



# Enzymes and Coenzymes 1+2

- Color Index:
- Important.
- Extra Information.
- Doctors slides.

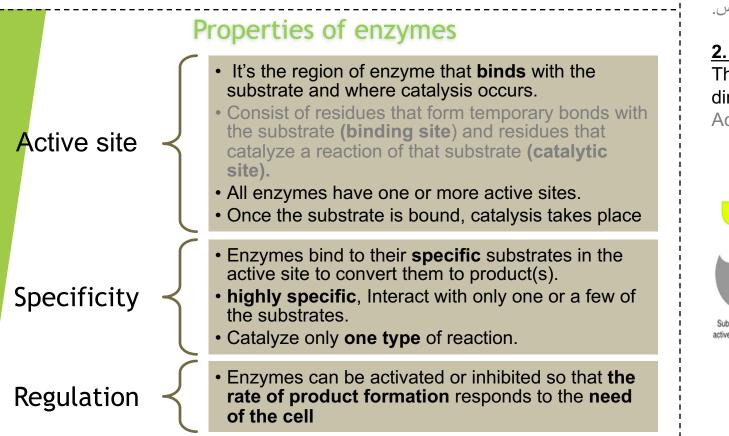
436 Biochemistry team

# **Objectives:**

- Understand how enzymes are able to speed up the rate of biochemical reactions in the body.
- Identify classes of enzymes based on the type of reactions they catalyze.
- Comprehend the basic terms of coenzymes, isoenzymes
- Understand enzyme activity and specificity
- Understand factors affecting enzyme activity.
- Understand the enzyme kinetics, types of inhibition and regulation of enzyme activity.
- Discuss the clinical role enzymes in the diagnosis of diseases.

### What are enzymes?

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



Objective: Understand enzyme activity and specificity

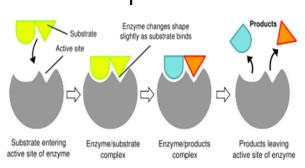
# Ways of Enzyme-substrate Binding طريقة الارتباط بالانزيم.

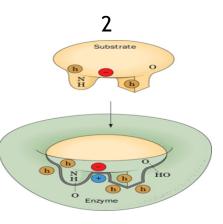
#### 1. Induct fit binding

After the binding of substrate the enzyme changes its shape to fit more perfectly with substrate "not fully complementary" زي القفاز ياخذ شكل اليد بعد ماينلبس.

#### 2. 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





# Classification of enzymes

Download from Dreamstime.com

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

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



|               | Classification    | Type of reaction catalyzed                 |
|---------------|-------------------|--|
| Omar          | 1.oxidoreductases | Oxidation- reduction reaction              |
| Tried         | 2.transferases    | Transfer of functional group               |
| Hard          | 3.hydrolases      | Hydrolysis reaction                        |
| Learning      | 4. lyases         | Group elimination to form double bonds     |
| International | 5. isomerases     | isomerization                              |
| Languages     | 6.ligases         | 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

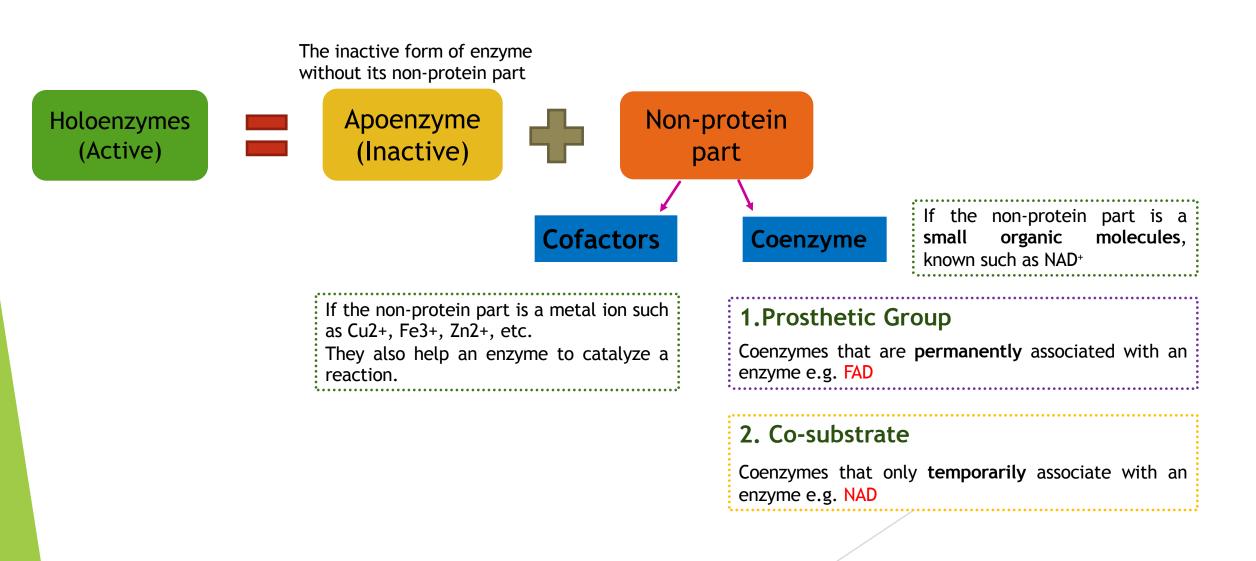
Class.Subclass.Sub-subclass.Enzyme number

Example: EC: 3.4.17.1 (carboxypeptidase A)

EC: Enzyme Commission (classification) ملاحظة: الأرقام ليست للحفظ

Some enzymes require **non-protein** groups to become active: Apoenzyme (inactive) + (Cofactor/coenzyme) \*non-protein part\* = Holoenzyme (active) Objective: Comprehend the basic terms of coenzymes, isoenzymes

# Holoenzymes



# Ribozymes, Isoenzymes and zymogens

### Ribozymes

are RNAs (Ribonucleic acid) with enzyme activity.

