CHAPTER- 4

ENZYMES

Introduction

- Enzymes are biological catalysts that let reactions to go faster.
  - Usually 10^5-10^{17} times

- The term '*enzyme'* was first suggested by Wilhelm Kühne (1867)
  - From Greek term '***ensimo***'

    -Meaning "**in leaven**", "***in ferment***" or "**in yeast**"

- More than 2,000 enzymes exist in our body

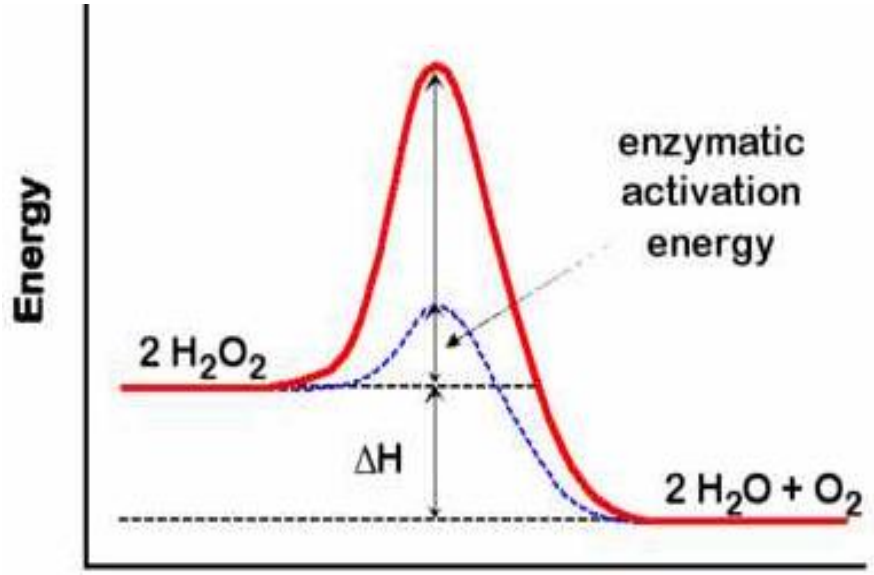
Characteristic of enzymes

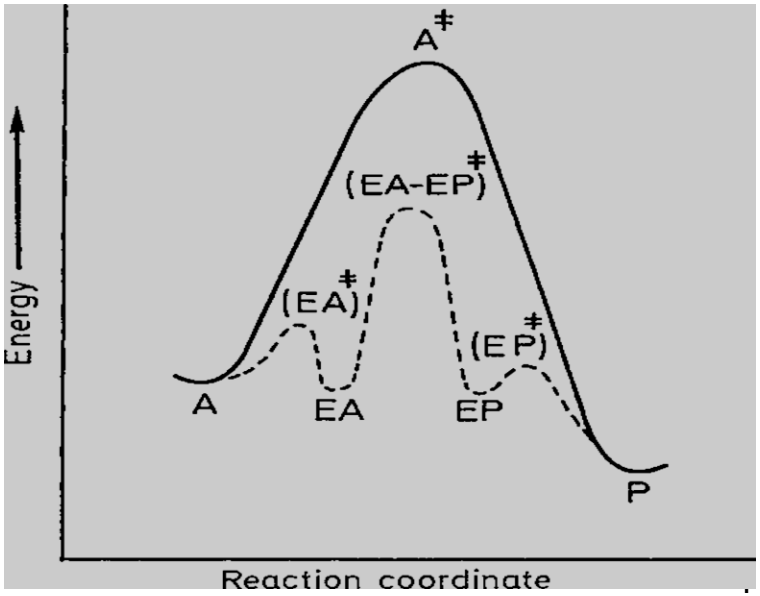
❖ Enzymes

- Are large **globular proteins**
  - ✓ Exception:- **Ribozyme** have NA as catalytic site
- Most require certain **non-protein** component (**cofactor**) for their action
- Possess certain structural component /**pocket site** (cleft and sinks) for binding – i.e **active site**
- Are **specific** in their action
  - ✓ Particularly to ***FG's, Linkages & Stereochemistry***
- Best function **under physiologic conditions**
- They **do not alter equilibrium condition**
- They can be **regulated**

How enzymes work (Enzyme Catalysis)

- Enzymes catalyze most reactions
  - By providing alternative easier pathway for the rxn to happen
    - ✓With lower activation energy barrier
- Enzymes will not have effect on equilibrium condition





Enzyme Nomenclature

Trivial names

- Classic names mostly offered after discoverer or their properties
- Doesn't have any relation with
  - ✓ Nature of their substrate(s) or
  - ✓ Nature of reaction they catalyse

  Examples: Trypsin, pepsin , lysozyme , chymotrypsin
- Only few names still exist (acceptable)

  Examples: Trypsin, pepsin

Enzyme Nomenclature

Official name

- Are those names to be approved by IUBMB
- Are derived in relation with
  - ✓ Nature of their substrate(s) or
  - ✓ Nature of reaction they catalyze

  } ***Hence unambigeous & informative***

- Have an ending 'ase'

NB:-and can be represented by four digit number (EC No)

e.g

EC No	Enzyme (activity)
1.1.1.1	**Alcohol dehydrogenase**-catalyse alcohol to aldehyde oxidation
2.27.1.2	**Hexokinase** – phosporylate hexose sugars
3.4.17.1	**Carboxypeptidase A**- Involve in C-terminal cleavage of peptide bond
4.1.1.1	**Pyruvate decarboxylase**-remove carboxyl group from pyruvate

Enzyme Classification

❖ **Based on type of reaction they catalyze enzymes are groped in to six categories**

S.N.	Enzyme class	Reactions catalyzed
1.	Oxidoreductases	Oxidation and reduction of substrates (usually involve hydrogen transfer)
	Dehydrogenases	Transfer of hydrogen atoms from substrate to NAD*
	Oxidases	Transfer of hydrogen atoms from substrate to oxygen
	Oxygenases	Partial incorporation of oxygen to substrate
	Peroxidases	Transfer of electrons from substrate to hydrogen peroxide
2.	Transferases	Transfer of a chemical group (such as a methyl group, amino group, phosphate group from one molecule to another
	Phosphorylases	Addition of orthophosphate to substrate
	Transaminases	Transfer of amino group from one substrate to another
	Kinases	Transfer of phosphate from ATP to substrate
3.	Hydrolases	Cleavage of bonds by the addition of water
	Phosphatases	Removal of phosphate from substrate
	Peptidases	Cleavage of peptide bonds
4.	Lyases	Addition of groups to double bond (-C=C-, C=O, -C=N-)
	Decarboxylases	Removal of carbon dioxide from substrate
5.	Isomerases	Rearrangement of atoms of a molecule
6.	Liagases	Formation of new bonds using energy from (simultaneous) breakdown of ATP
	Synthetases	Joining two molecules together

Enzyme kinetics

❖Study of kinetics of enzymatic catalyzed reaction helps to

- ➢ Understand how enzymes function and
- ➢ How their activity can be improved

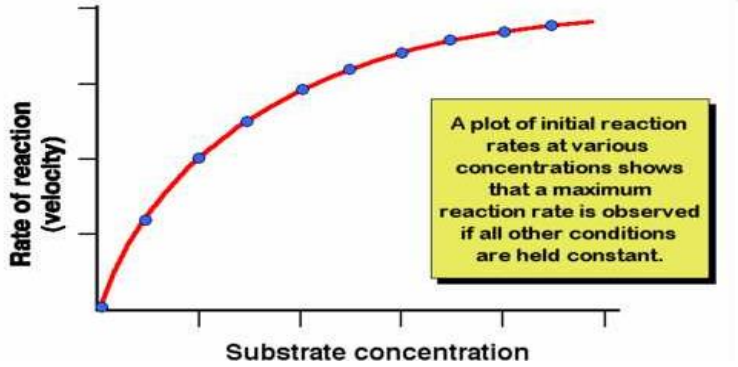
❖ **For non-catalyzed reactions**

Reaction rate increase with concentration.

