Enzymes

Learning objectives

- Explain biochemical nature of enzyme
- Enumerate the IUBMB enzyme classification and nomenclature with examples
- Describe various cofactors and coenzyme of vitamin B complex & mention the biochemical reactions in which they are involved.
- Describe various mechanisms of enzyme activity

- Describe the factors affecting the velocity of enzyme reaction and describe the importance of Vmax & Km.

- Describe various specificities of enzyme

- Describe reversible and irreversible types of enzyme inhibitors with examples

- Describe the isoenzyme with examples and clinical significance.

- Describe the alloenzyme with examples and clinical significance.

- Describe the diagnostic, therapeutic importance of various serum enzymes in various disorders

- Interpret the laboratory results of various serum enzymes of liver, cardiac, skeletal muscle, the biliary tract, and the pancreas in pathological conditions

- Explain biochemical nature of enzyme
- Enumerate the IUBMB enzyme classification and nomenclature with examples
- Describe various cofactors and coenzyme of vitamin B complex & mention the biochemical reactions in which they are involved.

- 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{sn}

- 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.

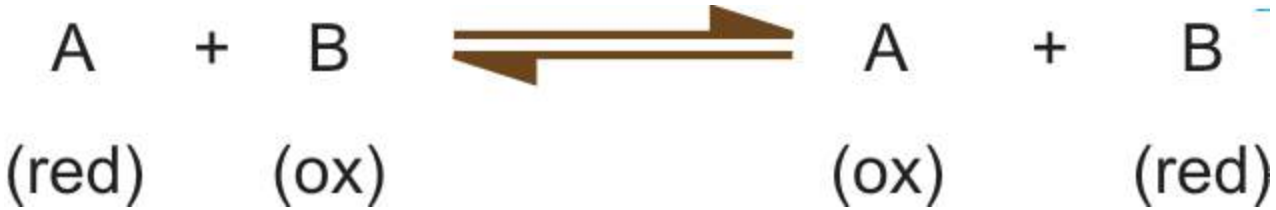
- According to the International Union of Biochemistry and Molecular Biology (IUBMB) system, enzymes are classified into seven major classes
- Enzymes are classified according to the type of reaction they catalyse.
- Each enzyme is assigned a four-digit 'EC' (Enzyme Commission) number, the first three digits of which define the reaction catalyzed and the fourth of which is a unique identifier (serial number).

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:

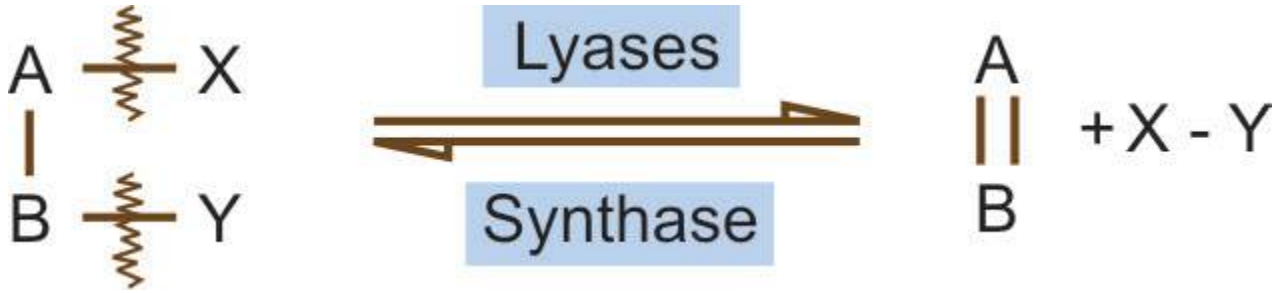
- All digestive enzymes like:

  - α-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.

TABLE 6.2: International IUBMB classification of enzymes.

Class	*Types of reaction catalyzed*	*Examples*
EC-1 Oxidoreductases	Oxidation-reduction reactions (transfer of electrons, hydride ions, or H atoms)	Lactate dehydrogenase (LDH) Glucose-6-phosphate dehydrogenase (G6PD) Ferroxidase (ceruloplasmin) Cytochrome oxidase Malate dehydrogenase
EC-2 Transferases	Groups (like amino, carboxyl, methyl, or phosphoryl, etc.) transfer reactions	Aspartate transaminase (AST) Alanine transaminase (ALT) Ornithine carbamoyltransferase Hexokinase 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 α-amylase Trypsin Chymotrypsin Lactase Sucrase Alkaline phosphatase Pepsin

EC-4 Lyases	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	Aldolase Porphobilinogen synthase Fumarase Argininosuccinase Carbonic anhydrase Cysteine desulfurase Decarboxylase
EC-5 Isomerases	Transfer of groups within molecules to yield isomeric forms	Phosphoglucomutase Triphosphate isomerase or mutase Phosphohexose isomerase Glucose 4-epimerase Retinal isomerase

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

- For example,

  - prothrombin,
  - proelastase
  - chymotrypsinogen,
  - trypsinogen,
  - pepsinogen

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.

TABLE 6.4: Some common coenzymes and their functions.

Vitamins	*Coenzymes*	*Coenzyme for*
Thiamine (vitamin B_1)	Thiamine pyrophosphate (TPP)	Oxidative decarboxylation and transketolase reaction
Riboflavin (vitamin B_2)	Flavin adenine dinucleotide and flavin mononucleotide (FAD and FMN)	Oxidation and reduction reactions
Niacin	Nicotinamide adenine dinucleotide (NAD) Nicotinamide adenine dinucleotide phosphate (NADP)	Oxidation and reduction reactions
Pyridoxine (vitamin B_6)	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_{12})	Methylcobalamin and deoxyadenosylcobalamin	Transfer of CH_3 group and isomerization

TABLE 6.3: Inorganic ions that serve as cofactors for enzymes.

Ions	*Enzymes*
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, and peroxidase
K^{+}	Pyruvate kinase, propionyl-CoA carboxylase, and acetyl-CoA thiolase
Mg^{2+}	Hexokinase, glucose-6-phosphatase, and pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo^{+}	Dinitrogenase, nitrate reductase
Ni^{2+}	Urease
Se	Glutathione peroxidase
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

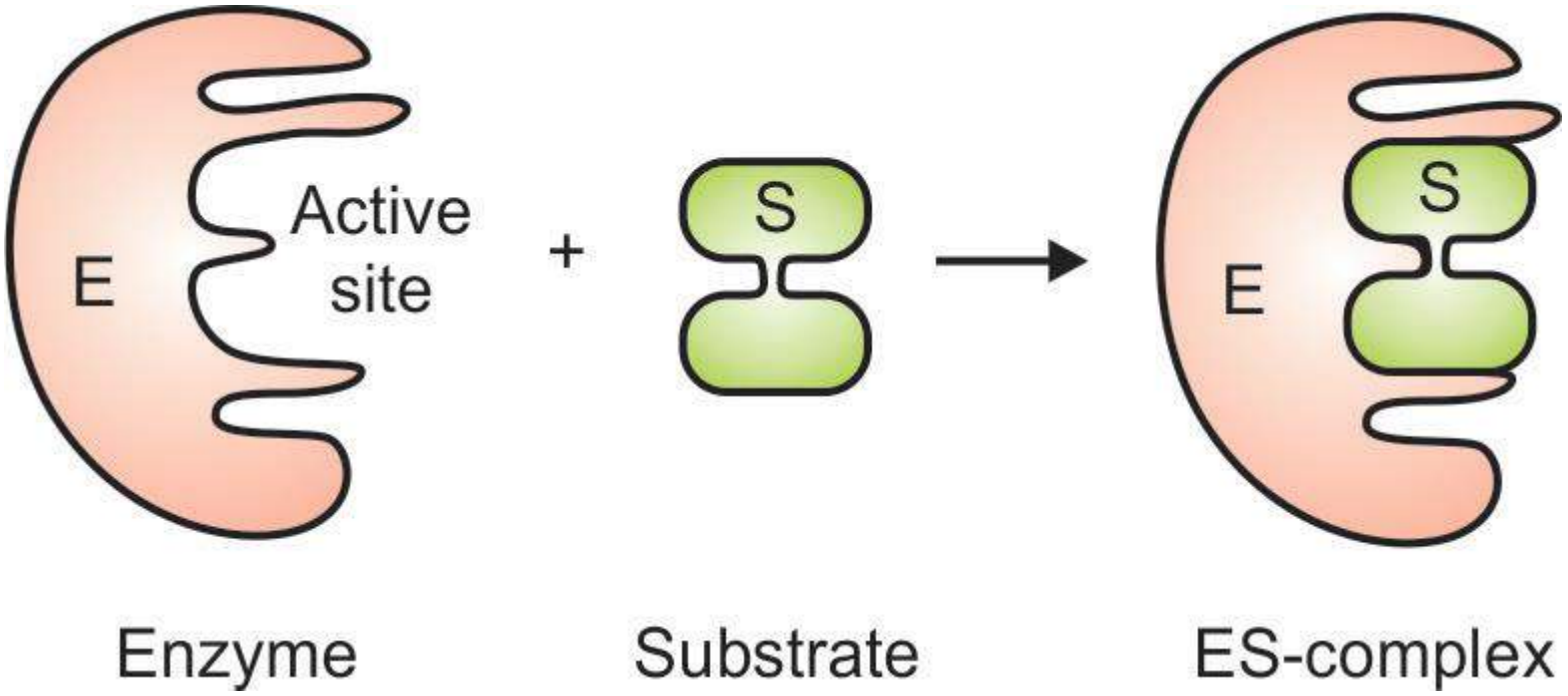
1. **Lock and key** model or rigid template model of Emil Fisher.

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.



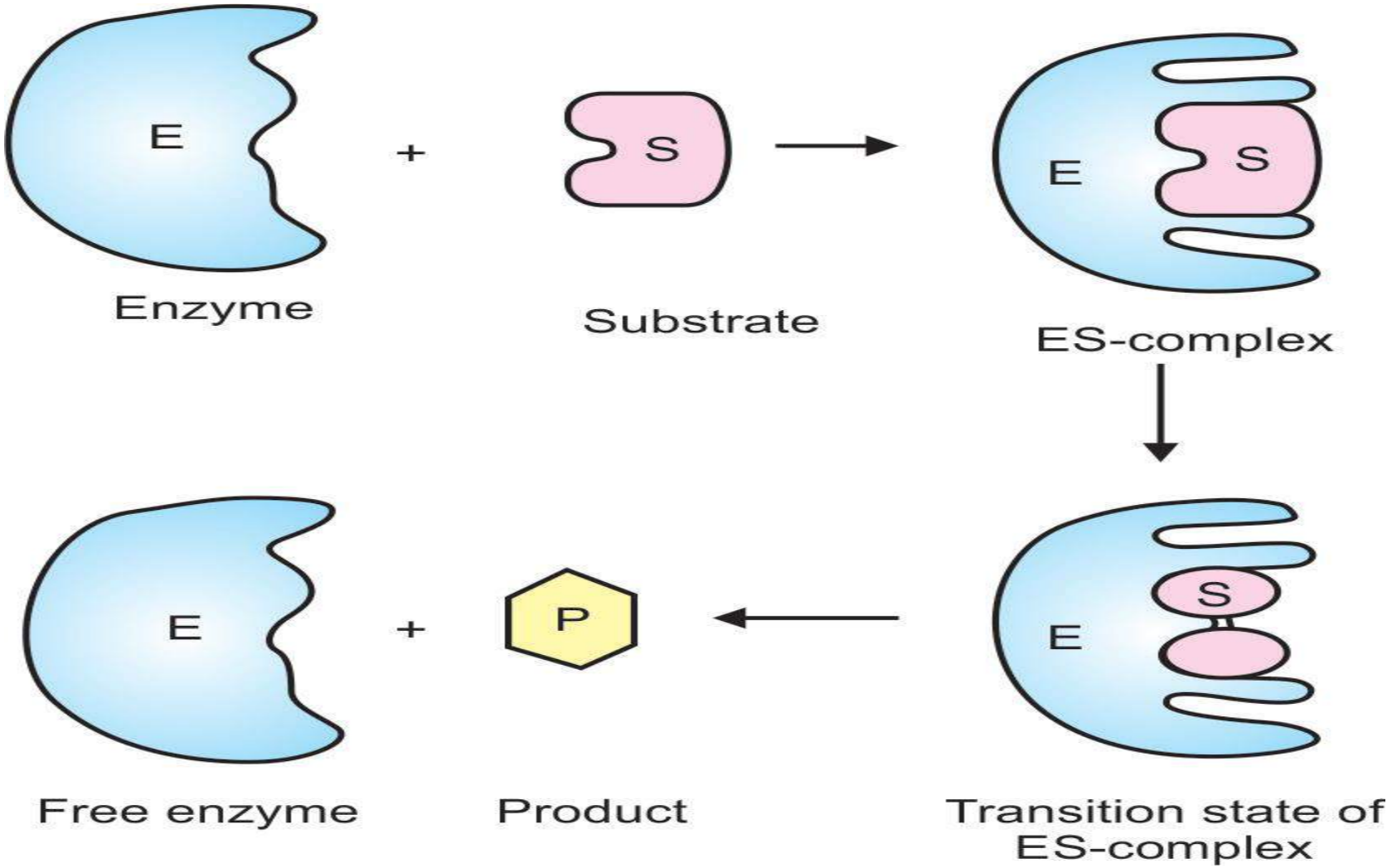
This model explains all mechanisms but do not explain the changes in the enzyme activity in the presence of modulator.

