Enzymes Chemistry

- **Definition: These are specific protein catalysts that accelerate the rate of chemical reactions.**

- Enzyme structure is not changed by entering the reactions.

- Enzyme does not affect the equilibrium constant (i.e. end products) of the reactions.

- **Cellular distribution of enzymes:**

A. Intracellular enzymes: Produced and act inside the cells e.g. metabolic enzymes.

B. Extracellular enzymes: Produced inside the cells and act outside the cells e.g. digestive enzymes.

Properties of ENZYMES

- The general properties of enzymes are those of proteins:

1. They are globular proteins.

2. They can be denatured by physical and /or chemical agents and they lose their biological function as the denaturation change their conformation.

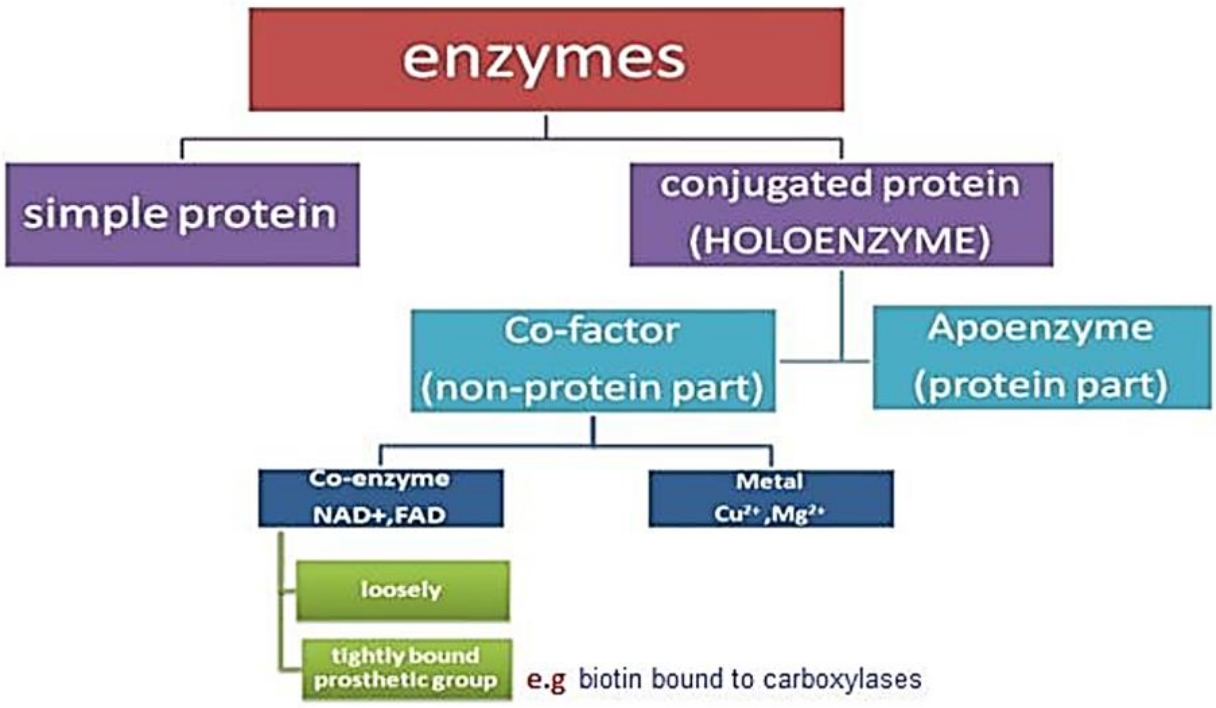
3. Enzymes are usually specific in action and the specificity varies in degree (see later).

4. Some enzymes are simple proteins, others are conjugated proteins.

5. Each enzyme has a characteristic tertiary structure and undergoes a conformational change suitable to the specific substrate

6. Some enzymes are secreted as Proenzymes (zymogens) then they are activated at the time of action.

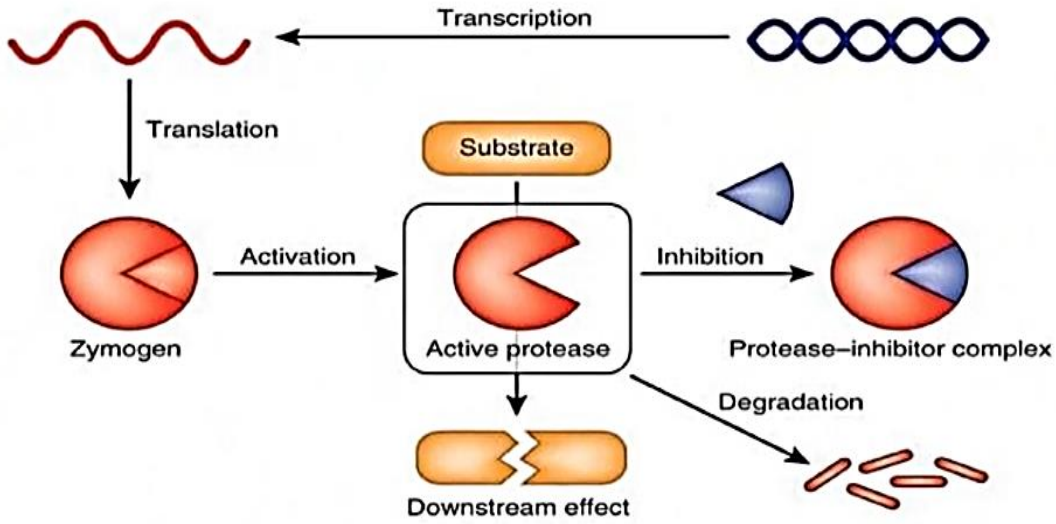
ENZYME STRUCTURE



Zymogens

They are inactive enzymes.

