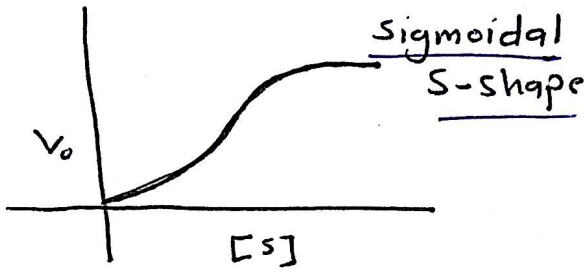
Chapter 4

Proteins / Enzymes

Allosteric (تتأثر بعوامل خارجية)

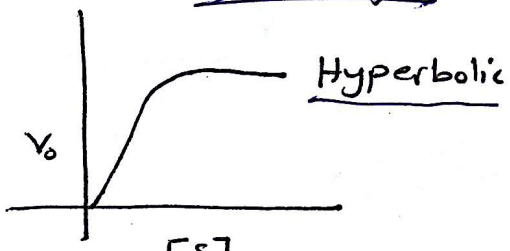
→ Ex: Hemoglobin
ATcase



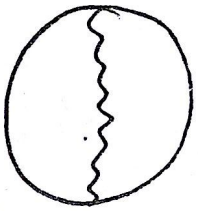
→ all of them are Quaternary proteins
→ Cooperative kinetic
* allosteric

Non-allosteric (لا تتأثر بعوامل خارجية)

→ Explained by M-M-equation
→ Ex: Myoglobin
Chemotrypsin



→ Not Quaternary
→ No cooperativity
→ Simple Kinetic



allosteric effectors: (بعض الروابط)
Substrate (S)
Inhibitor (I)
Activator (A)

ترتبط by non-covalent Bonds

الارتباط → يؤدي Changes the Quaternary Structure and the affinity (نشاط الانزيم)

* Cooperativity (حسنة مميزة لهذا النوع)

2 types

↑ affinity → ∴ Positive Cooperativity

↓ affinity → ∴ Negative Cooperativity

ارتباط اول مادة مع الانزيم يؤثر على affinity للارتباط الثاني لنفس المادة

* M-M equation is not (لا تطبق) applicable to allosteric Enzymes

$K_{0.5}$ instead of Km

Allosteric Effectors

المواد المؤثرة على هذه الانزيمات

→ Substances bind to the protein by Non-covalent bonds and change the (4°) Quaternary structure.

→ this change the affinity and behavior of the protein

(Activator, Inhibitor, Substrate)

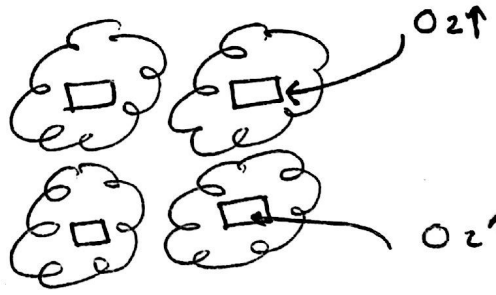
Enzymes التأثير على ينقسم إلى نوعين:

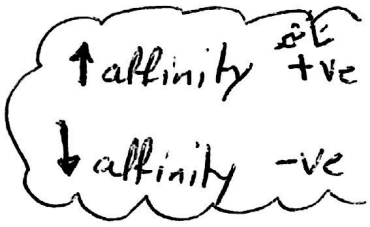
2 types

a. Homotropic : Identical molecules

اذا كانت المواد التي تؤثر على بعض متطابقة

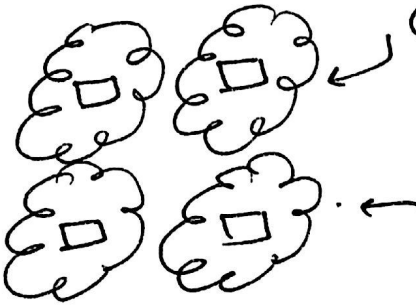
Exi- تأثير ارتباط اول O_2 على الثانية (O_2)





b. Heterotropic : Different molecules

اذا كانت المواد التي تؤثر على بعض مختلفة

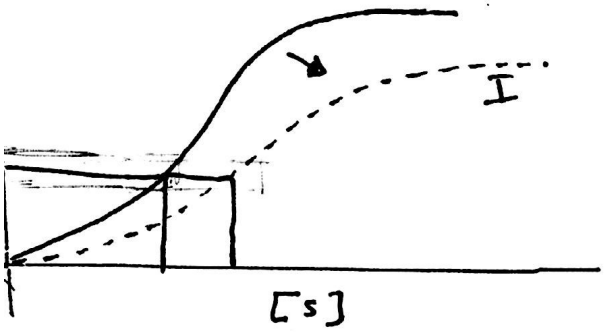


تأثير ارتباط الـ CO_2 على ارتباط O_2

* allosteric control لتنسيق في الانزيمات التحكم بهذه الارتباط (Non-covalent)

steric Inhibitors

* shift the curve to right more sigmoidal
* move substrate needed to reach any Velocity



