UNIVERSITY OF BASRAH
AL-ZAHRAA MEDICAL COLLEGE

Ministry of higher Education and Scientific Research

The module: Molecular, Gene and Diseases

Session 3: Lecture 1

Duration: 1hr

Lecture Title: Enzyme Activity: Kinetics & Inhibition

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This Lecture was loaded in blackboard and you can find the material in:

- Marks' Basic Medical Biochemistry Chapters 8, 9, 45
- Medical Biochemistry Chapters 5, 6
- Lippincott's Illustrated Reviews: Biochemistry Chapter 5

For more detailed instructions, any question, or you have a case you need help in, please post to the group of session

The Learning Objectives (LOs)

At the end of this lecture you should be able to:

1) Explain the effects of enzymes on chemical reactions. **(LO 3.1)**

2) Describe how reaction rates vary as a function of enzyme and substrate concentration. **(LO 3.2)**

3) Define the terms activity, international unit of enzyme activity, Km and Vmax. **(LO 3.3)**

4) Analyse and interpret kinetic data for enzyme-catalyzed reactions. **(LO 3.4)** → **SGS**

5) Describe the effects of enzyme inhibitors on enzyme kinetics and be able to distinguish between the two from simple graphs. **(LO 3.5)**

Enzyme (Catalyst): A substance (protein) that accelerates a chemical reaction but is not consumed or changed thereby.

Nearly, all of the chemical reactions that occur in the body are catalyzed by enzymes.

Effect of Enzyme on Chemical Reaction/ Example:

Glucose is consumed by a human (as a fuel), it releases free energy in **seconds** and converts to CO_2 and H_2O in the presence of oxygen. Without **catalysis** (Enzyme), chemical reactions could not occur **efficiently** and thus could not sustain life.

Enzyme Properties:

1. **All enzymes are proteins.**

With the **exception** of a few catalytic RNAs, all known enzymes (biological catalysts) are proteins.

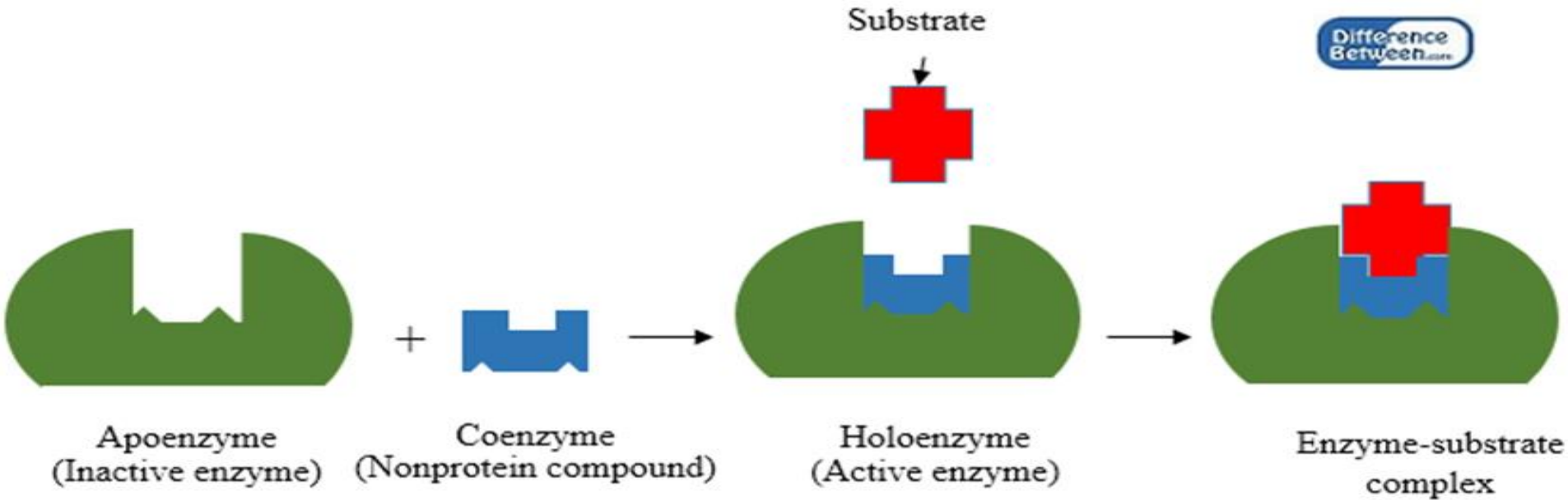
Some enzymes require the presence of additional chemical components (**non-peptide**) to catalyze reactions are known as:

- **Cofactors:** are inorganic ions (metals) such as Fe^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+}, Cu^{2+},…. etc.

- **Coenzymes:** are organic compounds that act as temporary carriers of groups in the reaction, e.g. nicotinamide adenine dinucleotide (NAD), Coenzyme A(CoA), vitamins.

Prosthetic groups (Cofactors & Coenzymes) that are covalently linked to the enzyme.

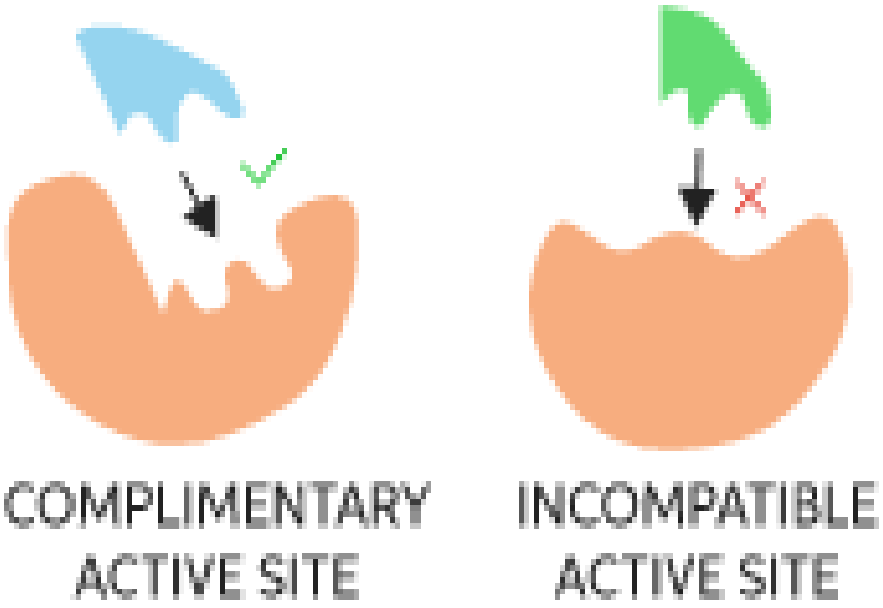
✓ **Apo- enzyme (Inactive)** + Cofactor, prosthetic group, or the coenzyme = **Holoenzyme (active)**



Enzyme Properties:

2. Enzymes are highly specific

- ✓ Interact with one or only a few substrates.
- ✓ Catalyze one type of reaction.

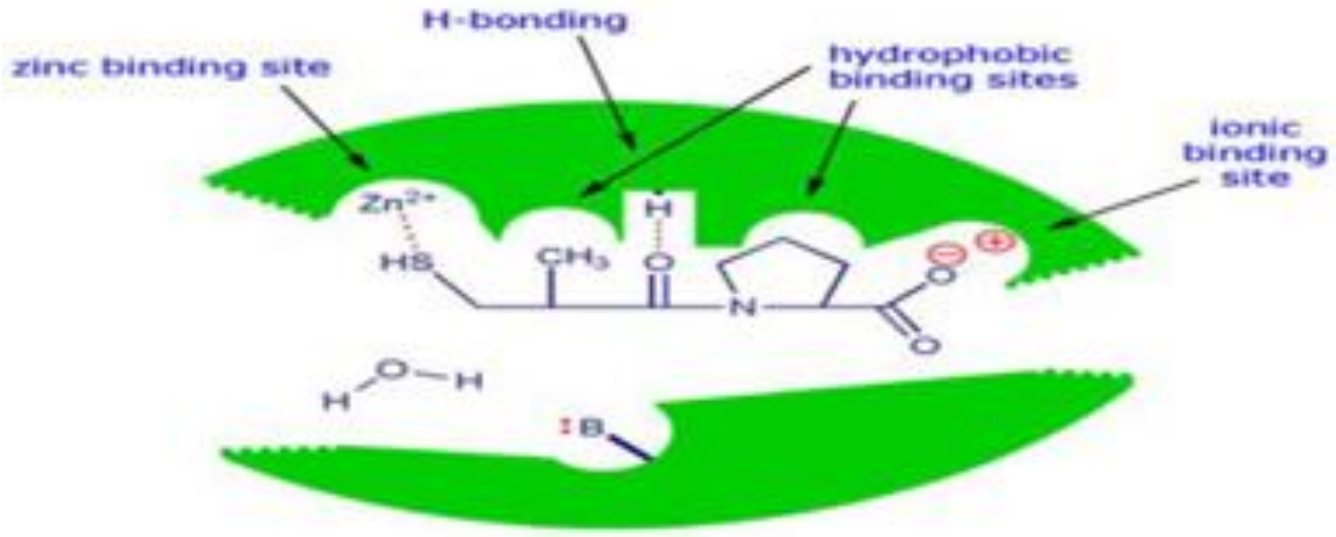


3. Enzymes increase the rate of a reaction by factors of 1 million or more.
They DO NOT affect the **equilibrium** of a reaction.