1. **Zymogens are inactive because their catalytic sites are masked by a polypeptide chain.**
2. **Activation of zymogen, into active enzyme is done by removal of the polypeptide chain to open the catalytic site for its substrate.**
3. **Examples of zymogens: are pepsinogen and trypsinogen.**



ENZYMES SPECIFICITY

1. **Relative specificity: One enzyme acts on compounds having the same type of bonds e.g. Lipase enzymes act on different TAG**
2. **Group specificity: the enzyme acts on a special type of bond at specific site and attached to specific groups e.g.:**

- *Pepsin:* acts on peptide bonds between amino group of aromatic amino acid and carboxylic group of another amino acid.

- *Trypsin:* acts on peptide bonds between carboxylic group of basic amino acid and amino group of another amino acid.

3. **Optical specificity:**

- Enzymes act on D or L isomers e.g.

D - Amino acid oxidase acts only on D-amino acids

L- Amino acid oxidase acts only on L-amino acids

- Enzymes act on specific type of linkages according to the type of linkage (α or β) of the compounds attached to it e.g.

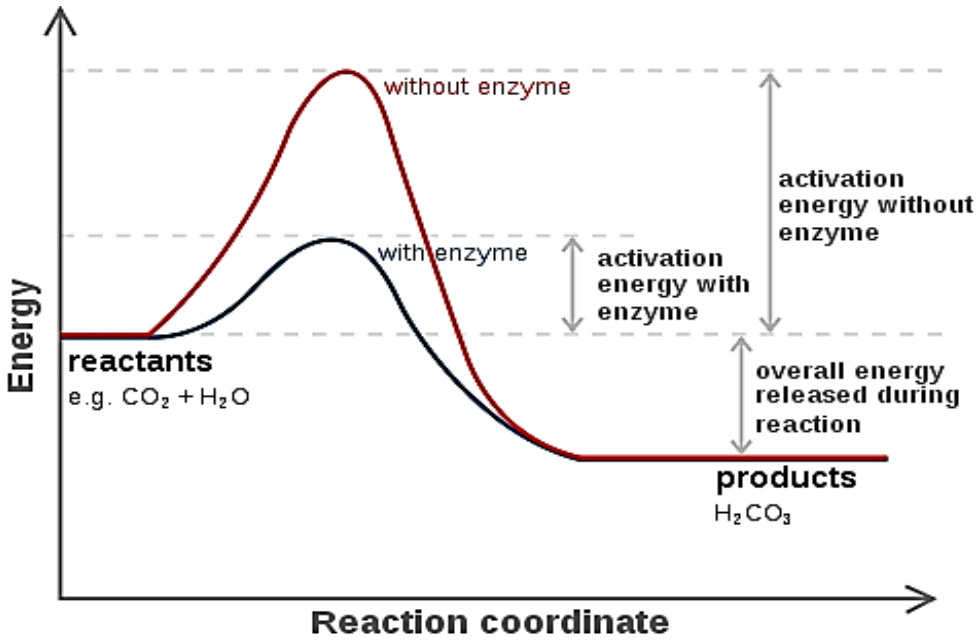
α Amylase hydrolyses α-1-4 glycosidic linkage of starch.

4. **Absolute specificity: One enzyme acts only on one substrate e.g. urease enzyme acts only on urea.**

MECHANISM OF ENZYME ACTION

A. Energy of activation:

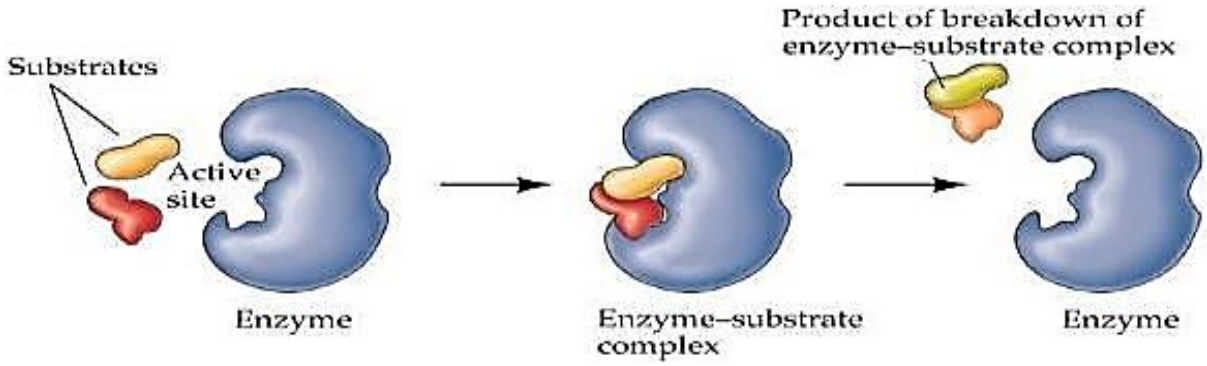
- **All the reactions that proceed from initial substrates (initial state) to products (final state) consume energy. This is called free energy of the reaction.**
- **However the substrates do not become products directly, but must be energized (absorb energy) to reach an activated or transition state. This energy is called activation energy.**
- **At transition state, there is a high probability that a chemical bond will be made or broken to form the product.**
- **The definition of activation energy: is the amount of energy required to raise 1mole of substance to the transition state.**
- **The effect of enzymes: is to decrease the energy of activation.**