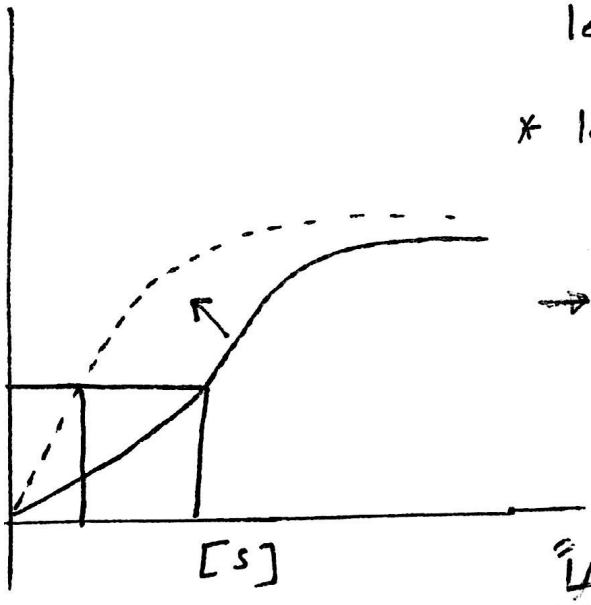
→ shift to higher Substrate Concentration

* ↑ cooperativity

هذه الصفة تكون أكثر وضوحاً بوجود Inhibitor

Allosteric Activator

* shift the curve to left less sigmoidal, more Hyperbolic
* less substrate needed to reach any velocity



→ shift to lower Substrate Concentration

* ↓ cooperativity

هذه الصفة تكون أقل وضوحاً بوجود Activator

Feed-back Inhibiton (End-product inhibition)

allosteric Enzymes — إنزيمات أخرى محيزة لـ

- any pathway : series of reaction — سلسلة

A $\xrightarrow{E_1}$ B $\xrightarrow{E_2}$ C $\xrightarrow{E_3}$ D $\xrightarrow{E_4}$ End product

-ve inhibit

if ↑ "excess" زيادة

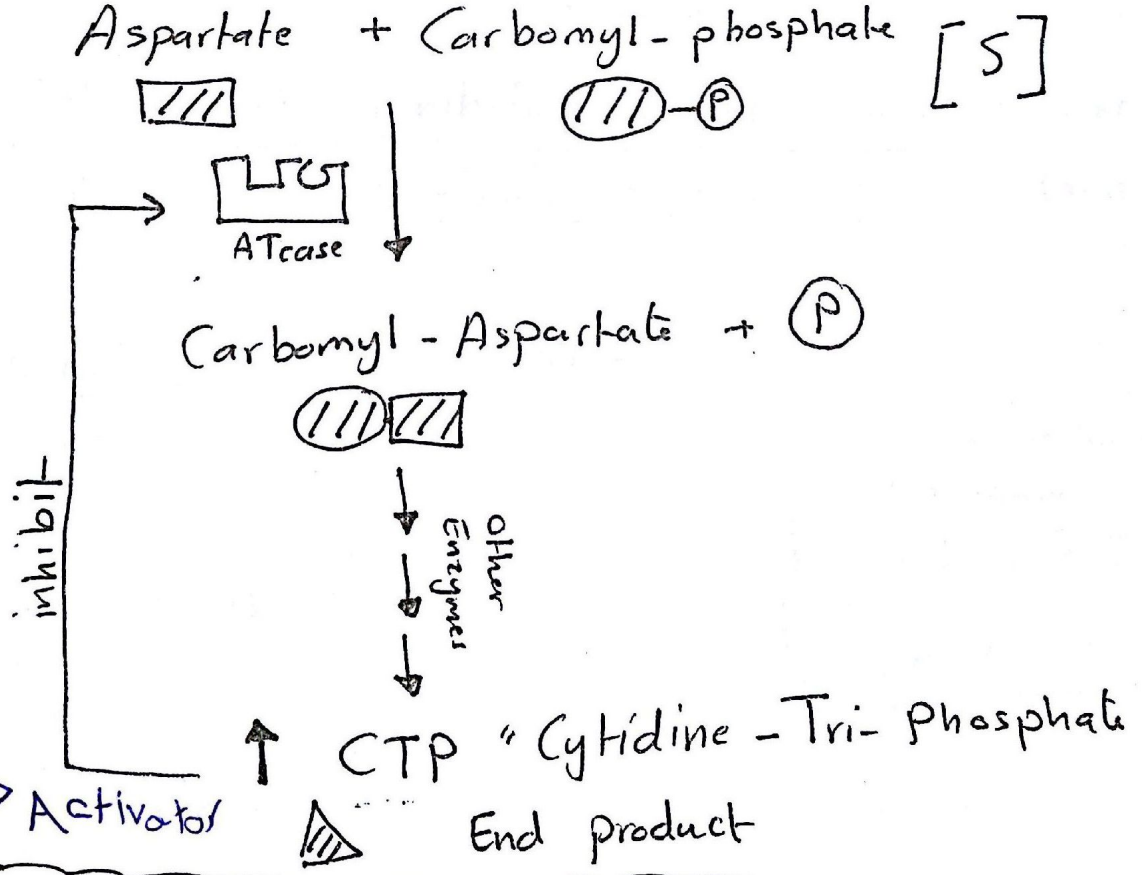
Then

→ the End-product inhibit the Enzyme Catalyzing the First Step — يثبط إنزيم الخطوة الأولى

