University of Diyala/ College of Science

Department of Biotechnology

4^{nd} **stage**

Enzymes
(Lecture 1)

Edited by

Dr. Zeyad Khalouf

Enzymes: are **protein molecules** , produced by all living organisms. Act as highly efficient catalysts in biochemical reactions, that is, they help a chemical reaction take place quickly and efficiently. Enzymes are highly efficient in increasing the reaction rate of biochemical processes that otherwise proceed very slowly, or in some cases, not at all. Enzymes are excellent catalysts, speeding up reactions 105 to 1020 fold. They speed up reactions without being used up or consumed it.

The human body uses thousands of enzymes to carry out a myriad of biochemical processes. One clear example of an enzyme-assisted process is digestion. Enzymes help break down carbohydrates, fats and proteins into simple compounds that the body can absorb and burn for energy or use to build or repair tissue.

These include:

- Amylase and lipase in saliva break down carbohydrates and fats.

- Proteases (pepsin) released in the stomach aid in digestion of proteins;

Lipases, amylases, and proteases are secreted in the small intestine and play

a pivotal role in completing the digestive process.

Enzymes efficiency include three major ways:

1- Catalytic power: Catalysts increase the rate of chemical reactions without being used up in the process.

Enzymes act like many other catalysts by lowering the activation energy of a reaction, allowing it to achieve equilibrium more rapidly. The catalyst is able to reduce the activation energy by forming a transition state in a more favorable manner, and create a more "**comfortable**" fit for the substrate of a reaction to progress to a transition state. **Enzymes can end up the reaction in seconds than it might take hours or weeks under laboratory.**

2- Specificity: enzymes are highly specific in the reactions they catalyze. An enzyme with stereochemical specificity catalyzes the reaction of only one of two possible enantiomers (D-amino acid oxidase catalyzes the reaction of D-amino acids, but not L-amino acids).

In general, there are four distinct types of specificity:

1- Absolute specificity - the enzyme will catalyze only one reaction.

2- Group specificity - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate.

3- Linkage specificity - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.

4- Stereochemical specificity - the enzyme will act on a particular steric or optical isomer.

3- Regulation: enzymes activity as catalysts can be regulated, the catalytic behavior of enzymes can be regulated. A relatively small number of all of the possible reactions which could occur in a cell actually take place, because of the enzymes which are present. The cell controls the rates of these reactions and the amount of any given product formed by regulating the action of the enzymes.

Cofactors (Coenzyme and activator)

Some enzymes require an additional non-protein component for its optimum activity. This additional component is called *cofactor* which may be either loosely or tightly bound to the protein of the enzyme.

These cofactors may be:

Organic compounds, called *coenzymes*

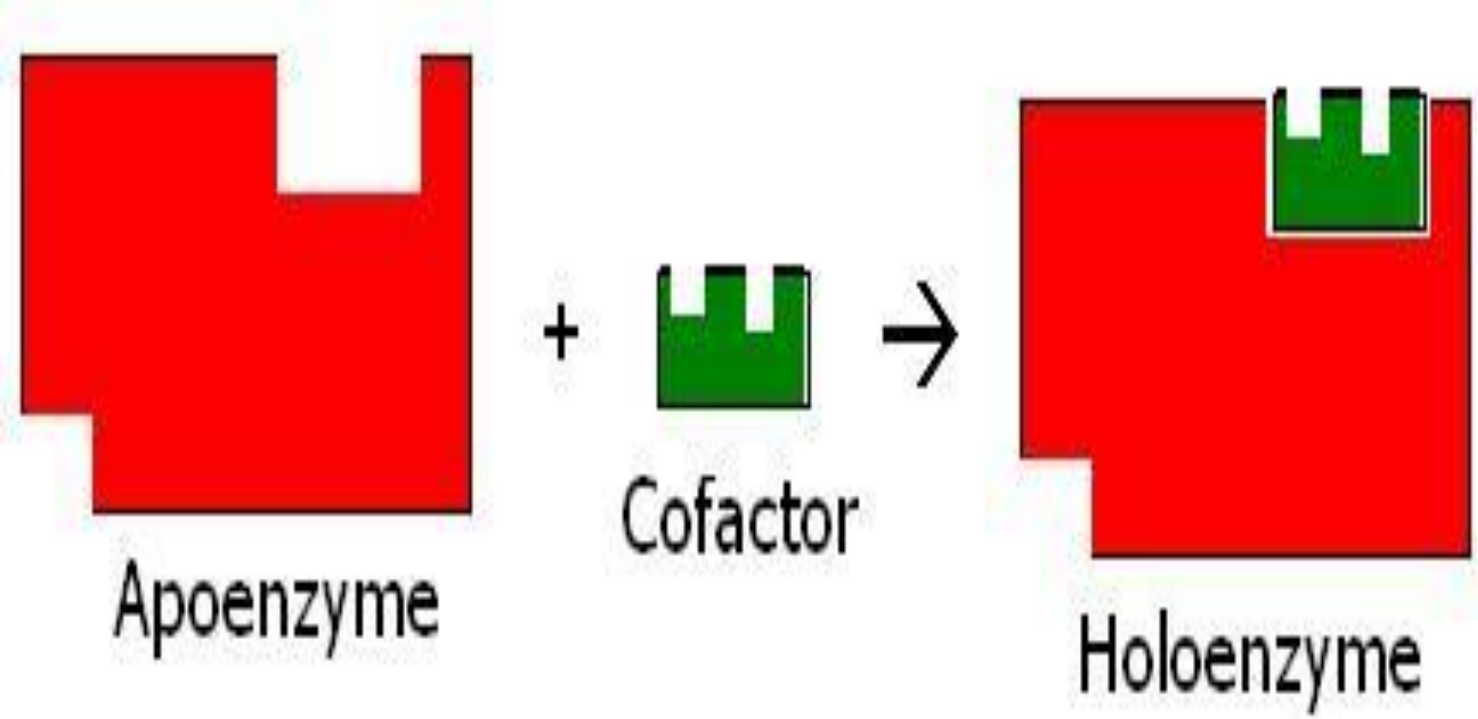
Inorganic ions, called *activators*.

Enzyme without its cofactor is referred to as an *apoenzyme*; the complete catalytically active enzyme is called *holoenzyme*.

Apoenzyme + cofactor = holoenzyme

Enzymes, like other proteins, have molecular weights ranging from about 12,000 to more than 1 million. Some enzymes require no chemical groups for activity other than their amino acid residues.

Others require an additional chemical component called a cofactor—either one or more inorganic ions, such as Fe2+ _, Mg2+ _, Mn+2 _, or Zn+2_, or a complex organic or metalorganic molecule called a coenzyme Some enzymes require both a coenzyme and one or more metal ions for activity.


Apoenzyme
+
Cofactor
Holoenzyme

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Enzymes
(Lecture 2)

Edited by

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Type of Enzyme

Intracellular enzymes

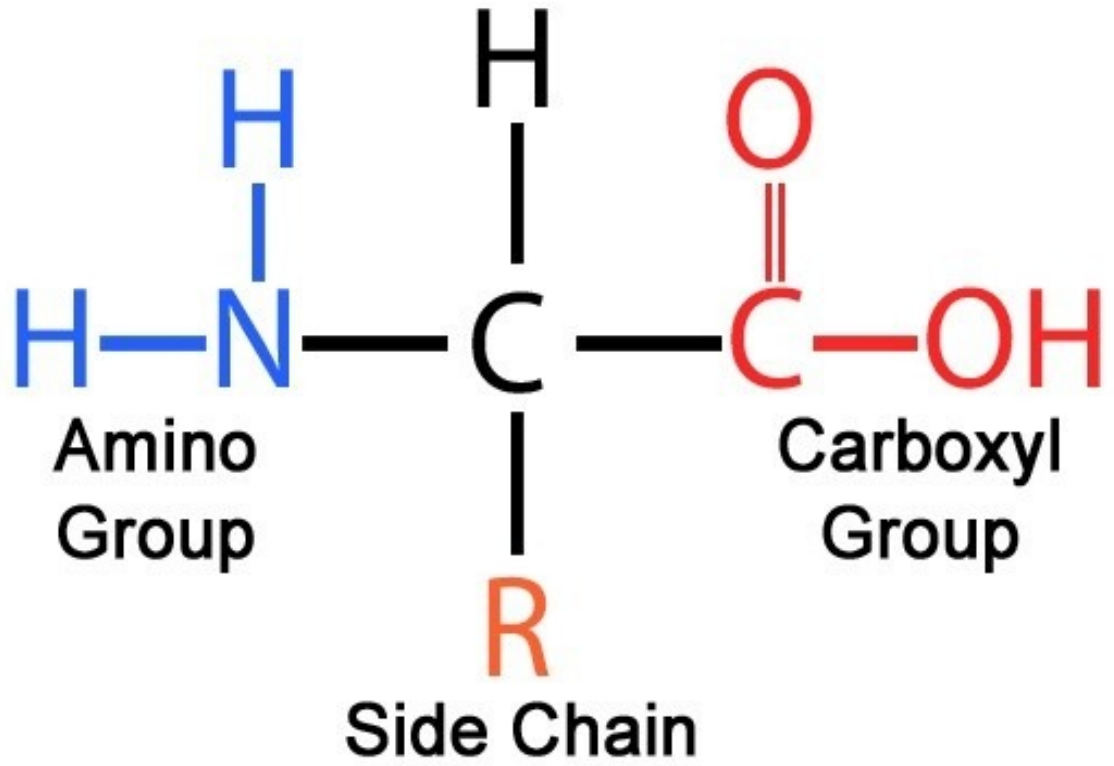
- The enzymes that act within the cells in which they are produced are called intracellular enzymes or endoenzymes.
- As these enzymes catalyze most of the metabolic reactions of the cell, they are also referred to as metabolic enzymes.
- Most of the enzymes in plants and animals are intracellular enzymes or endoenzymes.
- Intracellular enzymes usually break down large polymers into smaller chains of monomers.
- All intracellular enzymes undergo intracellular digestion during cell death.

Extracellular enzymes

- The enzymes which are liberated by living cells and catalyze useful reactions outside the cell but within its environment are known as extracellular enzymes or exoenzymes.
- Exoenzymes act chiefly as digestive enzymes, catalyzing the breakdown of complex macromolecules to simpler polymers or monomers, which can then be readily absorbed by the cell.
- These mostly act at the end of polymers to break down their monomers one at a time.
- Exoenzymes are enzymes found in bacteria, fungi, and some insectivores like *Drosera* and *Nepenthes*.
- Extracellular enzymes, unlike intracellular enzymes, undergo external digestion during cell death.

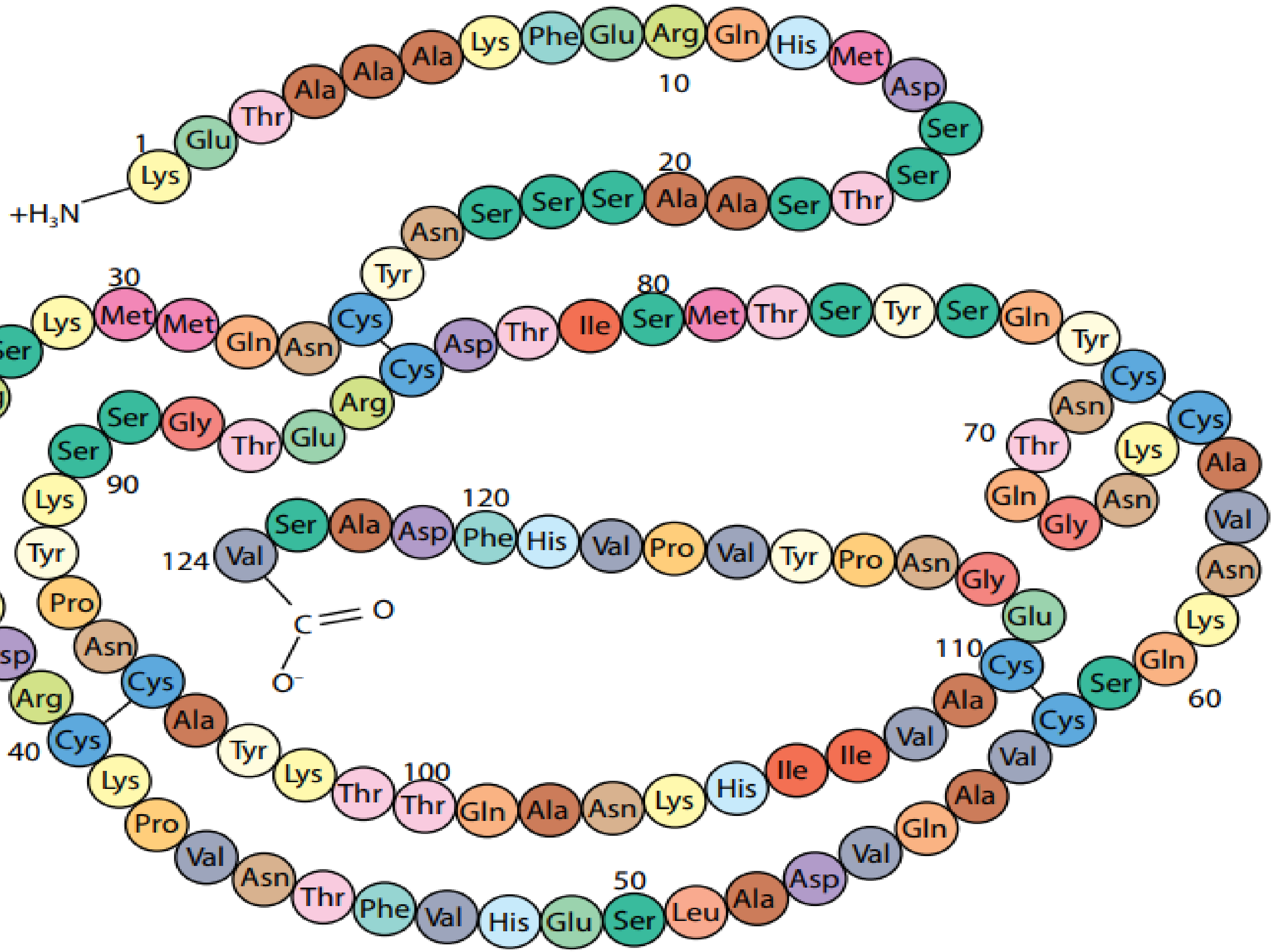
All enzymes are proteins composed of amino acid chains linked together by peptide bonds. This is the basic structure of enzymes. All enzymes have a highly specialized binding site, or active site, to which their substrate binds to form an enzyme-substrate complex. The three-dimensional structures of many proteins have been observed using X-ray crystallography. These structures vary from enzyme to enzyme. Examples of some enzymes and their structures include:

Amino acid



Ribonuclease (RNase)

- Ribonuclease is a small globular protein secreted by the pancreas into the small intestine, where it is involved in the catalysis of the hydrolysis of certain bonds in ribonucleic acids present in ingested food.
- This enzyme protein consists of a single polypeptide chain of 124 amino acid residues with lysine at the N-terminal and valine at the C-terminal.
- About 25% of the segments are in α-helix structure while the rest are β-sheets.
- The active site is present in the depression at the middle of the chain and the residues forming the active site are 6-8, 11, 12, 41, 42, 46-48, and 117-119.



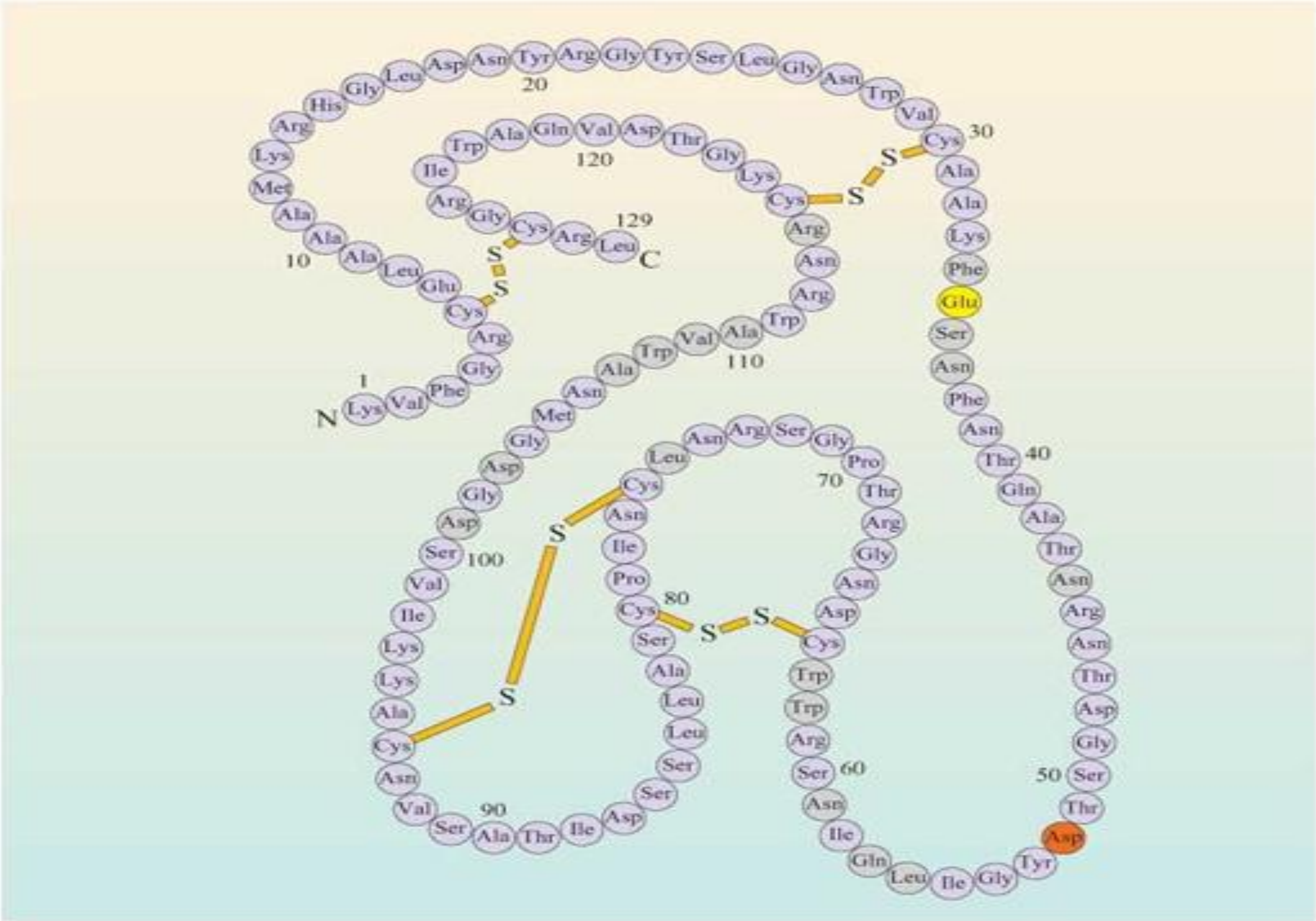
+H3N
1
Lys
Glu
Thr
Ala
Ala
Ala
Lys
Phe
Glu
Arg
10
Gln
His
Met
Asp
Ser
Ser
Thr
Ser
Ala
20
Ala
Ser
Ser
Ser
Asn
Tyr
Cys
Asn
Gln
Met
Met
30
Lys
Ser
Arg
Asn
Leu
Thr
Lys
Asp
Arg
Cys
40
Lys
Pro
Val
Asn
Thr
Phe
Val
His
Glu
Ser
50
Leu
Ala
Asp
Val
Gln
Ala
Val
Cys
Ser
Gln
60
Lys
Asn
Val
Ala
Cys
Lys
Asn
Gly
Gln
Thr
70
Asn
Cys
Tyr
Gln
Ser
Tyr
Ser
Thr
Met
Ser
80
Ile
Thr
Asp
Cys
Arg
Glu
Thr
Gly
Ser
Ser
90
Lys
Tyr
Pro
Asn
Cys
Ala
Tyr
Lys
Thr
Thr
100
Gln
Ala
Asn
Lys
His
Ile
Ile
Val
Ala
Cys
110
Glu
Gly
Asn
Pro
Tyr
Val
Pro
Val
His
Phe
120
Asp
Ala
Ser
Val
124
C
O
O⁻

Lysozyme

- Lysozyme is a hydrolytic enzyme that breaks down peptidoglycan, the main component of bacterial cell walls, especially in Gram-positive bacteria. Consists of 129 amino acids linked together to form the primary structure, and the first amino acid is lysine.
- The active site has six subsites that bind various substrates or inhibitors, and the amino acid residues located at the active sites are 35, 52, 59, 62, 63, and 107.
- Lysozyme is widely found in body tissues and fluids: Tears, Saliva, Respiratory and nasal mucus, Milk (especially breast milk), White blood cells and neutrophils, Egg whites (the main commercial source for its extraction).
- The enzyme has about 12% β-conformation and 40%-α helical segments.

Biological Importance:

- Innate immune defense: the first line of defense against pathogenic bacteria.
- Maintains microbial balance in mucous membranes.
- Uses in biomedicine:
  - As a food preservative.
  - In the pharmaceutical industry as a natural antibacterial.
  - In bacterial degradation research or cellular component extraction.
  - It has applications in nanobiotechnology due to its high stability and ease of purification.


N
1
Lys Val Phe Gly Arg Cys Glu Leu Ala Ala
10
Ala Met Lys Arg His Gly Leu Asp Asn Tyr
20
Arg Gly Tyr Ser Leu Gly Asn Trp Val Cys
30
Ala Ala Lys Phe Glu Ser Asn Phe Asn Thr
40
Gln Ala Thr Asn Arg Asn Thr Asp Gly Ser
50
Thr Asp Tyr Gly Ile Leu Gln Ile Asn Ser
60
Arg Trp Trp Cys Asp Asn Gly Arg Thr Pro
70
Gly Ser Arg Asn Leu Cys Asn Ile Pro Cys
80
Ser Ala Leu Leu Ser Ser Asp Ile Thr Ala
90
Ser Val Asn Cys Ala Lys Lys Ile Val Ser
100
Asp Gly Asp Gly Met Asn Ala Trp Val Ala
110
Trp Arg Asn Arg Cys Lys Gly Thr Asp Val
120
Gln Ala Trp Ile Arg Gly Cys Arg Leu
129
C

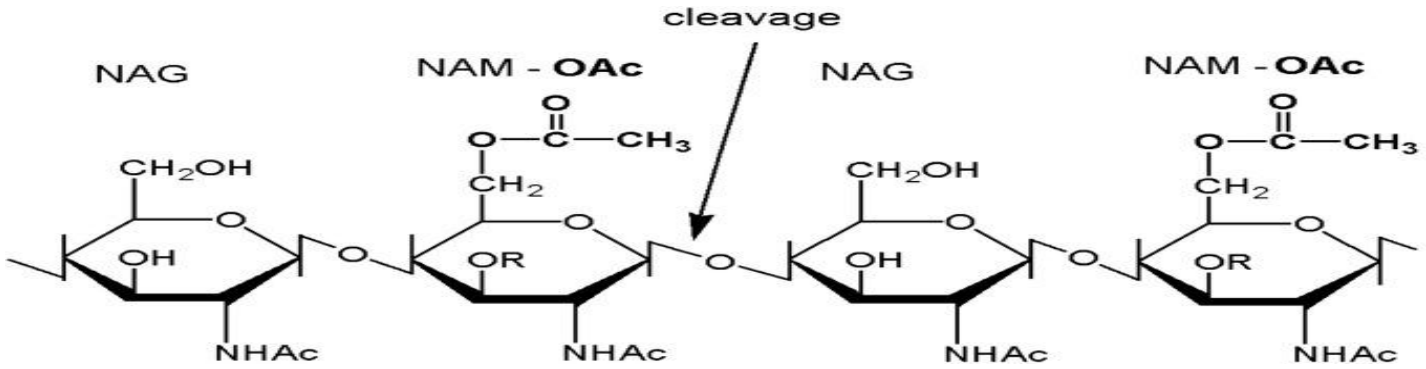
Mechanism of Action

The bacterial cell wall is composed of chains of **N- acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)** linked by **β-1,4-glycosidic bonds.**

Lysozyme breaks these bonds, leading to cell wall degradation and subsequent cell rupture and death (osmotic lysis).

In other words:

Lysozyme acts as "molecular scissors" that attack and destroy the bacterial cell wall.



Chymotrypsin

- Is a proteolytic enzyme belonging to the serine protease family. It is secreted in an inactive form from the pancreas as chymotrypsinogen and then activated in the small intestine.
- A molecule of chymotrypsin consists of 3 short polypeptide chains of 13, 131, and 97 amino acid residues respectively, supported by two interchain disulfide bonds.
- The secondary structure of chymotrypsin consists of several antiparallel β pleated sheet regions and a little α helical structure.