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

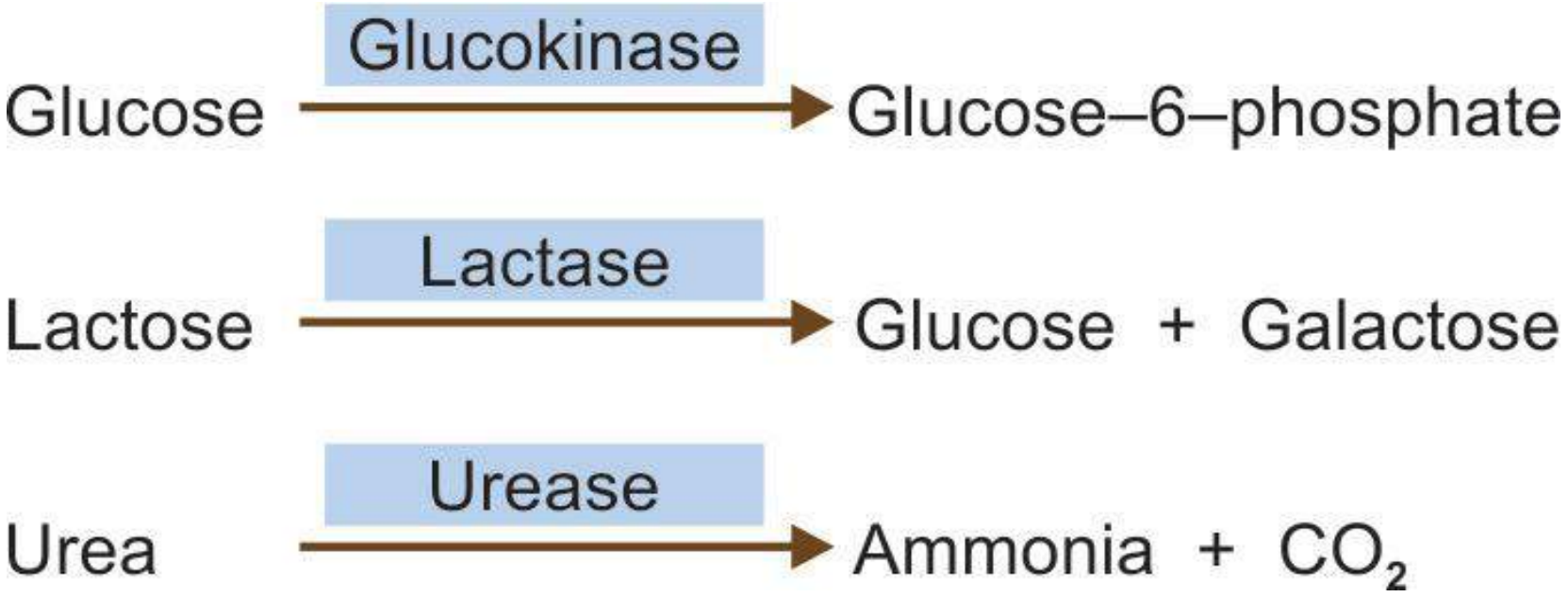
i. Absolute substrate specificity

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.


Glucokinase
Glucose
Glucose–6–phosphate
Lactase
Lactose
Glucose + Galactose
Urease
Urea
Ammonia + CO_2

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.

- α-amylase, cleaves α-(1→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.

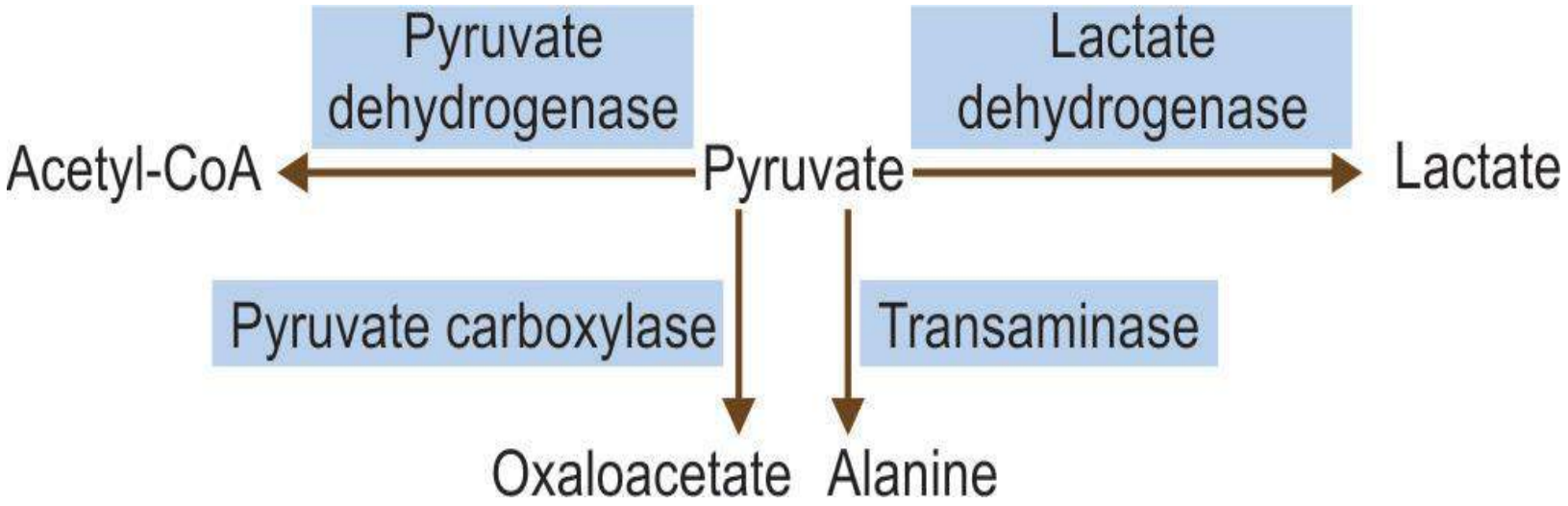


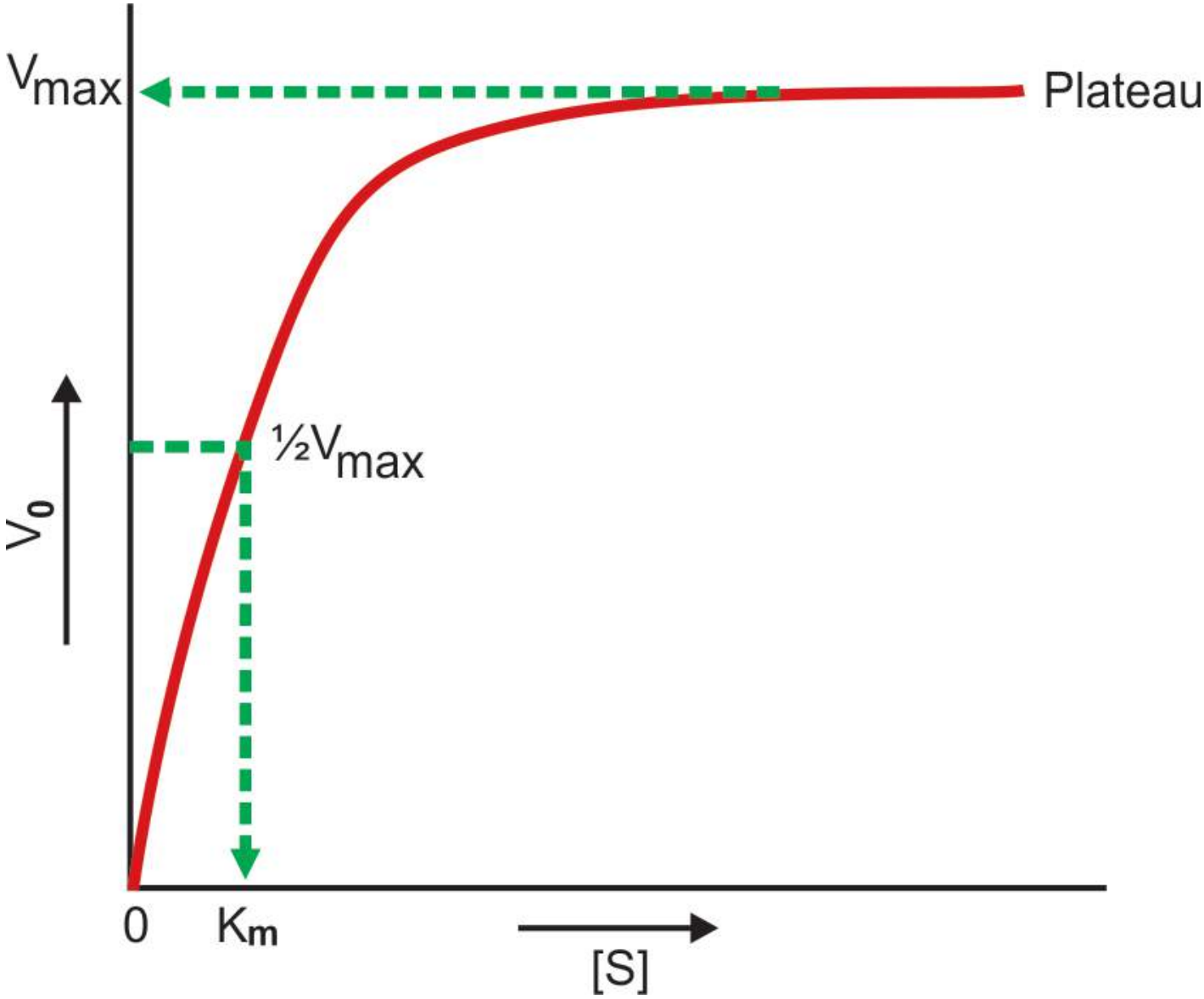
Figure 6.5: Example of reaction specificity.

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 α-amylase acts on the α-1,4 glycoside linkage and is inactive on β-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