→ Very efficient because it stops all the Series — فعّال — يوقف — السلسلة كل

→ So No accumulation of intermediates — تراكم — المركبات الوسطية

Ex : Aspartate-Trans Carbomylase (ATCase) "allosteric"



* CTP is an Inhibitor to ATcase

Case Enzyme

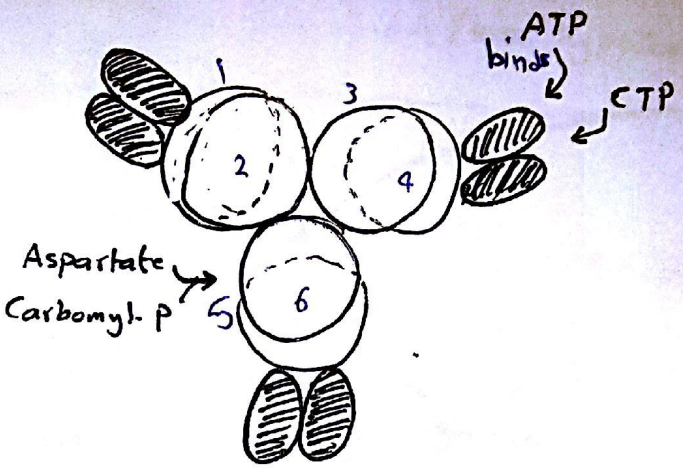
Consist of 2 parts

1 Catalytic Subunit

↓

2 trimers (6)

→ Bind to the substrates (Aspartate and Carbomyl-P) and do the reaction



2 Regulatory Subunit

3 dimmers (6)

→ Bind to regulatory Factors (Inhibitor and activator)

Can be Separated by p-hydroxymercuribenzoate

↓

loss control

↓

Become hyperbolic

→ CTP is an inhibitor → shift to right (more sig)

→ ATP is an activator → shift to left (less sig)

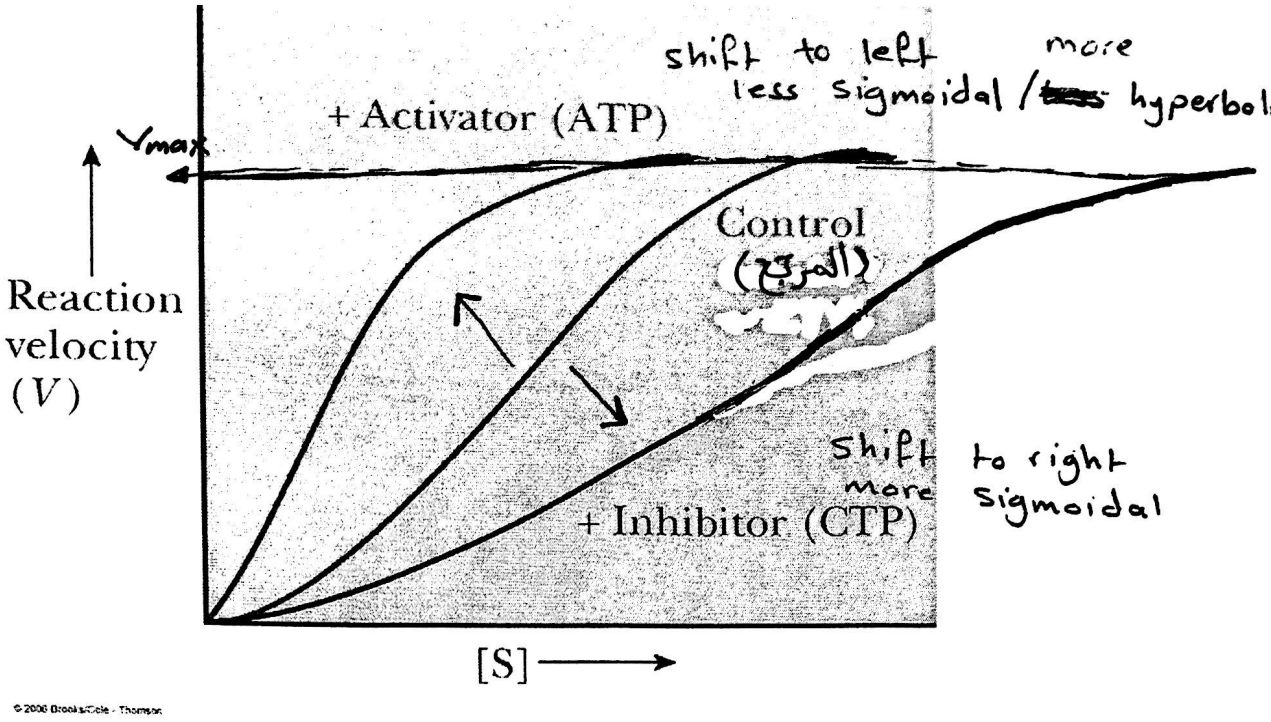
$\frac{CTP}{ATP}$ ratio is important

$\uparrow\frac{CTP}{ATP}$ increase ?

$\downarrow\frac{ATP}{CTP}$ decrease ?

* allosteric site

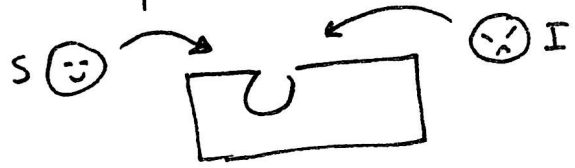
activator & inhibitor



what is the type of CTP inhibitor ??

