# ENZYMES I

**Color Index:**

- **Original slides.**
- Important.
- 436 Notes
- 438 notes
- **Extra information**


Biochemistry team 438

# Objectives:

Slide No.9

1. Understand how enzymes are able to speed up the rate of biochemical reactions in the body.

Slide No.5

2. Identify classes of enzymes based on the type of reactions they catalyze.

Slide No.6

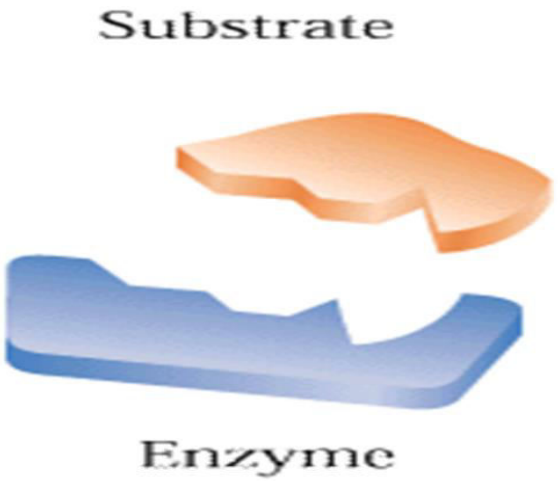
3. Comprehend the basic terms of coenzymes, isoenzymes, enzyme activity and specificity along with factors affecting their activity.

Slides (10-12)

4. Understand the enzyme kinetics, types of inhibition and regulation of enzyme activity.
5. Discuss the clinical role enzymes play in the diagnosis of diseases.

# What are Enzymes ?

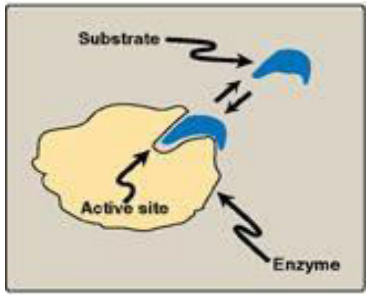
- Enzymes are biological catalysts that speed up the rate of a reaction without being -consumed- changed in the reaction.
- Enzymes are non-consumable molecules.
- All enzymes are protein in nature, but not all proteins are enzymes.
- Some enzymes have both active and regulatory sites.
- Substances upon which the enzymes act are called substrates.
- Enzymes bind to their specific substrates to convert them to product(s).



# Properties of Enzymes

**Active site**

- It's the region of enzyme that **binds** with the substrate and where catalysis occurs.
- All enzymes have one or more active sites.
- Once the substrate is bound, catalysis takes place.




**Specificity**

- Enzymes bind to their **specific** substrates in the active site to convert them to product(s).
- **Highly specific**, Interact with only one or a few of the substrates.
- Catalyze only **one type** of reaction.

**Regulation**

- Enzymes can be activated or inhibited so that **the rate of product formation** responds to the **need of the cell.**

# Models of enzyme-substrate binding

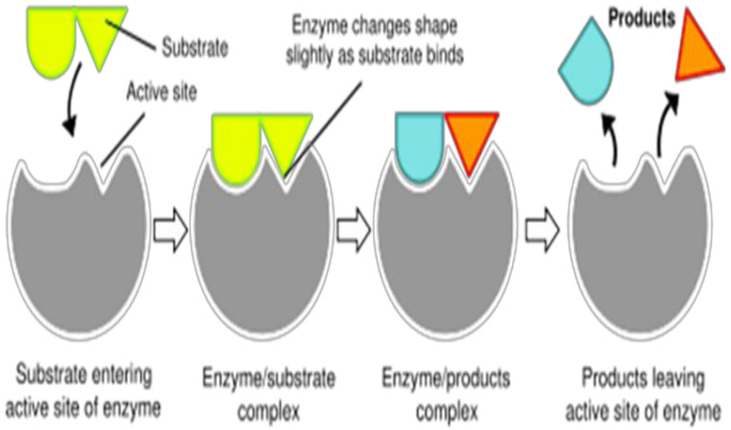
طريقة الارتباط بالإنزيم.

## Induct fit binding

After the binding of substrate the enzyme changes its shape to fit more perfectly with substrate

"not fully complementary"