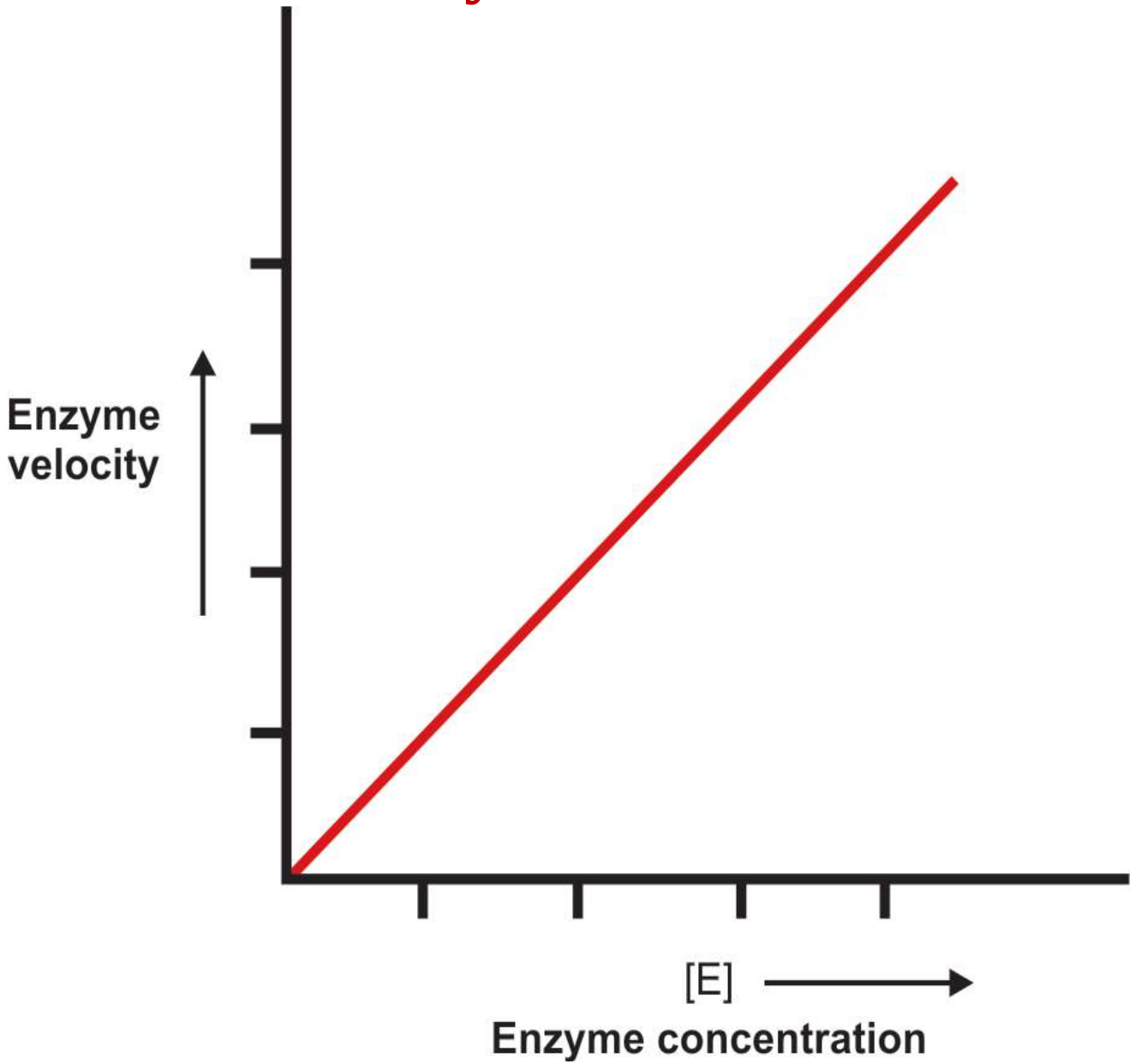
V_0 : initial velocity

V_{max} : maximum velocity

K_m : 1/2 Vmax = Michaelis Menten constant

[S] : substrate concentration

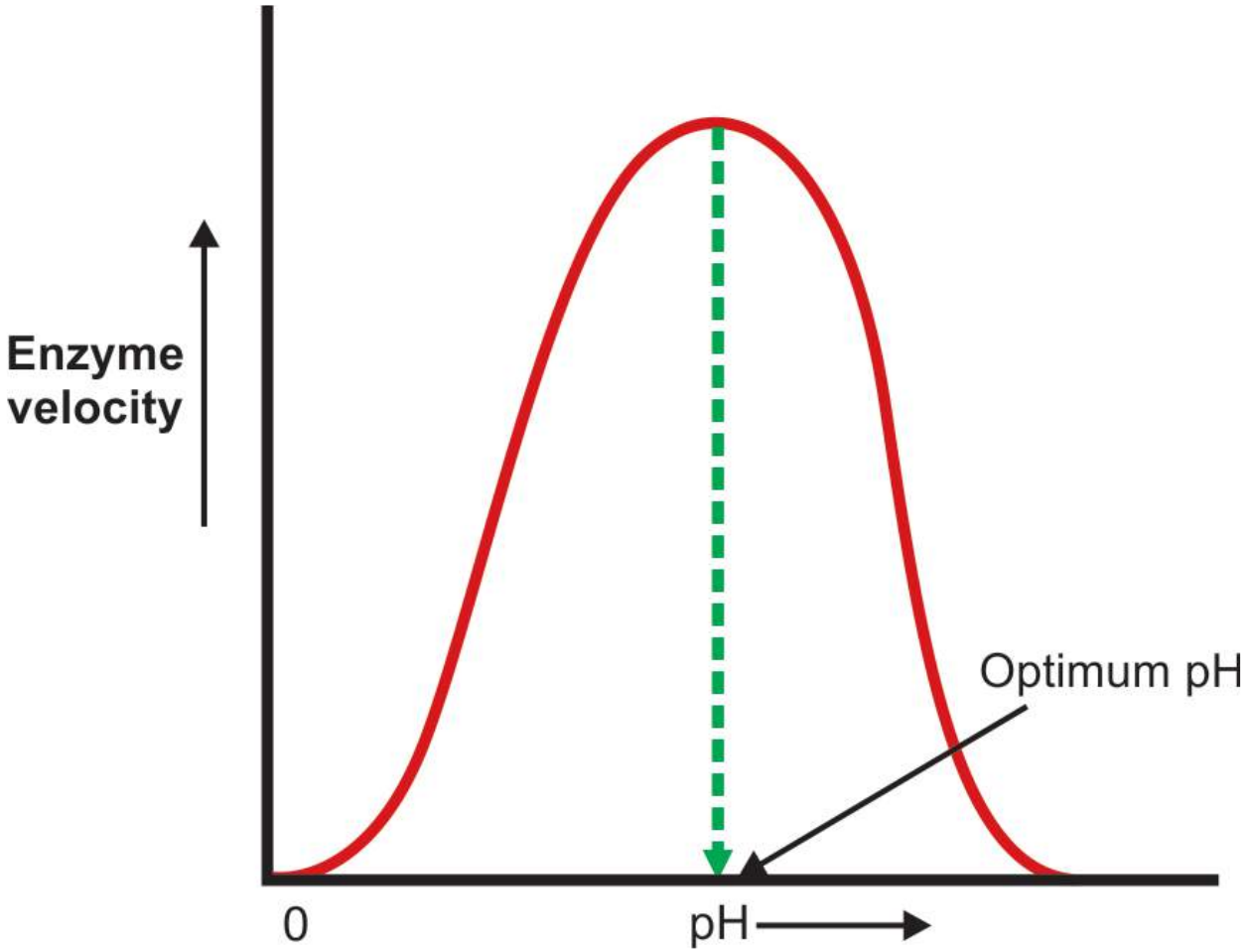
Effect of Enzyme Concentration



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



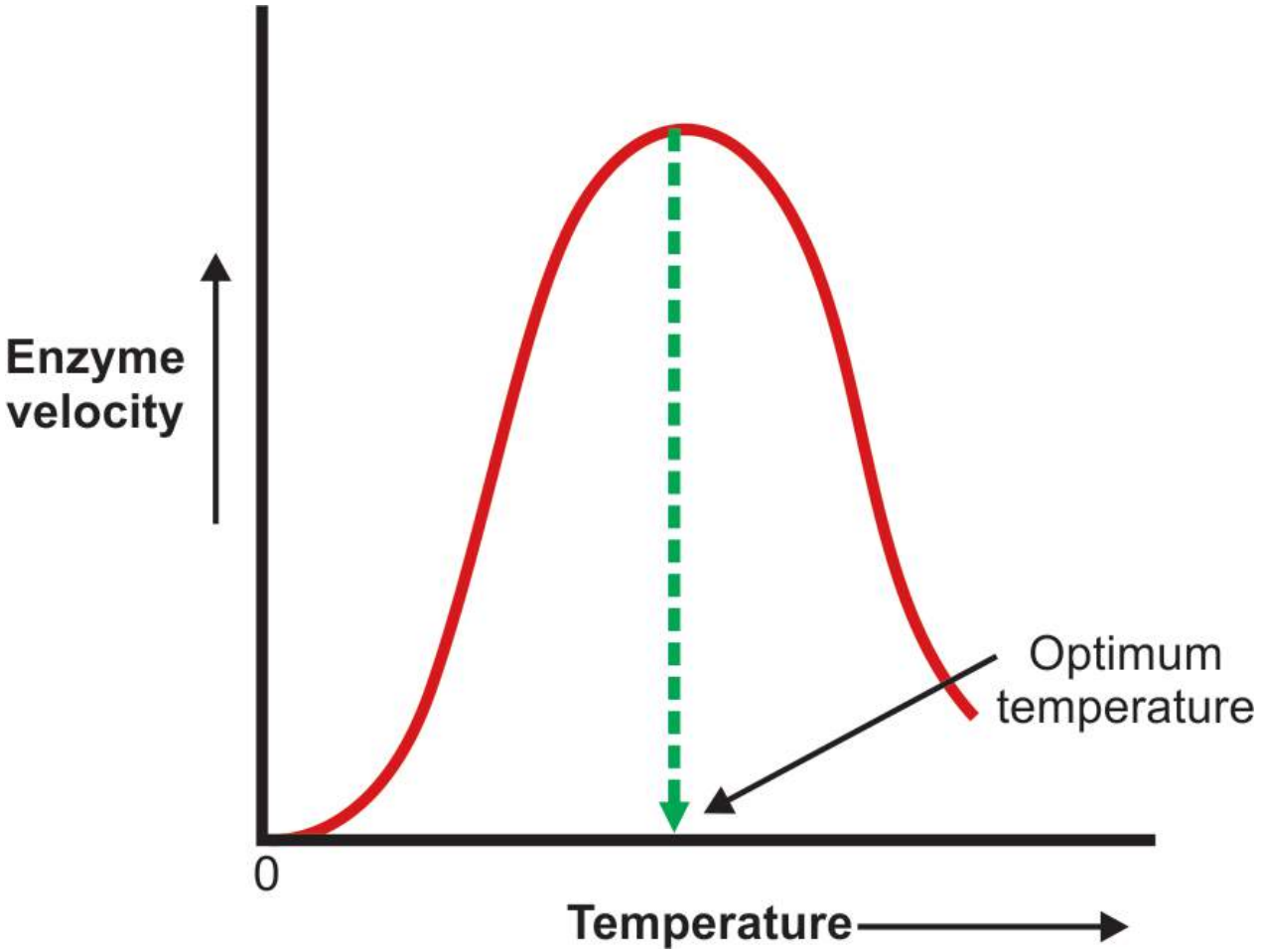
Changes in pH can alter the following:

- 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°C to 38°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].