B. Active site:

1) During the enzyme action, there is a temporary combination between the enzyme and its substrate forming enzyme-substrate complex. This occurs at active site of enzyme.

2) This is followed by dissociation of this complex into enzyme again and products.

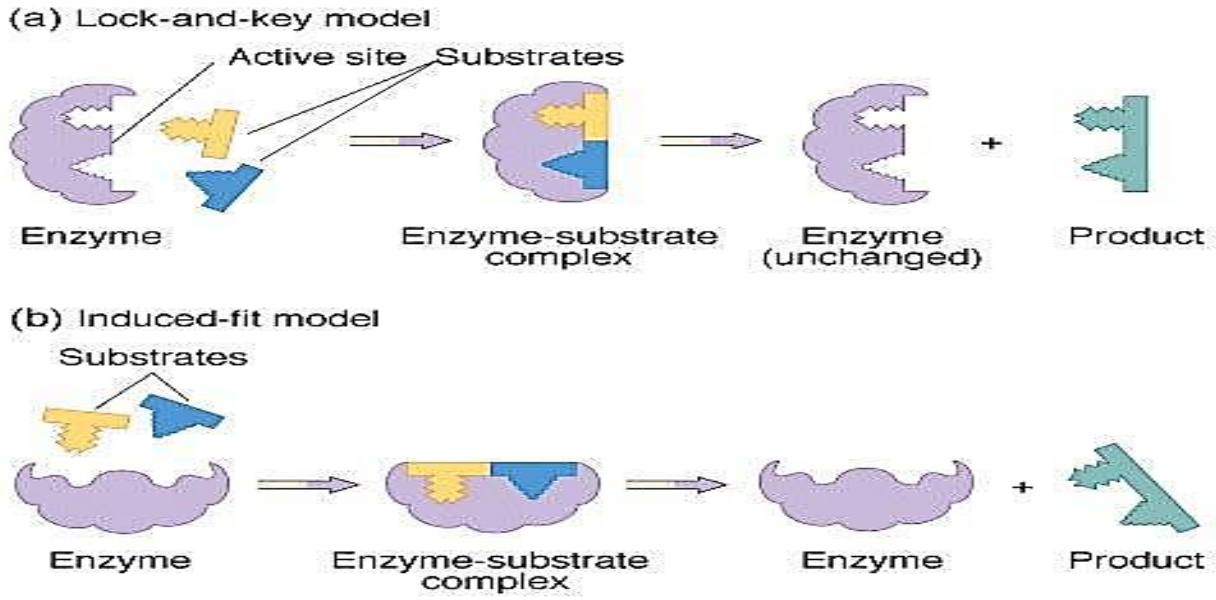


C. Theories of enzyme action:

Two theories have been proposed to explain the specificity of enzyme action:

a) The lock and key theory: The active site of the enzyme is complementary in conformation to the substrate so that enzyme and substrate "recognize" one another.

b) The induced fit theory: The enzyme changes shape upon binding the substrate, so that the conformation of substrate and enzyme protein are only complementary after the binding reaction.



