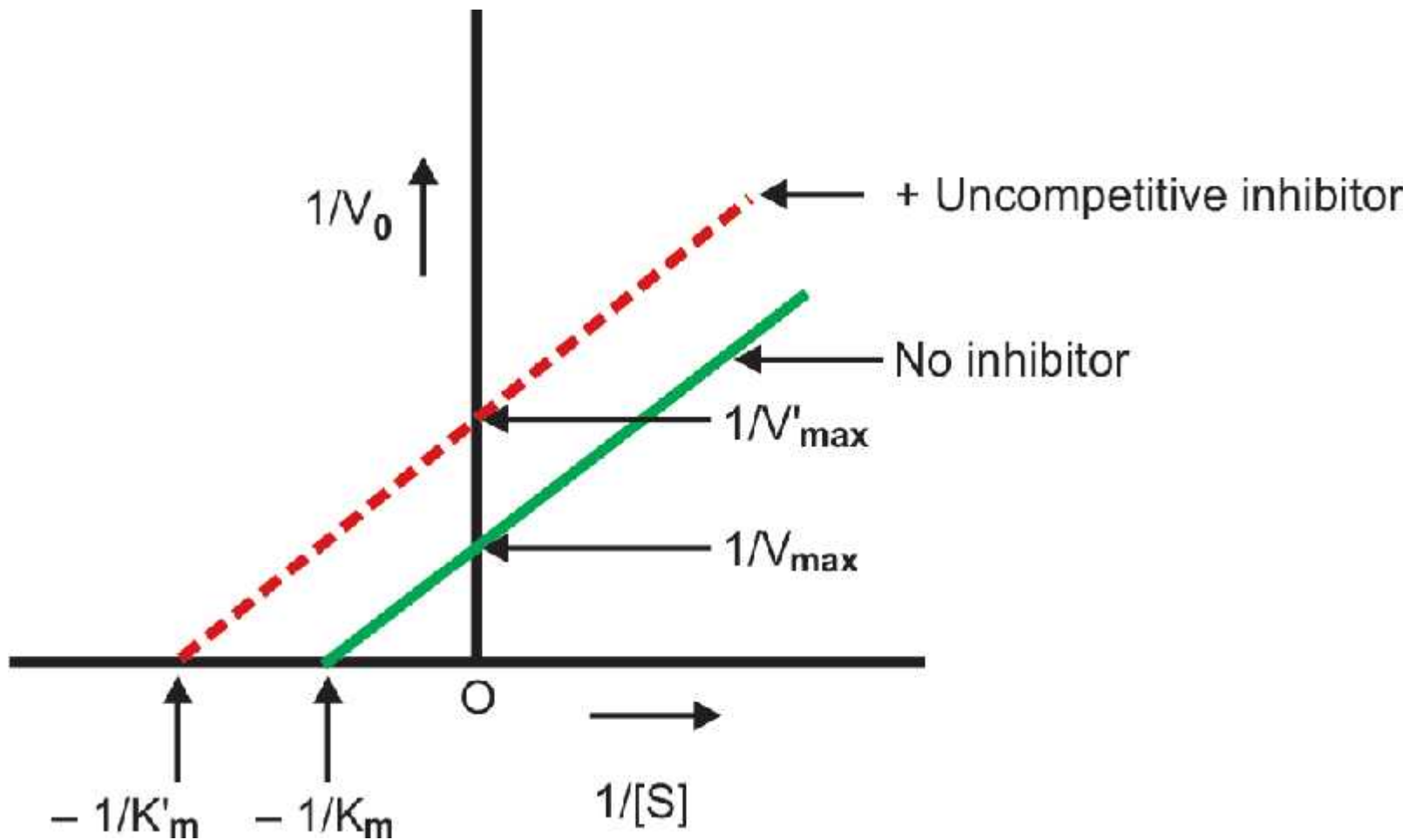
## Uncompetitive Inhibitor

- Uncompetitive inhibitor can bind only to the enzyme-substrate (ES) complex.
- It does not have affinity for free enzyme.
- Enzyme-substrate-inhibitor complex, ESI does not continue to form any product.

- Uncompetitive inhibition cannot be overcome by the addition of more substrate.
- Consequently  $V_{\max}$  cannot be attained, even at high substrate concentration.



Uncompetitive inhibitor binds only to enzyme-substrate complex.



Uncompetitive inhibitor decreases both  $V_{\max}$  and  $K_m$ .

- The herbicide **glyphosate**, also known as **Roundup**, is an uncompetitive inhibitor of an enzyme in the biosynthetic pathway for aromatic amino acids in bacteria
- Nontoxic in animals because they lack the enzyme.