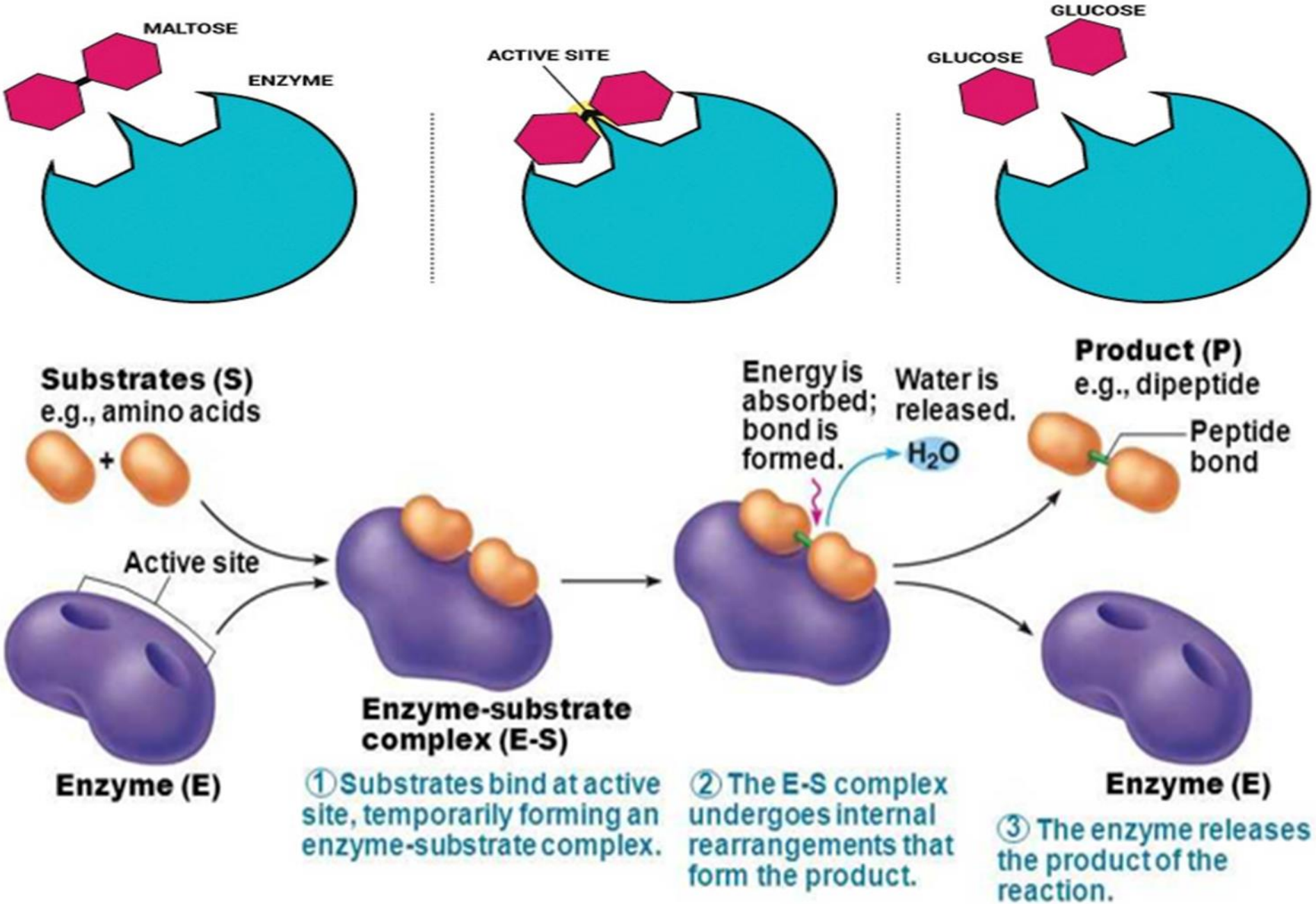
4. Enzymes are not changed after the reaction has occurred:

- ✓ Substrate binds in a pocket on the enzyme (**active site**), where reaction occur; forms a complex with enzyme (an ES complex).
- ✓ **The active site:** are usually clefts or crevices in the protein where the substrate bind by multiple weak bonds.
- ✓ ES is stabilized by weak interactions (hydrogen bonds, hydrophobic and ionic interactions), this forms part of the energy used for increase enzymatic rate.
- ✓ Multiple interactions is one reason of the large size of enzymes.

The active site

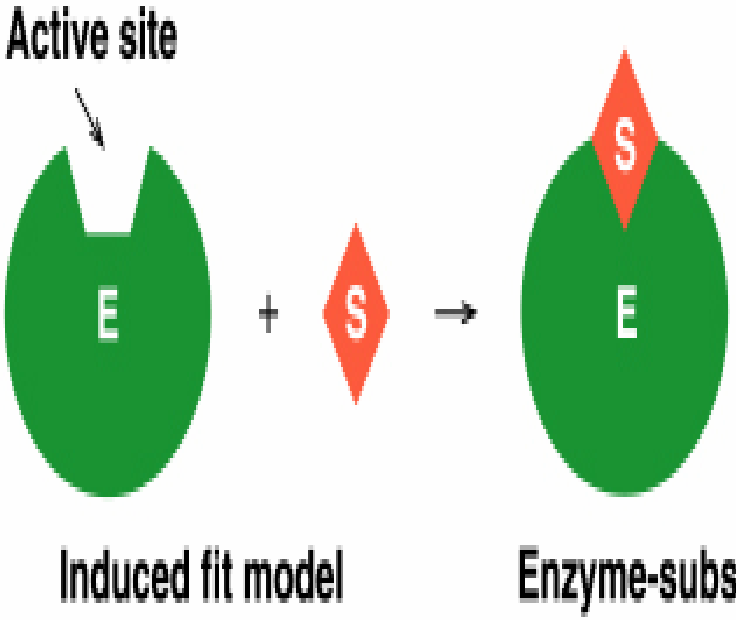


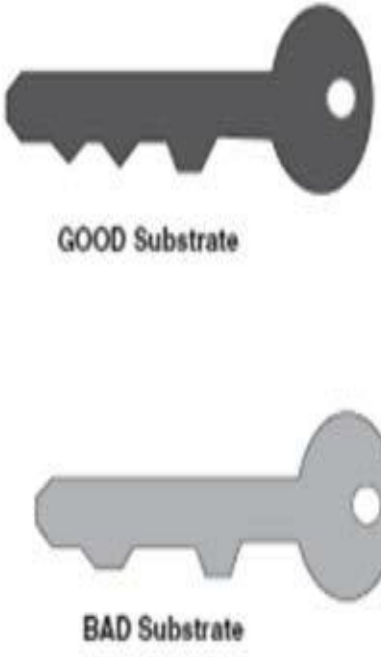
ENZYMES


MALTOSE
ENZYME
ACTIVE SITE
GLUCOSE
GLUCOSE
Substrates (S)
e.g., amino acids
+
Active site
Enzyme (E)
Enzyme-substrate complex (E-S)
Energy is absorbed; bond is formed.
Water is released.
H_2O
Product (P)
e.g., dipeptide
Peptide bond
Enzyme (E)
① Substrates bind at active site, temporarily forming an enzyme-substrate complex.
② The E-S complex undergoes internal rearrangements that form the product.
③ The enzyme releases the product of the reaction.

There are two theories that describe the substrate binding to an enzyme's active site: 1) Lock and Key Theory and 2) Induced Fit Theory.

- ✓ Highly specificity, only molecules that have a complementary shape to the active site will be able to bind **(Lock and Key Hypothesis).**
- ✓ The binding often results in changes in the shape of the enzyme to enhance binding **(Induced Fit Hypothesis).**