CLASSIFICATION OF ENZYMES		
1- Oxidoreductases (redox enzymes)	Oxidases Catalyze the removal of H or e^- from substrate but use only oxygen as a hydrogen acceptor and form **water** or H_2O_2 as a reaction product.	Oxidases forming water (H_2O): **e.g. cytochrome oxidase**
		2. Oxidases forming hydrogen peroxide (H_2O_2). They are **flavoproteins** containing either FMN or FAD
	Hpdroperoxidase Enzymes utilizing H_2O_2 as substrate.	**Catalase:** is present in all cells and tissues, especially liver, kidney and erythrocytes. In the catalase reaction, one molecule of H_2O_2 act as a **substrate** and the other molecule act as hydrogen donor.
		Peroxidase: present in RBCs, milk and leucocytes. Peroxidase uses H_2O_2 as substrate and an **organic substrate** as hydrogen donor. e.g. ascorbic acid, glutathione
	Dehydrogenases These remove hydrogen from one substrate to a hydrogen carrier. They cannot use oxygen as a hydrogen acceptor.	**a)** Dehydrogenases depend on **nicotinamide coenzymes** (NAD, NADP)
		b) Dehydrogenases depend on **riboflavin coenzymes** (FAD and FMN).
	Oxygenase They catalyze the direct incorporation of oxygen into the substrate.	**Dioxygenases:** Two atoms of oxygen molecule are incorporated into the substrate. As in tryptophan metabolism **Mono-oxygenase:** Only one atom of molecular oxygen is incorporated into the substrate in the form of hydroxyl group (termed hydroxylases or mixed function oxidases), they require a hydrogen donor
2- Transferases **These are enzymes which catalyze transfer of functional groups (G) other than hydrogen**	Transglycosylases (glycosyl transferases):	They catalyze transfer of an activated glycosyl (sugar) residue. Usually activation by UDP glucose
	Transphosphorylases (phosphotransferases):	They catalyze transfer of phosphoryl group 1. kinases 2. phosphoglucomutases
	Transacylases (acyl transferases)	They catalyze transfer of acyl group (R-CO) and they need coenzyme A (COA) as a carrier for acyl group
		These transfer NH_2 group from amino acid to

	Transaminases	α-ketoacid to produce a new amino acid, vitamin B_6 is a coenzyme
	Transmethylases (methyltransferases)	These transfer one carbon e.g. CH_3 group from methyl donor to methyl acceptor. The methyl donor is mainly S-adenosyl-methionine. The methyl carrier is methyl tefrahydrofolic acid ; and methyl-cobalamine
3- Hydrolases **These enzymes act by splitting (cleavage) of a certain bond by adding water.**	Glycosidases. act on glycosidic linkage causing its separation by hydrolysis	Amlyases α 1,4 glucosidase): Starch maltose
	Esterases These attack the ester linkage i.e. (linkage between acidic and alcoholic group)	Lipase: Triglyceride Glycerol + 3Fatty acids
	Peptidases **Split** the peptide linkage (CO-NH) of proteins.	**Endo-peptidase:** These act on intenal bond in the polypeptide chain e.g.: pepsin.
		Exo-peptidase: They are divided into: Carboxypeptidase: act on the carboxylic end Aminopeptidase: act at the amino end of protein chain.
4. Lyases:	These catalyze the addition or removal of groups e.g. H_2O , NH_3 or CO_2, **without hydrolysis, oxidation or reduction**.	**Dehydratases (or hydratases)**: they catalyze removal or addition of water or from the substrate.
		Decarboxylases. They catalyze splitting of CO_2.
5. Isomerases	They catalyse the interconversion of two isomers.	Aldose-ketose isomerases
6. ligases	These enzymes link two molecules using **energy from ATP**	(1) Amide synthetase: (C —N) (2) Carboxylase:

COENZYMES

1- According to the structural basis into:

A- Vitamins Coenzymes:

Vitamin	Coenzyme derivative	Group carried in activated form
Thiamine (vitamin B1)	Thiamine pyrophosphate(TPP)	Aldehyde
Riboflavin (vitamin B2)	Flavin adenine dinucleotide Flavin mononucleotide (FAD, FMN)	Hydrogen carrier
Nicotinate (niacin)	Nicotinamide adenine dinucleotide, Nicotinamide adenine dinucleotide	Hydrogen carrier
Pyridoxine, pyridoxal Pyridoxamine (vitamin B6)	Pyridoxal phosphate(PLP)	Amino group
Pantothenic acid	CO enzyme A	Acyl group
Biotin	Carboxbiotin	CO_2
Folate	Tetrahydrofolate(THFA)	One carbon unit
Cobalamin (vitamin B12)	Cobamide coenzymes	- Methylcobalamine - Deoxyadenosylcobalamine
Lipoic acid	Lipoamide	Acyl group, Hydrogen carrier
Quinone	Ubiquinone	Electrons
Vitamin C	L- ascorbic acid	Hydrogen carrier

B- Non Vitamins Coenzymes:

1- Nucleotide coenzymes (UDP-glucose, others nucleotide derivatives of carbohydrates): ATP.

2- Nucleoside: S adenosyl methionine (SAM) .

3- Peptide coenzymes (glutathione :GSH)

2- According to the functional basis into:

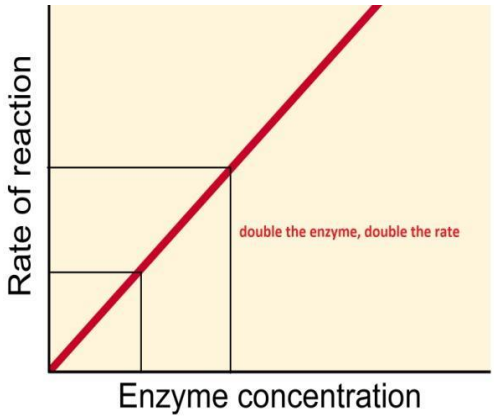
Coenzymes for transfer of H Hydrogen carriers	Coenzymes for group transfer Other than H
1. NAD+ and NADP+ 3. Lipoic acid 5. Vitamin C 2. FMN and FAD 4. Coenzyme Q 6. Glutathione.	1. ATP, GTP, CTP etc. 2. Thiamin pyrophosphate (TPP). 3. Coenzyme A (CoA) 5. Folic acid 4. Pyridoxal phosphate (B6) 6. Biotin