# **IRREVERSIBLE INHIBITOR**

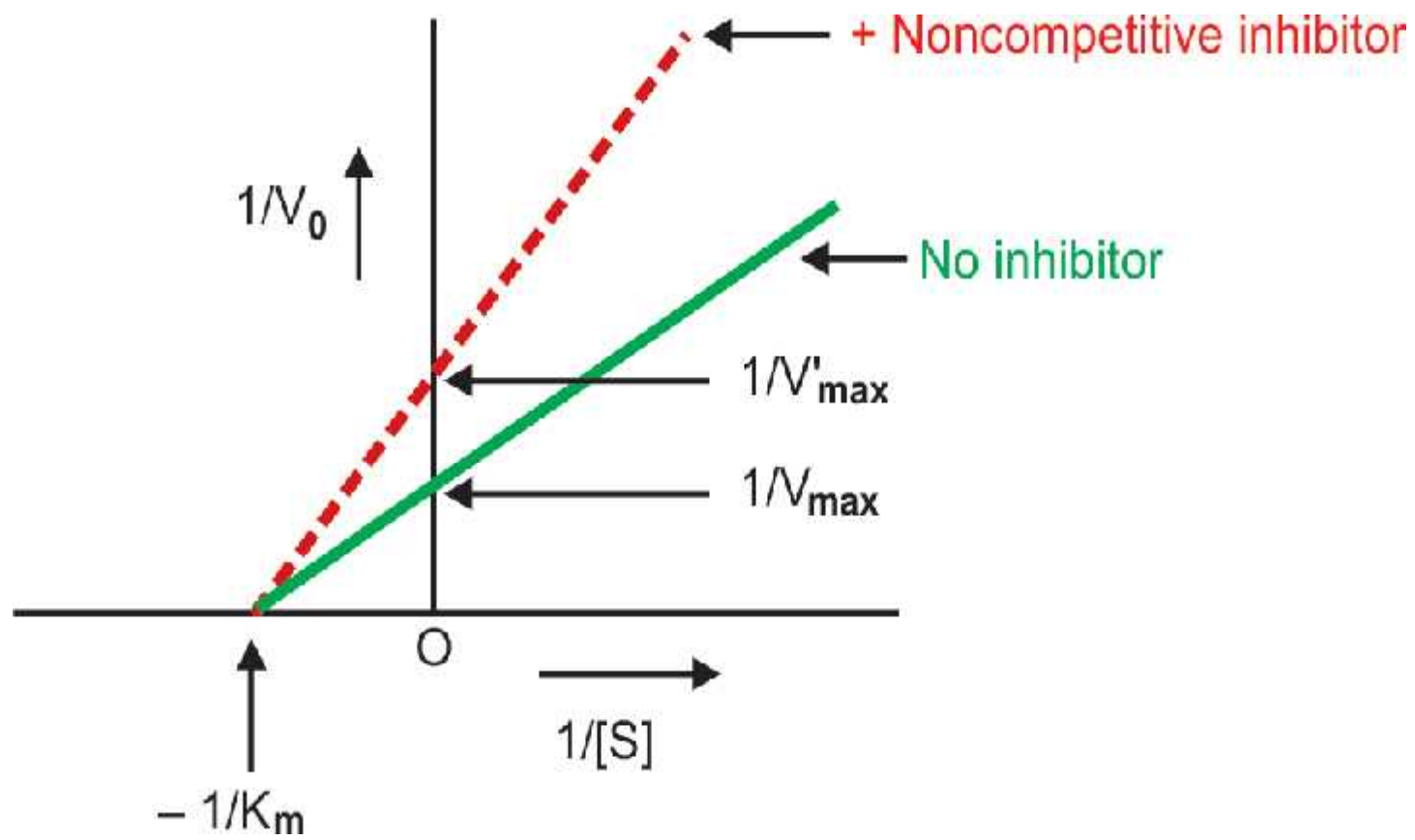
- An irreversible inhibitor binds with an enzyme tightly **covalently** and forms a stable complex.
- An irreversible inhibitor **cannot be released** by dilution or dialysis or simply by increasing the concentration of substrate.



Irreversible inhibitors can be divided into three categories:

- Substrate analogue inhibitor or affinity labels
- Group specific inhibitors
- Suicide inhibitor or mechanism based inactivation.

In terms of enzyme kinetics, the effect of an irreversible inhibitor is like that of the reversible non-competitive inhibitors resulting in a decreased  $V_{max}$  but having no effect on the  $K_m$



# Substrate Analogue Irreversible Inhibitor or Affinity Labels

- Substrate analogues or affinity labels are molecules that are **structurally similar** to the substrate.
- These substrate analogues possess a highly **reactive group** which is not present in the natural substrate.

- The reactive group of substrate analogues covalently reacts with amino acid residues of the active site of the enzyme and permanently block the active site of the enzyme
- 3-Bromoacetal phosphate (BAP) inhibits enzyme phosphotriose isomerase of glycolysis.

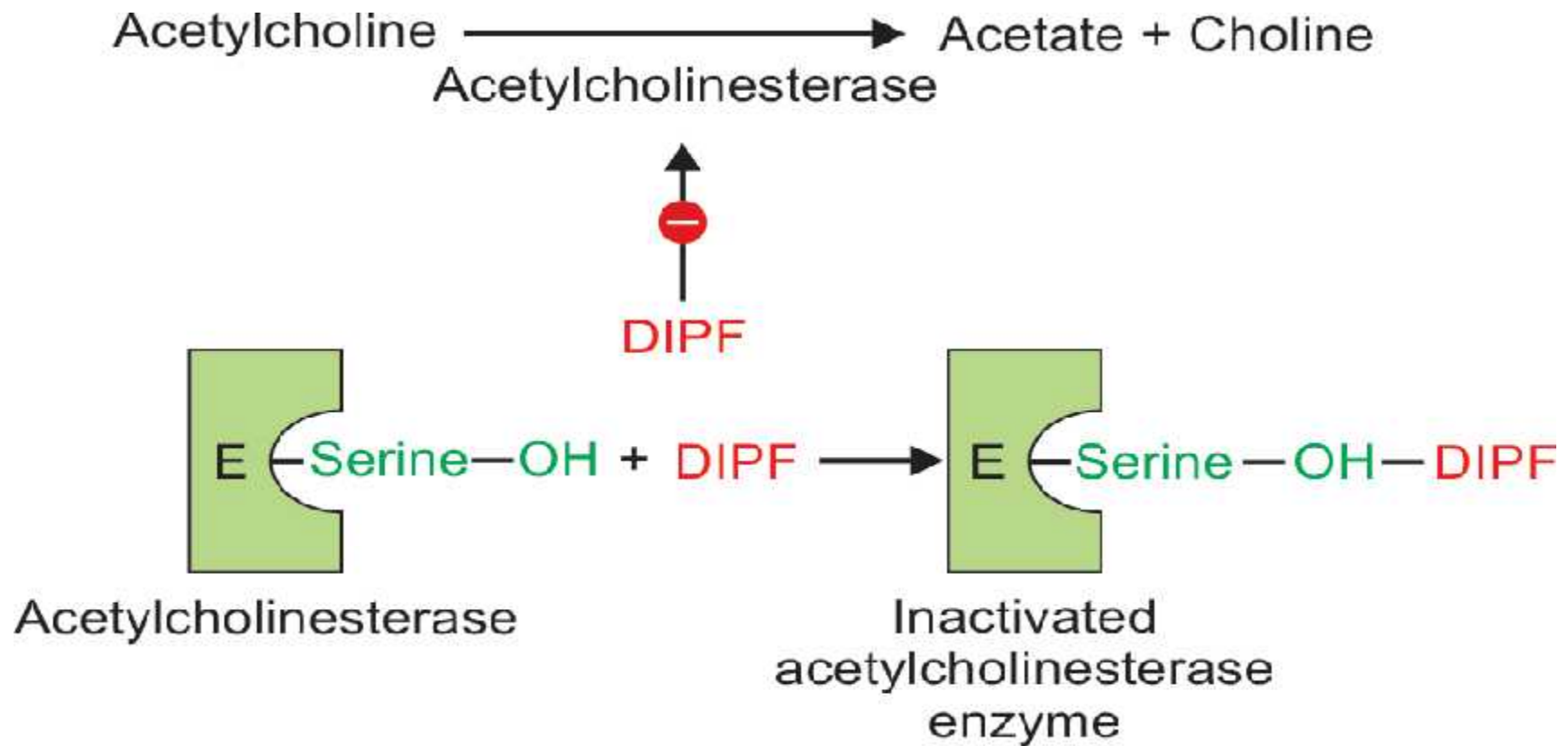
## Group Specific Irreversible Inhibitor