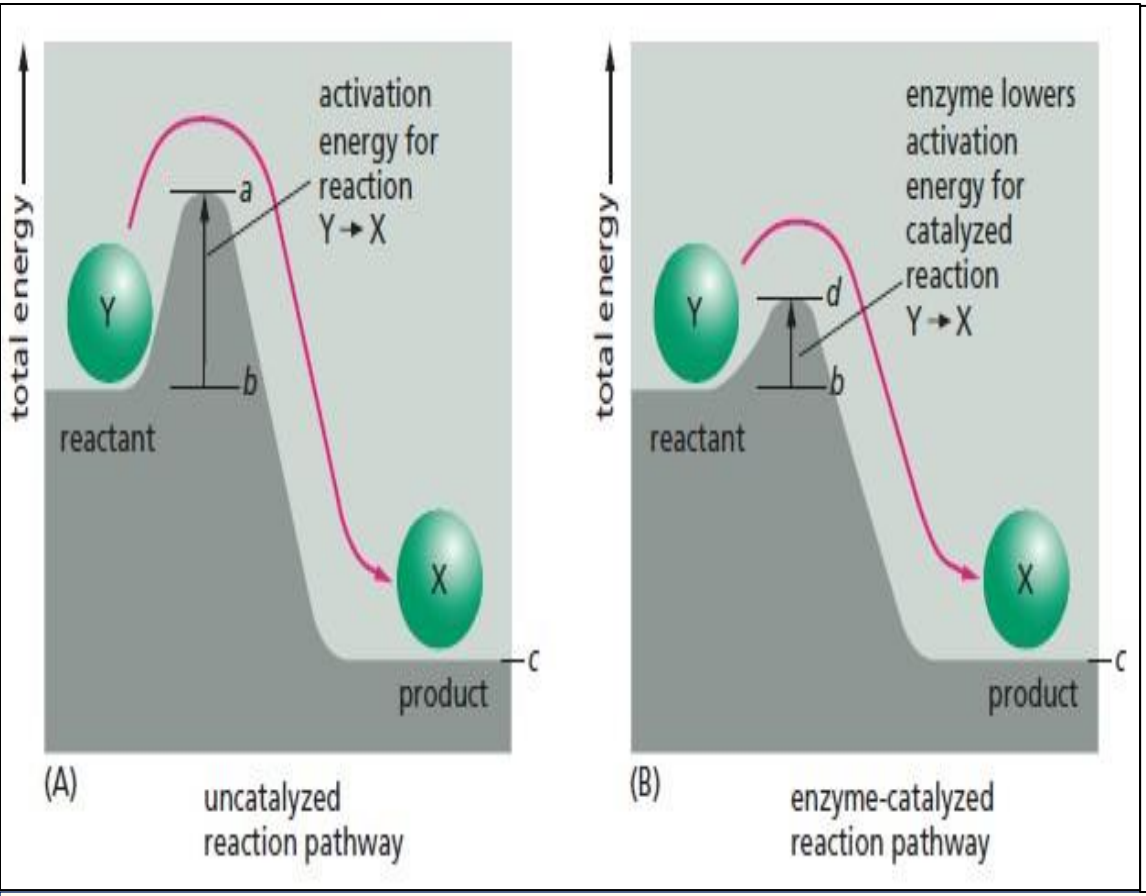


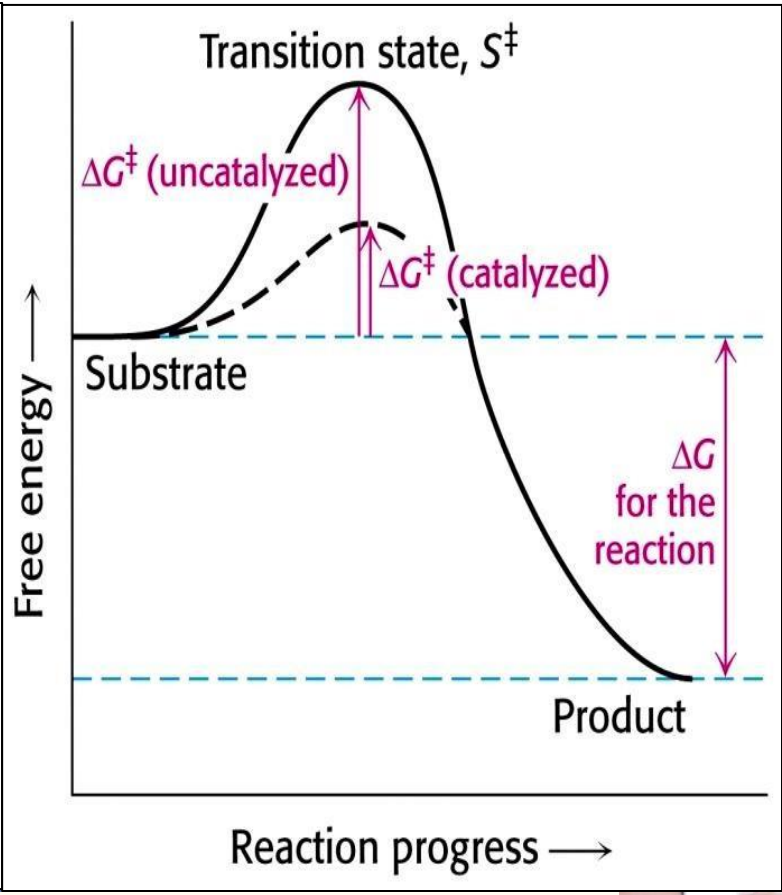


How do Enzymes Work?

LO 3.1

Enzymes work by **lowering the activation energy** needed for a reaction to occur.





❖ Enzymes Employ Multiple Mechanisms To Facilitate Catalysis:

- Catalysis often involves Multiple Mechanisms, to provide lower-energy reaction path.

✓ **Covalent catalysis:** formation of a **transient** covalent bond between a substrate and a residues in the enzyme active site or with a cofactor.

✓ **Metal ion catalysis**: A metal ion in the active site participates in catalysis by coordinating charge stabilization.

✓ **Catalysis by Proximity or strain:** an enzyme can accelerate a reaction between two species simply by holding the two reactants close together in an appropriate orientation.

✓ **Acid-base catalysis:** involve conformational change due to change in pH, so some ionic bond is broken or formed.

Nomenclature: Enzymes are named by 2 system (LO 3.1)

✓ **By adding the suffix " ase " to the name of substrate.**

Examples:- The enzyme catalyzes the hydrolysis of

Urea is called **Urease**

Arginine is called **Arginase**

Exceptions: trypsin, thrombin, pepsin.

This gives no idea of source, function or reaction catalyzed by the enzyme.

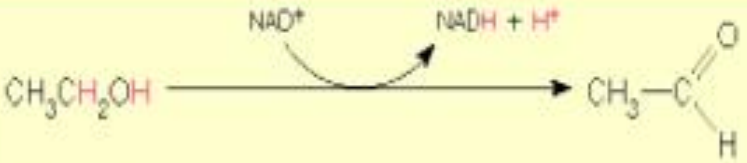
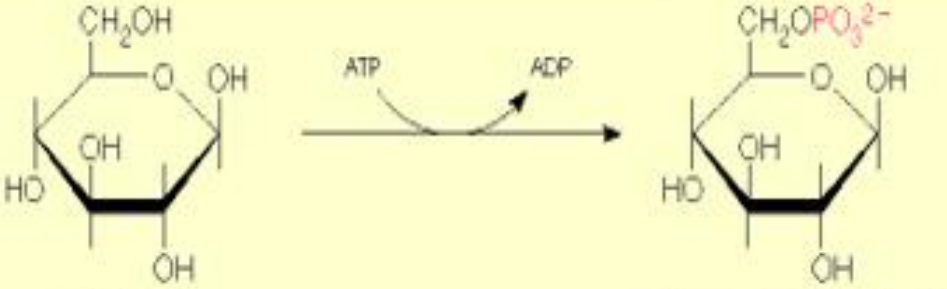
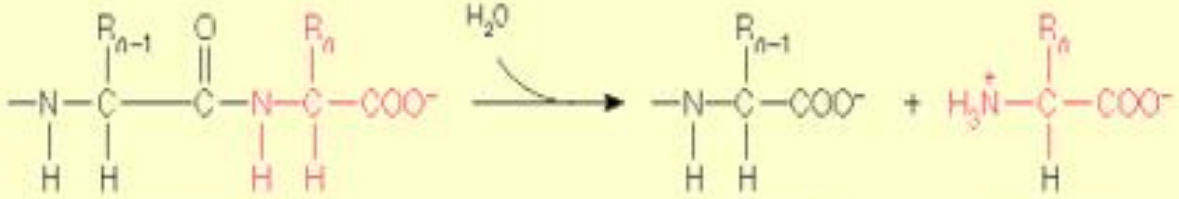
✓ **Systematic Name: IUB System:** According to the **I**nternational **U**nion of **B**iochemistry.

The enzyme name consists of 2 parts:

- The **1st** part: the name of substrate (s).
- The **2nd** part: the type of reaction (ending in – ase)

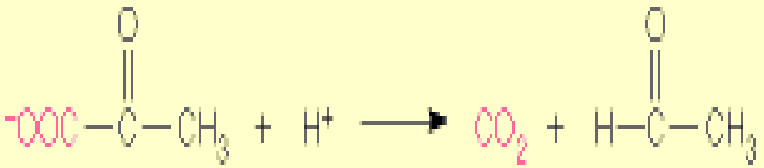
Examples :- Glutamate dehydrogenase, Aspartate aminotransferase.

Classification Of Enzymes (IUB system): Enzymes are classified into six different groups according to the **reaction being catalyzed**. **(LO 3.1)**

Class	Example (reaction type)	Reaction Catalyzed
1. Oxidoreductases	Alcohol dehydrogenase (EC 1.1.1.1) (oxidation with NAD^+)	
Oxidation-reduction reactions (i.e. transfer of electrons)		Ethanol → Acetaldehyde
2. Transferases	Hexokinase (EC 2.7.1.2) (phosphorylation)	
Transfer C-, N- or P- containing groups		D-Glucose → D-Glucose-6-phosphate
3. Hydrolases	Carboxypeptidase A (EC 3.4.17.1) (peptide bond cleavage)	
Catalyze cleavage of bonds by the addition of water		C-terminus of polypeptide → Shortened polypeptide + C-terminal residue

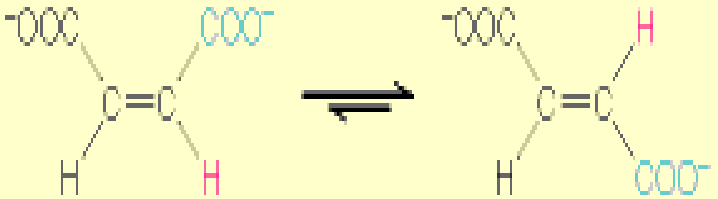
(LO 3.1)

4. Lyases — Pyruvate decarboxylase (EC 4.1.1.1) (decarboxylation)



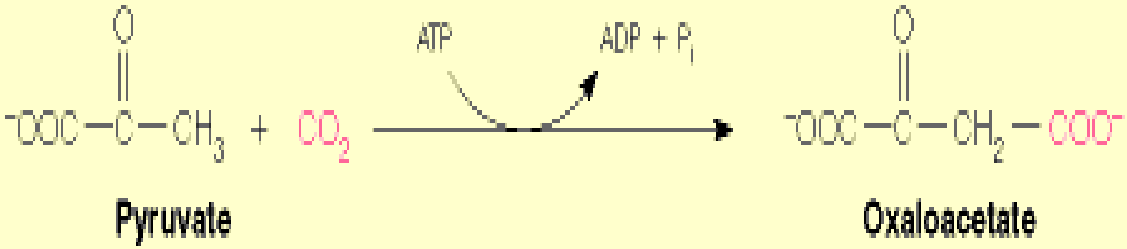
Addition or removal of groups

5. Isomerases — Maleate isomerase (EC 5.2.1.1) (*cis–trans* isomerization)



Transfer of groups within molecules to form isomers

6. Ligases — Pyruvate carboxylase (EC 6.4.1.1) (carboxylation)



Formation of bonds between C and O, S or N at the xpense of ATP

Enzyme Commission :

- Established the nomenclature for enzymes
- Numbers that follow E.C. gives the identity of the enzyme

Additional information: http://www.chem.qmul.ac.uk/iubmb/enzyme/index.html

The Michaelis- Menten Model for enzyme catalysis:

