Enzymes and Coenzymes I

- Objectives :-

- What are enzymes?
  - Classification of enzymes and naming.
  - Coenzymes, Cofactors, Isoenzymes.
- Enzyme activity and specificity.
- Factors affecting enzyme activity.
- Enzyme kinetics (Michaelis Menten equation).
- Enzyme inhibition and types.
- Regulation of enzyme activity.
- Enzymes in clinical diagnosis.

Enzymes and Coenzymes I

SIMPLE INFORMATION ABOUT ENZYME (IT IS KIND OF PROTEIN, IT IS CATALYST AND VERY IMPORTANT IN METABOLISM)

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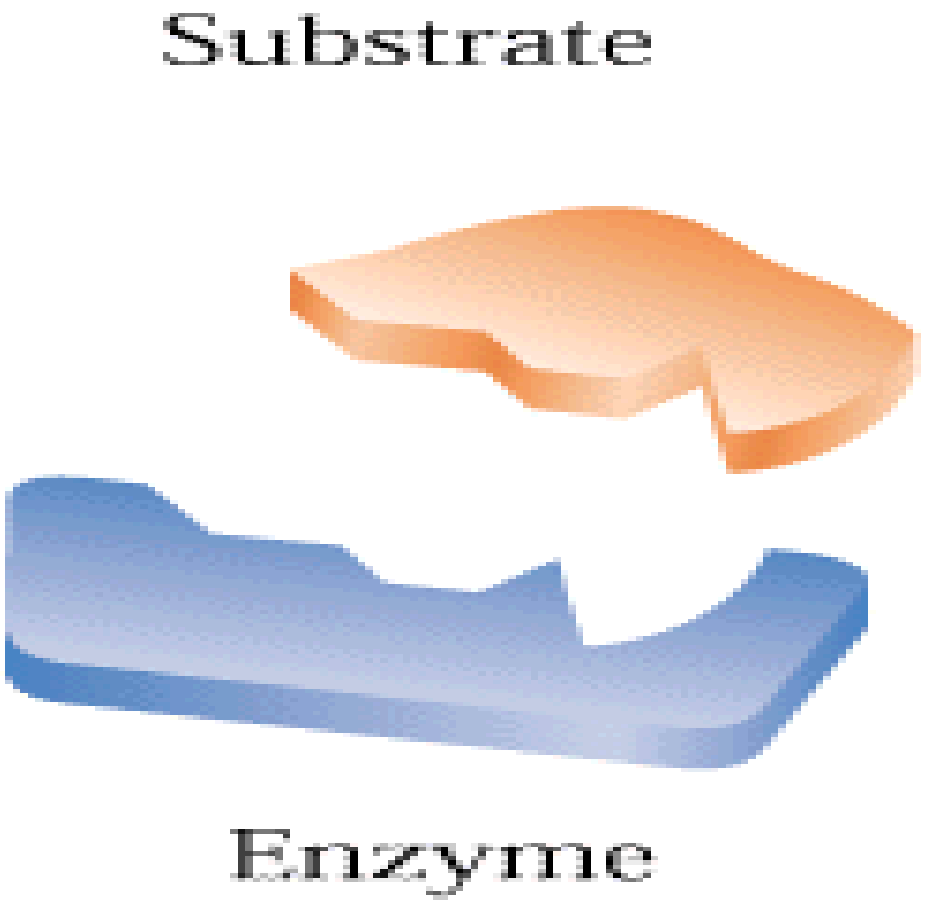
Catalysts= lower the energy need in any reaction

In any reaction,
Reactants(higher energy)
products(lower energy)

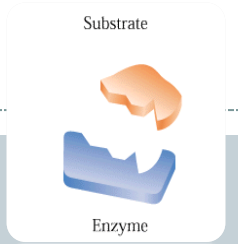
What are Enzymes?

- Enzymes are biological catalysts that speed up the rate of a reaction without being changed in the reaction(in another word , nothing happen to the enzymes after the reaction)
- All enzymes are protein in nature
- But all proteins are not enzymes
- Substance upon which the enzymes act are called substrates(reactanes)
- Enzyme converts substrates into product(s)
- All enzymes have one or more active sites.
- Some enzymes have both active and regulatory sites.

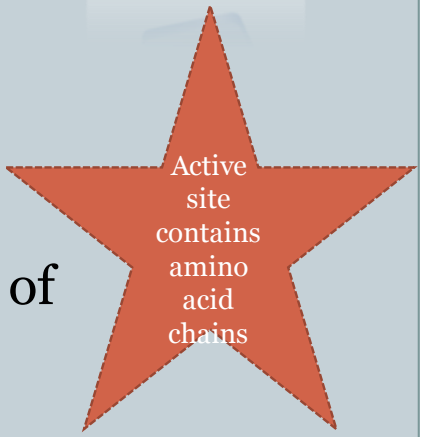
- When enzyme fits or combines with the substrate we call it enzyme substrate complex.



Properties of Enzymes



- Active site- (it composed of aa residues)
  - The region of enzyme that binds with the substrate and where catalysis occurs
  - All enzymes have one or more active sites
- Specificity-
  - Enzymes bind to their specific substrates(one type of substrates or 2) in the active site to convert them to product(s) (one enzyme catalysts one specific one type of reaction)
- Regulation-
  - Enzymes can be activated or inhibited(reduce or complete inhibition) so that the rate of product formation responds to the need of the cell

