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FIFTH EDITION



HANDBOOK OF
APPLIED BIOCHEMISTRY NUTRITION & DIETETICS
(for Nursing and Allied Health Students)

As per the Revised INC Syllabus for BSc Nursing, 2021

Shivananda Nayak B

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Chapter 2

Enzymes

LEARNING OBJECTIVES

At the end of this chapter students should be able to:

- Understand the classification and function of enzymes
- Know about the active site and mechanism of enzyme action
- Explain the factors affecting enzyme activity
- Describe the different types of inhibition and use of competitive inhibition technique in the drug therapy
- Know about the diagnostic importance of enzymes

Introduction

- Enzymes are biological catalysts produced by the living cells and they catalyze several reactions in the body.
- They are proteins in nature.
- They are specific in action, i.e., each enzyme can catalyze only one type of reaction.
- They are required in very small quantities.
- The loss of catalytic activity was observed when they are subjected to heat or strong acids or bases or organic solvents.
- The enzymes mainly catalyze the metabolic pathways in the human body.
- The deficiency of the enzyme leads to inborn errors of metabolism.
- Most of the enzymes are produced by the cells of a particular tissue and function within that cell. Such enzymes are called *intracellular enzymes*.
- *Example*: Enzymes of glycolysis, tricarboxylic acid (TCA) cycle, and fatty acid synthesis.
- There are certain enzymes, which are produced by the cells of a particular tissue from where these are liberated for use in the other tissues. Such enzymes are called *extracellular enzymes*.

Example: Various proteolytic enzymes of gastrointestinal tract (trypsin and chymotrypsin).

The enzyme binds with its specific substrate and forms an enzyme–substrate complex. At the end of the reaction, the substrate is converted into the product and the enzyme remains unchanged.

$$E + S - ES \rightarrow E + \text{Product}$$

Chemical Nature of Enzymes

The chemical nature of enzymes is as follows:

- Enzymes with two or more subunits (polypeptides) are called oligomeric enzymes.
- Several enzymes occur in the form of multienzyme complex. In this case, several enzymes occur in a single complex form, e.g., pyruvate dehydrogenase and fatty acid synthase complex.
- Some enzymes require the presence of certain additional organic or inorganic substances and are *conjugated proteins*. Such enzymes are called **holoenzymes**. The protein part is called *apoenzymes* and the nonprotein part is *prosthetic group*.
- **Holoenzyme:** Apoenzyme+prosthetic group (coenzyme).
- Several apoenzymes require the presence of metal ions such as Mg^{2+} (for hexokinase) and Zn^{2+} (for the activity of carboxypeptidase). Such inorganic ions are called **cofactors**. If the metal ion is the integral part of the enzyme, such enzymes are called **metalloenzymes**.

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Ribozymes

These are RNA molecules that can act as catalysts.

These ribozymes break RNA phosphodiester bonds at certain specific location in the RNA molecules, serving as ribonucleases and as peptidyl transferase (catalyzes the formation of peptide body).

These ribozymes are being considered as possible therapeutic agents for disorders caused by the inappropriate expression of RNA or the expression of a mutated RNA. However, further research is required for this to consider.

What are proenzymes?

Zymogens or Proenzymes

The protein-digesting enzymes (proteolytic enzymes) of gastrointestinal tract are produced in the form of precursor to prevent unwanted degradation of body self-protein. These inactive precursor forms of enzymes (zymogen) are converted into active form by HCl and trypsin.

For example:

- Pepsinogen → pepsin (HCl activates the pepsinogen).
- Trypsinogen → trypsin (trypsin and enteropeptidase activate the enzymes).
- Procarboxypeptidase to carboxypeptidase.
- Chymotrypsinogen to chymotrypsin.

What are coenzymes? Explain with examples.

Coenzymes

Coenzymes are dialyzable, thermostable, low-molecular-weight organic substances (also considered as cosubstrate or second substrate) **(Table 2.1)**.

They are small organic molecules that transport chemical groups from one enzyme to another.

They are usually regenerated and their concentrations maintained at a steady level inside the cell, e.g., NADPH is regenerated through the pentose phosphate pathway and *S*-adenosylmethionine by methionine adenosyltransferase.

Energy of Activation of Catalyzed and Uncatalyzed Reactions

All chemical reactions have an energy barrier, separating the reactants and the products. This barrier, called the free energy of activation, is the energy difference between the energy of the reactant and high-energy intermediates

Table 2.1: Common coenzymes and their functions.