❖ **Enzyme catalyzed reactions**

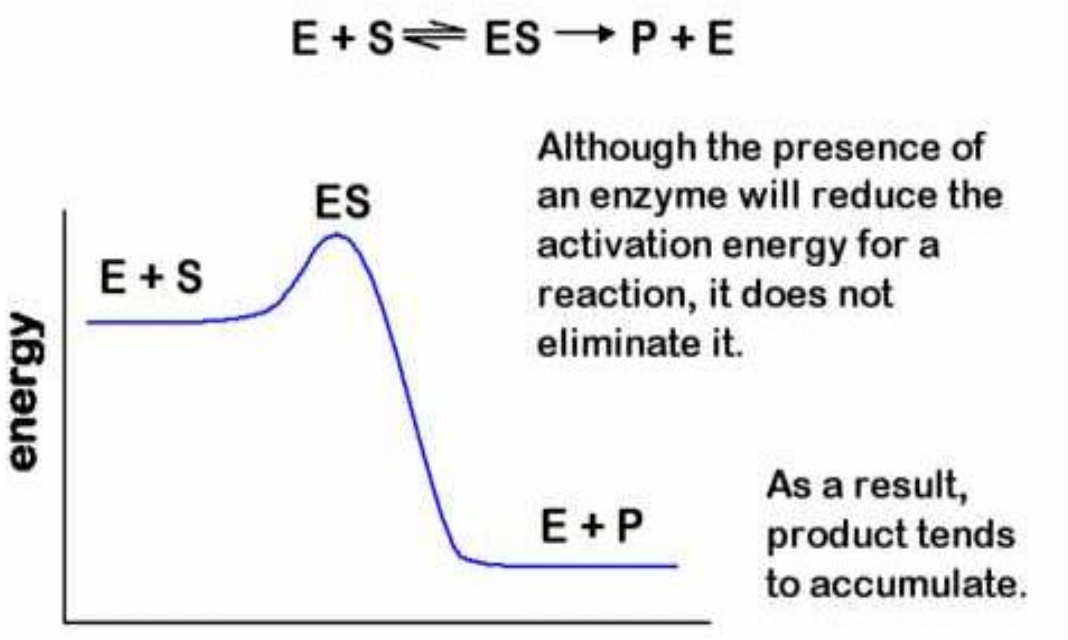
Also increase but only to a certain point.

V_{max} **maximum velocity (show saturation kinetics)**



Enzyme kinetics

- Mechanism for this type reaction was first formulated by Michaelis and Menten (1913)
- Initially enzyme reacts with its substrate (S) to form an activated complex (ES)
- The ES complex then decompose to a product (P) and the enzyme or back to substrate



Assumptions

- V_o = initial velocity (ignore reverse reaction)
- [ES] is constant
- Conversion of S to P is rate-limiting, $v_o = k_2$ [ES]

Enzyme kinetics

❖ Michaelis-Menten equation

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P + E$$

➢ Three rate expressions are used to describe the enzymatic reaction:

- $rate_f = k_1[E_o\text{-}ES][S]$ formation of ES
- $rate_d = k_2[ES]$ decomposition of ES
- $rate_p = k_3[ES]$ formation of product

Where E_o = initial enzyme concentration

NB : k_4 is neglected because its effect is very small during the initial stages of the reaction.

Enzyme kinetics

❖ Michaelis-Menten equation

- Typically, as this type of reaction proceeds, it reaches an equilibrium like condition where [ES] remains constant.

$$rate_f = rate_d + rate_p$$

- If we substitute in our rate expressions and rearrange, we end up with:

$$[ES] = \frac{k_1 [E_o] [S]}{(k_2 + k_3) \quad + k1[S]}$$

- We can simplify our equation by includin all of the rate constants in a single term.

$$K_m = \frac{k_2 + k_3}{k_1}$$

Where: Km=Michaelis constant

- In to $[ES] = [E_o]\frac{[S]}{K_M + [S]}$

Enzyme kinetics

❖ Michaelis-Menten equation

- The rate of product formation is then:

$$\text{rate}_p = k_3 [E_o] \frac{[S]}{K_M + [S]}$$

- Typically, it is the substrate that is to be measured so $k_3[E_o]$ will control the rate.

$$V_{max} = k_3[E_o]$$ **Maximum velocity**

- rate of product formation is then:

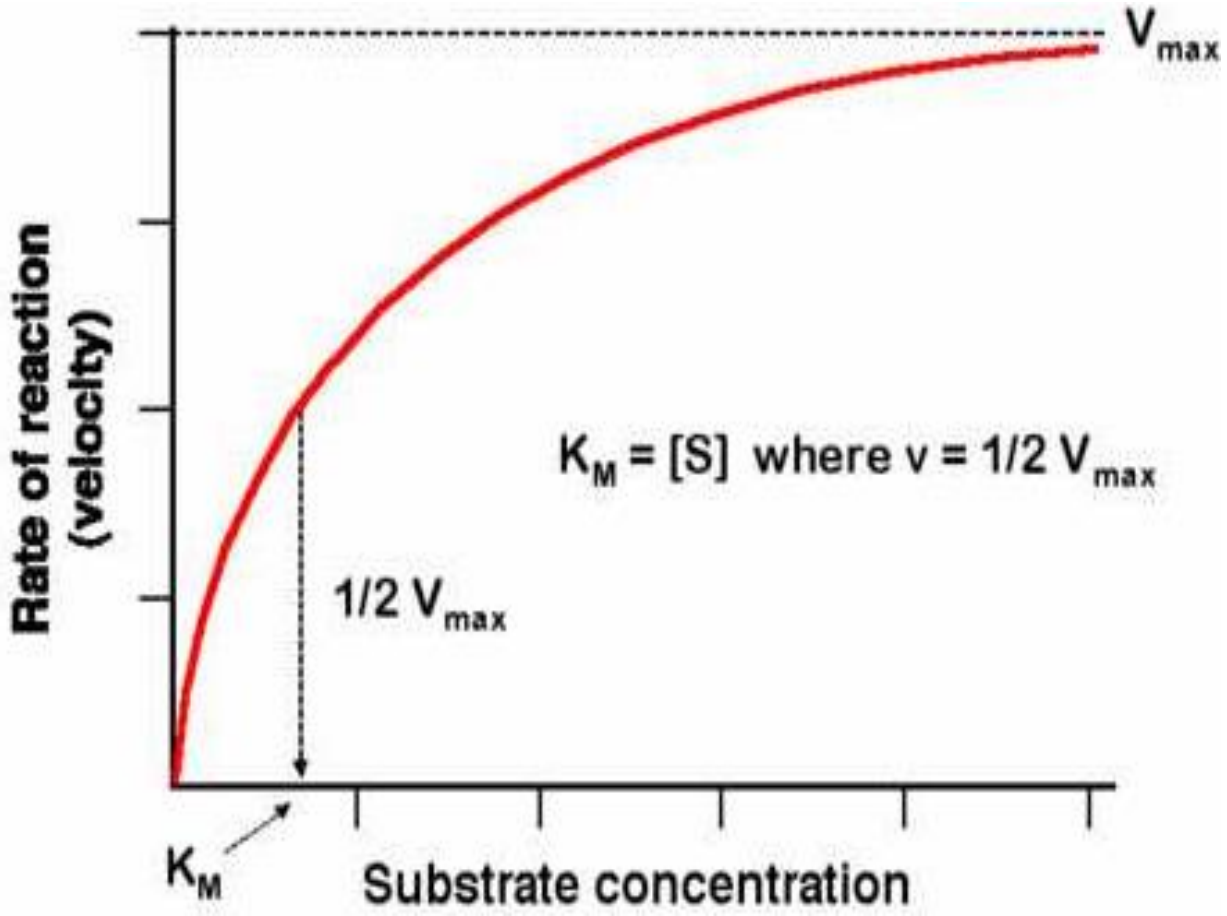
$$\text{rate}_p = V_{max} \frac{[S]}{K_M + [S]}$$

$$V = V_{max} \frac{[S]}{K_M + [S]}$$

Enzyme kinetics

❖ Michaelis-Menten equation

- The plot of **substrate concentration[S]** vs **rate of reaction[Vo]** gives rectangular hyperbolic plot



Enzyme kinetics

❖ Michaelis-Menten constant (Km)

- A mathematical interpretation of enzyme action
- Measures
  - ✓ Substrate concentration required to reach one half its Vmax

    i.e 1/2 of the enzyme is bound to S
  - ✓ "relative affinity/specificity" of an enzyme for its substrate
- Is a characteristic physical property for each enzyme (for a given substrate)
  - ✓ Large Km implies waek E-S interaction ⟶ ***Low affinity***
  - ✓ Small Km implies strong E-S interaction ⟶ ***High affinity***

e.g M + ATP $\xrightleftharpoons{\textit{Hexose Kinase}}$ M-6-P + ADP

Substrate	Km
Glucose	8×10^{-6}
Allose	8×10^{-3}
Mannose	5×10^{6}

Enzyme kinetics

❖ Turn over number (K*cat*)

- This is a measure of how rapidly an enzyme can process a substrate.

$$\text{turnover number} = k_3 = \frac{V_{max}}{[E_T]}$$

Example. A 10^{-9} M solution of catalase causes the breakdown of 0.4 M H_2O_2 per second.

$$k_3 = \frac{0.4 \text{ moles/liter } H_2O_2 \text{ per second}}{10^{-9} \text{ moles/liter catalase}}$$