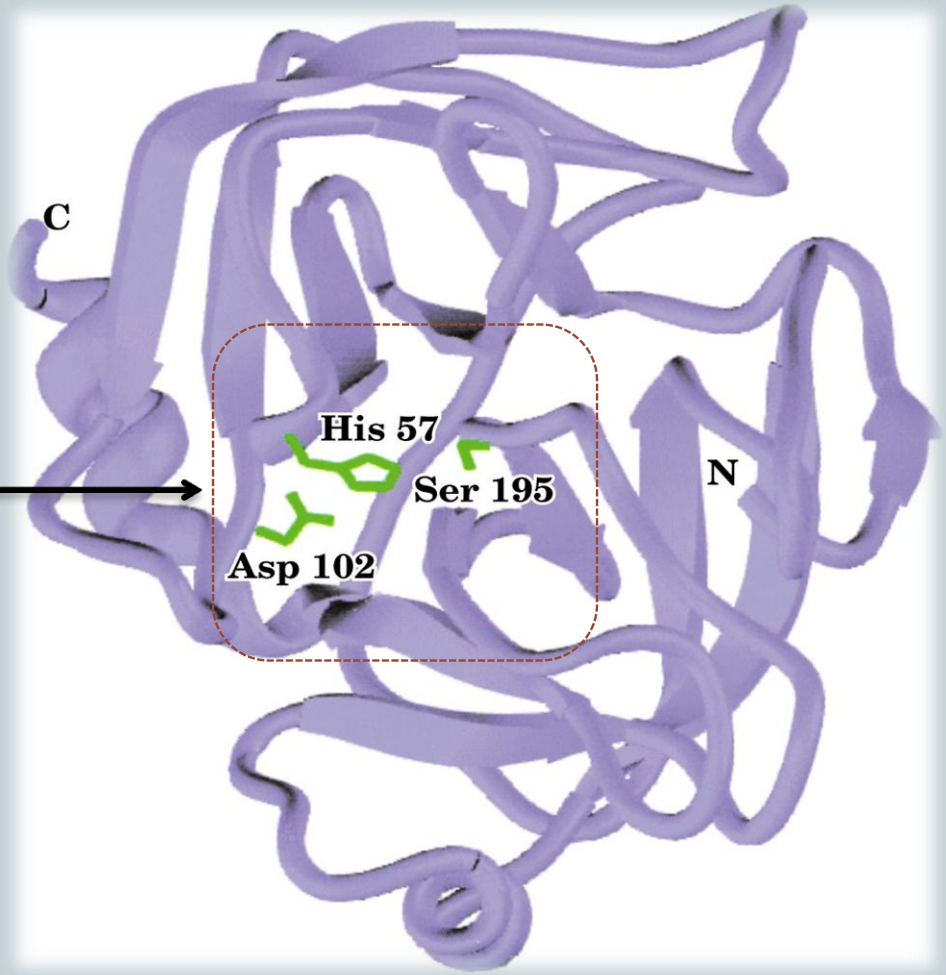


increase ↑ Enzyme incomplete inhibition (the activity of the enzyme is decreased)*

decrease ↓ Enzyme complete inhibition= when you stop the active site of the enzyme (the activity of the enzyme is completely stopped)

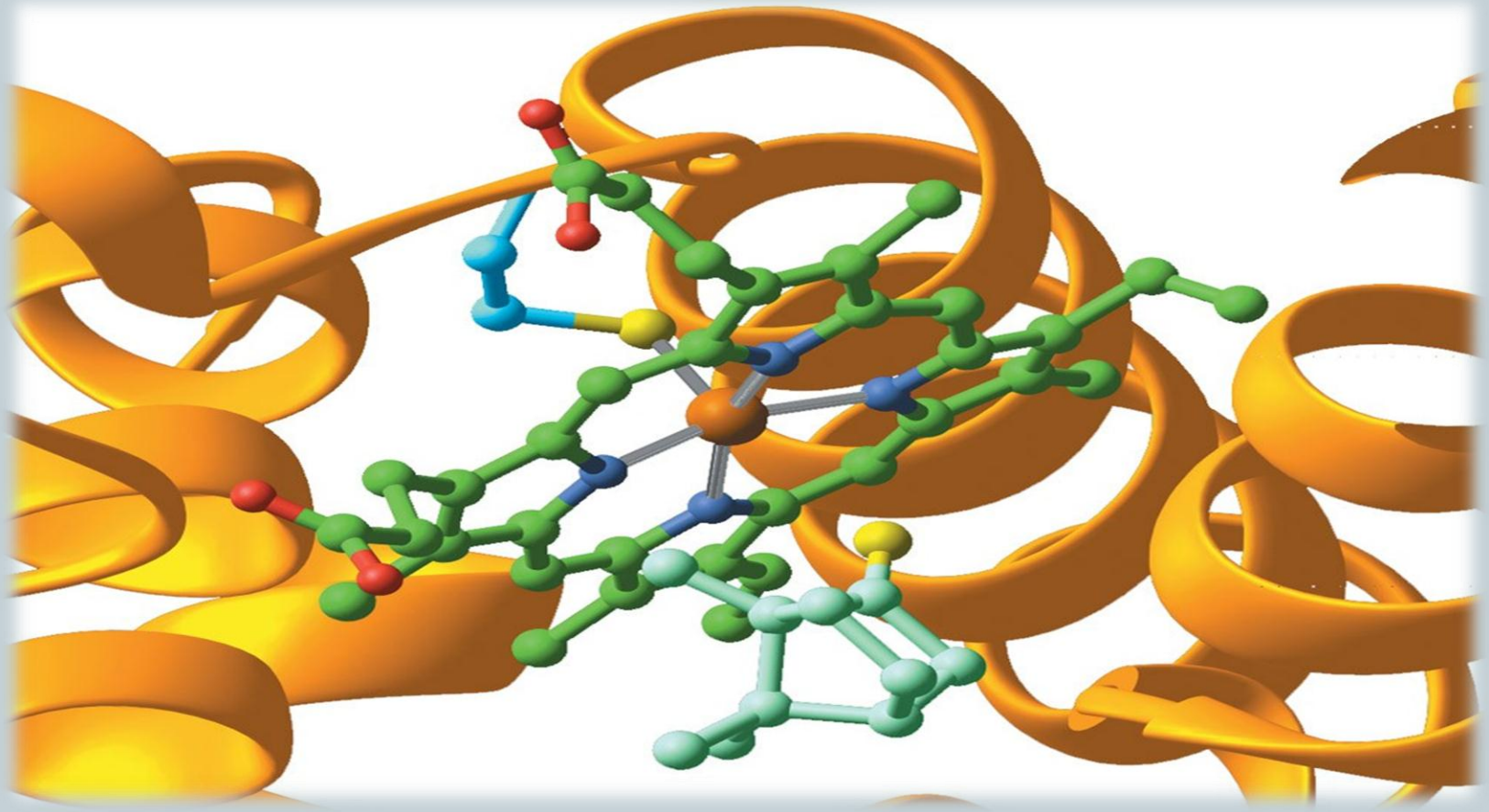
• Structure of trypsin enzyme

That is the active site(aa residues make 3-dimentional)