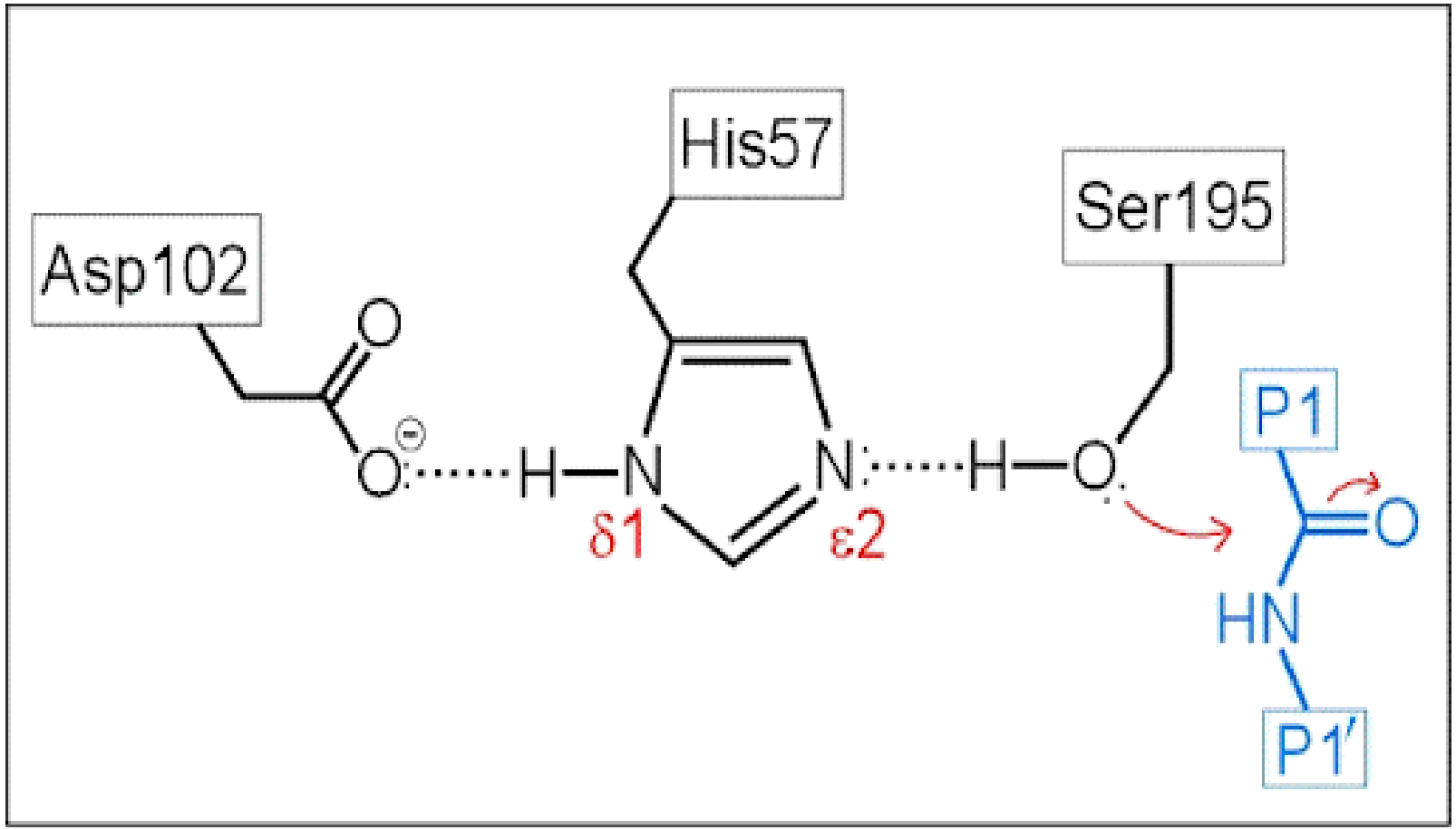
Biological and Research Importance:

- In the body: Responsible for the digestion of proteins in the small intestine into peptides and amino acids.
- In the laboratory: Widely used in:

Protein sequencing, catalytic mechanism studies, preparing peptides for industrial and pharmaceutical applications.

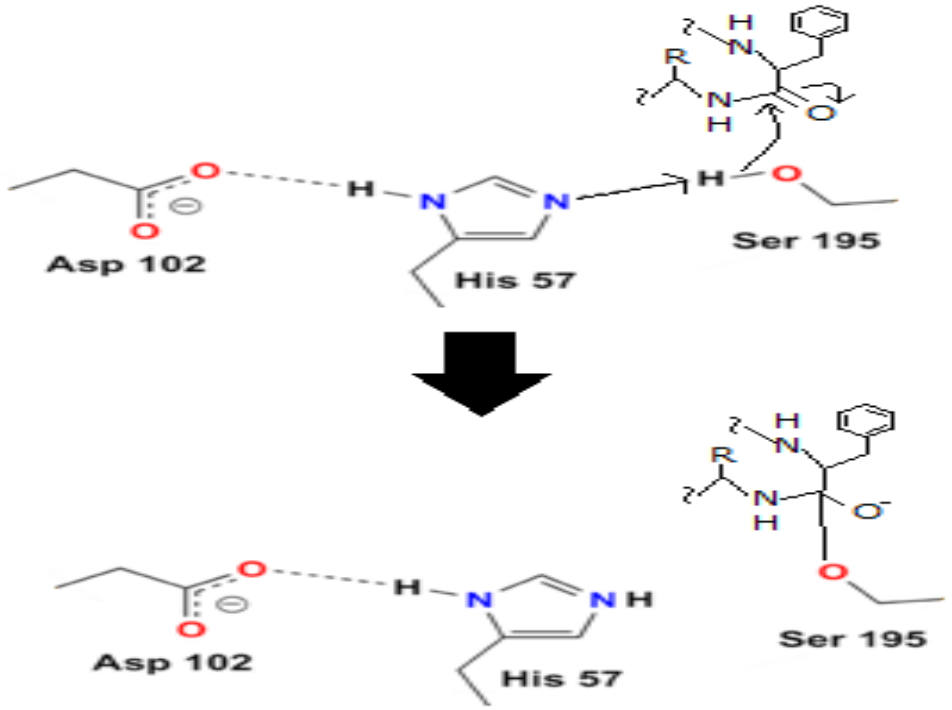
Medical Importance:

- It is sometimes used in topical treatments to reduce inflammation or accelerate tissue healing.
- Also, its deficiency or deficiency can lead to digestive disorders or protein malabsorption.


Asp102
His57
Ser195
P1
O
HN
P1′
δ1
ε2

It is secreted from pancreatic cells as inactive chymotrypsinogen to prevent digestion of the pancreatic tissue itself.

It is activated by the enzyme trypsin in the small intestine, converting chymotrypsinogen into active chymotrypsin.

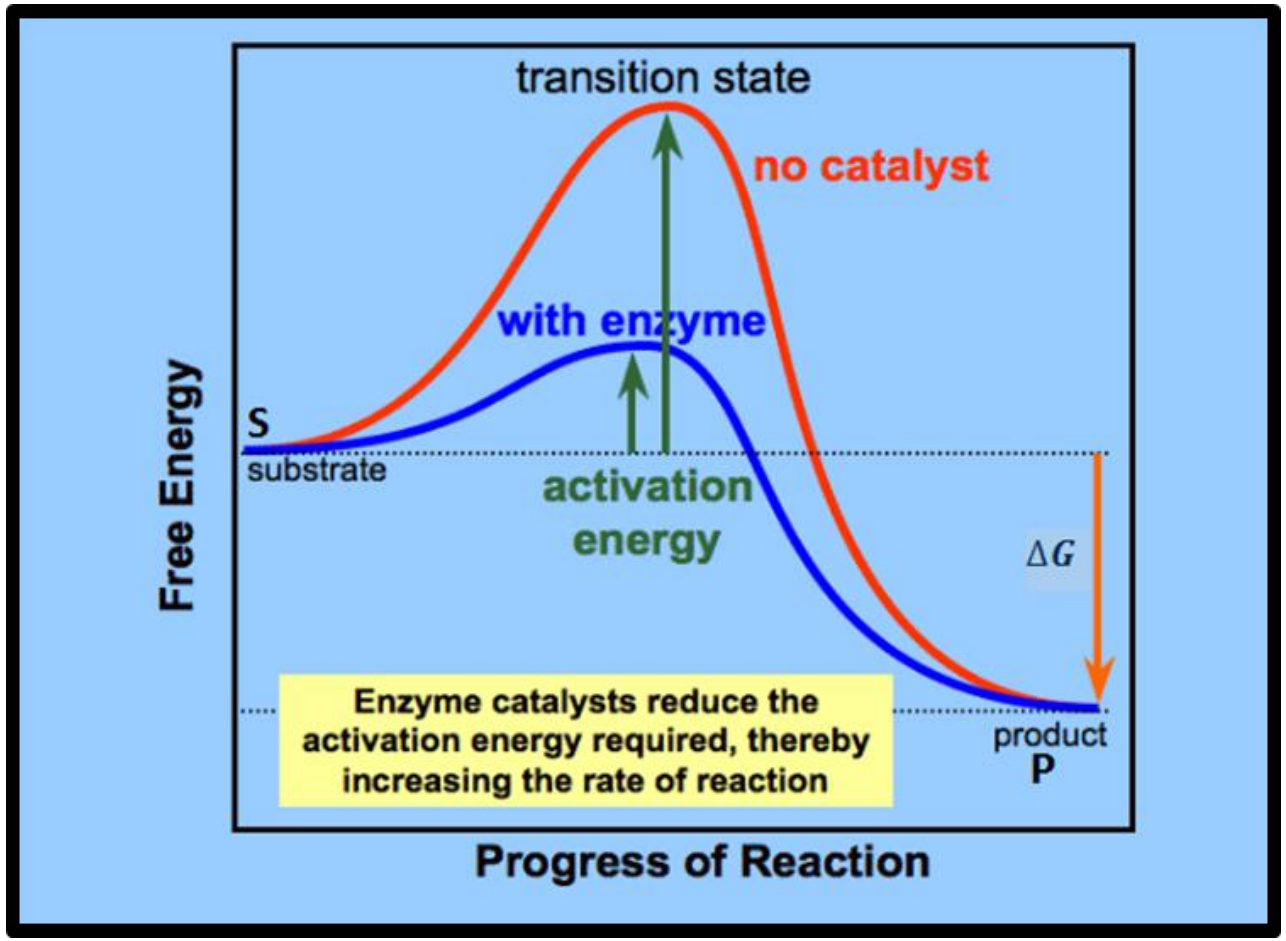


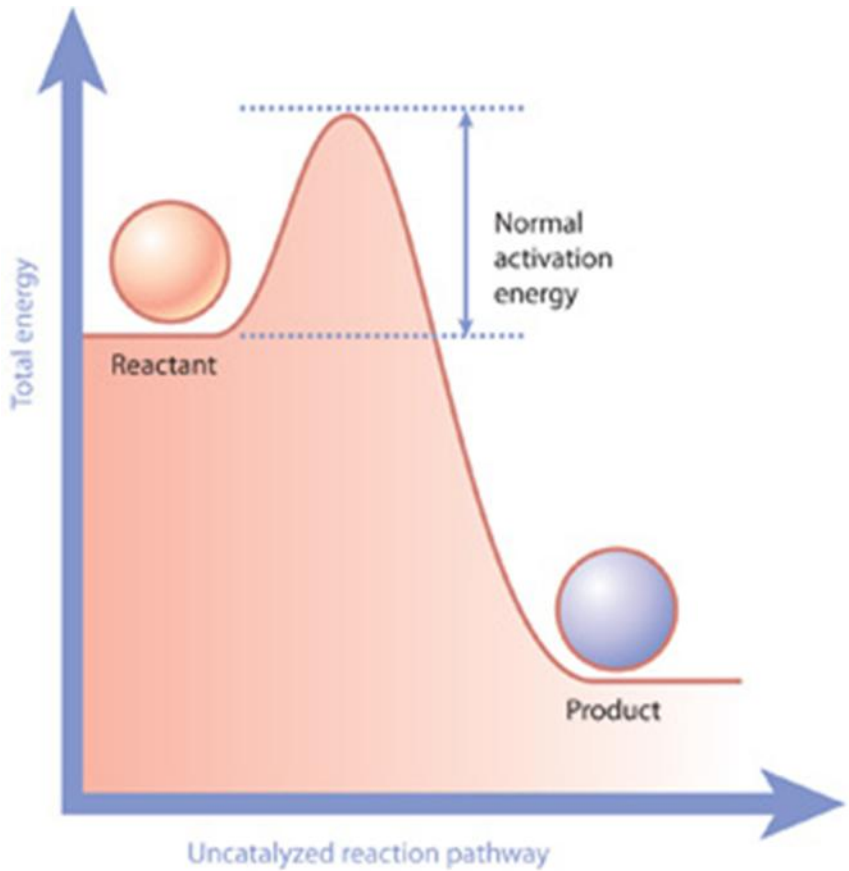
How Enzyme works:

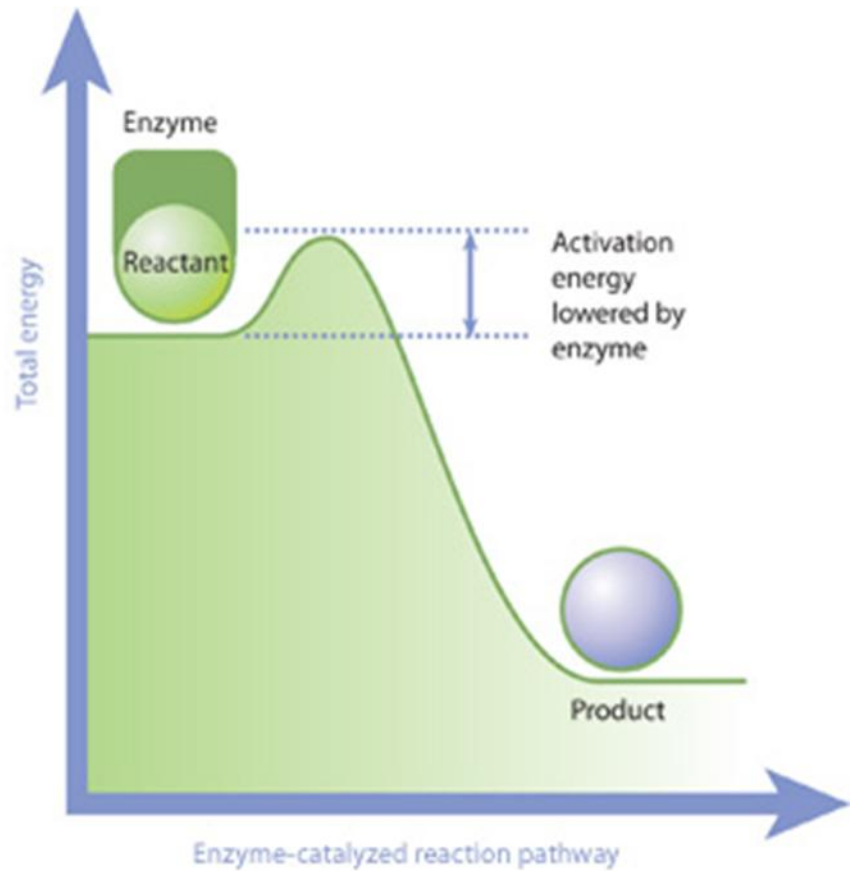
Almost all chemical reactions have an energy barrier, separating the reactants and the products. This barrier, called activation energy, is the energy difference between the energy of the reactant and high energy intermediates that occurs during the formation of a product. Bellow figure shows the changes in energy during the conversion of a molecule of reactant 'S' to product 'P' through the transition state.

As noticeable in Figure, the enzyme-catalyzed reactions lead to decrease the activation energy with no effect on free energy ΔG.

activation energy: is the energy required to start a reaction. The rate of reaction increases if the activation energy decrease.


transition state
no catalyst
with enzyme
S
substrate
activation
energy
ΔG
Enzyme catalysts reduce the activation energy required, thereby increasing the rate of reaction
product
P
Free Energy
Progress of Reaction


Total energy
Normal
activation
energy
Reactant
Product
Uncatalyzed reaction pathway

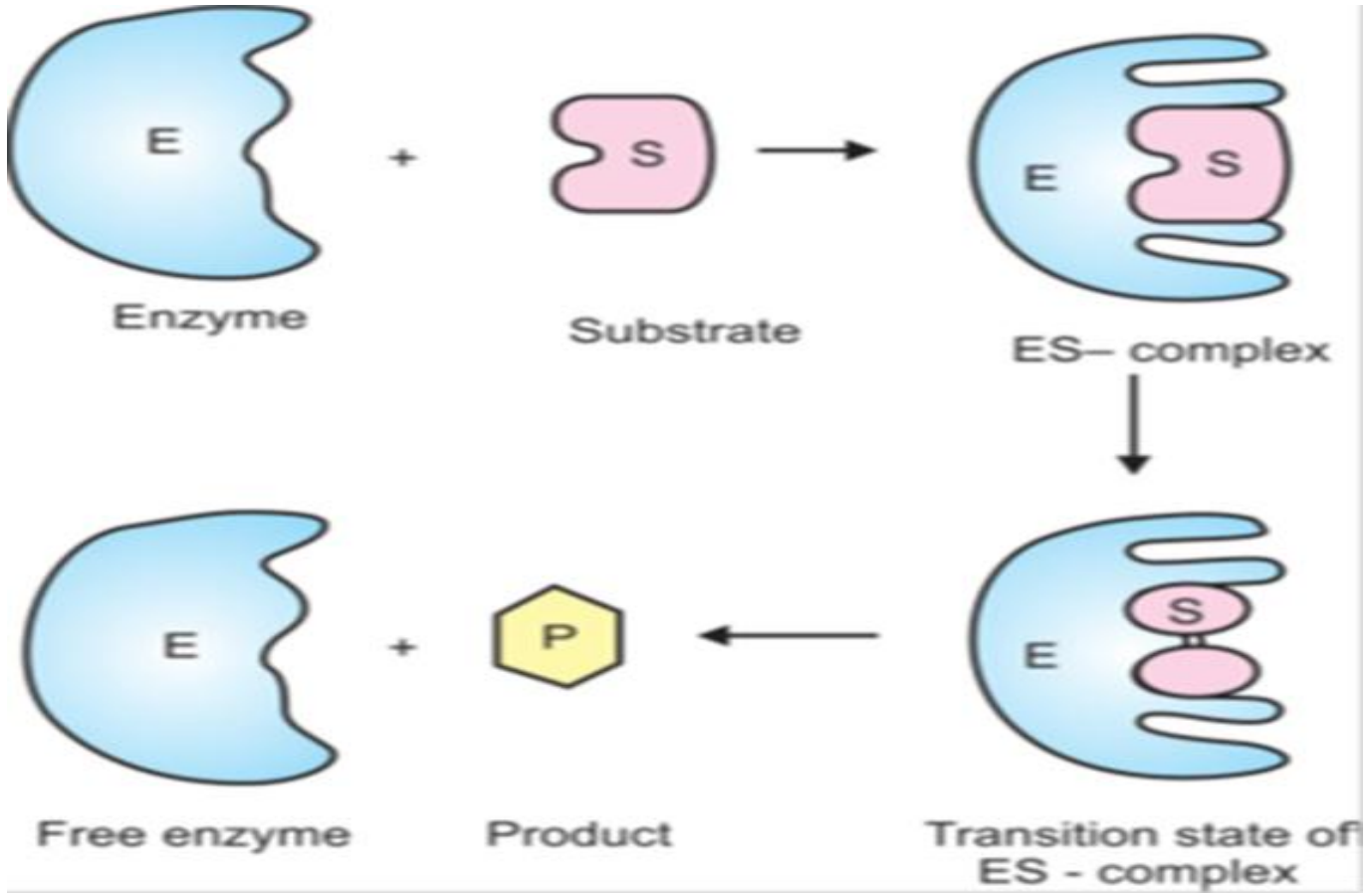

Enzyme
Reactant
Total energy
Activation
energy
lowered by
enzyme
Product
Enzyme-catalyzed reaction pathway

Mechanism of Enzyme action:

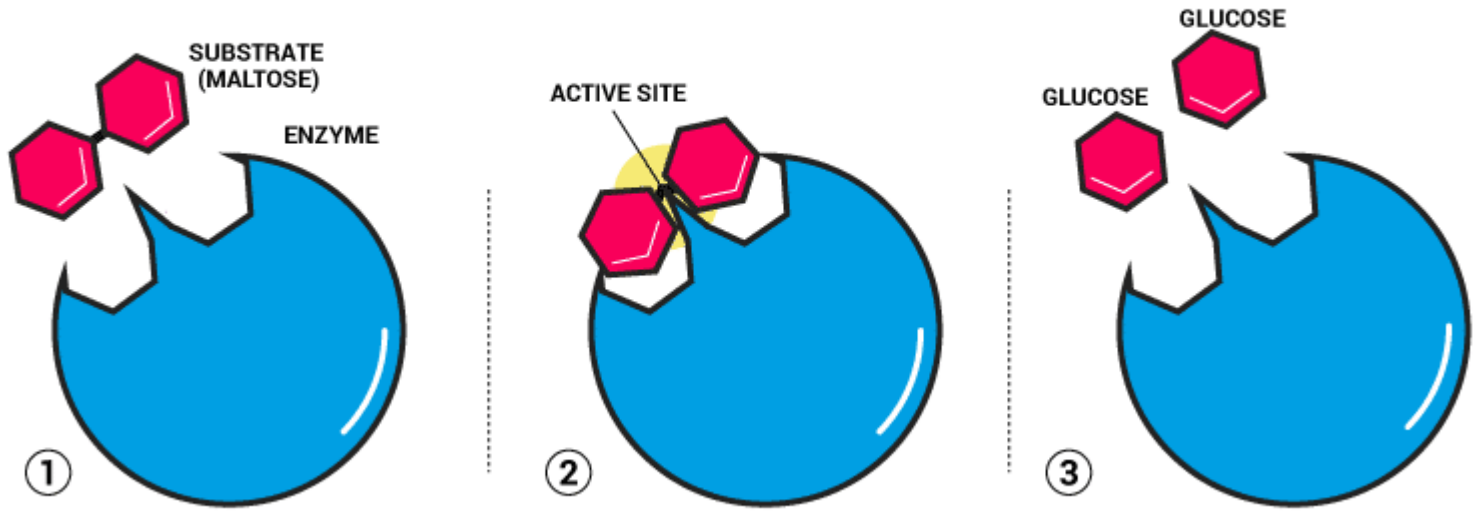
Formation of an enzyme-substrate (ES) complex is the first step in enzymatic catalysis.

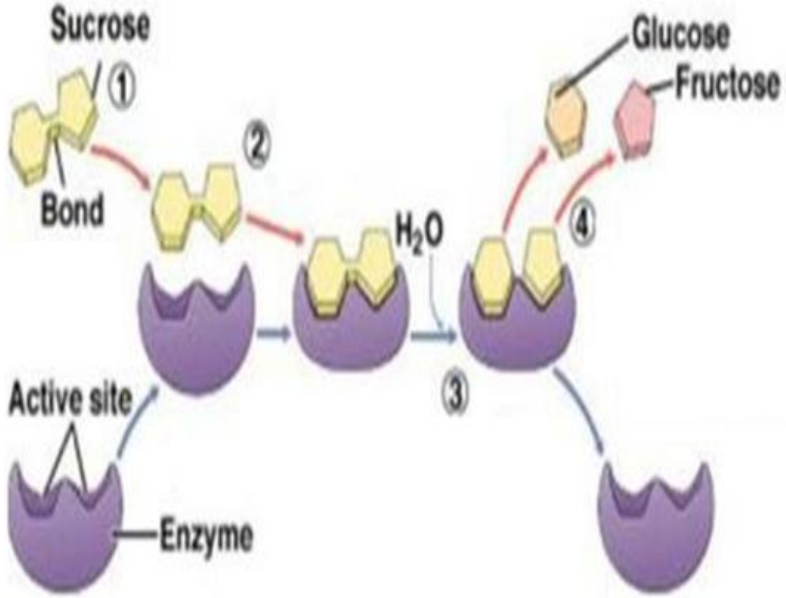
Substrate is bound through multiple non-covalent interactions at the active site* of the enzyme and forming an enzyme-substrate (ES) complex which is subsequently converted to product and free enzyme.

* Active site is the region of an enzyme where substrate molecules bind and undergo a chemical reaction.


