Biochemistry

Title = Enzyme/2
Lec no = 2
Done By = Baraa Safi

وقل رب زدني علما

Biochemistry lecture 2: enzymes 2 of 3

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Enzymes II	1.Effect of substrate concentration on rate of enzymatic reaction 2. Understanding enzyme kinetics 3. Michaelis-Menten equation 4. What are Km and V max values? 5.Enzyme activation and inhibition 6.Irriversible and reversible inhibitors 7.Kinetics of reversible inhibitors
Enzymes III	1. What are isozymes? 2. Application of isozymes in diagnosis 3.Control of enzyme activity a. Allosteric regulation b. Covalent modification

Factors affecting the rate of enzymatic reaction

1. Enzyme concentration
2. Substrate concentration
3. Product concentration
4. Temperature
5. Hydrogen ion concentration (pH)
6. Presence of activators
7. Presence of inhibitors
8. Presence of repressor or derepressor.
9. Covalent modification

MICHAELIS–MENTEN THEORY

- In 1913, Michaelis and Menten put forward the Enzyme–Substrate complex theory.
- In this model, the enzyme (E) reversibly combines with its substrate (S) to form an ES complex that subsequently yields product (P), regenerating the free enzyme.

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \qquad \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

MICHAELIS–MENTEN THEORY

- S is the substrate.
- E is the enzyme.
- ES is the enzyme–substrate complex
- P is the product.
- k1, k−1, and k2 (or, kcat) are rate constants.
  - **kcat** is the turnover number and this describes **how many substrate molecules** are transformed into products **per unit time** by a single enzyme.

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

MICHAELIS–MENTEN EQUATION

ما بنا نتعمق بالموضوع

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

Vo = initial reaction velocity

Vmax = maximal velocity

Km = Michaelis constant

[S] = substrate concentration

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

Effect of enzyme concentration

- **Rate of a reaction or velocity** (V) is **directly proportional** to the **enzyme concentration**, when sufficient substrate is present.

- This is true up to a point when a further increase in the enzyme concentration is not accompanied by an increase in the velocity of the reaction
  - At this point the **substrate is said to be the limiting factor**

- This property is made use of in determining the level of particular enzyme in plasma, serum or tissues
  - Known volume of serum is incubated with substrate for a fixed time
  - Then reaction is stopped, and product is quantitated
  - Since the **product formed will be proportional to the enzyme concentration**, the latter could be assayed.

شرح طريقة تحديد مستوى انزيمات معينة في بلازما الدم :-

1) بنجيب حجم معين محروق من البلازما اللي بيحتوي على (E) معين بدي احسبه

2) يتم وضعه مع نسبة معينة من الـ (S) لفترة معينة

3) ونظرًا لأنه ما معي كمية لا نهائية من الـ (S) فالتفاعل سيتوقف

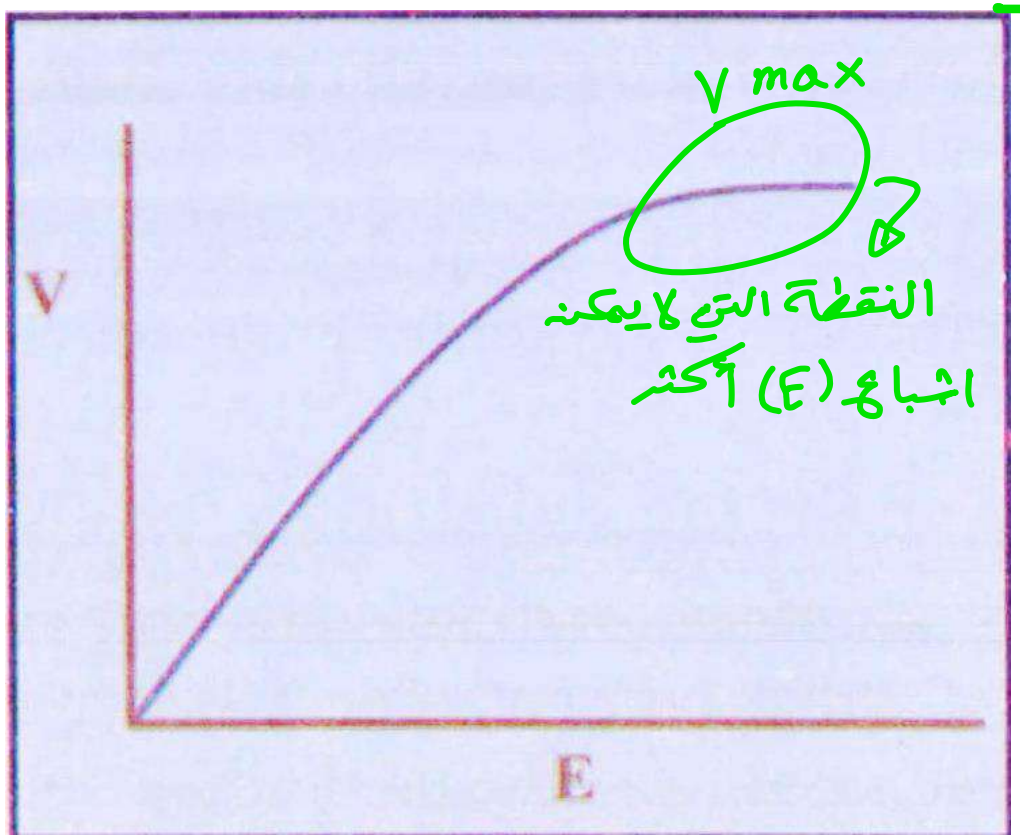
4) في هذه اللحظة أنا كأنه معي (ES) complix وبعد كما سيتحول إلى (P) و (E)

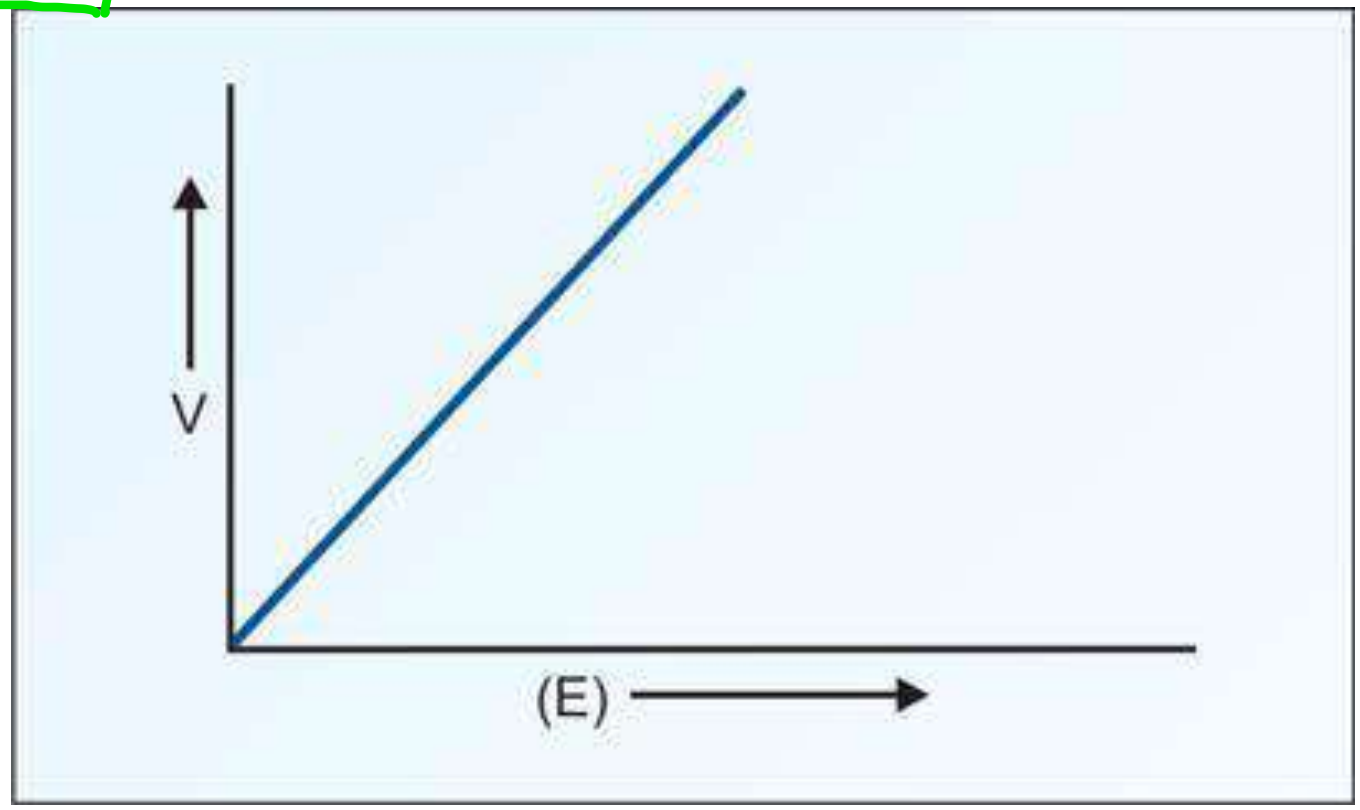
5) أنا أستطيع حساب الـ (P) والذي يتناسب مع (Enzyme concentration)

6) بالتالي أستطيع حساب الـ (E) المعين في البلازما في الدم / كما الأنسجة

على افتراضه أنا عندي (S) بتتصرف وأقل من (E) زادت (V) لحد نقطة معينة خلصه فيها (S) فتوقف التفاعل

* كل ما عمر بزيد (E) كل ما عمر بزيد على افتراضه أنه (S) لا نهائي (عندي كمية لا نهائية منه (S)) وكل ما بزيد (E) رح تزيد الـ (V) لحد معين وهو الإشباع





* احنا رح نركز على الرسمة اللي على اليسار لأنه احنا بنفترض أنه (S) دائما كافي ولا نهائي

Effect of substrate concentration on the (V)

- The velocity of the reaction ↑ as the **substrate concentration** ↑ up to point where the enzyme is saturated.

  If the enzyme is saturated with substrate—meaning that as soon as a product molecule is released, a new substrate is bound—then the reaction will reach a maximum rate, or velocity.