Vitamin	*Coenzyme*	*Function*
Thiamine (B_1)	TPP	Oxidative decarboxylation and transketolase reaction
Riboflavin (B_2)	FAD	Oxidative and reductive reaction
	FMN	
Niacin	NAD	Oxidative and reductive reaction
	NADP	
Pyridoxine (B_6)	PLP	Transamination, deamination, and decarboxylation reactions
Biotin	Biocytin	Carboxylation reactions
Folic acid	THF	Carrier of one carbon
Pantothenic acid	Coenzyme A	Acyl carrier
Cyanocobalamin (B_{12})	Methylcobalamin and deoxyadenosylcobalamin	Transfer of CH_3 group and isomerizations

(FAD: flavin adenine dinucleotide; FMN: flavin mononucleotide; NAD: nicotinamide adenine dinucleotide; NADP: nicotinamide adenine dinucleotide phosphate; PLP: pyridoxal phosphate; THF: tetrahydrofolate; TPP: thiamine pyrophosphate).

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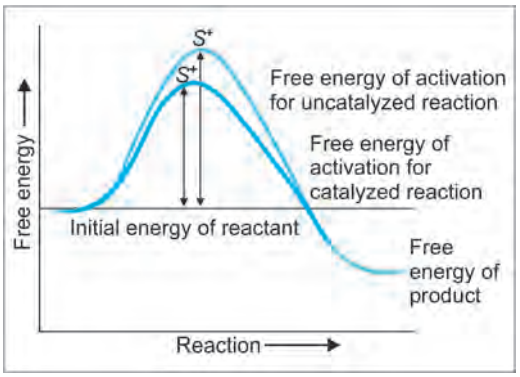

Fig. 2.1: Free energy of activation of a catalyzed and uncatalyzed reaction.

that occurs during the formation of a product **(Fig. 2.1)**.

$$S \longleftrightarrow S^* \longleftrightarrow P$$

[Reactant] [Product]

The peak of free energy activation, represents the transition state, in which the high-energy intermediates (S^*) are formed during the conversion of a reactant to a product.

Due to the effect of activation energy, the rates of unanalyzed chemical reactions are slow.

An enzyme lowers the energy required for activation to the transition state.

With an enzyme as a catalyst, the reaction may easily proceed at the normal physiological temperature, otherwise addition of heat energy is required for the reaction to occur.

Active Site of an Enzyme

- The active site of an enzyme is the region where substrate binds.
- This active site contains the specific amino acid residues (binding and catalytic residues) and possesses three-dimensional structure.
- The amino acid residues at the active site of an enzyme have two functions:
  1. The binding amino acid residues recognize and bind the correct substrate to form enzyme–substrate (ES) complex.
  2. The catalytic residues create a chemical environment that enhances the rate of reaction and ES complex is converted to an enzyme (E) and a product (P).
- A change in the primary, secondary, tertiary, or quaternary structure may alter the three-dimensional shape of the active site to reduce its binding and catalytic activity.

List and explain the factors that affect the enzyme activity.

1. pH
2. Temperature
3. Concentration of substrate
4. Concentration of enzyme.

Effect of PH

Each enzyme has an optimum pH at which the activity of the enzyme is maximum **(Fig. 2.2)**. Either decreased or increased pH causes a decrease in enzyme activity.

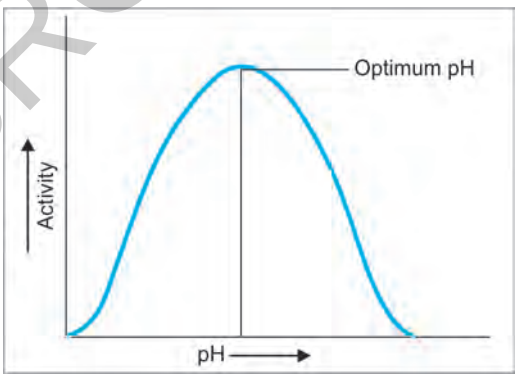

Fig. 2.2: Effect of pH on enzyme activity.

For example:

1. Pepsin has an optimum pH at 1.2 (its activity is maximum at this pH).
2. Optimum pH for amylase is 6.8.
3. Optimum pH for alkaline phosphatase (ALP) is 9.0.
4. Optimum pH for acid phosphatases (ACP) is 5.0.

Effect of Temperature

The temperature at which the enzyme activity is more is called optimum temperature **(Fig. 2.3)**. Any strong change in the optimum temperature results in the loss of enzyme activity.

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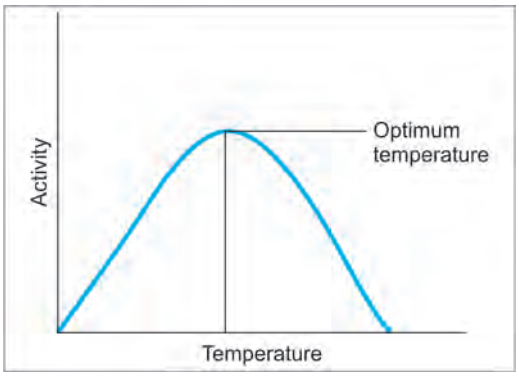

Fig. 2.3: Effect of temperature on enzyme activity.