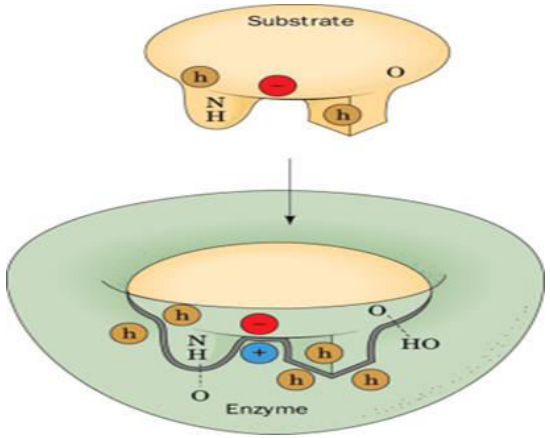
زي القفاز يأخذ شكل اليد بعد ما ينلبس

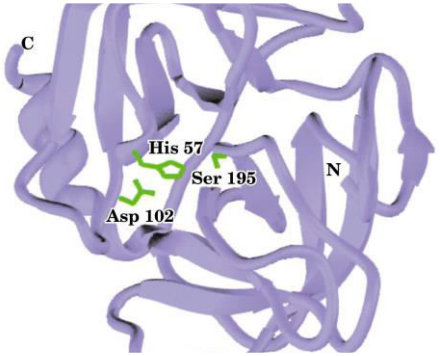



## Lock and key binding

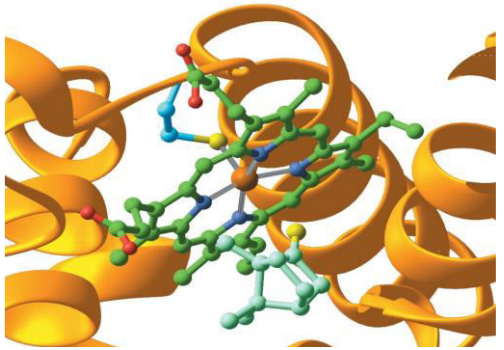
The enzyme has an active site that fits **the exact** dimensions of the substrate

Active site is **complementary** to the substrate



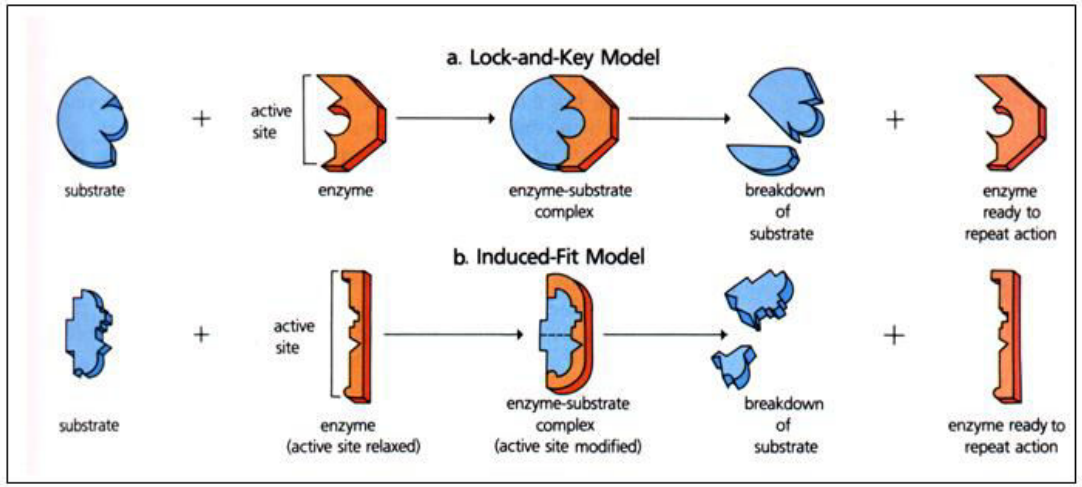




Structure of trypsin enzyme



An enzyme with its active site

A graph illustrating the differences between the 2 models:



**Objective 2:** Identify classes of enzymes based on the type of reactions they catalyze.

# Classification of enzymes:

They are classified into 6 types, according to the type of chemical reaction catalyzed.

Omar Tried Hard Learning International Languages

| Classification | Type of reaction catalyzed |
|---|---|
| 1. **oxidoreduct**ases | Oxidation – Reduction reaction |
| 2. **transfer**ases | Transfer of functional groups |
| 3. **hydrol**ases | Hydrolysis reactions |
| 4. **ly**ases | Group elimination to form double bonds |
| 5. **isomer**ases | isomerization |
| 6. **liga**ses | Bond formation coupled with **ATP** hydrolysis |

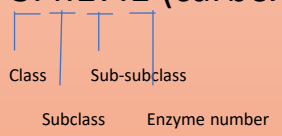
ملاحظة: لازم نحفظهم بالترتيب!

## enzyme Nomenclature (naming):

It is based on the rules given by IUBMB (international union of biochemistry and molecular biology)

Class.Subclass.Sub-subclass.Enzyme number

Example:
EC: 3.4.17.1 (carboxypeptidase A)




EC: Enzyme Commission (classification)

ملاحظة: الأرقام ليست للحفظ

**Objective 3:** Comprehend the basic terms of coenzymes, isoenzymes

# Holoenzymes:

Some enzymes require **non-protein** groups to become active:

Holoenzymes (Active) = Apoenzyme (Inactive) + Non-protein part

The inactive form of enzyme without its non-protein part

Non-protein part

**Coenzyme**

If the non-protein part is a **small organic molecules**, known such as $NAD^+$

**Cofactors**

- If the non-protein part is a metal ion such as $Cu^{2+}$, $Fe^{3+}$, $Zn^{2+}$, etc.
- They help an enzyme catalyze a reaction.

**1.Prosthetic Group**

Coenzymes that are **permanently** associated with an enzyme e.g. FAD

**2. Co-substrate**

Coenzymes that only **temporarily** associate with an enzyme e.g. NAD

# Ribozymes, Isoenzymes and zymogens

## Ribozymes

are RNAs (Ribonucleic acids) with enzymatic activity.

## Isoenzymes

are enzymes that catalyze the same chemical reaction but they have slightly different structures.