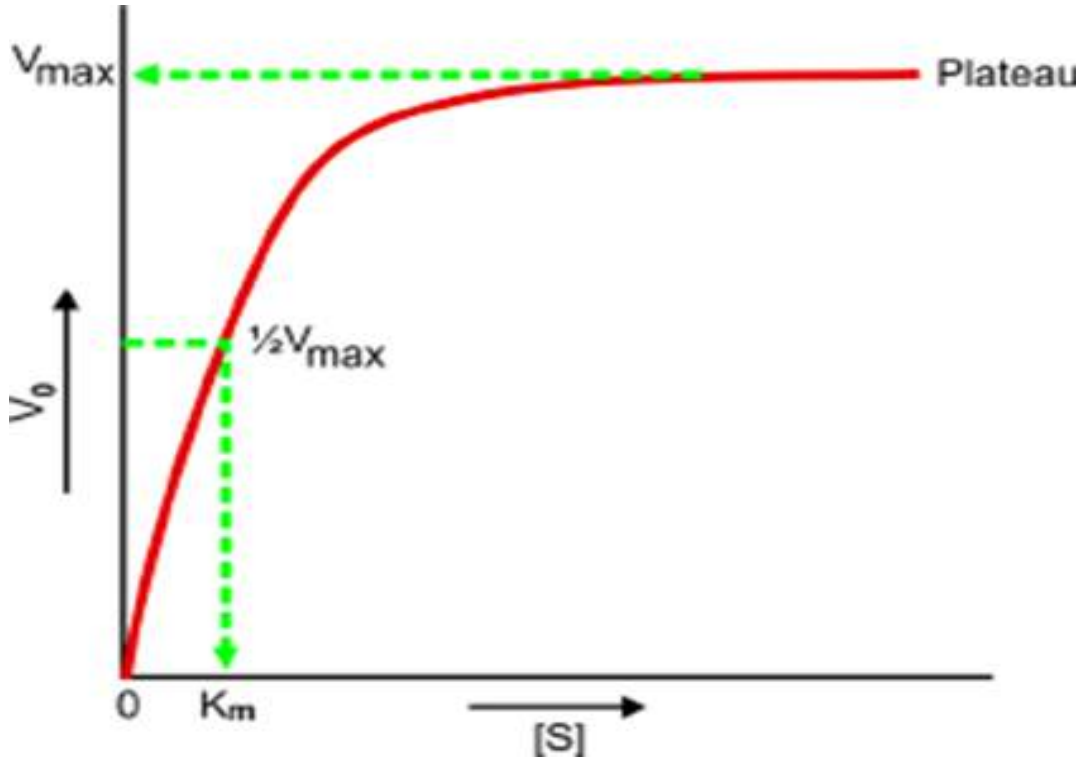
Effect of Substrate Concentration

V_0 : initial velocity

V_{max} : maximum velocity

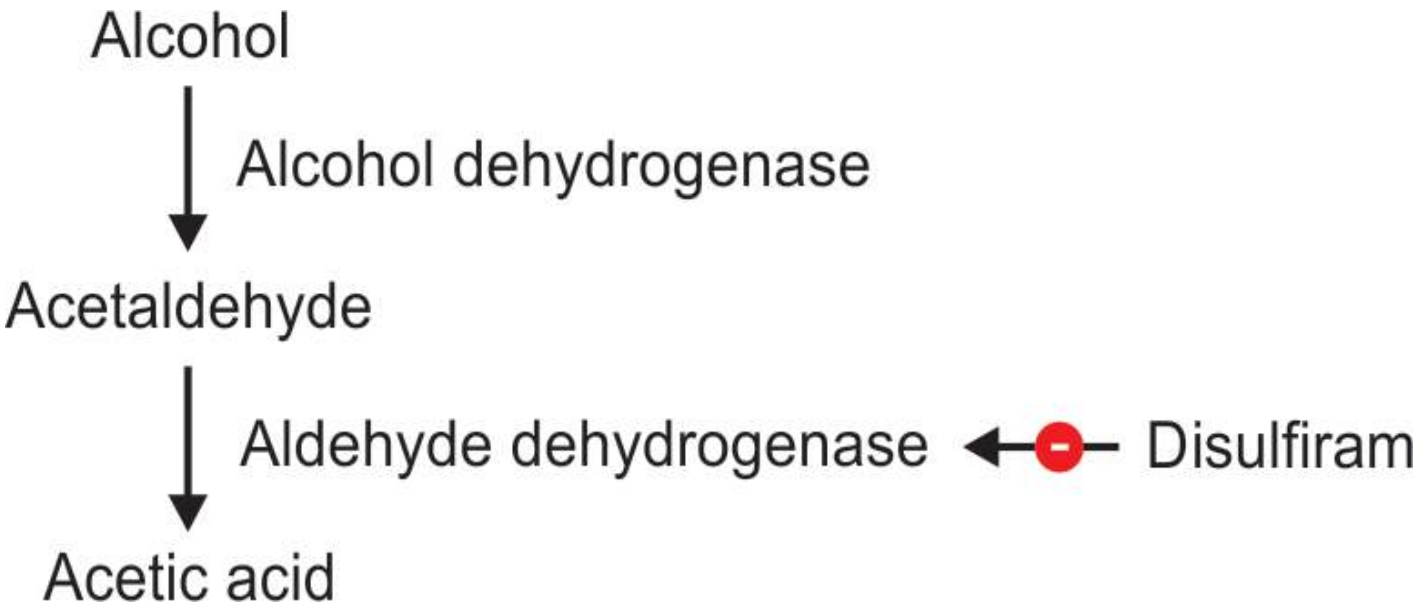
K_m : 1/2 Vmax = Michaelis Menten constant

[S] : substrate concentration



Significance of Km (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.



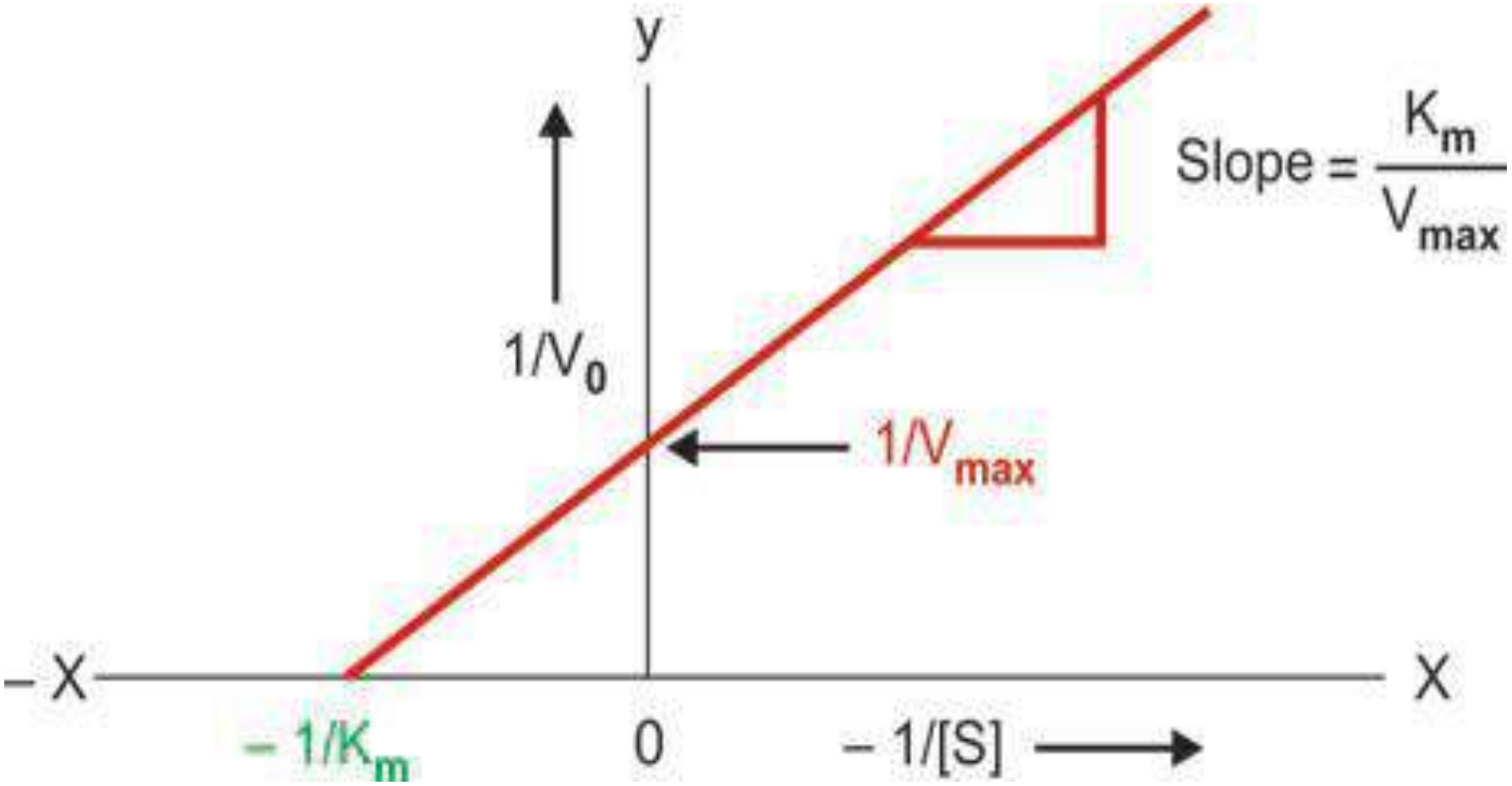
- A low Km mitochondrial form
- A high Km cytosolic form.

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.

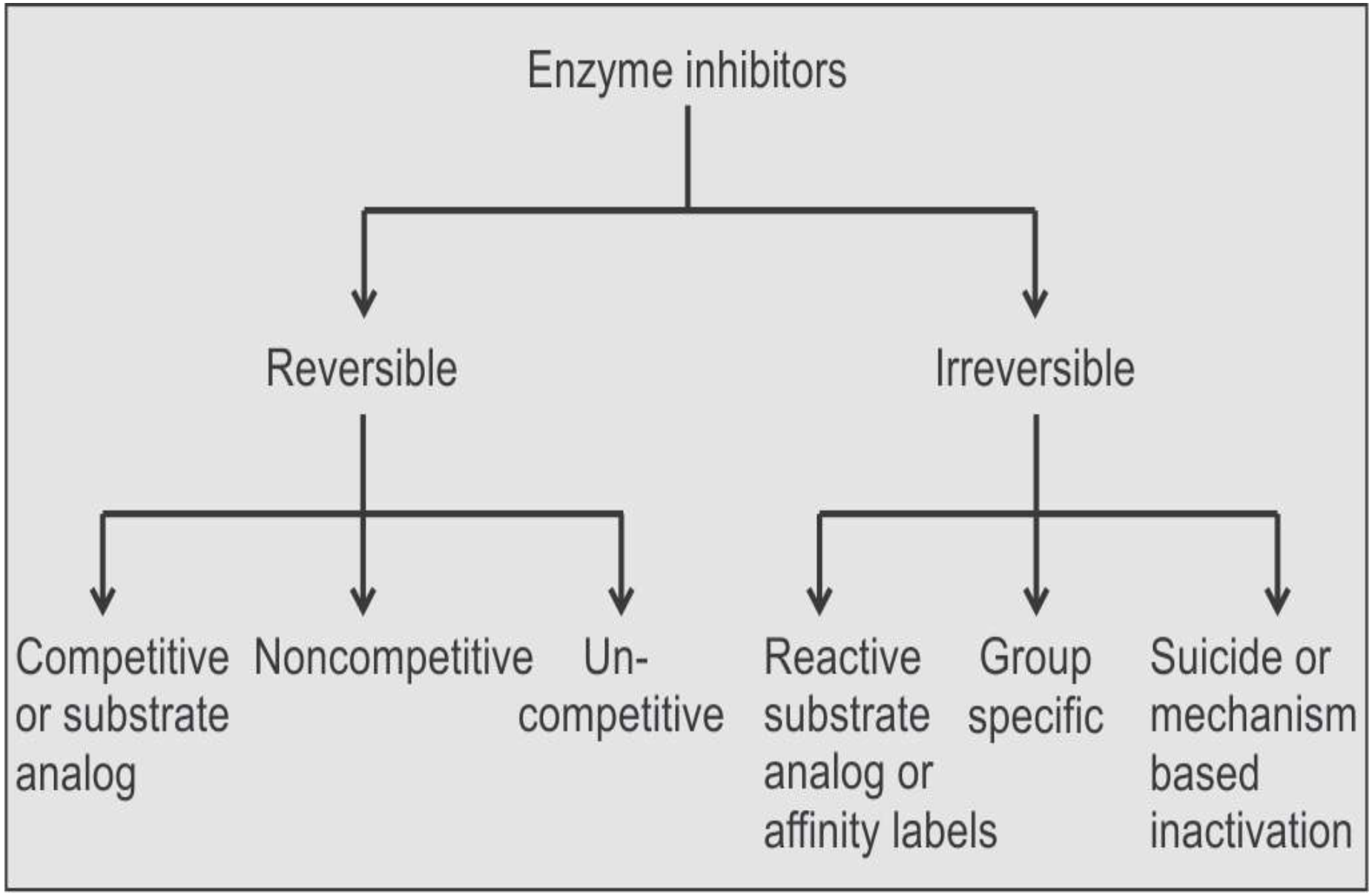
Lineweaver-Burk plot
(Double reciprocal plot)



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
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:

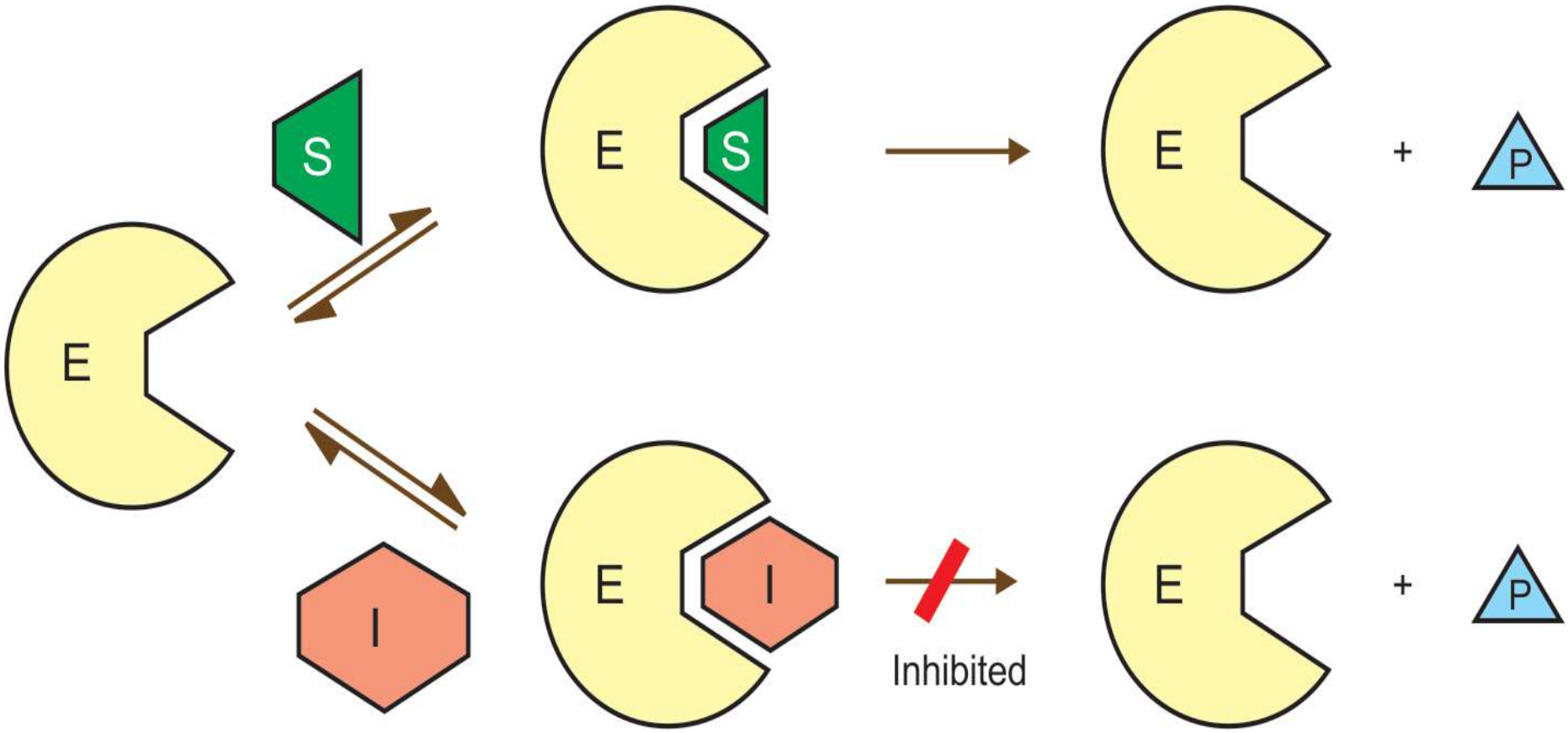
i. Competitive or substrate analogue inhibitor

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.