Remember
2 types of inhibetors:

* Compitiitive inhibitors



يشبه شكل الـ S

Same Vmax
↑ Km

for Non-allosteric Enzymes.

* Non-Compitiitive inhibitor

تختلف شكل الـ S

↓ Vmax
Km نفسه

CTP → has different structure from aspartate and C.P and bind to other site

NOT Competitive

Because these are for non allosteric enzymes.

→ Vmax نفسه
CTP يوجد

NOT non-Compitiitive

stead of Competitive and non-competitive
بدلاً من

Allosteric proteins have 2 system

a. K-System (as in allosteric / as in non allosteric Competitive)

inhibitor ↑ $K_{0.5}$
activator ↓ $K_{0.5}$

- Same Vmax
- Change $K_{0.5}$

Ex:- ATcase

b. V-System (as non-Competitive)

- Same $K_{0.5}$
- Change Vmax

inhibitor ↓ Vmax
activator ↑ Vmax

Q: what happens when K-acting inhibitor is added to an allosteric enzyme system?

a. the appearant $K_{0.5}$ increase
b. the appearant $K_{0.5}$ decrease
c. the appearant Vmax increase
d. the appearant Vmax decrease.

Enzyme Kinetics falls into two general Catagories, Simple Saturation and cooperative kinetics

True False

Q: The Term $K_{0.5}$ is analogous to Km

True False

Q: In Comparison of allosteric and non-allosteric Enzymes

a. it's always possible to define Km

b. it's always possible to define Vmax

c. Competitive inhibition is always a possibility

d. much of the termenology is completely unchanged

Q: In reactions Catalyzed by allosteric Enzymes:-

a. Substrate, activator, and inhibitors all compete for the same binding site on the enzyme

b. there is no distinction between catalytic and regulatory subuni

c. activator make the plot of reaction rate against [S] less ~~cooperative~~

d. inhibitor make the plot of reaction rate against [S] less cooperative

: where do allosteric inhibitors and activators bind ?

a. They always bind at a site different from the active site

b. They always bind to the active site

c. They can bind either active site or another site.

Q: How do each of these compound affect function of ATCase ?

a. ATP is a K-effector and CTP is a V-effector

b. ATP V-effector and CTP K-effector

c. Both ATP and CTP are K-effectors

d. Both ATP and CTP are V-effectors.

Q: The final product Q, will most likely inhibit reaction 1

Q: if this pathway is freely reversible, which two enzymes would be the most likely to be regulated