Page 519

• An enzyme with its active site



Page 533

• Enzyme specificity

- Enzymes are highly specific
- Interact with only one or a few of the substrates
- Catalyze only one type of reaction

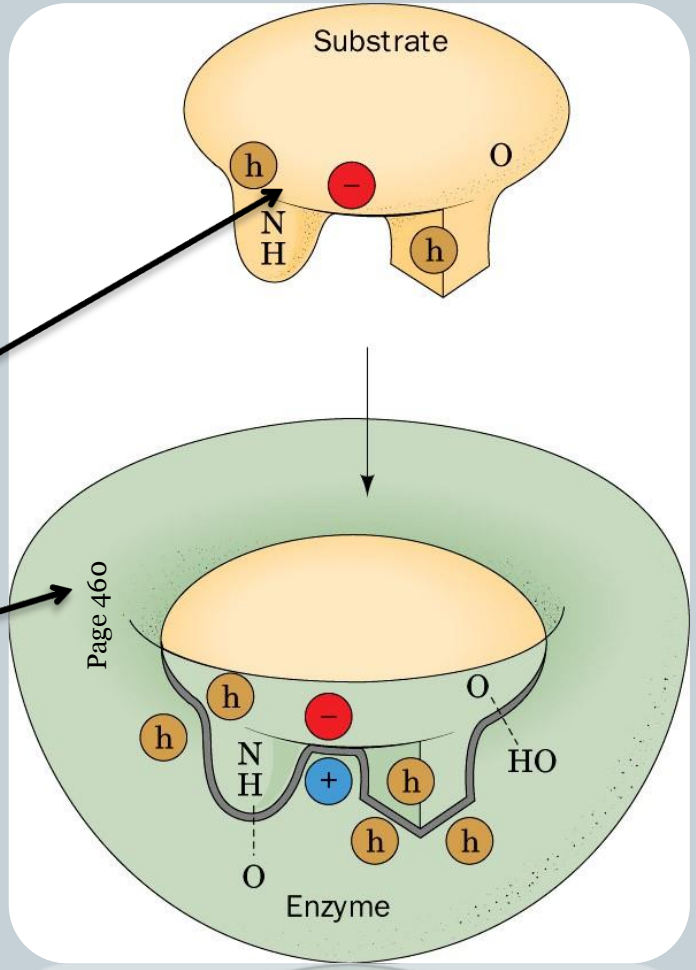
• Enzyme-substrate binding

(when enzyme and substrate bind together)

- Two models have been proposed
  - Lock(represent the active site of the enzyme) and key(represent a specific substrate) binding
  - Induced fit binding
  - The two models occur both or only one

• Lock and key binding

- The enzyme has an active site that fits the exact dimensions of the substrate.
- They are structurally similar complementary to each other.
- They form 3D cleft.



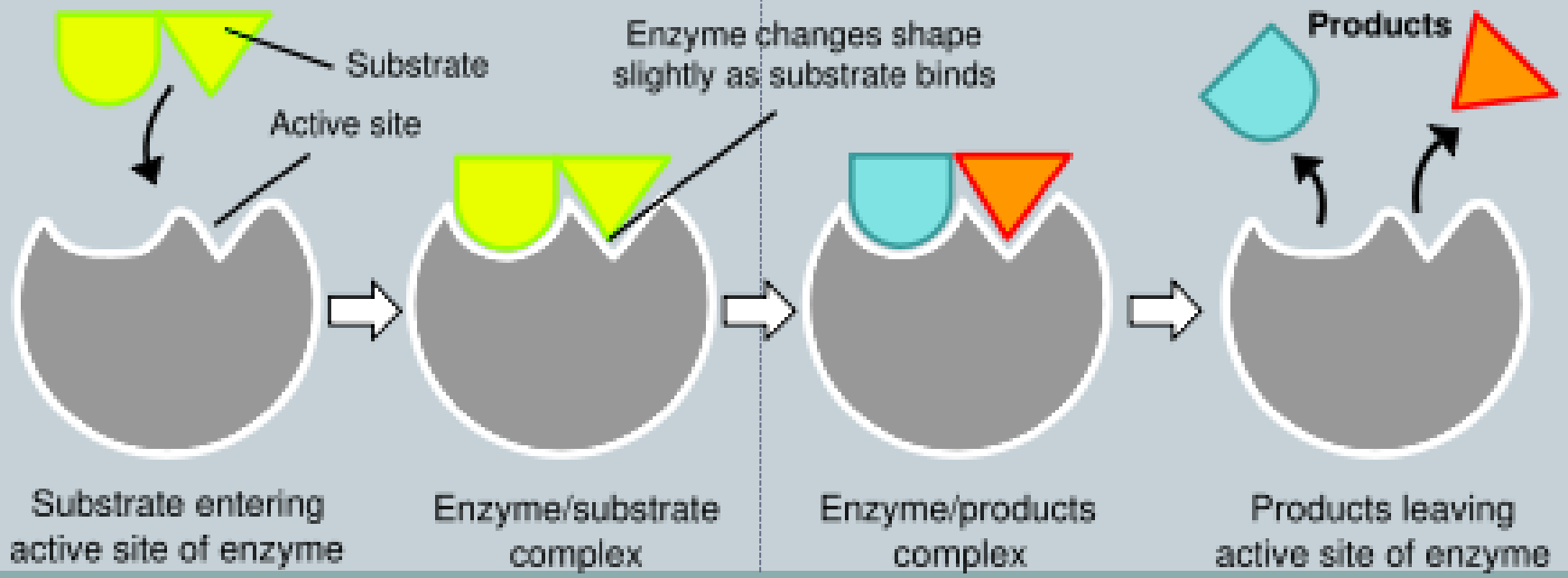
Key substrate*

The lock active site*

• Induced fit binding

Wearing gloves fits the hand shape

- After the binding of substrate, the enzyme changes its shape to fit more perfectly with substrate(after formation and releasing the products , the enzyme come back to it is original shape).