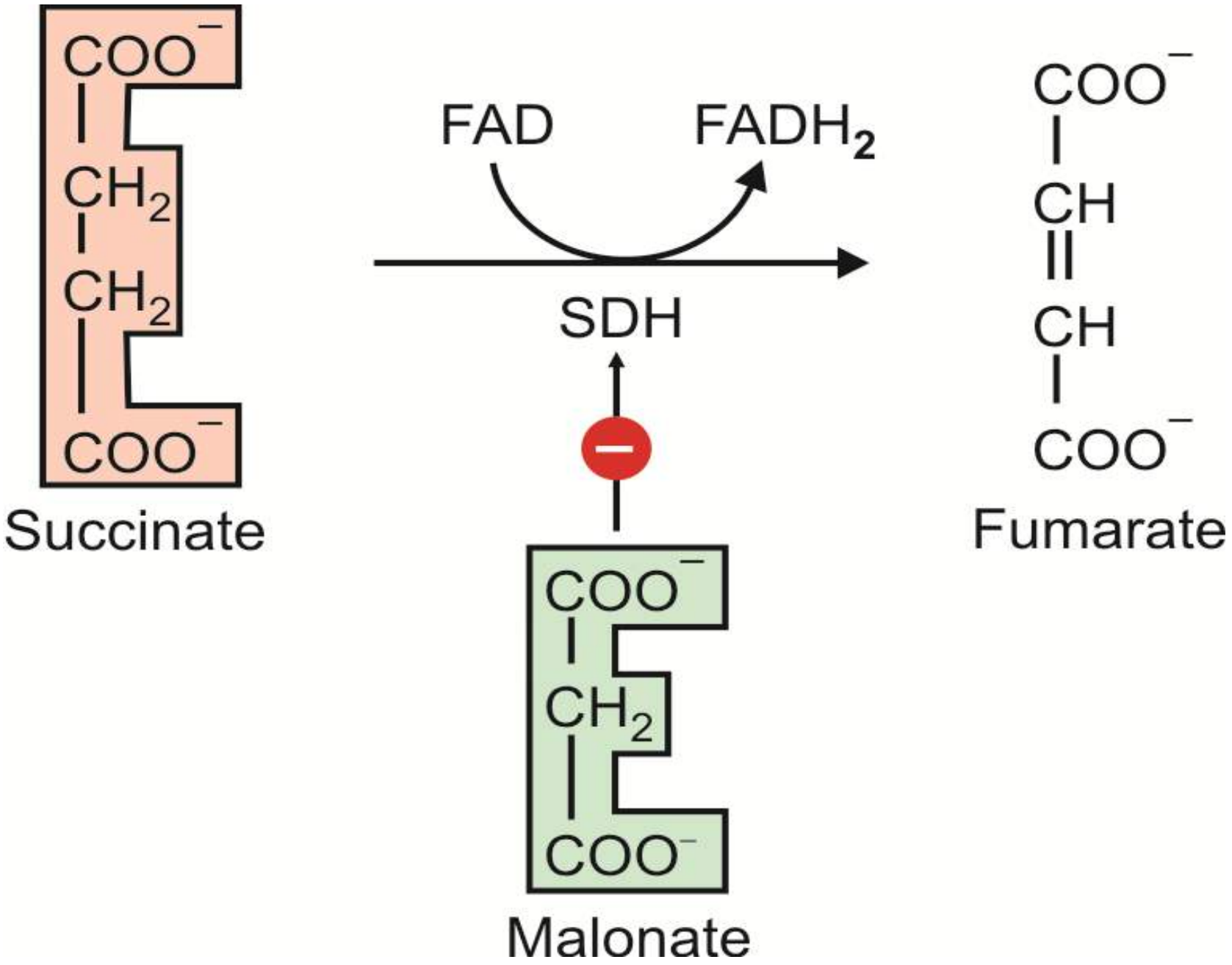


Diagrammatic representation of competitive inhibition

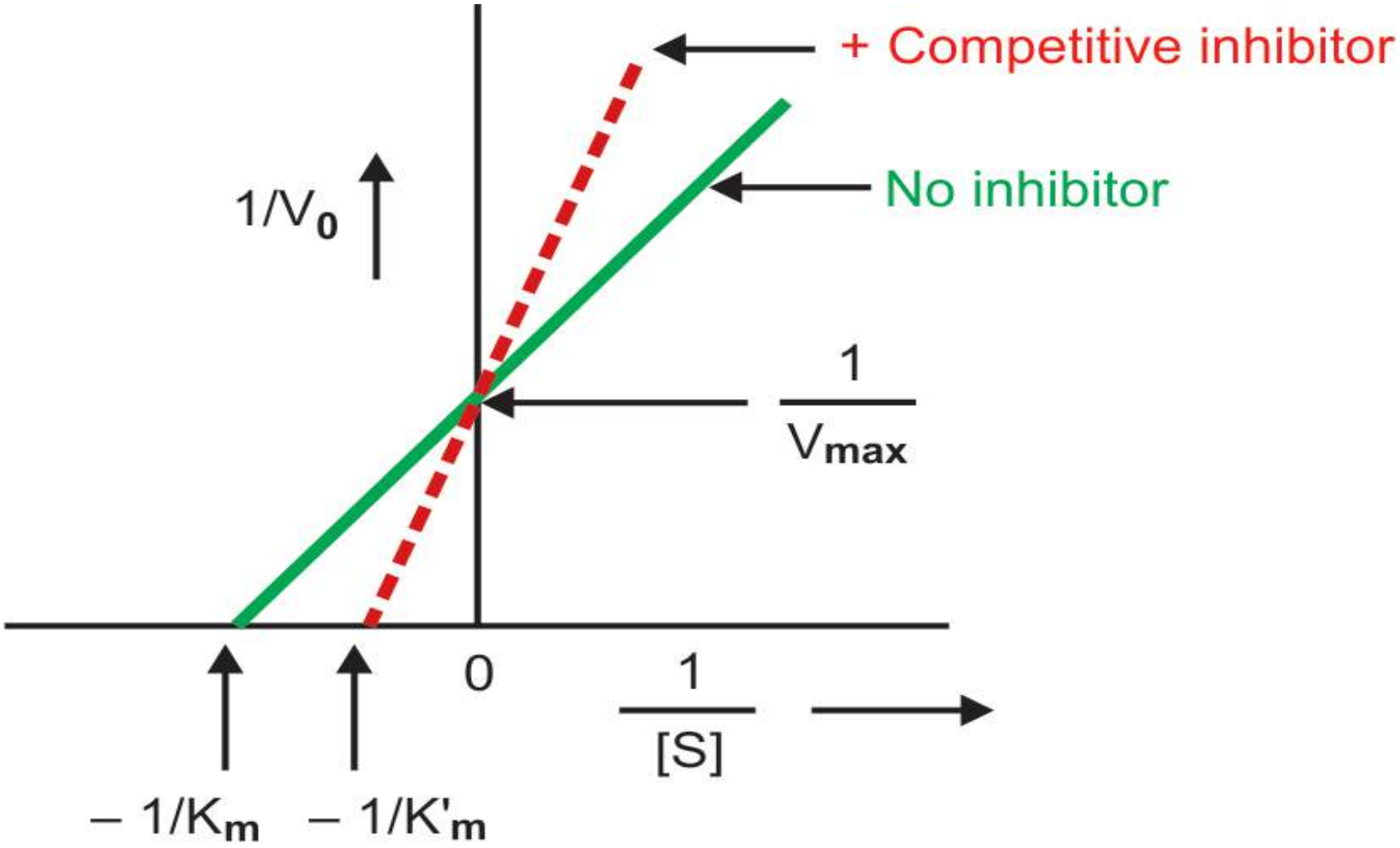
E: Enzyme; S: Substrate; I: Competitive inhibitor; P: Product

The inhibition could be overcome by increasing

substrate concentration


COO⁻
CH2
CH2
COO⁻
Succinate
FAD
FADH2
SDH
COO⁻
CH2
COO⁻
Malonate
COO⁻
CH
CH
COO⁻
Fumarate

Enzyme kinetics of competitive inhibitor



Enzyme kinetics of competitive inhibitor

Vmax is unaltered

Km 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

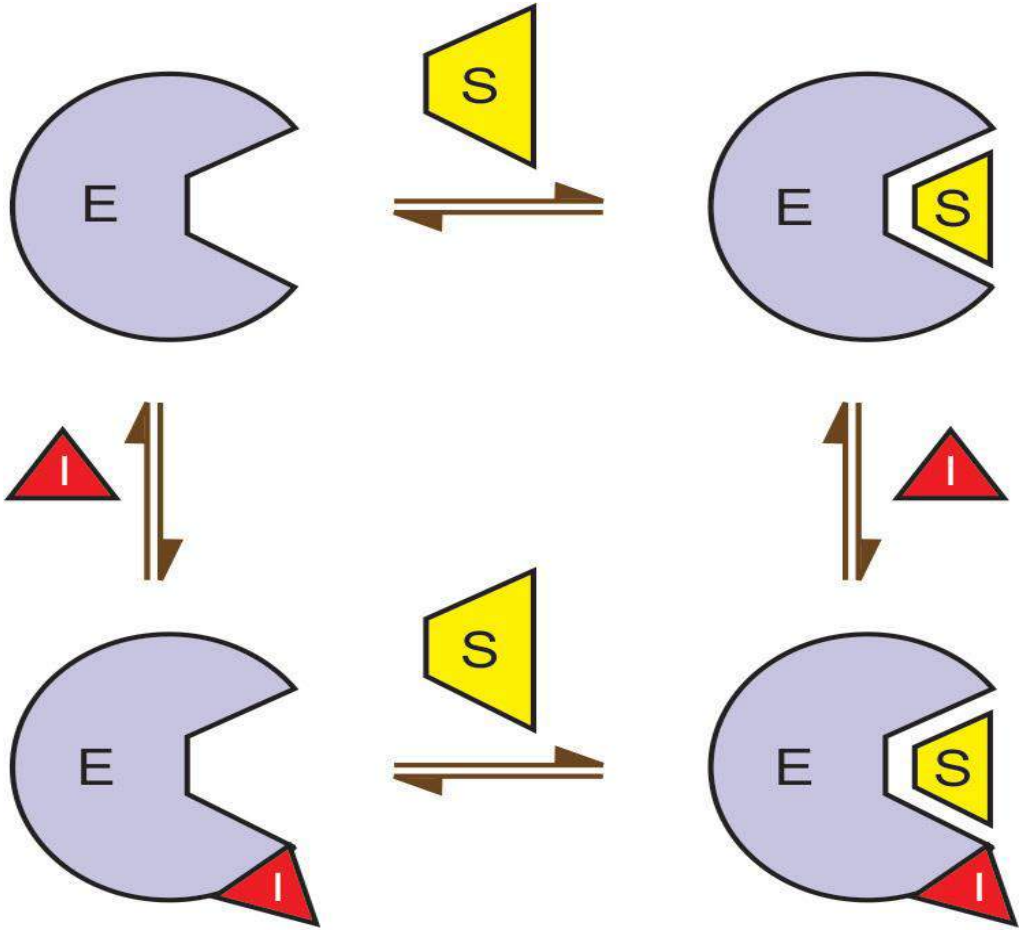
It inhibits acetylcholinesterase and use to treat glaucoma and myasthenia gravis.

Drugs such an **ibuprofen** (anti-inflammatory drug), **statin** (cholesterol lowing 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.