## Zymogens

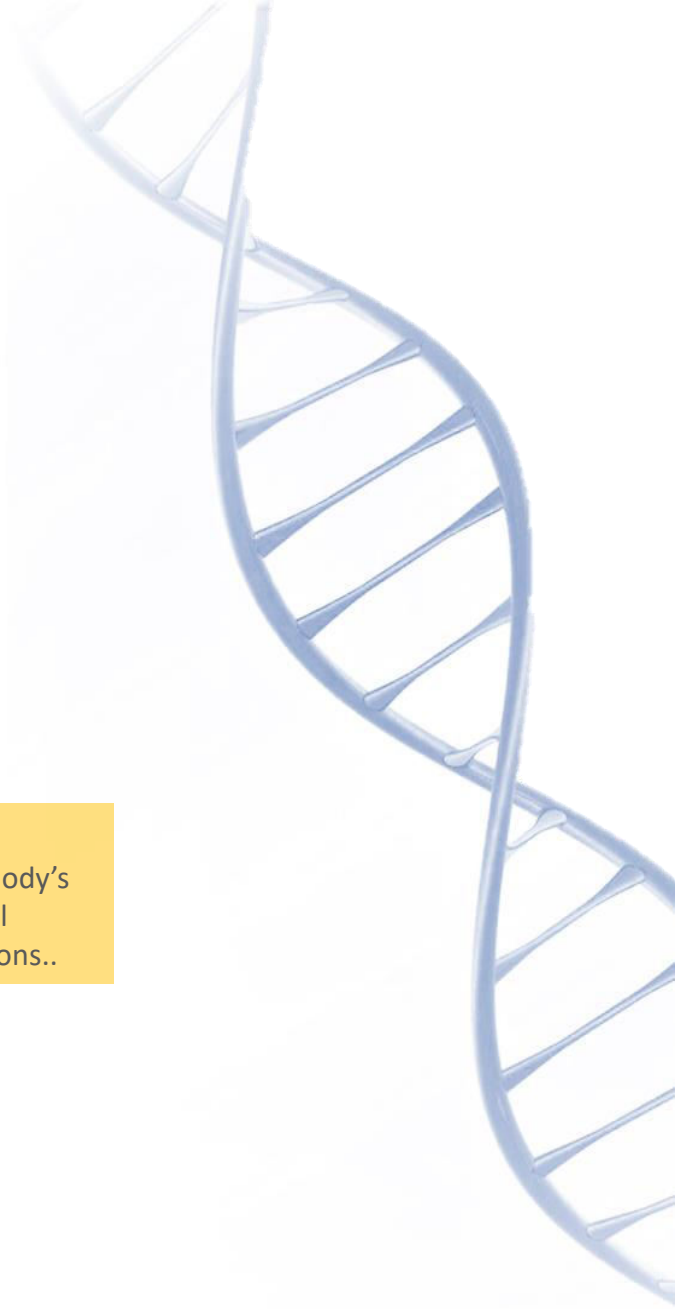
are inactive enzyme precursors (inactive enzymes in male slides) that require a biochemical change to become active e.g. cleavage of a peptide blocking the active site.

They are activated when needed.

Why do they exist?

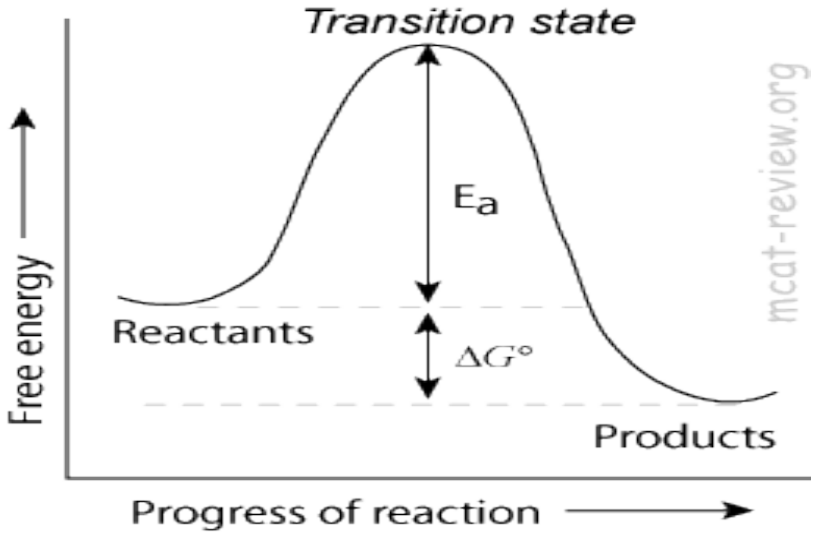
To cover the excessive body's demand of this chemical reaction in some situations..

- RNA or antibodies could act as enzymes.
- Inhibitors are structurally similar to enzymes → to control the action.