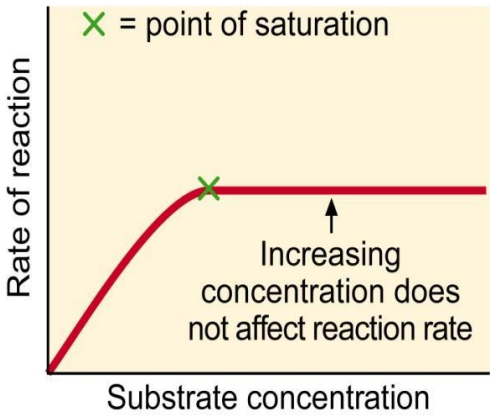
FACTORS AFFECTING ENZYME ACTIVITY

1. Concentration of enzyme: The initial velocity of a reaction is directly proportional to the amount of the enzyme present, provided that all other conditions remain constant.

2. Concentration of substrate:

The initial velocity of a reaction is directly proportional to the amount of substrate present till it reaches a maximum point known as maximum velocity (Vmax), where any further increase in the amount of substrate causes no increase in the velocity of the reaction. This is true if all other conditions especially enzyme concentration remain constant.





a) Definition of maximum velocity (V max): It is the maximum point in substrate velocity curve where any further increase in the amount of substrate causes no increase in the velocity of the reaction due to enzyme saturation.

c) Michaelis constant Km - Def: It is substrate concentration that produces half maximum velocity.

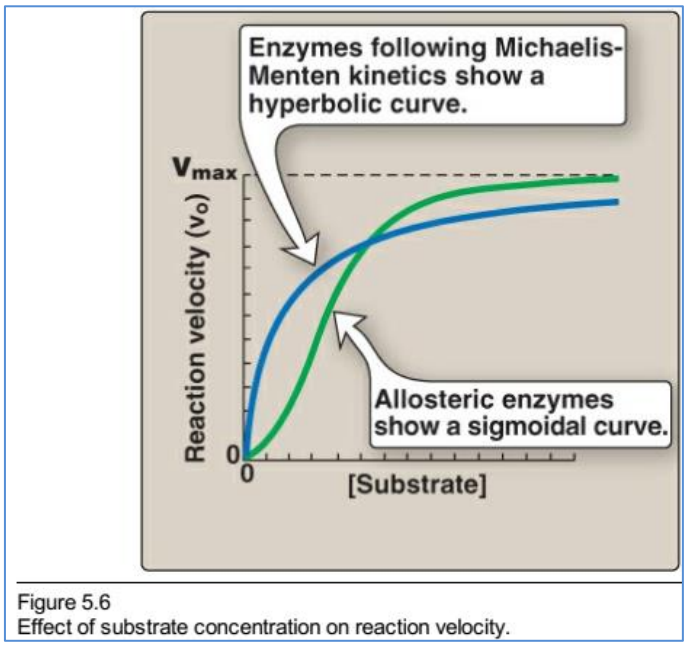


Figure 5.6
Effect of substrate concentration on reaction velocity.

d) Important conclusions about Michael is-Menten kinetics:

- Km is a constant, characteristic of an enzyme and a particular substrate. Km reflects the affinity of the enzyme for the substrate.

- The smaller the Km value → the more active the enzyme:

i- Small (low) Km reflects a high affinity of the enzyme for substrate i.e. low concentration of substrate is needed to half saturate the enzyme.

ii- Large (high) Km reflects a low affinity to the enzyme for substrate i.e. high concentration of substrate is needed to half saturate the enzyme.

3. Effect of temperature:

- The optimal temperature for enzymatic activity in human body is 37 °C i.e. the temperature of the cells.

- At zero temperature, the enzyme is inactive. The reaction velocity increases with increase of temperature until a maximum velocity is reached.
- Further elevation of the temperature results in a decrease in reaction velocity. At 55°C - 60°C, most enzymes are denaturated and become permanently inactive.

4. Effect of pH:

- The optimal pH for enzyme activity is that pH at which the enzyme acts maximally.
- Above or below this pH, the ionic state of both enzyme and substrate will be changed, and the rate of reaction will therefore decrease.
- Each enzyme has its own optimal pH e.g.
  - Salivary amylase 6.8.
  - Pepsin 2
  - Trypsin 8
  - Alkaline phosphatase 8.4
- Extremes of pH can also lead to denaturation of the enzyme.