• Classification of Enzymes (according to the type of reaction catalyzed)

You should memorize it in sequence

Enzymes suffix=ase

Could be any group

Classification	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization
6. Ligases	Bond formation coupled with ATP hydrolysis

joining

• Enzyme nomenclature (Naming)

- Enzyme nomenclature is based on the rules given by IUBMB (International Union of Biochemistry and Molecular Biology)
- EC 3.4.17.1 (carboxypeptidase A , it is the common name)

  EC = Enzyme Commission(know it comes from IUBMB)

Class.Subclass.Subsubclass.Enzyme number

3 is the class (hydrolases) *

- There benefits are : knowing the type ,function of enzyme and the substrate for it

• Holoenzymes

- Some enzymes require non-protein groups to become active (because the enzymes in their nature are not active)

- The inactive form of enzyme without its non-protein part is called an apoenzyme

- Apoenzyme (inactive) + nonprotein part = Holoenzyme (active)

• Cofactors, Coenzymes

- Cofactors or Coenzymes are The non protein part
  - If the non-protein part is a metal ion such as Cu^{2+}, Fe^{3+}, Zn^{2+}, etc., it is called cofactor
  - If small organic molecules, known as coenzymes such as NAD^{+}
    - Prosthetic groups (permanently associated with enzyme)
    - Cosubstrates (temporary associated with enzyme)

• Coenzymes

- Coenzymes that are permanently associated with an enzyme known as prosthetic groups e.g. FAD

- Coenzymes that only temporarily associate with an enzyme known as cosubstrates e.g. NAD

Needs not protein part

Apoenzyme (inactive) + Cofactor/coenzyme = Holoenzyme (active)

Metal ion

Prosthetic

cosubstrates

• Ribozymes(RNA (ribo nuclic acid)), Isoenzymes and zymogens

- Ribozymes are(some) RNAs with enzyme activity

ex:catalyzing the cleavage and the synthesis of phosphodiester bonds

- Isoenzymes are enzymes that catalyze the same chemical reaction (same function)but they have slightly different structures

- Zymogens(we keep them ,when needed they become active) are inactive enzyme precursors that require a biochemical change to become active e.g. cleavage of a peptide blocking the active site

Inactive → precursor →active enzyme

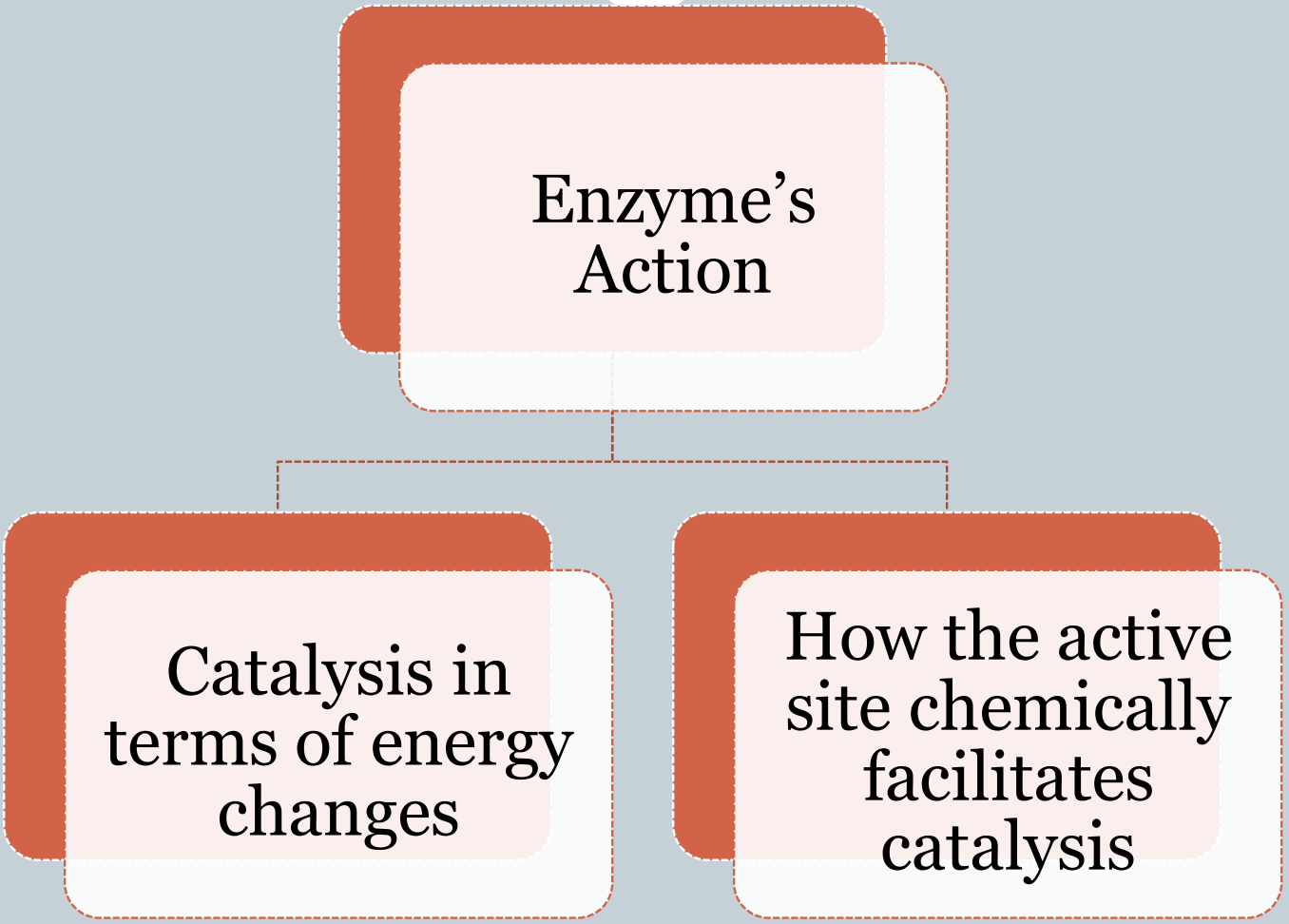
The precursors

- The precursors (it is a step or form in synthesis enzyme which come before enzyme formation)
- e.g pepsiongen(the precursors) change into pepsin (enzyme) when the body need it in digestion .