E
+
S
Enzyme
Substrate
E
S
ES– complex
E
S
Transition state of
ES - complex
E
+
P
Free enzyme
Product

ENZYME ACTION





An example is the action of the enzyme **sucrase** hydrolysing sucrose into glucose and fructose (*see left*)

Two models for substrate binding to the active site of the enzyme have been proposed to explain the specificity that an enzyme has for its substrate:

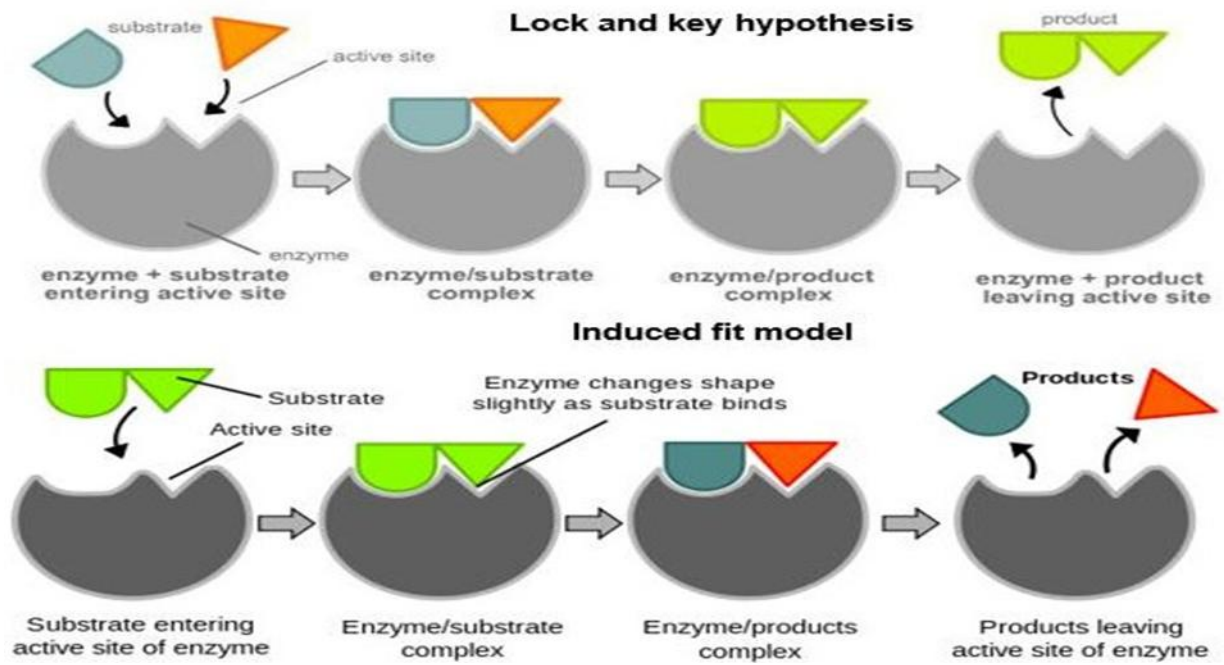
1. Lock and Key Model or Rigid Template

In this model, the active site of enzyme has a rigid structure that is complementary to that of the substrate. This model is called lock and key model, because in this model the substrate fits into the active site in much the same way that a key fits into a lock.

2. Induced Fit Model or Hand-in-glove Model

Experimental evidence indicates that many enzymes have flexibility in their shapes. They are not rigid and static; there is constant change in their shape. The induced-fi t model allows for small changes in the shape or geometry of the active site of an enzyme to bind with substrate. It can be said: when substrate approaching the enzyme leads to inducing the active site to make a fit shape for a substrate.

This flexibility is similar to the changes that occur in the shape of a glove when a hand is inserted into it. The induced fit is a result of the enzyme's flexibility; it adjusts to accept the incoming substrate.


Lock and key hypothesis
substrate
active site
enzyme
product
enzyme + substrate
entering active site
enzyme/substrate
complex
enzyme/product
complex
enzyme + product
leaving active site
Induced fit model
Substrate
Active site
Enzyme changes shape
slightly as substrate binds
Products
Substrate entering
active site of enzyme
Enzyme/substrate
complex
Enzyme/products
complex
Products leaving
active site of enzyme

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Enzymes
(Lecture 3)

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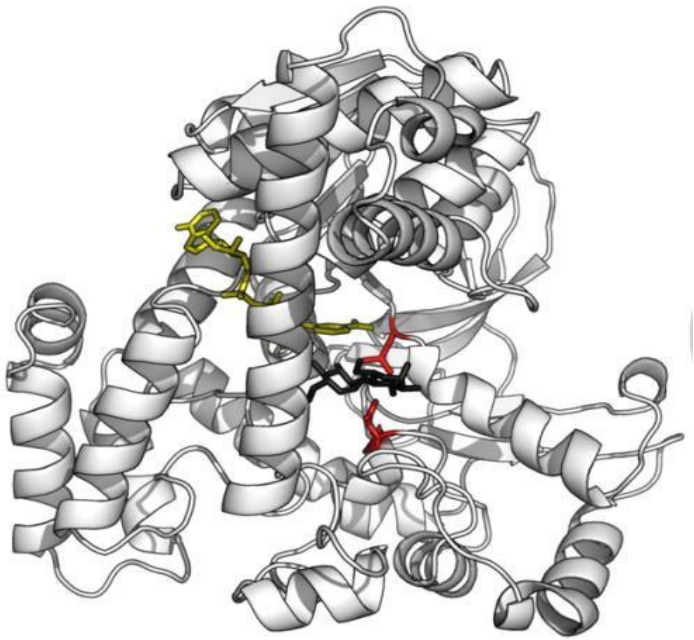
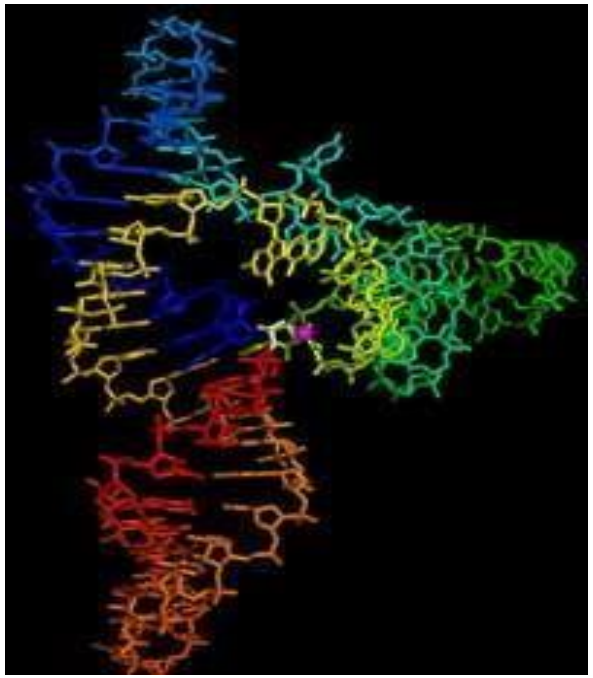
- **Enzymes nature**

- It was believed that all enzymes were proteins, but in the 1980s it was found that some ribonucleic acid (RNA) molecules are also able to exert catalytic effects. These RNAs, which are called ribozymes, play an important role in gene expression. Thomas Cech in the US was the first to discover an RNA molecule with enzymatic activity.



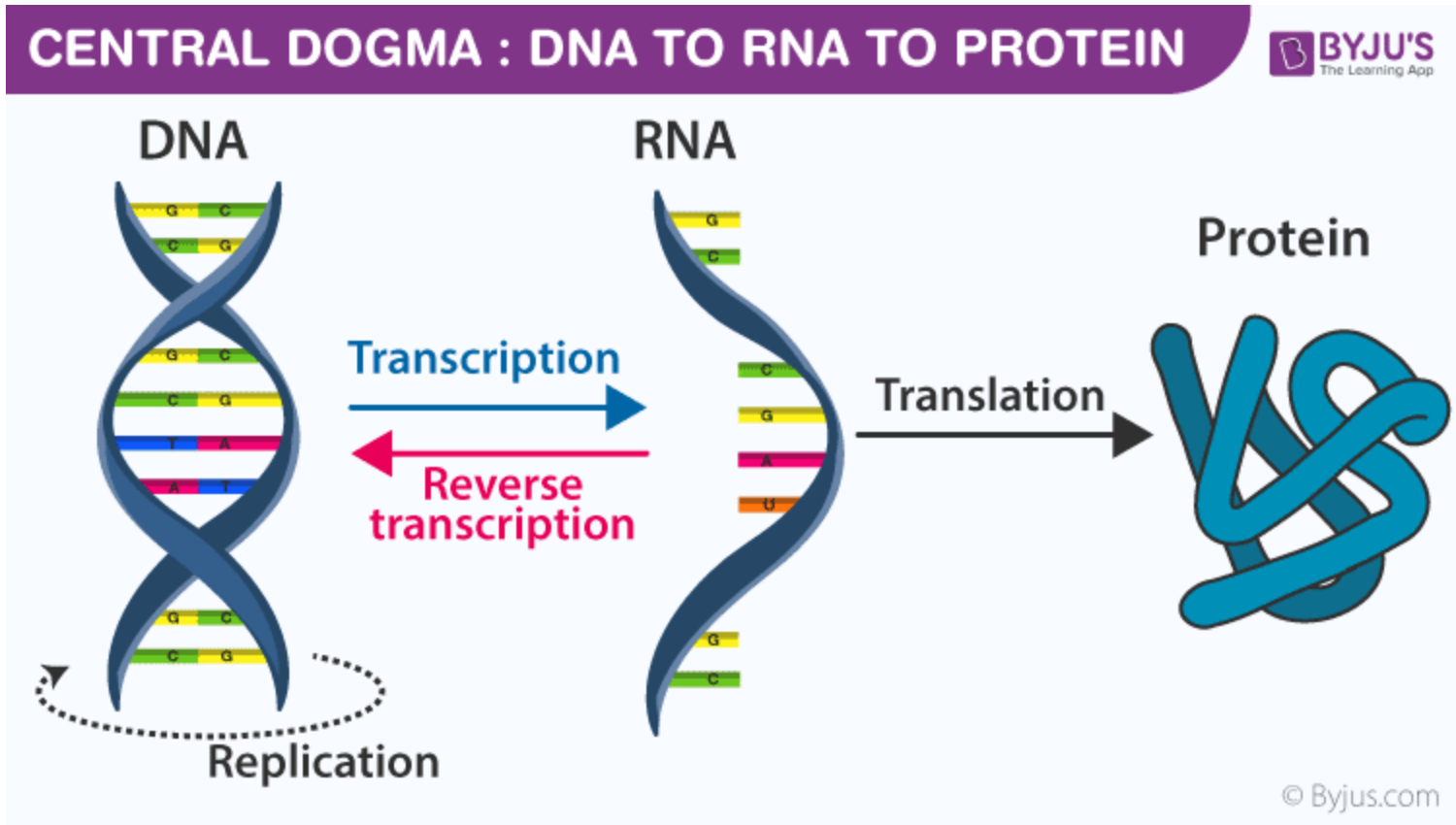
Pretentious enzyme



RNA enzyme

Enzyme chemistry: Enzymes are built of proteins folded into complicated shapes. Some proteins have structural roles (e.g. actin in the muscles), other have catalytic (chemical-reaction-making) activity and are called enzymes. their activities depends on the 3D structure of the amino acids that compose them.

The Biosynthesis of Proteins: Genetic information is stored in cells as the base sequence of DNA. The information may be transcribed into RNA structure and then translated into protein structure in such a way that the amino acid sequence of each protein synthesized is determined by the base sequence of a section of DNA known as a gene.


CENTRAL DOGMA : DNA TO RNA TO PROTEIN
BYJU'S
The Learning App
DNA
RNA
Protein
Transcription
Reverse transcription
Translation
Replication
© Byjus.com

The Structure of Proteins:

Proteins consist of L-amino acid residues linked by peptide bonds. The sequence of amino acids in each polypeptide chain constitutes the primary structure of the protein. Regular, repeating three-dimensional features constitute the secondary structure. The overall three-dimensional structure of each polypeptide chain is termed the tertiary structure. Proteins may consist of one or more polypeptide chains, the complete structure being called the quaternary structure. The primary structure of a protein consists of amino acid residues linked by covalent peptide bonds. Covalent disulphide bridges (-S-S-), linking cysteine residues, are often involved in the maintenance of tertiary structure. In a very few instances, disulphide bridges may also link the separate polypeptide components of a protein

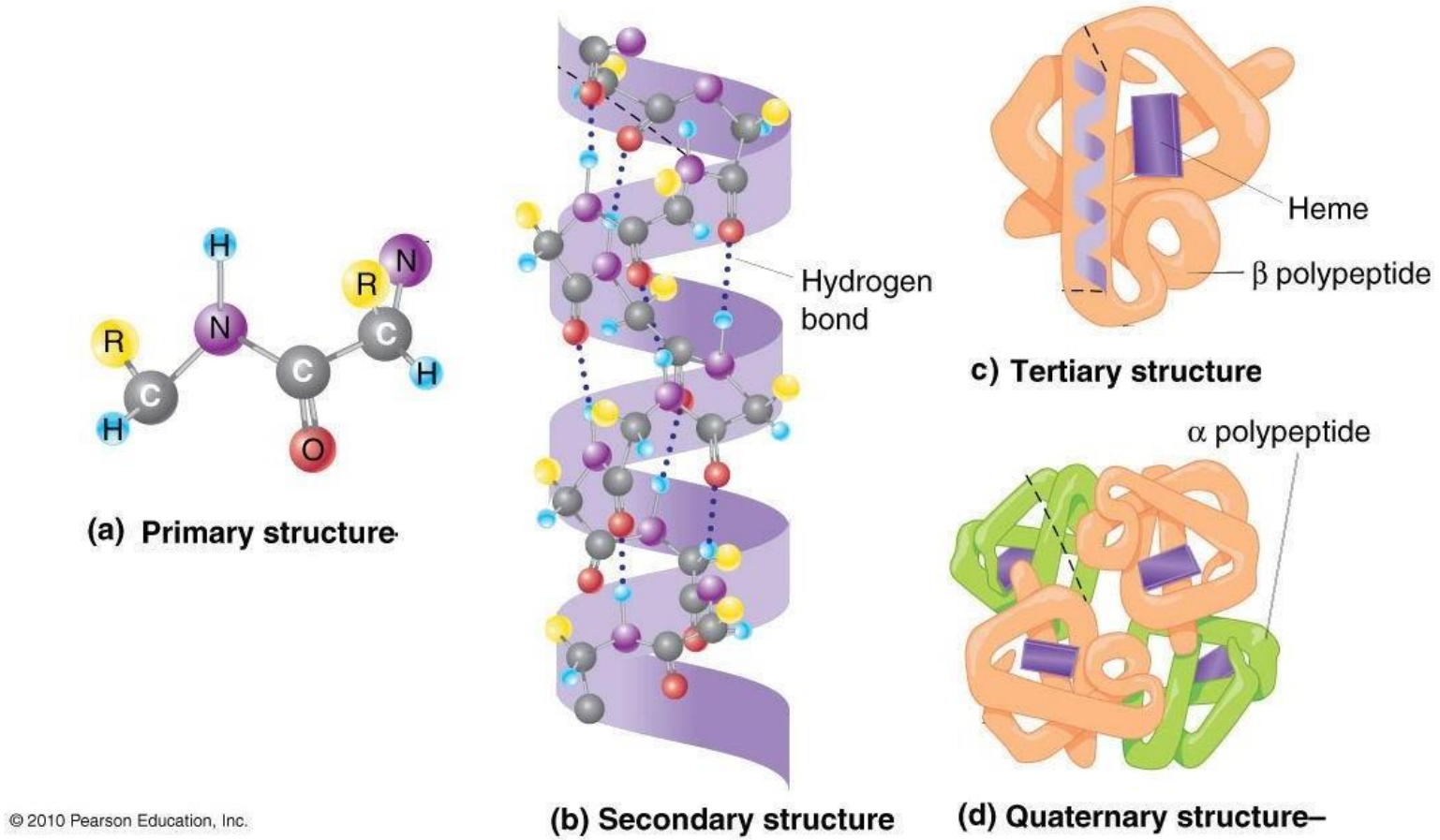


Figure 2. protein structure

Features of enzyme active site :

1. It also contains the residues that directly participate in the making and breaking the bonds called the catalytic groups.
2. In essence, the interaction of the enzyme and substrate at the active site promotes the formation of the transition state.
3. The active site takes up a relatively small part of the total volume of an enzyme .
4. Substrates are bound to enzymes by multiple weak attractions as products needs to be released after the completion of the reactions.
5. Active site has a precise amino acid sequence that is never changed.
6. A change in amino acid sequence in active site generally renders enzyme into a non functional form.

The amino acids around the active site attach to the substrate molecule and hold it in position while the reaction takes place. This makes the enzyme specific for one reaction only. As the catalytic amino acids, are directly involved in the catalysis of the biochemical reaction and form the catalytic site. The substrate(s) is bound in the active site by multiple weak forces (electrostatic interactions, hydrogen bonds, van der Waals bonds, hydrophobic interactions; and in some cases by reversible covalent bonds.

Factors affecting on enzyme activity :

Various factors that affect enzyme activity are:

1.Substrate concentration

2.Enzyme concentration

3.pH

4. Temperature

5.Time

6.Inhibitors

1. Effect of Substrate Concentration

For a given quantity of enzyme, the velocity of the reaction increases as the concentration of the substrate is increased. At first, this relationship is almost linear but later, the reaction curve becomes hyperbolic in shape. This change in curve happen because all the free enzymes will have been converted into **ES** form so that any further increase in substrate concentration has no effect on the rate and the reaction achieves a steady state.

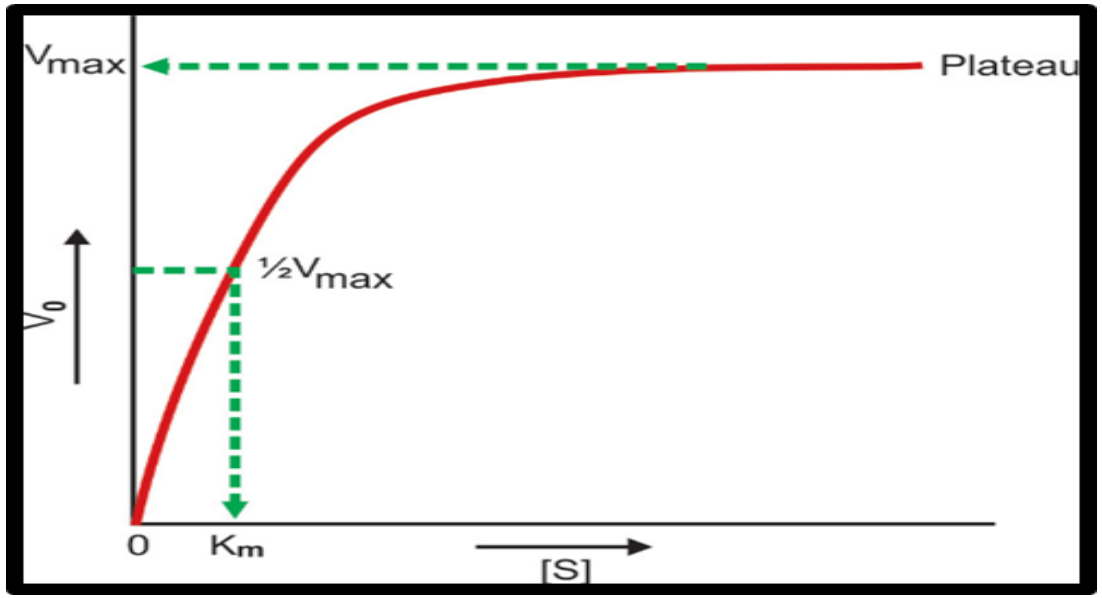


Figure : Effects of substrate concentration [S] on enzyme activity keeping enzyme concentration constant

Where, V_0: initial velocity

V_{max}: maximum velocity

Km: 1/2 Vmax = Michaelis Menten constant

[S]: substrate concentration

2. Effect of Enzyme Concentration

The velocity of a reaction is directly proportional to the amount of enzyme present as long as the amount of substrate is not limiting.

3. Effect of pH

Each enzyme has an optimum pH at which the enzyme activity is maximum. Below or above this pH, enzyme activity is decreased. The optimum pH differs from enzyme to enzyme. The proper ionic form is found at optimum pH. A bell-shaped curve is obtained when we plot the enzyme velocity Vs pH.

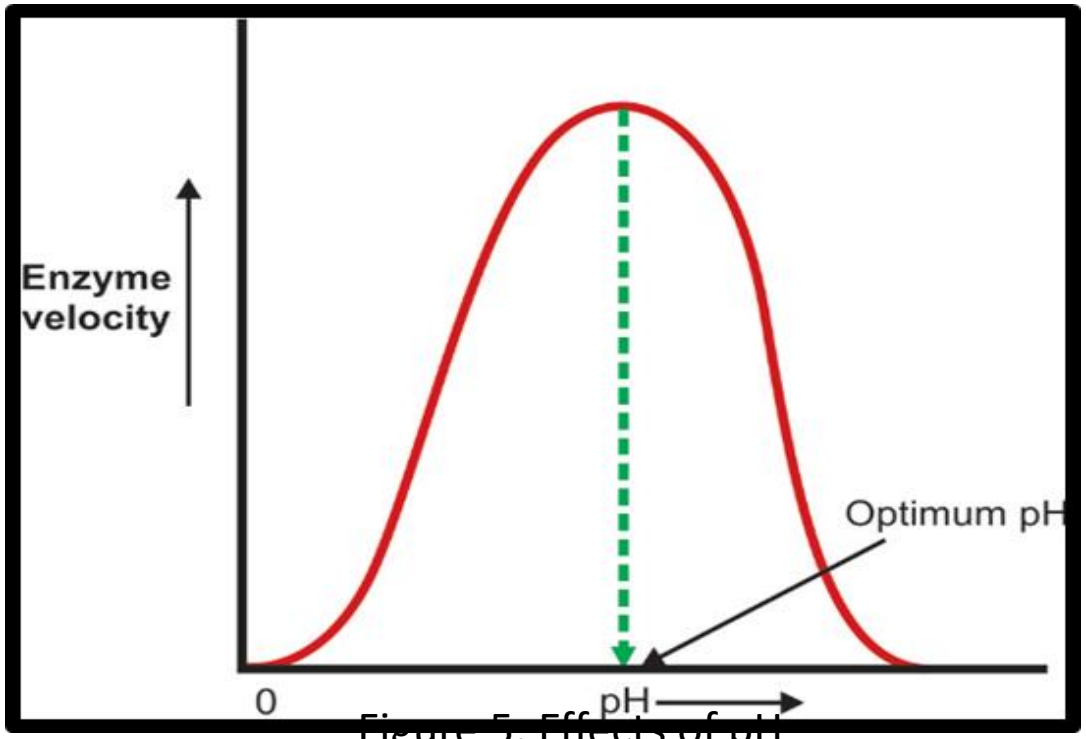


Figure 5: Effects of pH

4. Effect of temperature

Enzyme catalyzed reactions show an increase in rate with increasing temperature at reaching the optimum temperature. Above optimum temperature the denaturation of enzyme is happen. A bell-shaped curve is obtained when we plot the enzyme velocity Vs. temperature as shown in figure (6).

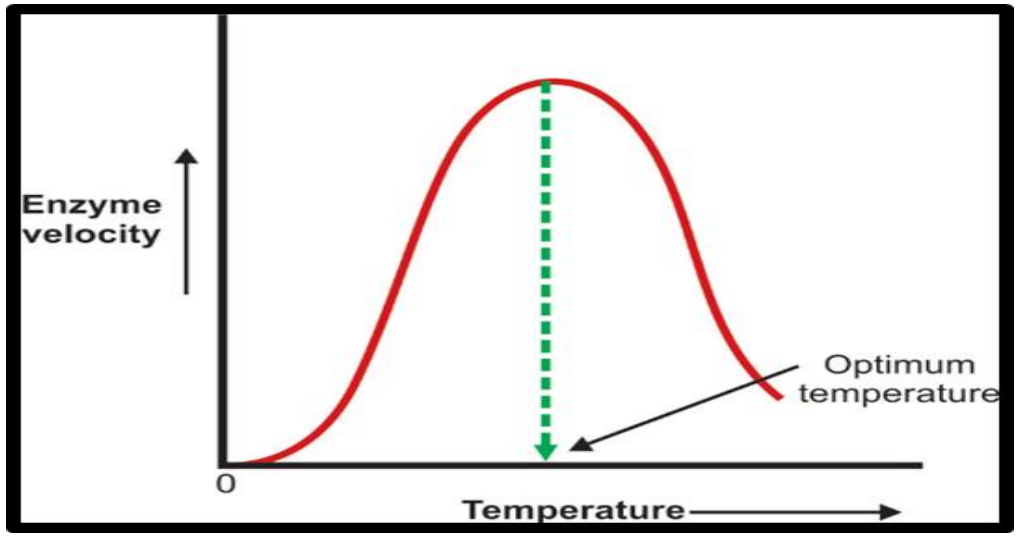


Figure 6: Effects of temperature

5. Effect of time

Under optimum conditions of pH and temperature, time required for an enzyme reaction is less. The time required for the completion of an enzyme reaction increases with changes in temperature and pH from its optimum.

6. Effect of inhibition

The substances which stop the enzymatic reaction are called inhibitors. Presence of these substances in reaction medium decreases the rate of enzyme reaction. There are three types of inhibition

- Competitive inhibition
- Uncompetitive inhibition
- Non- competitive inhibition

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Enzymes
(Lecture 5)

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Enzyme activity and enzyme assay:

To measure the activity of an enzyme one must measure how much product is formed over a given time or, in some cases, how much substrate has been used up, which should be the same thing. By international agreement 1 unit of enzyme activity(I.U) is defined as the amount of enzyme causing transformation of 1.0 µ mol of substrate per minute at 25 _Cº under optimal conditions of measurement.

The term activity refers to the total units of enzyme in a solution. The specific activity is the number of enzyme units per milligram of total protein I.U= µmol/min specific activity =I.U /mg of protein = µmol/min of protein. The specific activity is a measure of enzyme purity: it increases during purification of an enzyme and becomes maximal and constant when the enzyme is pure.