Optimum temperature of enzymes in the human body is 37°C.

Effect of Substrate Concentration

At low substrate concentration enzyme, molecules are free initially and the ES complex (ES=enzyme–substrate) formation is proportional to the substrate concentration. This means the rate of velocity is directly proportional to the [*S*] and follows first-order kinetics. At higher concentration, all the enzyme molecules are saturated with substrate. There will be no change in the activity further **(Fig. 2.4)** and followed by hyperbolic curve; this is zero-order kinetics.

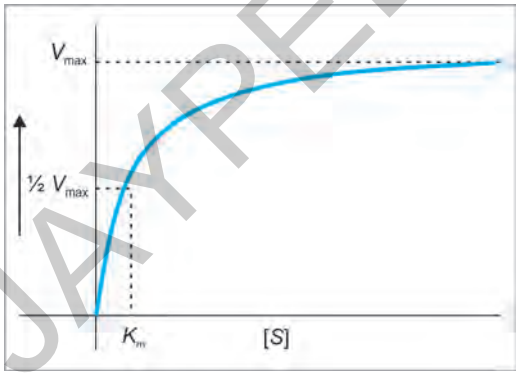

Fig. 2.4: Effect of substrate concentration.

Effect of Enzyme Concentration

The velocity of the enzyme reaction is directly proportional to the enzyme concentration **(Fig. 2.5)**.

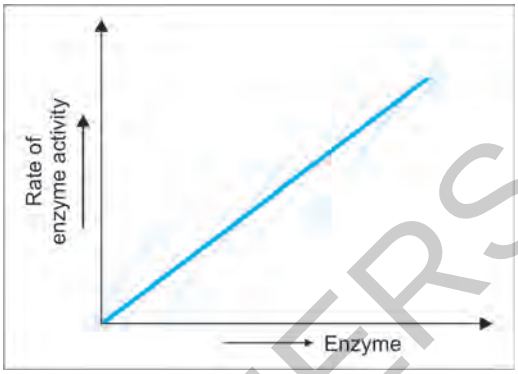

Fig. 2.5: Effect of enzyme concentration.

Classification of Enzymes with Examples

Enzymes are classified into six groups **(Table 2.2)** according to the International Union of Biochemistry.

Enzyme Specificity

The most important property of enzyme is its specificity. They exhibit several types of specificity **(Table 2.3)**.

Briefly explain the mechanism of enzyme actions using different models.

Mechanism of Enzyme Catalysis

In the first step in enzyme catalysis, the substrate binds noncovalently at the active site of the enzyme and forms enzyme–substrate (ES) complex. This complex is subsequently converted to product and free enzyme.

Two models have been proposed to explain the binding mechanism of substrate to the active site of the enzyme.

- Lock and key model
- Induced fit model or hand-in glove model of Koshland.

Lock and Key Model (Proposed by Emil Fischer)

In this model, the enzyme is preshaped and the active site has a rigid structure that is complimentary to substrate.

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Table 2.2: Classification of enzymes.	
Class	*Examples*
Oxidoreductases: Enzymes involved in oxidation-reduction reactions	Lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase
Transferases: Transfer a specific group from one substrate to another	Alanine transaminase and hexokinase
Hydrolases: Hydrolyze the substrate with the addition of water molecule	Glucose-6-phosphatase amylase and pepsin
Lyases: Catalyze the removal of a small molecule from a large substrate without the addition of water	Fumarase and enolase
Isomerases: Isomerize substrates	Racemases and isomerase
Ligases: Synthesize substance by joining two substrates with the utilization of energy	Glutamine synthetase

Table 2.3: Enzyme specificity.
1. *Stereospecificity:* The group of enzyme catalyzes either L- or D-isomer
2. *Reaction specificity:* One enzyme catalyzes only one type of reaction
3. *Substrate specificity:* Pepsin hydrolyzes residues of only aromatic amino acids while trypsin hydrolyzes residues of the basic amino acids only a. *Absolute specificity:* Glucokinase acts on glucose only b. *Group specificity:* Hexokinase catalyzes hexoses
4. *Bond specificity:* Refers to the action of proteolytic enzymes. Peptidase and glycosidase act on peptide bonds of proteins and glycosidic bonds of carbohydrates, respectively. Lipases act on ester bonds of lipids

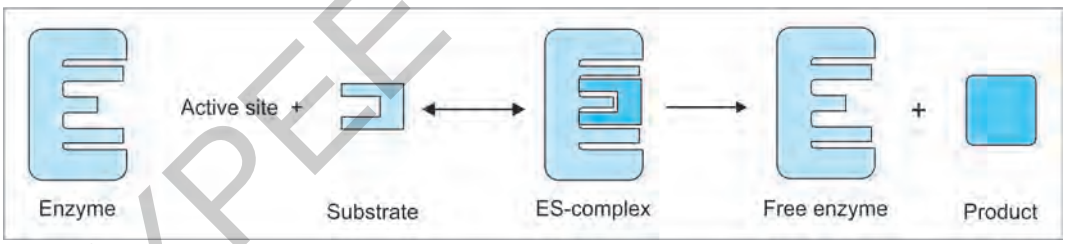

Fig. 2.6: Lock and key model.