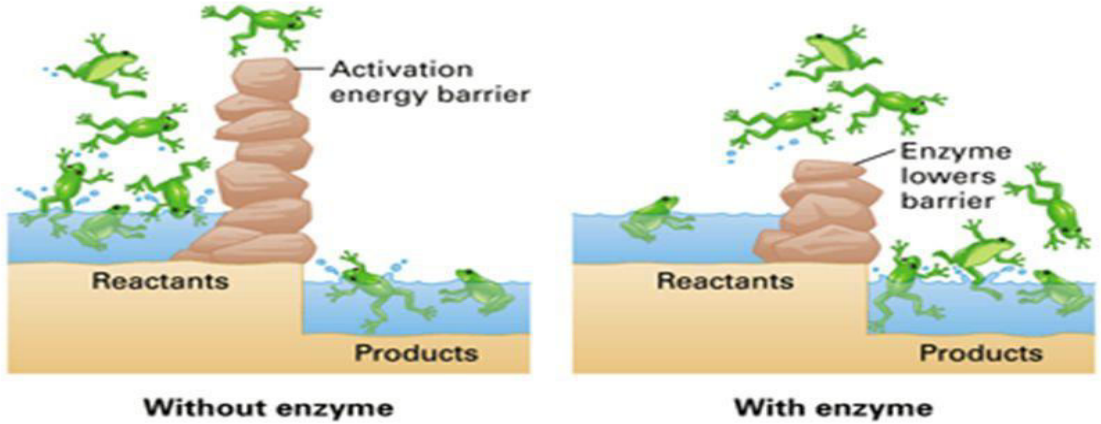
# Activation energy

- In every chemical reaction, the reactants pass through a transition state that has greater energy than that of the reactants or products alone (the highest point as shown in the figure, it's also called high-energy intermediate)
- activation energy (Ea): The difference in energy between the reactants and the transition state.
- If the activation energy is available then the reaction can proceed forming products




For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. In the absence of of enzyme, only a small amount of molecules may posses enough energy to achieve the transition state between reactants and products.
So, the lower activation energy, the more molecules have sufficient energy to pass through the transition state, and therefore, the faster the rate of the reaction.

- Enzyme induction → increases enzyme activity.
- Enzyme inhibition → decreases enzyme activity.




**The activation energy barrier is like a wall between two parts of a pond. If an enzyme lowers the wall, more frogs have enough energy to reach the other side**

**Objective 1:** Understand how enzymes speed up the rate of biochemical reactions in the body

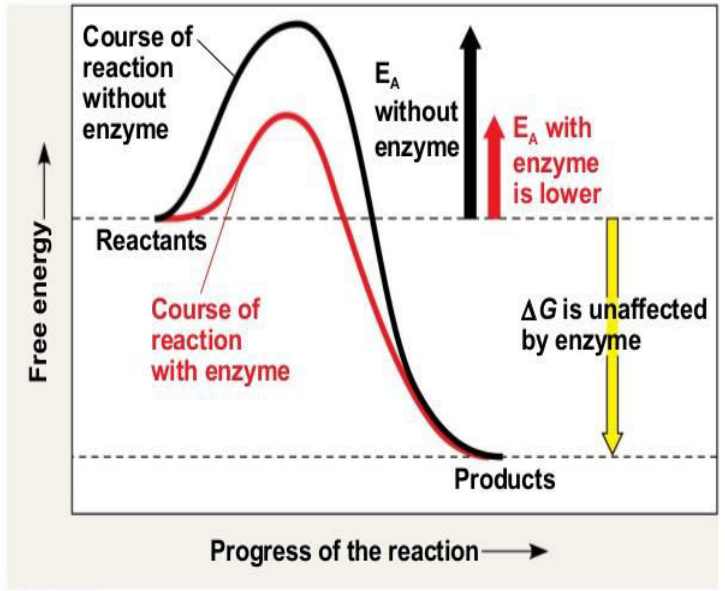
# How do enzymes work?

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.

Enzymes decrease the activation energy but they do not alter the free energy (ΔG) (available energy).

(ΔG remains the same, whilst Ea is reduced) i.e. enzymes do not change the equilibrium of the reaction. However, they accelerate the rate by which equilibrium is reached.

Figure 8.13



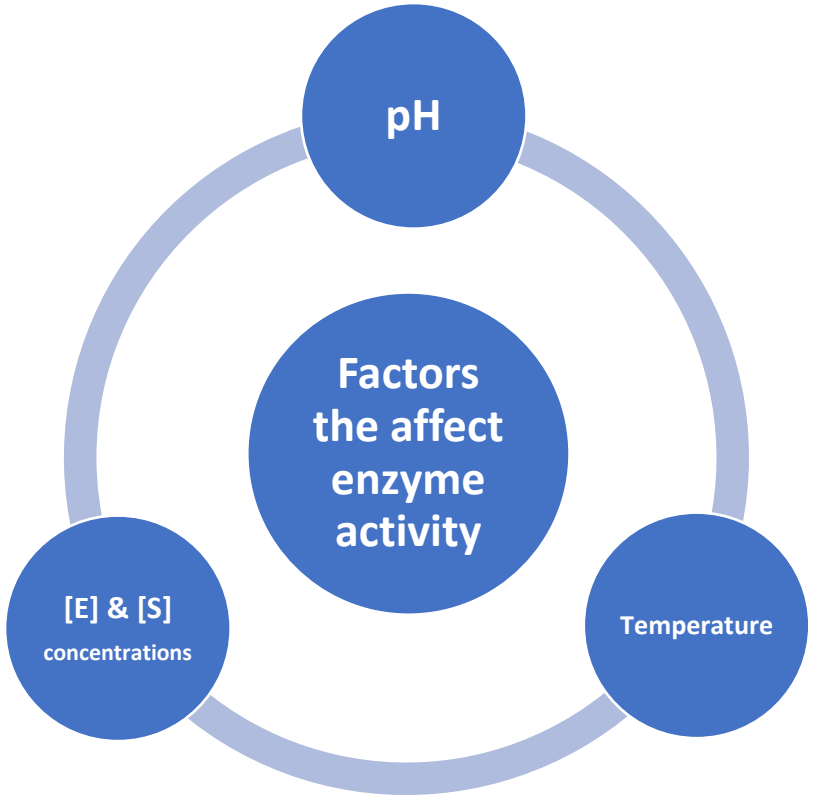

**The difference between Activation energy (Ea) and free energy (ΔG)**

- Activation energy is reduced.
- Free energy remains the same.