a. 1 and 2

b. 2 and 3

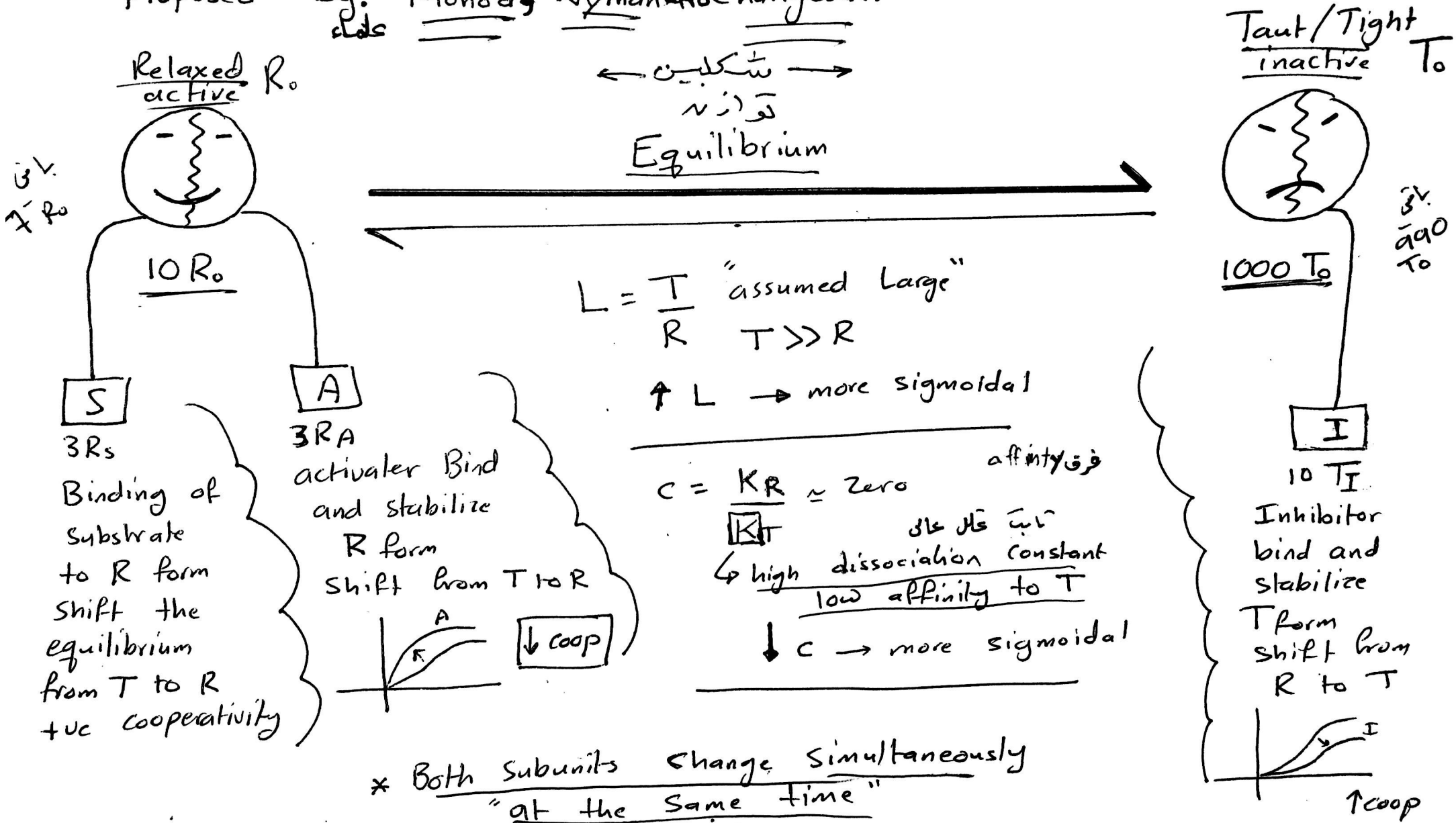
c. 3 and 4

d. 1 and 4

2 models to explain the behavior of allosteric Enzymes

[1] Concerated Model / Simple, don't Explain -ve cooperativity

Proposed by: Monod, Wyman and Changeux.



$L = \frac{T}{R}$ "assumed Large" $T \gg R$

↑ L → more sigmoidal

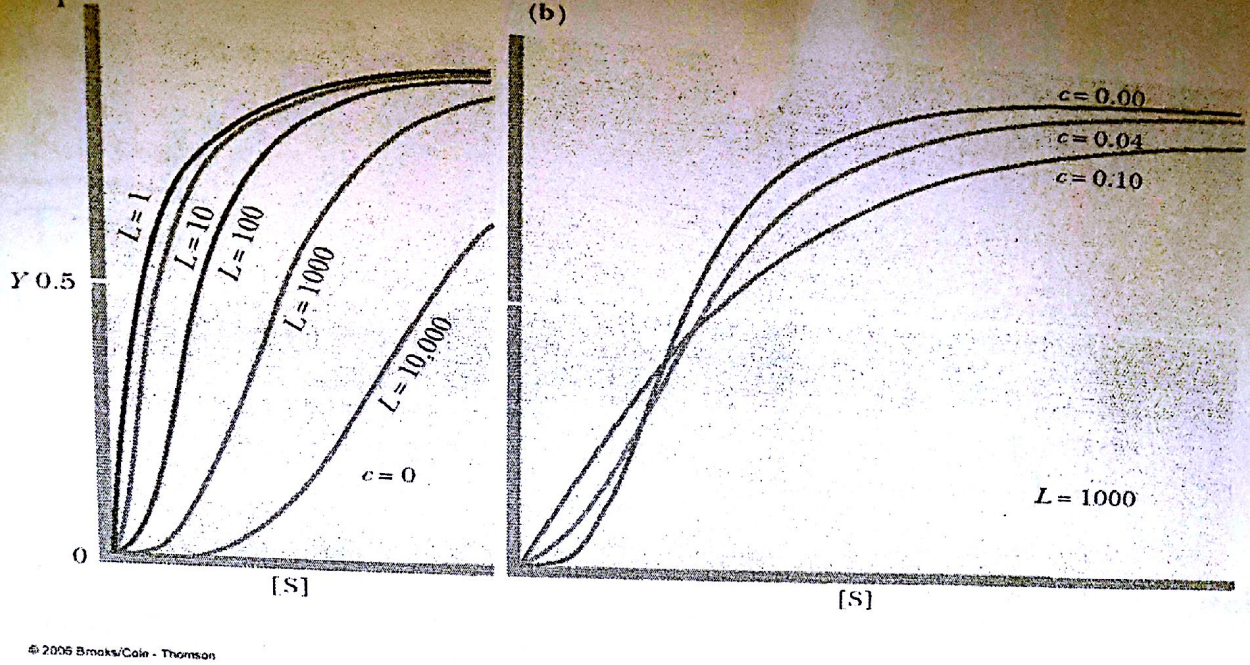
$C = \frac{K_R}{K_T} \simeq$ Zero

فرق affinty

high dissociation constant → low affinity to T

↓ C → more sigmoidal

* Both Subunits change Simultaneously "at the Same time"



The Monod–Wyman–Changeux (or concerted) model.
(a) As L (the ratio of the T/R form) increases, the shape becomes more sigmoidal.

→ high difference between the affinity of R and T to S

Small $c = \frac{K_R}{K_T} \rightarrow \frac{1}{10000}$ → very high affinity between S and R
→ very low affinity between S and T

↑ $c = \frac{1}{2}$ → low difference between the affinity of R and T to S

The effect of activator on L

$L = \frac{T\downarrow}{R\uparrow}$ ⇒ L decreas.