This model is called lock and key model, because in this model, the substrate fits into the active site in the same way as a key fits into a lock **(Fig. 2.6)**.

This model gives the idea about the specificity of the enzymes, which binds only a specific substrate not another compound with an almost identical structure.

For example: The enzymes of glycolysis can bind D-isomer than L-isomer (which differs only in the configuration around a single carbon atom).

Induced Fit Model

This model explains the specificity of the enzyme as well as the changes taking place during catalysis.

Koshland explained that the enzymes are flexible and shapes of the active site can be modified by the binding of the substrate.

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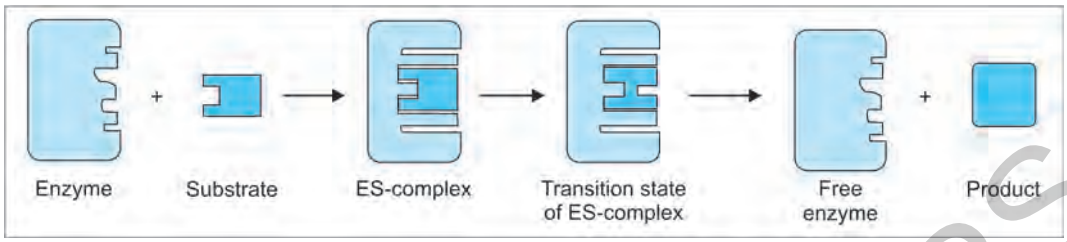

Fig. 2.7: Induced fit model.

In the induced fit model, the substrate induces a conformational change in the enzyme, in the same way in which placing a hand (substrate) into a glove (enzyme) induces changes in the glove's shape. Therefore, this model is also called a hand-in glove model **(Fig. 2.7)**.

This arranges catalytic residues, which participate in catalysis. The enzyme in turn induces reciprocal changes in its bound substrate that changes their orientation and configuration and strains the structure of the bound substrate. Such changes help to bring the ES complex into its transition state.

The intrinsic binding energy due to the substrate–enzyme interaction is made available for the transformation of the substrate into product.

Enzyme Kinetics

Study of the impact made on the rate of an enzyme-catalyzed reaction by changes in experimental conditions is known as enzyme kinetics.

Knowledge of kinetics can be a very useful tool in understanding the mechanism by which an enzyme carries out its catalytic activity.

The effect of substrate concentration on the initial rate of an enzyme-catalyzed reaction is the main concept in enzyme kinetics. The substrate concentration is the important factor affecting rate of a reaction catalyzed by enzyme.

The effect on initial velocity (V_i) of varying substrate concentration [S], when enzyme concentration is held constant is shown below **(Fig. 2.8)**.

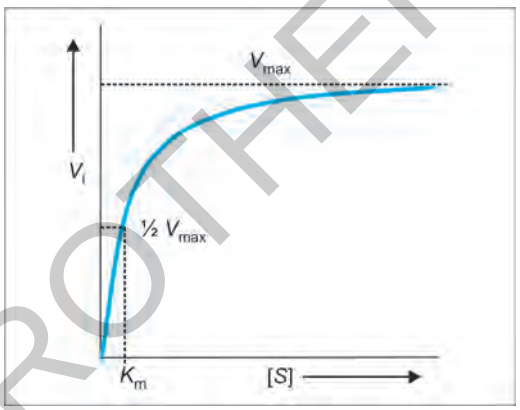

Fig. 2.8: Initial reaction velocity versus substrate concentration for Michaelis–Menten equation.

V_i = initial velocity
[S] = substrate concentration
V_{max} = maximum velocity
K_m = substrate concentration when V_i is one half of V_{max} (Michaelis–Menten constant).

There are several modern ways to explain the way in which the Michaelis–Menten equation is derived, and one is mentioned below.

For most enzymes, if increased the substrate concentration [S_z] and hold enzyme concentration [E] constant, the resulting initial velocities, or reaction rates, of the reaction (V_i) produce a hyperbolic curve. In other words, V_i increases rapidly at first as we increase substrate concentration [S]. This is first-order kinetics.

At higher substrate concentrations, V_i increases by smaller amounts in response to increase in [S].

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Then, the rate of increase in V_i decreases, and V_i approaches a limit of the reaction rate, called V_{max}. No further increases in [S] will increase velocity. This condition is known as zero-order kinetics.

We already learned that in an enzyme-catalyzed reaction, the enzyme exists in two forms, that is free [E] and the combined form [ES].

At low substrate concentration, most of enzyme will be in freeform *E*. In this condition, the rate is proportional to [S].