### Isoenzymes -

are enzymes that catalyze the same chemical reaction but they have slightly different structures. Why do they exist? To cover the excessive body's demand of this chemical reaction in some situations..

### Zymogens

are inactive enzyme precursors that require a biochemical change to become active e.g. cleavage of a peptide blocking the active site.

They are activated when needed.

• RNA or antibodies could act as enzymes.

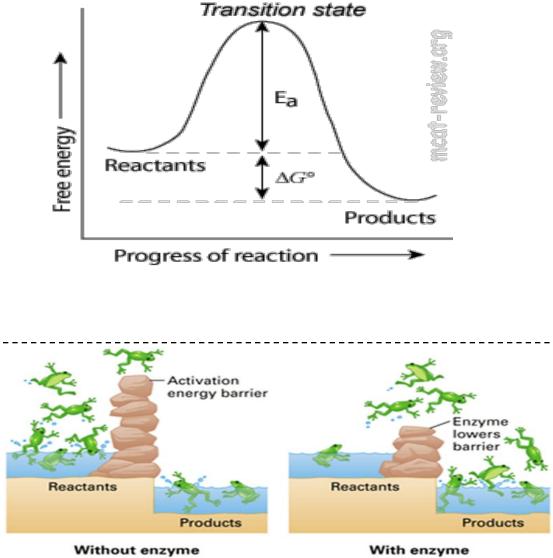
 Inhibitors are structurally similar to enzymes → to control the action.

# **Understanding Activation Energy**

- In every chemical reaction, the reactants pass through a <u>transition state</u> 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)
- <u>activation energy</u> (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.

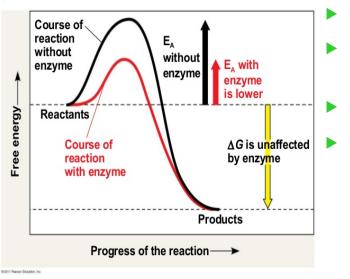
| •       | Enzyme induction $\rightarrow$ increases enzyme activity.  |
|---------|--|
| •       | Enzyme inhibition $\rightarrow$ decreases enzyme activity. |
| • • • • |  |



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

# How do enzymes work?

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



- An enzyme reduces the activation energy required for a reaction
- It provides an alternative transition state of lower energy called the <u>enzyme-substrate complex</u> and thus speeds up the reaction.
  - Enzymes decrease the activation energy but they do not alter the change in the free energy ( $\Delta G$ )

(it remains the same) and it does not change the equilibrium of the reaction. It does however accelerate the rate by which equilibrium is reached.

The difference between Activation energy (EA) and free energy ( $\Delta G$ ) • Activation energy is reduced.

- Erec operative remains the same
- Free energy remains the same.

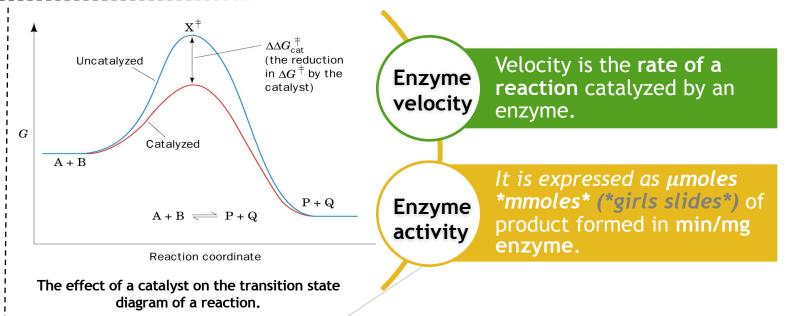
#### Notes :

Figure 8.13

Enzymes operate by lowering the amount of energy needed to get a reaction going (the activation energy). When this energy is lowered, the nature of the bonds is changed, so they are more easily broken.

Therefore, the lower activation energy required, the faster rate of the reaction.

عدما يعمل الأنزايم فإن الترانسيشن ستايت راح تقل طاقتها وعليه: 1 - طاقة النتشيط ستقل (لأنها الفرق بين طاقة المتفاعلات وطاقة الترانسيشن ستليت، وطاقة الترانسيشن ستايت قلّت!) 2 - لن يحدث أي تغيير في دلتا جي (لأنها الفرق بين طاقة المتفاعلات وطاقة النواتج ولم يحدث أي تغيير فيهم)



# Factors that affect enzyme activity:

Objective: Understand factors affecting enzyme activity.

Temperature PH [E] and [S]

### 1. Effect of temperature

Every enzyme has an <u>optimal temp</u> for catalyzing a reaction

(In humans: most enzyme have an optimal temperature of 37 C.)