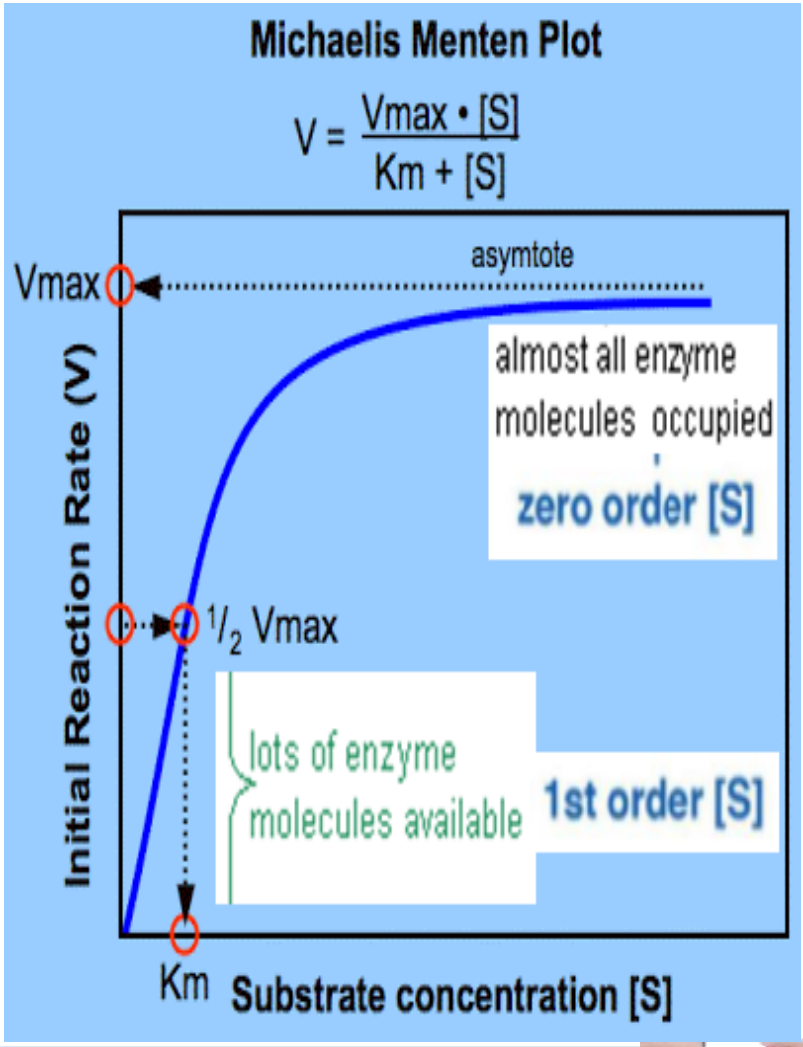
LO 3.2 & 3.3

An enzyme, E, combines **reversibly** with substrate, S, to form an enzyme-substrate intermediate, ES. ES can then break down to form free enzyme and the reaction product, P.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Substrate binding — Catalytic step

- The relationship between **[S]** and **Vo** has the general shape for most enzymes **(hyperbolic)**, which can be expressed algebraically by the **Michaelis- Menten equation.**



- ✓ V_0 = initial reaction velocity
- ✓ [S] = substrate concentration
- ✓ Vmax = the maximal velocity of an enzyme catalyzed
- ✓ reaction.
- ✓ Km = Michaelis constant, the substrate concentration that will give half the maximal rate (Vmax).

* **Low Km** means high affinity of enzyme to substrate.

* **High Km** means low affinity of enzyme to substrate.

The Lineweaver-Burk plot:

LO 3.2 & 3.3

Inverting the M-M Equation yields Lineweaver-Burke Equation:

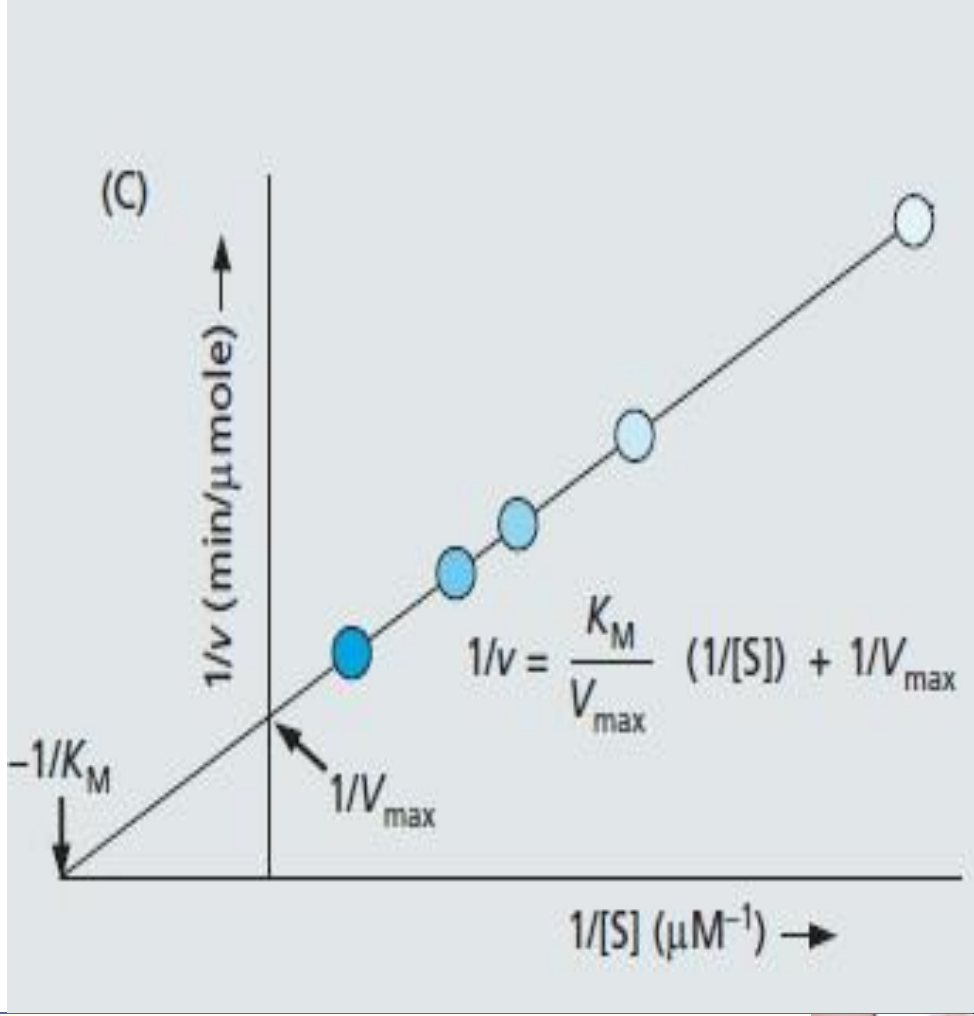
$$\frac{1}{V_O} = \frac{K_m + (S)}{V_{max}(S)}$$

$$\frac{1}{V_O} = \frac{K_m}{V_{max}(S)} + \frac{(S)}{V_{max}(S)}$$

$$\frac{1}{V_O} = \frac{K_m}{V_{max}(S)} + \frac{1}{V_{max}}$$

Straight line equation Y = ax +b

- **By plotting 1/ V as a function of 1/[S],**
- A linear plot is obtained:
- Slope = Km/Vmax
- Y-intercept = 1/Vmax