How do enzymes work?

- In every chemical reaction, the reactants pass through a transition state that has greater energy than that of the reactants or products alone
- The difference in energy between the reactants and the transition state is called the activation energy(in another word, it is needed by reactants to arise the transition state)
- If the activation energy is available then the reaction can proceed forming products(but if it is not available the reactants would not reach the products)
- Enzymes decrease activation energy of a reaction.

• How do enzymes work?

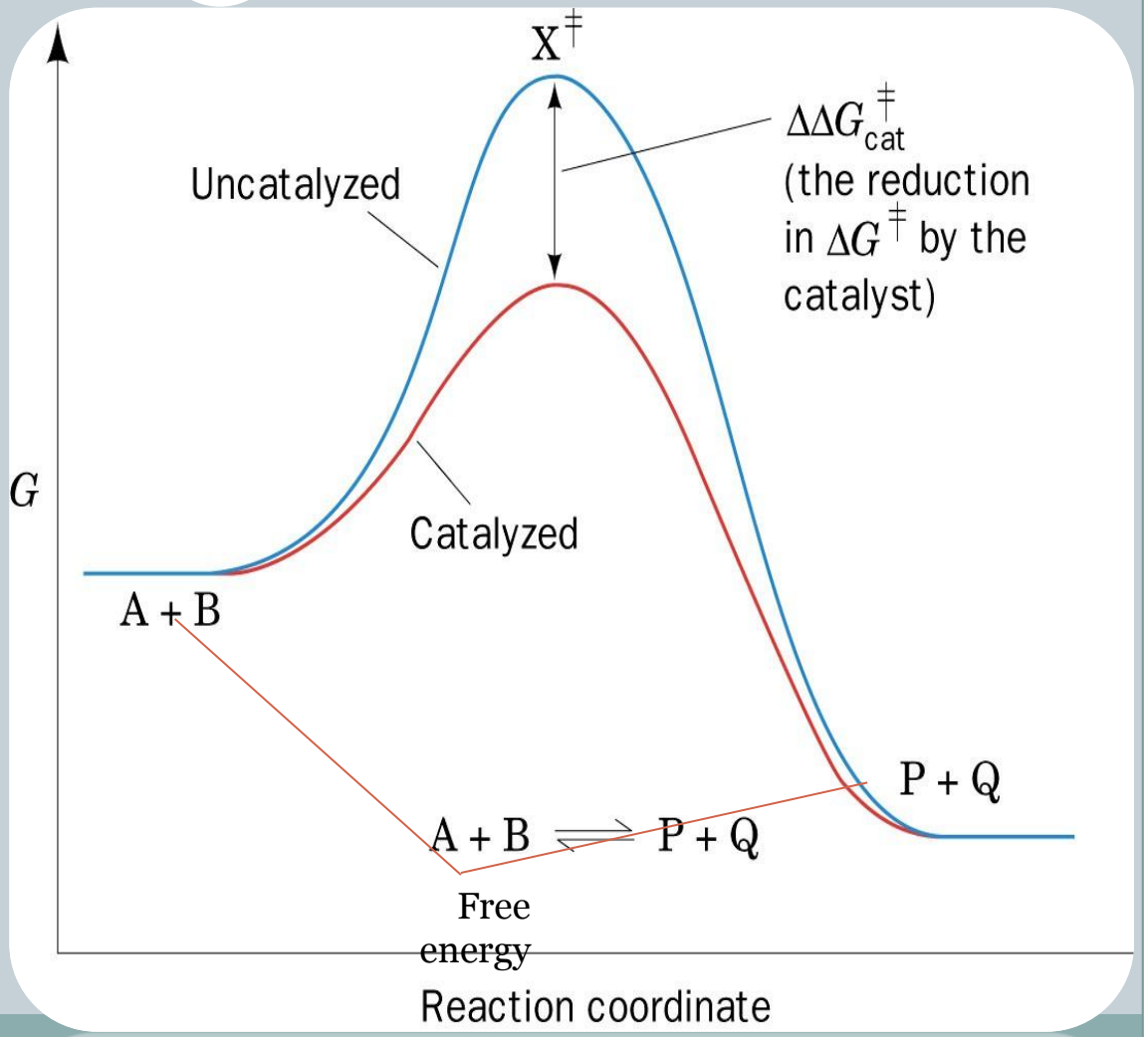


How do enzymes work?

- An enzyme reduces the activation energy required for a reaction
- It provides an alternative transition state of lower energy called the enzyme-substrate complex and thus speeds up the reaction(because the enzyme itself react with the substrate)
- Enzymes decrease the activation energy but they do not alter the change in the free energy (ΔG)(free energy is the difference in energy between substrate and products)

The effect of a catalyst on the transition state diagram of a reaction

- X represents the transition state which is instable state
- The change in energy after using enzyme it is only happen in activation energy



Lowering energy more reactants converted so more products

High free energy = uncatalyzed chemical reactions are slow and vice versa

Page 477

• Enzyme Activity (number of products formed) or Velocity

- Velocity is the rate of a reaction catalyzed by an enzyme

- Enzyme activity is expressed as:

*M*moles (micrmoles) of product formed during min per mg enzyme

Ex: mm=100 time=1 min mg=10

Enzyme activity = 10

• Factors that affect enzyme activity

- Effect of temperature (the temperature give the reaction energy)
  - Every enzyme has an optimal temp(it is the temp when the enzyme maximally active). for catalyzing a reaction
  - The rate of an enzyme reaction initially increases with rise in temperature
  - At high temp. enzymes are denatured and become inactive
  - In humans most enzyme have an optimal temp. of 37°C