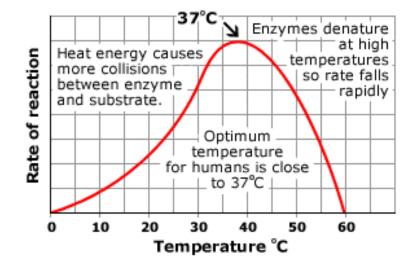
The rate of an enzyme reaction **<u>initially</u>** increases (in velocity) with rise in temperature until it reaches a specific temperature that is considered

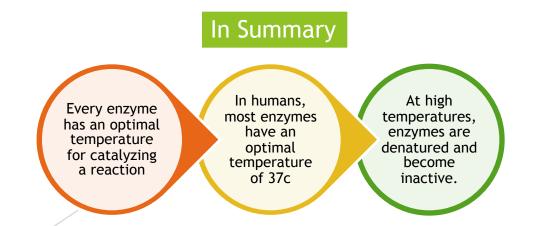
too high for the enzyme to work because it denatures the enzyme, After about 40c.

( الشيء اذا زاد عن حده ينقلب ضده )

#### #Remember

**Denaturation** is a process in which proteins or nucleic acids lose the quaternary structure, tertiary structure and secondary structure which is present in their native state, by application of some external stress.

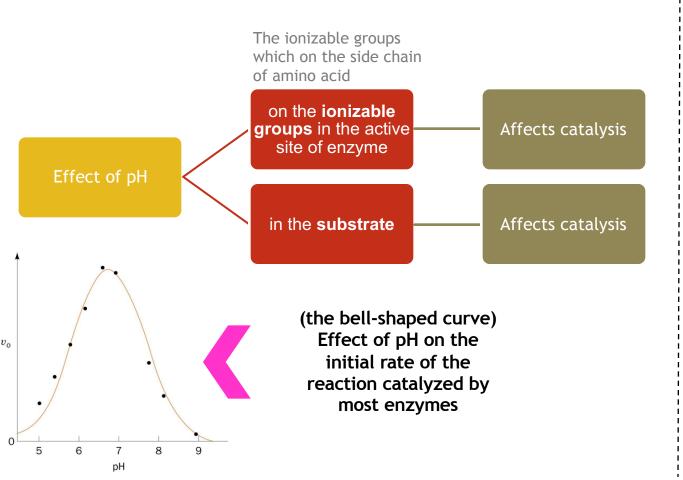




## 2.Effect of pH

Every enzyme has an <u>optimal pH</u> 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



### 3. Effect of [E] and [S]

Higher

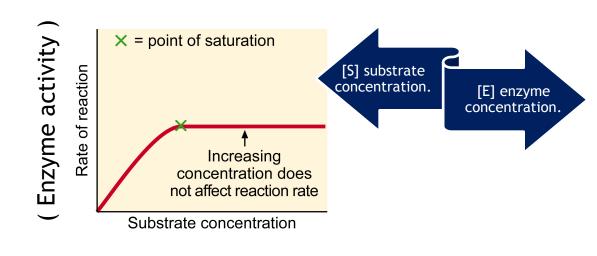
than

\*If [S] > enzyme  $\rightarrow$  the rate of enzyme reaction will be directly proportional to the concentration of enzyme.

The reaction velocity increases *initially* with increasing [S]

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).

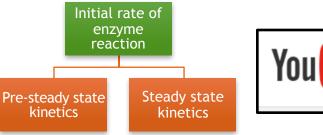
#### \*At low [S] $\rightarrow$ the reaction rate is proportional to [S]





## **Enzyme Kinetics**

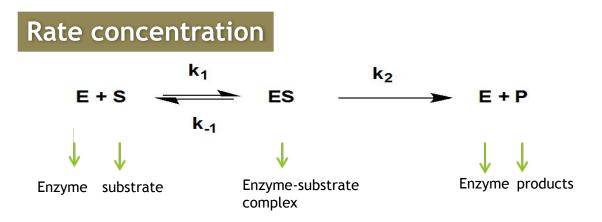
Objective: Understand the enzyme kinetics



You Tube

مهم

- The model of **enzyme kinetics** was first proposed by ٠ Michaelis and Menten in 1913 and later modified by Briggs and Haldane.
- The equation of Michaelis Menten describes the relationship of: Initial rate of enzyme reaction to [S]



#### Notes:

- Michaelis–Menten kinetics is one of the best-known models of enzyme kinetics.
- What does the equation describe? the rate of enzymatic reactions.
- How? by relating reaction rate v
- to [S] the concentration of a substrate.

#### 1. Pre-steady state:

Enzyme + high concentration of substrate  $\rightarrow$  an initial short period of time (a few hundred microseconds) during which intermediates of products gradually build up. NO PRODUCT DURING THIS PHASE.

2. Steady state reaction: occurs after initial state: when the reaction rate and the concentration of intermediates change slowly with time.