# Cont. How do enzymes work?

## Enzyme Activity or Velocity

- Velocity is the rate of a reaction catalyzed by an enzyme
- Enzyme activity is expressed as: μ moles of product formed/min/mg enzyme



# Factors that affect enzyme activity:

## 1. The effect of temperature:

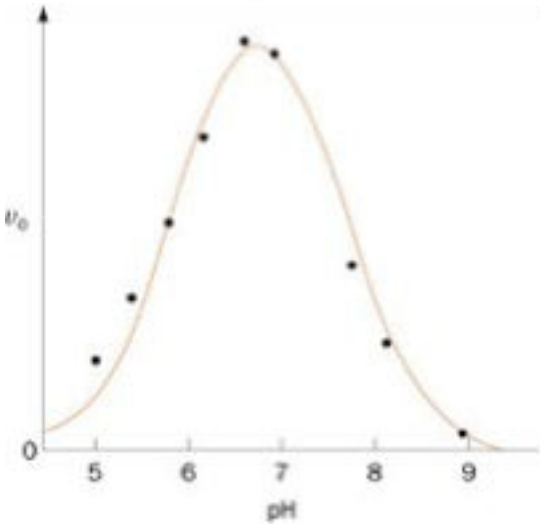
- The rate of an enzyme reaction initially **increases** with rise in temperature (increase in velocity).
- Every enzyme has an **optimal temp**. for catalyzing a reaction (In humans most enzyme have an optimal temp. of **37C** )
- At high temp. enzymes are **denatured** and become inactive.

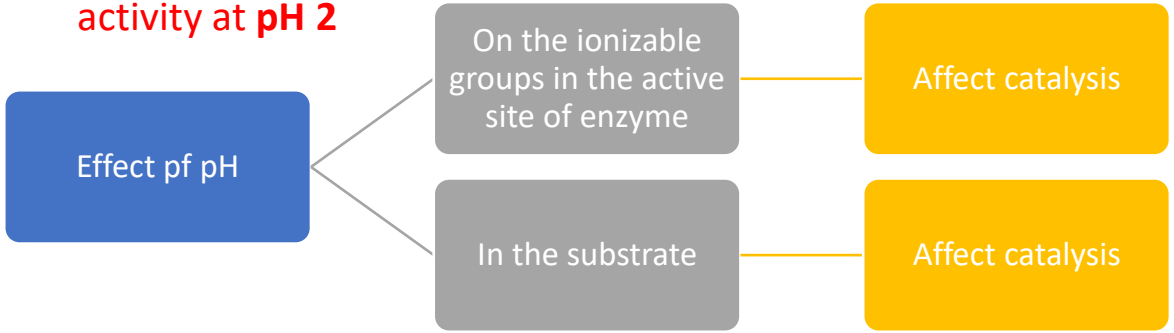
كل انزيم لـه درجه حراره محدده يعمل فيها وكل ما زادت درجة الحراره يزداد rate of reaction ولكن اذا وصلت درجة حراره عالىه مره راح يتأثر الانزيم وبالتالي ما راح يشتغل (الشيء اذا زاد عن حده انقلب ضده)

## 2. The effect of pH:

- Every enzyme has an optimal pH for catalyzing a reaction
- Most enzymes have highest activity between **pH 6 and pH 8**
- Pepsin (digestive enzyme in the stomach) has highest activity at **pH 2**

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




حامضية او قاعدية الوسط المناسب لعمل الإنزيم تعتمد على مكان عمله اذا كان يعمل في بيئة حامضية مثل المعدة، يكون الوسط المناسب له حامضي؛ او قاعدية مثل الأمعاء يكون الوسط المناسب له قاعدي

## 3. The effect of [E] and [S] Concentrations:

- 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).
- The rate of an enzyme reaction is directly proportional to the conc. of enzyme if the substrate concentration [S] is higher than enzyme.


Until excess substrate causes the reaction velocity to be constant *Further addition of substrate has no effect on enzyme velocity (v)* (because enzyme is saturated).

إذا كان[S] أكثر من[E] في هذي الحالة سرعة التفاعل راح تعتمد بشكل طردي على[E] فكل ما زادت تركيز الإنزيمات يكون التفاعل أسرع ولو كان العكس[E] أكثر من[S] في هذي الحالة تعتمد سرعة التفاعل على[S]

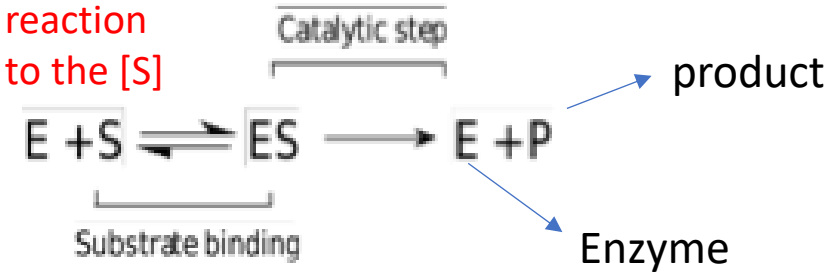
Increase of the substrate concentration will increase the rate of the enzyme; until the enzymes reaches saturation and then further increase of the [S] will have no effect. “Is no longer a limiting factor”

Increase of the enzyme concentration will increase the rate of the enzyme; until all substrate are used up and bound to their enzymes; then further increase of enzyme concentration will have no effect “ no longer a limiting factor”