- The Vi increases to a maximum value Vmax. → where (E) is saturated

- The substrate concentration that produces half the maximal velocity is termed **Michaelis constant or Km.**

  يعني أنا أوصل نصف (Vmax) يعني أنا بوقف وبحكي هاد (Km) وعندها يكون ½ مشبع (E)

- **Km** is a **substrate concentration** and is the amount of substrate it takes for an **enzyme** to reach Vmax/2

  Km : (S) مع (E) قدرة ارتباط ال

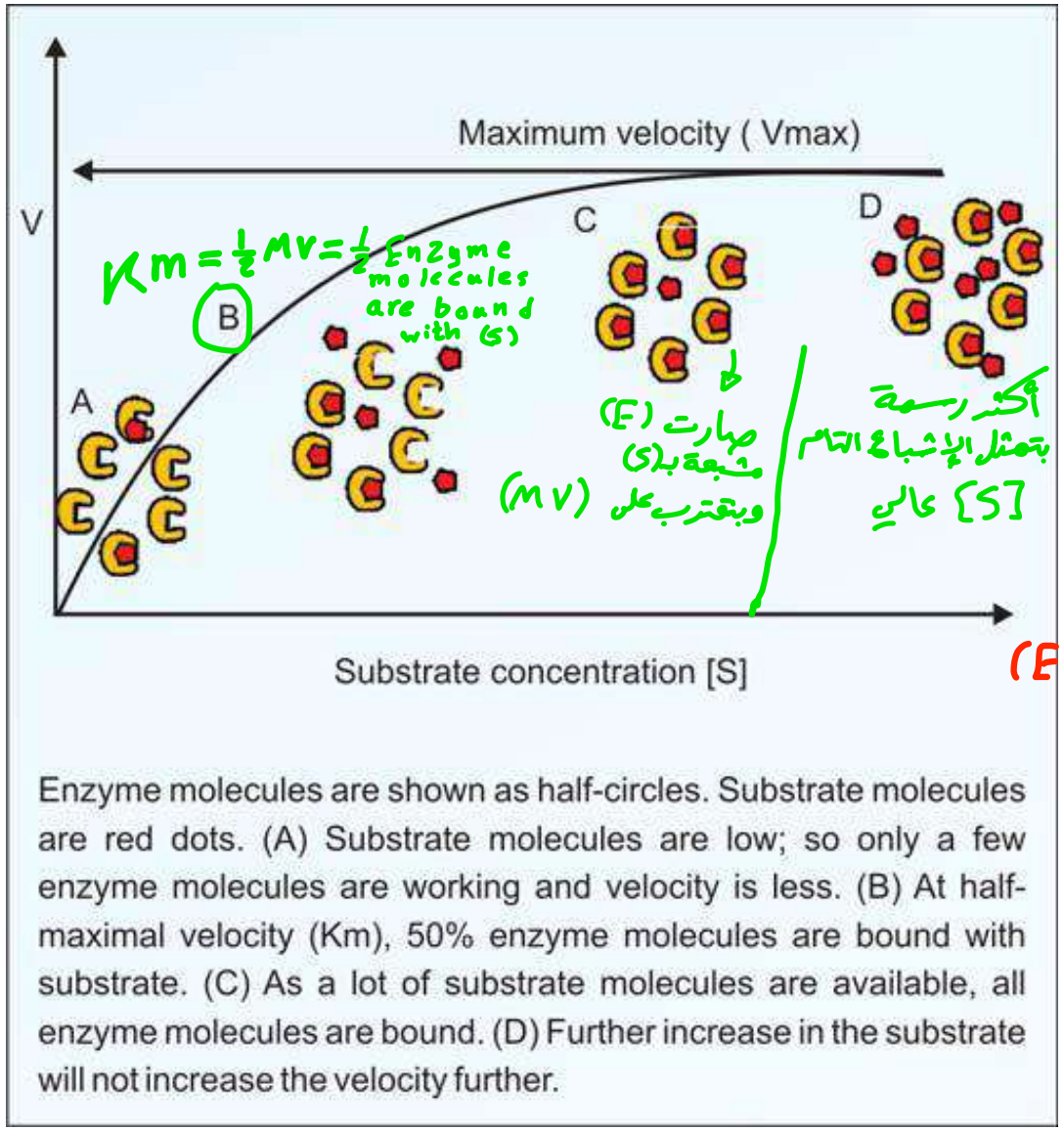
- When [S] is approximately equal to Km, Vi is very responsive to changes in [S], and the enzyme is working at half-maximal velocity

سؤال منه الدكتورة وقالت ممكن يجي في الامتحان:

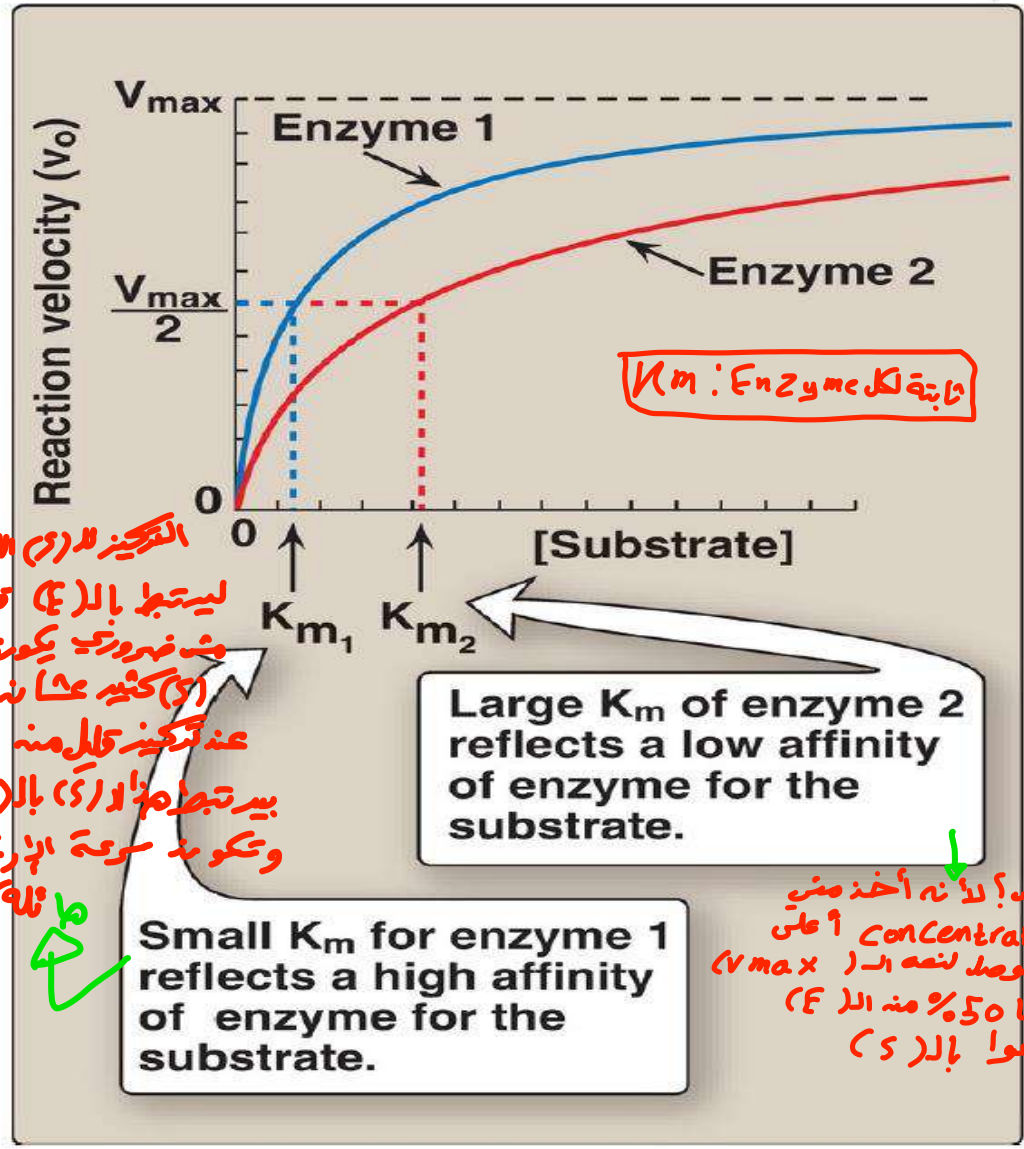
س: ليش Km هي (Affinity)؟

ج: (Km) يعني أنه الأنزيم أشبع نصف اشباع بالـ(S)، ويعني أنني وصلت إلى نصف الـ(V.max) وعند زيادة الـ(Km) هذا يعني أنني أحتاج لزيادة تركيز(S) للوصول إلى الـ(Vmax) وهذا يعني أنه قدرة ارتباط الـ(E) مع (S) ضعيفة لذلك زاد تركيز(S) وزاد (Km) للوصول لنفس قيمة (Vmax) وهذه القدرة على الإرتباط هي الـ(Affinity) وتتناسب تناسب عكسيًا مع (Km)

* ملاحظة: عادي لو ما فهمت لأنه في شرح لهم لقدام فارجع بس تخلص المحاضرة



Enzyme molecules are shown as half-circles. Substrate molecules are red dots. (A) Substrate molecules are low; so only a few enzyme molecules are working and velocity is less. (B) At half-maximal velocity (Km), 50% enzyme molecules are bound with substrate. (C) As a lot of substrate molecules are available, all enzyme molecules are bound. (D) Further increase in the substrate will not increase the velocity further.



Michaelis Menten Constant (Km)

- Describes the behavior of enzymes as substrate concentration is changed.

- **Km** denotes the affinity of enzyme for substrate

- **The lesser the numerical value of Km, the affinity of the enzyme for the substrate is more**

Michaelis Menten Constant (Km)

- Km is independent of enzyme concentration
  - If enzyme concentration is doubled, the Vmax will be doubled
  - But the Km will remain exactly same

* حابب هنا بس نزيد (E) أكيد بتزيد V و بتزيد (Vmax) طب ليش ما بتزيد الـ Km ؟
بباطة (Km) ثابتة لكل (E) وهي لو تغير بتغير قدرة الإرتباط وبتغير التركيز المطلوب منه (S) احنا ما بدنا نعمل هيك بس بدنا نزيد الـ (E).

- According to Michaelis Menten Constant:
  - the enzyme– substrate complex is a reversible reaction
  - the breakdown of the complex to enzyme + product is irreversible.