An intermediate changes into steady state when the rate of its

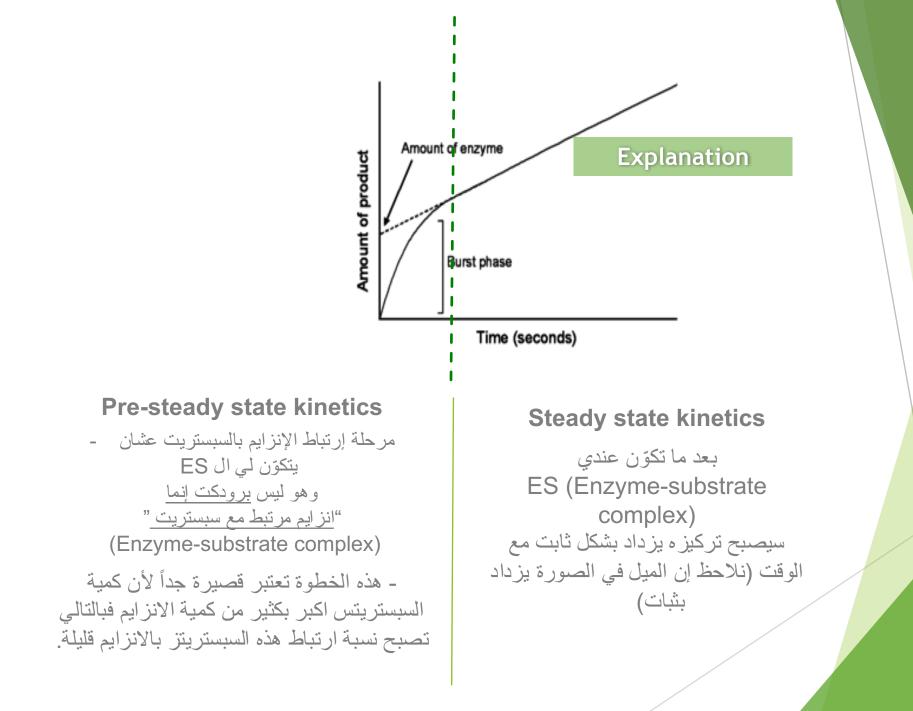
synthesis becomes equal to its rate of degradation.

The rate of formation of ES = the breakdown of ES to (E+S) & (E+P)

بعني سرعة تكونES تساوى انحلاله وتفككه.

#### Notes:

- Pre-steady-state kinetics is concerned with the formation and consumption of enzyme-substrate intermediates until their steady-state concentrations are reached.
- reactive intermediate: a short-lived, high-energy, highly reactive molecule. When generated in a chemical reaction, it will quickly convert into a more stable molecule.



### **Michaelis Menten Equation**

# •It measures the **initial velocity** $(v_o)$ of an enzyme reaction.

Note: initial velocity is also described as "rate of a reaction."

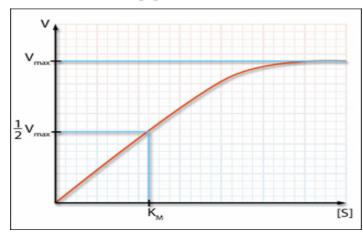
#### [S] = Substrate concentration

#### $\mathbf{K}_{m}$ = Michaelis constant

The constant  $K_{\rm m}$  is a measure of how efficiently an enzyme converts a substrate into product.

V<sub>max</sub> = Maximum velocity achieved by the system when the enzymes will be saturated & full up with substrate, and won't able to react anymore quickly..

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



 $oldsymbol{V_o} = rac{V_{ ext{max}}[S]}{K_{ ext{M}} + [S]}.$ 

 $K_{m}$ : a substrate concentration where the initial rate is half of the maximum rate (½  $V_{max}$ ) Meaning: It is the [S] required to saturate

half of all of the active sites of an enzyme

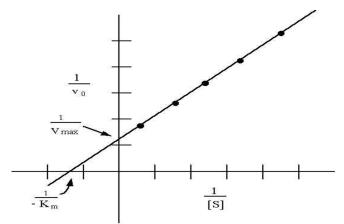
The  $K_{\rm m}$  value of a substrate depends on its affinity with the enzyme

High K<sub>m</sub> → low affinity with enzyme (Therefore higher concentration of substrate is needed to saturate the enzyme)

Low  $K_m \rightarrow$  high affinity with enzyme (less substrate needed to saturate the enzyme)

Note: The meaning of affinity is "natural liking."

### Lineweaver-Burk plot (doublereciprocal plot)



obtained by taking reciprocals of the Michaelis Menten equation
It is plotted to:

\*calculate the **Km and Vmax values** \*determine the **mechanism of action** of enzyme **inhibitors** 

لللرّبط الله الما يكون الشخص صاحب مستوى عالي بمنصب مثل لما يكون الشخص صاحب مستوى عالي بمنصب أو غيره ومشغول طول الوقت هنا يصير إحتكاكه مع فلذلك يحتاج إنه يقوّي علاقته أكثر فيحتاج مستريتس أكثر عشان يتشبّع. اسبستريتس أكثر عشان يتشبّع. هنا العكس اللي يكون منصبه بسيط فبكذا عنده وقت أكثر وإحتكاكه مع أصدقائه عالي فبيحتاج عدد أقل من السبستريتس عشان يتشبّع الإنزايم.