$k_3 = 40{,}000{,}000\ H_2O_2$ per mole of catalase per second

Values of k_{cat} (Turnover Number) for Some Enzymes

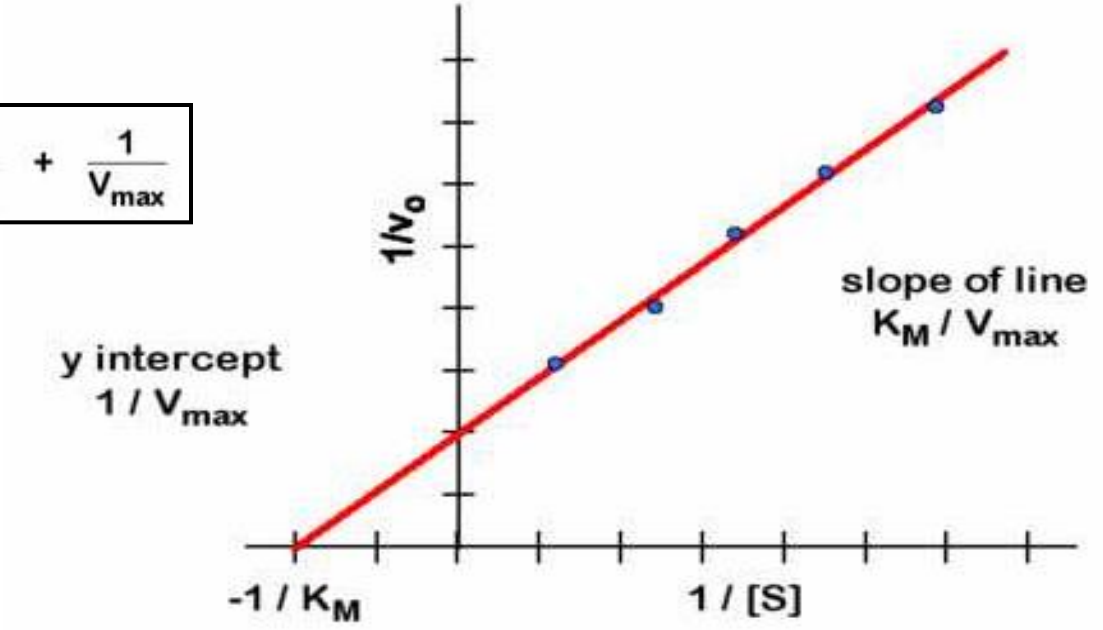
Enzyme	k_{cat} (sec^{-1})
Catalase	40,000,000
Carbonic anhydrase	1,000,000
Acetylcholinesterase	14,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Lysozyme	0.5

Enzyme kinetics

❖ Lineweaver-Burk equation

- Using the Michaelis-Menten equation can be difficult to determine V_{max} from experimental data.
- An alternate approach was proposed by Lineweaver and Burk that results in a linear plot of data.

$$\frac{1}{v_o} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$



Factors affecting enzyme activity

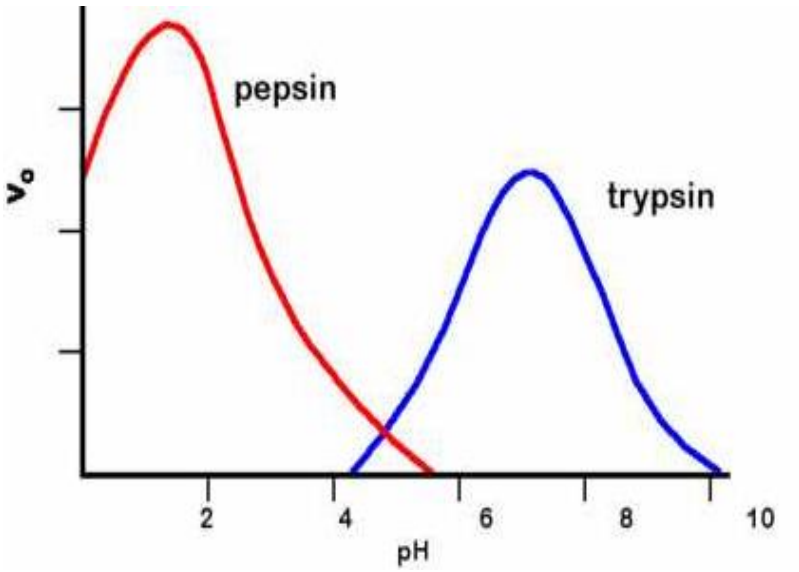
- **Factors that alter performance of enzyme activity are**
  - **Enzyme concentration**
  - **Substrate concentration**
  - **Reaction environment**:- Temprature & pH
  - **Modulators :-Activators (Cofactors) and inhibitors**

Factors affecting enzyme activity

❖ Effect of pH on enzyme activity

- **Enzymes have optimum working pH**

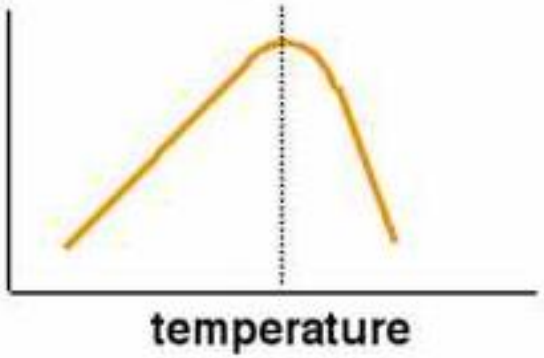
Enzyme	Source	Optimum pH
pepsin	gastric mucosa	1.5
sucrase	intestine	6.2
catalase	liver	7.3
arginase	beef liver	9.0
alkaline phosphatase	bone	9.5



Factors affecting enzyme activity

❖ Effect of temprature on enzyme activity

- ✓ **Enzymes have optimum working temperature**
- ✓ Exceeding normal temperature ranges always reduces enzyme reaction rates.



- ✓ Optimum temperature is usually 25 - 40ºC but not always.

Factors affecting enzyme activity

❖ Activators(Cofactors)

- Some enzymes require a second species to be present in order to do their job.

For Cofactor type enzymes:

Apoenzyme - protein portion of enzyme
- almost ready to work.

Cofactor - prosthetic group needed to 'activate' the apoenzyme. **(Coenzymes)**
- usually a metal ion that holds protein in the proper shape.

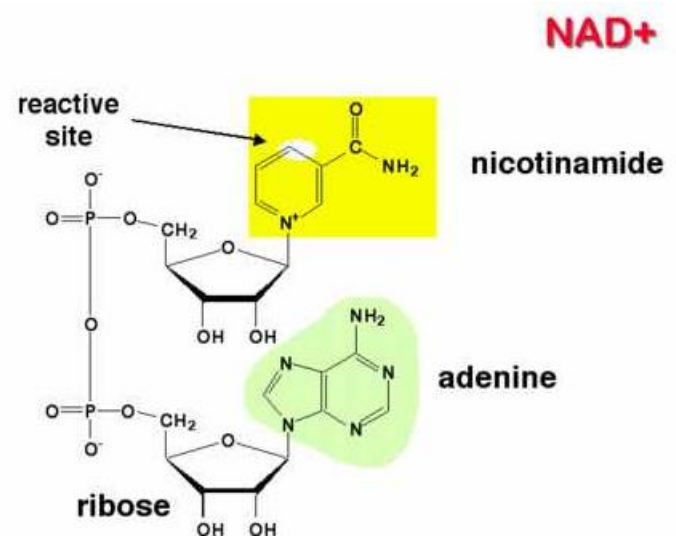
Factors affecting enzyme activity

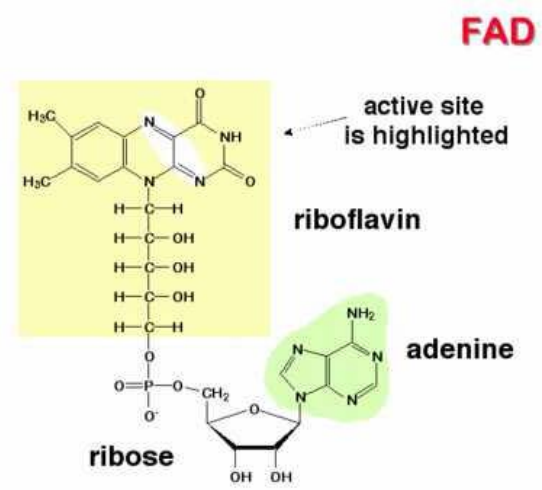
❖ Coenzymes /Prostetic groups

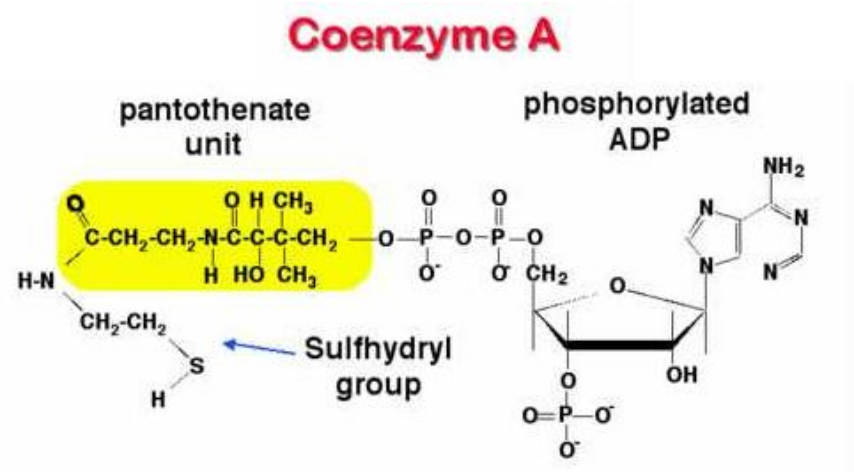
- Organic or organometallic molecule that assists an enzyme.
- are covalently linked or noncovalently bound very tightly to an enzyme partner.
- Part of each coenzyme structure is made from a vitamin.
- **Serve as transient carriers of specific atoms or functional groups**

Coenzyms/prosthetic groups	Chemical groups transfered	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Factors affecting enzyme activity







Factors affecting enzyme activity

❖ Metal ions

➢ Metal ions associated with an enzyme or substrate often participate in catalysis.