Types of Enzyme Assays:

1- Continuous Assay: Measures the enzyme activity in real-time, either by tracking the increase in product formation or the decrease in substrate concentration over time. This is often done using spectrophotometry (monitoring changes in absorbance) if the product or substrate has distinct light absorption properties.

Example: Monitoring the formation of NADH in reactions involving dehydrogenases by measuring absorbance at 340 nm.

2- Discontinuous Assay: The reaction is stopped at specific time intervals, and the amount of product formed or substrate consumed is measured.

This often involves chemical or physical steps to quench the reaction,

such as a change in pH or the addition of a stopping reagent.

3- Coupled Assay: Used when the product of the enzymatic reaction is not easily measurable.

In this type of assay, the product of the first reaction is used as a substrate for a second,

easily measurable reaction. For instance, ATP production in one reaction can be coupled

to a luciferase assay that produces light.

4- Radioactive Assay: Involves the use of radioactive substrates, and enzyme activity is measured by detecting the incorporation or release of radioactivity. This is particularly useful for reactions that do not lend themselves to direct optical detection methods.

5- Fluorometric Assay: Enzyme activity is measured by tracking the formation of a fluorescent product or the decrease of a fluorescent substrate. These assays are highly sensitive and useful for detecting low levels of enzyme activity.

Practical Applications of Enzyme Assays:

Medical Diagnostics: Enzyme assays are used to diagnose diseases by measuring the levels of specific enzymes or their activity in biological samples. For instance, elevated levels of liver enzymes (e.g., alanine transaminase) can indicate liver damage.

Pharmaceutical Industry: Enzyme assays are crucial for screening potential drugs, particularly enzyme inhibitors that may serve as therapeutic agents.

Biotechnology: Enzyme assays are used in the production of industrial enzymes and in optimizing conditions for enzymatic reactions in bioprocesses.

Enzyme and the diagnosis of disease:

Diagnostic enzymology is principally concerned with changes in activity of serum enzymes that are predominantly intracellular and that are normally present in serum at low activity. By measuring the activities of these enzymes, clinical information about the location and nature of the pathological changes in the tissues can be extracted.

All of the hundreds of different enzymes present in the human body are synthesized intracellularly, and most carry on their function within the cells in which they are formed. Enzymes found in the blood can be classified into two:

1- Plasma specific enzymes:

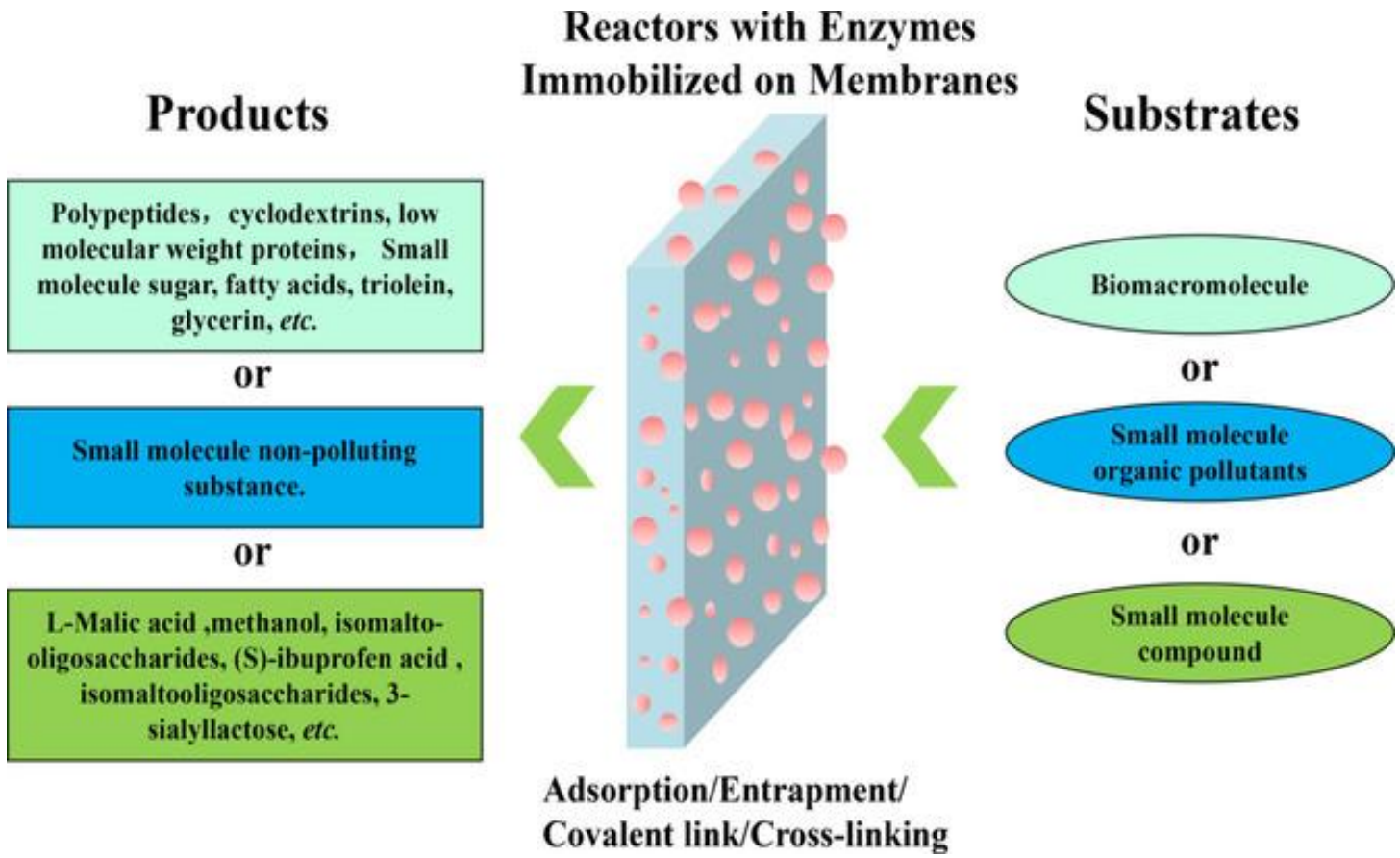
- Plasma is their normal sit of action
- Synthesized by the liver and are constantly
- liberated into plasma to maintain a steady state concentration.
- Plasma level below normal is indication of impaired function or inborn metabolic error.
- E.g. enzyme involved in blood coagulation, and immune response.

2- Non plasma specific enzymes :

- Have no important physiological function in the plasma (plasma is deficient in activator or coenzymes)
- Relased into plasma as a result of leakage or cell death.
- Present in plasma at concentrations much lower than in cells
- Have diagnostic importantance when their concentration is increased above a certain level.

Immobilization:

is the process of attaching enzymes to a solid support to restrict their mobility, which enhances their stability, reusability, and allows for easier separation from products



Advantages of Enzyme Immobilization

1. **Reusability of Enzymes**
   Immobilized enzymes can be used repeatedly in several reaction cycles, which reduces the overall cost of industrial processes.
2. **Improved Stability**
   Immobilized enzymes usually show higher stability against changes in temperature, pH, and chemical conditions. This makes them more reliable in long-term applications.
3. **Easy Separation from Products**
   Because the enzyme is attached to a solid support, it can be easily removed from the reaction mixture. This leads to cleaner products and simpler purification steps.
4. **Suitable for Continuous Processing**
   Immobilized enzymes can be used in continuous-flow systems, allowing industries to run reactions for long periods without interruption.
5. **Reduced Product Contamination**
   Since the enzyme does not mix with the final product, the risk of contamination is low, which is important in food, pharmaceutical, and biotechnology industries.

Disadvantages of Enzyme Immobilization

1. **High Initial Cost**

   The materials and methods used for immobilization may be expensive, making the initial setup costly.

2. **Possible Loss of Enzyme Activity**

   Some enzymes lose part of their activity during the immobilization process due to changes in their structure or environment.

3. **Mass Transfer Limitations**

   The movement of substrates and products to and from the immobilized enzyme can be slower, which may reduce the reaction rate.

4. **Not Suitable for All Enzymes**

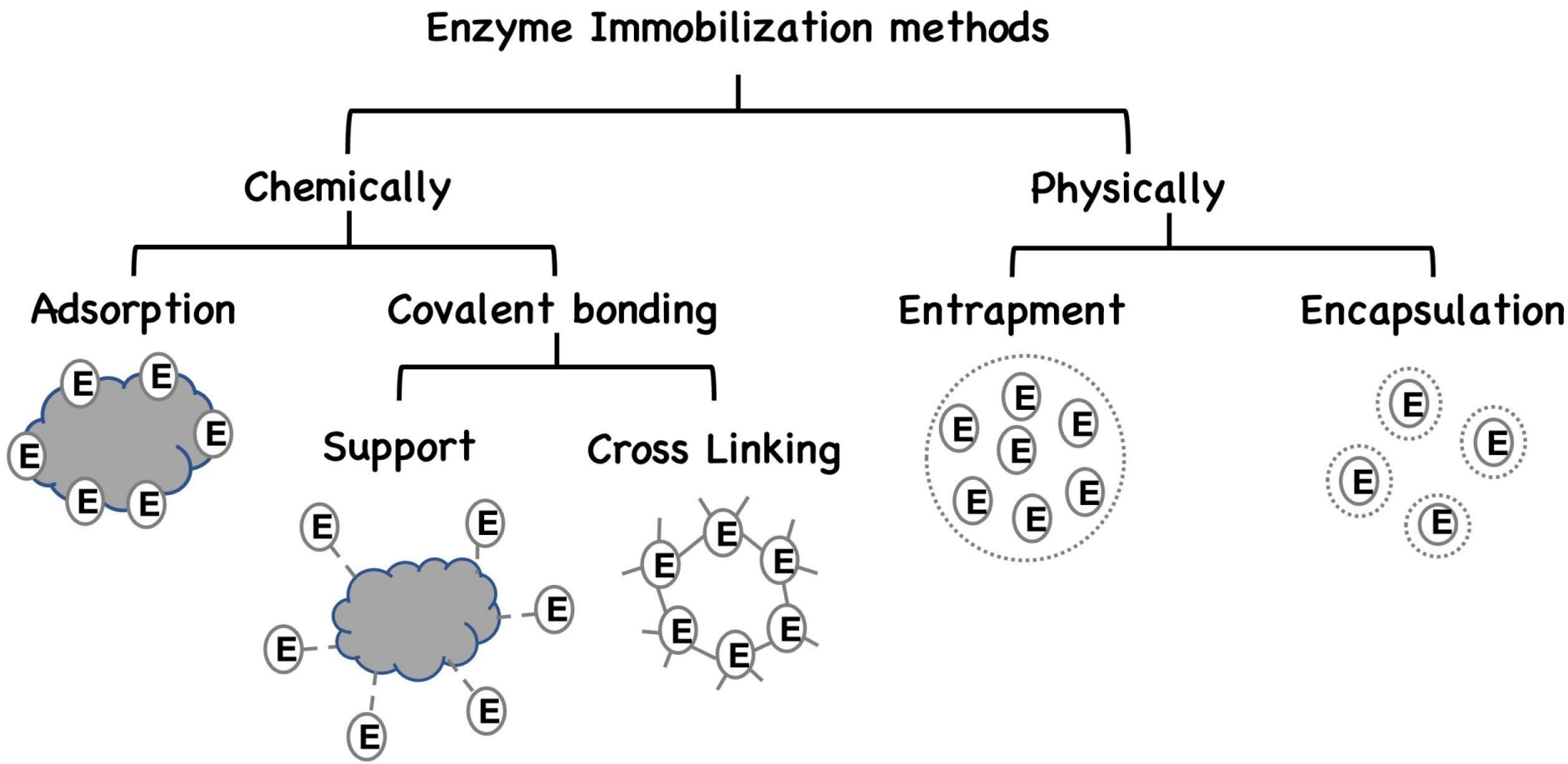
   Certain enzymes do not perform well after immobilization because they require more flexibility or specific environmental conditions.

5. **Support Material Damage**

   The solid support that holds the enzyme may degrade over time, especially in large-scale industrial processes, leading to decreased efficiency.

Methods of Immobilization

- 1. Adsorption
- 2. Covalent Binding
- 3. Entrapment
- 4. Encapsulation


Enzyme Immobilization methods
Chemically
Physically
Adsorption
Covalent bonding
Entrapment
Encapsulation
Support
Cross Linking
E

Adsorption

Advantages

- Simple and inexpensive method.
- Mild procedure that does not damage the enzyme.
- Enzymes can often be reused multiple times.

Disadvantages

- Weak binding forces lead to enzyme leakage during use.
- Not suitable for long-term industrial applications.
- Enzyme activity may decrease if environmental conditions change.

Covalent Binding

Advantages

- Very strong and stable attachment of the enzyme to the support.
- Minimal enzyme leakage during reaction.
- Suitable for continuous and long-term industrial processes.

Disadvantages

- More expensive and chemically complex.
- The enzyme may lose part of its activity during binding.
- Difficult to regenerate or reuse the support after deactivation.

Entrapment (Encapsulation)

Advantages

- Protects the enzyme from harsh environmental conditions.
- Mild technique that keeps most of the enzyme’s natural activity.
- Suitable for sensitive enzymes and biomedical applications.

Disadvantages

- Mass transfer limitations: substrates move slowly into the matrix.
- Possible leakage of small enzyme molecules.
- Difficult to control the pore size of the gel.

Cross-Linking (CLEAs: Cross-Linked Enzyme Aggregates)

Advantages

- No need for a solid support (cost-effective).
- High enzyme concentration increases activity and efficiency.
- Good thermal and chemical stability.

Disadvantages

- Requires chemical reagents such as glutaraldehyde.
- May cause partial loss of enzyme activity.
- Not suitable for all types of enzymes.

Applications

- Food industry (cheese production, juice clarification)
- Pharmaceutical manufacturing
- Bioremediation
- Biofuel production

University of Diyala/ College of Science

Department of Biotechnology

4^{nd} **stage**

Enzymes
(Lecture 4)

Edited by

Dr. Zeyad Khalouf

Enzyme naming and classification:

Enzyme naming and classification:

Each enzyme is a signed two names. The first is its short, recommended name, useful for everyday use. The second is the more complete systematic name, which is used when an enzyme must be identified without doubt.

Recommended name

Most commonly used enzyme names have the suffix “ase” attached to the substrate of the reaction (for example, urease) or to a description of the action performed (for example, lactate dehydrogenase).

[Note: Some enzymes hold their original names, which give no hint of the associated enzymatic reaction, for example, trypsin and pepsin.]

Systematic name (enzymes classification):

In the early days, the enzymes were given names by their discoverers in an arbitrary manner. For example, the names pepsin, trypsin and chymotrypsin convey no information about the function of the enzyme or the nature of the substrate on which they act. Sometimes, the suffix-ase was added to the substrate for naming the enzymes e.g. lipase acts on lipids; nuclease on nucleic acids; lactase on lactose. These are known as trivial names of the enzymes which, however, fail to give complete information of enzyme reaction (**type of reaction, cofactor requirement etc.)** Enzymes are sometimes considered under two broad categories: (a) Intracellular enzymes – They are functional within cells where they are synthesized. (b) Extracellular enzymes – These enzymes are active outside the cell; all the digestive enzymes belong to this group.

The International Union of Biochemistry (IUB) appointed an Enzyme Commission in 1961. This committee made a thorough study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes. Since 1964, the IUB system of enzyme classification has been in force. Enzymes are divided into six major classes (in that order). Each class on its own represents the general type of reaction brought about by the enzymes of that class

Each class in turn is subdivided into many sub-classes which are further divided. A four-digit Enzyme Code (E.C.) number is assigned to each enzyme representing the class **(first digit)**, sub-class **(second digit),** sub-sub class **(third digit)** and the individual enzyme **(fourth digit).** Each enzyme is given a specific name indicating the substrate, coenzyme (if any) and the type of the reaction catalyzed by the enzyme.

Although the IUB names for the enzymes are specific and unambiguous, they have not been accepted for general use as they are complex and cumbersome to remember. Therefore, the trivial names, along with the E.C. numbers as and when needed, are commonly used and widely accepted.

According to the IUB system, enzymes are classified into six major classes as follows:

1. EC-1: Oxidoreductases

2. EC-2: Transferases

3. EC-3: Hydrolases

4. EC-: Lyases

5. EC-5: Isomerases

6. EC-6: Ligases

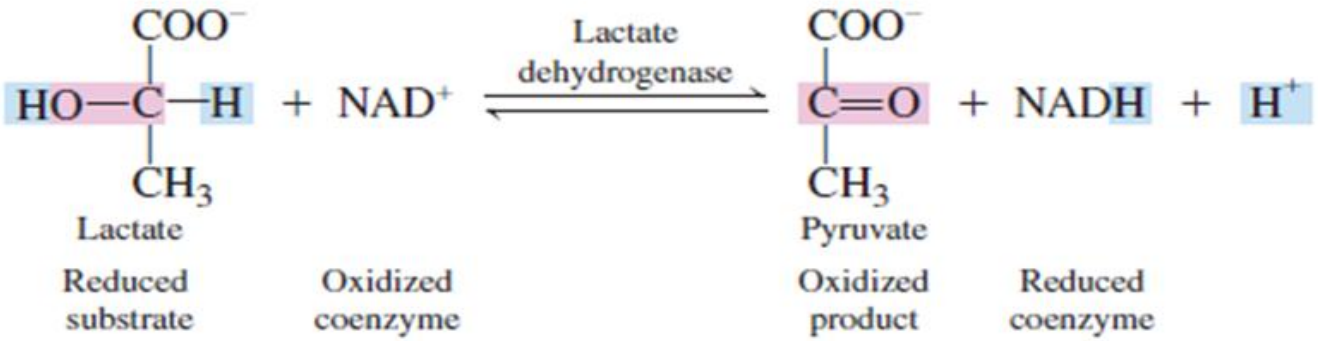
classification of enzyme

Enzyme class with examples*	Reaction catalysed
1. **Oxidoreductases** Alcohol dehydrogenase (alcohol : NAD^+ oxidoreductase E.C. 1.1.1.1.), cytochrome oxidase, L- and D-amino acid oxidases	Oxidation ⟶ Reduction $AH_2 + B \longrightarrow A + BH_2$
2. **Transferases** Hexokinase (ATP : D-hexose 6-phosphotransferase, E.C. 2.7.1.1.), transaminases, transmethylases, phosphorylase	Group transfer $A - X + B \longrightarrow A + B - X$
3. **Hydrolases** Lipase (triacylglycerol acyl hydrolase E.C. 3.1.1.3), choline esterase, acid and alkaline phosphatases, pepsin, urease	Hydrolysis $A - B + H_2O \longrightarrow AH + BOH$
4. **Lyases** Aldolase (ketose 1-phosphate aldehyde lyase, E.C. 4.1.2.7), fumarase, histidase	Addition ⟶ Elimination $A - B + X - Y \longrightarrow AX - BY$
5. **Isomerases** Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, E.C. 5.3.1.1), retinol isomerase, phosphohexose isomerase	Interconversion of isomers $A \longrightarrow A'$
6. **Ligases** Glutamine synthetase (L-glutamate ammonia ligase, E.C. 6.3.1.2), acetyl CoA carboxylase, succinate thiokinase	Condensation (usually dependent on ATP) $A + B \xrightarrow[ATP]{ADP + Pi} A - B$

For one enzyme in each class, systematic name along with E.C. number is given in the brackets.

1) Oxidoreductases:

These are enzymes which catalyse the oxidation – reduction reactions between two substrates. which are involved in electron transfer; ex: Dehydrogenase (Alcohol Dehydrogenase), Oxidase (Cytochrome Oxidase), Peroxidase (Glutathione Peroxidase)



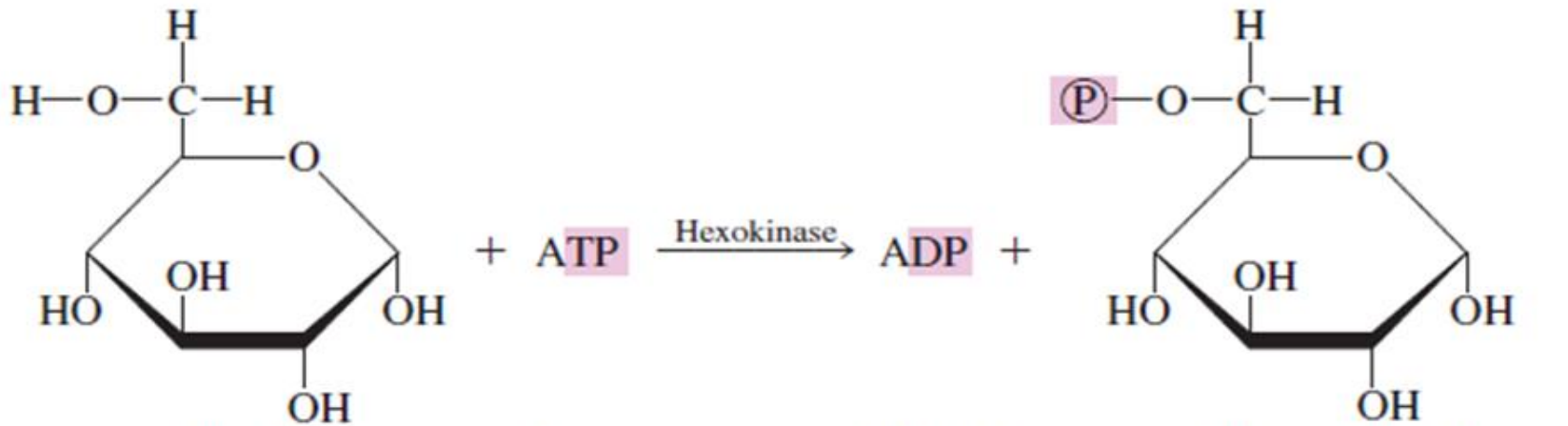
2) Transferases:

which transfer a chemical group from one substance to another

These are enzymes which catalyse the transfer of certain groups such as phosphate, amino or acetyl groups from one substrate to another.

Ex: Transaminase:

They catalyse the transfer of amino group from amino acid to keto acid. Example: Glutamate oxaloacetate transaminase (GOT) or Aspartate transaminase (AST). This enzyme catalyses the transfer of amino group from glutamic acid to oxaloacetic acid. It requires pyridoxal phosphate (PLP) as coenzyme for its activity.



Glucose

Adenosine triphosphate

(3 phosphate groups present)

Adenosine diphosphate

(2 phosphate groups present)

Glucose 6-phosphate

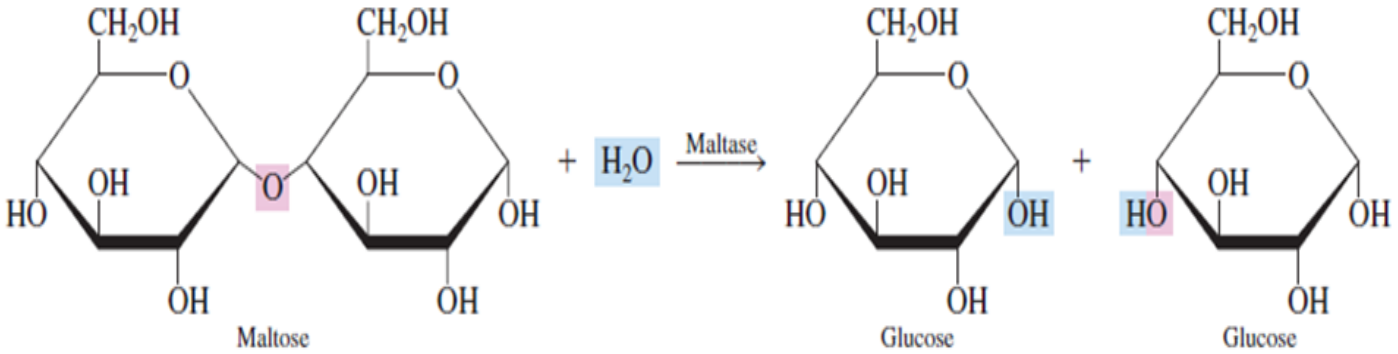
The symbol Ⓟ is a shorthand notation for a PO_3^{2-} unit.

3) Hydrolase:

which cleave the substrate by uptake of a water molecule (hydrolysis);

These are enzymes which catalyse the hydrolysis of substrates. They bring about the hydrolysis by adding water.

Example : a) Maltase b) Urease c) Glycosidase.