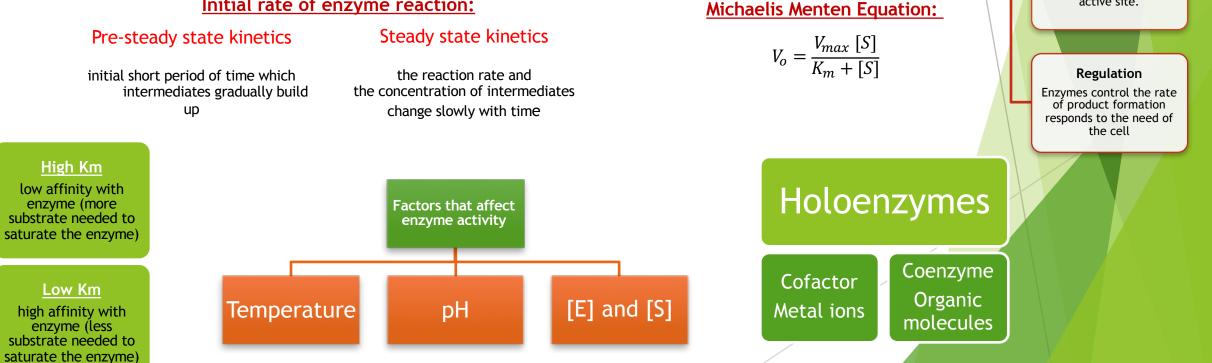
Enzyme

**Reacts with** to form

#### Substrate **Products**

- Enzymes are biological catalysts that speed up the rate of a reaction without being ٠ changed in the reaction
- All enzymes are protein in nature ٠
- Apoenzyme (inactive) + nonprotein part = Holoenzyme (active) ٠

#### Initial rate of enzyme reaction:



**3** Properties

of Enzymes:

Active site

region of binding and

catalyzing

Specificity

Enzymes bind to their specific substrates in the active site.

# MCQs + Videos



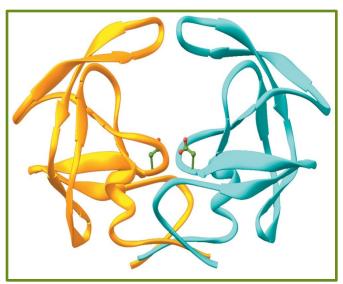
- https://youtu.be/j00Ep0Byu0Y
- https://youtu.be/7u2MkbsE\_dw
- https://youtu.be/X\_YXTWU2maY

# **Enzyme inhibition**

Objective: Understand types of inhibition

Inhibition is a process in which the enzyme activity is regulated or controlled or stopped.
Ki ( inhibitor constant ) is a measure of the affinity of the inhibitor for the enzyme. Also, known as <u>dissociation</u> constant.

An enzyme **<u>without</u>** inhibitor



An enzyme <u>with</u> inhibitor

To inhibit

means to stop



#### Note:

-affinity is the tendency of a molecule to associate with another. -if enzymes worked randomly, our whole system will be damaged.

#### 436 Biochemistry team

| Tł | nere are three types of enzyme inhibition:                                |  |  |  |
|----|---|--|--|--|
|    | <u>Competitive</u><br>- Inhibitor has a similar structure to<br>substrate |  |  |  |
|    | - It competes with substrate for binding to active site of enzyme.        |  |  |  |
|    | Noncompetitive  |  |  |  |
|    | - Inhibitor does not have similar structure to substrate                  |  |  |  |
|    | -No competition exists between<br>inhibitor and substrate                 |  |  |  |
|    |   |  |  |  |

Uncompetitive (this type of inhibition was only mentioned in boys slides).

# **COMPETITIVE INHIBITION**

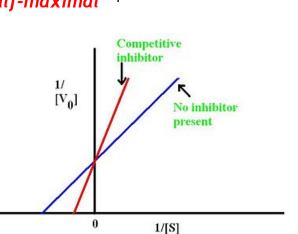
- The inhibitor is a structural analogue (similar) \*to the substrate\* that competes with the substrate for binding to the same active site of enzyme (competitive). Thus prevent the enzymatic reactions.
- Competitive inhibition is always reversible reaction.

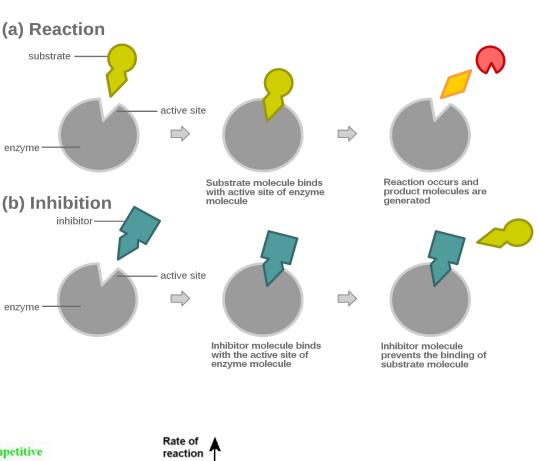
Two reactions are possible: Enzyme + Substrate ↔ ES complex →Product + Enzyme (active) OR Enzyme + Inhibitor ↔ EI (inactive)

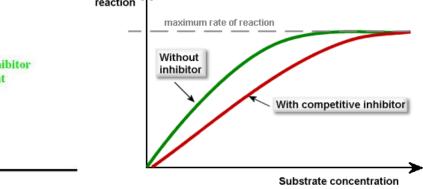
- Vmax  $\rightarrow$  unchanged in the presence and the absence of inhibitor.
- Km → increased, because Substrate and Inhibitor compete for binding at the same site.

\*A higher concentration of substrate is required to achieve half-maximal velocity\*

Which means : The competitive inhibition prevents the reaction by preventing the substrate from binding to the active site.







# NON-COMPETITIVE INHIBITION

- Inhibitor doesn't have a similar structure to the substrate.
- It binds somewhere other than the active site.
- Non-competitive inhibition could be irreversible or reversible.