# Enzyme Kinetics:

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]




# Michaelis Menten Equation:

It measures the initial velocity (Vo) of an reaction enzyme

$$V_O = \frac{V_{max}[S]}{K_m + [S]_m}$$

*[S] = substrate concentration*
*$V_{max}$ = maximum velocity*
*$K_m$ = Michaelis constant*

You might be asked to either find (Vo, or Vmax, Km or [S] ) using this equation

# Initial rate of enzyme reaction

The time they take to get arranged

**1. Pre-steady state:**

*Enzyme + high concentration of substrate= an initial short period of time (a few hundred microseconds) during which intermediates of products gradually build up.*

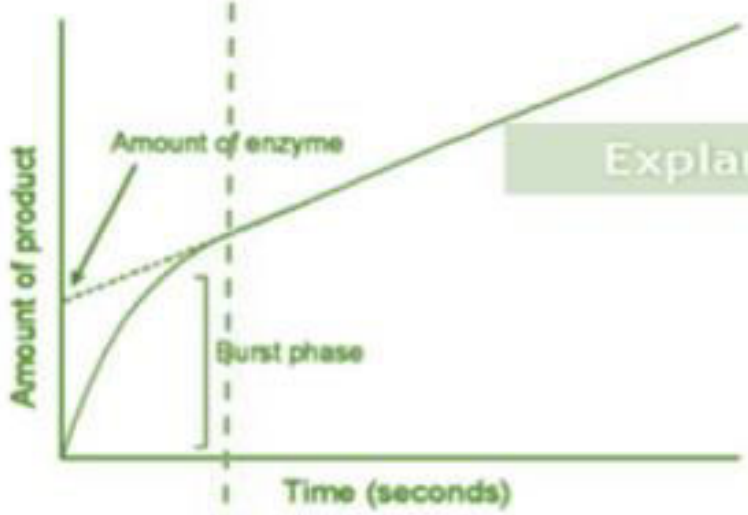
***NO PRODUCT DURING THIS PHASE.***
Enzyme-substrate complex formation only

**2. steady state:**

*occurs after initial state, when the reaction rate and the concentration of intermediates changes slowly with time.*

*An intermediate changes into steady state when the rate of its synthesis becomes **equal** to its rate of degradation.*

# Extra Info:




Explanation

## Pre-steady state kinetics

- مرحلة إرتباط الإنزايم بالسبستريت عشان يتكوّن لي ال ES
وهو ليس بروديكت إنما "انزايم مرتبط مع سبستريت"
(Enzyme-substrate complex)
- هذه الخطوة تعتبر قصيرة جداً لأن كمية السبستريتس اكبر بكثير من كمية الانزايم فبالتالي تصبح نسبة ارتباط هذه السبستريتز بالانزايم قليلة.

## Steady state kinetics

بعد ما تكوّن عندي ES (Enzyme-substrate complex)
سيصبح تركيزه يزداد بشكل ثابت مع الوقت (نلاحظ إن الميل في الصورة يزداد بثبات)

Thanks to TEAM 436

# $K_m$ (Michaelis Constant):

- *K*m is the substrate concentration at which the initial rate is one-half of the maximum rate (1⁄2 Vmax)
- It is the [S] (substrate concentration) required to saturate half of all of the active sites of an enzyme

**The $K_m$ 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)

Affinity= tendency to bind to a substrate

# Lineweaver-Burk Plot

Definition:

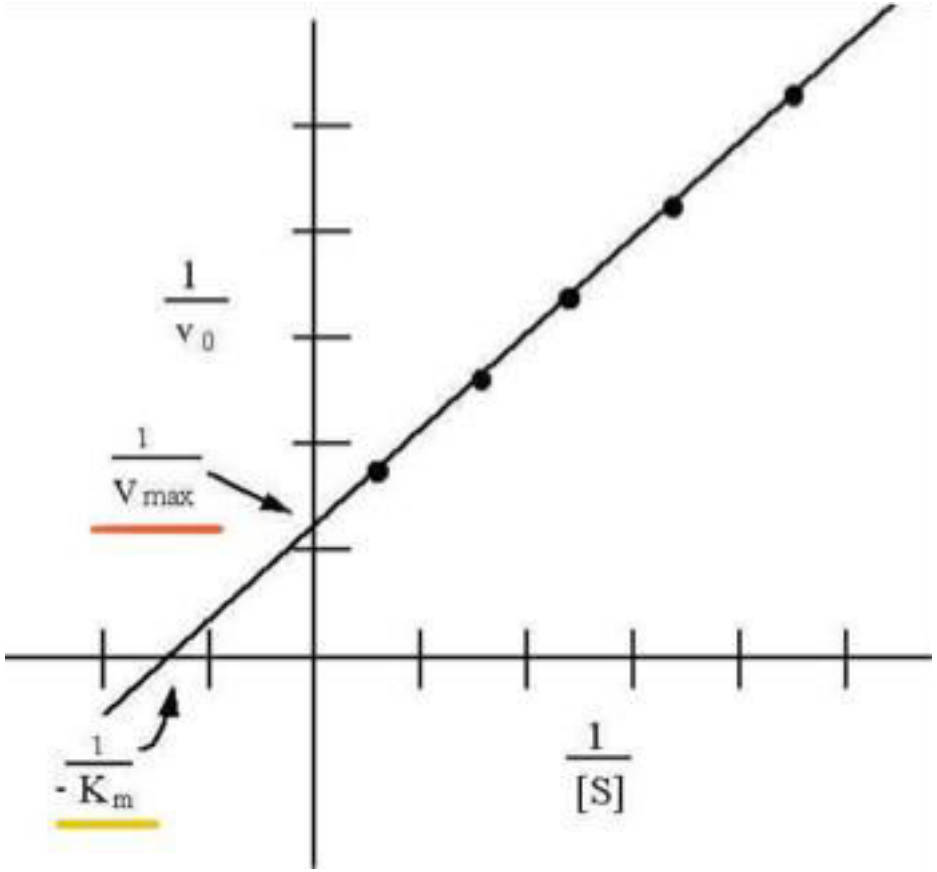
- Also called the double-reciprocal plot, obtained by taking reciprocals of the Michaelis Menten equation