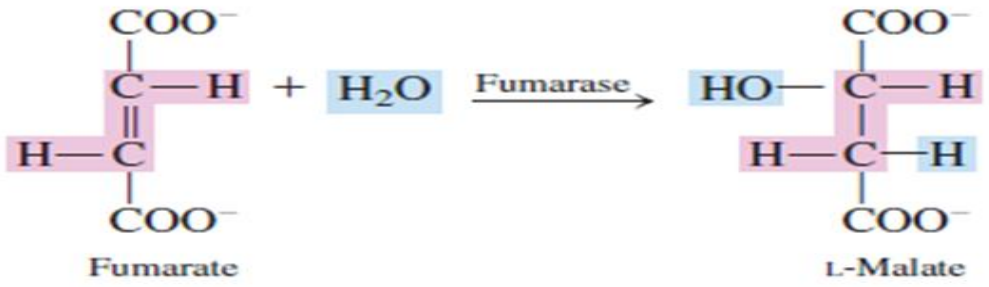


4) Lyases:

which form double bonds by adding or removing a chemical group

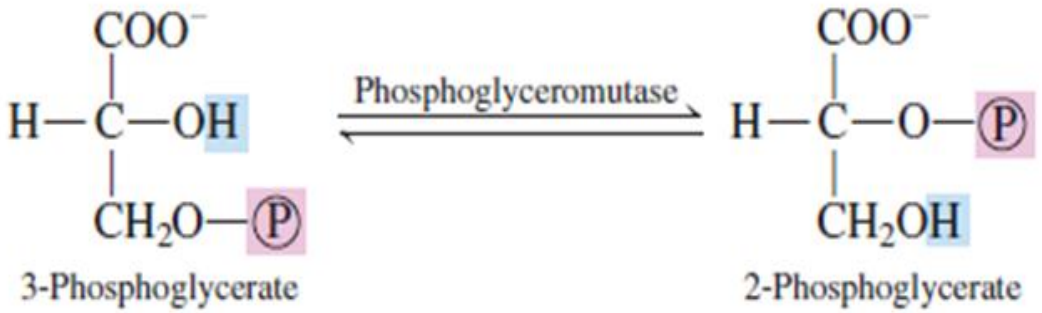
These enzymes catalyse the addition or elimination of groups like H2O, CO2, and NH3 etc.

Example: Aldolase, decarboxylase



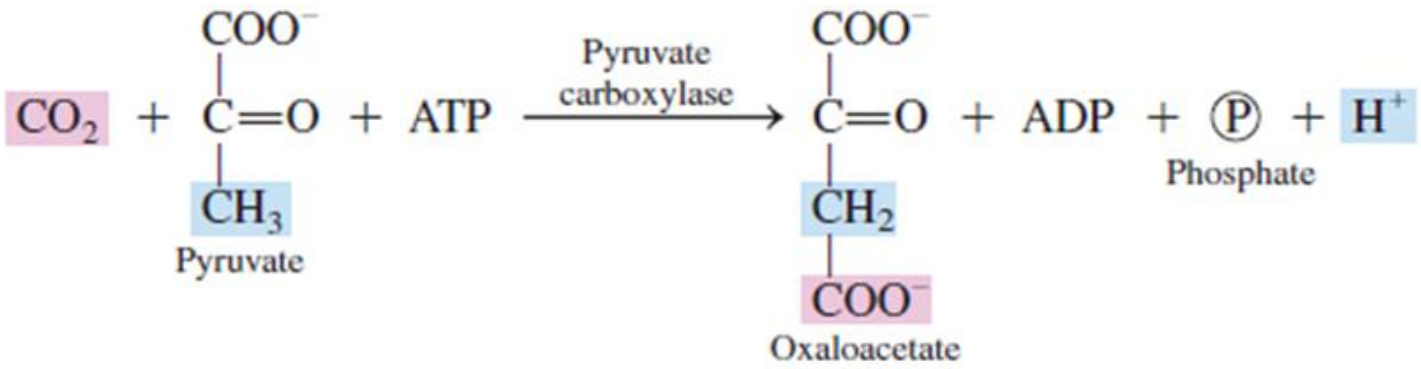
5) Isomerases:

An isomerase is an enzyme that catalyzes the isomerization (rearrangement of atoms) of a substrate in a reaction, converting it into a molecule isomeric with itself



6) Ligases:

A ligase is an enzyme that catalyzes the bonding together of two molecules into one with the participation of ATP



Isozyme

Isoenzymes:

are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. Now called “isoform” of an enzyme .Some enzyme are oligomerse (i.e:- proteins constructs from multiples of the same type of subunits others are constructed from multiples of different subunits . One of the first enzymes found to have isozymes was lactate dehydrogenase (LDH) , which, in vertebrate tissues, exists as at least five different isozymes separable by electrophoresis.

- **Function:**

They all catalyze the same chemical reaction.

- **Structure:**

They have different amino acid sequences, which results in variations in their structure, size, or charge.

- **Location:**

They are often organ-specific or tissue-specific, meaning a particular isozyme is found in higher concentrations in certain tissues.

- **Properties:**

The structural differences give them distinct kinetic parameters and different regulatory properties.

- **Origin:**

Isozymes can arise from different genes or from different modifications of the same gene product.

Why are isozymes important?

Tissue-specific needs:

They allow for the fine-tuning of metabolism to meet the specific metabolic requirements of different tissues or developmental stages.

Stress response:

Cells can use different isozymes to adapt to various conditions, such as a stress response or a change in growth conditions.

Clinical relevance:

They can be used in diagnostic medicine. For example, elevated levels of specific isozymes in the blood can indicate damage to a particular organ.

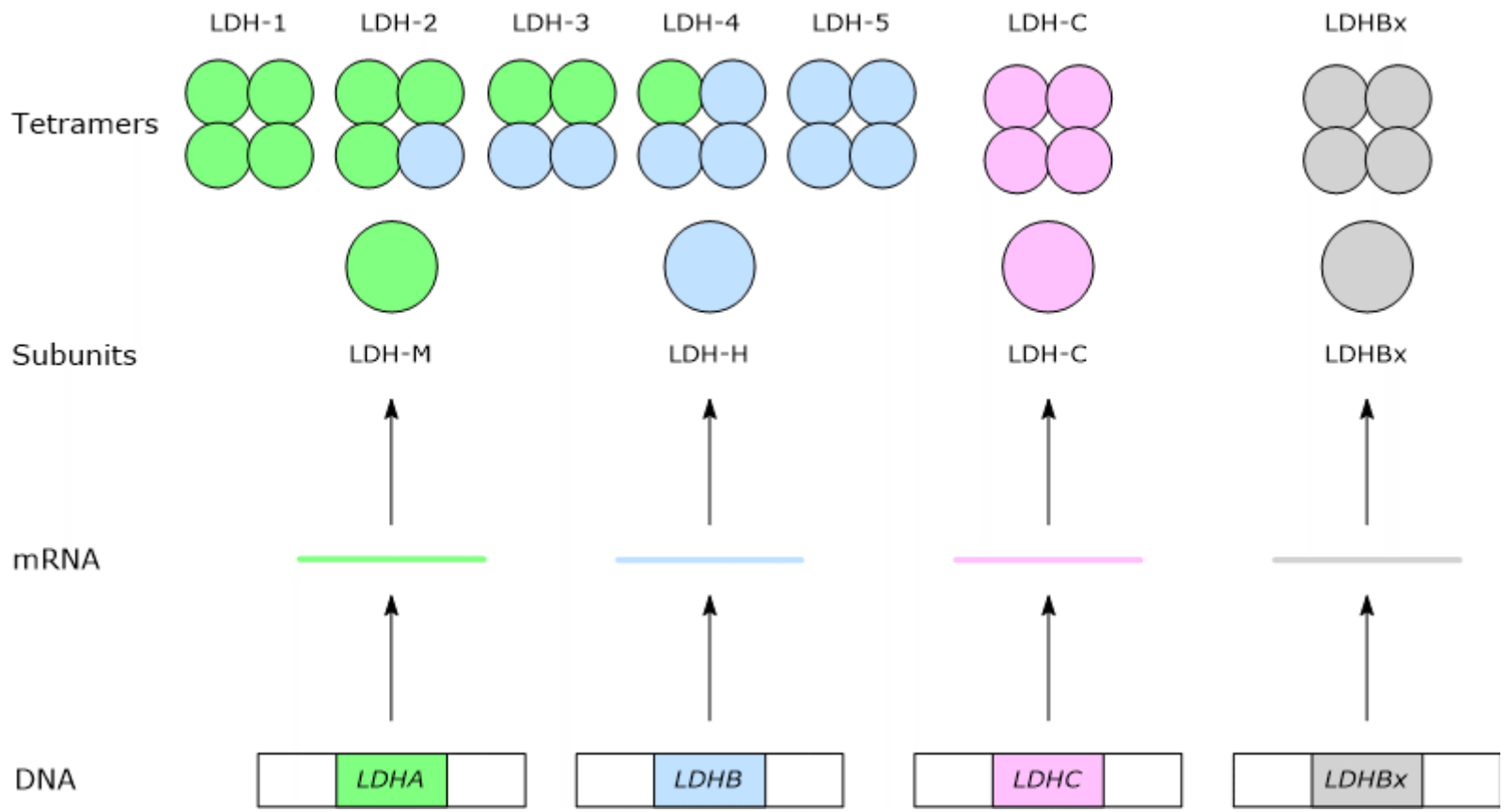
Disease research:

Studying isozyme changes can provide insights into diseases, such as cancer, where fetal isozymes may reappear in tumors.

All LDH isozymes contain four polypeptide chains (each of Mr 33,500), each type containing a different ratio of two kinds of polypeptides. The M (for muscle) chain and the H (for heart) chain are encoded by two different genes. In skeletal muscle the predominant isozyme contains four M chains, and in heart the predominant isozyme contains four H chains. Other tissues have some combination of the five possible types of LDH isozymes:

Type Composition Location:

- LDH1 HHHH Heart and erythrocyte
- LDH2 HHHM Heart and erythrocyte
- LDH3 HHMM Brain and kidney
- LDH4 HMMM Skeletal muscle and liver
- LDH5 MMMM Skeletal muscle and liver


LDH-1
LDH-2
LDH-3
LDH-4
LDH-5
LDH-C
LDHBx
Tetramers
Subunits
LDH-M
LDH-H
LDH-C
LDHBx
mRNA
DNA
LDHA
LDHB
LDHC
LDHBx

These differences in the isozyme content of tissues can be used to assess the timing and extent of heart damage due to myocardial infarction (heart attack). Damage to heart tissue results in the release of heart LDH into the blood. Shortly after a heart attack, the blood level of total LDH increases, and there is more LDH2 than LDH1.

After 12 hours the amounts of LDH1 and LDH2 are very similar, and after 24 hours there is more LDH1 than LDH2. This switch in the LDH1/LDH2 ratio, combined with increased concentrations in the blood of another heart enzyme, creatine kinase, is very strong evidence of a recent myocardial infarction.

University of Diyala/ College of Science

Department of Biotechnology

4^{nd} **stage**

Enzyme kinetics

(Lecture **6**+7)

Edited by

Dr. Zeyad Khalouf

Enzyme kinetics:

Enzyme kinetics is the study of the factors that affect the speed of enzyme-catalyzed reactions. Is the field of biochemistry concerned with:

1. The quantitative measurement of the rates of enzyme-catalyzed reactions
2. The factors that affect these rates.

Enzyme Kinetics

Enzyme = The active worker

Is a specialized protein that speeds up chemical reactions

Active site

Specific area on enzyme best suited for substrate

Studying enzyme kinetics

Measuring the enzyme's productivity

Understanding the factors that affect activity

Substrate = Raw material

Substrate is the molecule that the enzyme works on

Factors affecting activity

No. of workers = Enzyme concentration

Working environment

Presence of inhibitors

Why Enzyme kinetics are important ?

1- Understanding Biological Regulation and Function:

Enzyme kinetics provides the quantitative data necessary to elucidate how metabolic pathways are controlled within living organisms:

- **Pathway Analysis:** By determining key kinetic parameters (V_{max}), (K_m), and (k_{cat})), scientists can predict how changes in substrate concentration or enzyme activity will affect the overall flow (flux) of a metabolic pathway. This is crucial for understanding normal cellular function, such as how glucose is metabolized or how DNA is synthesized .
- **Physiological Insights:** Kinetic studies reveal how enzymes respond to environmental changes (temperature, pH) and regulatory signals (activators and inhibitors), thus explaining how organisms adapt to different conditions and maintain homeostasis .

2- Rational Drug Design and Pharmacology:

A primary application of enzyme kinetics is in the development of pharmaceuticals. Most drugs function by modulating the activity of specific enzymes:

Identifying Targets: Kinetics helps identify which enzymes are critical targets for treating diseases.

Physiological Insights: Kinetic studies reveal how enzymes respond to environmental changes (temperature, pH) and regulatory signals (activators and inhibitors), thus explaining how organisms adapt to different conditions and maintain homeostasis .

Mechanism of Action: By studying how a potential drug molecule interacts with an enzyme (e.g., competitive, non-competitive, or uncompetitive inhibition), researchers can determine its precise mechanism of action.

Efficacy and Dosage: Kinetic data allows for the quantification of drug binding affinity (e.g. K_i values) and efficacy, informing optimal dosage and predicting potential side effects or drug interactions.

3- Elucidating Disease Mechanisms:

Many diseases, including metabolic disorders, cancers, and neurodegenerative conditions, result from the malfunction of specific enzymes.

Diagnosing Disorders: Kinetic analysis can pinpoint the exact nature of an enzyme deficiency or mutation. For example, understanding how a mutation affects an enzyme's (Km) can explain why a specific genetic disorder develops.

Biomarker Development: Measuring the kinetic properties of enzymes in biological samples (blood, tissue) can serve as diagnostic biomarkers for various pathologies .

4- Industrial and Biotechnology Applications:

Enzymes are widely utilized in various industrial processes, and kinetic studies are vital for optimizing their performance:

Industrial Biocatalysts: In the food, detergent, and chemical industries, enzymes are used to produce specific products efficiently. Kinetic analysis helps engineers select the most appropriate enzyme for a given process and optimize reaction conditions (e.g., temperature, substrate concentration, pH) to maximize yield and efficiency .

Enzyme Engineering: When modifying enzymes for specific industrial uses (e.g., creating more heat-stable enzymes for detergents), kinetic assays are the primary tool used to evaluate the success of the engineering efforts .

5- Mechanistic Enzymology and Basic Research:

At the most fundamental level, enzyme kinetics is essential for understanding the basic principles of catalysis itself:

Reaction Mechanisms: Kinetic experiments, often involving steady-state and pre-steady-state kinetics, are indispensable for mapping out the step-by-step molecular mechanism by which an enzyme converts a substrate into a product .

Structure-Function Relationships: Kinetics links the three-dimensional structure of an enzyme to its functional behavior. Understanding how changes in structure (via mutagenesis) affect kinetic parameters provides deep insight into fundamental chemical principles .

Factors affecting the rate of enzyme reactions

1. Substrate concentration
2. Enzyme concentration
3. Temperature
4. pH
5. Cofactors or co- activators
6. Inhibitors

1- Substrate Concentration and Reaction Rate

- The rate of reaction increases as substrate concentration increases (at constant enzyme concentration)

- The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time.

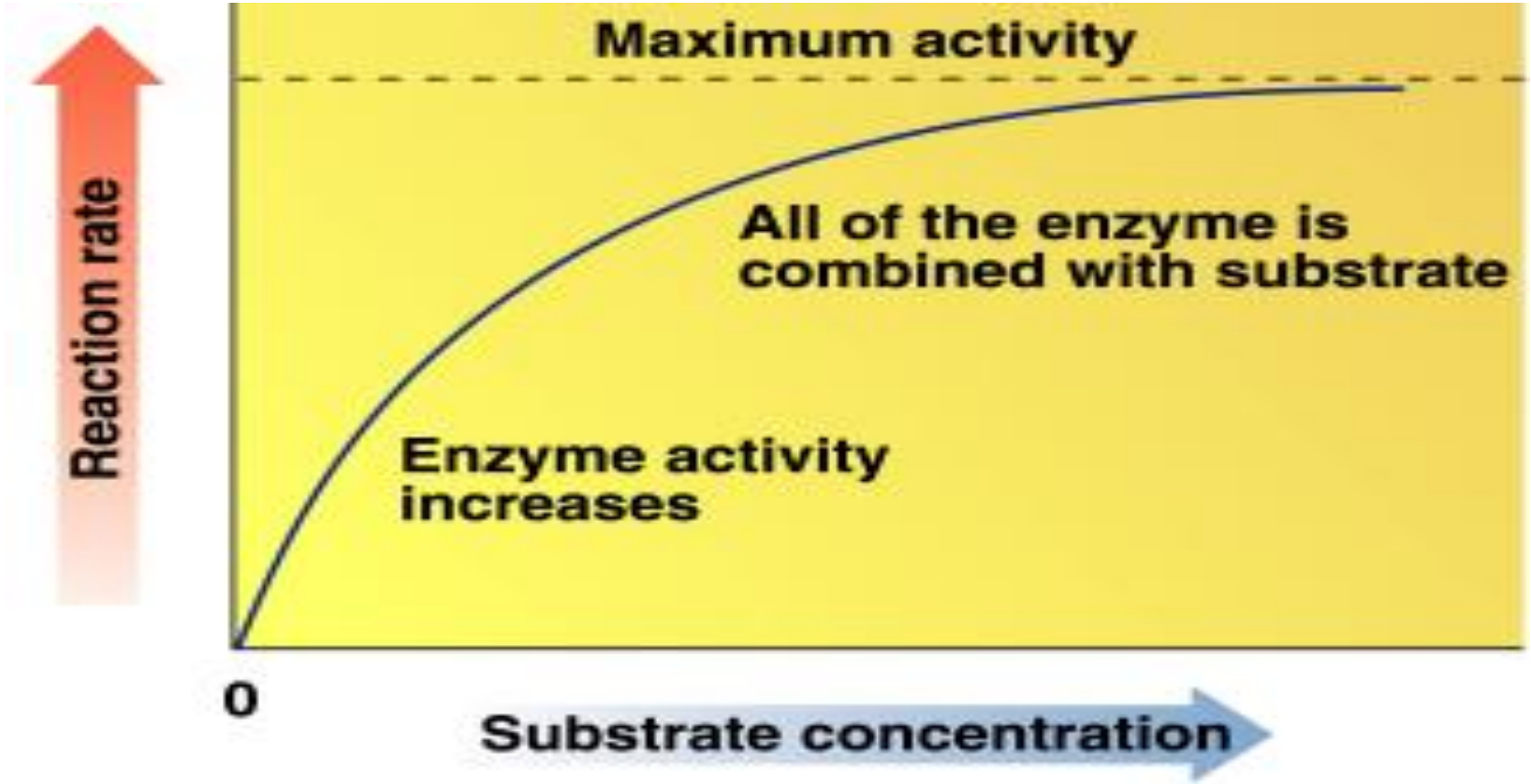
- velocity is usually expressed as μ mol of product formed per minute.

- The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity is reached .

- The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.

- Maximum velocity occurs when the enzyme is saturated (when all enzymes are binding substrate)

Substrate Concentration and Reaction Rate



They increase the rate by stabilizing the transition state (i.e. lowering the energy barrier to forming the transition state (they do not affect the energetics of either the reactant(s) or product(s)

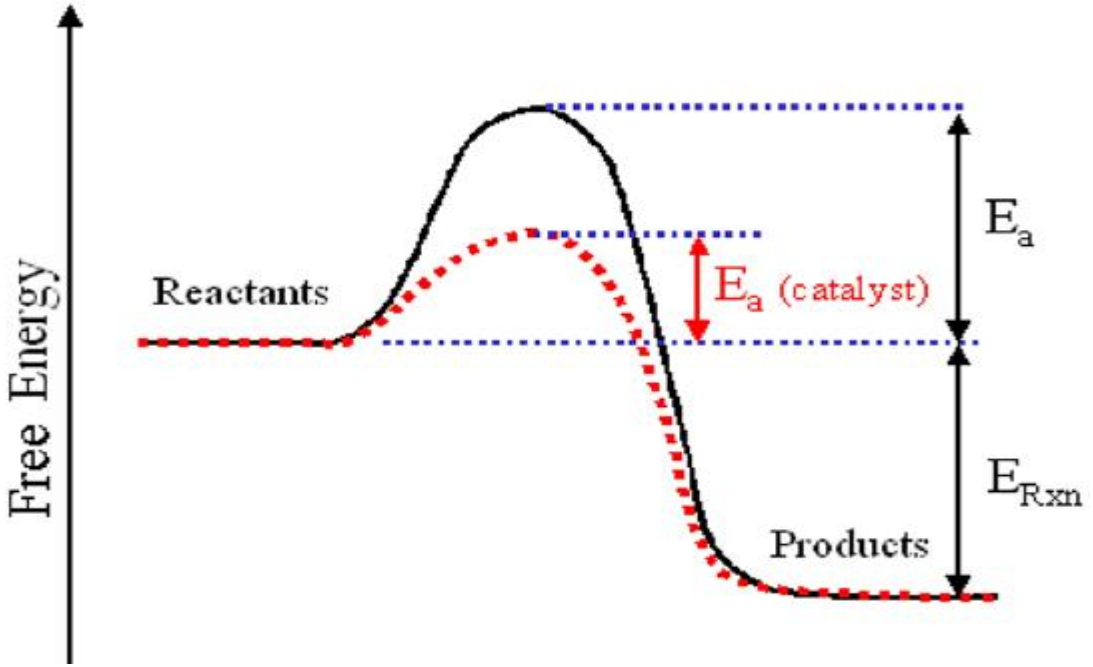


Figure 1: Catalyst activity

REGULATION OF ENZYMES BY SUBSTRATE AND PRODUCT CONCENTRATION:

- The velocity of all enzymes is dependent on the concentration of substrate.
- The hypothesis of enzyme kinetics assumes the rapid, reversible formation of a complex between an enzyme and its substrate .
- It also assumes that the rate of formation of the product, P, is proportional to the concentration of the complex.
- The **velocity of such a reaction is greatest when all the sites at** which catalytic activity can take place on the enzyme molecules (active sites) are filled with substrate; i.e. **when the substrate concentration is very high.**

Michaelis-Menten derivation for simple steady-state kinetics:

The Michaelis-Menten equation is a **mathematical model** that is **used to analyze simple kinetic data**. The model has certain **assumptions**, and as long as these assumptions are correct, it will accurately model your experimental data. The derivation of the model will highlight these assumptions.

- Michaelis and Menten proposed a simple model that accounts for most of the features of enzyme-catalyzed reactions.

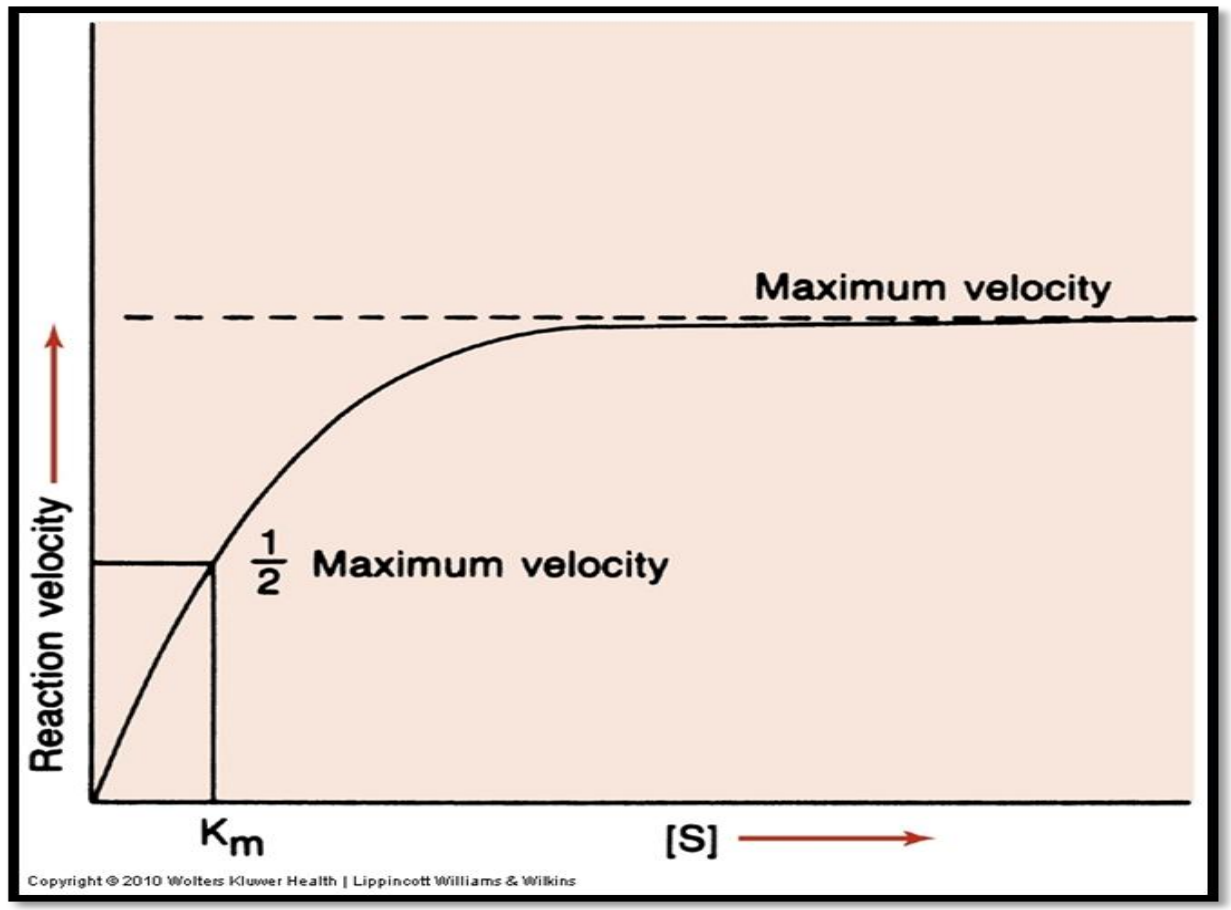
- In this model, the enzyme reversibly combines with its substrate to form an ES complex that subsequently breaks down to product, regenerating the free enzyme

The Michaelis constant is the substrate concentration at which the reaction rate is half of Vmax.