Common metal ions:

Na^+, K^+, Mg^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}

➢ They assist by one of the following actions.

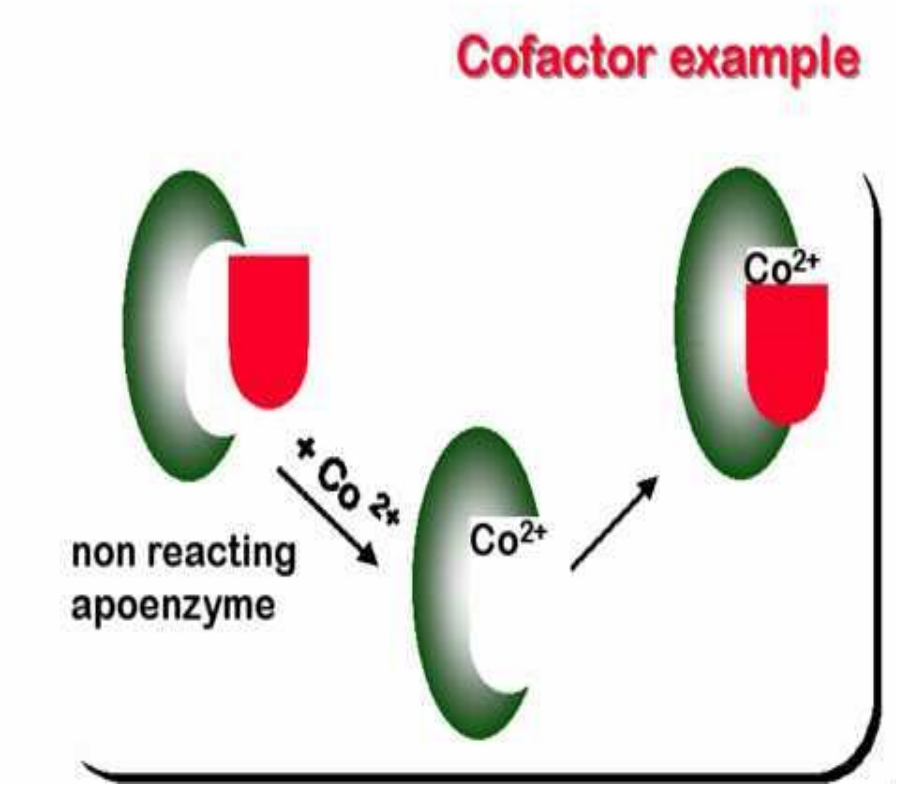
★ Properly holding substrate in place using coordinate covalent bonds

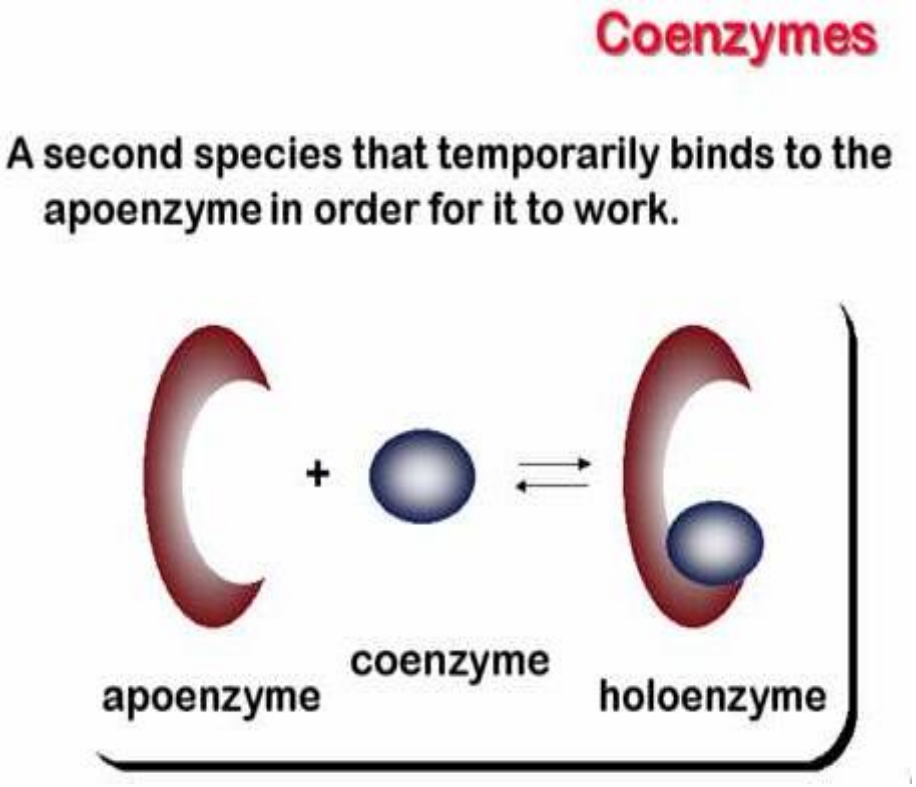
★ Enhance a reaction by polarizing the scissile bond or stabilizing a negatively charged intermediate.

★ Participate in an oxidation-reduction reaction.

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^+	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Factors affecting enzyme activity





Catalytic mechanism of enzyme

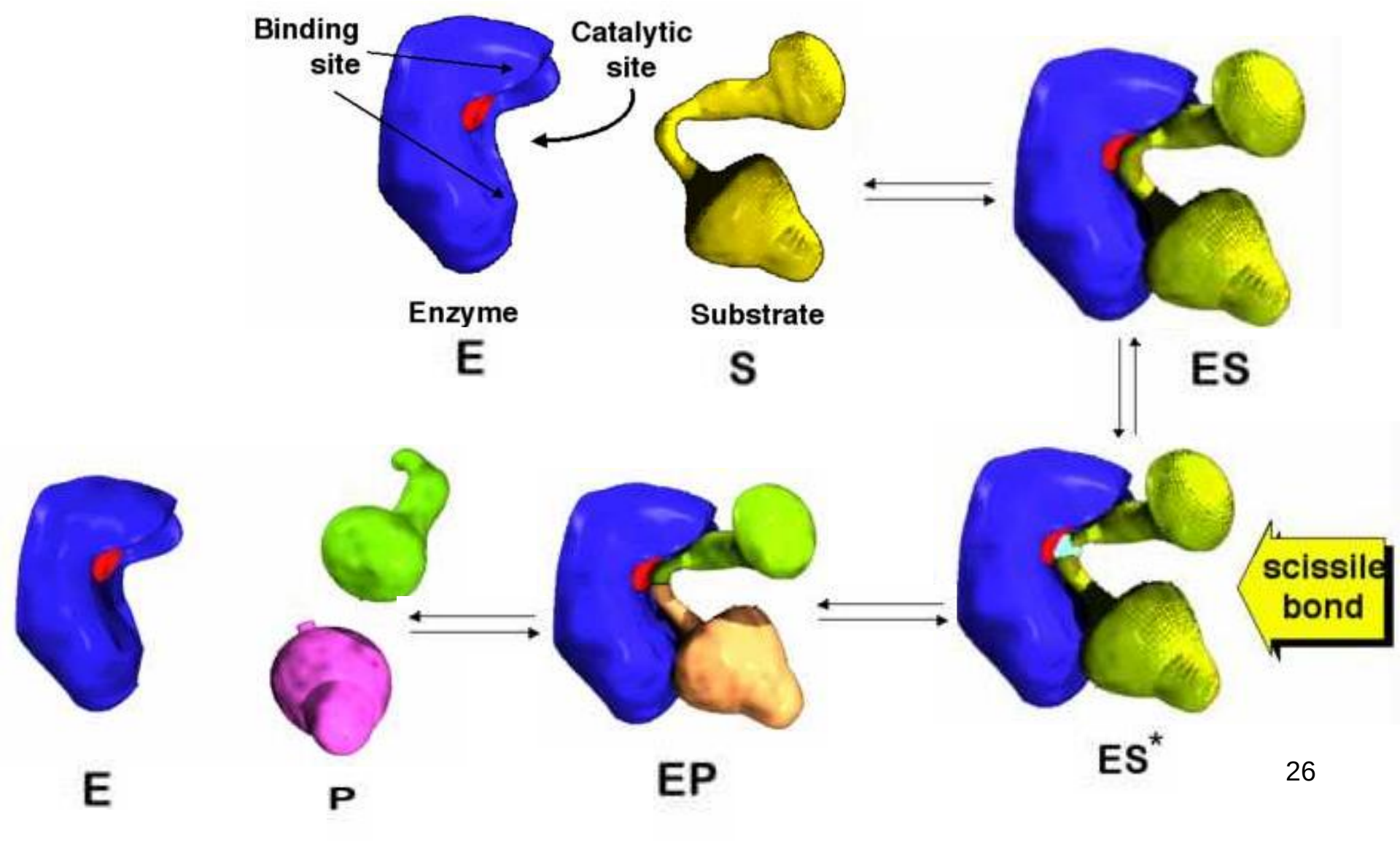
❖ **Steps in enzymatic reaction**

1. Enzyme and substrate combine to form a complex.
2. Complex goes through a transition state - not quite substrate or product
3. A complex of the enzyme and the product is produced
4. Finally the enzyme and product separate

All of these steps are equilibria.