ES

E and P

(E) الـ من ٪50 ÷ مرتبط يكون ف/ Concentration of substrate الـ

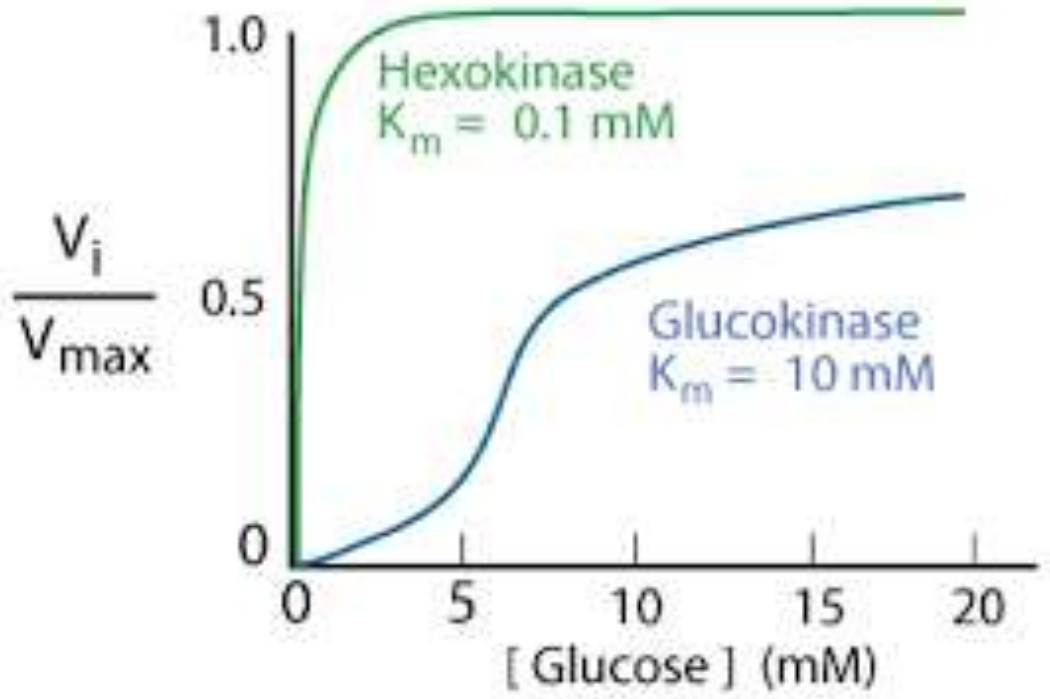
Salient Features of (Km)

- **Km value is substrate concentration** (expressed in moles/L) **at half-maximal velocity**

- **It denotes that 50% of enzyme molecules are bound with substrate molecules at that particular substrate concentration**

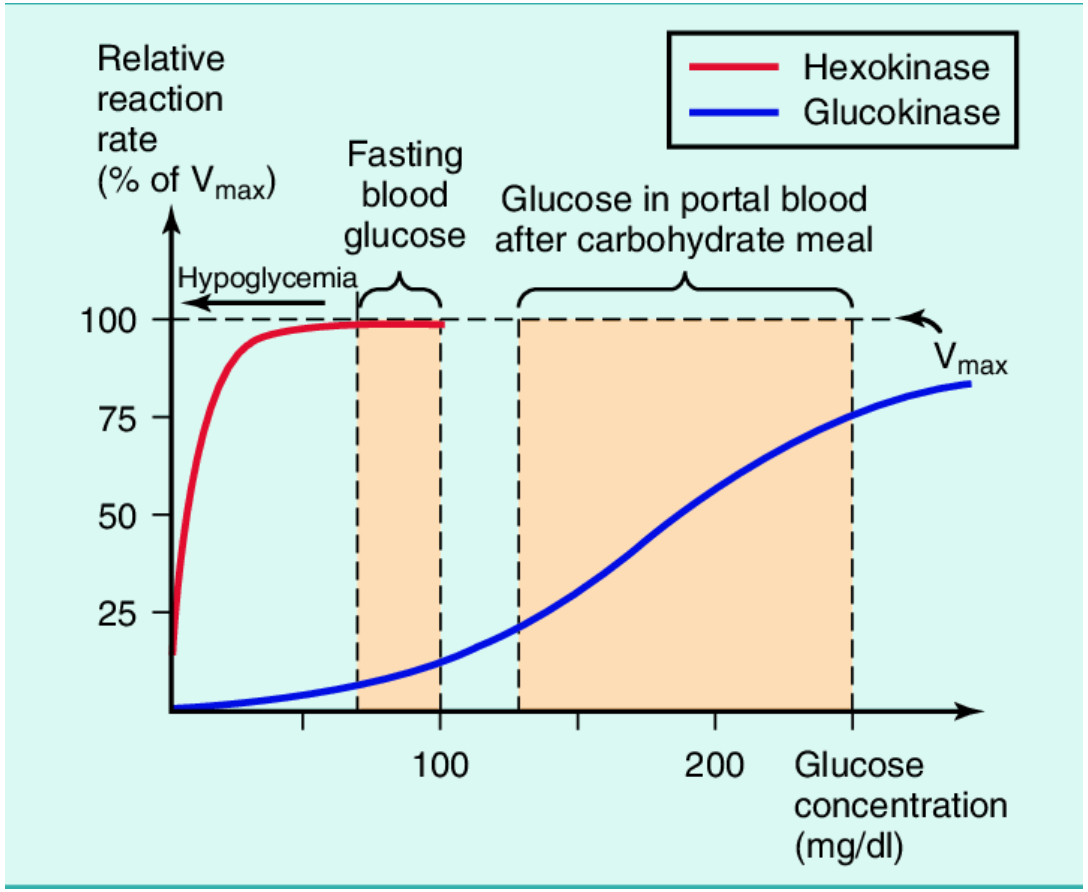
(E) يعني نسبة بتكون

- **Km is the Signature of the Enzyme**
  - Km value is thus a constant for an enzyme
  - It is the **characteristic feature of a particular enzyme for a specific substrate**



(Glucokinase and Hexokinase)

انزيمين بيشتغلوا على الجلوكوز



؟ (Glucokinase ②) و (Hexokinase ①) هو الفرق بين

① it breaks down glucose to use energy, mostly when we were fasting. (Km) is very low, and its affinity is very high.

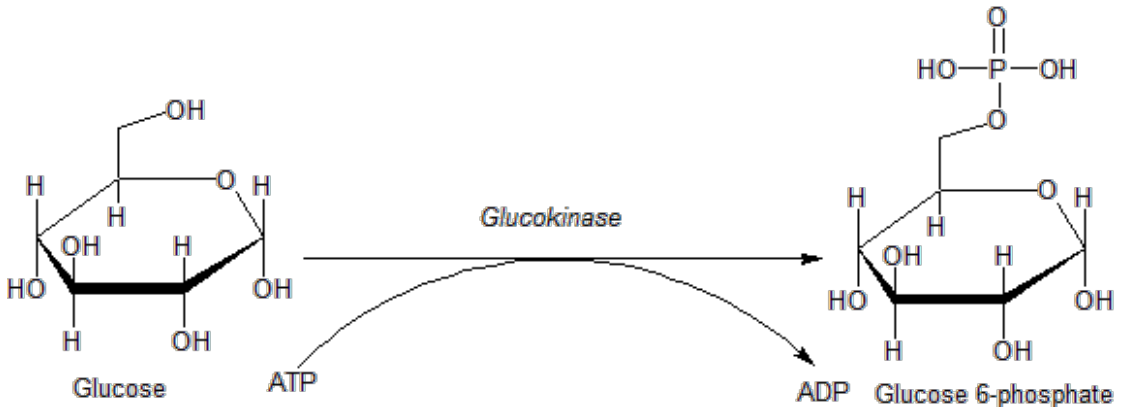
① (goes into Hexokinase) ← فلما يكون الواحد صايم ويكون عنده نسبة قليلة من (Glucose) الأنزيم بقول أنا رح أحولهم لطاقة عشان الـ (Affinity) عالية

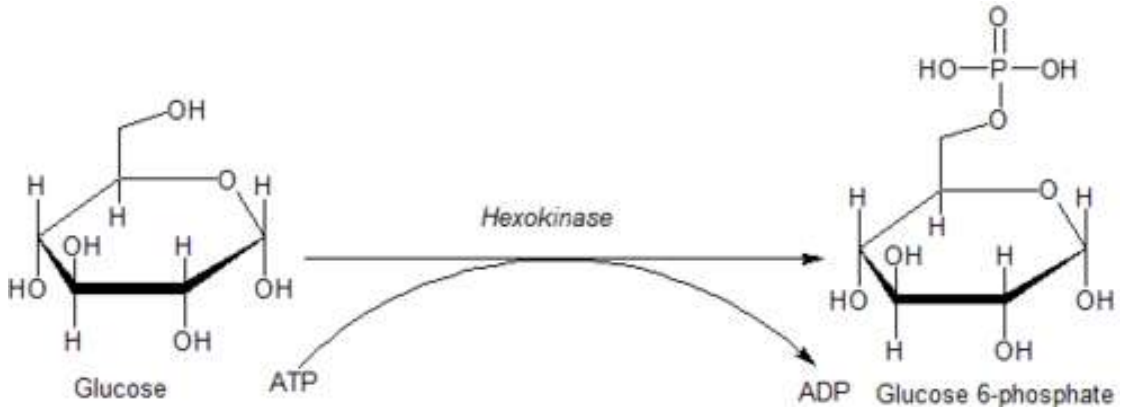
② it turns glucose to glycogen, But its Affinity is very low, and its (Km) is very high.

② ← goes into glycogenisis

- وعشان الـ (Affinity) تاعتها قليلة إذا متى بحاجة عندي جلوكوز وبدأ أحوله لجلايكوجين؟

لما أكون أكلت أكثر ويكون عندي فائض من الـ (Glucose)


Glucose
Glucokinase
ATP
ADP
Glucose 6-phosphate


Glucose
Hexokinase
ATP
ADP
Glucose 6-phosphate

كل ماتزيد درجة الحرارة كل ما زادت سرعة التفاعل لكنه تصل لـ(MV) وبعدين
يتبلش السرعة تنزل لأنه بس أضل أزيد الحرارة شو بصير بالأنزيم؟ بصيرله تشوه

Effect of temperature

- The velocity of enzyme reaction **increases** when temperature of the medium is increased → reaches a maximum and then falls **(Bell shaped curve)**

- **Optimum temperature:** Temperature at which maximum amount of the substrate is converted to the product per unit time

احنا بنعرف أنه كل ما زادت الحرارة بتزيد الـ (energy)

- As temperature is increased, more molecules get activation energy, or molecules are at increased rate of motion

  → their collision probabilities are increased and so the **reaction velocity is enhanced**