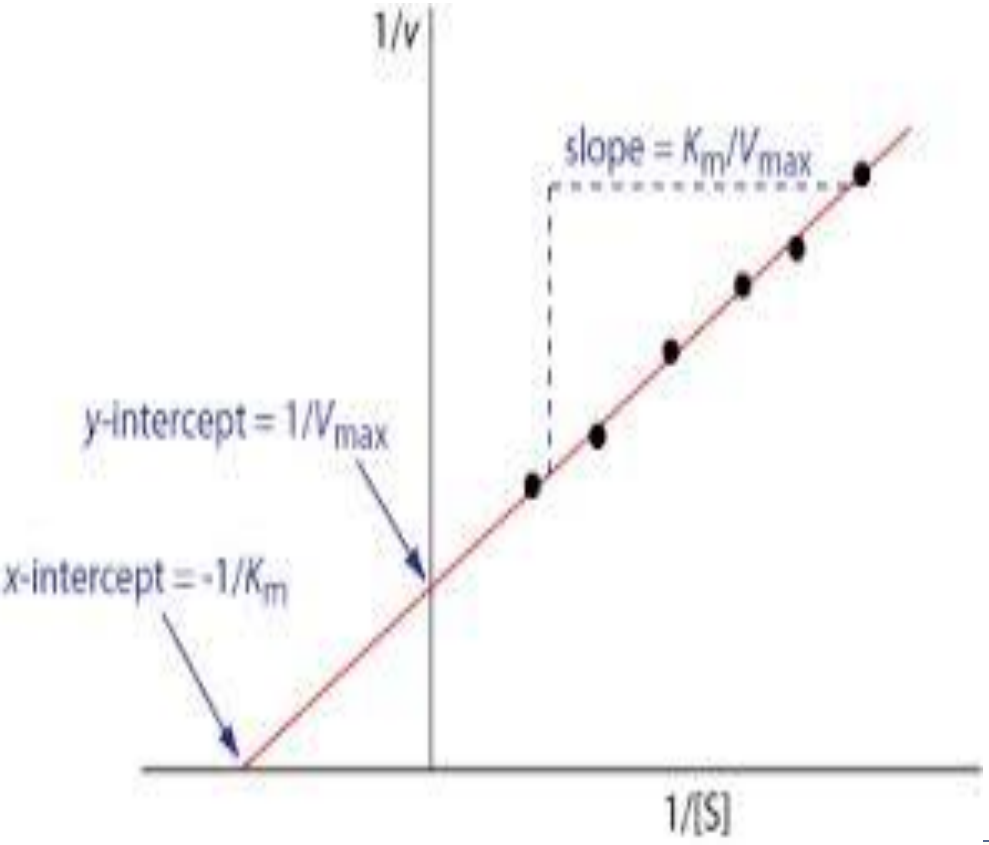


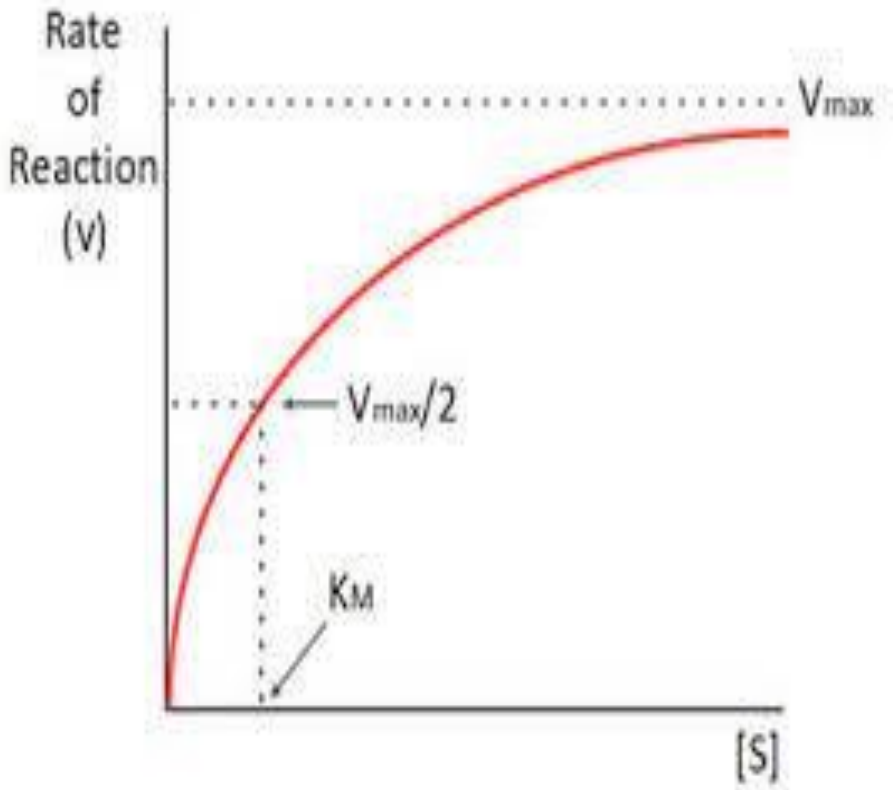
Lineweaver – Burke plot

Advantages of Lineweaver - Burke plot:-

1. Allow accurate determination of Vmax & Km.

2. Give valuable information on enzyme inhibition.





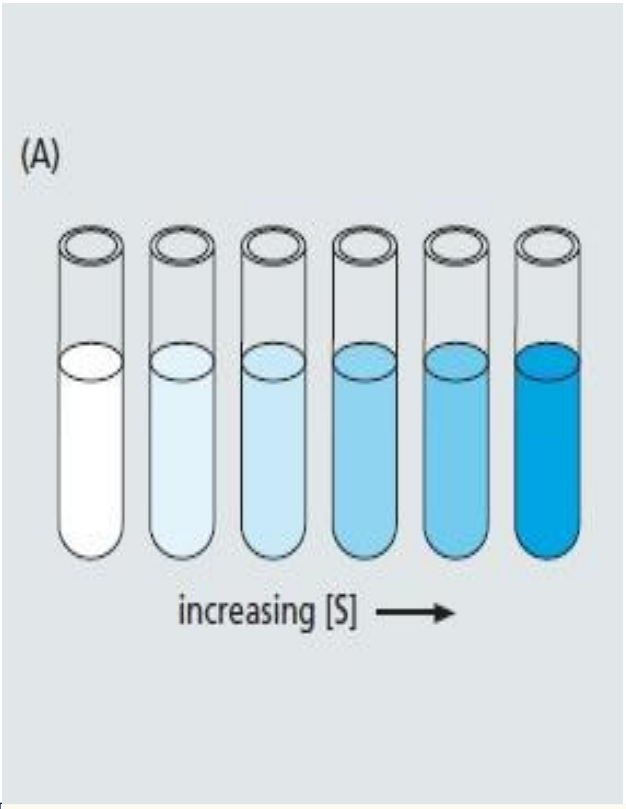
❖ Factors affecting enzyme activity (Reaction Rates):

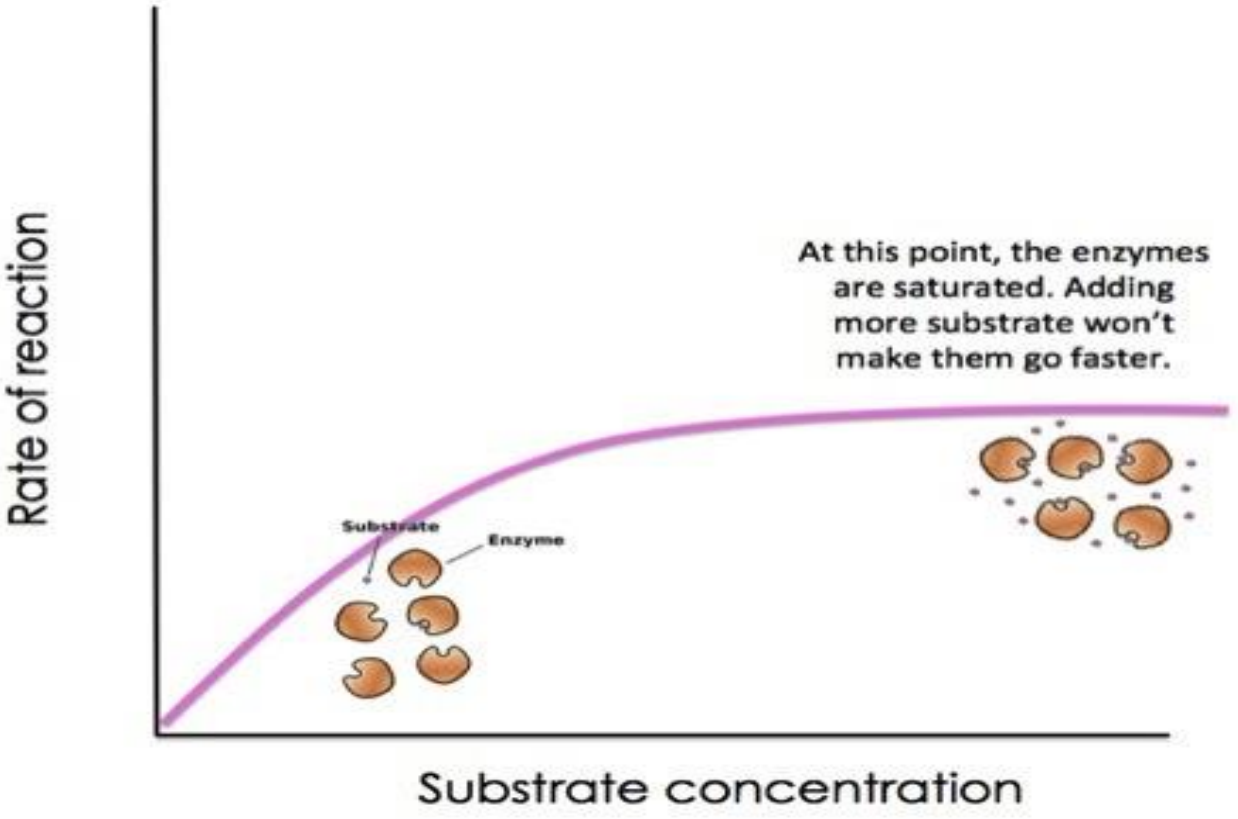
1. Substrate concentration
2. Enzyme concentration
3. Temperature
4. pH
5. Activators & inhibitors

1- Reaction Rates is affected by Substrate Conc.

LO 3.2 & 3.3

- Increasing substrate concentration also increases the rate of reaction to a certain point. Once all of the enzymes have bound, any substrate increase will have no effect on the rate of reaction, as the available enzymes will be saturated and working at their maximum rate.