- These inhibitors react with specific R-groups (side chain) of amino acid residues in the active site of enzyme.

- Examples of group specific irreversible inhibitors:
  - Di-isopropylphosphofluoride (DIPF)
  - Iodoacetamide
  - Heavy metals

- DIPF can inhibit an enzyme **acetylcholine esterase** by covalently reacting with **hydroxyl group** of a **serine** residue present at the active site of the enzyme
- DIPF has also been found to inhibit trypsin, chymotrypsin, elastase and phosphoglucomutase





Irreversible inhibition of acetylcholinesterase by a group-specific inhibitor, diisopropylphosphofluoride (DIPF).

- Iodoacetamide and heavy metals like,  $\text{Pb}^{2+}$ ,  $\text{Ag}^{+}$ ,  $\text{Hg}^{2+}$ , etc. which react with **sulfhydryl (-SH) group of cysteine** residues present at the active site of the enzyme and makes them inactive.

## **Suicide Inhibitor or Mechanism Based inactivation**

- These compounds are relatively **unreactive** until they bind to the active site of a specific enzyme.
- On binding to the active site of the enzyme they carry out the first few catalytic activities of the normal enzyme reaction.

- Instead of being transformed into a normal product, however, the inhibitor is converted to a very reactive compound that combines irreversibly with the enzyme leading to its irreversible inhibition
- These are also called **mechanism based inactivation** because they utilize the normal enzyme reaction mechanism to inactivate the enzyme

## Example Suicide Inhibitor

### ➤ Penicillin

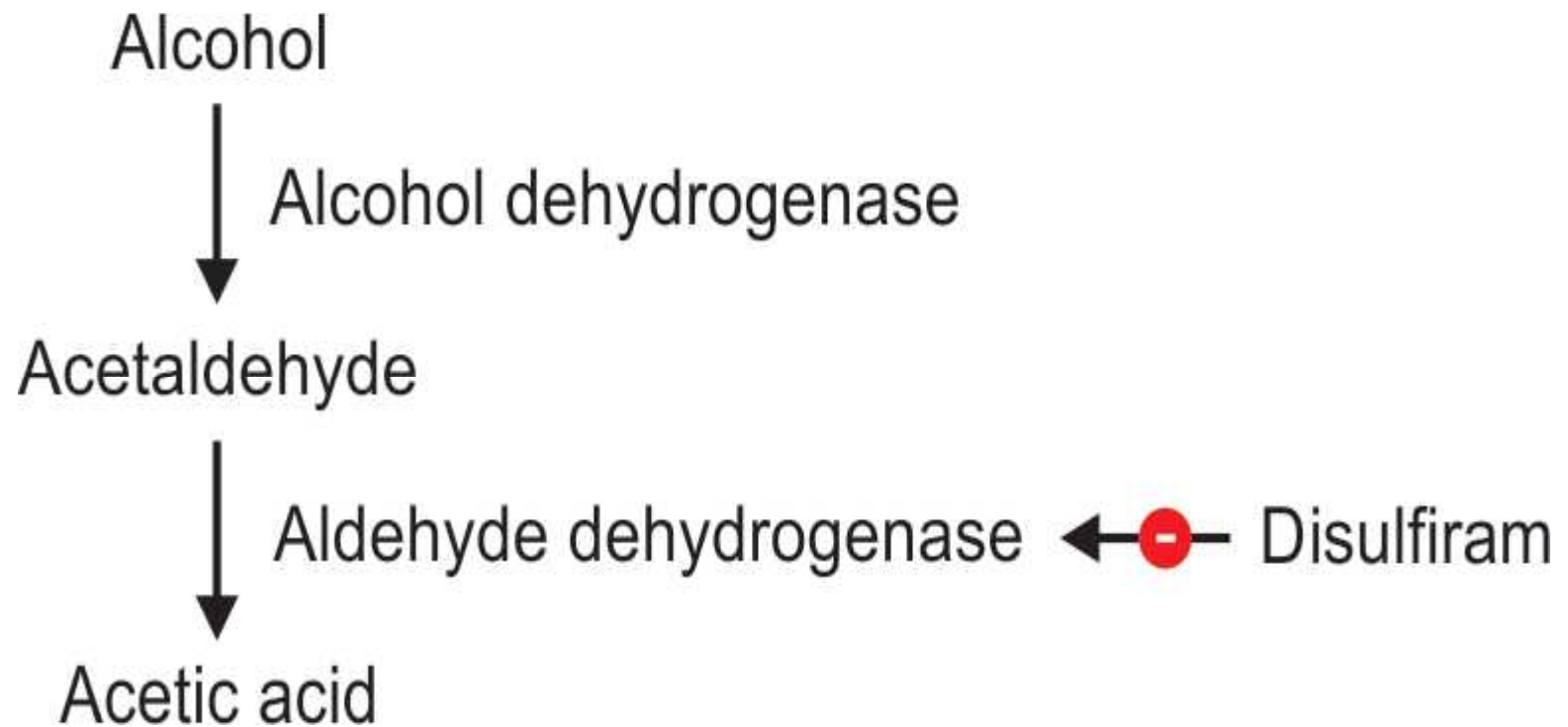
Inactivates bacterial enzyme **glycopeptidyl transpeptidase** involved in the formation of bacterial cell wall.