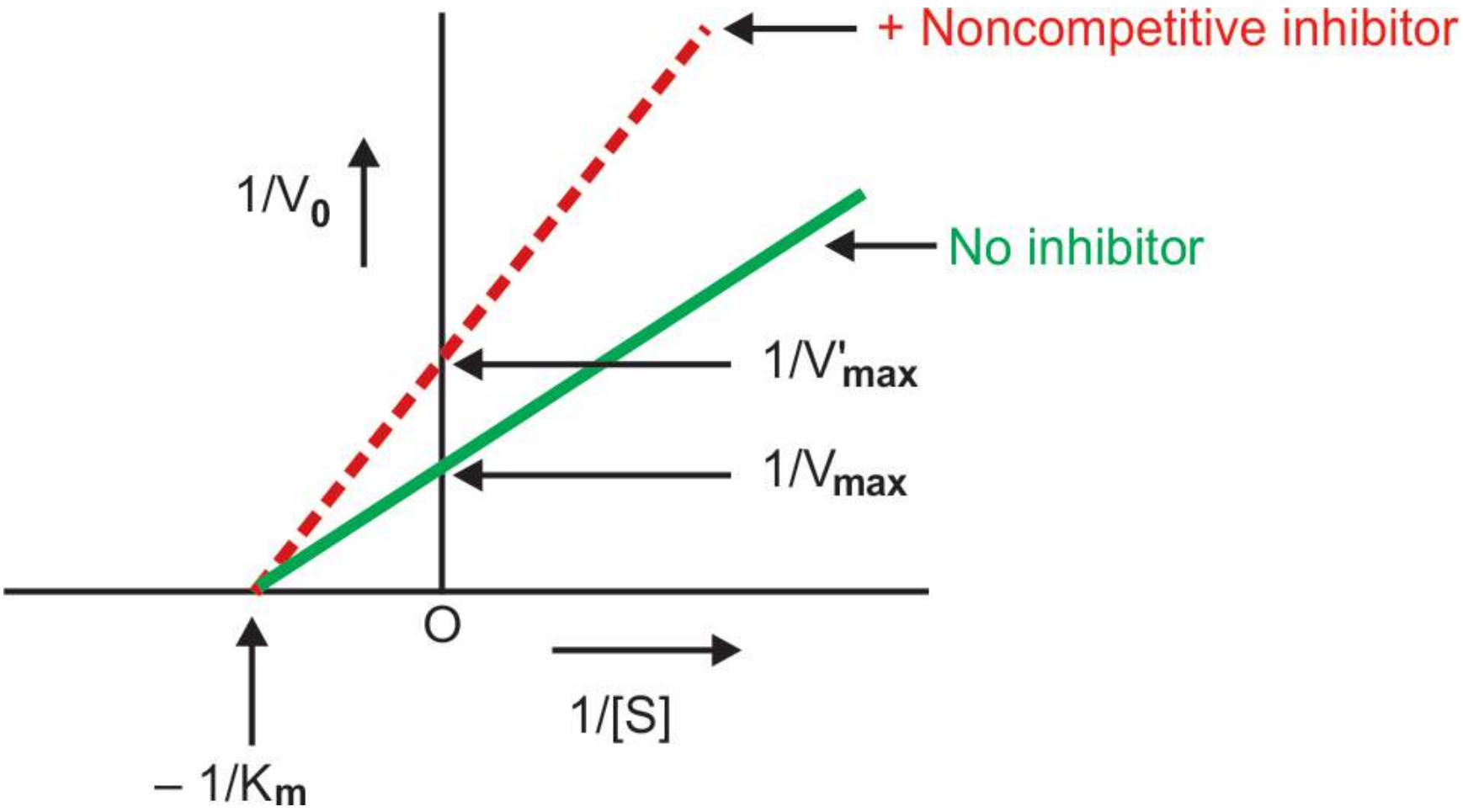
(E = Enzyme; S = Substrate; I = Non-competitive inhibitor; P = Product)

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.

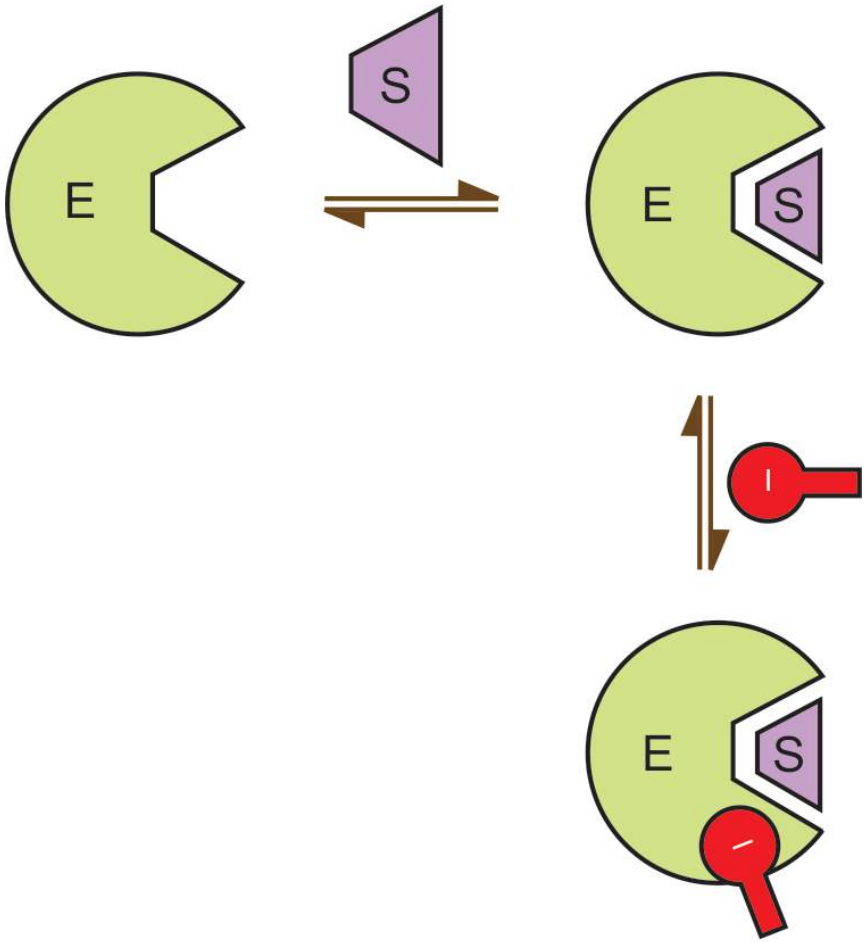


For non-competitive inhibition, the Km value is unchanged while Vmax is lowered

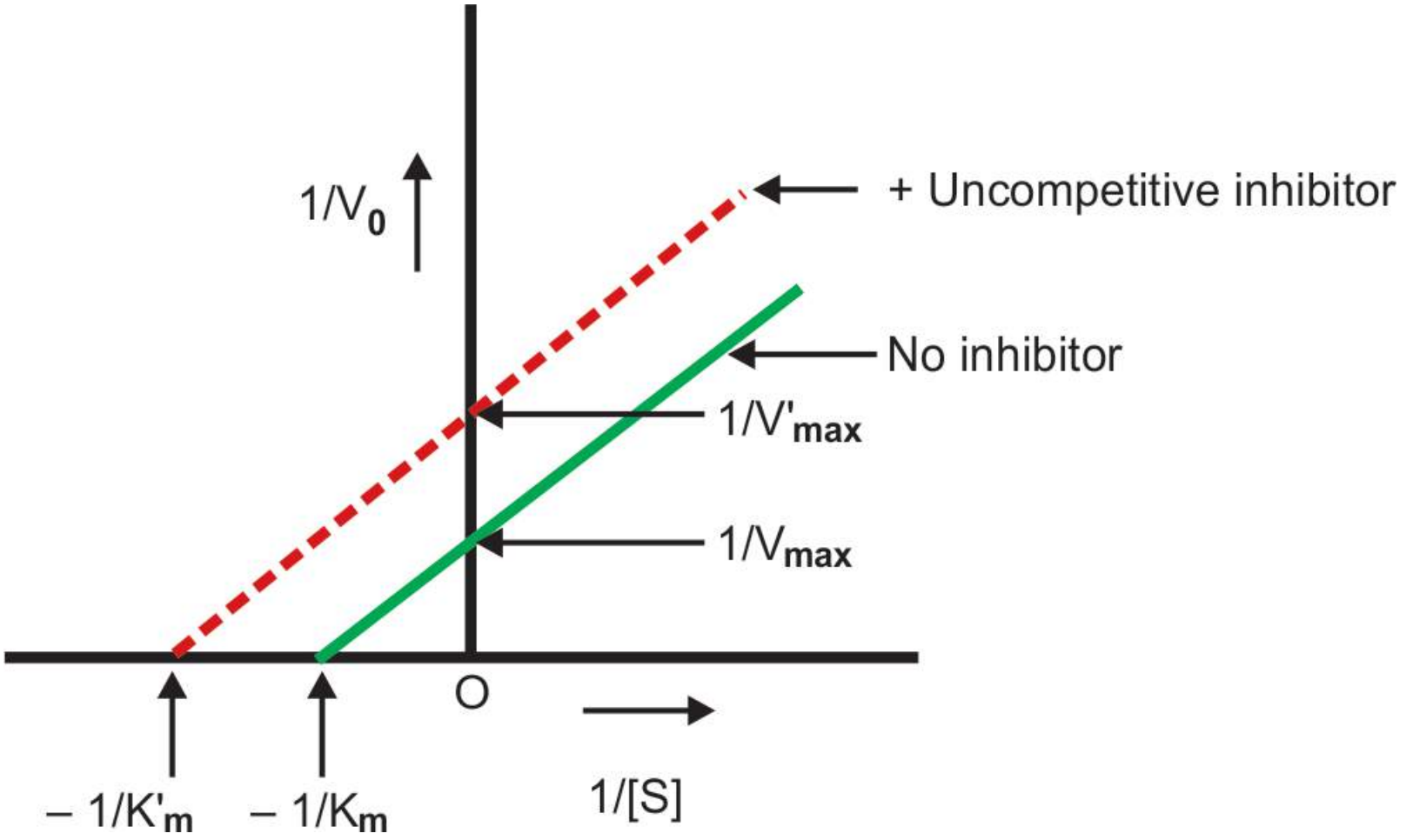
Uncompetitive Inhibitor

- 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 Vmax cannot be attained, even at high substrate concentration.



Uncompetitive inhibitor binds only to enzyme-substrate complex.



Uncompetitive inhibitor decreases both Vmax and Km.

- 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.

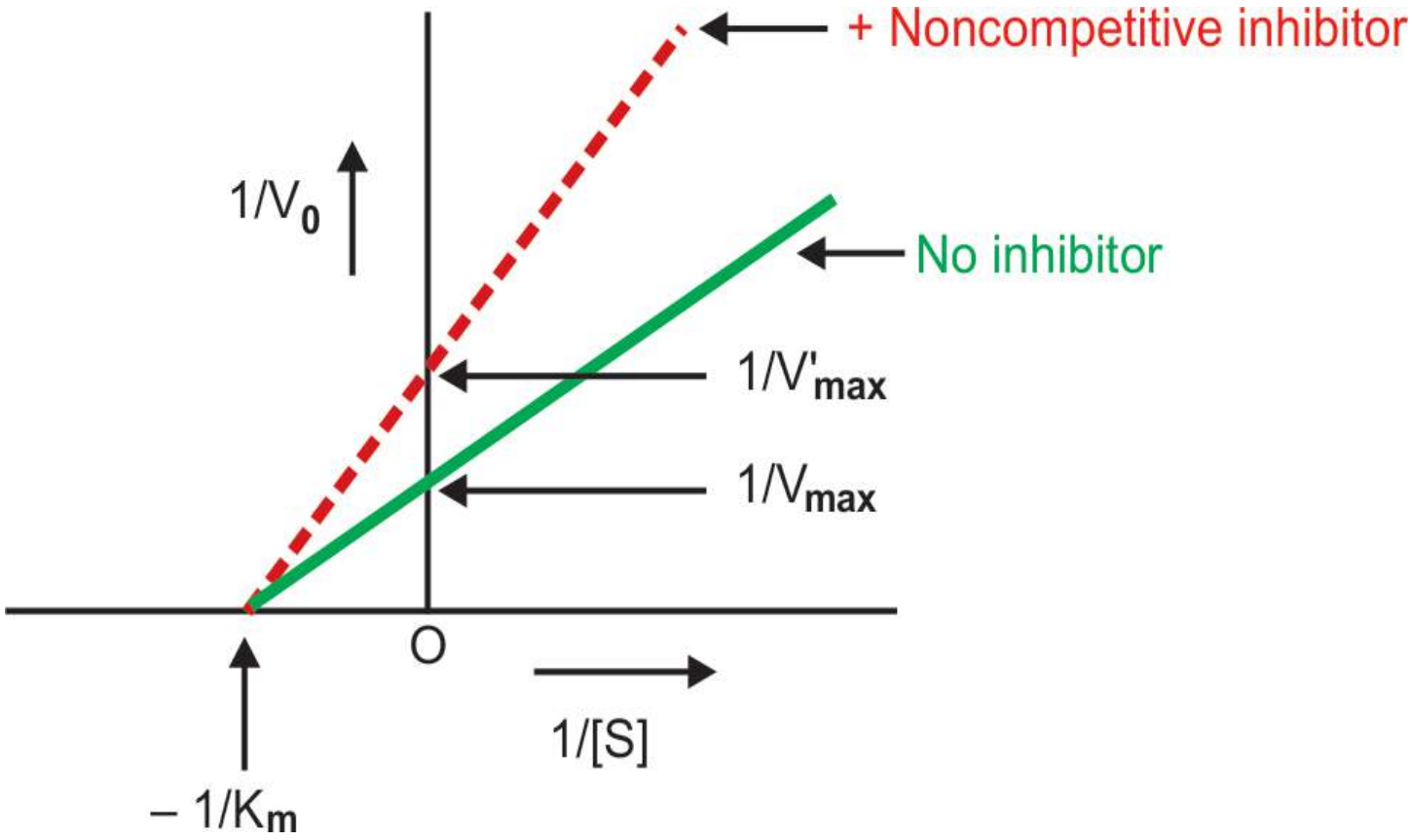
IRREVERSIBLE INHIBITOR

- 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 in Vmax but having no effect on the Km