The effect of Inhibitor on L

$L = \frac{T\uparrow}{R\downarrow}$ ⇒ L increase

· In the Concerated model, which state binds the Substrate more tightly?

a. R-state

b. T-State

c. Both states binds equally well

Q: According to the concerated model of allosteric behavior an allosteric activator :-

a. Favors the taut form of the enzyme

b. Favors the relaxed form of the enzyme

c. Can Only bind to the enzyme if the S is already bound

d. Can Only bind to the enzyme if the S has not already bound

Q: -In the concerated model for allosteric enzymes :-

(c) affinity وزوجية

a. the relative affinities of Substrate for T and R conformations plays an important role in the cooperativity of the reaction

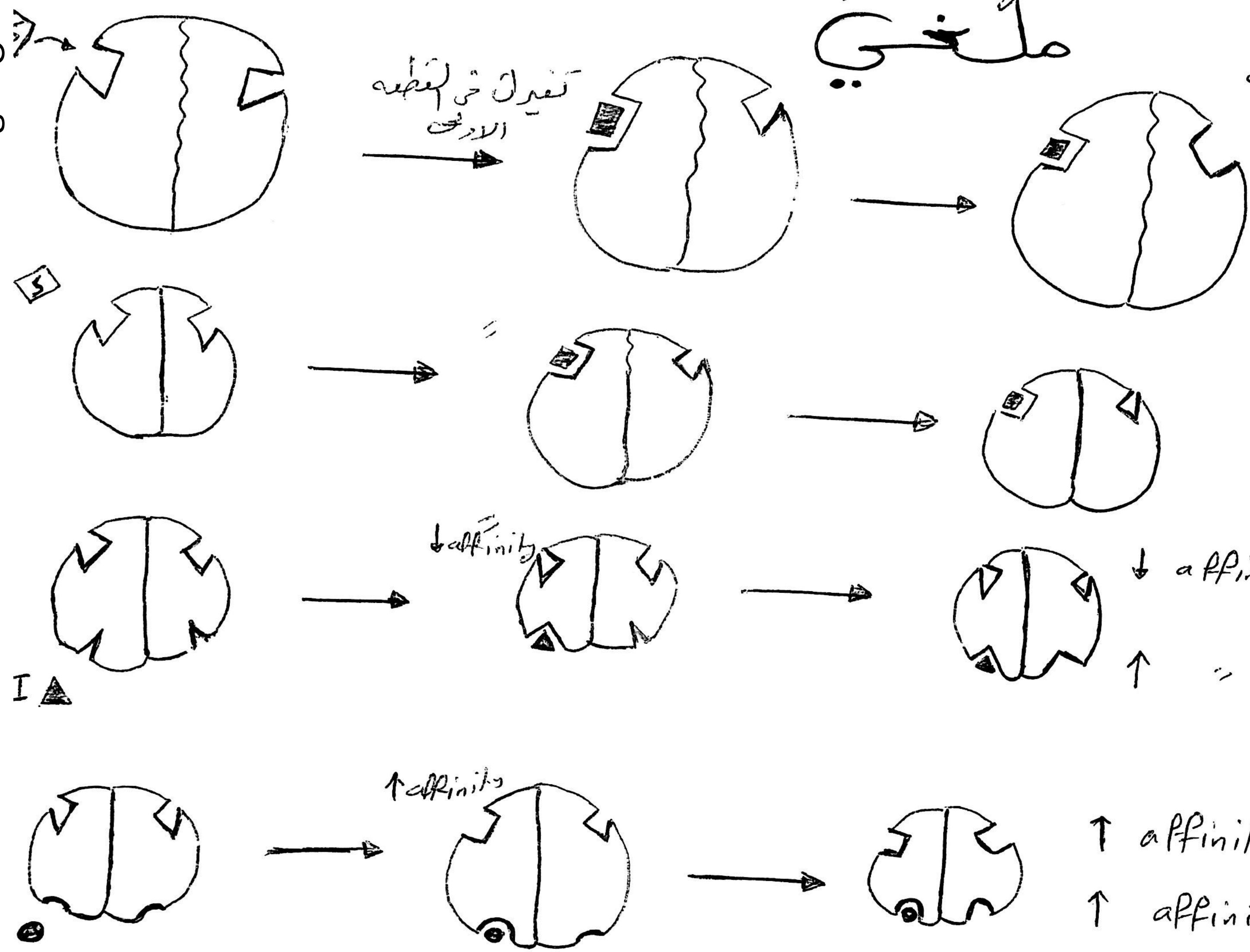
b. the equilibrium between T and R forms plays a minor role

c. the enzymatic activity of the T-form is considerably higher than R-form

d. it's possible to describe the reactions of all allosteric enzymes accurately.

Sequential Model :- - Complex
- Explain -ve cooperativity / proposed by Daniel Kosh.

ملغي



تغيرات في القطعة الثانية
↑ affinity

+ve Cooperati

↓ affinity

-ve cooperativity

↓ affinity of binding S
↑ " of biniding other I

↑ affinity of binding S
↑ affinity of binding other A

The change in the 2 subunits is sequential تسلسلي

Example of Enzyme showing -ve cooperativity "Tyrosyl-tRNA Synthatase"