### ➤ Aspirin

Inactivates an enzyme **cyclo-oxygenase** required for the synthesis of **prostaglandins** .

## ➤ Disulfiram (antabuse)

- Inhibits aldehyde dehydro- genase enzyme resulting in accumulation of acetaldehyde.
- **Antabuse**, or **disulfiram** is, a medicine for treatment of **alcohol abuse** and alcohol dependence
- Antabuse is prescribed to people who want to quit drinking.



## Clinical Application of Enzyme Inhibitor

- Enzyme inhibitors have therapeutic applications.
- Most antibiotics and anticancer drugs that are used therapeutically are either competitive inhibitor or mechanism based suicide inhibitor.



**TABLE 6.7: Commonly used drugs that are enzyme inhibitors.**

| <i>Drugs</i>               | <i>Types of inhibition</i> | <i>Target enzymes</i>                                        | <i>Therapeutic uses</i>             |
|----------------------------|----------------------------|--------------------------------------------------------------|-------------------------------------|
| Mevinolin and lovastatin   | Competitive                | HMG-CoA reductase (3-hydroxy-3-methylglutaryl-CoA reductase) | Hypercholesterolemia                |
| Allopurinol                | Competitive                | Xanthine oxidase                                             | Gout                                |
| Methotrexate               | Competitive                | Dihydrofolate reductase                                      | Cancer                              |
| Captopril and enalapril    | Competitive                | Angiotensin-converting enzyme (ACE)                          | High blood pressure                 |
| 5-fluorouracil             | Suicide                    | Thymidylate synthase                                         | Cancer                              |
| Aspirin                    | Suicide                    | Cyclooxygenase                                               | Anti-inflammatory                   |
| Penicillin                 | Suicide                    | Bacterial transpeptidase                                     | Antibacterial                       |
| N,N-dimethylpropargylamine | Suicide                    | Monoamine oxidase                                            | Antidepressant, Parkinson's disease |
| (-) Deprenyl               | Suicide                    | Monoamine oxidase                                            | Antidepressant, Parkinson's disease |
| Clavulanic acid            | Suicide                    | Bacterial $\beta$ -lactamases                                | Antibacterial                       |

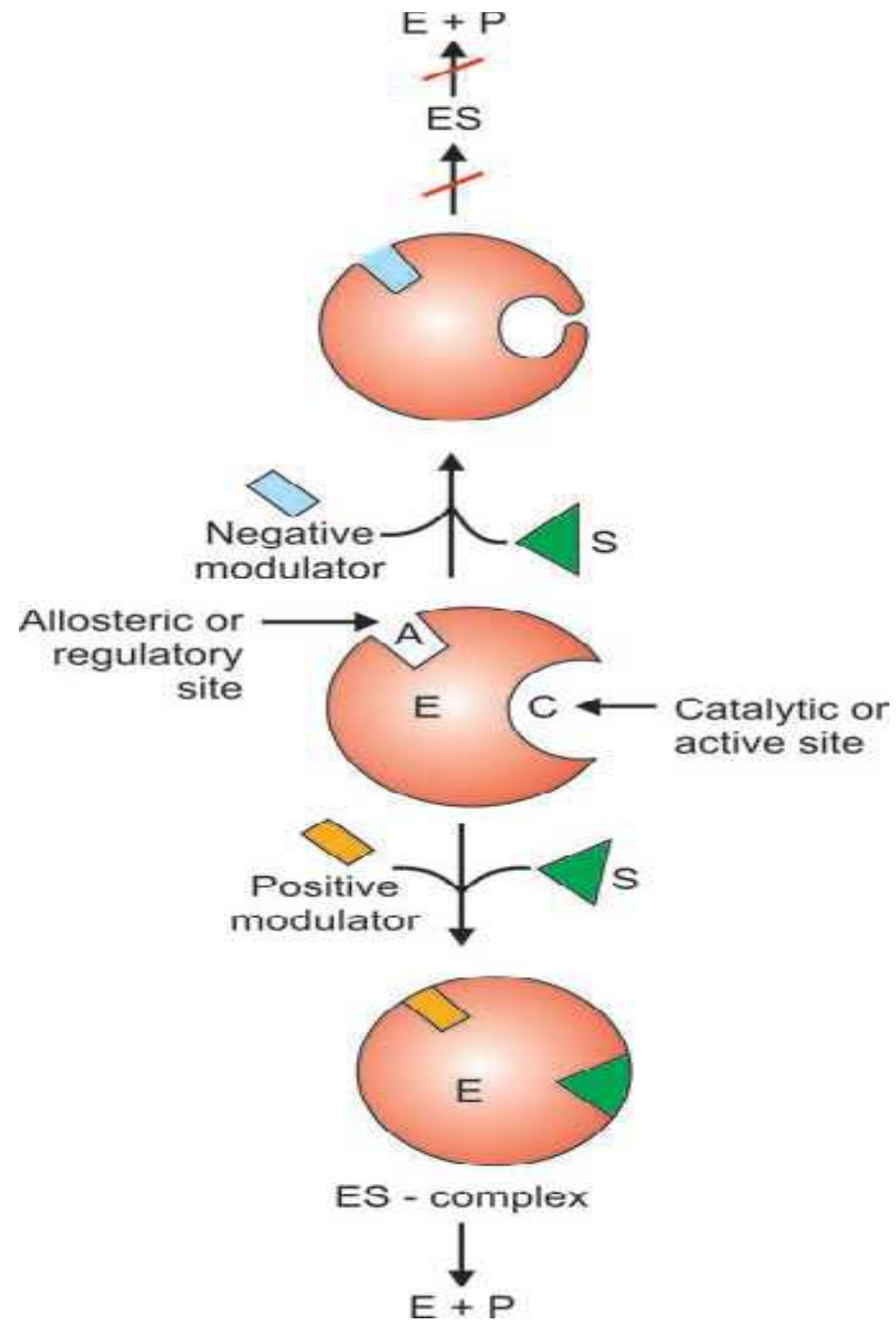
# ALLOSTERIC ENZYME

- Allosteric enzyme is a **regulatory** enzyme.
- The term allosteric derives from Greek word, **allo** means **other** and **steros** means **space** or **site**.