Catalytic mechanism of enzyme

❖ **Steps in enzymatic reaction**



Catalytic mechanism of enzyme

❖ **Catalytic and binding site of enzymes**

Catalytic site

- Where the reaction actually occurs.

Binding site

- Area that holds substrate in proper place.
- Enzymes use weak, non-covalent interactions to hold the substrate in place based on R groups of amino acids.
- Shape is complementary to the substrate and determines the specificity of the enzyme.
- Sites are pockets or clefts on the enzyme surface.

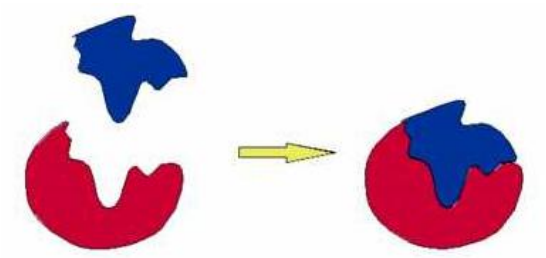
Catalytic mechanism of enzyme

❖ Models in enzyme catalysis

- ➢ Are explanations for enzyme's mechanism of action
- ➢ Two of these are

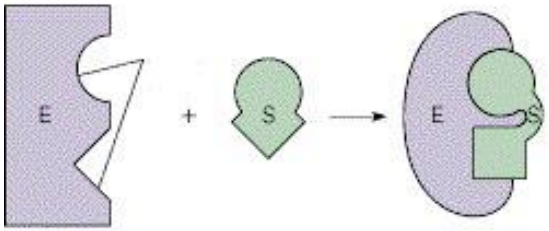
Lock and key model (Emil Fisher,1890)

✓**Assume that only substrate of proper shape could fit with the enzyme**



Induced fit model (Daniel Koshland,1958)

✓**Assume that enzymes have flexible structure**



Catalytic mechanism of enzyme

❑ Mechanism of enzyme catalysis

❖ **Enzymes catalyze reactions in various ways**

- ***Acid-base catalysis***
- ***Covalent Catalysis***
- ***Metal ion catalysis*** *(reading assignment)*
- ***Transition state stabilization****(reading assignment)*

Catalytic mechanism of enzyme

❖ Mechanism of enzyme catalysis

Acid-base catalysis

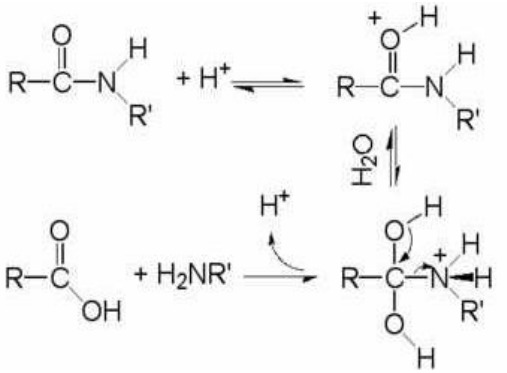
- Here an enzyme *avoids unstable charged intermediates in reaction* (which would have high free energies) by having groups(from its aa residues) appropriately located to:
  - ✓ **donate a proton** (act as a ***general acid***), or
  - ✓ **accept a proton** (abstract a proton, act as a ***general base***)
- Often used in the hydrolysis of ester/ peptide bonds, phosphate group reactions, addition to carbonyl groups, etc.

Catalytic functions of reactive groups of ionizable amino acids

Amino acid	Reactive group	Net charge at pH 7	Principal functions
Aspartate	$-COO^{\ominus}$	−1	Cation binding; proton transfer
Glutamate	$-COO^{\ominus}$	−1	Cation binding; proton transfer
Histidine	Imidazole	Near 0	Proton transfer
Cysteine	$-CH_2SH$	Near 0	Covalent binding of acyl groups
Tyrosine	Phenol	0	Hydrogen bonding to ligands
Lysine	$NH_3^{\oplus}$	+1	Anion binding; proton transfer
Arginine	Guanidinium	+1	Anion binding
Serine	$-CH_2OH$	0	Covalent binding of acyl groups

What side-chains of aa's can act donate or accept protons?

e.g

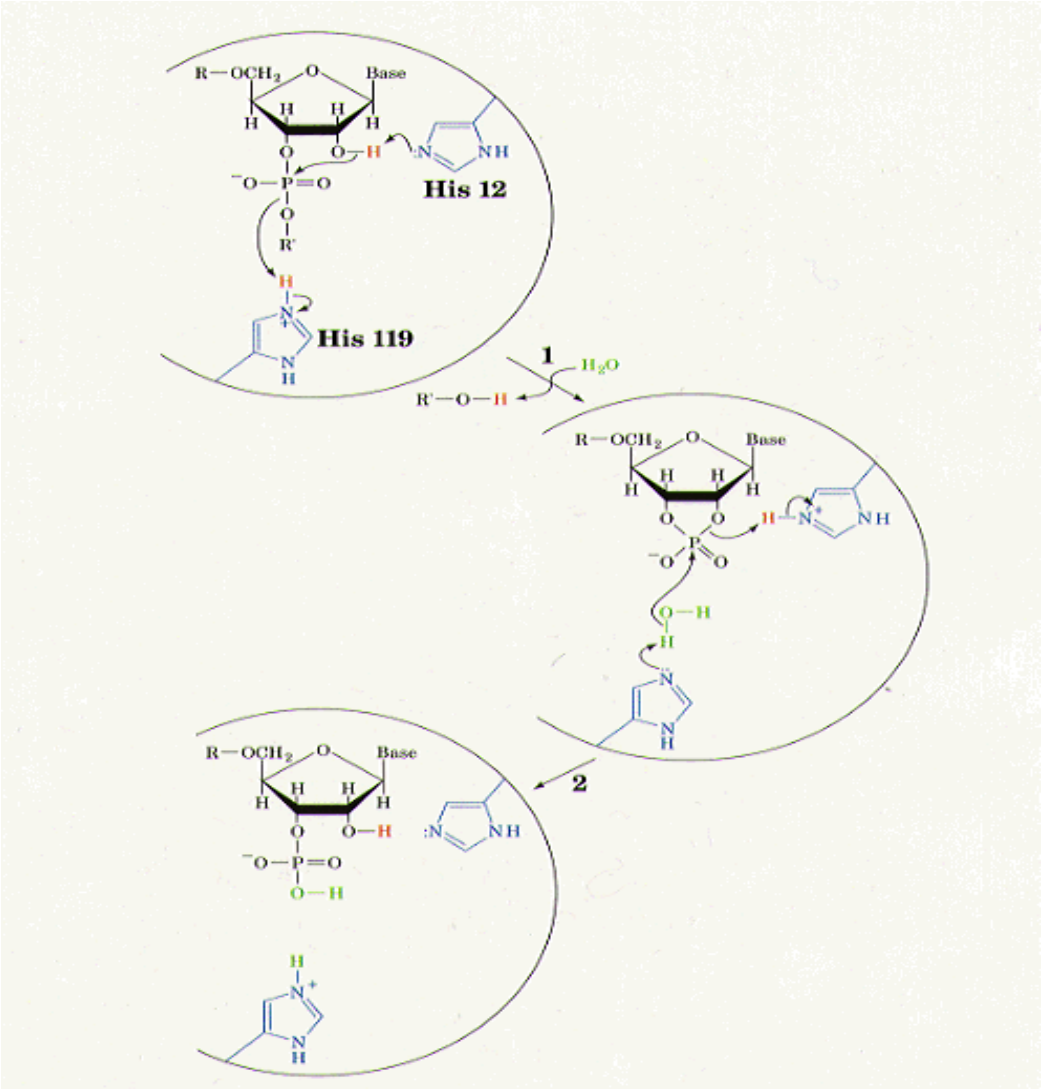


Catalytic mechanism of enzyme

❖ **Mechanism of enzyme catalysis**

Acid-base catalysis

RNase (Bovine pancrease)