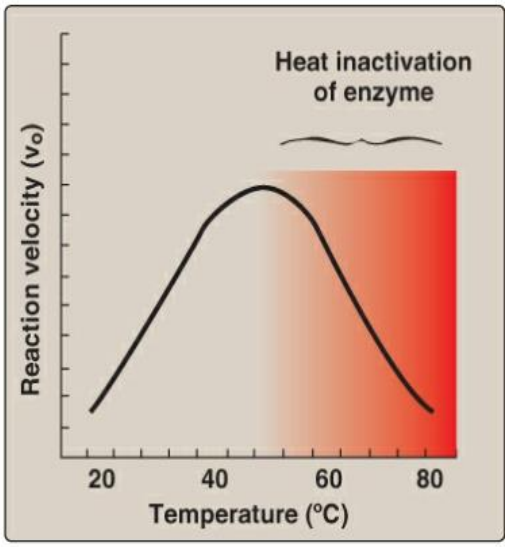


Figure 5.7
Effect of temperature on an enzyme-catalyzed reaction.

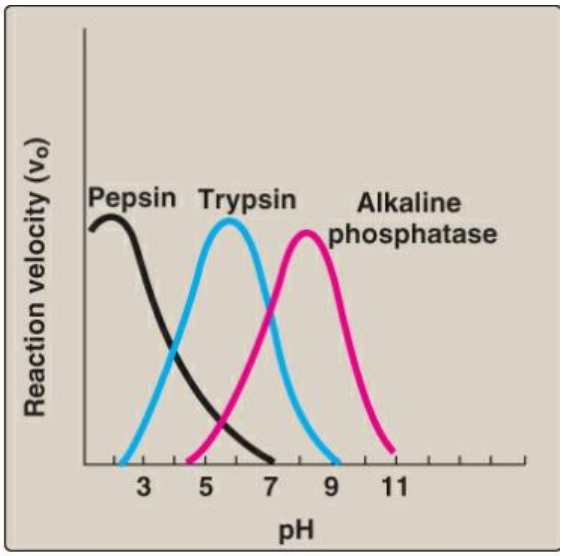


Figure 5.8
Effect of pH on enzyme-catalyzed reactions.

5. Effect of Co-enzymes concentration:

- Co-enzymes concentration has the same effect and gives the same curve of substrate concentration on enzymatic activity, as NAD.

6. Effect of physical agents:

- Red and blue lights increase the enzyme activity.
- Heating, shaking stirring inhibit enzyme activity by denaturation.
- Ultra violet rays and infrared rays inhibit enzyme activity.

7. Effect of time;

- In all the previous factors, time must be taken into consideration, and its effect on enzyme kinetics must be measured against time.

8. Effect of product concentration:

- **Increased product concentration decreases enzyme activity, this may be due to:**
  1. **Change in the pH of the medium.**
  2. **The product is more or less similar to the substrate, so it may compete it to catalytic site of the enzyme**
  3. **The product may bind to the enzyme at the allosteric Site (in case of allosteric enzyme).**

9. Enzyme activators:

- **Activators increase the rate of enzyme catalyzed reactions.**
- **Velocity of the reaction depends on activator concentration.**
- **Some enzymes are activated by different ways:**

A. Removal of peptide converts inactive forms of the enzyme (zymogen) to active.

e.g. Pepsinogen $\xrightarrow{\text{Pepsin or HCl}}$ pepsin

B.Some enzymes containing SH groups e.g. glyceraldehyde 3-P dehydrogenase require reducing agents (vitamin C) to be activated.

C. Some enzymes require minerals; they are called metal activated enzymes eg.

- **Cl^- for amylases (nonmetal ions)**
- **Mg^{++} for kinases**

D. Allosteric activators (Allosteric modifiers): The binding of allosteric activator produces Conformational changes in the protein structure of the enzyme resulting in increased velocity of the reaction

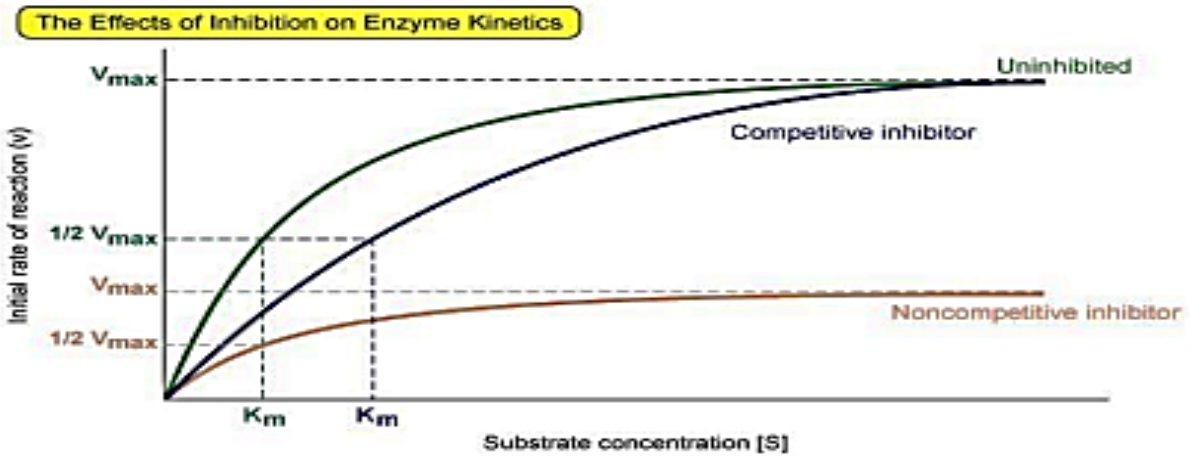
e.g. AMP is an allosteric activator of phosphofructokinase enzyme.