The maximum velocity of the catalyzed reaction is observed when the entire enzyme is present as the enzyme–substrate complex and concentration of *E* is vanishingly low. Therefore, at this condition all the enzymes will be saturated with its substrate, and all the free enzymes will be converted into ES form. So that any increase in the [S] has no effect on the rate and the reaction immediately reaches a steady state, in which [ES] remains approximately constant. The ES complex breaks down to yield the product and the enzyme is free to bind another substrate molecule.

Michaelis–Menten Equation

With some assumptions, the Michaelis–Menten equation describes the relationship between [S] and reaction rate (V_i), which is as follows:

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

K_m is Michaelis–Menten constant and is equal to the [S] where $V_i = \frac{V_{max}}{2}$.

K_m is also an indicator of the enzyme's affinity for the substrate. The lower the K_m value, the higher the affinity, so it takes less substrate to reach half of V_{max} and the enzyme is a better catalyst for the reaction.

By considering a simple reaction in which one substrate, *S*, in the presence of an enzyme, *E*, converts to one product, *P*. Michaelis and Menten hypothesized that the enzyme catalyzes the reaction by reacting with the substrate

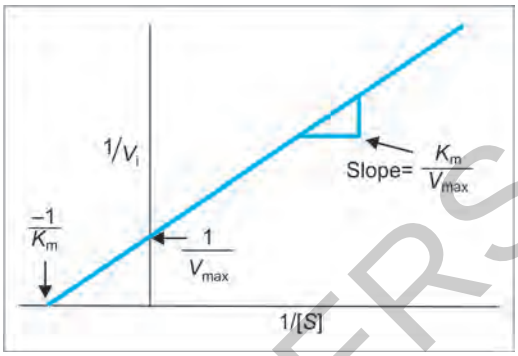

Fig. 2.9: Double reciprocal or Lineweaver–Burk plot.

to form an intermediate enzyme–substrate complex, ES. This complex experiences a catalytic reaction to form the enzyme, *E*, and the product, *P*. The following diagram represents the situation, where K_1, K_2, K_3, and K_4 are rate constants:

$$E + S \underset{K_2}{\xrightarrow{K_1}} ES \xrightarrow[K_4]{K_3} E + P$$

To derive their model, Michaelis and Menten made the following simplifying assumptions:

Moreover, considering $x = 1/[S]$ to be an independent variable and $y = 1/V_i$ to be a dependent variable, the equation has the form of a line, $y = mx + b$. Thus in the graph **(Fig. 2.9)** of $1/V_i$ versus $1/[S]$, the slope is:

1. The reaction rate is determined before much product is formed. Consequently, the reverse reaction from $E + P$ to ES is negligible.
2. K_3 is small in comparison to K_1 and K_2, i.e., the rate of product formation is slow in comparison to the rate of ES formation and the rate of ES dissociation to $E + S$.
3. [S] is much greater than [E], so that [S] is virtually constant.
4. [E]+[ES] is constant.

Under these assumptions, the Michaelis–Menten equation models reaction (1) as follows:

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

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Although the Michaelis–Menten equation captures the relationship of reaction velocity to substrate concentration, K_m and V_{max} are difficult to ascertain from its graph. Hans Lineweaver and Dean Burk reorganized the equation into a form that is more helpful for determination of these constants. Taking the reciprocal of both sides, they solved for $1/v$ in terms of $1/[S]$, as follows:

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

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

$$\frac{1}{V_i} = \left(\frac{K_m}{V_{max}}\right) + \frac{1}{[S]} + \frac{1}{V_{max}}$$

$\frac{K_m}{V_{max}}$ and $\frac{1}{V_{max}}$ are constants

Moreover, considering $x = 1/[S]$ to be an independent variable and $y = 1/V_i$ to be a dependent variable, the equation has the form of a line, $y = mx + b$. Thus in the graph **(Fig. 2.9)** of $1/V_i$ versus $1/[S]$, the slope is and the vertical intercept is $\frac{K_m}{V_{max}}$ and the vertical intercept is $\frac{1}{V_{max}}$.

Setting $1/V_i$ equal to zero, we find that the horizontal intercept is $\frac{-1}{K_m}$. Such a plot is called double reciprocal or Lineweaver–Burk plot.

What is enzyme inhibition and what are its types? Explain with specific examples.

Enzyme Inhibition

The phenomenon of the decrease in the rate of enzymatic reaction brought about by the addition of a chemical substance is called enzyme inhibition. The substances, which inhibit the enzyme, are called inhibitors.

Competitive Inhibition

The competitive inhibitor (reversible inhibitor) closely resembles with that of substrate.