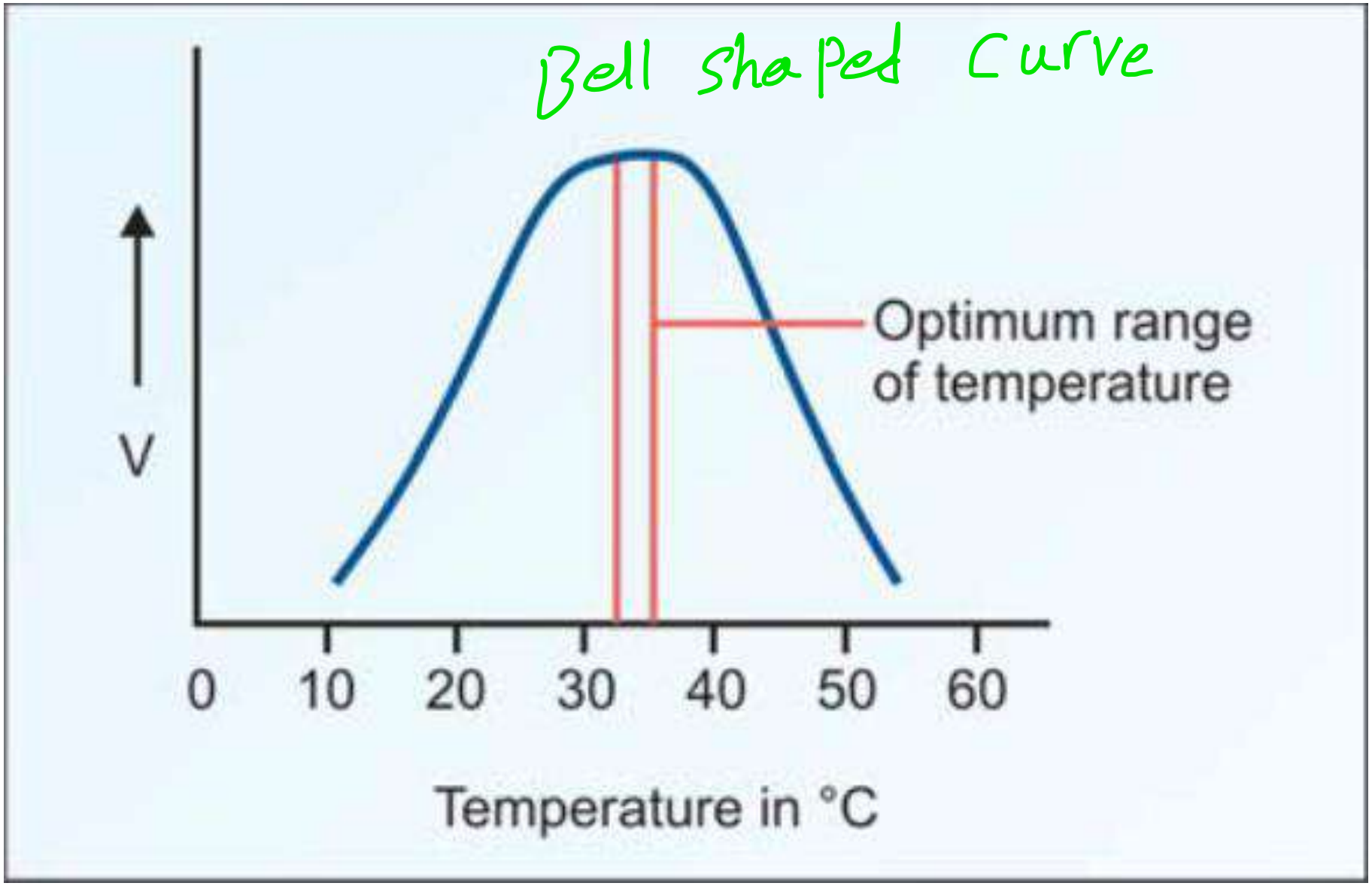
Effect of temperature

- But when **temperature is more than 50°C,** heat **denaturation** and consequent loss of tertiary structure of protein occurs: and loss of function
  Activity of the enzyme is decreased

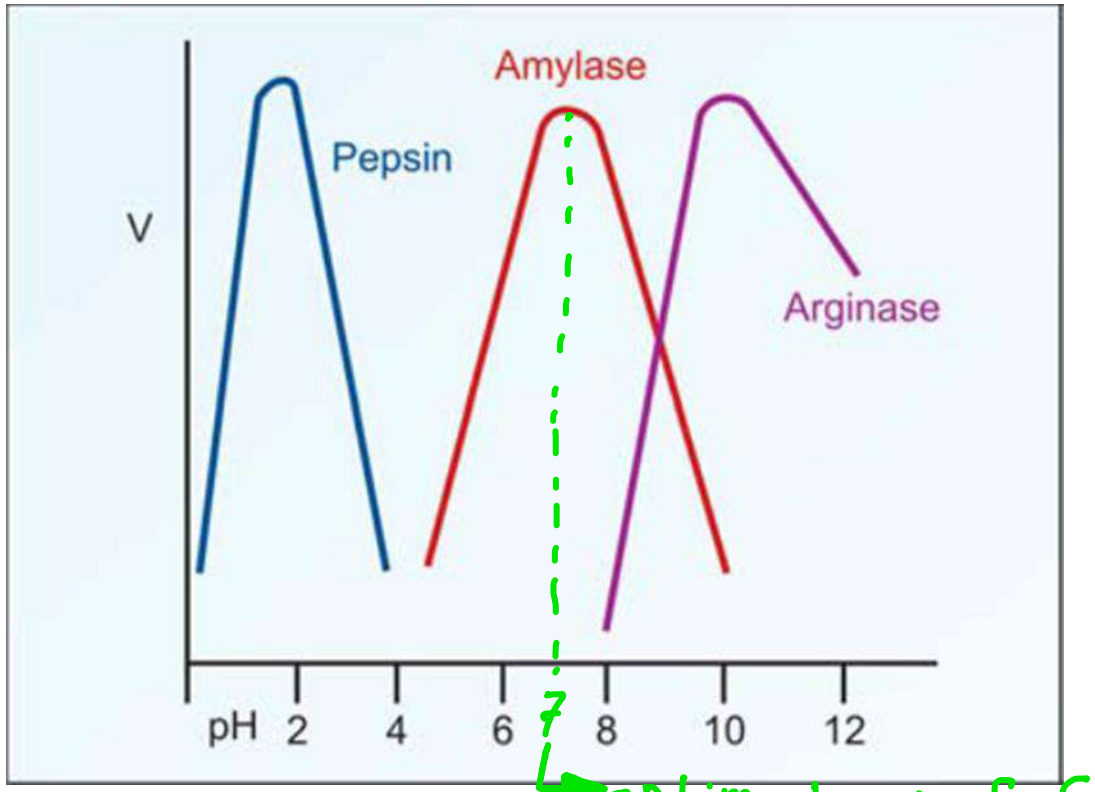
- Most **human** enzymes have the optimum temperature around 37°C

- **Plants:** optimum temperature around 50°C

- Certain **bacteria** living in hot springs will have enzymes with optimum temperature near 100°C


Bell shaped Curve
V
Optimum range
of temperature
0
10
20
30
40
50
60
Temperature in °C

Effect of pH



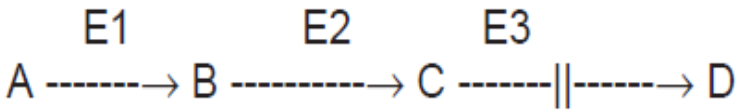
- Each enzyme has an optimum pH at which it shows maximal activity
- Activity decreases as we go away from the optimum pH
- Activity virtually (تقريبا) stops about 2 units of pH above or below this pH

optimal pH of Enzyme activity that shows maximal activity

Effect of pH on the rate of reaction

- Slight changes in pH causes marked changes in enzyme activity due to **alteration of the charges on the substrate and on the catalytic site of the enzyme** (Active site)
- Extreme changes of pH cause <u>denaturation</u> and <u>irreversible inhibition</u> of enzyme action
- Usually, enzymes have the optimum pH between 6 and 8
- Some important exceptions are:
  - Pepsin (with optimum pH 1-2)
  - Alkaline phosphatase (optimum pH 9-10)
  - Acid phosphatase (optimum pH 4-5)

Effect of Concentration of Products



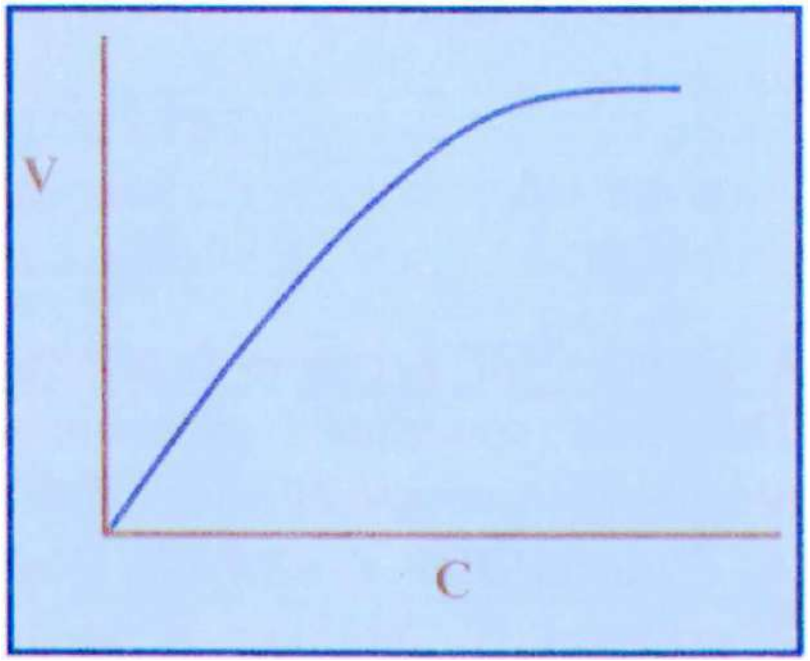
If E3 enzyme is absent, C will accumulate, which in turn, will inhibit E2. Consequently, in course of time, the whole pathway is blocked.

- In a reversible reaction (S ↔ P), when equilibrium is reached:
  - The reaction rate is slowed down

- When <u>product concentration is ↑</u>, the reaction is **slowed** or **stopped** → **feedback inhibition**

Effect of cofactor concentration

لها وجوده طردي مع سرعة التفاعل حتى نقطة معينة



- If the enzyme requires a cofactor (coenzyme or activator) for its activity:
  - The velocity of the **reaction will be directly proportional to the concentration of the cofactor**

حتى

- This is true till a certain point

- After this point, any increase in cofactor concentration will **not** increase the velocity of the reaction:
  - The **enzyme concentration is the limiting factor**

* يعني شوصلنا بالنقطة ؟ هنا لو زدت الـ (cofactor) وصار أكثر من الـ (E) اللي بتستقبله الـ بتزيد سرعة التفاعل ؟ أكيد لا مارح تزيد

Enzyme activation

- Some enzymes show higher activity in presence of inorganic ions
  - chloride ions activate salivary amylase
  - calcium activate lipases

- Another type of activation is the conversion of an inactive pro-enzyme or **zymogen** to the active enzyme
  - By splitting a single peptide bond & removal of a small polypeptide from trypsinogen → active trypsin is formed
  - This results in unmasking of active center

Zymogen = pro-enzyme = enzyme but it is inactive state.

ليش صار (inactive) ؟

لأنه صار الـ (E) وتحديد الـ (Active site) مغطى بال (Amino Acid) فهذا الـ (E) ما عاد قادر يرتبط بالـ (S) .