+ Noncompetitive inhibitor
No inhibitor
1/V0
1/V'max
1/Vmax
O
1/[S]
– 1/Km

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

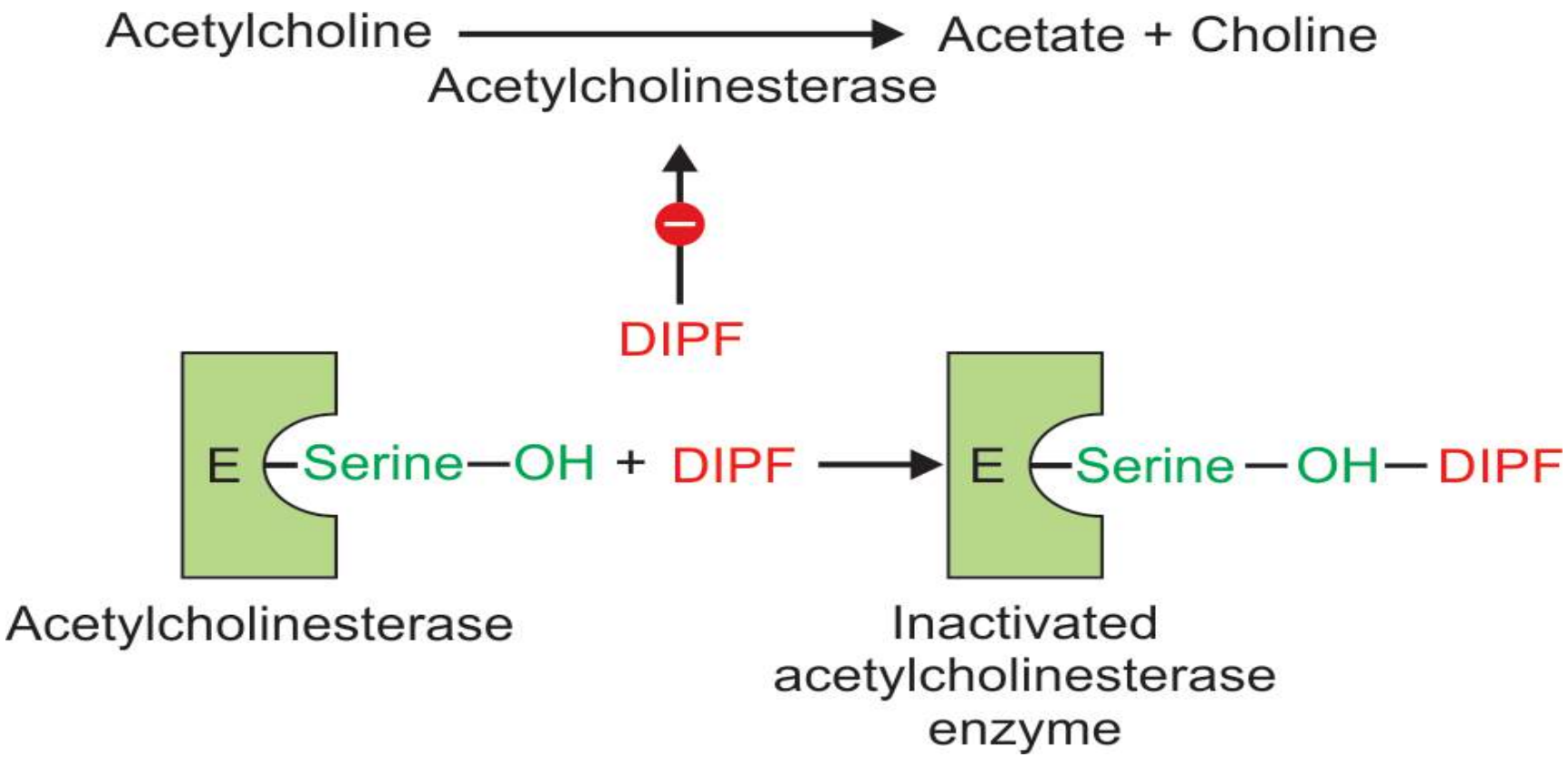


Figure 6.20: Irreversible inhibition of acetylcholinesterase by a group-specific inhibitor, diisopropylphosphofluoride (DIPF).

- Iodoacetamide and heavy metals like, Pb^{2+}, Ag^{+}, 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

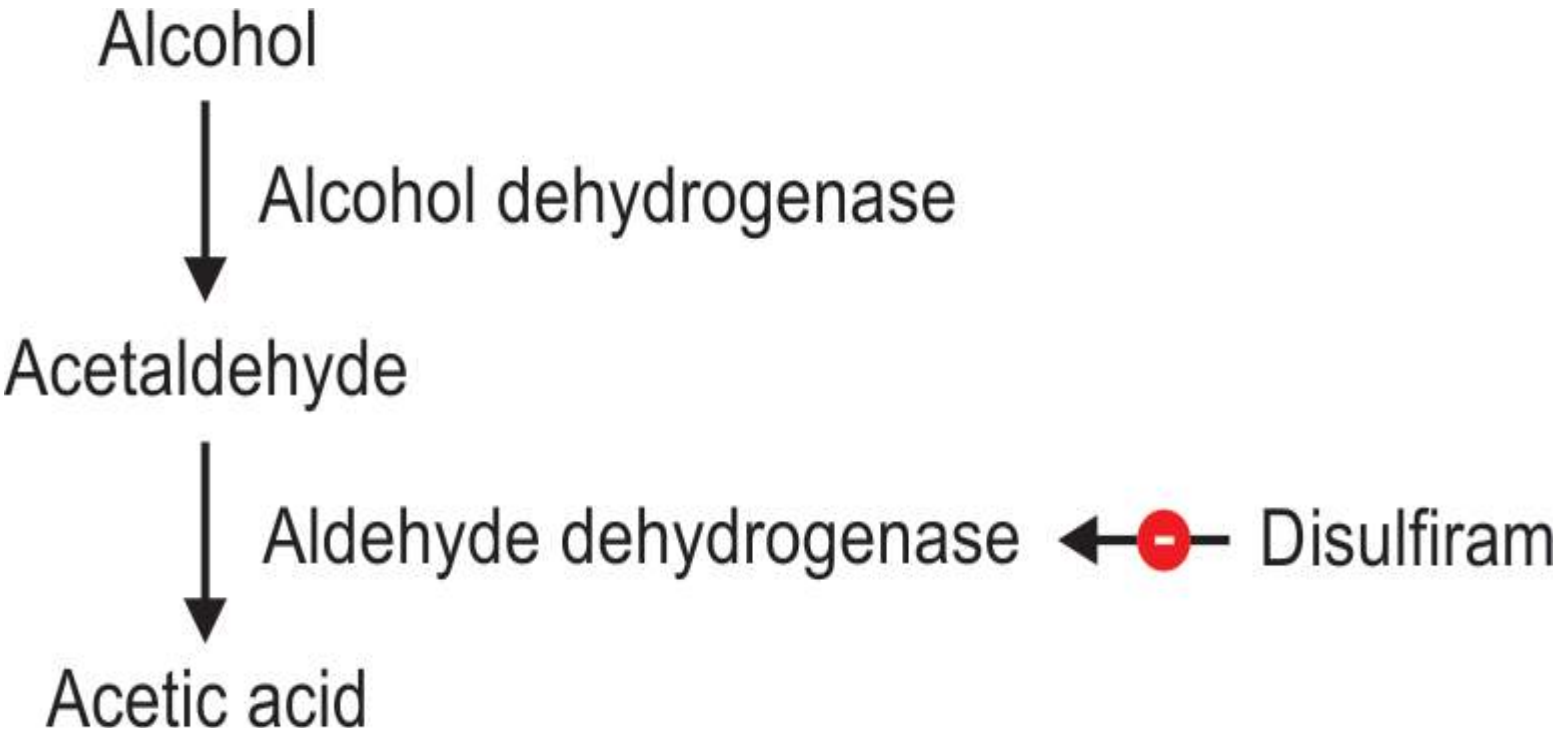
Inactivates bacterial enzyme glycopeptidyl transpeptidase involved in the formation of bacterial cell wall.

- Aspirin

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

➢ Disulfiram (antabuse)

- Inhibits aldehyde dehydrogenase 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.


Alcohol
Alcohol dehydrogenase
Acetaldehyde
Aldehyde dehydrogenase
Disulfiram
Acetic acid

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.

Drugs	*Types of inhibition*	*Target enzymes*	*Therapeutic uses*
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 β-lactamases	Antibacterial

ALLOSTERIC ENZYME

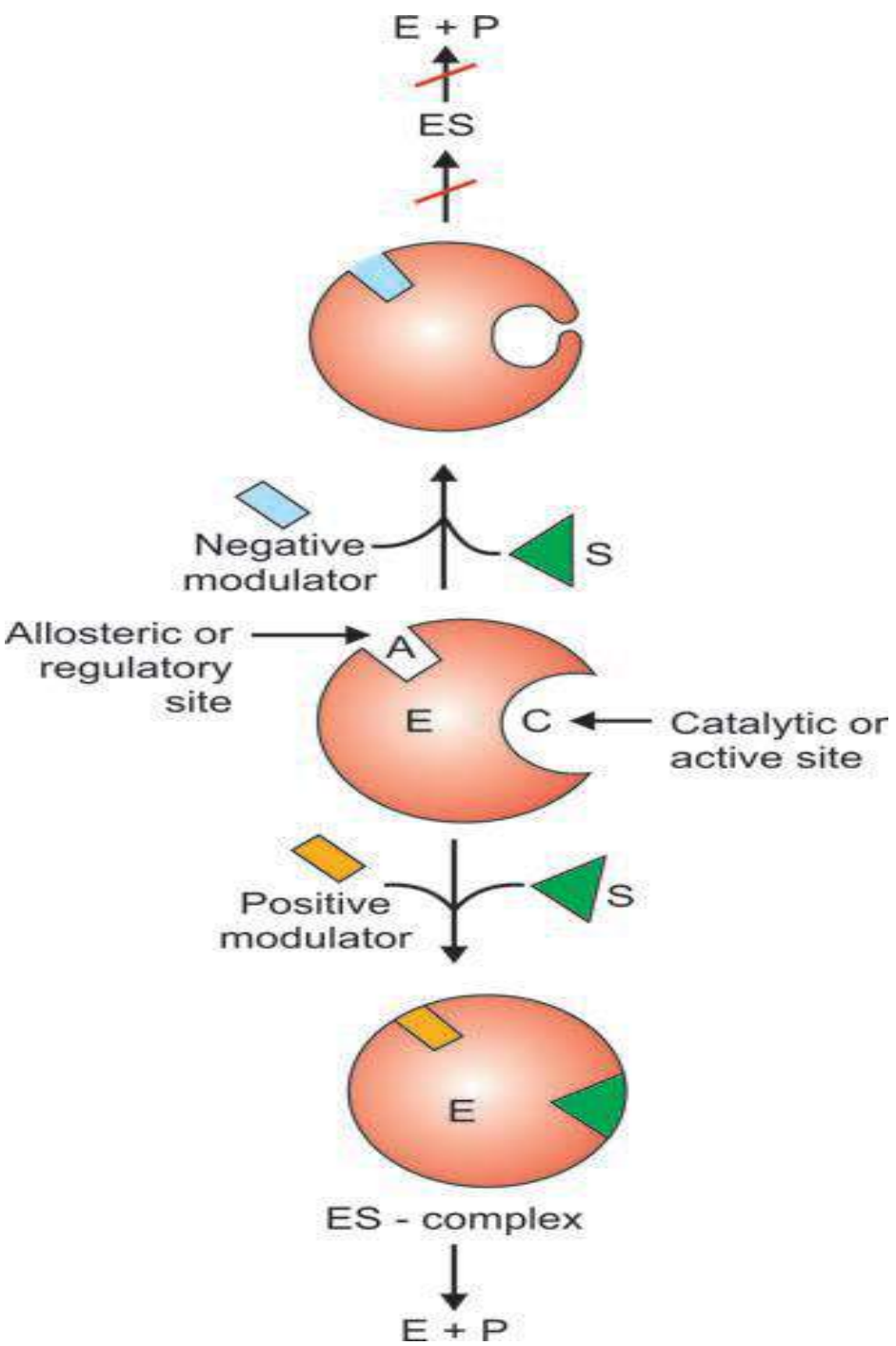
- Allosteric enzyme is a regulatory enzyme.
- The term allosteric derives from Greek word,