1. Substrate concentration

LO 3.2& 3.3

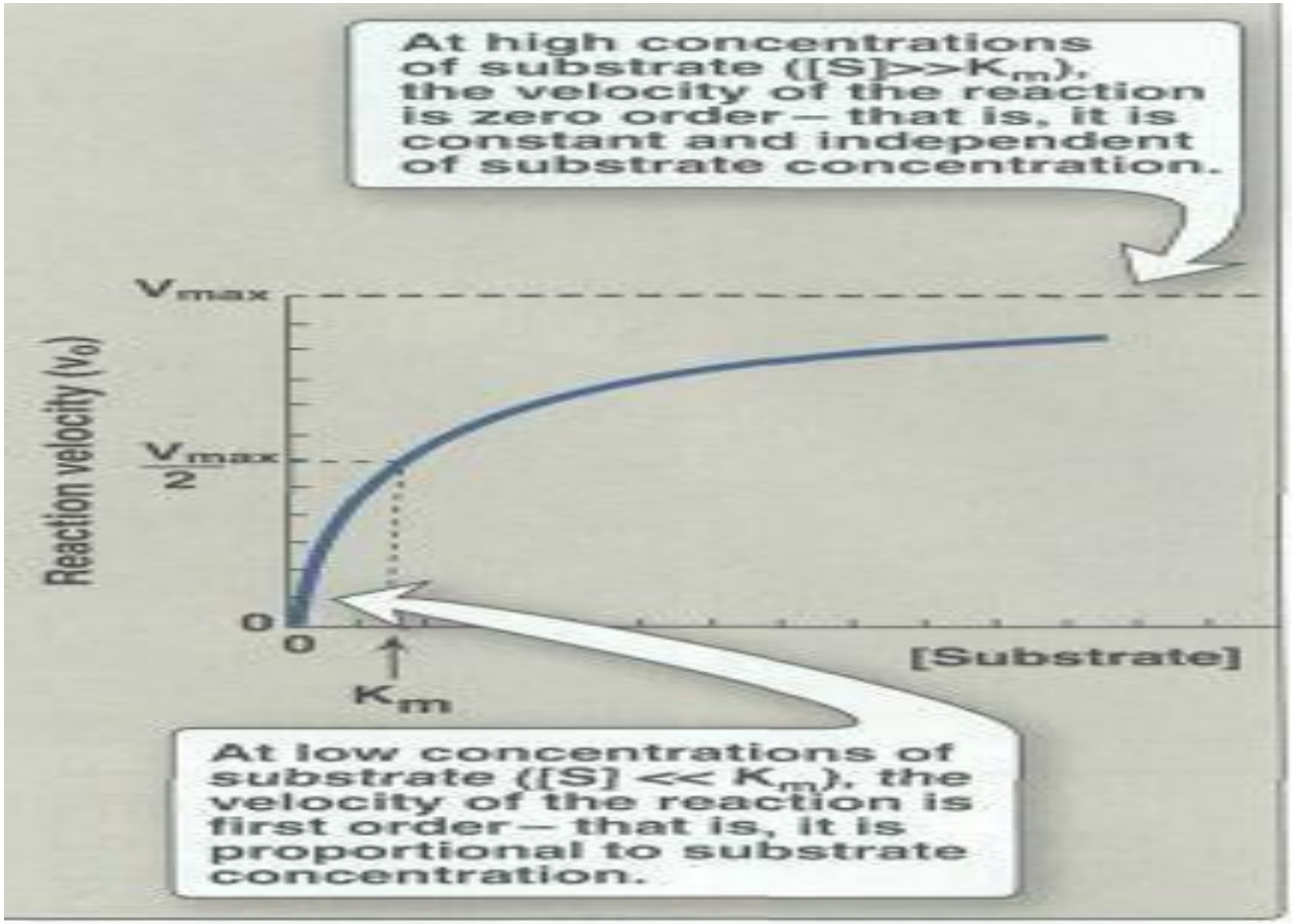


Figure 5.10
Effect of substrate concentration on reaction velocity for an enzyme-catalyzed reaction.

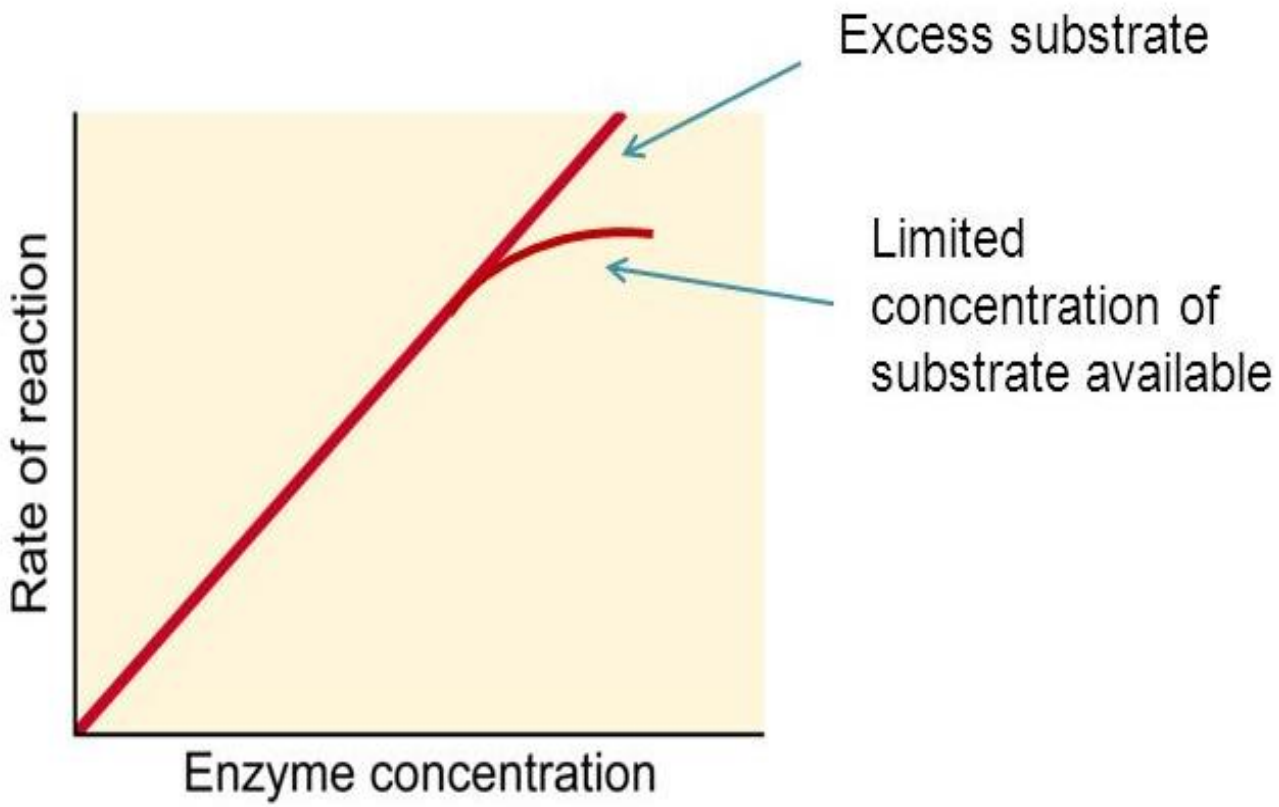
2- Reaction Rates is affected by Enzyme Conc.

LO 3.2 & 3.3

The rate of the reaction (velocity) is **directly proportional** to the enzyme concentration at all substrate concentrations.

Double the amount of enzyme → Double the amount of product/min

If substrate amount is limited, the rate of reaction no longer increases and the curve flattens out.



❖ 3. Effect of Temp.

↑temp lead to ↑rate of any reaction.
At more than 50°c most enzymes are denatured and inactivated, this lead to ↓rate of reaction.

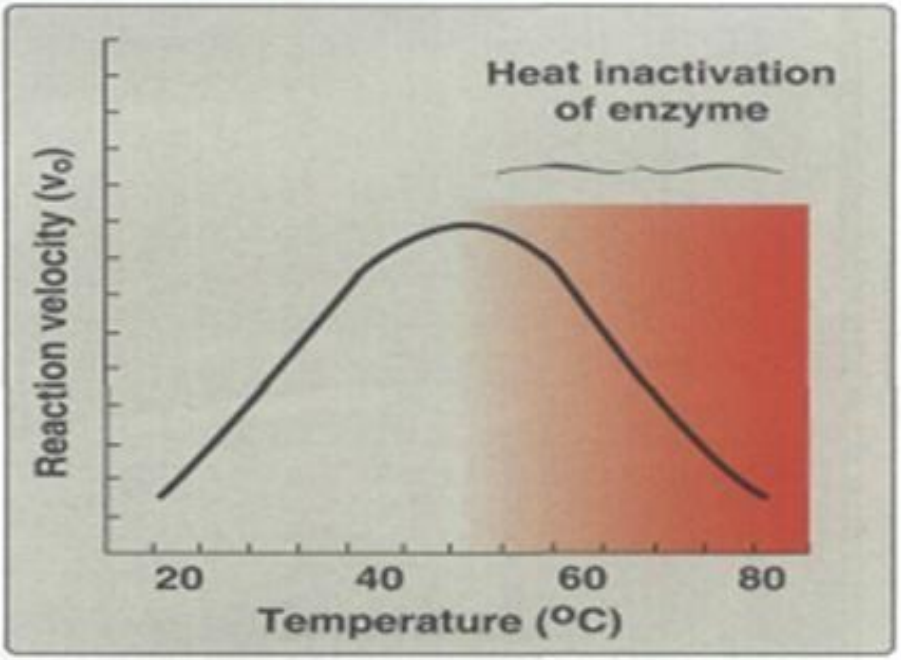


Figure 5.7
Effect of temperature on an enzyme-catalyzed reaction.

❖ 4. Effect of pH

LO 3.2 & 3.3

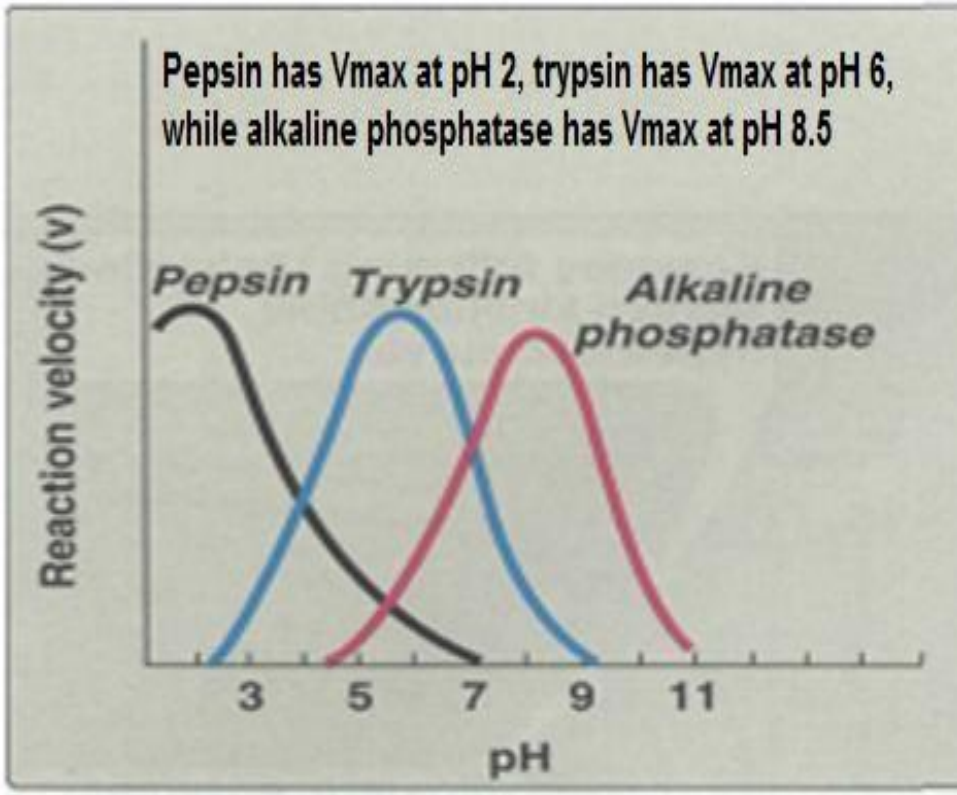


Figure 5.8
Effect of pH on enzyme-catalyzed reactions.