• Factors that affect enzyme activity

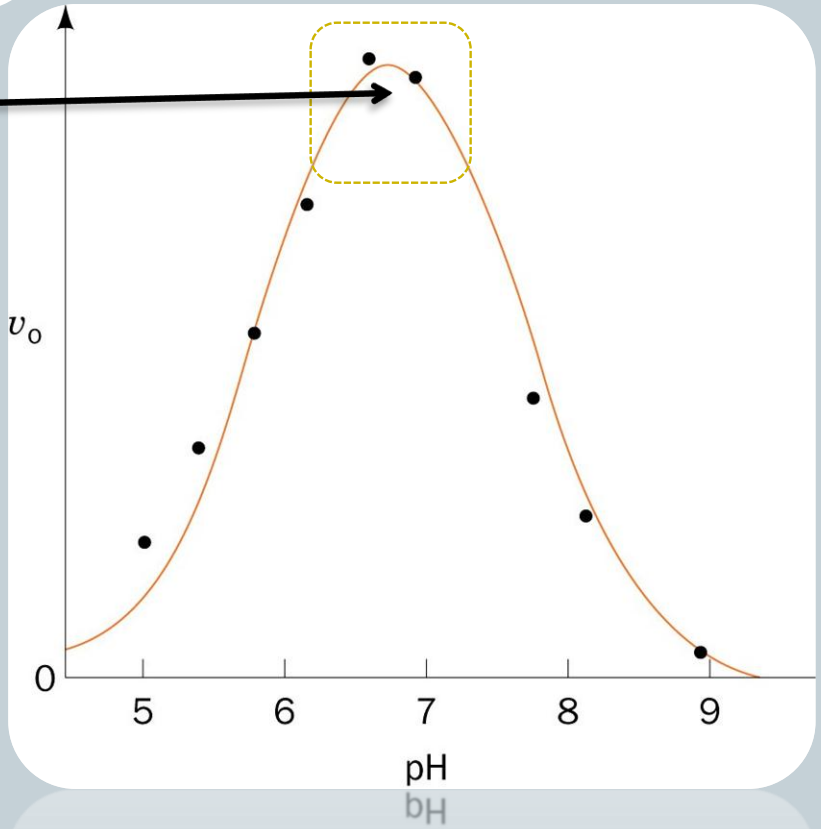
❖ Effect of pH (the ph that surround the enzyme)

- Effect of pH on the ionizable groups in the active site of enzyme or in the substrate affect catalysis
- Every enzyme has an optimal pH for catalyzing a reaction
- Most enzymes have highest activity between pH 6 and pH 8 (that is the range)
- Pepsin has highest activity at pH 2 (because it is in the stomach and the stomach is acidic)
  - If an enzyme works in 6 ph , and we put it in 2 ph for example it will denature or reduce its activity

• Effect of pH on the initial rate of the reaction catalyzed by most enzymes (the bell-shaped curve)

- The maximum activity

- In the other point the activity is reduced because of the change in the ph



Page 487

• Factors that affect enzyme activity

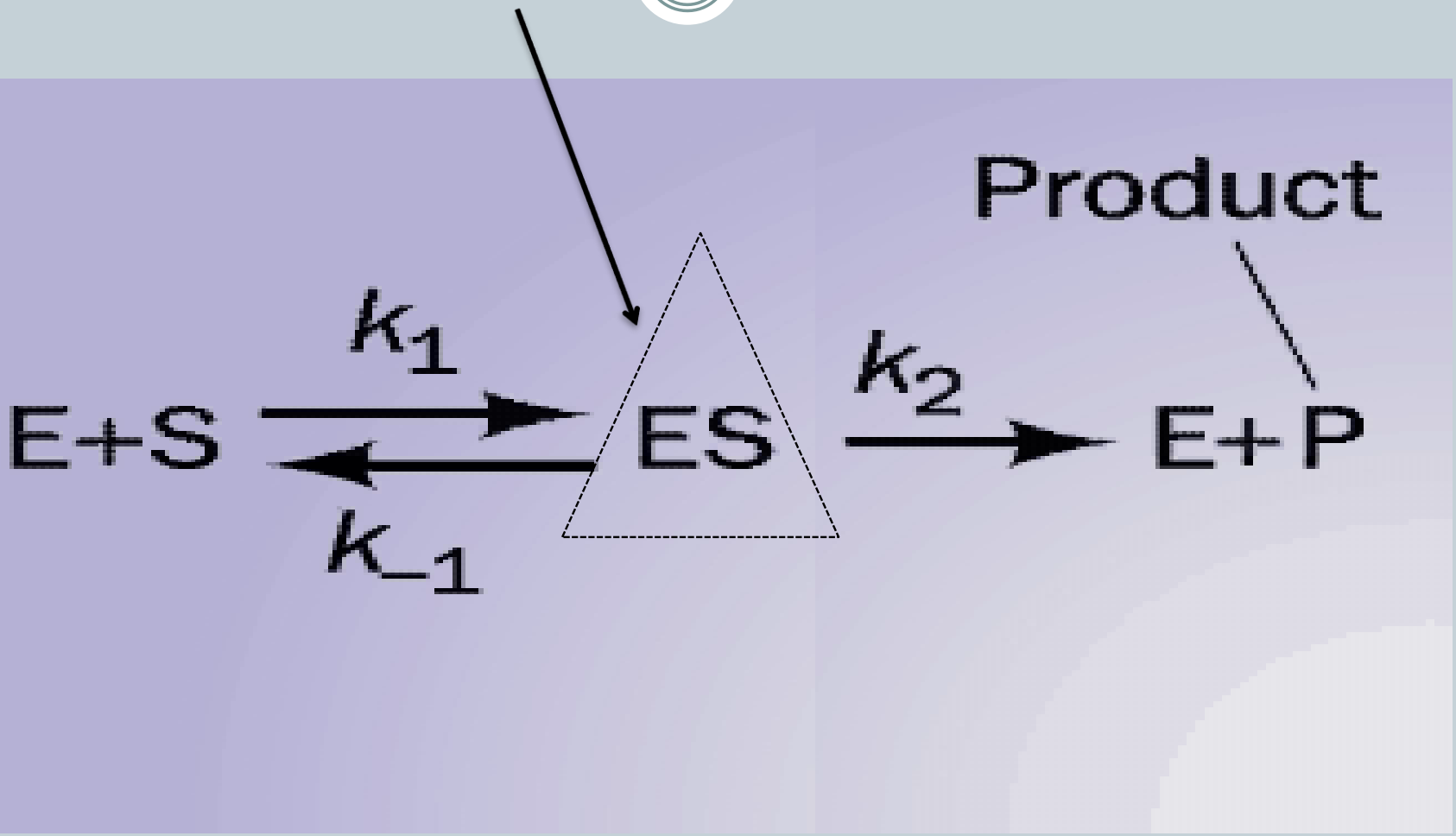
- Effect of [E](enzyme concentration) and [S] (substrate concentration)
  - The reaction velocity increases initially with increasing [S]
  - At low [S], the reaction rate is proportional to [S]
  - Further addition of substrate has no effect on enzyme velocity (v) (because the enzymes are saturated already with substrate)
  - The rate of an enzyme reaction is directly proportional to the conc. of enzyme if the substrate concentration [S] is higher than enzyme ⟶ total percentage of bounding is small
  - (but in normal body enzyme is more than substrate)