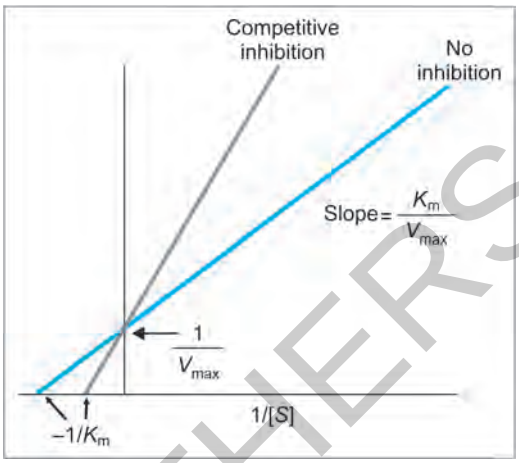


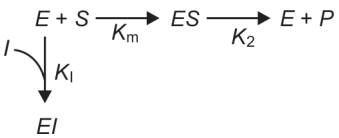
Fig. 2.10: Competitive inhibition.

Hence, the inhibitor competes with the substrate for substrate-binding sites of the enzyme. This type of inhibition can be overcome by sufficiently high concentrations of substrate, i.e., by out-competing the inhibitor **(Fig. 2.10)**.

Competitive inhibitors can bind to *E*, but not to ES. Competitive inhibition increases K_m (i.e., the inhibitor interferes with substrate binding) but does not affect V_{max} (the inhibitor does not hamper catalysis in ES because it cannot bind to ES).

For example: Inhibition of succinate dehydrogenase by malonate.

The Michaelis–Menten equation for competitive inhibition is:



where K_i is the dissociation constant for the EI complex. EI does not react to form *E+P*, and the enzyme is unable to bind both *S* and I at the same time.

The Michaelis–Menten equation for competitive inhibition is:

$$V = \frac{V_{max}[S]}{[S] + K_m\left(1 + \frac{[I]}{K_i}\right)}$$

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Table 2.4: Commonly used drugs that are competitive inhibitors.

Drug	*Enzyme*	*Substrate*	*Therapeutic use*
Methotrexate	Dihydrofolate reductase	FH_2	Treatment of cancer
Allopurinol	Xanthine oxidase	Hypoxanthine	Treatment of gout
Acetazolamide	Carbonic anhydrase	H_2CO_3	To treat hypertension
Mevinolin and lovastatin	HMG CoA reductase	HMG CoA	To treat hypercholesterolemia
Captopril and enalapril	ACE	Angiotensin	To treat high blood pressure

(ACE: angiotensin converting enzyme; HMG CoA: β-hydroxy-β-methyl-glutaryl CoA).

The Lineweaver–Burk equation for competitive inhibition is:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \frac{1}{[S]} \left(1 + \frac{[I]}{K_i}\right) + \frac{1}{V_{max}}$$

For example: Succinate dehydrogenase is the enzyme catalyzing the conversion of succinate to fumarate.

The malonate has the close structural resemblance to succinate.

That is why the malonate tries to occupy the active site of the enzyme.

Some of the competitive inhibitors made use in the treatment of disorders are as follows:

List the therapeutic use of competitive inhibition.

Competitive Inhibition Technique in Drug Therapy

The competitive inhibitors available are mostly synthetic compounds, which are designed in such a way that it should have similarities with the substances present in the human body. So this similarity helps that compound to inhibit the enzymes that act on the particular substrates and finally block the reaction. Such type of inhibitors or drugs inhibits the important enzyme reactions in a bacteria or virus to control the infection. This type of treatment with chemicals or drugs to control infection is called chemotherapy.

To understand this, we should go through some of the examples **(Table 2.4)**.

a. *Treatment with sulfa drugs*: Bacteria can synthesize folic acid from para-aminobenzoic acid (PABA).
   - Sulfa drugs such as sulfonamide have a structure similar to PABA.
   - When a person is treated with sulfa drugs, it inhibits the synthesis of folic acid in bacteria.
   - The folic acid is an important vitamin required for bacterial multiplication.
   - When sulfa drugs block the synthesis folic acid, the bacterial multiplication is inhibited and infection is controlled.

b. *Treatment for gout by allopurinol*:
   - Allopurinol is the drug of choice for the treatment of gout.
   - It has a structure similar to hypoxanthine.
   - So allopurinol competitively inhibits xanthine oxidase, the enzyme that converts hypoxanthine to xanthine and then to uric acid.

c. *Control of cancer by amethopterin and aminopterin*:
   - Amethopterin and aminopterin are antifolic compounds having structural similarity with folic acid **(Fig. 2.11)**.
   - Coenzyme (tetrahydrofolic acid) of this folic acid helps in the transfer of one

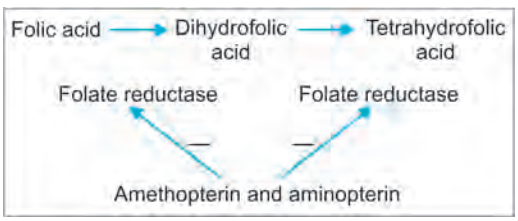

Fig. 2.11: Action of amethopterin and aminopterin.