* كل اشي اخره (ogen) يكون (Zymogen)

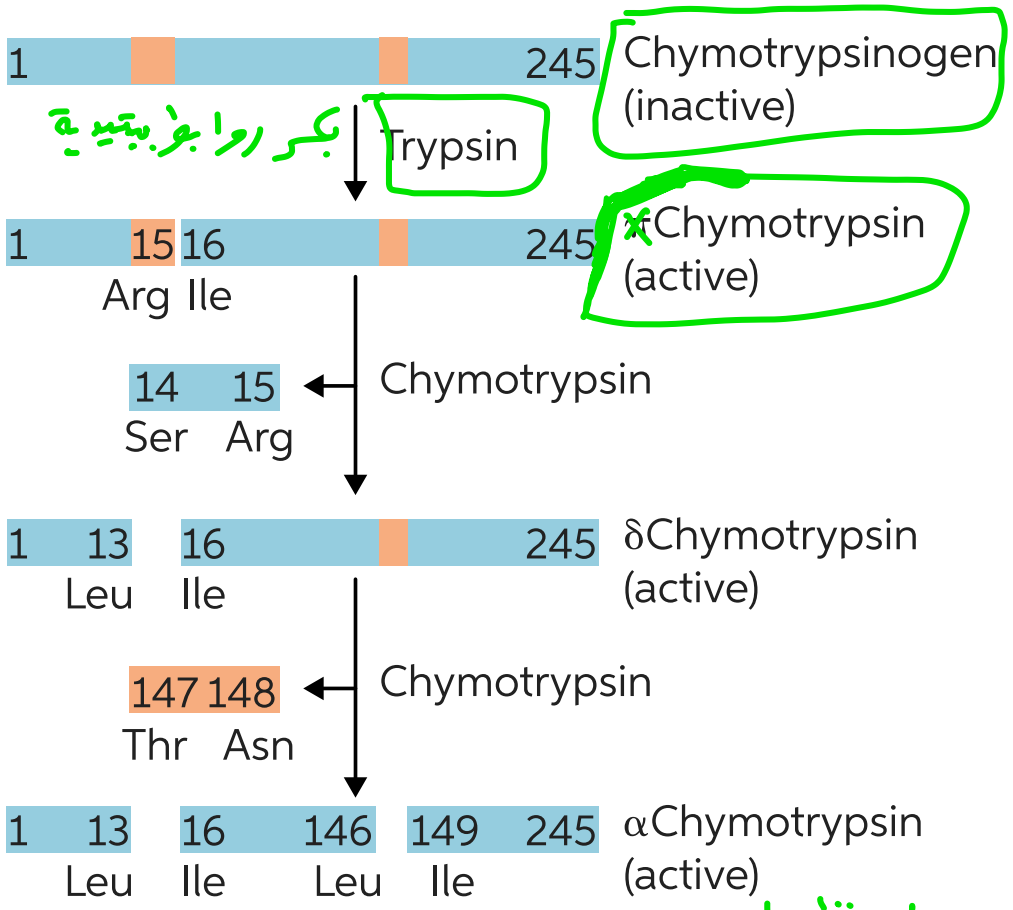
Enzyme activation

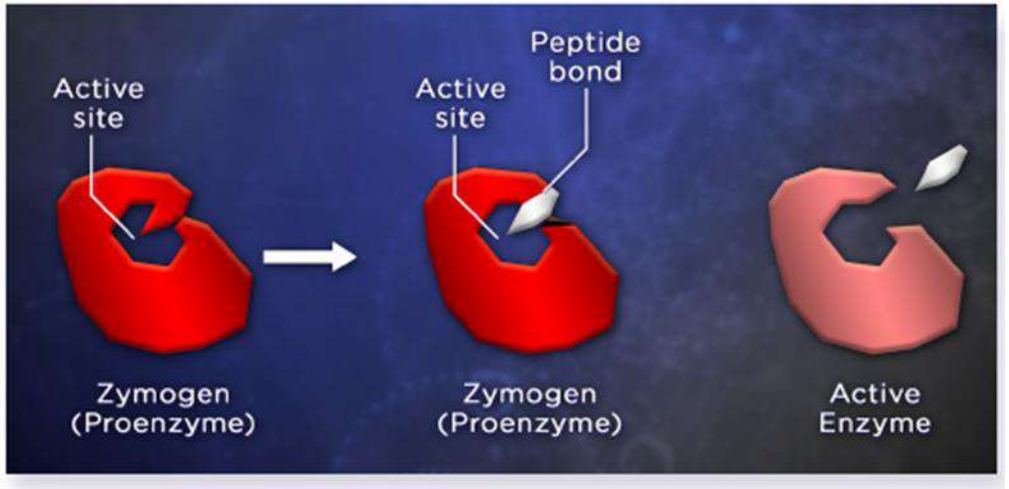
- Trypsin activates **chymotrypsinogen**, to form active chymotrypsin and two peptides (A and B peptides)

- All the gastrointestinal enzymes are synthesized in the form of pro-enzymes, and only after secretion into the alimentary canal, they are activated. This **prevents autolysis of cellular structural proteins.**

- **Coagulation factors** are seen in blood as zymogen form.

+ Activation of Chymotrypsin





Enzyme inhibition

Two main types:

1. Reversible
   - **Competitive inhibition**

2. Irreversible
   - **Non-competitive inhibition**
   - **un-competitive inhibition**

تذكر أنه ممكن يجي من
Extreme changes of pH

تنافسي

Competitive inhibition (reversible)

- A competitive inhibitor is **structurally similar to that of substrate**:

  It competes with substrate to bind reversibly at active or catalytic site

- The degree of inhibition depends on the **ratio** of the concentration of the inhibitor:substrate and not on the **absolute** concentration

نسبة التركيز إلى نسبة تركيز

مش لازم تكون نسبة التركيز نفس الشيء

- The inhibition also depends on the relative affinity of the substrate and the inhibitor to the enzyme

مين الـ (Affinity) عنده أعلى
و (Km) عند أقل هو اللي رح يرتبط

Competitive inhibition (reversible)

No Effect on V max:

- Effect of a competitive inhibitor is reversed by ↑ [S]
- At a sufficiently high [S] concentration, the reaction velocity reaches the Vmax observed in absence of inhibitor

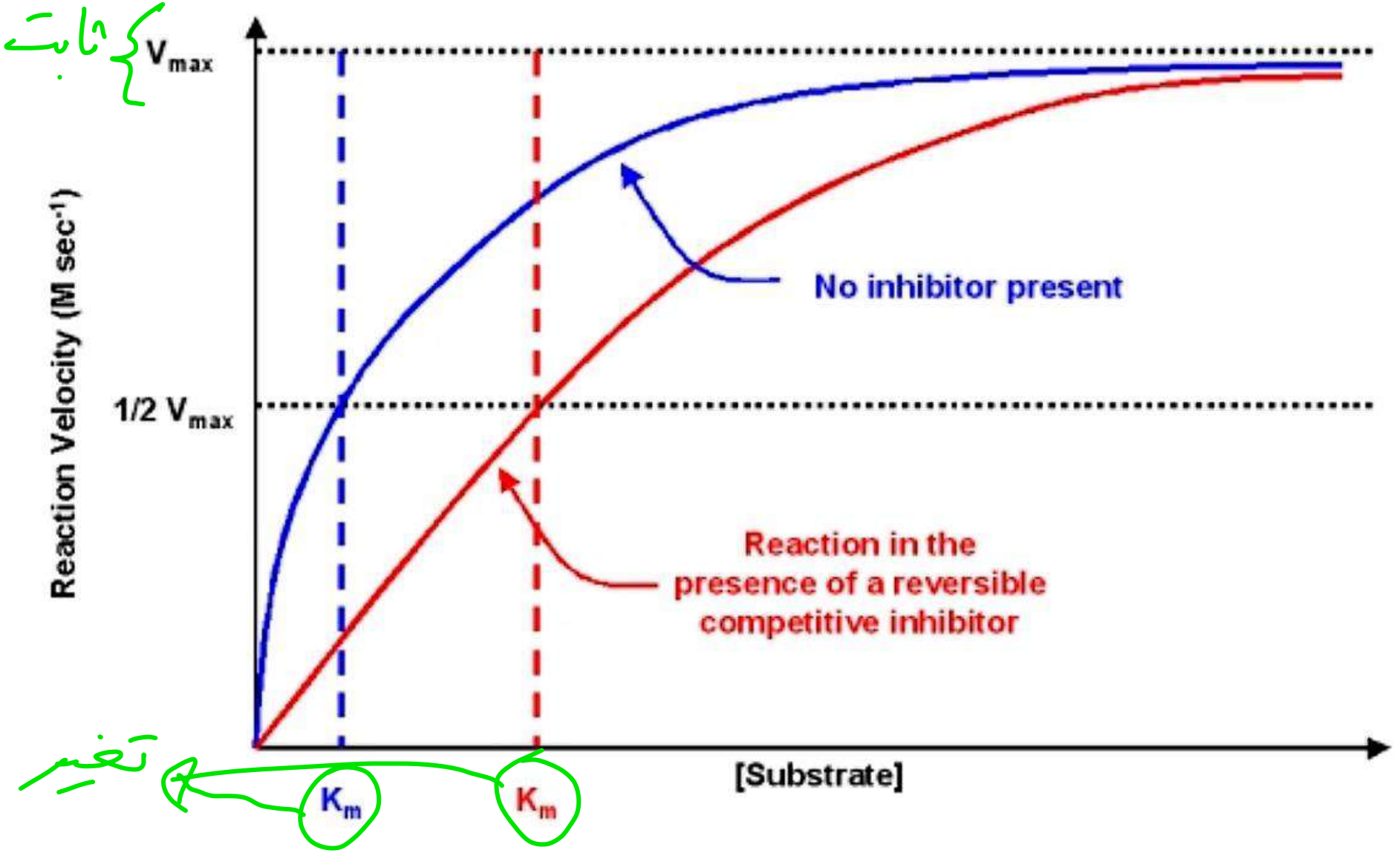
صحيح احنا دائما بنفترض أنه عند تركيز كافي من (S) بالتالي قد مازدنا (inhebitor) ولكن عندنا تركيز (S) كتير

Increase of Km:

بالتالي ز تعجبي كل المواقع و ما يتأثر ال (Vmax)

- A competitive inhibitor increases the apparent Km for a given [S]
- This means that, in the presence of a competitive inhibitor, more [S] is needed to achieve ½ Vmax