Usage:

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

graphs are for further understanding,
but you should be able to recognize each graph

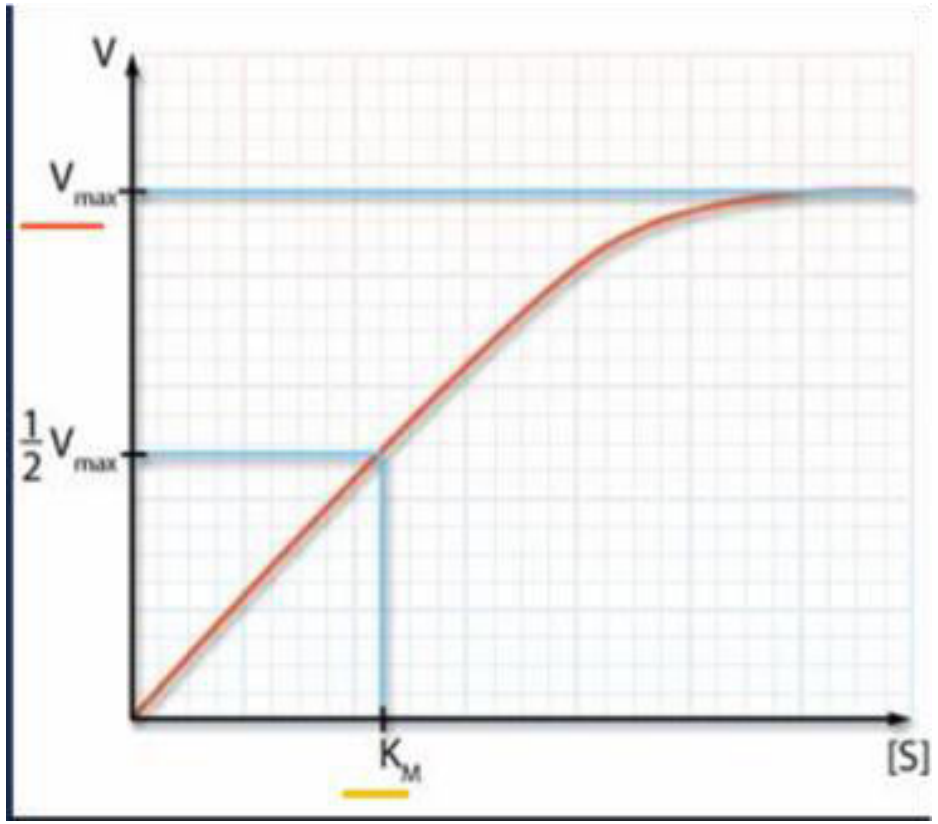
## Lineweaver-Burk plot

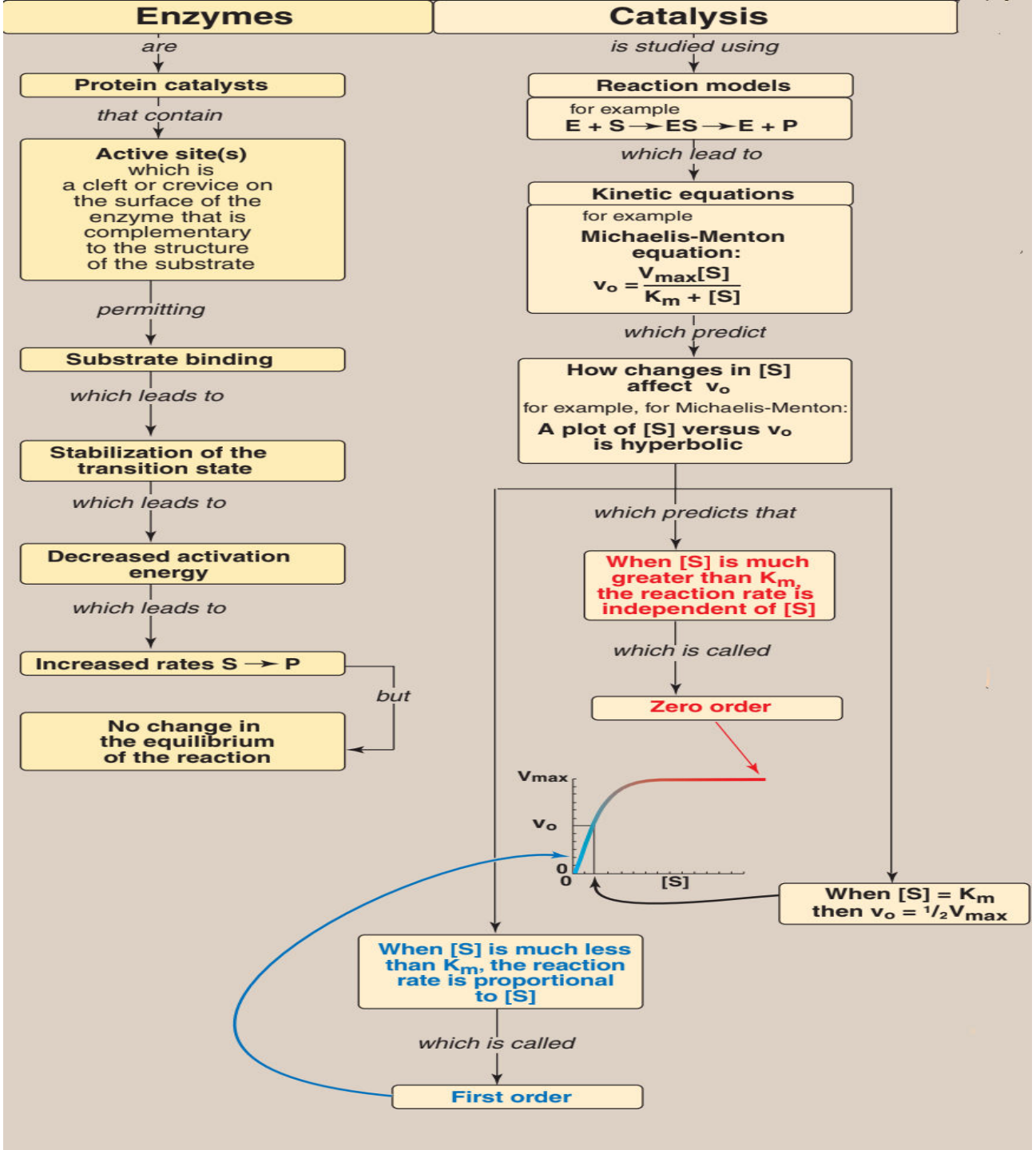



## Initial velocity (Vo) of a simple Michaelis- Menten reaction

*Vs*

## the substrate concentration (S)




Enzymes
Catalysis
are
Protein catalysts
that contain
Active site(s) which is a cleft or crevice on the surface of the enzyme that is complementary to the structure of the substrate
permitting
Substrate binding
which leads to
Stabilization of the transition state
which leads to
Decreased activation energy
which leads to
Increased rates S → P
but
No change in the equilibrium of the reaction
is studied using
Reaction models
for example
E + S → ES → E + P
which lead to
Kinetic equations
for example
Michaelis-Menton equation:
$v_o = \frac{V_{max}[S]}{K_m + [S]}$
which predict
How changes in [S] affect $v_o$
for example, for Michaelis-Menton:
A plot of [S] versus $v_o$ is hyperbolic
which predicts that
When [S] is much greater than $K_m$, the reaction rate is independent of [S]
which is called
Zero order
Vmax
vo
0
0
[S]
When [S] = $K_m$ then $v_o = {}^1/_2V_{max}$
When [S] is much less than $K_m$, the reaction rate is proportional to [S]
which is called
First order

# Review

# MCQs:

**1- an enzyme is:**

a) Lipid
b) Protein
c) Nucleic acid
d) Carbohydrate

**2- Enzymes that are having slightly different molecular structures but performing identical activity are**:

a) holoenzymes
b) Apoenzymes
c) coenzymes
d) isoenzymes

**3- The Michaelis constant depends on?**

a) The affinity of an enzyme to a receptor.