Enzyme kinetics *In specific time (when the enzyme velocity is proportional with the substrate concentration before saturation)

- The model of enzyme kinetics was first proposed by Michaelis and Menten in 1913 and later modified by Briggs and Haldane

- The Michaelis Menten equation describes the relationship of initial rate of an enzyme reaction to the [S]

- When it is pass the transition state , it is not reversible anymore



• Initial rate of enzyme reaction

- ✓ Pre-steady state kinetics(before start forming products)
  - When an enzyme is mixed with high concentration of substrate, there is an initial short period of time (a few hundred microseconds) during which intermediates leading to the formation of product gradually build up(more in number)

• Michaelis Menten Equation

•It measures the initial velocity (υ_o) of an enzyme reaction

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

*المعادلة ليست للحفظ.

[S] = substrate concentration

V_{max} = maximum velocity

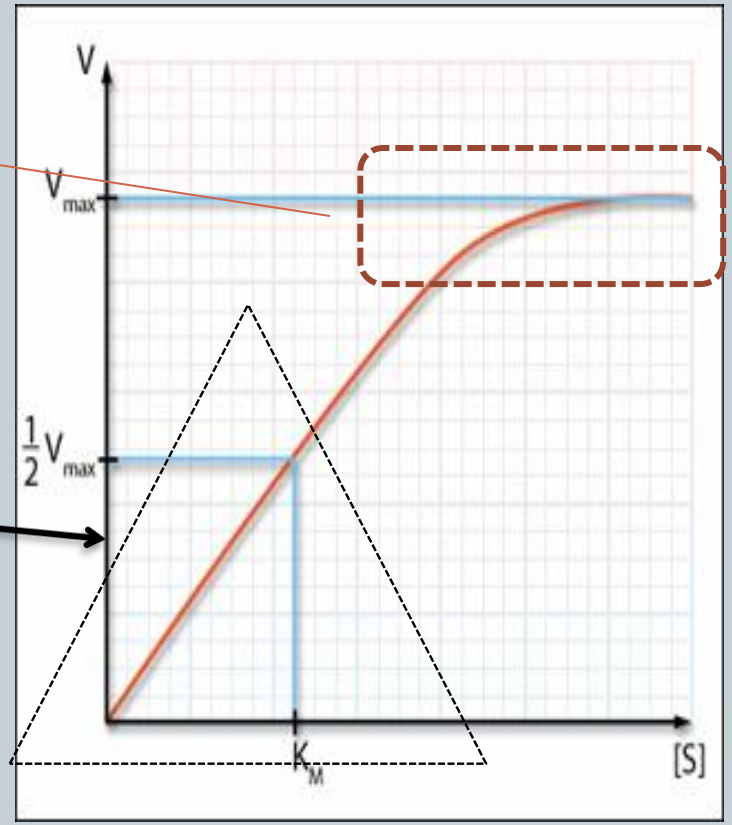
K_m = Michaelis constant

Vo= intial velocity

Initial velocity vo of a simple Michaelis–Menten reaction versus the substrate concentration[S]

- The rate of enzyme catalyzed reaction increases with substrate concentration till maximum velocity

- The proportional region



• K_m (Michaelis Constant)

- K_m is the substrate concentration at which the initial rate is one-half of the maximum rate (½ V_{max})

*Km doesn't vary with the concentration of the enzyme

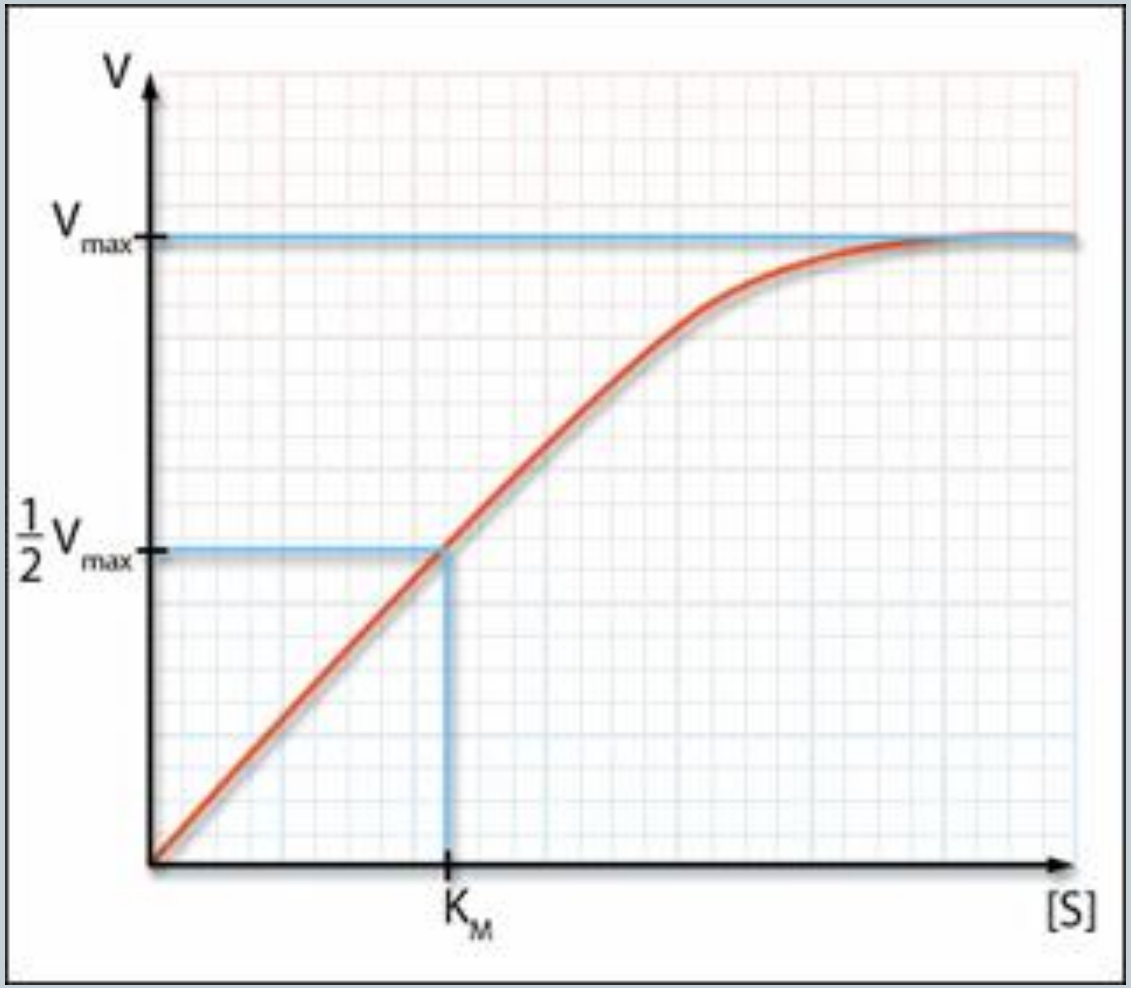
- It is the [S] required to saturate half of all of the active sites of an enzyme