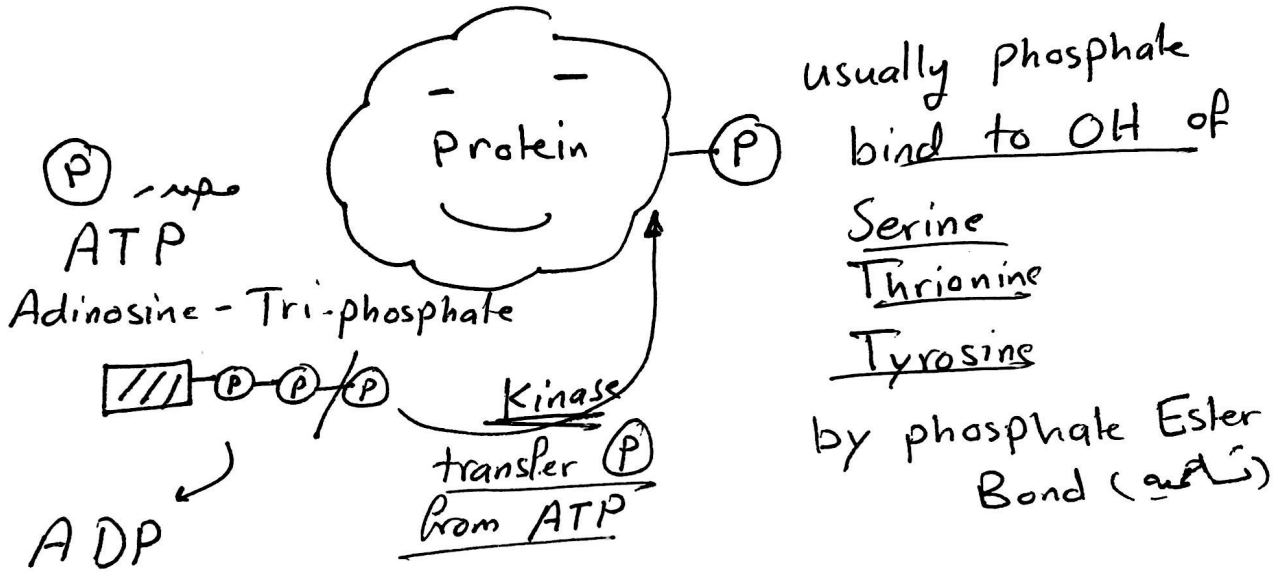
مهم في صناعة البروتينات

Phosphorylation of proteins اضافة فوسفات

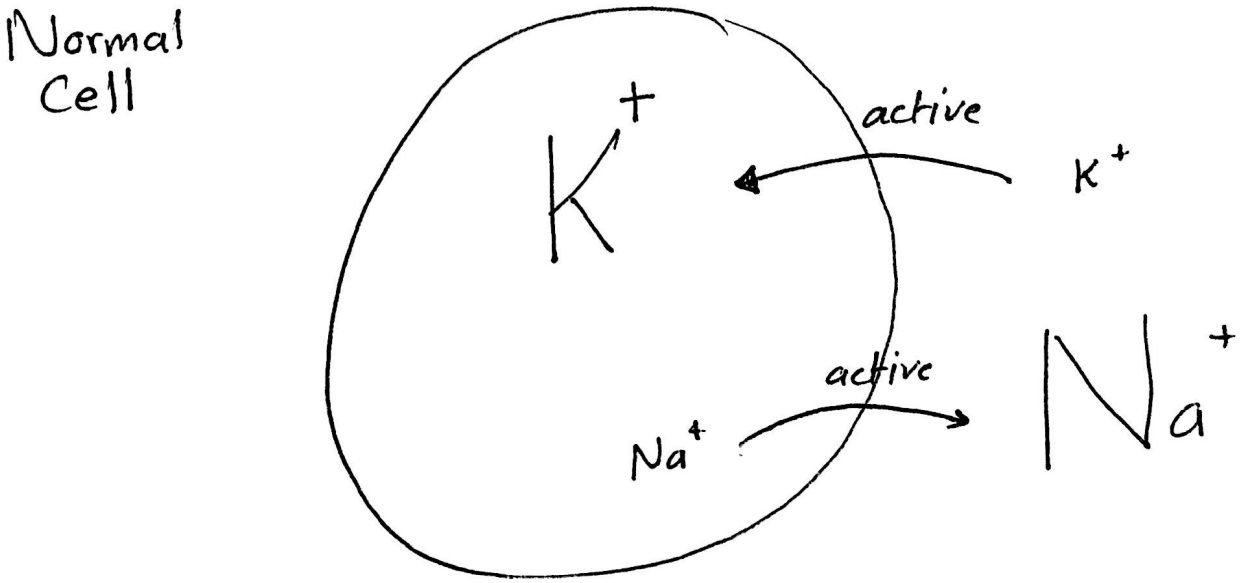
→ binding of phosphate group to the protein by Covalent Bond (because phosphate bound via covalent bond)