Remember: \*No competition exists between the inhibitor and the substrate\*

The inhibitor can bind to: a free enzyme or to an enzymesubstrate complex. In both cases the complex is catalytically inactive

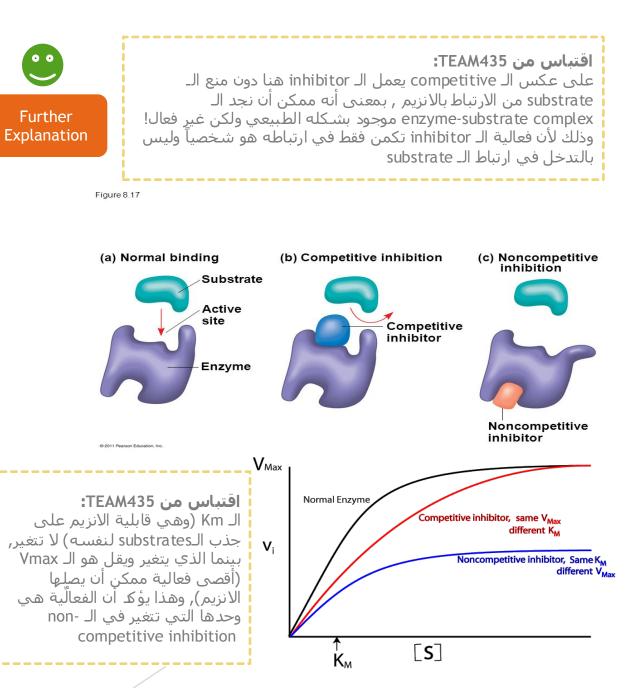
```
Two reactions are possible:

ES + I ↔ ESI (inactive)

Or

E + I ↔ EI (inactive)
```

- Vmax  $\rightarrow$  decreased by the inhibitor.
- Km → unchanged because the affinity of Substrate for Enzyme is unchanged





# **COMPETITIVE VS NON-COMPETITIVE**

| مُقتبس من تیم ٤٣٥                | Competitive   | Non-competitive  |
|----------------------------------|---|--|
| structure                        | Similar to substrate  | Different from substrate   |
| Binding site                     | Active site of enzyme   | Away from active site  |
| Competition<br>exists bet. S & I | Yes   | No   |
| K <sub>m</sub>                   | increased   | Unchanged  |
| V <sub>max</sub>                 | Unchanged   | Decreased  |
| Possible<br>reactions            | $E + S \leftrightarrow ES \text{ complex } P \text{ (ACTIVE)}$<br>or<br>$E + I \leftrightarrow EI \text{ (INACTIVE)}$ | $ES + I \leftrightarrow ESI (INACTIVE)$<br>or<br>$E + I \leftrightarrow EI (INACTIVE)$ |
| Reversibility                    | Always reversible   | Sometimes reversible & sometimes<br>irreversible                                       |

# Regulation of enzyme activity

Objective: understand regulation of enzyme activity.

- Regulatory enzymes usually catalyze <u>first</u> or <u>an early reaction in a metabolic pathway.</u>
- They catalyze a rate-limiting reaction (<u>slowest</u> <u>step</u>) that controls the overall pathway.
- They may also catalyze a reaction unique to that pathway known as <u>committed step</u>.
- After the committed step reactants in the pathway become committed and will end up as the final product of the pathway.

#### Notes:

-The overall rate of a reaction is often determined by the slowest step, known as the rate-limiting step.

-The committed step is an effectively irreversible enzymatic reaction.



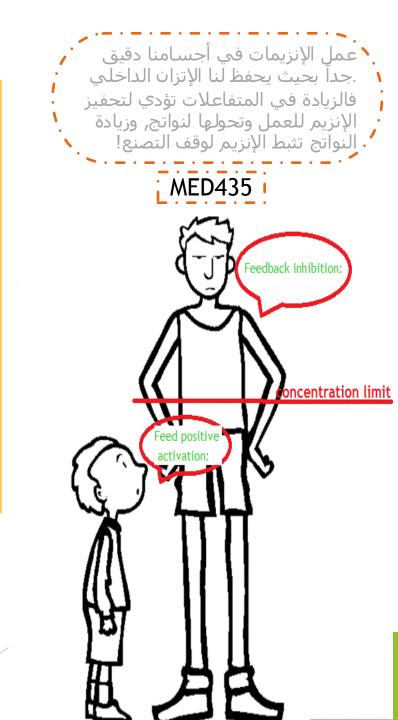
#### أحد التطبيقات العملية على الإنزيمات هي كونها من أهم أجزاء الـ pathways metabolicالتي تتم في أجسـامنا عـلى مدار الثانية. الإنزيمات تحفز أو تثبط التفاعلات بشـكل عام, ولكن بشكل خاص هناك جزء من الإنزيمات تسمى enzymes Regulatory وهو المنظم الأسـاسـي لاتجاه سـير التفاعل (كإشـارة المرور في الطرقات) وعدم توفره يعني عدم وجود الـ pathway من الأسـاس