• Lineweaver-Burk plot

- Also called the double-reciprocal plot, obtained by taking (Km and Vmax) reciprocals of the Michaelis Menten equation

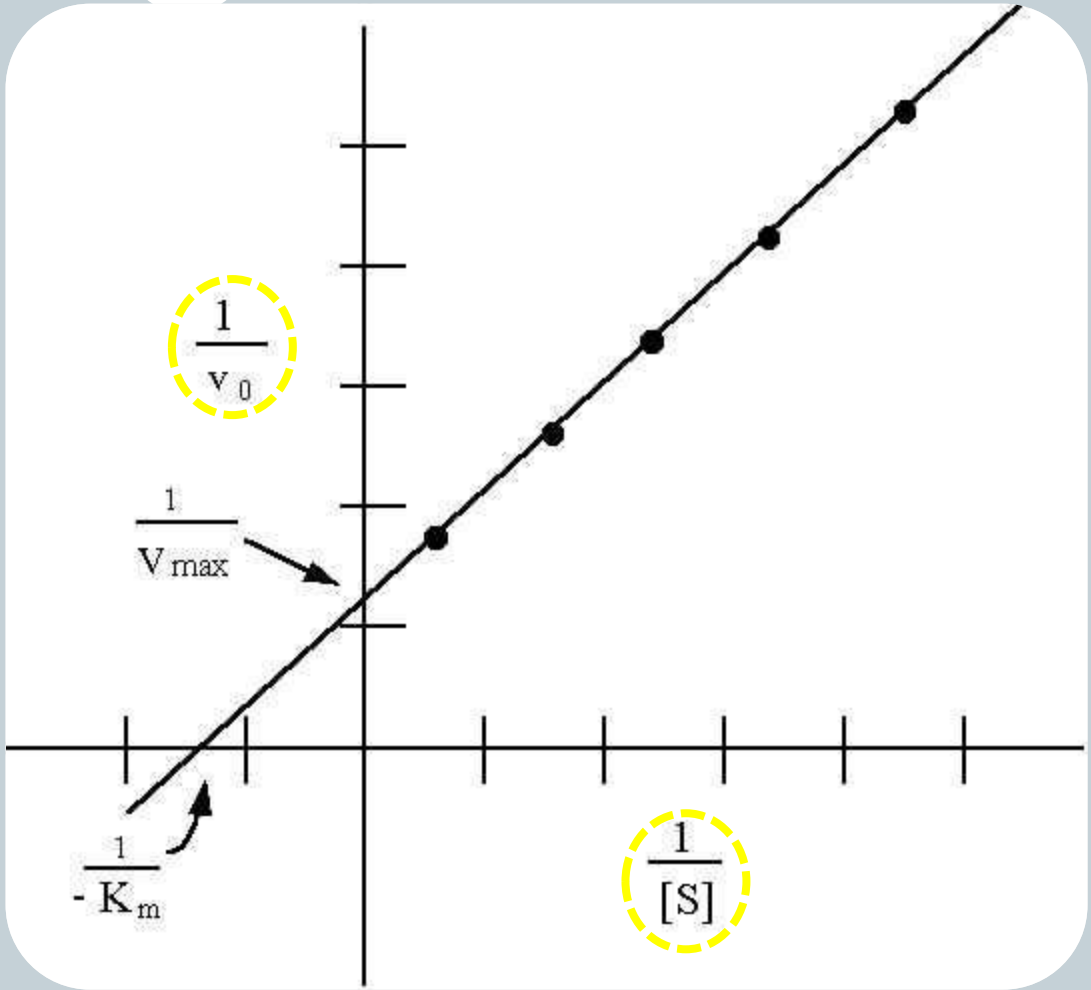
- It is plotted to calculate the Km and Vmax values and to determine the mechanism of action of enzyme inhibitors

- Initial velocity v_0 of a simple Michaelis–Menten reaction versus the substrate concentration[S]



• Lineweaver-Burk plot

- 1/v0 is plotted versus 1/[S] = straight line is obtained



- The Km value of a substrate depends on its affinity with the enzyme

  - High K_m means low affinity with enzyme (more substrate needed to saturate the enzyme)
  - Low K_m means high affinity with enzyme (less substrate needed to saturate the enzyme)

High Km → more substrates needed to staturate the enzyme → ↓ affinity

Low Km → less substrates needed to staturate the ezyme → ↑ affinity

• Questions :-

1. The non protein part :

The answer is : D

A. Cofactor.

B. Coenzyme.

C. Enzyme.

D. A and B.

2. which one of these is correct :

The answer is : B

A. All proteins are enzymes.

B. All enzymes have one or more active sites.

C. Enzyme converts products into substrates.

D. All enzymes have both active and regulatory sites.