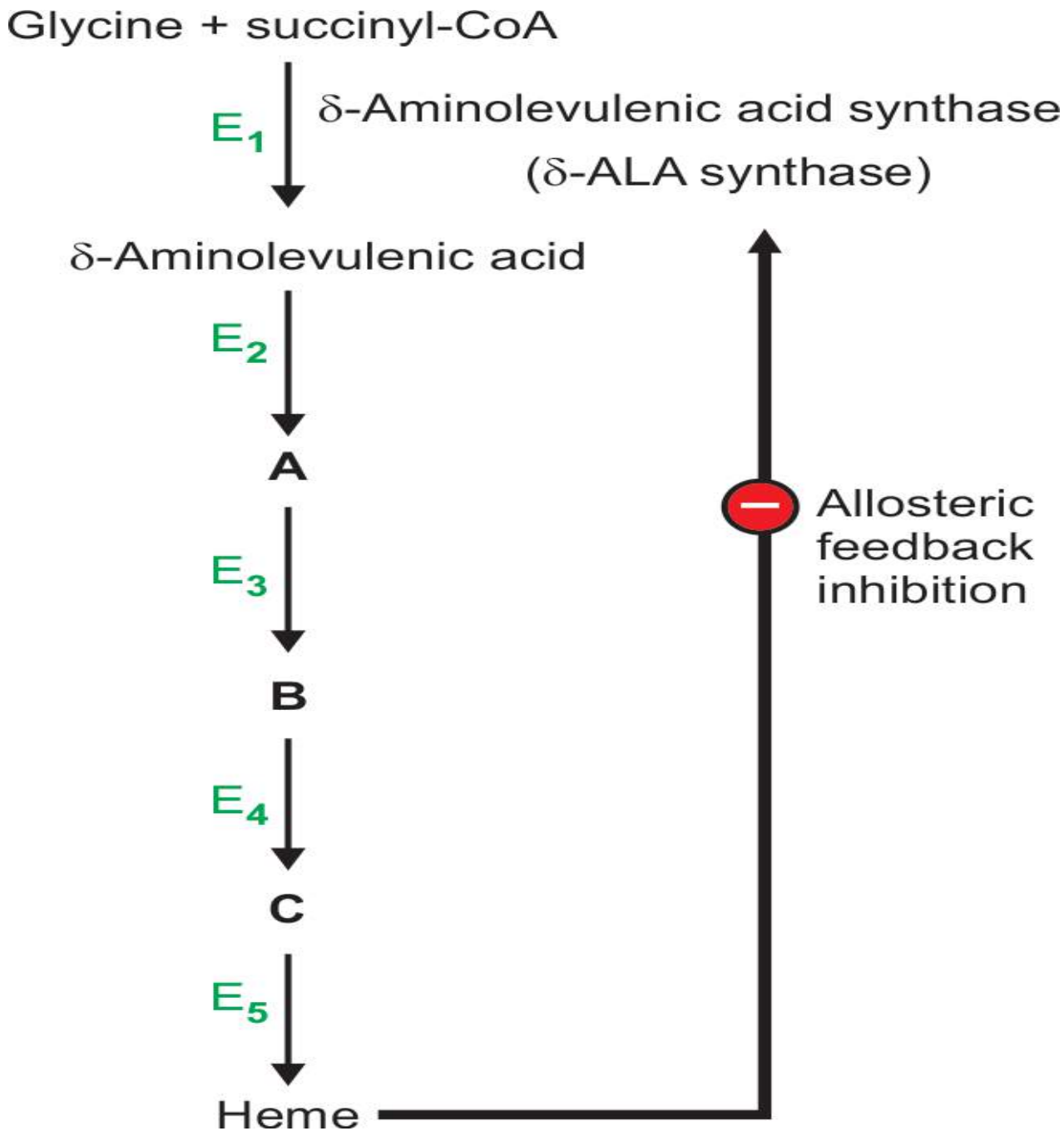
  allo means other and steros means space or site.

- 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*.


E + P
ES
Negative
modulator
S
Allosteric or
regulatory
site
A
E
C
Catalytic or
active site
Positive
modulator
S
E
ES - complex
E + P


Glycine + succinyl-CoA
E1
δ-Aminolevulenic acid synthase
(δ-ALA synthase)
δ-Aminolevulenic acid
E2
A
E3
B
E4
C
E5
Heme
Allosteric
feedback
inhibition

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)

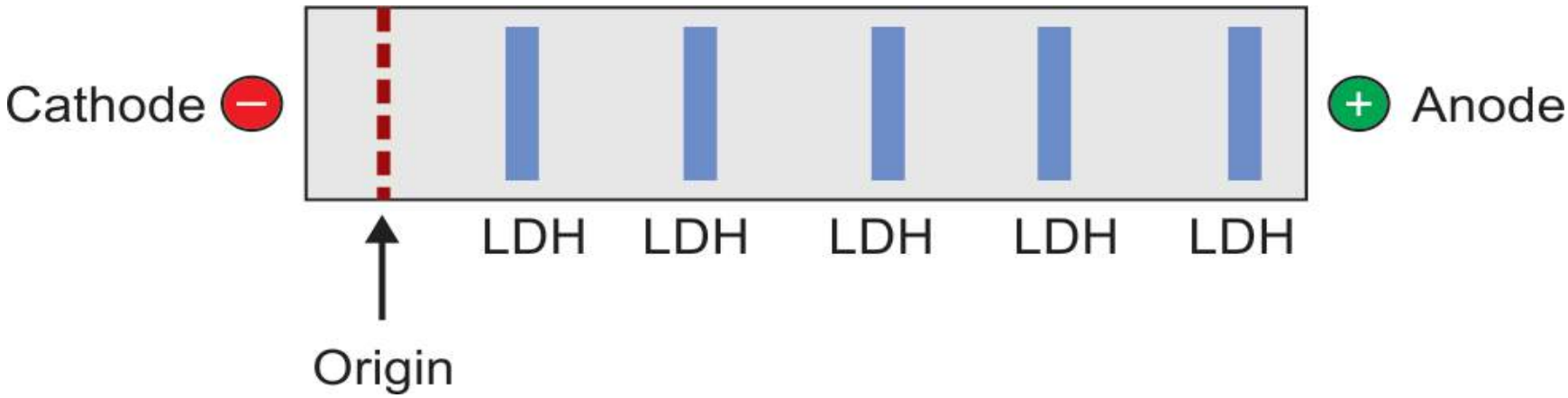
2. Creatine kinase (CK)

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_1
  - LDH_2
  - LDH_3
  - LDH_4
  - LDH_5.

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



- LDH_1 is the fastest moving fraction towards the anode and LDH_5 is the slowest moving isoenzyme of LDH.

- LDH_1 predominates in cells of cardiac muscle, and erythrocytes and 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.

TABLE 6.13: Type, composition, location, and diagnostic importance of lactate dehydrogenase (LDH) and creatine kinase (CK) isoenzymes.

Type	*Composition*	*Location*	*Diagnostic importance (cause of elevated level)*
LDH_1	HHHH	Heart, RBC	Myocardial infarction
LDH_2	HHHM	Heart, RBC	Kidney diseases, megaloblastic anemia
LDH_3	HHMM	Brain, kidneys	Leukemia, malignancy
LDH_4	HMMM	Lung, spleen	Pulmonary infarction
LDH_5	MMMM	Liver, muscle	Liver diseases, muscle damage/diseases
CK_1	BB	Brain, prostate gland, GI tract, lung, bladder, and uterus	Neurological injury, tumor marker
CK_2	BM	Heart	Myocardial infarction
CK_3	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.

TABLE 6.14: Enzymes of diagnostic importance.

Enzymes	*Locations*	*Clinical applications*
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
γ-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.

TABLE 6.15: Enzymes as tumor markers and their associated types of cancer.

Enzymes	*Types of cancer*
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
γ-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.

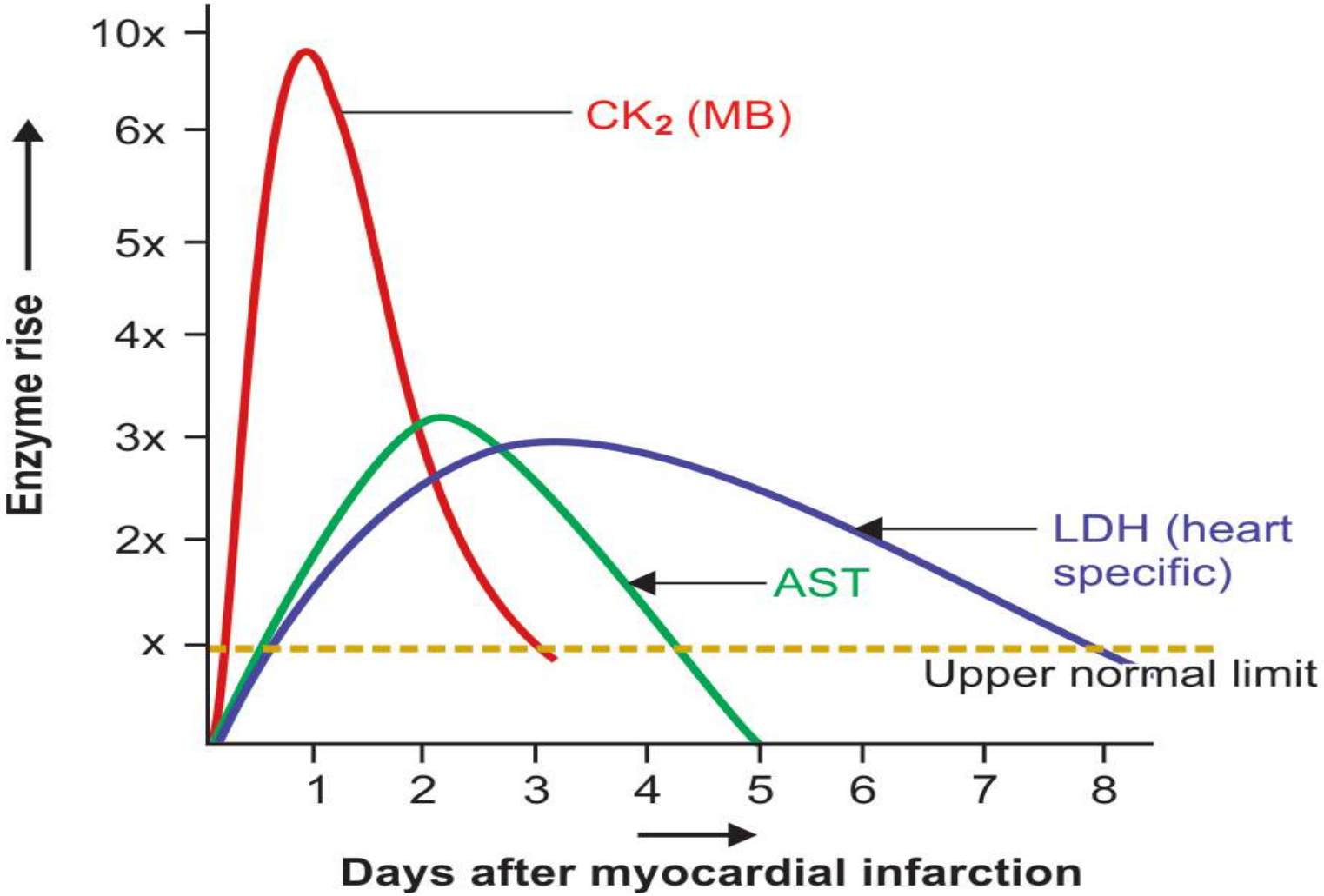
Nonenzyme proteins includes :

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

TABLE 6.16: Cardiac markers with time course after onset of acute myocardial infarction.

Markers	*Abnormal activity detectable (hours)*	*Time for maximum rise (hours)*	*Time for return to normal (days)*
CK_2 (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

Figure 6.28: 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
   - γ-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

TABLE 6.17: List of enzymes used in the clinical laboratory as analytical reagents for investigations.

Enzymes as reagents	*Investigations*
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

TABLE 6.18: Some important therapeutic enzymes.

Enzymes	*Uses*
Asparaginase	Leukemia
Chymotrypsin	Inflammation and edema
Collagenase	Skin ulcers
Fibrinolysin	Blood clot
Glutaminase	Leukemia
Hyaluronidase	Heart attack
Lysozyme	Antibiotic
Rhodanase	Cyanide poisoning
Ribonuclease	Antiviral
β-lactamase	Penicillin allergy
Streptokinase	Blood clots
Trypsin	To dissolve the blood clot
Uricase	Gout
Urokinase	Blood clots