➤ Allosteric enzymes are those having other site in addition to active site for binding of *modulator* (regulatory metabolites).

➤ Allosteric enzymes may be inhibited or stimulated by their modulators

Modulators that inhibit enzyme activity are termed *negative modulators*. Whereas those that increase enzyme activity are called *positive modulators*.



Glycine + succinyl-CoA

$E_1$  ↓  $\delta$ -Aminolevulinic acid synthase  
( $\delta$ -ALA synthase)

$\delta$ -Aminolevulinic acid

$E_2$  ↓  
**A**

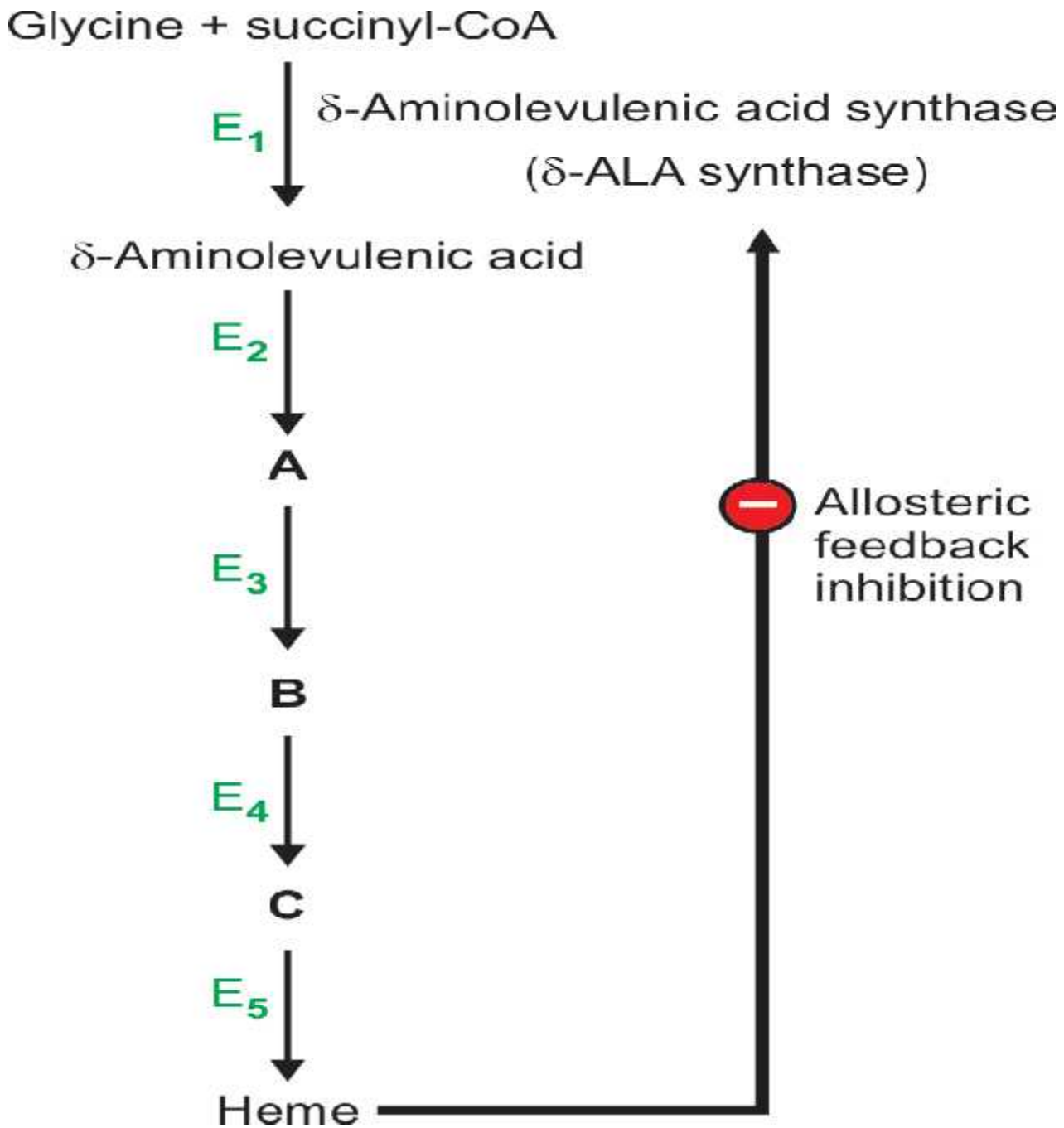
$E_3$  ↓  
**B**

$E_4$  ↓  
**C**

$E_5$  ↓  
Heme

↑  
○  
Allosteric  
feedback  
inhibition

Heme



## ISOENZYME

- Isoenzymes or isozymes are **multiple forms** (isomers) of the same enzyme that catalyze the **same biochemical reaction**.
- Isoenzymes show **different chemical** and **physical properties** like **electrophoretic mobility** and **kinetic properties**.
- Only those enzymes, which are in polymeric form demonstrate isoenzyme.