- Michaelis–Menten saturation curve for an enzyme reaction showing the relation between the substrate concentration and reaction rate.

- The Michaelis-Menton equation describes how reaction velocity varies with substrate concentration

- Most enzymes show Michaelis-Menten kinetics, in which the plot of initial reaction velocity against substrate concentration [S], is hyperbolic


Maximum velocity
Reaction velocity
1/2 Maximum velocity
Km
[S]
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Derivation of Michaelis-Menten equation:

- Enzymes are **protein catalysts,** they influence the **kinetics** but **not the thermodynamics** of a reaction
- Increase the rate of a chemical reaction
- Do not alter the equilibrium

The Michaelis-Menton equation describes how reaction velocity varies with substrate concentration

$$v0 = \frac{Vmax\,[S]}{Km + [S]}$$

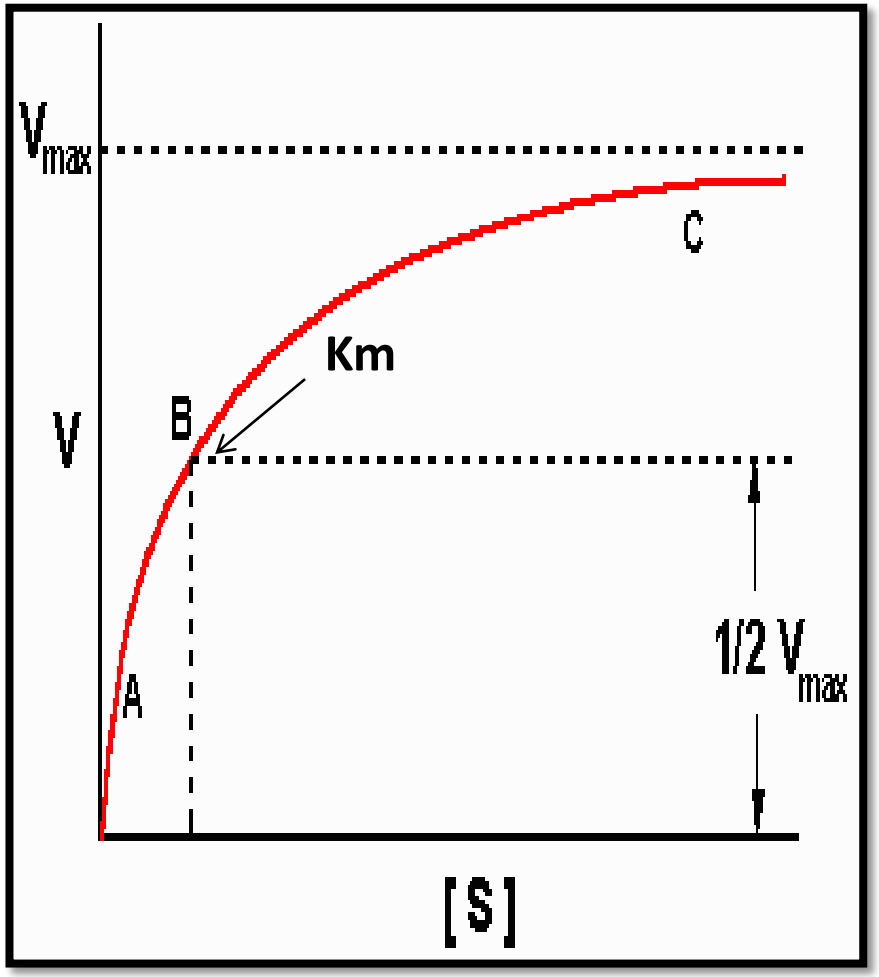
V0 = initial velocity, Vmax= maximum velocity
Km= (k-1 + k2)/k1

The Michaelis–Menten constant (Km):

- The Michaelis-Menten constant (Km) = is the substrate concentration required for an enzyme to reach one half its maximum velocity.

- Each enzyme has a characteristic Km for a given substrate.
  - **Small Km: A numerically small (low) Km reflects a high affinity of the enzyme for substrat**e, because a low concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is 1⁄2Vmax .

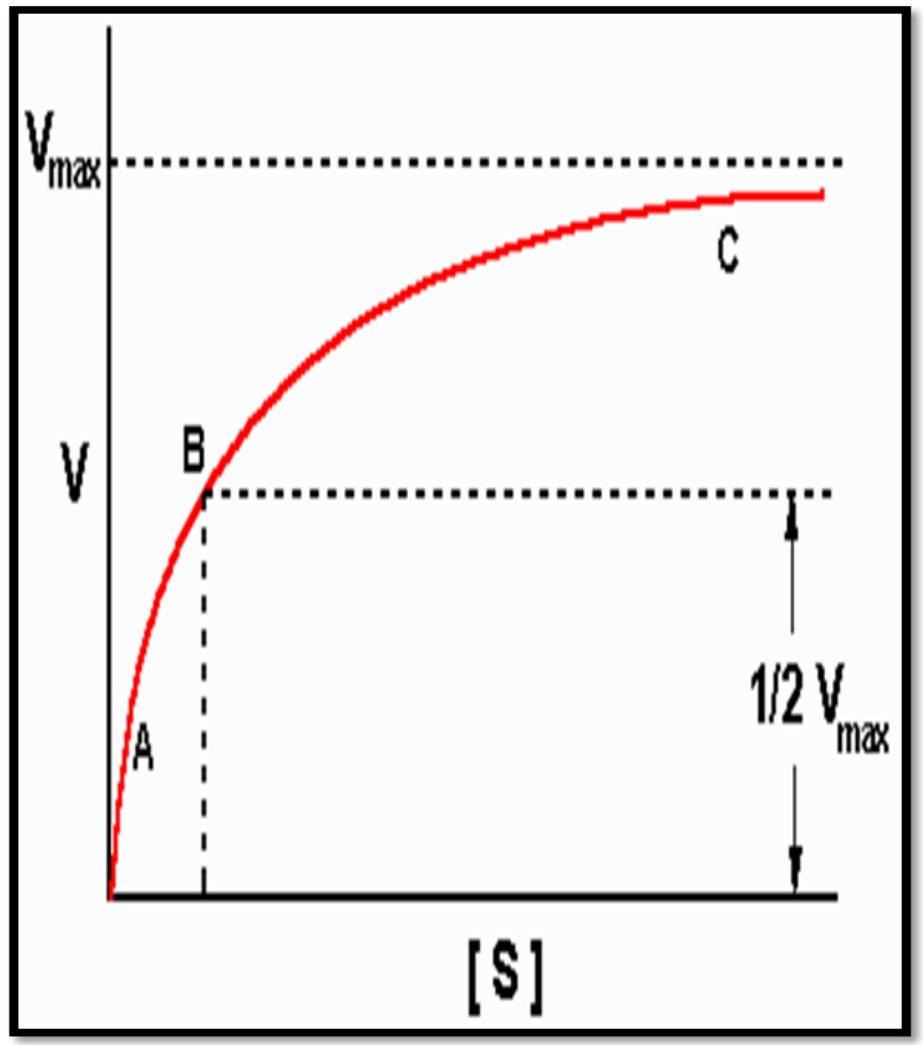
  - **Large Km: A numerically large (high) Km reflects a low affinity of enzyme for substrate** because a high concentration of substrate is needed to half-saturate the enzyme


Vmax
C
Km
V
B
1/2 Vmax
A
[S]

Important conclusions about Michaelis-Menten kinetics:

- The initial velocity V0= the rate of the reaction is measured as soon as enzyme & substrate are mixed , at that time the concentration of product is very small & therefore the rate of back reaction can be ignored thus the dependence of intial reaction velocity on S & Km can be evaluated under three conditions :

1. [S] << Km point A
2. [S] = Km point B
3. [S] >> Km point C


Vmax
C
V
B
A
1/2 Vmax
[S]

1. [S] << Km point A

- When **[S]** is much less than Km , the term (km+ [S]) will be equal to Km only :
- Replacing Km + **[S]** by km reduce the equation to:

$$V0 = \frac{Vmax\,[S]}{Km + [S]} = \frac{Vmax\,[S]}{Km} = \left[\frac{Vmax}{Km}\right]\,[S]$$

$$\left[\frac{Vmax}{Km}\right] \text{ is constant} \longrightarrow V0 = k\,[S]$$

- Initial velocity is directly proportional to [S]

2. [S] = Km point B

$$V0 = \frac{Vmax\,[S]}{Km + [S]} = \frac{Vmax\,[S]}{2\,[S]}$$

$$V0 = \frac{Vmax}{2}$$

- **Initial velocity is equal to half of the maximum velocity**

3. [S] >> Km point C

- When **[S]** is much grater than Km , the term **(km+ [S])** will be equal to **[S]** only :
- Replacing Km + **[S]** by **[S]** reduce the equation to:

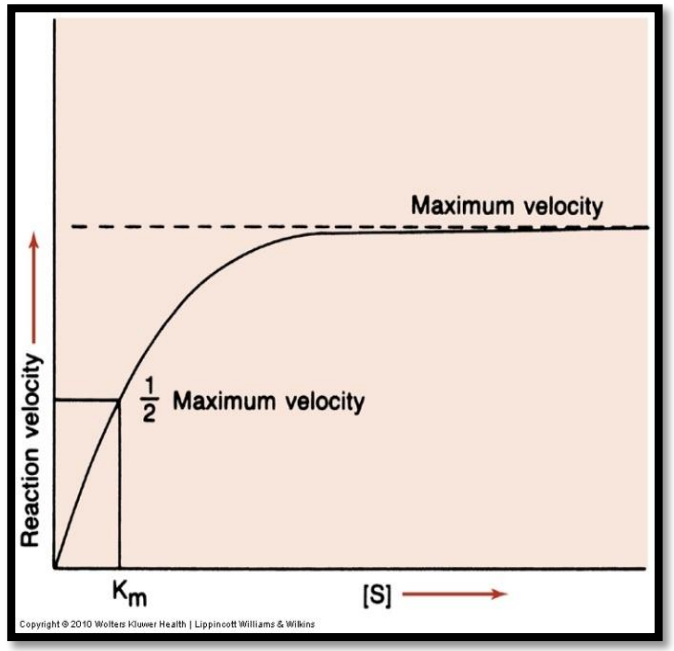
$$Vo = \frac{Vmax\,[S]}{Km + [S]} = \frac{Vmax\,\cancel{[S]}}{\cancel{[S]}} = Vmax$$

- **Initial velocity is equal to the maximum velocity**

Michaelis-Menten Equation Utility:

- Equation used to distinguish different kinds of inhibition

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]}$$



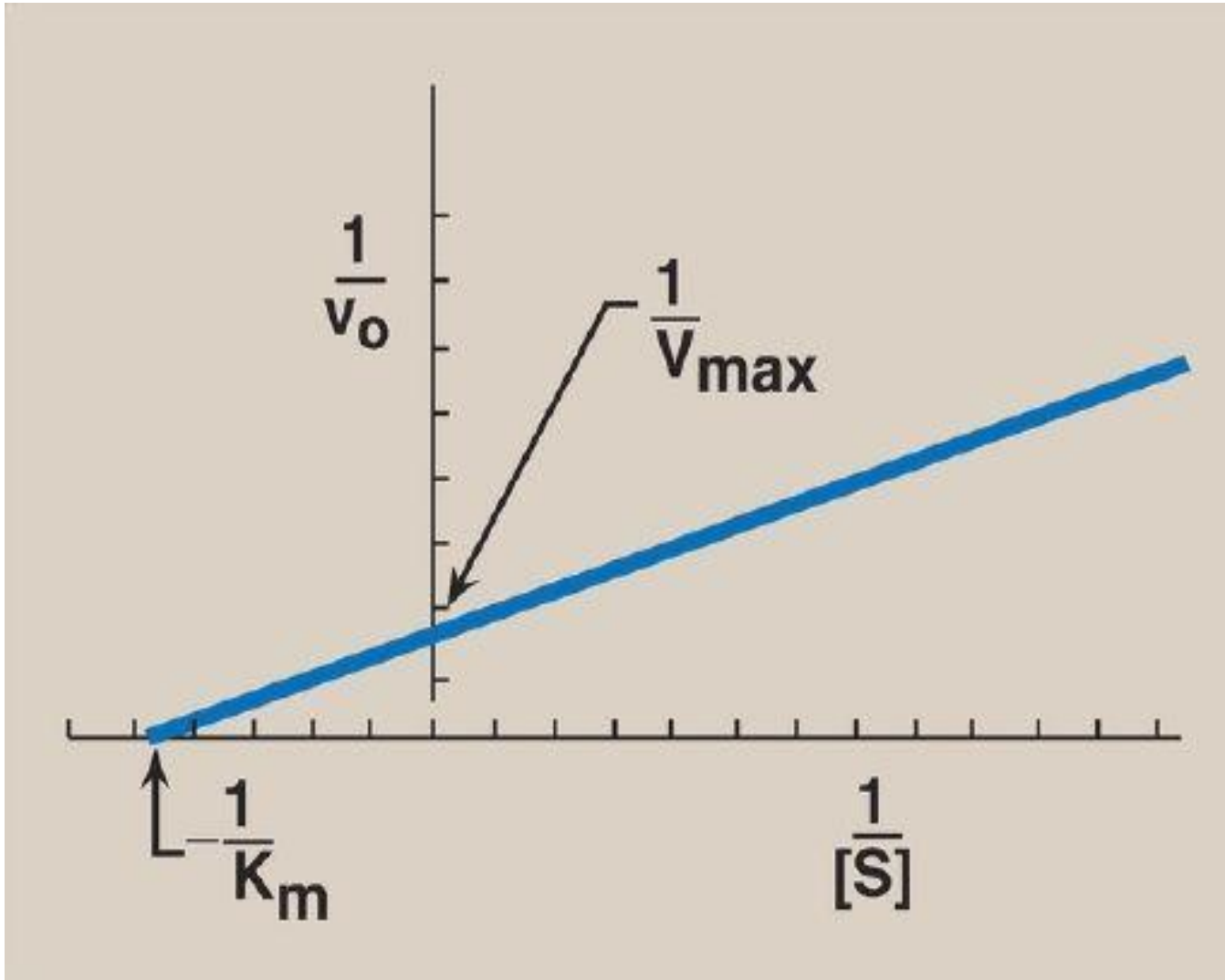
- Whereas:
  - V_0: velocity/rate of enzymatic activity
  - V_{max}: The maximal rate of reaction when the enzyme is saturated
  - K_m: (constant)the substrate concentration that produces ½ of the maximal velocity
  - S: substrate concentration

Lineweaver-Burke (the "*double reciprocal*" plot)

Another commonly-used plot in examining enzyme kinetics is the Lineweaver-Burk plot, in with the inverse of the reaction rate, $1/r$, is plotted against the inverse of the substrate concentration $1/[S]$. Rearranging Equation.

It gives a straight line, with the intercept on the y-axis equal to 1/Vmax, and the intercept on the x-axis equal to Km/Vmax. The slope of the line is equal to Km/Vmax.

Vmax and Km can be determined experimentally by measuring V0 at different substrate concentrations. Then a double reciprocal or Lineweaver–Burk plot of 1/V0 against 1/[S] is made.


1/v_o
1/V_max
−1/K_m
1/[S]

Slope Derivation Equation is :

Y= mx + b

Taking the reciprocal gives,

$$\frac{1}{V} = \frac{Km+[S]}{Vmax[S]}$$

$$\frac{1}{V} = \left(\frac{km}{Vmax}\right)\left(\frac{km}{[S]}\right) + \left(\frac{1}{Vmax}\right)$$

$$Y = m \quad x \quad + \quad b$$

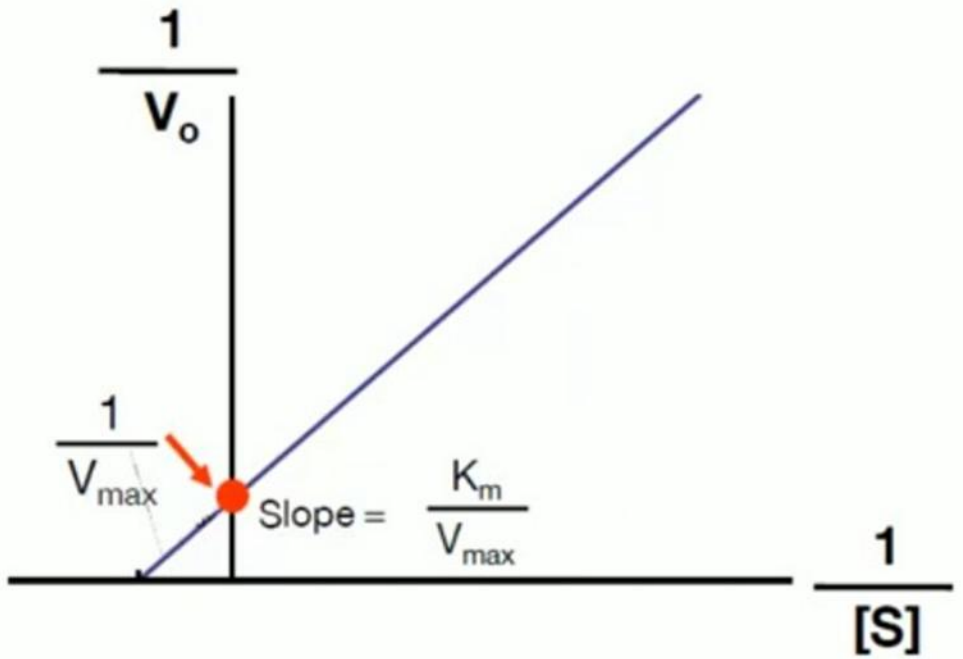
The y-intercept of such a graph is equivalent to the inverse of Vmax ; the x-intercept of the graph represents −1/Km . It also gives a quick, visual impression of the different forms of enzyme inhibition.

Lineweaver Burk: y-intercept

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

≈ 0

$$\rightarrow \frac{1}{V_o} = \frac{1}{V_{max}}$$



Lineweaver Burk: x-intercept

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

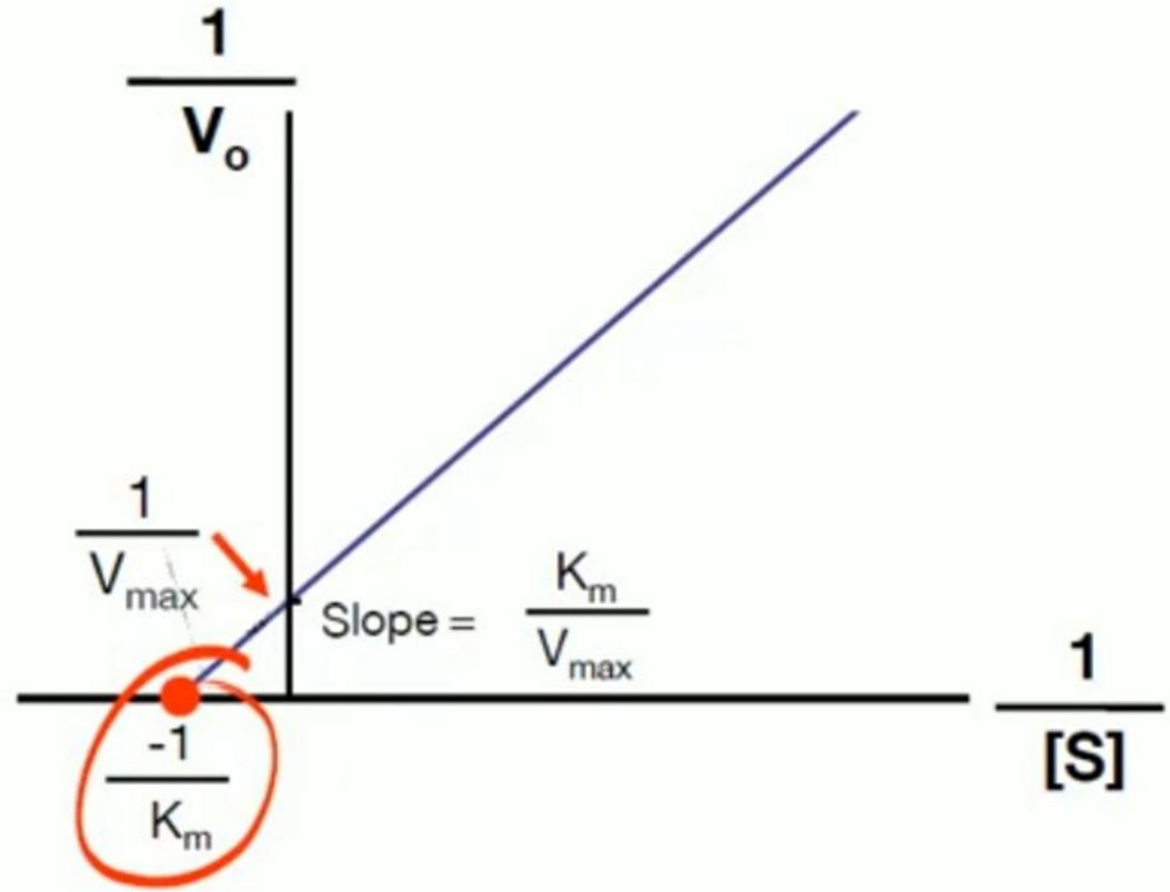
≈ 0

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

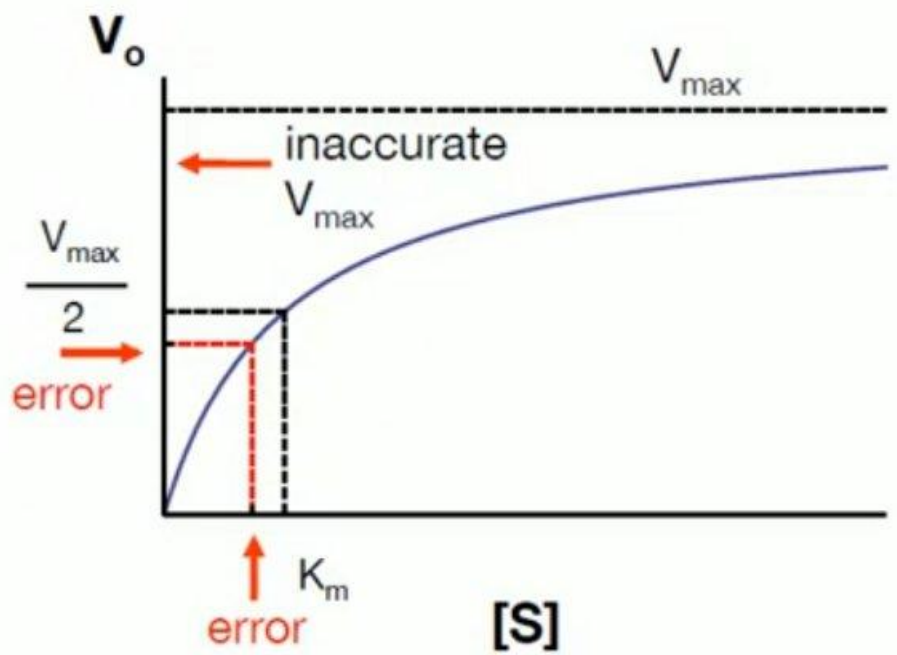
$$\rightarrow \frac{K_m}{[S]} = -1$$

$$\rightarrow \frac{1}{[S]} = \frac{-1}{K_m}$$

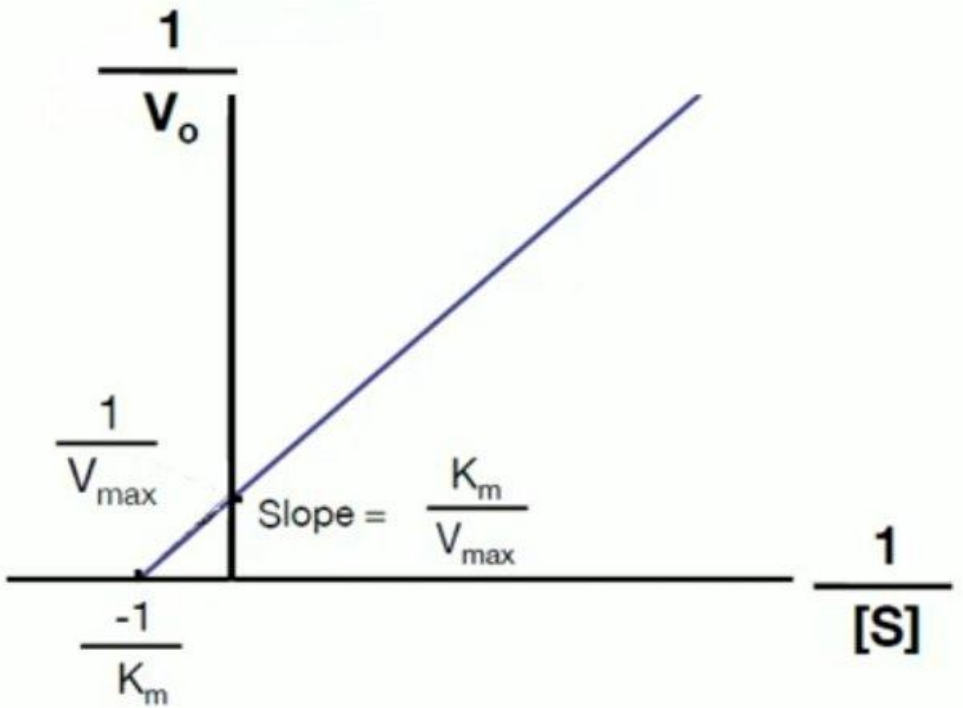
Lineweaver Burk



Michaelis Menten



Lineweaver Burk

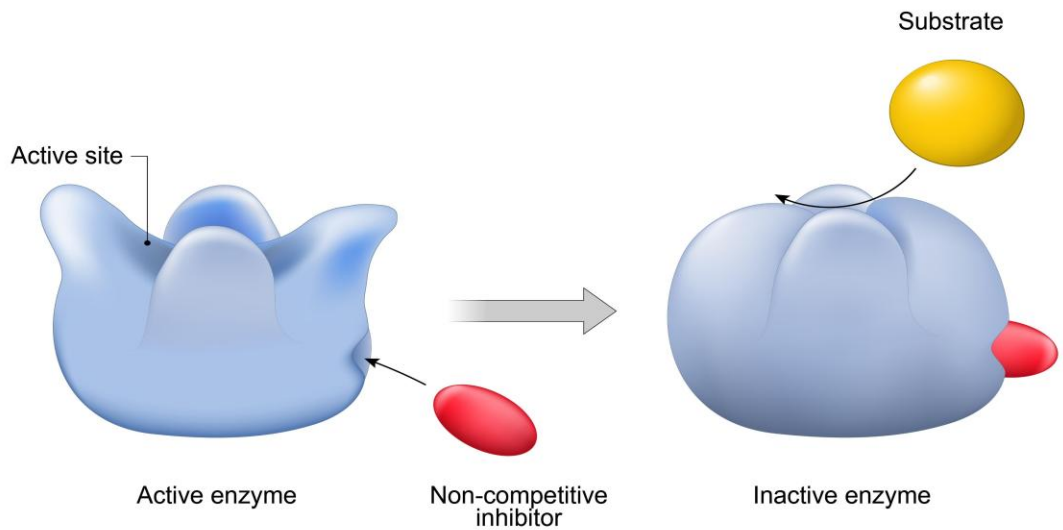


less error when determining Km

- Reversible enzyme inhibitors can be classified as either competitive or noncompetitive, and can be distinguished via a Lineweaver–Burk plot.
- It is a useful way of determining how an inhibitor binds to an enzyme.
- Competitive inhibition can be recognized by using a Lineweaver–Burk plot if V_0 is measured at different substrate concentrations in the presence of a fixed concentration of inhibitor.