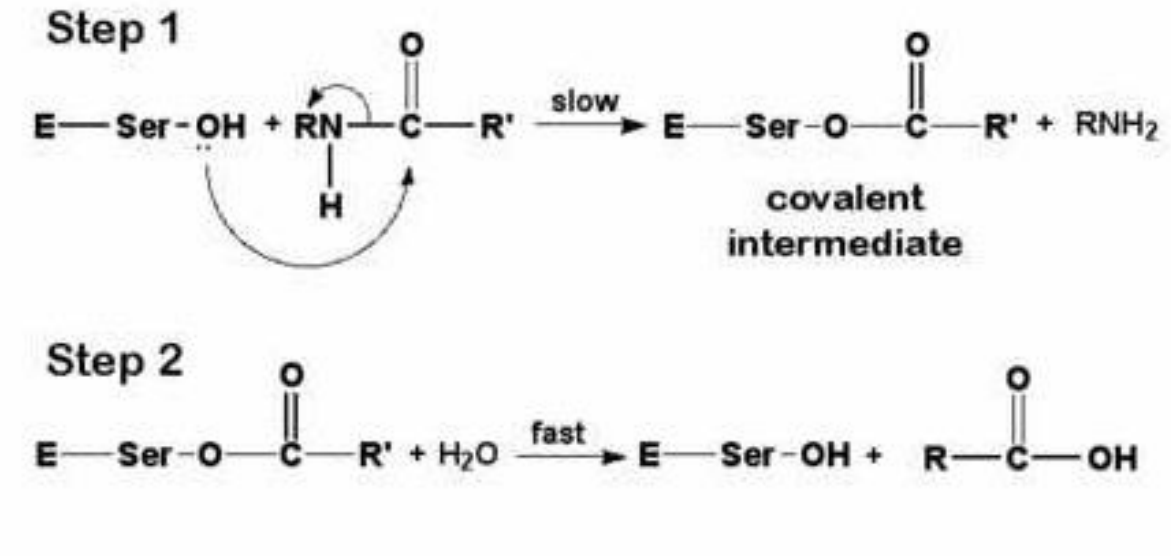
Catalytic mechanism of enzyme

❖ Mechanism of enzyme catalysis

➢ Covalent catalysis

- ✓ **Occurs when a nucleophilic functional group on enzyme reacts to a substrate**
  - **Mostly involve two steps**
- ✓ **Leads to highly reactive intermediate form**
  - **eg. Protein kinases, Serine proteases**

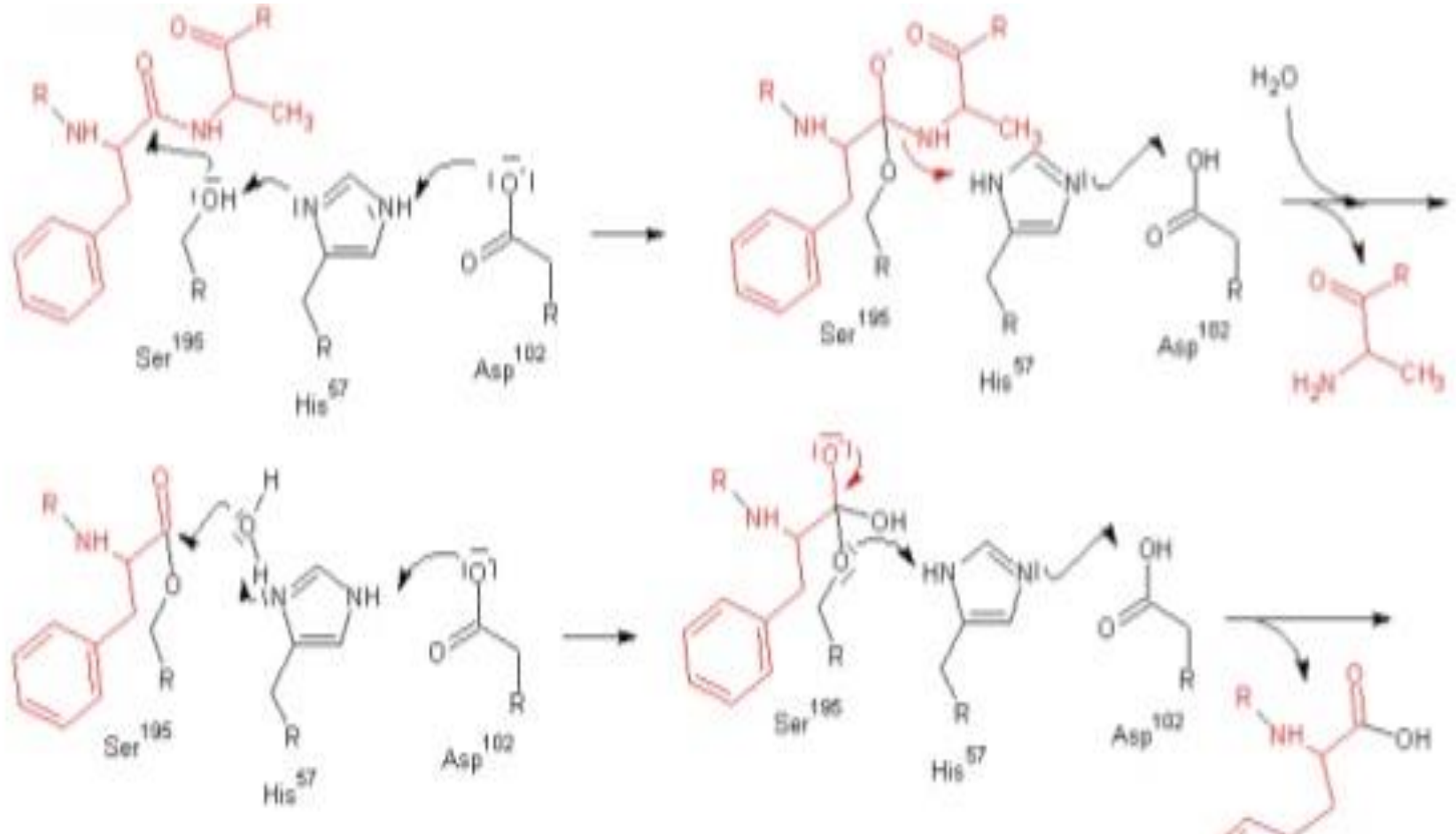
Serine proteases



Catalytic mechanism of enzyme

- **Mechanism of enzyme catalysis**
  - **Covalent catalysis**

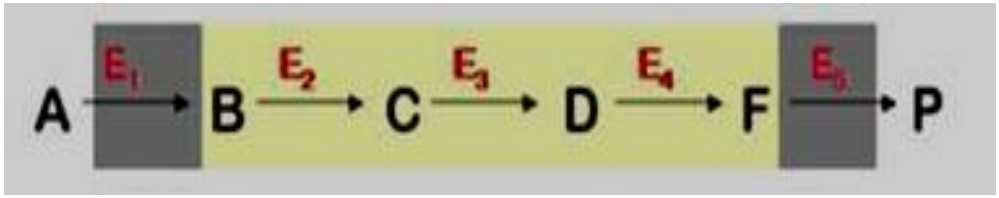
Chymotrypsin



Methods of enzymes regulation

Regulation of key enzyme in multistep reactions

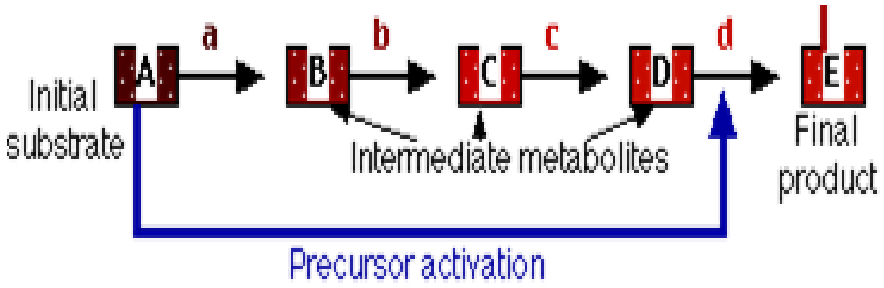
❖**Many of reactions in metabolic processes are arranged in sequences (multi-step)**



❖**Regulation of such processes are commonly achieved through:-**

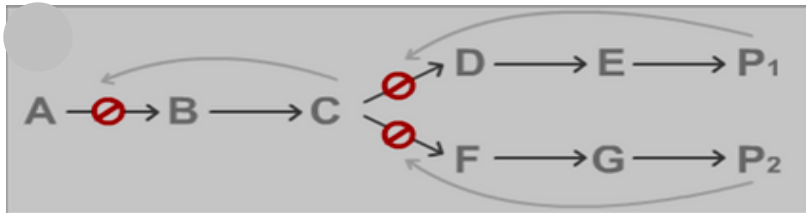
➢ **Precursor activation:-** accumulation of a substance may specifically activate specific enzyme (s) in a sequence .