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carbon unit (in the reactions like the synthesis of purines and pyrimidines).
- Purines and pyrimidines are required for the synthesis of nucleic acids for growth and cell multiplication.
- Aminopterin or amethopterin competitively inhibits folate reductase and interferes with the synthesis of tetrahydrofolate.
- Thus these compounds are used in the treatment of blood cancer where in there is excessive production of WBC.
- Because of the coenzyme deficiency, the multiplication of WBC is inhibited.

d. *Dicumarol to thromboembolic condition*:
- Vitamin K is involved in the γ-carboxylation (see details in Vitamin K) of glutamic acid residues of the clotting factors such as prothrombin, proconvertin factor, Christmas factor, and Stuart–Prower factor.
- There are various anticoagulants to treat the thromboembolic conditions.
- Important and useful clinically are coumarins and heparins. Dicumarol and warfarin are the coumarins to treat the thromboembolic condition.
- When the patient is treated with dicumarol, it competes with vitamin K and decreases the formation of prothrombin by liver.

e. *Isonicotinic acid hydrazide (INH) treatment for tuberculosis:*
- The INH drug has structural similarity with pyridoxine.
- This drug interferes with the formation of pyridoxal phosphate, a coenzyme of pyridoxine used by TB bacillus. That is the reason why patients treated with INH always supplemented with vitamin B_6.

Noncompetitive Inhibition

In this type, the inhibitor does not resemble the substrate and does not bind to the substrate-binding site of the enzyme. It binds to the enzyme other than the active site **(Fig. 2.12)**, e.g., inhibition of enzymes by heavy metals such as Hg^{2+}, iodoacetamide, and diisopropylphosphofluoride (DIPF).

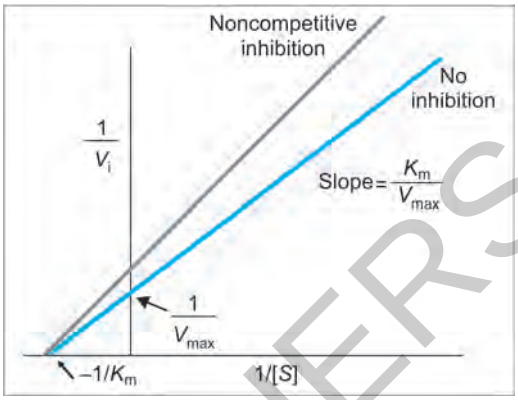


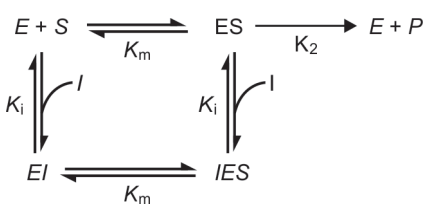
Fig. 2.12: Noncompetitive inhibition.

Iodoacetamide reacts with sulfhydryl groups of cysteine residues or with the imidazole group of histidine residues of the enzyme.

DIPF can inhibit acetylcholine esterase by covalently reacting with the hydroxyl group of a serine residues present at the active site of an enzyme.

A noncompetitive inhibitor lowers the V_{max} with no change in the K_m value.

Noncompetitive inhibitors have identical affinities for E and ES ($K_i = K_i'$). Noncompetitive inhibition does not change K_m (i.e., it does not affect substrate binding) but decreases V_{max} (i.e., inhibitor binding affects catalysis).



where K_i is the dissociation constant for either the EI complex or the IES complex. Neither of these complexes can react to form $E + P$.

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The Michaelis–Menten equation for non-competitive inhibition is:

$$V = \frac{V_{max}[S]}{([S]+K_m)\left(1+\frac{[I]}{K_i}\right)}$$

The Lineweaver–Burk equation for non-competitive inhibition is:

$$\frac{1}{V} = \frac{K_m}{V_{max}}\frac{1}{[S]}\left(1+\frac{[I]}{K_i}\right)+\frac{1}{V_{max}}\left(1+\frac{[I]}{K_i}\right)$$

Uncompetitive Inhibition

It occurs when the inhibitor binds only to the enzyme–substrate complex, not to the free enzyme; the EIS complex is catalytically inactive. Inhibitor binds to ES complex at locations other than the catalytic site. This mode of inhibition is rare and causes a decrease in both V_{max} and the K_m value **(Fig. 2.13)**.

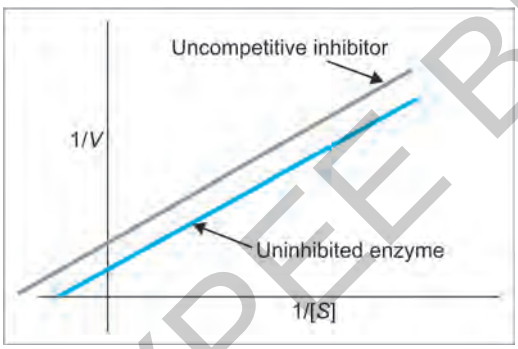

Fig. 2.13: Uncompetitive inhibition.