- A competitive inhibitor increases the slope of the line on the Lineweaver–Burk plot, and alters the intercept on the x-axis (since Km is increased), but leaves the intercept on the y- axis unchanged (since V_{max} remains constant).
- Noncompetitive inhibition can also be recognized on a Lineweaver–Burk plot since it increases the slope of the experimental line, and alters the intercept on the y-axis (since V_{max} is decreased), but leaves the intercept on the x-axis unchanged (since Km remains constant).

Non-competitive inhibition



Uses of Lineweaver–Burk Plot:

1. Used to determine important terms in enzyme kinetics, such as K_m and V_{max}, before the wide availability of powerful computers and non-linear regression software.
2. Gives a quick, visual impression of the different forms of enzyme inhibition.

S	1/[S]	V0	1/V
0		0	
1.25	0.8	1.18	0.8475
1.67	0.6	1.51	0.6623
2	0.5	1.65	0.6061
2.5	0.4	1.91	0.5236
3.33	0.3	2.34	0.4274
5	0.2	2.75	0.3636
10	0.1	3.57	0.2801
150	0.07	3.64	0.2747

University of Diyala/ College of Science

Department of Biotechnology

4^{nd} **stage**

ENZYME INHIBITION

(Lecture **8**)

Edited by

Dr. Zeyad Khalouf

ENZYME INHIBITION :

Enzymes catalyze virtually every process in the cell. The catalytic activity of certain enzymes is altered by certain inorganic and organic molecules called modifiers. Those molecules which increase the enzyme activity are called activators (Positive modifiers) and those which decrease the enzyme activity are called inhibitors (Negative modifiers).

Compounds which convert the enzymes into inactive substances and thus adversely affect the rate of enzyme-catalyzed reaction are called enzyme inhibitors. Such a process is known as enzyme inhibition. Two broad classes of enzyme inhibitions are generally recognized : Reversible and Irreversible , depending on whether the enzyme inhibitor complex dissociates rapidly or very slowly.

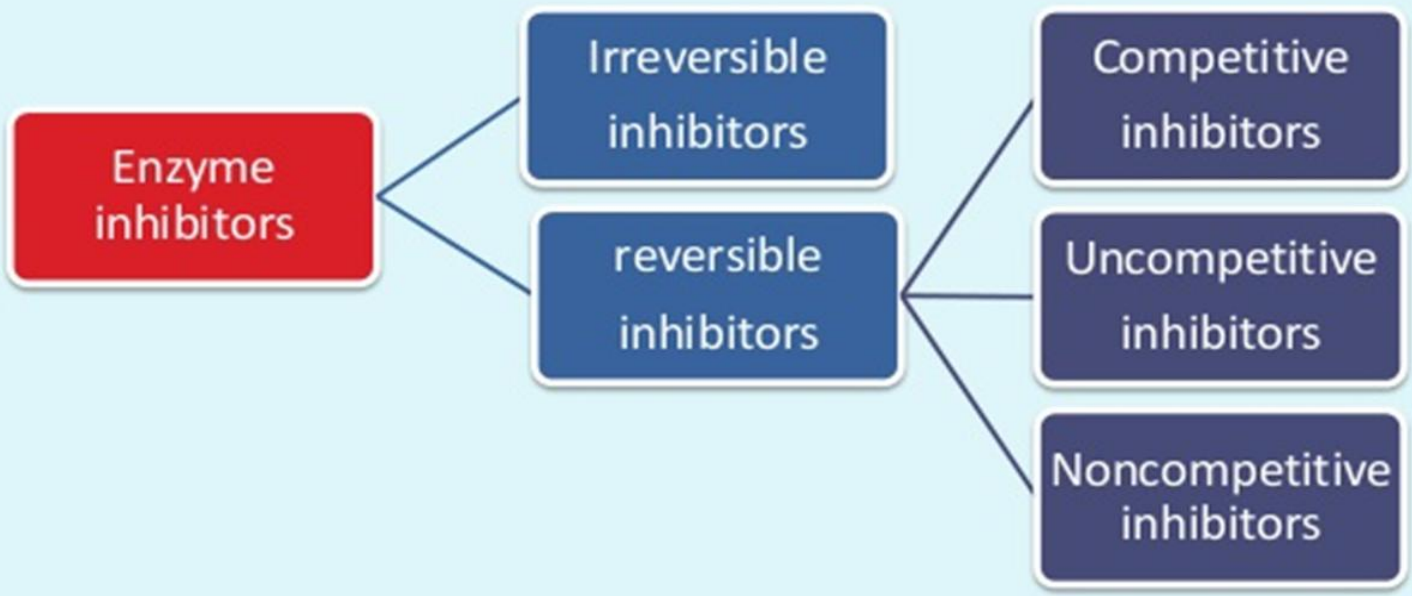
Inhibiton :

The prevention of an enzyme process as a result of interaction of inhibitors with the enzyme.

Inhibitors:

Any substance that can diminish the velocity of an enzyme catalyzed reaction is called an inhibitor.

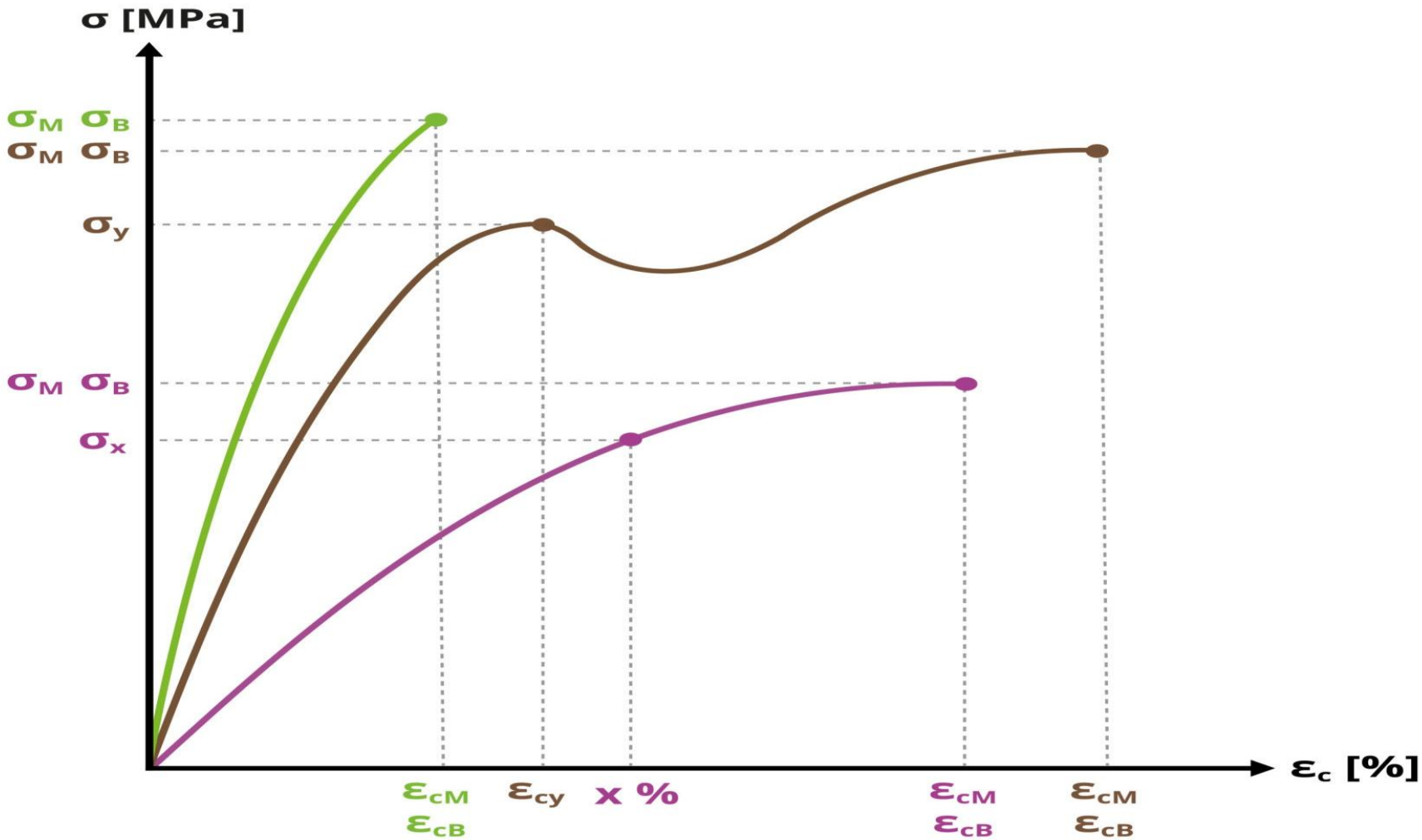
Enzyme inhibitors



Types of Enzyme Inhibitions

Reversible	Irreversible
1. Enzymes do follow Michaelis- Menten rate equation [hence Lineweaver-Burk plot also] and exhibit Rectangular hyperbolic curve when [V] is plotted against [S].	1. Enzymes usually do not follow Michaelis-Menten rate equation [hence Lineweaver-Burk plot also] and exhibit Sigmoidal curve when [V] is plotted against [S].
2. A reversible inhibitor dissociates very rapidly from its target enzyme because it becomes very loosely bound with the enzyme.	2. An irreversible inhibitor dissociates very slowly from its target enzyme because it becomes very tightly bound to its active site, thus inactivating the enzyme molecule. The bonding between the inhibitor and enzyme may be covalent or noncovalent in case of this type modification of enzymes which are commonly called as Regulatory enzymes also.
3. Three general types of inhibition are distinguished depending on three factors : (i) Whether the inhibition is or is not overcome by increasing the concentration of the substrate. (ii) (ii) Whether the inhibitor binds at the active site or at allosteric site. (iii) (iii) Whether the inhibitor binds with the free enzyme only, or with the enzyme-substrate complex only, or with either of the two.	3. Two general types of inhibition / modulation are distinguished depending on two factors : (i) Catalytic activity is modulated through the noncovalent binding of a specific metabolite at a site on the protein other than the catalytic site – Allosteric enzyme. (ii) Catalytic activity is interconverted between active and inactive forms by the action of other enzymes – Covalently modulated enzymes.

COMPRESSIVE STRESS/STRAIN CURVES



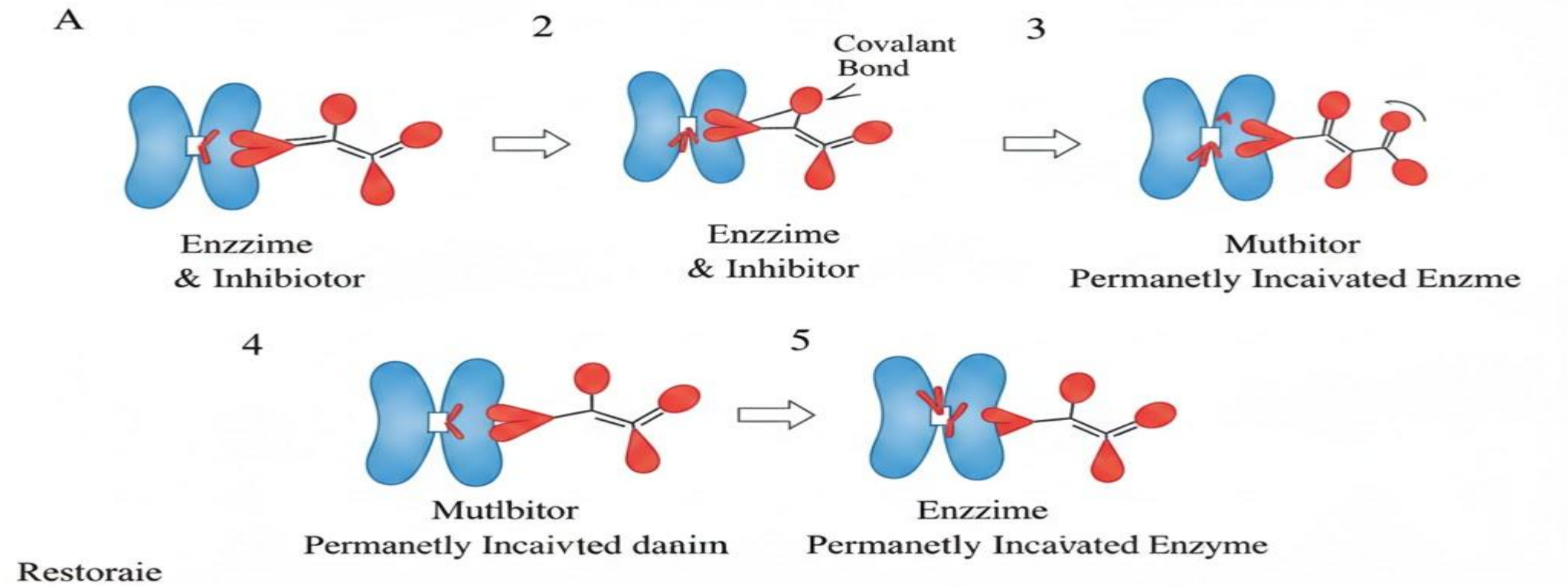
Competitive	Uncompetitive	Non-competitive
1. The inhibitor can combine with the free enzyme in such a way that it competes with the normal substrate for binding at the active site. It is also called as Substrate analogue Inhibition	1.The inhibitor does not combine with the free enzyme or affects its reaction with its normal substrate; however, it does combine with the enzyme-substrate complex.	1.The inhibitor does not combine with the free enzyme or affects its reaction with its normal substrate; however, it does combine with the enzyme-substrate complex.
2. An enzyme-inhibitor complex is formed [EI], analogous to the enzyme-substrate complex [ES]. Higher substrate concentration can lower the rate of inhibition.	2. An inactive enzyme- substrate inhibitor complex [ESI] is formed here which cannot undergo further reaction to yield the normal product.	2. Inhibitors often to deform the enzyme, so that these do not form the [ES] complex at its normal rate and once formed, the [ES] complex does not decompose at the normal rate to yield products. Two inactive complex's, [ESI] & [EI] are formed.
3. The degree of inhibition depends on the relative concentrations of the substrate and the inhibitor.	3. The degree of inhibition may increase when the substrate concentration is increased.	3. The degree of inhibition is not reversed by increasing the substrate concentration.
4. Example : Enzyme – Succinate Dehydrogenase Substrate – Succinate Inhibitors – Malonate,Glutarate, Oxalate etc.	4. Example : Rare in one-substrate reaction; but common in Bi-substrate reaction.	4. Example : Metal ion-requiring enzymes can be inhibited by chelating agents like EDTA.
5- Kinetics : Slope [Km/Vmax] is changed; Intercept on ordinate [1/Vmax] is not changed.	5. Kinetics : Slope [Km/Vmax] is not changed; Intercept on ordinate [1/Vmax] is changed.	5. Kinetics : Slope [Km /Vmax] is changed; Intercept on ordinate [1/Vmax] is also changed.

Irreversible inhibition

This type of inhibition involves the covalent attachment of the inhibitor to the enzyme.

The catalytic activity of enzyme is completely lost.

It can only be restored only by synthesizing molecules.


A
2
Covalant
Bond
3
Enzzime
& Inhibiotor
Enzzime
& Inhibitor
Muthitor
Permanetly Incaivated Enzme
4
5
Mutlbitor
Permanetly Incaivted danim
Enzzime
Permanetly Incavated Enzyme
Restoraie

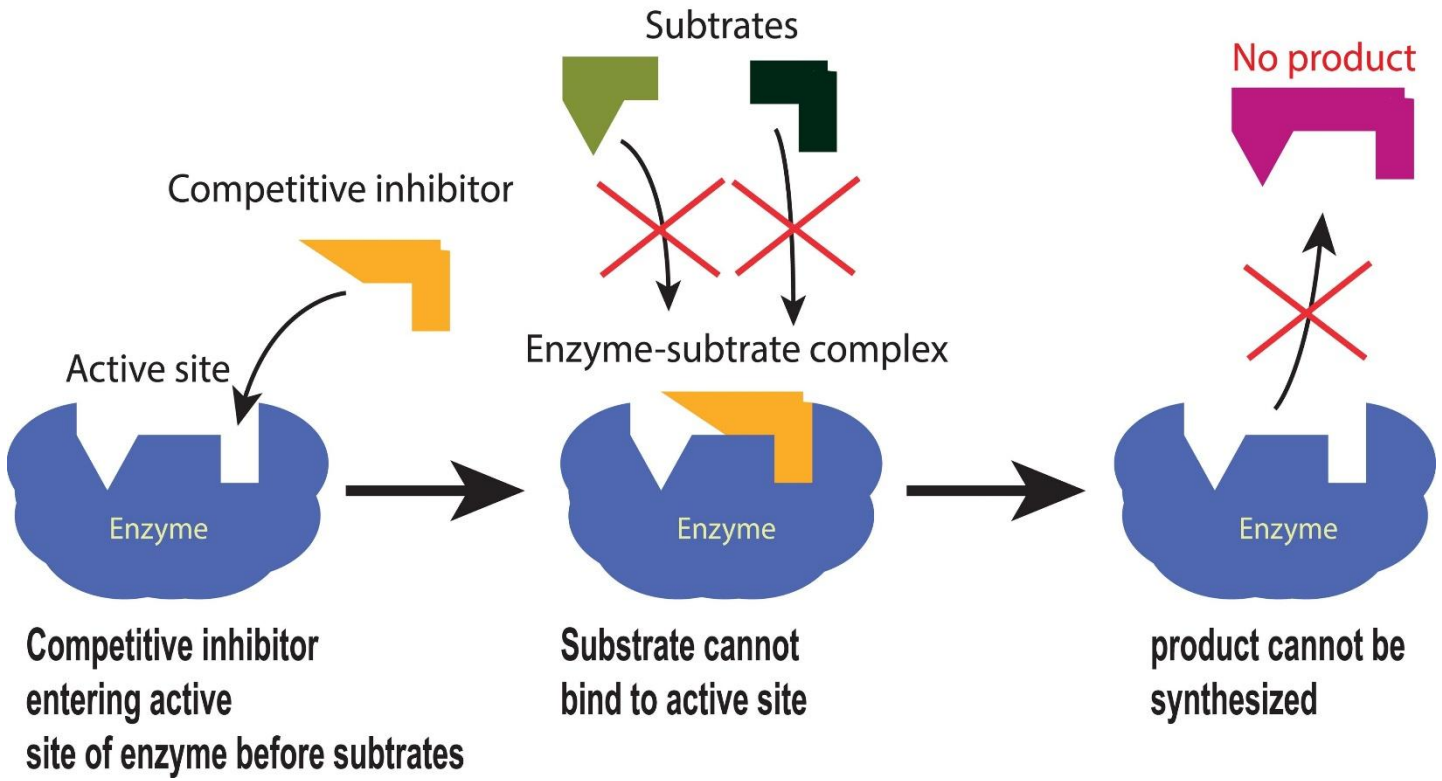
Irreversible Inhibition: Poisons

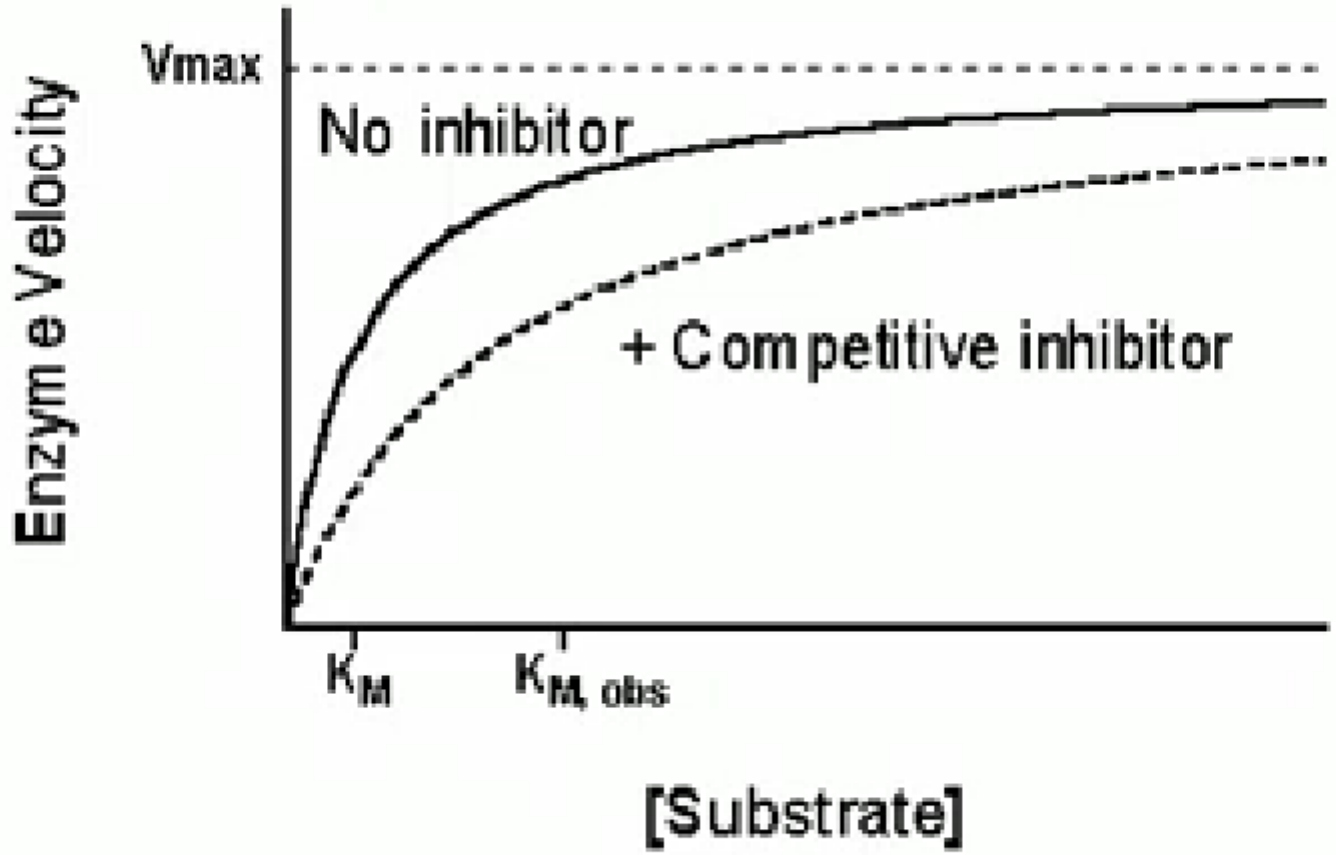
An irreversible inhibitor inactivates an enzyme by bonding covalently to a particular group at the active site. The inhibitor-enzyme bond is so strong that the inhibition cannot be reversed by the addition of excess substrate. The nerve gases, especially Di isopropyl fluorophosphate (DIFP), irreversibly inhibit biological systems by forming an enzyme-inhibitor complex with a specific OH group of serine situated at the active sites of certain enzymes. The peptidases trypsin and chymotrypsin contain serine groups at the active site and are inhibited by DIFP.