✓ This reduces concentration of the initial substrate



➢ **Feedback inhibition:-** accumulation of a product in a series may specifically inhibit the action of previous enzymes

✓ This inhibit further production of the product



Methods of enzymes regulation

Anchoring/sequestering enzymes on membranes

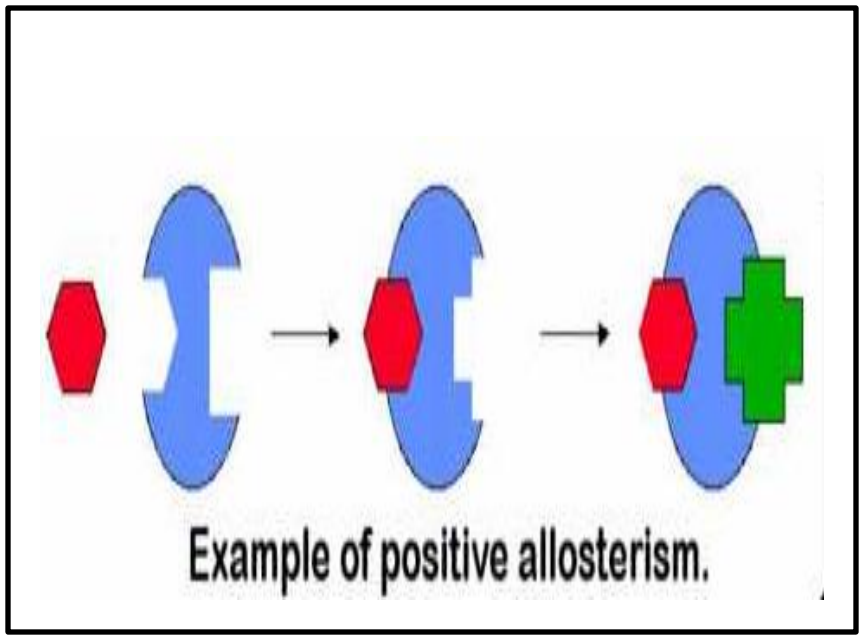
- Activity many enzymes are regulated by sequestering them into
  - Plasma membrane
  - Membranes of mitochondria and chloroplasts
  - Membranes of the endoplasmic reticulum
  - Nuclear envelope
- Hence access to their substrates is limited

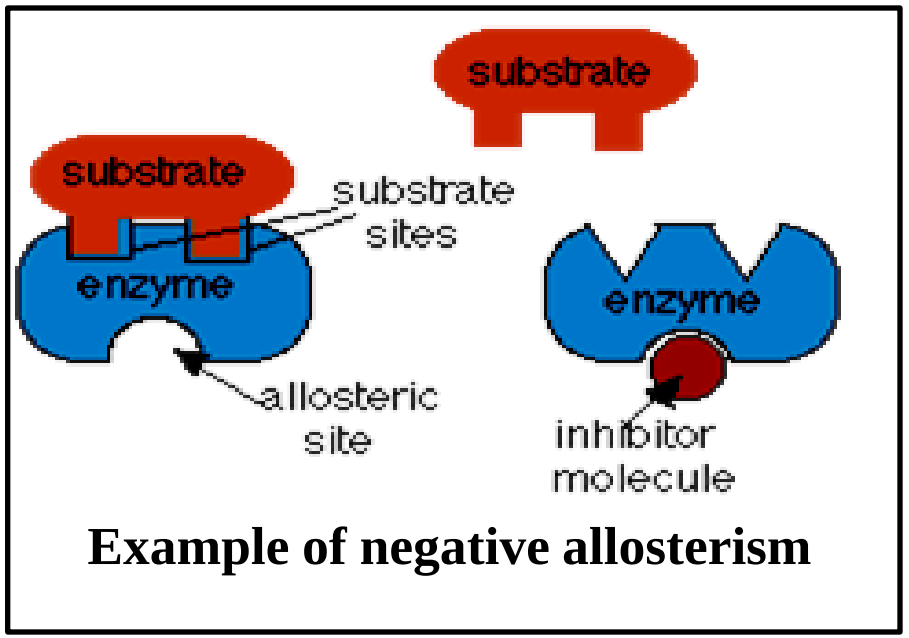
e.g Proteolysis of cell proteins and glycolipids by responsible enzymes is controlled by sequestering these enzymes within the lysosome.

Methods of enzymes regulation

Allosteric regulators

- The regulator molecule binds to the enzyme at a **different site (allosteric site)**
  - ✓ Thereby **alter shape** of the enzyme active site (**similar to coenzymes)**
  - -Substrates may be **promoted** or **inhibited** for binding





Methods of enzymes regulation

Covalent modification

- Involve reversible covalent changes to specific aa chain
- Common alterations include ***phsphorylation, acetylation, adenylation, reduction*** etc…

★ Phosphorylation of hydroxyl groups in serine, threonine or tyrosine.	⟹ ***Phosphorylation***
★ Attachment of an adenosyl monophosphate (AMP) to a similar hydroxyl group.	⟹ ***Adenylation***
★ Reduction of cysteine disulfide bonds.	⟹ ***Reduction***

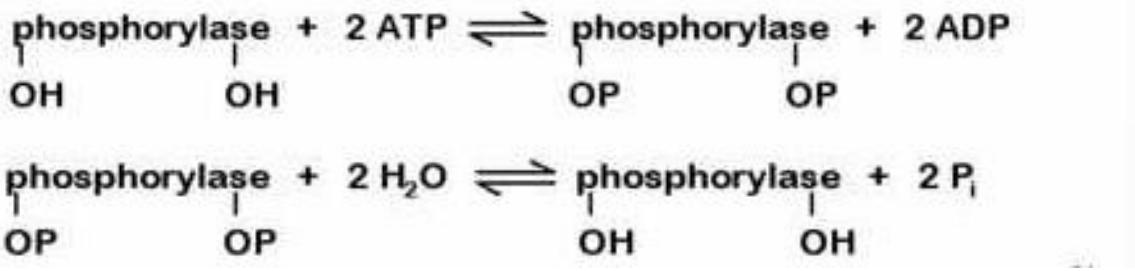
- This may result in activation or deactivation of enzymes

Methods of enzymes regulation

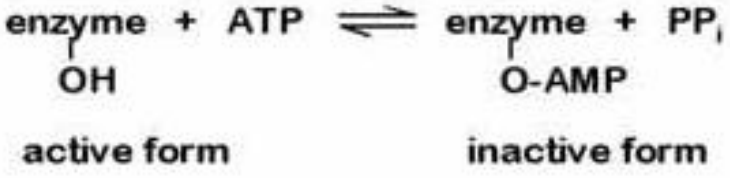
Covalent modification

Examples

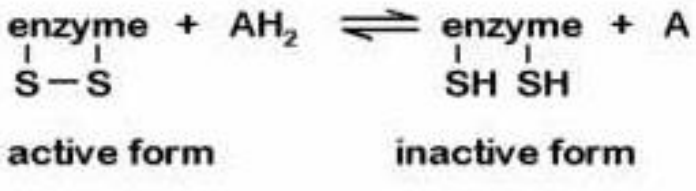
i) Control of glycogen phsphorylase



ii) Attachment of AMP to glutamine synthetase



iii) Reduction of cysteine disulfide bonds by AH_2



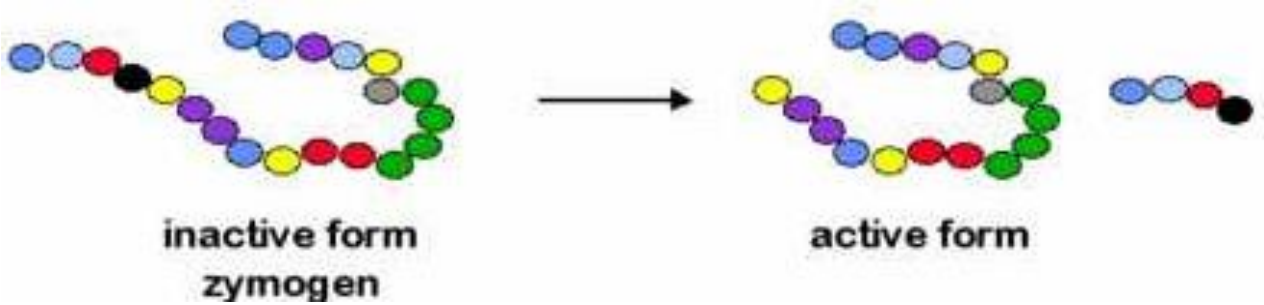
Methods of enzymes regulation

Proenzyme activation

- Some enzymes are synthesized as their inactive form (**Proenzyme/zymogen)** and are stored in secretory granules

e.g **Pepsinogen, trypsinogen and chymotrypsinogen**

- Upon release from their storage sites; they are **covalently activated**
  - ✓ That remove portion of the protein (irreversibly)
    - Mostly in **pH dependent** fashion



Enzyme inhibition

- Many substances can inhibit enzyme activity.

  substrate analogs, toxins,
  drugs, metal complexes

- Inhibition studies can provide:
  - Information on metabolic pathways.
  - Insight on how drugs and toxins exert their effects.
  - Better understanding of enzyme reaction mechanisms.