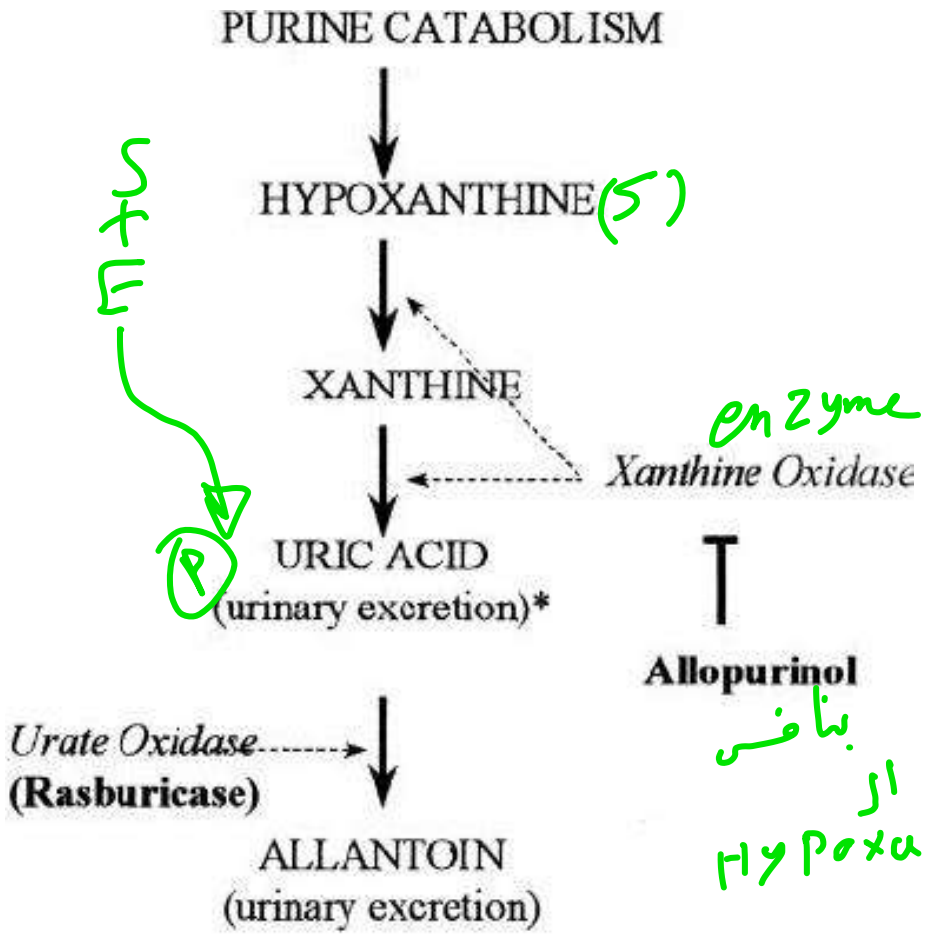
صحيح أنا اجتجت تركيز أعلى من (S) عشان نه أصل لنصف ال Vmax اللي هو Km بسبب وجود (inhhibitor) فقلت ال (affinity) وبالتالي زاد (Km)


V_{max}
1/2 V_{max}
Reaction Velocity (M sec^{-1})
No inhibitor present
Reaction in the
presence of a reversible
competitive inhibitor
[Substrate]
K_m
K_m

[illegible]ble 5.5. Clinically useful Competitive Inhibitors

Dru[illegible]	Enzyme inhibited	Clinical use	Ref c[illegible]ter
1. Allo[illegible]inol	xanthine oxidase	gout	39
2. Dicou[illegible]rol	vit.K-epoxide-reductase	anti-co[illegible]lant	33
3. Penicillin	transpeptidase	[illegible]cteria	2
4. Sulphonam[illegible]	pteroid syntheta[illegible]	bacteria	34
5. Trimethoprim	[illegible]H2-reductas[illegible]	bacteria	34
6. Pyrimethamine	[illegible]	malaria	34
7. Methotrexate	d[illegible]	cancer	51
8. 6-mercapto-purine	ade[illegible]succinate sy[illegible]se	cancer	51
9. 5-fluorouracil	[illegible]mid[illegible]te [illegible]nthase	cancer	51
10. Azaserine	phosphor[illegible]syl-amidotrans[illegible]se	cancer	51
11. Cytosine arabin[illegible]e	DNA polymer[illegible]	cancer	51
12. Acy[illegible]ir	DNAP of virus	antiviral	42
13. N[illegible]igmine	ACh-esterase	[illegible]yesthenia	23
14. [illegible]ha-[illegible]ethyl dopa	dopa-decarboxylase	[illegible]ertension	17
1[illegible] Lovastatin reductase	HMGCoA-lowering	cho[illegible]erol	12
16. Oseltamiver (Tamiflu)	Neuraminidase	Influenz[illegible]	

* هما احنا بنستفيد منه (competitive inhibitor
في ال (drugs) فاحنا
بنعمل (drug)
structurally similar to (S)
فبنعمل (inhibitor) معين
لـ (E) معينة لأني ما بدي
هاد ال (E) يشتغل



* A normal endpoint of purine metabolism in humans

* مثال كتير مهم
هو Allopurinol
على موضوع
Competitive inhibition

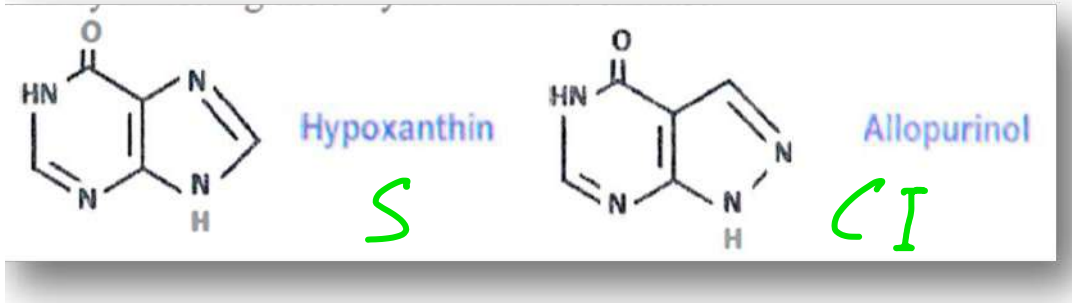
enzyme

S

P

I

بنافس ال Hypoxanthine



* مادام ما بنافس إذا بترتبط على موقع مختلف عنه الأنزيم

Non-competitive inhibitor (irreversible)

ما بنافس

- **No competition occurs between substrate and inhibitor** to bind at active site of enzyme

- Inhibitor is **not structurally related to substrate**
  - Inhibitor binds to a site **different than the active site** of enzyme

- The inhibitor can bind either the free enzyme (***non-competitive***) or the enzyme substrate (ES complex; ***un-competitive***)

قبل ما يرتبط بـ (S)

بعد ما يرتبط بـ (S)

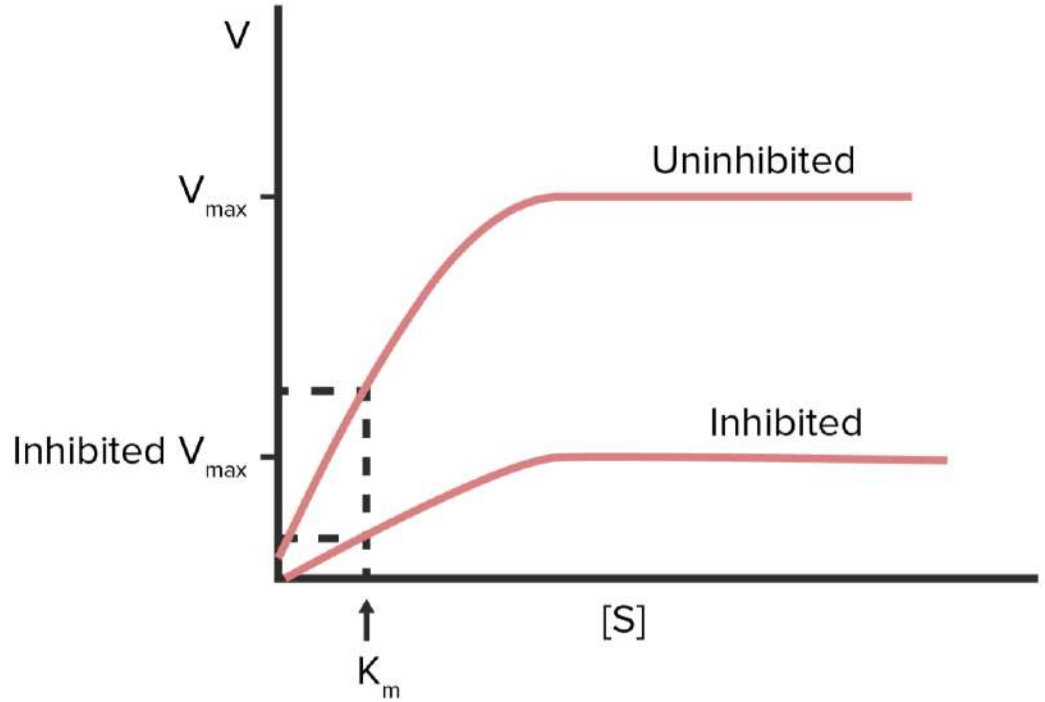
- Increase in the substrate concentration generally **does not relieve this inhibition.**

ما يخفف

Non-competitive inhibitor

- **Effect on Vmax:**
  - Non-competitive inhibition cannot be overcome by increasing the concentration of substrate
  - → non-competitive inhibitors decrease the Vmax

- **Effect on Km:**
  - **Non**-competitive inhibitors do not interfere with the binding of substrate to enzyme
  - the enzyme shows the same Km in the presence or absence of the non-competitive inhibitor
    - Remaining enzyme has same affinity for substrate