b) The concentration of substrates.
c) The affinity of a substrate to an enzyme.
d) The dissociation rate of a substrate to an enzyme.

**4-** ***Km* is the substrate concentration at which:**

a) initial rate is one half the maximum rate
b) initial rate is one third the maximum rate
c) initial rate is one quarter the maximum rate

**5-** ***a single enzyme can catalyze:***

a) 6 types of reactions
b) 1 type of reaction
c) 2 types of reactions
d) 3 types of reactions

**6-** ***an enzyme increases reaction velocity by:***

a) Increasing activation energy
b) decreasing activation energy
c) Increasing free energy
d) decreasing activation energy

## Answer key:

1) B
2) D
3) C
4) A
5) B
6) B

# SAQs

Q1: how enzymes are able to speed up the rate of biochemical reactions in the body ?

By reducing the activation energy required for the reaction

Q2: Name 3 types of enzymes

e.g. transferases, ligases, hydrolases (classification of enzymes is in slide No. 5 )

Q3: what does an Apoenzyme require to be active?

**non-protein** groups.

Q4: Most enzymes have highest activity between ……. And …….. , But pepsin have highest activity at …… ?

pH 6 , pH 8 , pH 2

## Girls team:

- أجيد آل رشود
- الوتين البلوي
- إيلاف المسيحل
- جود الخليفة
- جود العتيبي
- ريم القرني
- سارة الهلال
- شهد السلامه
- طيف العتيبي
- عبير الخضير
- غيداء البريثن
- لينا العصيمي
- نورة التركي
- نورة المزروع
- نوف الحميضي
- هيفاء الوايلي

## Boys team:

- بدر الشهري
- حميد حميد
- سهيل باسهيل
- عمر الغامدي
- مهند القرني
- نايف السبر
- **ضيف الشرف: محمد الحمود**

## Team leaders:

ديما المزيد

رائد العجيري


Special thanks to:

BIO T AM

Biochemistry Team

Biochemistry team 436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