International unit of Enzyme Activity

LO 3.3

- **Activity:** the amount of product formed by per unit time.

  ✓ **Activity = amount of product / time**

- By international agreement, **1.0 unit of enzyme activity** is defined as the amount of enzyme causing transformation of 1.0 micromole of substrate per minute, under optimal conditions of measurement.

  ✓ **1 unit of activity = 1 µmol/min**

Inhibition of enzyme activity

LO 3.5

❖ Many drugs work by inhibiting the activity of enzymes.

- **The inhibitions may be**

1. Irreversible inhibitors: bind covalently to the enzyme molecule to destroy enzyme function.

2. Reversible inhibitors: bind weakly to enzyme.

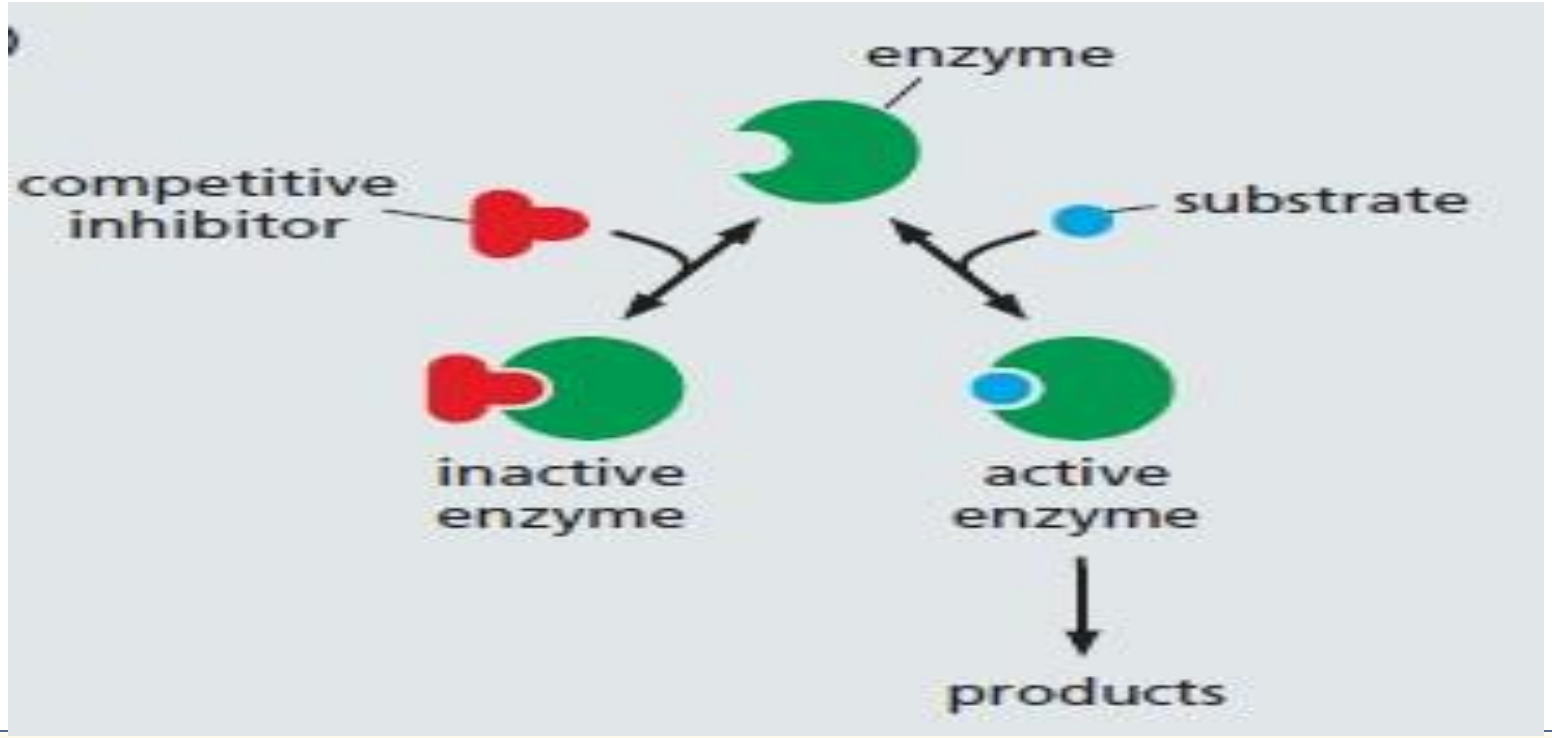
- The Reversible inhibition can be classified into **2 types**:
  - **Competitive inhibitors**
  - **Non-competitive inhibitors**

Examples?: Self learning

Competitive inhibitors:

LO 3.5

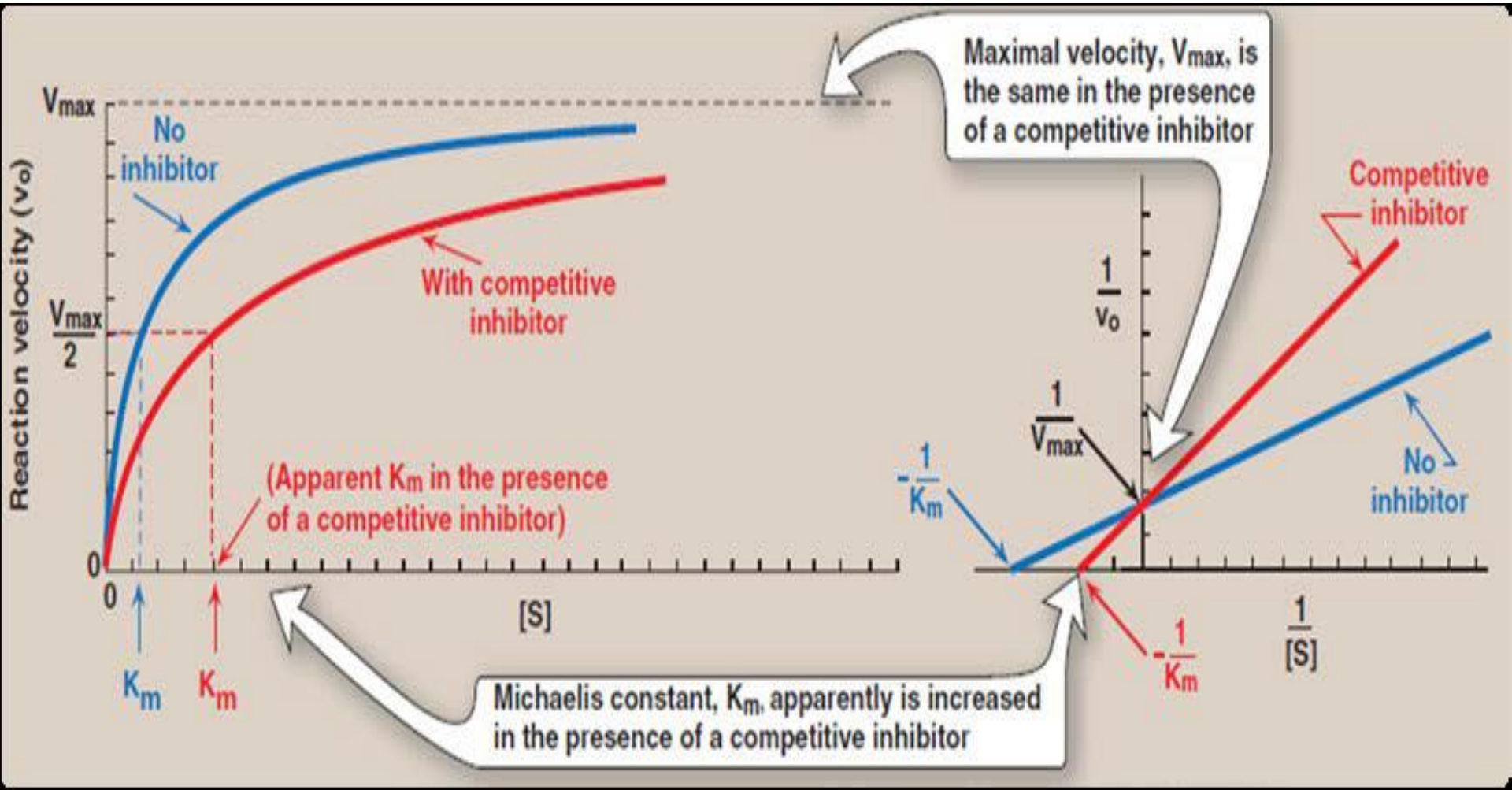
- The inhibitor **competes** with the substrate for binding at the active site.
- Competitive inhibitor has some structural similarity with the substrate and bind to the active site preventing the substrate from binding.