### Feedback inhibition

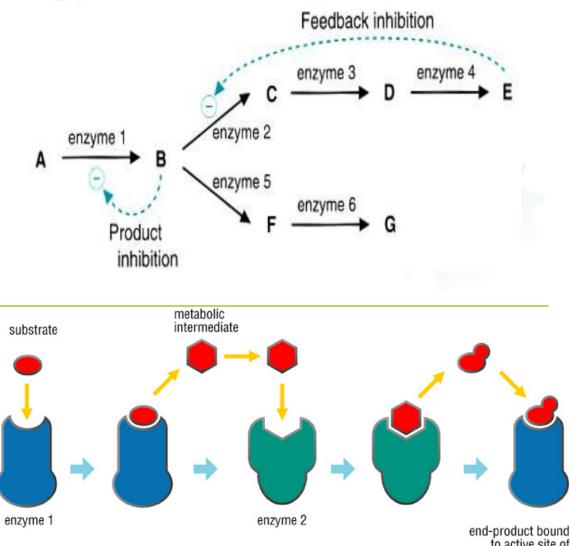
When the end product of a metabolic pathway <u>exceeds</u> its concentration limit, it **inhibits** the regulatory enzyme to normalize the pathway

### Feed positive activation

When the end product of a metabolic pathway is <u>below</u> its concentration limit, it **activates** the regulatory enzyme to normalize the pathway.



# Feedback Inhibition



Still can't get it ?

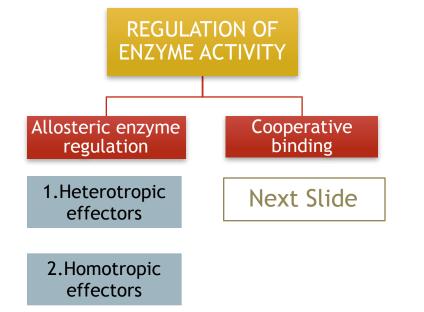
-Feedback Inhibition of biochemical pathways :

https://www.youtube.com/watch?v=n3 i7XMQTZ\_s

Feedback Inhibition or End
 Product Inhibition of Enzymes:

https://www.youtube.com/watch?v=bR GsmNLMn4I

nd-product bound to active site of enzyme 1 shuts down pathway



### Regulation of allosteric enzymes

The term "allosteric" came from Greek word "allos" meaning "other"

#### Allosteric Enzymes:

The enzymes in metabolic pathways whose activities can be regulated by certain compounds (ligand) that bind to enzyme other than the catalytic site (active site)

-NOTE : Allosteric enzymes are regulated by molecules called effectors (also called modifiers) that bind non-covalently at a site other than the active site. These enzymes are usually composed of multiple subunits, and the regulatory (allosteric) site that binds the effector may be located on a subunit that is not itself catalytic. The presence of an allosteric effector can alter the affinity of the enzyme for its substrate , or modify the maximal catalytic activity of the enzyme, or both.

**Homotropic:** Effect of one ligand on the binding of the same ligand (a regulatory enzyme modulated by its own substrate).

When the substrate itself serves as an effector the effect is said to be homotropic.

Most often, an allosteric **substrate** f<u>unctions as a **positive effector**</u>. In such a case, the presence of a **substrate** molecule at one site on the enzyme **enhances(+)** the **catalytic** properties of the other substratebinding sites—that is, their binding sites exhibit cooperativity.

#### Heterotropic: Effect of one ligand on the binding of a different ligand.

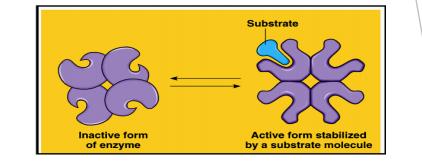
The **effector** may be **different** from the **substrate**, in which case the effect is said to be heterotropic

https://youtu.be/qgHr0dWKhSc?t=2m1s (2:00-4:20)

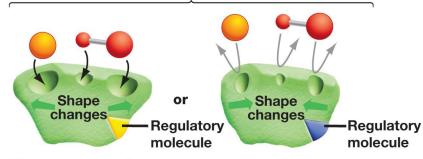
Definition: a ligand is an ion or molecule (functional group) that binds to a central metal atom to form a coordination complex.

### Cooperative binding

- It is the process by which binding of a ligand to a regulatory site affects binding of the same or of another ligand to the enzyme.
   سنا المادة مع الانزيم في منطقة وحدة يحفّز المناطق الأخرى بنفس الإنزيم.
- Binding of an allosteric modulator causes a change in the **conformation** of the enzyme
- This causes a change in the binding affinity of enzyme for the substrate
- The effect of a modulator may be positive (activation) or negative (inhibition)
- Positive: increased E, S affinity
- Negative decreased E, S affinity
- Most allosteric enzymes are oligomers (two or more polypeptide chains or subunits)
- The subunits are known as protomers