- Two broad classes of inhibitors have been identified based on the extent of interaction.

Irreversible

Forms covalent or very strong noncovalent bonds. The site of attack is an amino acid group that participates in the normal enzymatic reaction.

Reversible

Forms weak, noncovalent bonds that readily dissociate from an enzyme. The enzyme is only inactive when the inhibitor is present.

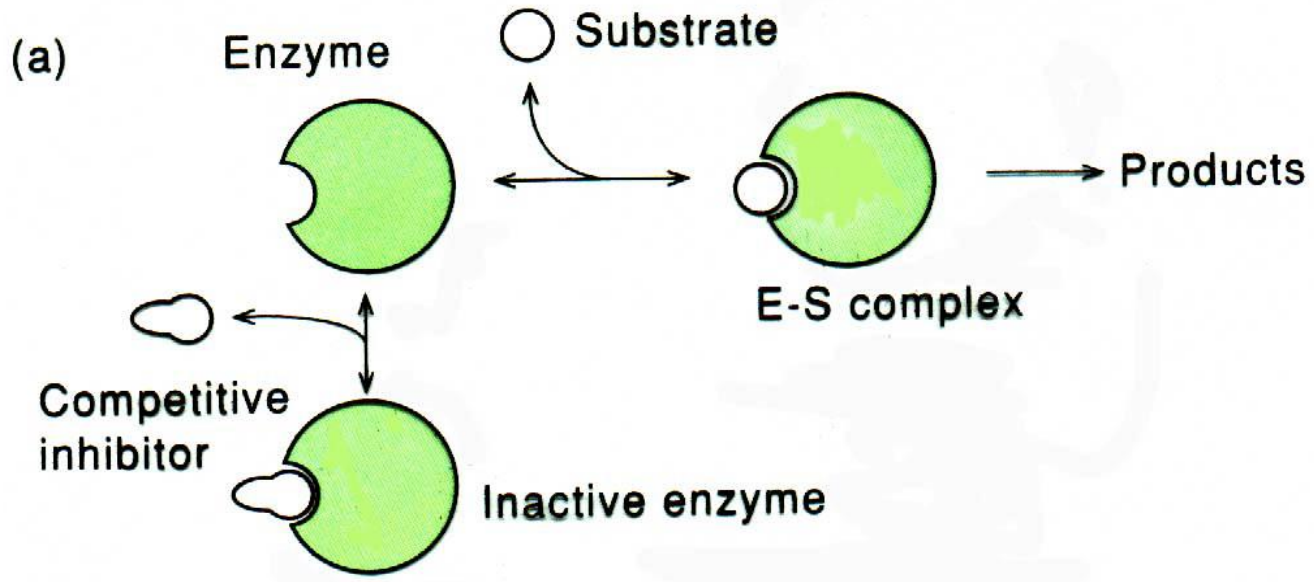
Reversible enzyme inhibition

Competitive reversible inhibitors

- Resemble substrate in structure & competes with it for binding site
- Effect could be reversed by increasing substrate concentration

Note :- Competitive inhibition can also occur in allosteric enzymes

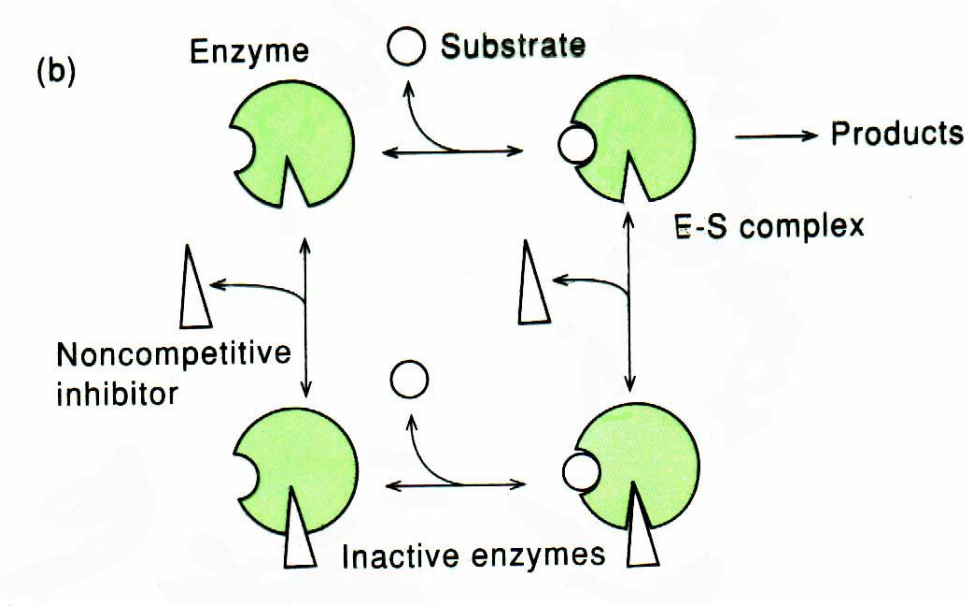
- Called nonclassical competitive inhibition)



Reversible enzyme inhibition

Noncompetitive reversible inhibitors

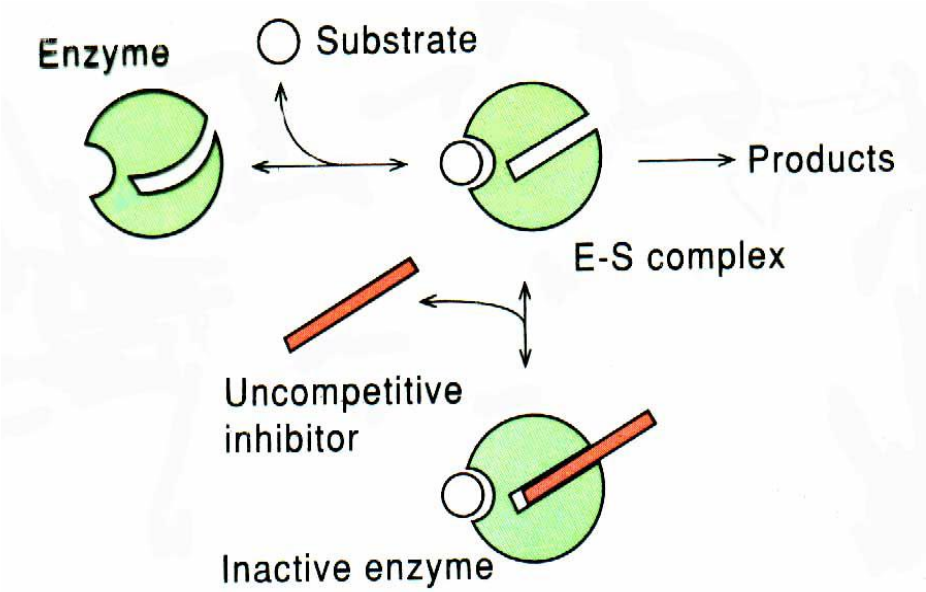
- Is typically observed in allosteric enzymes
- Inhibitor binds to site other than active site
  - ✓ And changes catalytic property of the enzyme
    - Enzyme denies from binding substrate
- Addition of excess substrate will not overcome the inhibition



Reversible enzyme inhibition

Uncompetitive reversible inhibitors

- Typically seen in multi-substrate reactions
- Similar to noncompetitive inhibitor but only binds to ES complex (not free E)
  - ✓affecting conversion of ES to E + P



Irreversible enzyme inhibition

Irreversible inhibitors

- Irreversibly bind to (or destroys) enzyme catalytic site (by covalent interaction)
- Bear no structural similarity with substrates
- Addition of excess substrate will not overcome the inhibition

Examples

- ✓**Heavy metal ions** (e.g Ag, Hg & Pb)
- ✓**Malathion** - specific for insect acetylcholinesterase (organophosphorus inhibitor)
- ✓**Diisopropylfluorophosphate**(nerve gas) - irreversibly binds to catalytic sites that contain Serine (OH)
- ✓**Cyanide** - binds to transition metals that are used as cofactors (Fe, Cu, Zn, etc.)
- ✓**Iodoacetate**- reacts with cyteine SH, imidizol, carboxy & R-S-R groups

Application of enzymes

- Metabolism
- Industrial processing
- Clinical diagnosis of diseases
- Therapeutics (drug targets, enzyme therapy)
- Food science and nutrition
- Organic synthesis
- Peptide sequencing
- Biomedical sciences (molecular biology, biotechnology, genetic engineering …)
- Forensic sciences
- As detergents
- Photography etc…