Michaelis-Menten
Non-competitive Inhibition

Examples of non-competitive inhibitors

- **Cyanide** and **carbon monoxide** inhibits cytochrome oxidase

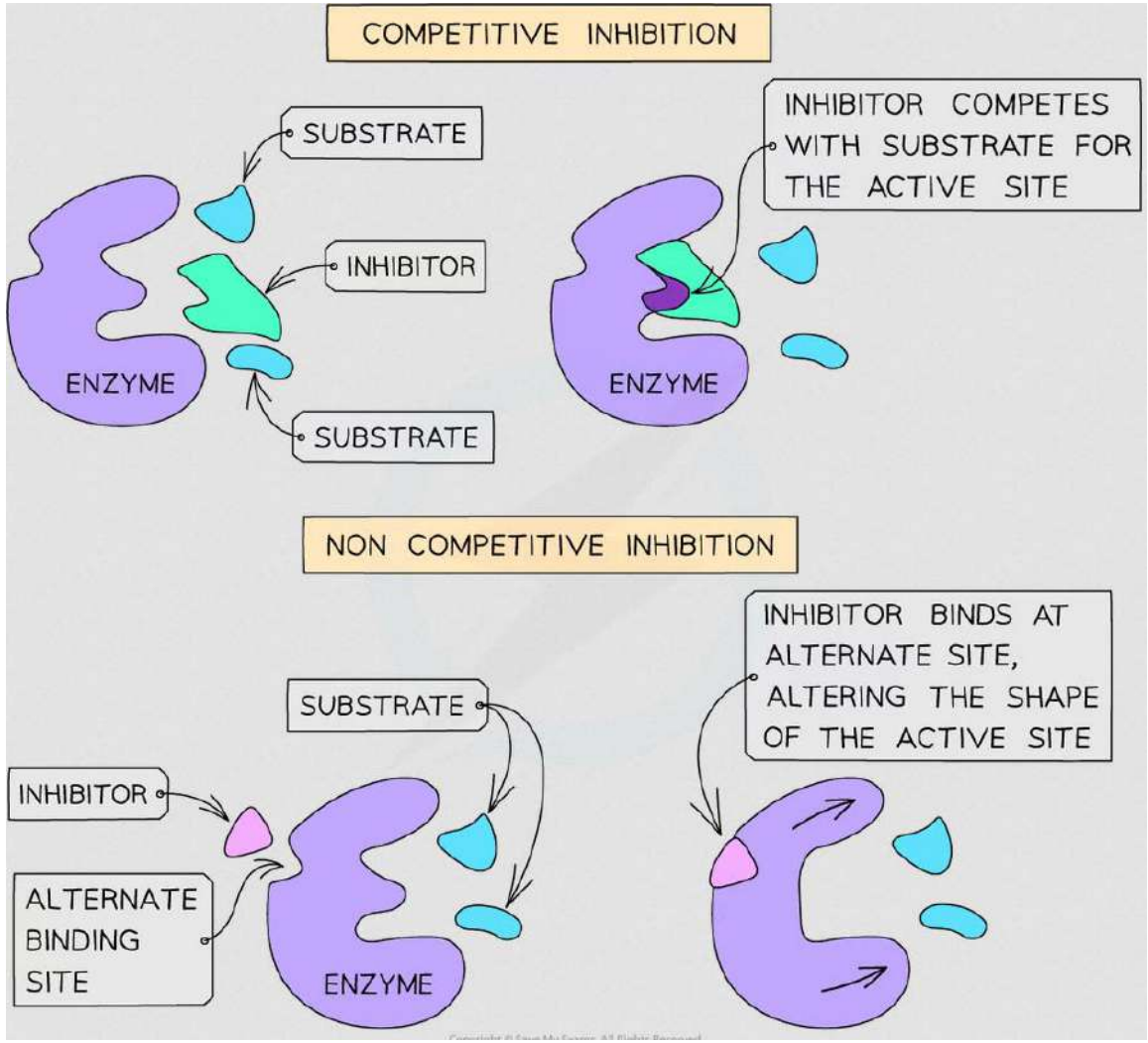
F
- **Fluoride** will remove magnesium and manganese ions and so will inhibit the enzyme, enolase, and consequently the glycolysis

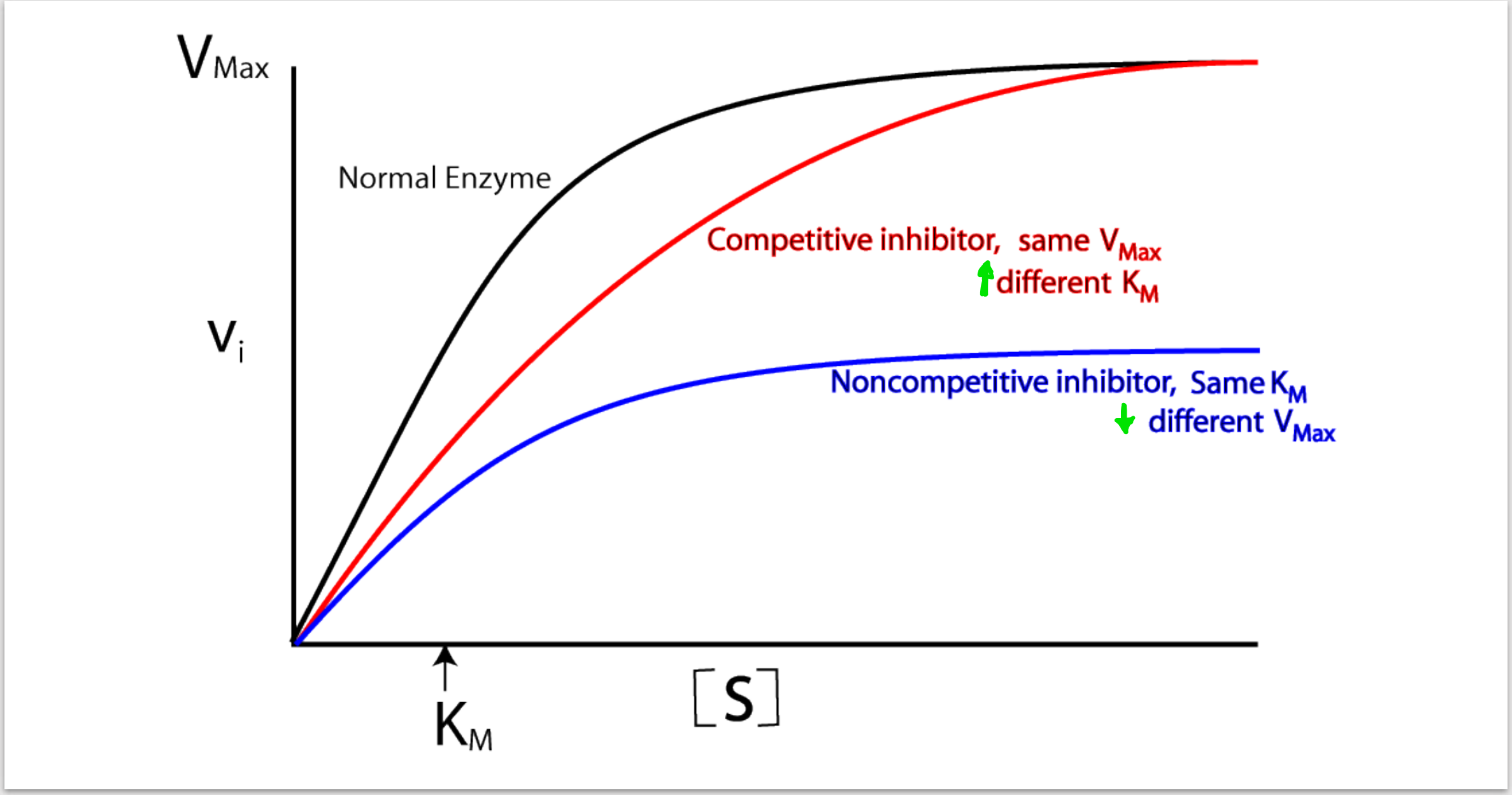
I
- **Iodoacetate** would inhibit enzymes having-SH group in their active centers

B
- **BAL** (British Anti Lewisite; dimercaprol) is used as an antidote for heavy metal poisoning
  - The heavy metals act as enzyme poisons by reacting with the SH group
  - BAL has several SH groups with which the heavy metal ions can react and thereby their poisonous effects are reduced.

Table 5.6. Comparison of two types of inhibition

	Competitive inhibition	Non-competitive inhibition
Acting on	Active site	May or may not
Structure of inhibitor	Substrate analog	Unrelated molecule
Inhibition is	Reversible	Generally irreversible
Excess substrate	Inhibition relieved	No effect
Km	Increased	No change
Vmax	No change	Decreased
Significance	Drug action	Toxicological


COMPETITIVE INHIBITION
SUBSTRATE
INHIBITOR
ENZYME
SUBSTRATE
INHIBITOR COMPETES WITH SUBSTRATE FOR THE ACTIVE SITE
ENZYME
NON COMPETITIVE INHIBITION
SUBSTRATE
INHIBITOR
ALTERNATE BINDING SITE
ENZYME
INHIBITOR BINDS AT ALTERNATE SITE, ALTERING THE SHAPE OF THE ACTIVE SITE


V_{Max}
Normal Enzyme
Competitive inhibitor, same V_{Max}
different K_M
V_i
Noncompetitive inhibitor, Same K_M
different V_{Max}
K_M
[S]

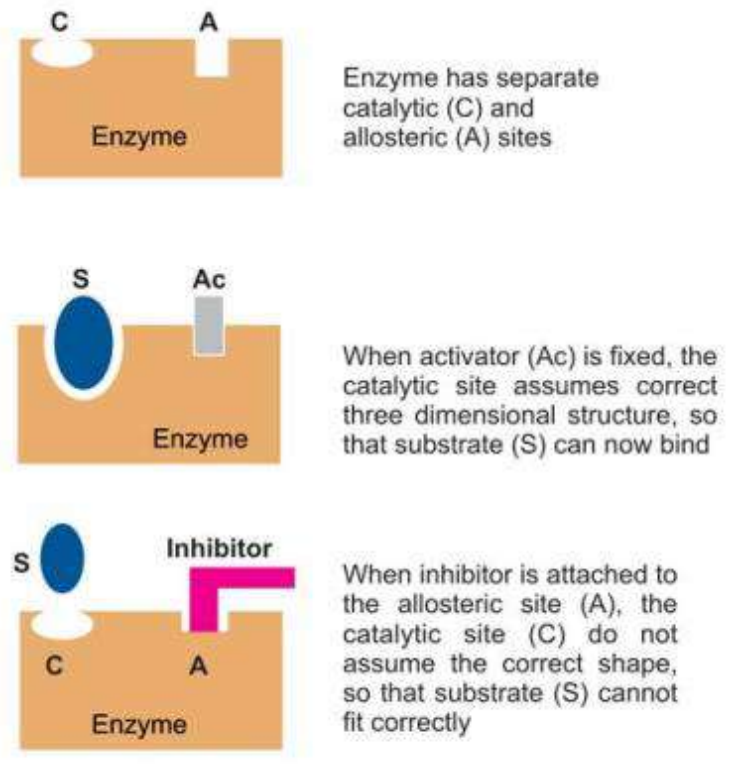
أنه أنا في عندي (E) وهاد الـ (E) يحتوي على موقع إضافي مختلف عنه الـ (Active site) بنسميها هاد الموقع (Allosteric site) هاد الموقع بكون مناسب إلو رتباط إما (Activator/inhibitor) شو بعملو بس يرتبطوا؟ بعملوا (conformational changes) وهاد بغيرمنه تاعت الـ (E) (catalytic properties)

Allosteric regulation

- Allosteric enzymes are enzymes that have an **additional** binding site for effector molecules other than the active site.
- The binding brings about conformational changes, thereby changing its catalytic properties. The effector molecule can be an inhibitor or activator.
- An allosteric site is a region of an enzyme that allows activator or inhibitor molecules to bind to the enzyme and either activate or inhibit enzyme activity.