Substrate and Product Inhibition

Substrate and product inhibition is where either the substrate or product of an enzyme reaction inhibits the enzyme's activity. This inhibition may follow the competitive, uncompetitive, or mixed patterns. In substrate inhibition, there is a progressive decrease in activity at high substrate concentrations. This may indicate the existence of two substrate-binding sites in the enzyme. At low substrate, the high-affinity site is occupied and normal kinetics is followed. However, at higher concentrations, the second inhibitory site becomes occupied inhibiting the enzyme. Product inhibition is often a regulatory feature in metabolism and can be a form of negative feedback. *Example*: Hexokinase inhibition by glucose-6-phosphate and inhibition of HMG CoA reductase by cholesterol.

What are allosteric enzymes? Explain with and example.

Allosteric enzymes: These enzymes have more than one subunit with a catalytic and regulatory site. Allosteric activator or inhibitor binds to the regulatory site and regulate the activity of the enzyme, e.g., HMG CoA reductase enzyme is a regulatory enzyme of cholesterol biosynthesis.

ADP and glucose-6-phosphate are the allosteric activator and inhibitor of hexokinase, respectively.

What are isoenzymes? Briefly explain with examples.

Isoenzymes

They are defined as the different forms of a single enzyme and exist in the same species, which have same catalytic activity but differ structurally, physically, electrophoretically, and chemically.

For example: Lactate dehydrogenase (LDH)—LDH (has five different forms) and creatine phosphokinase—CK (has three different forms).

LDH: LDH has five different forms and each consists of four subunits (polypeptide chains). It is made up of two types of subunits (H and M) and contains four of them in different proportions as LDH_1, LDH_2, LDH_3, LDH_4, and LDH_5.

CK: It is a dimeric enzyme having two types of subunits (B and M). There are three isoenzyme forms of CK [CK_1 (BB), CK_2 (MB), and CK_3 (MM)].

What are diagnostic enzymes and list them with the clinical importance.

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Diagnostic Enzymes

The cells produce enzymes and they remain within the cells. Very small amount of the enzymes are released into the bloodstream, due to the normal breakdown of the cells. Hence, the enzymes are present even in the blood in very small amounts under normal conditions. The levels of these enzymes are greatly increased in blood under certain diseased conditions, which leads to breakdown of cells. Estimation of these enzyme levels in blood or plasma is useful in the diagnosis of various diseases. These enzymes indicate the organ from which they are released and based on this it is easy to find out which organ is affected **(Table 2.5)**.

The diagnostic enzymes are grouped according to the organ they belong. Important groups are as follows:

1. Liver enzymes: AST, alanine transaminase, ALP, and gamma-glutamyl transferase (GGT)
2. Cardiac enzymes: LDH, LD_1, CK, creatinine kinase myocardial bond (CKMB), and AST
3. Muscle enzymes: CK, LDH, and AST
4. Pancreatic enzymes: Amylase and lipase
5. Bone enzymes: ALP and ACP.

Lactate Dehydrogenase

LDH is present in almost all the tissues of the body. Among different forms of LDH, which are known as isoenzymes, it is one of the best examples for isoenzymes.

Table 2.5: Diagnostic importance of certain serum enzymes.

Serum enzymes	*Diagnostic significance*
1. Acid phosphatase	Increases in carcinoma of prostate
2. Alkaline phosphatase	Increases in obstructive jaundice and bone disorder such as rickets, Paget's disease, and hyperthyroidism
3. Amylase	Increases in acute pancreatitis, intestinal obstruction, decreases in acute liver disease
4. Creatine phosphokinase	
CPK 1 (BB)	Brain disorder
CPK 2 (MB)	Myocardial infarction (within 4 hours of onset)
CPK 3 (MM)	Muscular dystrophy
5. Ceruloplasmin	Increases in liver cirrhosis, decreases in Wilson's disease
6. Choline esterase	Increases in nephrotic syndrome and decreases in acute liver disease and organophosphorus poisoning
7. Glutamate oxaloacetate transaminase	Increases in myocardial infarction, toxic liver cell necrosis
8. AST and ALT	Increases in viral hepatitis and other liver diseases
9. Lactate dehydrogenase	
LDH_1, LDH_2	Myocardial infarction
LDH_4, LDH_5	Acute viral hepatitis
10. Lipase	Increases in acute pancreatitis
11. 5 Nucleotidase	Increases in liver disease and obstructive jaundice
12. γ-Glutamyl transpeptidase	Alcoholic liver disease

(ALT: alanine transaminase; AST: aspartate aminotransferase; GPT: glutamate pyruvate transaminase).

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