Competitive inhibitors:

LO 3.5

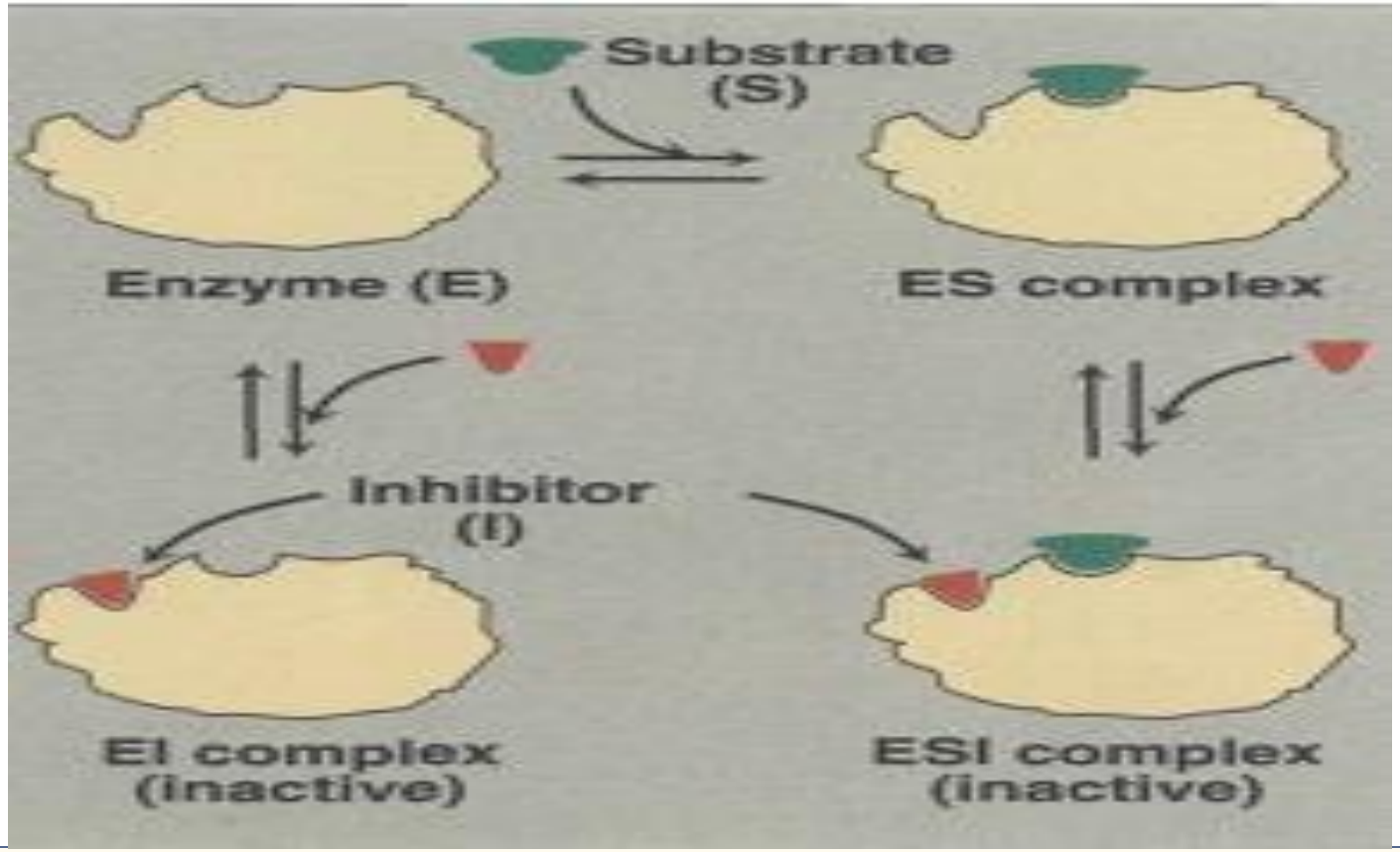
- ✓ Affects Km not Vmax **(Vmax: not changed / Km: increased)**
- ✓ Can be overcome by increasing the substrate concentration.
- ✓ Y- intercept is identical for both curves.



Non-Competitive inhibitors:

LO 3.5

- The inhibitor binds at a site other than the active site. So the inhibitor & substrate can bind at the same time, but at different sites, to the enzyme.



Non-Competitive inhibitors:

- ✓ Affects Vmax not Km (**Vmax: decreased /Km: not changed**) **LO 3.5**
- ✓ Km of the reaction is **not** changed in the presence of the non- competitive inhibitor.
- ✓ Cannot be overcome by increasing the substrate concentration.
- ✓ X- intercept is identical for both curves.

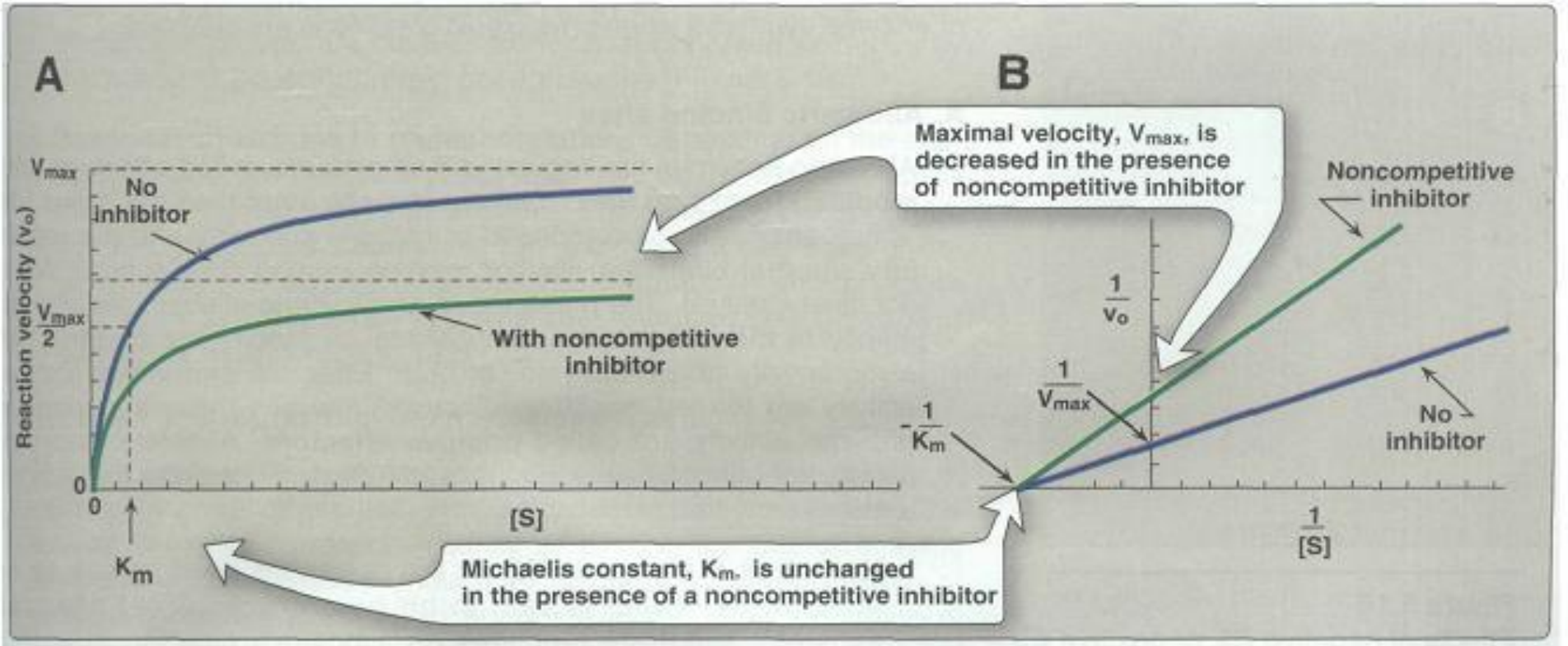


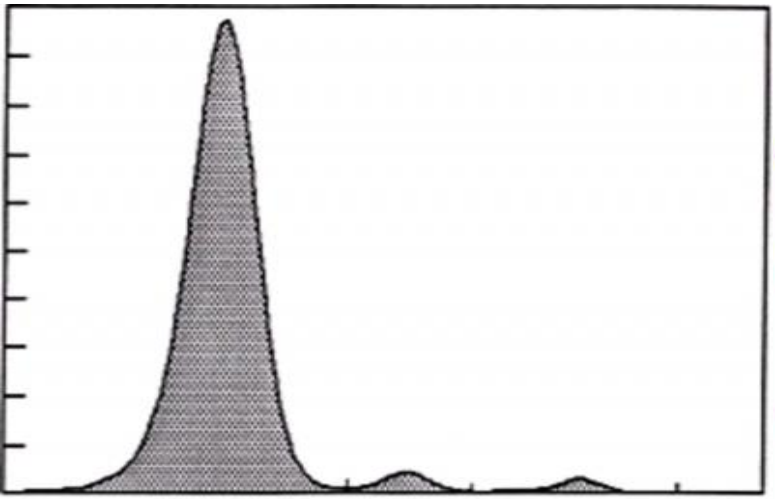
Figure 5.14
A. Effect of a noncompetitive inhibitor on the reaction velocity (v_o) versus substrate [S] plot. B. Lineweaver-Burke plot of noncompetitive inhibition of an enzyme.

❑ Isozymes

LO 3.3

Isozymes (also known as isoenzymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction.

In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes.



Fraction	%	U/L
CK-MM	95.3	7241
CK-MB	2.9	217
CK-BB	1.9	140
total CK		7598

Creatine kinase (CK) isoenzymes

Isoenzyme name	Composition	Present in	Elevated in
CK-1	BB	Brain	CNS diseases brain tumors
CK-2	MB	Heart	Acute myocardial infarction
CK-3	MM	Skeletal muscle	Skeletal muscle diseases

LO 3.3

Clinical significance:

- In some diseases, there may be a deficiency, absence, or excessive activity of one or more enzymes.
- Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in **diagnosing** certain illnesses.

Examples: Self learning
AST, ALT, Amylase

"Tell me and I **forget**, teach me and I may **remember**, involve me and I **learn**."

Thank you