10. Enzyme inhibitors:

Enzyme inhibitors

- **Definition:** These are substances that can diminish the velocity of enzymatic reactions.

Enzyme inhibitors		
Reversible inhibitors - Bind to enzymes through **non covalent** bonds. - Dilution of the enzyme-inhibitor complex dissociates the reversibly bound inhibitor and recovery of enz. activity.		**Irreversible inhibitors**
A. Competitive inhibitors	**B. Non-competitive**	
- **Similar** to substrate. - compete with substrate for **active site** of the enzyme. - Both substrate (S) and inhibitor (I) can bind with the catalytic site of the enzyme to form either **Enz-S-complex** or **Enz-I-complex**. - The combination between enzyme and substrate or inhibitor depends on: **1)** Concentration of substrate. **2)** Concentration of inhibitor. **3)** Affinity of both inhibitor and substrate to the active site of the enzyme. **- Example of competitive inhibitors:** **1)** Malonate and Succinate: both compete for the catalytic site of Succinate DH. **2)** Allopurinol and hypoxanthine: both compete on xanthine oxidase that oxidizes hypoxanthine into xanthine then to uric acid.. **3)** Dicumarol & Warfarin and vitamin K: both compete for the catalytic site of epoxide reductase enzyme. **- Effect of competitive inhibitor on Vmax and Km:** **a) Effect on Vmax:** A competitive inhibition **does not** affect Vmax. **b) Effect on K:** A competitive inhibition **increases** the Km of substrate.	- Inhibitor and substrate bind to different sites on the enzyme. - The inhibitor **does not** alter the catalytic site. - There is **no** structural similarity between substrate and inhibitor. - The inhibitor can bind either free enzyme or the enzyme-substrate complex. Both enzyme inhibitor complex and enzyme substrate inhibitor complex are inactive. **- Effect of noncompetitive inhibitor on Vmax and K:** a) Vmax is **decreased.** b) Km is **unchanged**.	-This type of inhibition cannot be reversed by adding more substrate - The inhibitor **alters** the catalytic site. - Irreversible inhibitors include the **following:** 1. All compounds that produce Denaturation of proteins. 2. Inhibitors of sulfhydryl group 3. Antienzymes: e.g. **Antithrombin III** 4. Removal of catalytic ions: by addition of EDTA. 5. Inhibition by phosphorylation and dephosphorylation 6. Cyanide and carbon monoxide inhibit cytochrome oxidase.

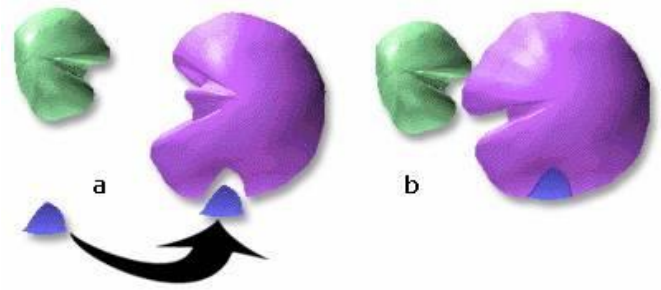


Regulation of enzyme activity

Enzyme activity is regulated by many mechanisms.

A. Allosteric regulation of enzyme activity:

1. **Allosteric enzymes generally catalyze the irreversible steps in metabolic pathways.**
2. **The term allosteric means "other site", It indicates that a molecules called effectors (also called modifiers or modulators) can bind non-covalently at a site other than active site.**

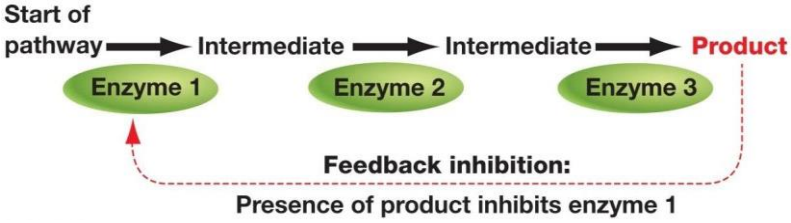


a) **Effectors are positive if they stimulate catalytic reaction and negative if they inhibit the reaction.**

b) **Effectors may be the end product of a metabolic pathway. If it inhibits the reaction (negative regulation), it is called: feedback inhibition.**

B. Feedback inhibition:

It means that the end product of a series of reactions directly inhibits the first enzyme of that series.