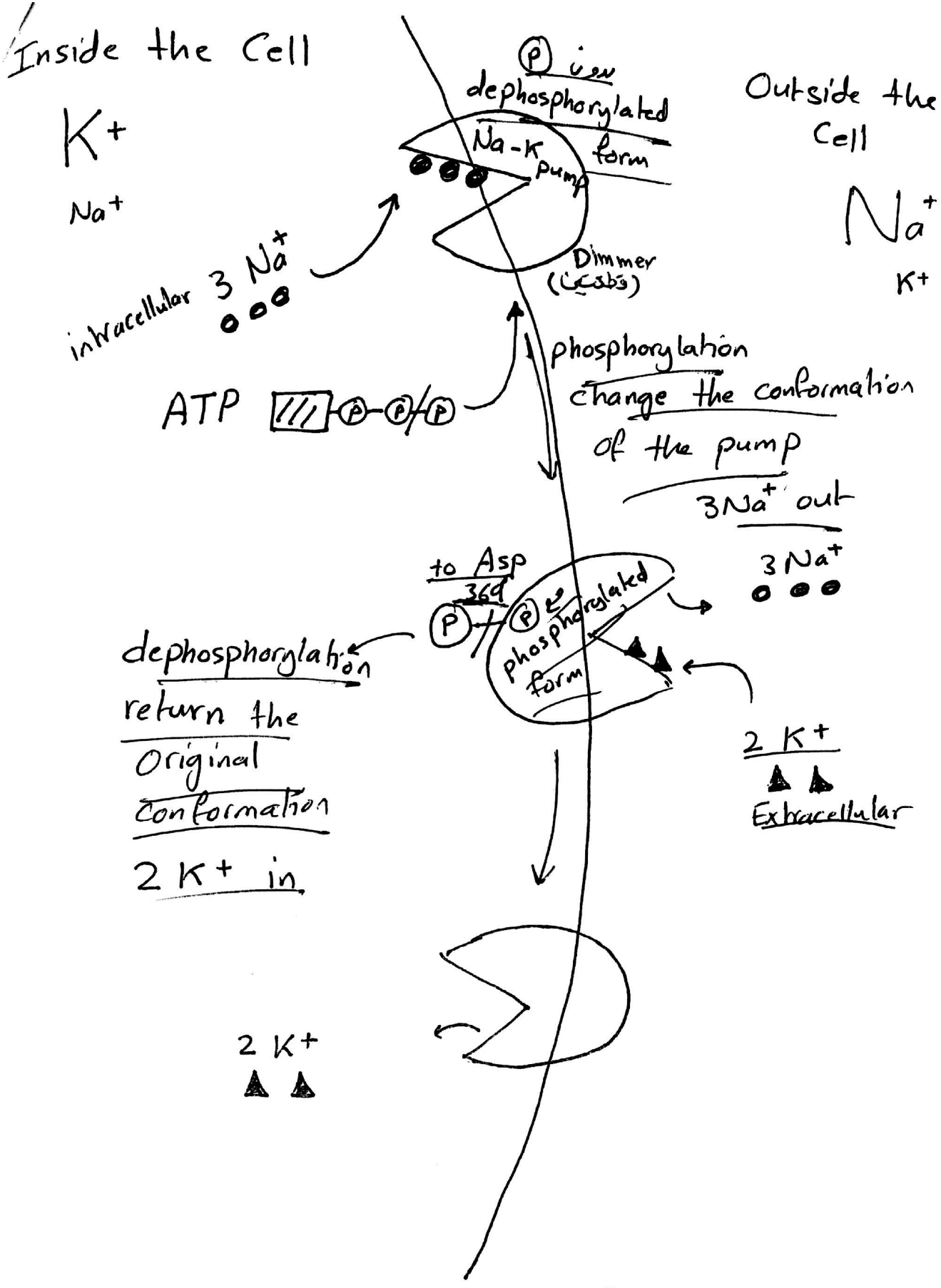
→ Covalent Control, NOT allosteric

→ Phosphorylation < activation / inactivation — تفعيل او تثبيط



Ex1: Sodium - Potassium Pump (Sodium - Potassium ATPase)
$Na^+ - K^+$ pump



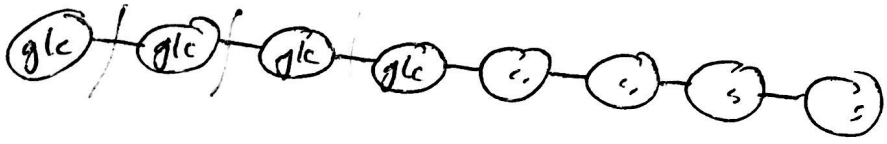


Glycogen phosphorylase.

2 subunits.

→ First step in Glycogen degradation

Glycogen



↓ Glucose Energy. → Glucose

in liver

X Muscles.

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dephosphorylated GP (b) — Covalent Control — phosphorylated GP (a) (more active)

phosphorylase Kinase (P) (P)

phosphoprotein phosphatase

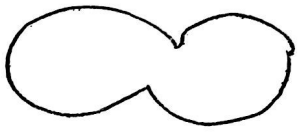
Ib

AMP

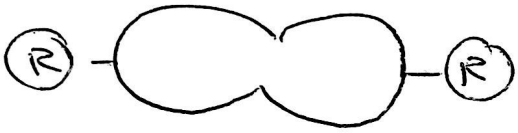
Glucose
G-6-P
ATP
Caffien.

Non-Covalent Control

Glucose
Caffien.



Rb



Ra

⇒ a form is more active

Phosphorylated.

which of the following is true?

a. phosphorylation always increase enzyme activity
b. Kinases often use AMP as a co-substrate in their phosphorylation reaction
c. Some enzymes are activated by phosphorylation, while others are inhibited
d. ADP is the most common substrate for kinase reaction

Q:- ATP is a negative allosteric effector for glycogen phosphorylase, This is an example of :-

a. feed-back inhibition
b. positive cooperativity
c. negative cooperativity
d. competitive inhibition.

Best Wishes
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