Competitive inhibition

In this type of inhibition competitive with the substrate for the active site. Formation of **E.S** complex is reduced while a new **E.I** complex is formed.

Enzyme and the Competitive inhibitor

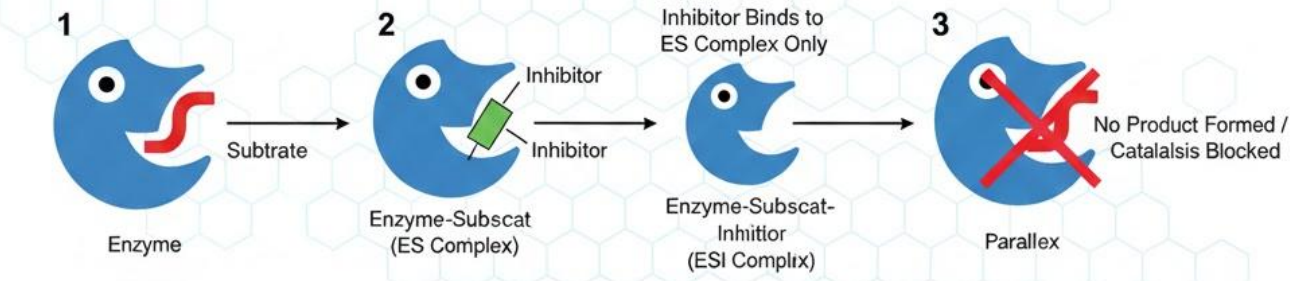



Vmax
No inhibitor
+ Competitive inhibitor
Enzyme Velocity
K_M
$K_{M, obs}$
[Substrate]

Uncompetitive inhibition

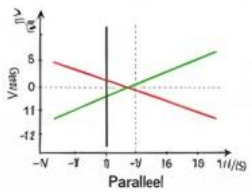
In the type of inhibition, inhibitor dose not with the substrate for the active site of enzyme instead it binds to another site known as **allosteric site.**

Uncomptticve Inhibition



V_{max} Decreases: ⬇

Km Decreases: ⬇ Lineweaver-Burk Plot



Examples of uncompetitive inhibition:

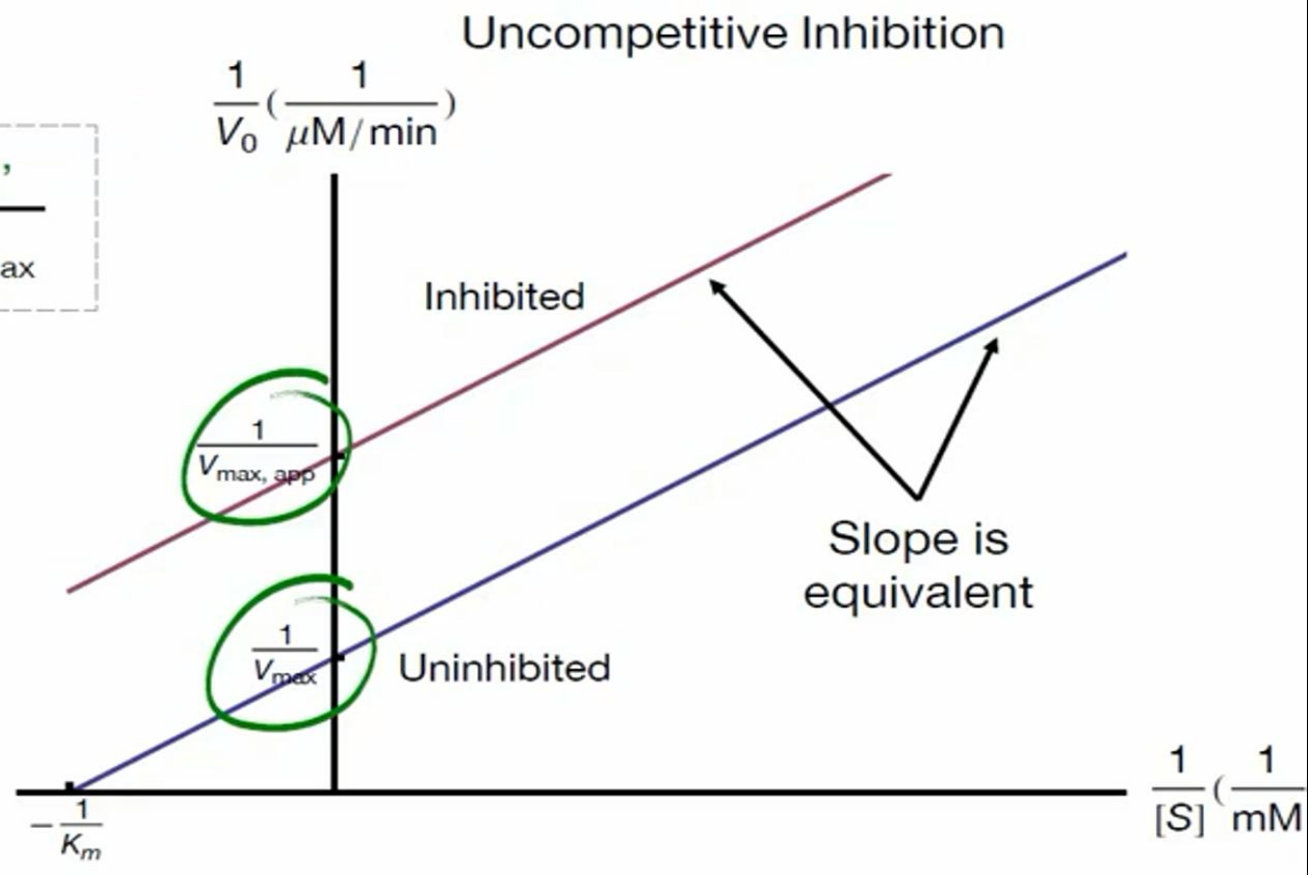
- Drugs to treat cases of poisoning by methanol or ethylene glycol act as uncompetitive inhibitors.
- Tetramethylene sulfoxide and 3- butylthiolene 1-oxide are uncompetitive inhibitors of liver alcohaldehydrogenase .

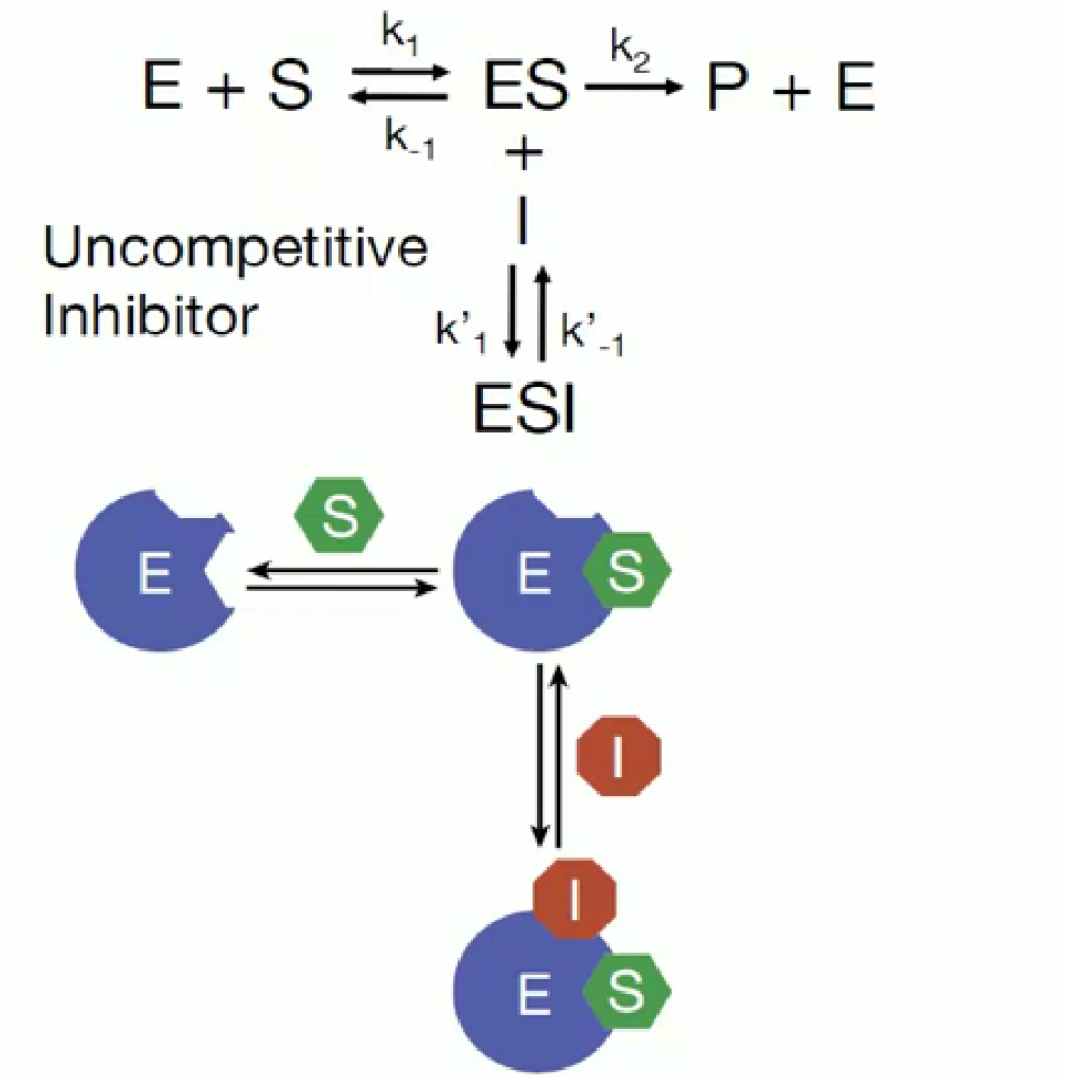
$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

$$\alpha' = 1 + \frac{[I]}{K'_I}$$

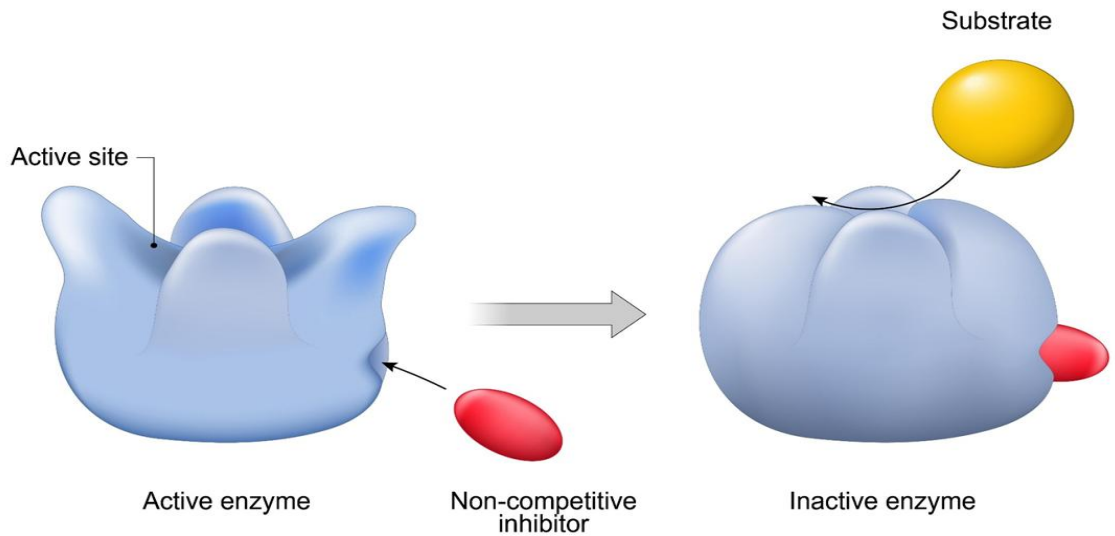
Decreased K_m

Decreased V_{max}




E + S
k1
k-1
ES
k2
P + E
+
I
Uncompetitive
Inhibitor
k'1
k'-1
ESI
E
S
E
S
I
I
E
S

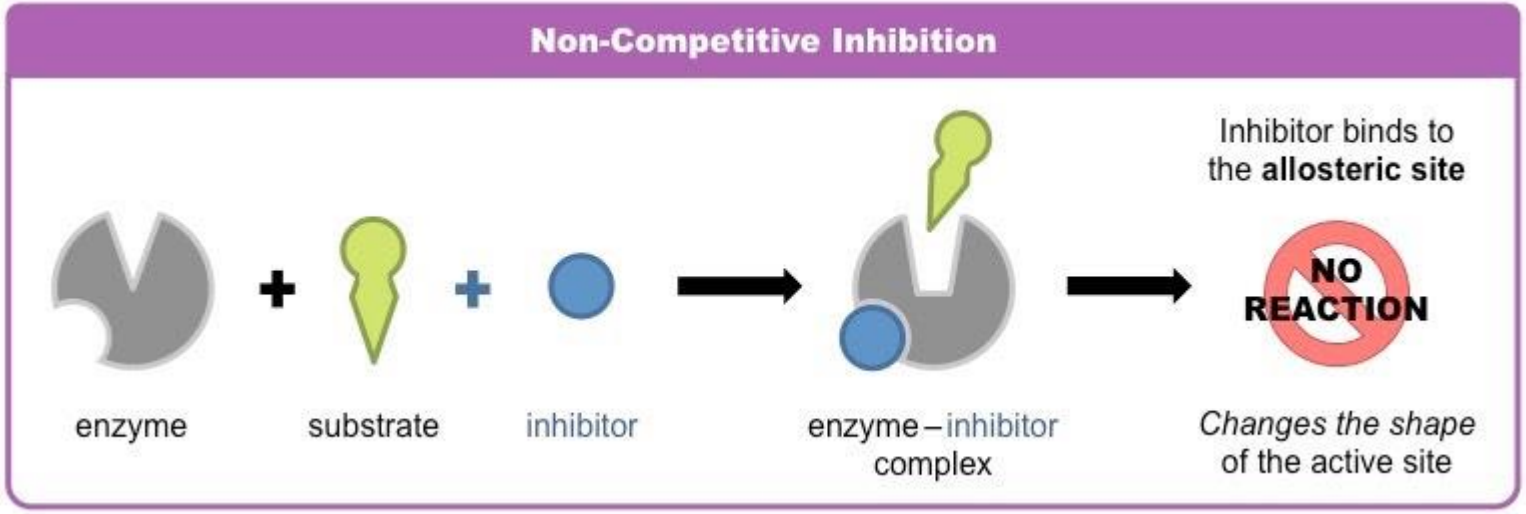
Non-competitive inhibition



Noncompetitive inhibitor:

can bind to an enzyme with or without a substrate at different places at the same time.

It changes the conformation of an enzyme as well as its active site, which makes the substrate unable to bind to the enzyme effectively so that the efficiency decreases.

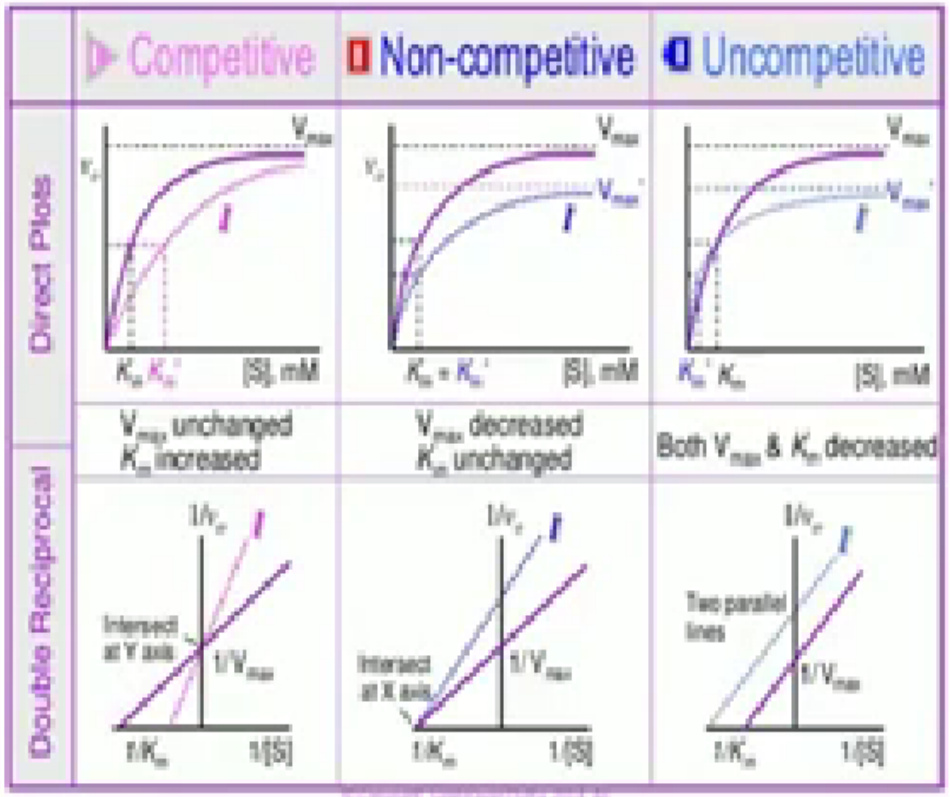


A noncompetitive inhibitor binds to a different site that is not the active site of the enzyme and changes the structure of the enzyme; therefore, it blocks the enzyme from binding to substrate, which stops enzyme activity. Thus, it decreases the rate of the chemical reaction of enzyme and substrate, which can not be changed by increasing concentration of substrate.

E+S+I→EI NR (no reaction) (competitive inhibitor) (Inhibition in the active site)
E+S⇌ES+I →ESI (no reaction) (uncompetitive inhibitor) (Inhibition in the allosteric site)
E+S+I→EI NR (no reaction) (noncompetitive inhibitor) (Inhibition in the allosteric site)

- where E is enzyme, I is inhibitor, ES is enzyme-substrate complex,. ESI is the molecule after the inhibitor is bound to the enzyme-substrate complex. ESI cannot form any products, so the later reaction is not allowed (or, no reaction).

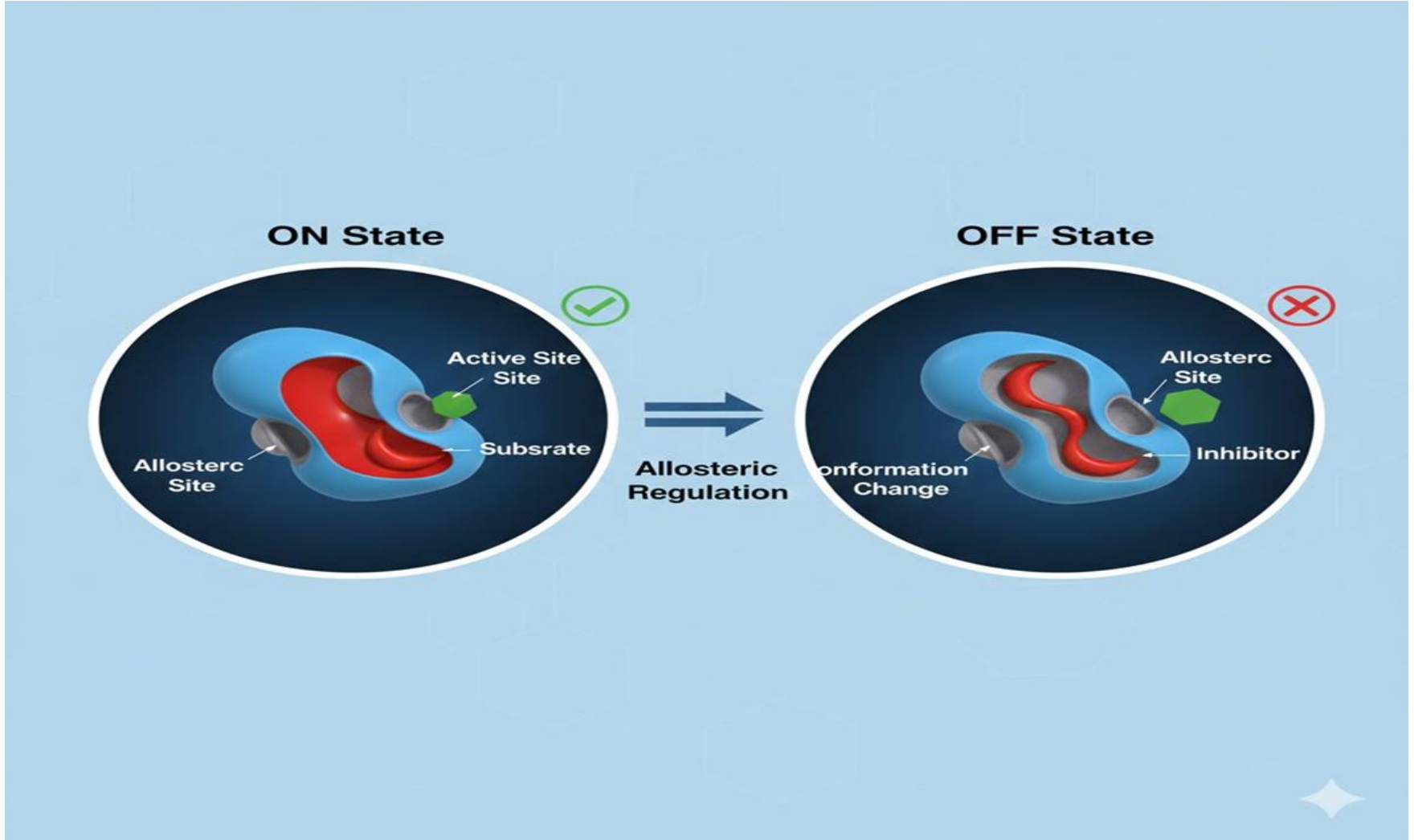
Enzyme Inhibition (Plots)



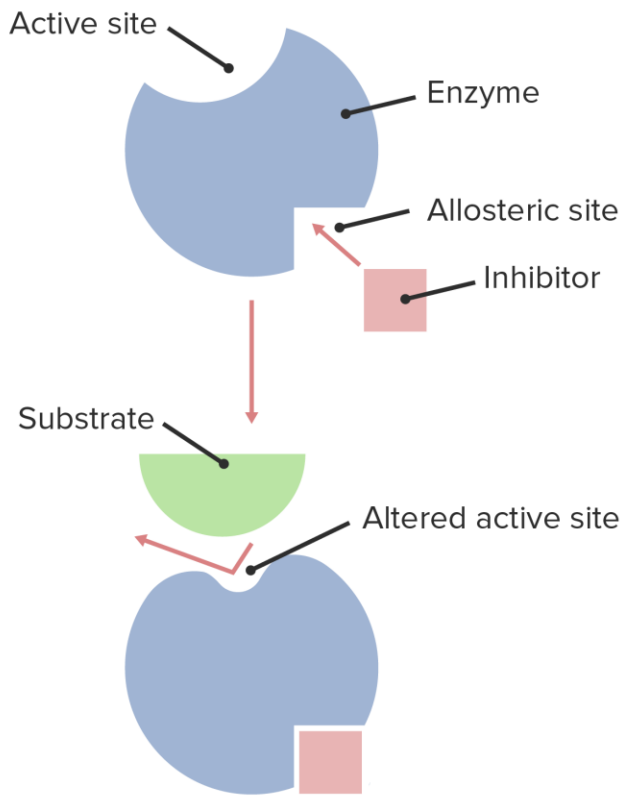
TYPE OF INHIBITOR	K_m VALUE	V_{max} VALUE	BINDING SITE	SUBSTRATE RESEMBLANCE
Competitive	Increases	Unchanged	Active Site ONLY	Yes
Non-Competitive	Unchanged	Decreases	Free Enzyme OR E-S Complex	No
Uncompetitive	Decreases	Decreases	E-S Complex ONLY	No
Mixed	Increase OR Decrease	Decreased	Free Enzyme OR E-S Complex	No

Allosteric Enzymes:

- Allosteric enzymes have one or more allosteric sites. Allosteric sites are binding sites distinct from an enzyme's active site or substrate-binding site
- Molecules that bind to allosteric sites are called effectors or modulators
- -Binding to allosteric sites alters the activity of the enzyme. This is called cooperative binding. Effectors may be positive or negative
- Regulatory enzymes of metabolic pathways are allosteric enzymes (eg: feedback inhibition)


ON State
Active Site
Site
Subsrate
Allosterc
Site
Allosteric
Regulation
OFF State
Allosterc
Site
Inhibitor
onformation
Change

Allosteric inhibition



Allosteric activation