➤ For example:

1. Lactate dehydrogenase (LDH)

2. Creatine kinase (CK)



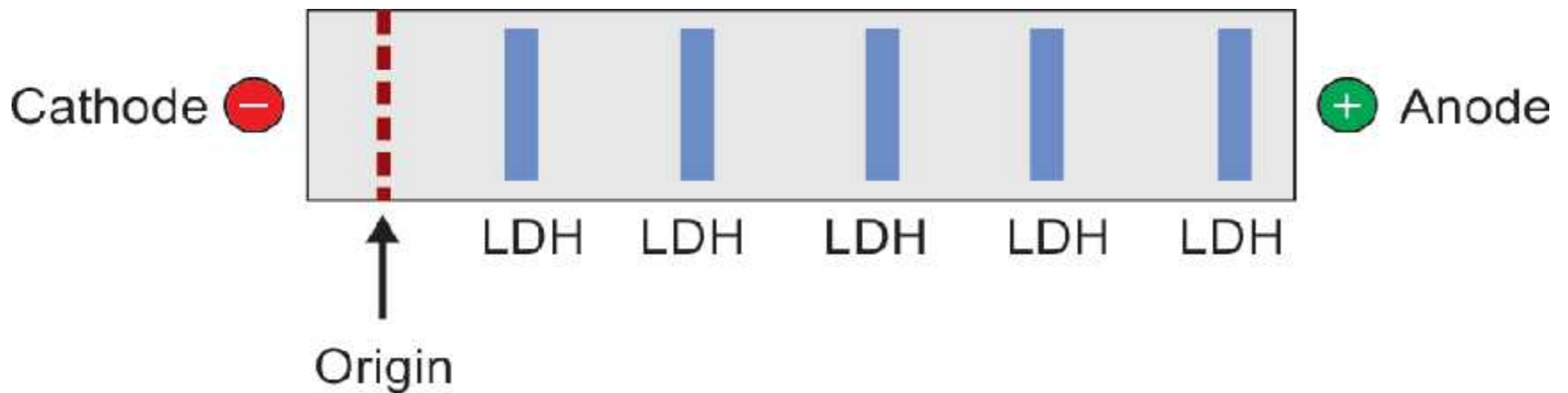
## Lactate Dehydrogenase (LDH)

- Lactate dehydrogenase is a **tetrameric** enzyme that catalyzes the oxidation of L-lactate to pyruvate.
- LDH is made up of two types of polypeptide **M** (*muscle*) type and **H** (*heart*) type.

➤ LDH has five isoenzymes:

- LDH<sub>1</sub>
- LDH<sub>2</sub>
- LDH<sub>3</sub>
- LDH<sub>4</sub>
- LDH<sub>5</sub>.

- Five isoenzymes of LDH can be detected by electrophoresis as they have different electrophoretic mobilities.



- $\text{LDH}_1$  is the **fastest** moving fraction towards the anode and  $\text{LDH}_5$  is the **slowest** moving isoenzyme of LDH.
- $\text{LDH}_1$  predominates in cells of **cardiac muscle**, and **erythrocytes** and  $\text{LDH}_5$  is the most abundant form in the **liver** and in **skeletal muscle**

## *Clinical Applications of LDH*

1. Significant elevation of LDH1 and LDH2 occurs within 24 to 48 hours after myocardial infarction.
2. Predominant elevation of LDH2 and LDH3 occur in leukaemia.
3. LDH3 elevated in malignancy of many tissues.
4. Elevation of LDH5 occurs after damage to the liver or skeletal muscle.

**Type, composition, location, and diagnostic importance of lactate dehydrogenase (LDH) and creatine kinase (CK) isoenzymes.**

| <b>Type</b>      | <b>Composition</b> | <b>Location</b>                                            | <b>Diagnostic importance (cause of elevated level)</b> |
|------------------|--------------------|------------------------------------------------------------|--------------------------------------------------------|
| LDH <sub>1</sub> | HHHH               | Heart, RBC                                                 | Myocardial infarction                                  |
| LDH <sub>2</sub> | HHHM               | Heart, RBC                                                 | Kidney diseases, megaloblastic anemia                  |
| LDH <sub>3</sub> | HHMM               | Brain, kidneys                                             | Leukemia, malignancy                                   |
| LDH <sub>4</sub> | HMMM               | Lung, spleen                                               | Pulmonary infarction                                   |
| LDH <sub>5</sub> | MMMM               | Liver, muscle                                              | Liver diseases, muscle damage/diseases                 |
| CK <sub>1</sub>  | BB                 | Brain, prostate gland, GI tract, lung, bladder, and uterus | Neurological injury, tumor marker                      |
| CK <sub>2</sub>  | BM                 | Heart                                                      | Myocardial infarction                                  |
| CK <sub>3</sub>  | MM                 | Skeletal muscle                                            | Muscular dystrophies and myopathies                    |

## Creatine Kinase (CK)

Creatine kinase isoenzymes are **dimer** that are made up of two types of polypeptide chains, which may be either **M** (*muscle*) type or **B** (*brain*) type, generating three isoenzymes.

- 1      **CK1 (BB)** : present in the brain
- 2      **CK2 (MB)** : present only in Cardiac tissue
- 3      **CK3 (MM)** : present in skeletal muscle

## *Clinical Application*

1. CK1 may be elevated in neonates particularly in damaged brain or very low birth weight new-born
2. Increased level of CK2 occurs in myocardial infarction Cardiac tissue is the only tissue which has mixed MB (CK2) isoenzyme.
3. CK-MB isoenzyme starts to increase within 4 hours after an acute myocardial infarction (AMI) and reaches a maximum within 24 hrs.
4. Elevated levels of CK3 in serum occur in dystrophies and myopathies.



# CLINICAL SIGNIFICANCE OF ENZYMES

Certain enzymes are used:

- For the diagnosis of the disease
- As therapeutic agents
- As analytical reagents.

## Diagnostic Use of Enzymes

The enzymes that are found in plasma can be categorized into two major groups:

- ❖ Plasma specific enzyme
- ❖ Plasma nonspecific enzyme.

The plasma specific enzymes are:

- The enzymes involved in blood coagulation
- Ferroxidase
- Pseudocholinesterase
- Lipoprotein lipase.

These enzymes are clinically of interest when their concentration decreases in plasma.

- The **plasma nonspecific** enzymes are present in very high concentration in **tissues** than in the plasma.
- Estimation of plasma nonspecific enzymes is very important for the **diagnosis of several disease**.