Allosteric regulation - Control of enzyme activity

- Allosteric enzyme has one catalytic site where the substrate binds and another **separate allosteric site** where the modifier binds (*allo* = other)



Allosteric regulation - Control of enzyme activity

- Allosteric and substrate binding sites may or may not be physically adjacent

- The binding of the regulatory molecule can either:
  - Enhance the activity of the enzyme (allosteric activation) → **positive modifier**, or
  - Inhibit the activity of the enzyme (allosteric inhibition) → **negative modifier**

Allosteric regulation - Control of enzyme activity

- The inhibitor/ activator is **not** a substrate analog
- When an inhibitor binds to the allosteric site, the configuration of catalytic site is modified such that substrate cannot bind properly
- **Km** is usually increased & **Vmax** is reduced when inhibitor binds
- It is partially reversible, when excess substrate is added
- The effect of allosteric modifier is maximum at or near substrate concentration equivalent to Km

Examples of allosteric enzymes

Enzyme	allosteric inhibitor	allosteric activator
1. ALA synthase	heme	
2. Aspartate transcarbamoylase	CTP	ATP
3. HMGCoA-reductase	Cholesterol	
4. Phospho-fructo kinase	ATP, citrate	AMP, F-2,6-P
5. Pyruvate carboxylase	ATP	AcetylCoA
6. Acetyl CoA carboxylase	Acyl CoA	Citrate
7. Citrate synthase	ATP	
8. Carbamoyl phosphate synthetase I	NAG	
Carbamoyl phosphate synthetase II	UTP	

عنه نفسي كمية محذوف
أنتو حددوا
ما الى دخل بها دلالة
بس هو أول عنه آخر مثال
الأصل محذوف وعند نفسي مش دارسه

إضافة أو إزالة مجموعات (groups) عن طريق covalent bonds

Covalent modification - Control of enzyme activity

- The activity of enzymes may be **increased** or **decreased** by covalent modification
- Either addition of a group to the enzyme protein by a covalent bond; or removal of a group by cleaving a covalent bond
- **Zymogen activation** by partial proteolysis is an example of covalent activation
  - Addition or removal of a particular group brings about covalent modification of enzyme protein. This is a reversible reaction.
- Commonest type of covalent modification is the reversible **protein phosphorylation** and **ADP ribosylation**.

إضافة للفوسفات على البروتين عن طريق Kinase

إضافة ADP ribose البروتين عكسي

Examples of covalent modification

Enzyme	Phosphorylated enzyme
Acetyl-CoA carboxylase	Inactive
Glycogen synthase	Inactive
Pyruvate dehydrogenase	Inactive
HMG-CoA reductase	Inactive
Pyruvate kinase	Inactive
PFK2	Inactive
Glycogen phosphorylase	Active
Citrate lyase	Active
Phosphorylase b kinase	Active
HMG-CoA reductase kinase	Active
Fructose-2,6-bisphosphatase	Active

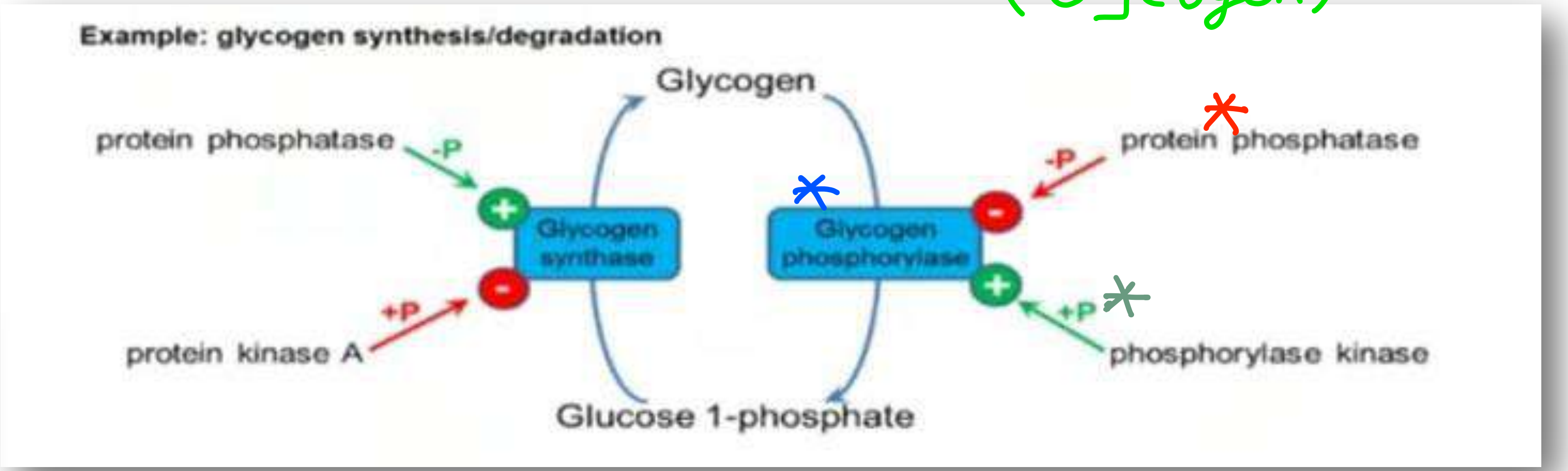
فقط امثلة

عند نفسي كميه محذوف
أنتو حددوا
ما الى دخل بهاد اللاية
بس هو أول عند آخر مثال
الأصل محذوف وعند نفسي مش دارسه

* الفوسفا = قروب يخلي الجلوكوز (inactive) اللي ضافها (protein kinase A)

وعشان نرجعه (Active) لازم أشيلها والقروب عن طريق (protein phosphatase)

هاد الكلام لما أصنع (Glycogen)

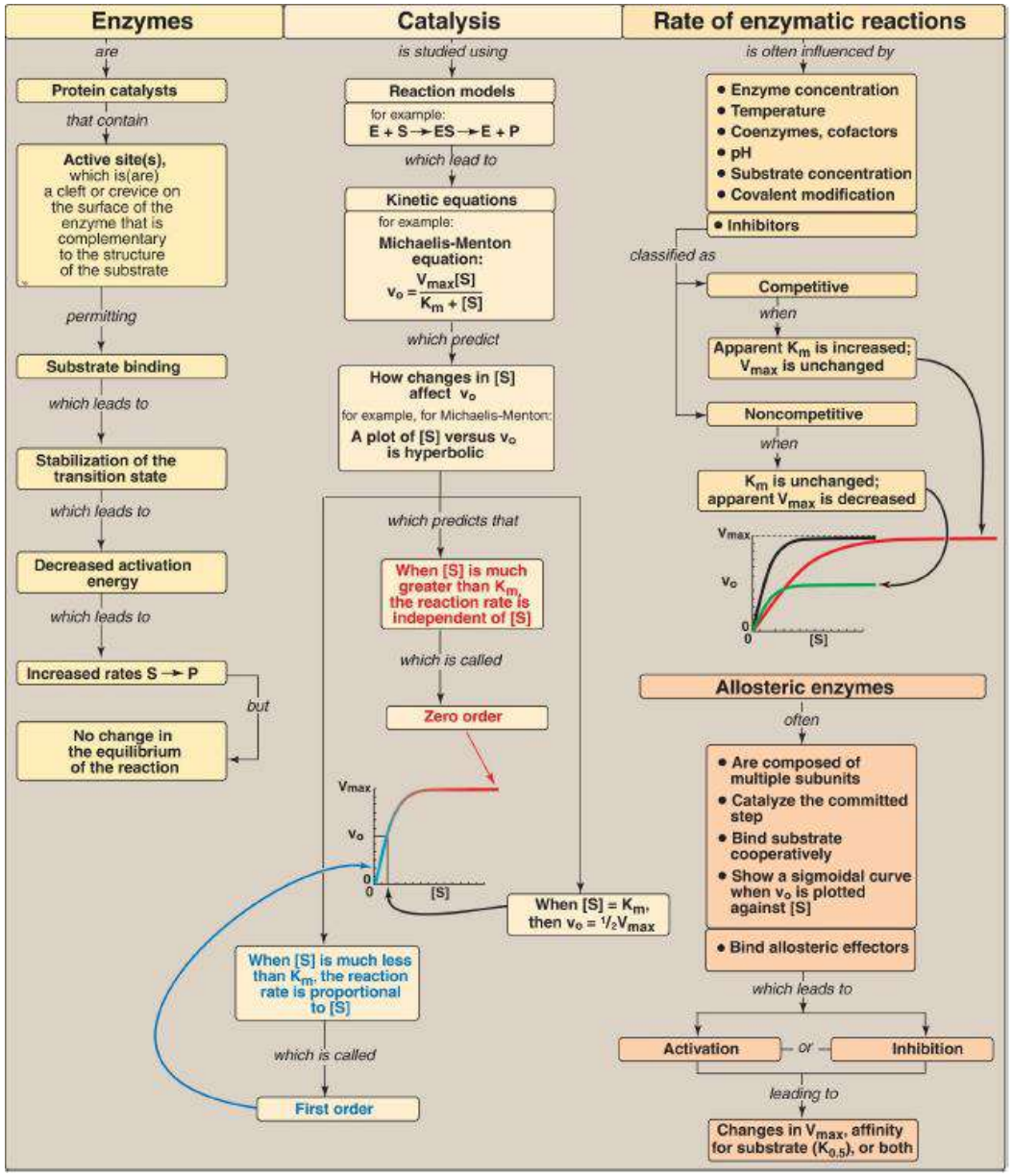


Insulin (well fed state): works via phosphatase → activates synthase and inactivates phosphoylase

Glucagon (fasting): works via kinase → activates phosphoylase and inactivates synthase

* طيب أثناء الصيام بدي أحول الجلايكوجين إلى جلوكوز عن طريق (*) فبخليه (inactive)

لما أشيل الفوسفات عن طريق (*) وبصير (Active) لما أضيف فوسفات عن طريق (*)


Enzymes
are
Protein catalysts
that contain
Active site(s), which is(are) 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
Catalysis
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 $v_o = \frac{1}{2}V_{max}$
When [S] is much less than K_m, the reaction rate is proportional to [S]
which is called
First order
Rate of enzymatic reactions
is often influenced by
• Enzyme concentration
• Temperature
• Coenzymes, cofactors
• pH
• Substrate concentration
• Covalent modification
• Inhibitors
classified as
Competitive
when
Apparent K_m is increased; V_{max} is unchanged
Noncompetitive
when
K_m is unchanged; apparent V_{max} is decreased
Vmax
Vo
0
0
[S]
Allosteric enzymes
often
• Are composed of multiple subunits
• Catalyze the committed step
• Bind substrate cooperatively
• Show a sigmoidal curve when v_o is plotted against [S]
• Bind allosteric effectors
which leads to
Activation
or
Inhibition
leading to
Changes in V_{max}, affinity for substrate ($K_{0.5}$), or both