#### (b) Allosteric regulation

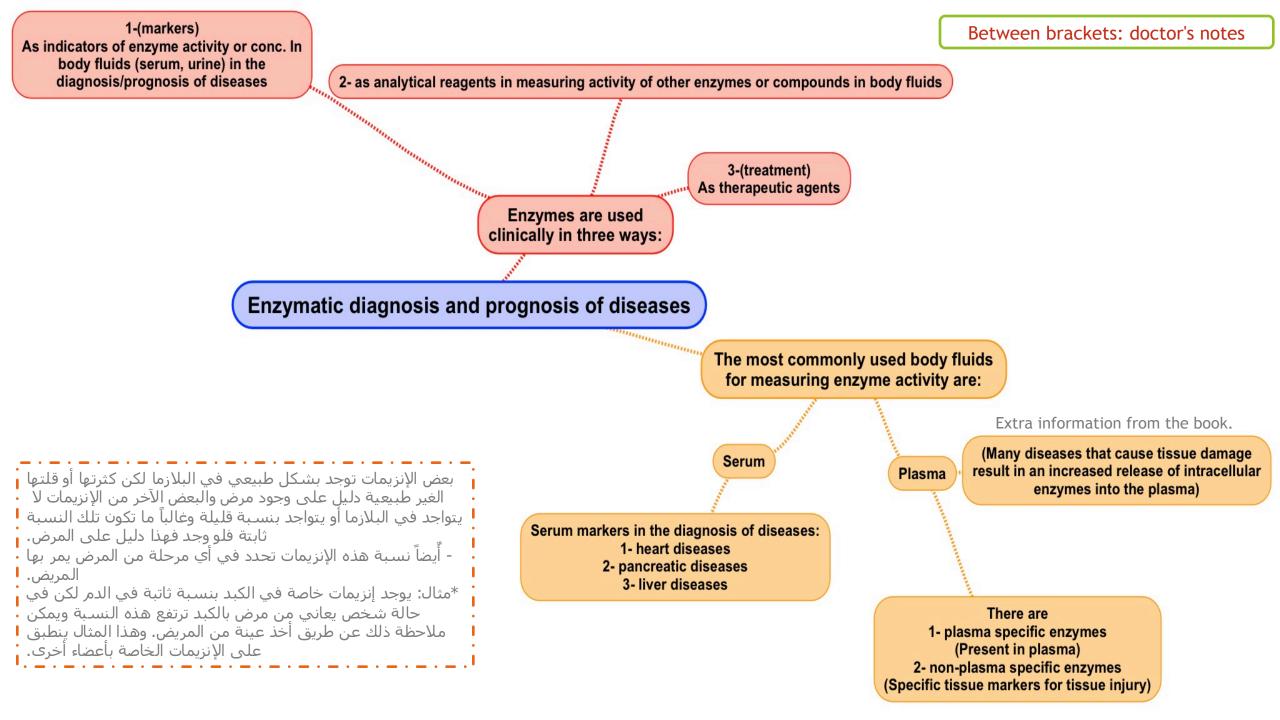


#### Allosteric activation

Allosteric deactivation

The active site becomes available to the substrates when a regulatory molecule binds to a different site on the enzyme. The active site becomes unavailable to the substrates when a regulatory molecule binds to a different site on the enzyme.

Effectors that inhibit enzyme activity are termed negative effectors, whereas those that increase enzyme activity are called positive effectors.



# Summary

- ✓ -Regulatory enzymes usually catalyze the first or an early reaction in a metabolic pathway.
- ✓ -There are two types of regulation allosteric enzyme regulation and cooperative binding
- Feedback inhibition inhibits the regulatory enzyme to normalize the pathway (feedback inhibition) while the feedback positive activiation
- $\checkmark\,$  activates the regulatory enzyme to normalize the pathway.
- ✓ -Two types of interactions occur in allosteric enzymes:
- ✓ Homotropic: Effect of one ligand on the binding of the same ligand.
- Heterotropic: Effect of one ligand on the binding of a different ligand.
- Enzymes are used clinically in three ways.

|                  | Competitive<br>inhibition  | Noncompetitive   |
|------------------|--|--|
| meaning          | similar to the structural<br>of the substrate and<br>competes with it for<br>binding at the active<br>site | The inhibitor does not have<br>structural similarity to the<br>substrate.The inhibitor<br>binds to the enzyme at a<br>site away from the<br>substrate binding site |
| V <sub>max</sub> | V <sub>max</sub> is unchanged<br>in the presence<br>and the absence<br>of inhibitor                        | The value of V <sub>max</sub> is<br>decreased by the inhibitor   |
| K <sub>m</sub>   | K <sub>m</sub> is increased because<br>substrate and inhibitor<br>compete for binding at<br>the same site  | K <sub>m</sub> is unchanged<br>because the affinity of<br>S for E is unchanged   |

# MCQs + Videos

- Most commonly used body fluids for measuring enzyme activity are :
- A- serum B- interstitial C-plasma D-A&B E-A&C
- Heterotropic: Effect of one ligand on the binding site of the same ligand
- A- TRUE B- FALSE
- Ki is a measure of affinity of the inhibitor for the enzyme
- A- TRUE B- FALSE
- In competitive inhibition ..... is unchanged in the presence and the absence of inhibitors
- A- Kmax B- Vmax C- Vavg D- V
  - ► <u>Competitive inhibition</u>
  - ► <u>Ki ( dissociation constant )</u>
  - Allosteric enzyme regulation
  - Cooperative binding

### Girls team members:

10- جومانا القحطاني. 11- نجود العنزي. 12- شذا الغيهب. 13- سارة الشمراني. 14- لجين الزيد. 15- روان الوادعي. 16- منيرة الضفيّان. 17- بثينة الماجد.

1- زينة الكاف. 2- هيفاء الوعيل. 3- ريم السرجاني. 4- سمية الغامدي. 5- لمى الفوزان. 6- مها الغامدي. 7- نورة الشبيب. 8- أسيل السليماني. 9- شهد السويدان.

### Boys team members:

1- محمد المهوس.

-Team leaders:

نوره السهلي. عبدالله المانع.

### -Contact us:

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