# Enzymes useful for the diagnosis of diseases

## *Alanine transaminase (ALT)*

- Alanine transaminase was known formerly as **glutamate pyruvate transaminase (GPT)**.
- The plasma ALT normal value for adult is **10 to 40 U/L**.
- ALT level is elevated in **liver diseases** (viral or toxic hepatitis), **jaundice** and **cirrhosis of liver**.

- *Aspartate transaminase (AST)*
- It was known formerly as **glutamate oxaloacetate transminase (GOT)**.
- The plasma AST normal value for adults is **10 to 30 U/L**.
- Increased AST level occurs after **myocardial infarction**.
- It is moderately elevated in **liver disease**.

The plasma AST level starts increasing after 6 to 8 hours after the onset of chest pain with peak values 18 to 24 hours and the values fall to normal level by the fourth or fifth day.

## *Alkaline phosphatase (ALP)*

- ALP hydrolyzes organic phosphate at alkaline pH.
- Normal serum level for adults is 3-13 KA units/dl.
- It is elevated in certain bone and liver disease.
- Very high levels may be noticed in obstructive jaundice, bone diseases such as Paget's disease, rickets, osteomalacia, carcinoma of bone and hyperparathyroidism



## *Acid phosphatase (ACP)*

- It hydrolyzes phosphoric acid ester at pH 5 to 6.
- Normal serum value for ACP is 0.5 to 4 KA units/dL.
- Acid phosphatase enzyme is useful for the diagnosis and prognosis of prostate cancer. ACP is therefore an important tumor marker.

## *Amylase*

- It catalyzes hydrolysis of starch and glycogen.
- Normal serum value is 50-120 U/L.
- The activity of serum amylase is increased in acute pancreatitis, chronic pancreatitis, mumps and obstruction of pancreatic duct.

*Creatine kinase (CK) : Refer isoenzyme.*

*Lactate dehydrogenase (LDH) : Refer isoenzyme.*

## Enzymes of diagnostic importance.

| <i>Enzymes</i>                 | <i>Locations</i>                                                        | <i>Clinical applications</i>                                                   |
|--------------------------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Acid phosphatase               | Prostate, erythrocyte                                                   | Prostatic cancer                                                               |
| Alanine aminotransferase       | Liver, skeletal muscle, and heart                                       | Hepatic parenchymal disease                                                    |
| Aldolase                       | Skeletal muscle, heart                                                  | Muscle diseases                                                                |
| Alkaline phosphatase           | Liver, bone, kidney, intestinal mucosa, and placenta                    | Bone disease, hepatobiliary disease                                            |
| Amylase                        | Salivary glands, pancreas                                               | Pancreatic diseases, peptic ulcer                                              |
| Aspartate transaminase         | Liver, skeletal muscle, heart, kidney, and erythrocytes                 | Myocardial infarction, hepatic parenchymal disease, muscle disease, and anemia |
| Cholinesterase                 | Liver                                                                   | Organophosphorus insecticide poisoning, hepatic parenchymal diseases           |
| Creatine kinase                | Skeletal muscle, brain, heart, and smooth muscle                        | Myocardial infarction, muscle diseases                                         |
| $\gamma$ -glutamyl transferase | Liver, kidney                                                           | Hepatobiliary disease, alcoholism                                              |
| Lactate dehydrogenase          | Heart, liver, skeletal muscle, erythrocytes, platelets, and lymph nodes | Myocardial infarction, hemolysis, and hepatic parenchymal diseases             |
| 5'-nucleotidase                | Hepatobiliary tract                                                     | Hepatobiliary disease                                                          |
| Prostate-specific antigen      | Prostate                                                                | Prostate cancer                                                                |
| Trypsin                        | Pancreas                                                                | Pancreatic disease, cystic fibrosis                                            |

## **Enzymes as Tumor Marker**

Elevated enzyme levels may signal the presence of malignancy.

## Enzymes as tumor markers and their associated types of cancer.

| <i>Enzymes</i>                       | <i>Types of cancer</i>                                 |
|--------------------------------------|--------------------------------------------------------|
| Aldolase                             | Liver                                                  |
| Alkaline phosphatase                 | Bone, liver, leukemia, and sarcoma                     |
| Placental alkaline phosphatase       | Ovarian, lung, gastrointestinal, and Hodgkin's disease |
| Amylase                              | Pancreatic                                             |
| Creatine kinase                      | Prostate, lung, breast, colon, and ovarian             |
| $\gamma$ -glutamyl transferase (GGT) | Liver                                                  |
| Lactate dehydrogenase (LDH)          | Liver, lymphomas, and leukemia                         |
| 5'-nucleotidase                      | Liver                                                  |
| Prostate-specific antigen            | Prostate                                               |
| Prostatic acid phosphatase           | Prostate                                               |

## Enzyme Assays in Myocardial Infarction/Cardiac Markers

Diagnostic enzymes include:

- Creatine kinase
- Lactate dehydrogenase
- Serum aspartate aminotransferase, also called
- serum glutamate oxaloacetate transaminase.

Nonenzyme proteins includes :