C. Feedback regulation:

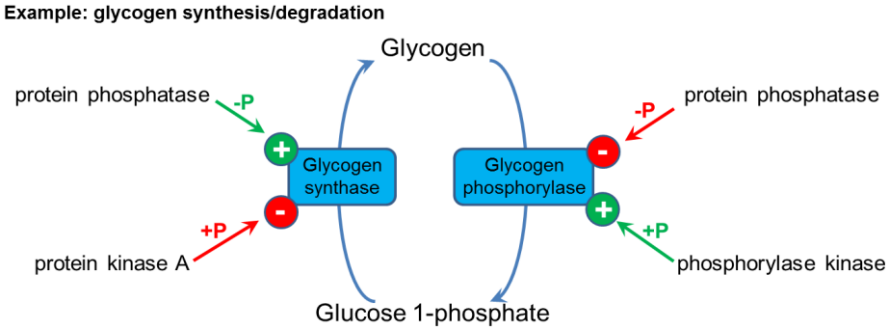
It means that the end product of a series of reactions has *no* inhibitory effect on the first enzyme. It rather affects the gene(s) that code for the formation of that enzyme preventing its synthesis

D. Covalent modification: phosphorylation / de phosphorylation:

1. Some enzymes may be regulated by covalent modification, most frequently by the addition or removal of phosphate groups from the enzymes.

2. Phosphorylation reactions are catalyzed by a family of enzymes, called: protein kinase. It utilizes ATP as a phosphate donor.

3. Phosphate groups are removed from Phosphorylated enzymes by the action of phosphor protein phosphatase



Isoenzymes

- Definition: Isoenzymes are different molecular forms of the enzyme that activate the same reaction, use the same coenzyme and same substrate but they are different in chemical protein structure. This leads to:

1. **Different immunological reactions.**
2. **Different Km and Vmax.**
3. **Different physical properties.**

- Example:

1. Lactate dehydrogenase enzyme (LD)

- Is a tetramer i.e. contains 4 polypeptide chains. These 4 chains are a mixture of different proportions of 2 chains H and M (H. after heart & M after muscle).

- There are 5 isoenzymes of LD enzyme having a Diagnostic importance:

Determination of different isoenzymes helps in diagnosis of diseases e.g.

LDI_1	HHHH	Heart	Serum LDI_1 increases in certain heart diseases (myocardial infarction).
LDI_2	HHHM	Red cells	Acute leukemia
LDI_3	HHMM	Lungs	Acute leukemia
LDI_4	HMMM	Other tissues	
LDI_5	MMMM	Liver	Serum LDI_5 increases in certain liver diseases (infective hepatitis)

2. Creatine kinase (CK) (CPK):

- **CK-BB: in brain.**
- **CK-MB: in Skeletal muscle.**
- **CK-MM: in myocardium.**

3. Isocitrate dehydrogenase: (cytosolic & mitochondrial)

4. Phosphodiesterases: 1-5

CLINICAL IMPORTANCE OF ENZYMES

A- Diagnosis of diseases

- **Enzymes are intracellular and when there is cellular damage, they are released into the circulation**
- **The measurement of these enzymes in the serum can be used in the diagnosis of certain diseases.**

Enzymes in plasma are classified into:

A. Functional plasma enzymes: These are enzymes present normally in blood to perform certain physiological functions. They are characterized by:

1. **They are synthesized in the liver.**
2. **They are present in blood in higher concentration than tissues.**
3. **Their substrates are present in the circulation.**
   - **Examples: Proenzymes of blood clotting, lipoprotein lipase.**

B. Non-functional plasma enzymes: These enzymes are present in a very low concentration in blood due to tissue turn over and increase in case of tissue damage.

They are characterized by:

1. **They perform no physiological function in blood,**
2. **Their substrates, are absent from blood.**
3. **Their level is normally low and they increase in tissue damage**
   - **Examples: for these enzymes are:**

1. **Alkaline phosphatase: increases in obstructive jaundice, hyperparathyroidism, rickets and metastatic carcinoma to bone.**
2. **Transaminases:**

- Aspartate aminotransferase (AST) or glutamic oxaloacetic transaminase (GOT)

Increases in heart disease

- Alanine aminotransferase (ALT) or glutamic pyruvic transaminase (GPT)

Increases in liver diseases.

B- TUMOR MARKERS:

Definition of tumor markers:

- Tumor markers are macromolecules mostly proteins whose appearance or changes in concentration in blood or other body fluids is indicative to the presence, extent or progress of a malignant tumor.

- Tumor markers may be tumor antigens, hormones or enzymes.

- Alterations of serum enzymes in malignancy may be due to:

1. **Production of increased amounts of enzymes by tumor cells.**
2. **Release of intracellular enzymes due to cell damage.**

Enzymes used as tumor markers:

1. **Alkaline phosphatase (ALP). It increases in bone metastasis.**
2. **Creatine kinase (CK): The isoenzyme fraction of the brain (CKBB) diagnose breast tumors, prostatic carcinoma, colonic cancer, and transitional cell carcinoma of bladder.**
3. **Lactic dehydrogenase (LDH). It is generally increased in malignancy.**

- Also decreased level of some enzymes can be used to diagnose inborn errors of metabolism e.g.

1. **Glucose-6-phopshate dehydrogenase in Favism.**
2. **Galactosyl transferase in galactosemia**

C- Treatment of diseases:

Enzymes are used in treatment of some diseases e.g.

1. **Fibrinolysins in treatment of infarctions (streptokinase).**
2. **Digestive enzymes in treatment of maldigestion.**
3. **α-chymotrypsin for treatment of intraocular haemorrhage.**