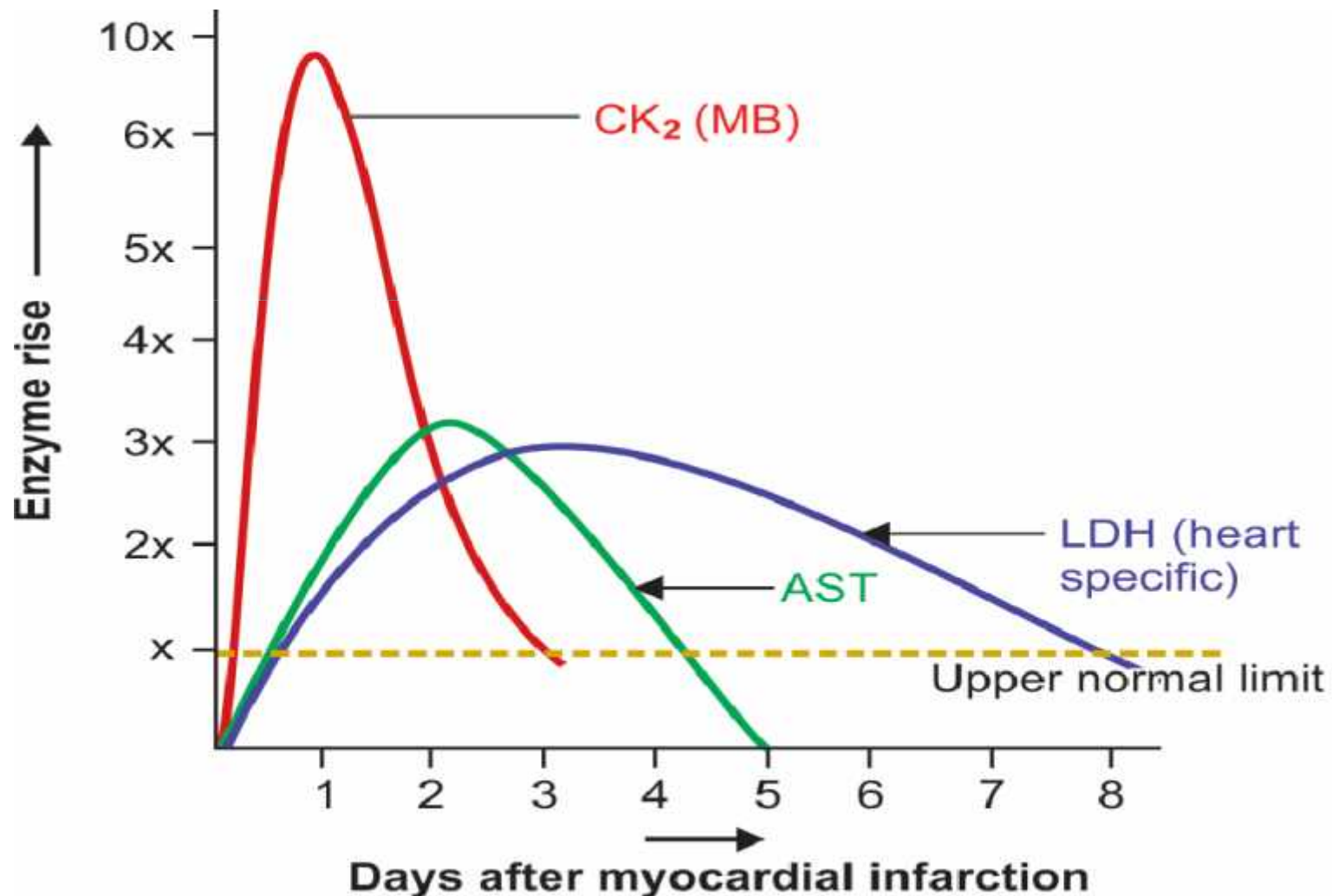
- Myoglobin (Mb)
- Cardiac troponin T and I (cTnT and cTnI).



**Cardiac markers with time course after onset of acute myocardial infarction.**

| <i>Markers</i>       | <i>Abnormal activity detectable (hours)</i> | <i>Time for maximum rise (hours)</i> | <i>Time for return to normal (days)</i> |
|----------------------|---------------------------------------------|--------------------------------------|-----------------------------------------|
| CK <sub>2</sub> (MB) | 3–10                                        | 10–24                                | 2–3                                     |
| AST/SGOT             | 6–12                                        | 24–48                                | 4–6                                     |
| LDH (heart specific) | 8–16                                        | 48–72                                | 7–12                                    |
| Myoglobin (Mb)       | 1–3                                         | 6–9                                  | 1                                       |
| Troponin-I (cTnI)    | 3–8                                         | 24–48                                | 3–5                                     |
| Troponin-T (cTnT)    | 3–8                                         | 72–100                               | 5–10                                    |

Various enzyme assays and their time course after onset of acute myocardial infarction.



## ***Enzyme Assays in Liver Diseases***

### **1. Enzymes in hepatocyte damage:**

- Aspartate aminotransferase
- Alanine aminotransferase.

ALT is the more liver-specific enzyme.

### **2. Enzymes in cholestasis:**

- Alkaline phosphatase
- 5'-nucleotidase
- $\gamma$ -glutamyl transferase.

## Enzyme Assays in Pancreatitis

- Serum Amylase
- Urine amylase
- Lipase

# Use Of Enzymes In Laboratory Investigations (Enzyme-based Assays)

Enzymes can be used as analytical laboratory reagents

List of enzymes used in the clinical laboratory as analytical reagents for investigations.

| <i>Enzymes as reagents</i>                                    | <i>Investigations</i> |
|---------------------------------------------------------------|-----------------------|
| Alcohol dehydrogenase                                         | Ethanol               |
| Lactate dehydrogenase                                         | Lactate               |
| Glucose oxidase and peroxidase                                | Glucose               |
| Hexokinase and glucose-6-phosphate dehydrogenase              | Creatine kinase       |
| Uricase                                                       | Uric acid             |
| Urease                                                        | Urea                  |
| Cholesterol oxidase and peroxidase                            | Cholesterol           |
| Lipase, glycerol kinase, and glycerol phosphate dehydrogenase | Triacylglycerol       |

# Therapeutic Use of Enzymes

Some enzymes are used in the treatment of some diseases  
of human being

### Some important therapeutic enzymes.

| <i>Enzymes</i>     | <i>Uses</i>                |
|--------------------|----------------------------|
| Asparaginase       | Leukemia                   |
| Chymotrypsin       | Inflammation and edema     |
| Collagenase        | Skin ulcers                |
| Fibrinolysin       | Blood clot                 |
| Glutaminase        | Leukemia                   |
| Hyaluronidase      | Heart attack               |
| Lysozyme           | Antibiotic                 |
| Rhodanase          | Cyanide poisoning          |
| Ribonuclease       | Antiviral                  |
| $\beta$ -lactamase | Penicillin allergy         |
| Streptokinase      | Blood clots                |
| Trypsin            | To dissolve the blood clot |
| Uricase            | Gout                       |
| Urokinase          | Blood clots                |