22

BIOCHEMISTRY

Faculty of medicine – JU2018

◉ Sheet ○ Slides

DONE BY

محمد & ياسمين

CONTRIBUTED IN THE SCIENTIFIC CORRECTION

محمد السيد & ياسمين خالد

CONTRIBUTED IN THE GRAMMATICAL CORRECTION

محمد السيد & ياسمين خالد

DOCTOR

Mamoun Ahram

Allosteric enzyme

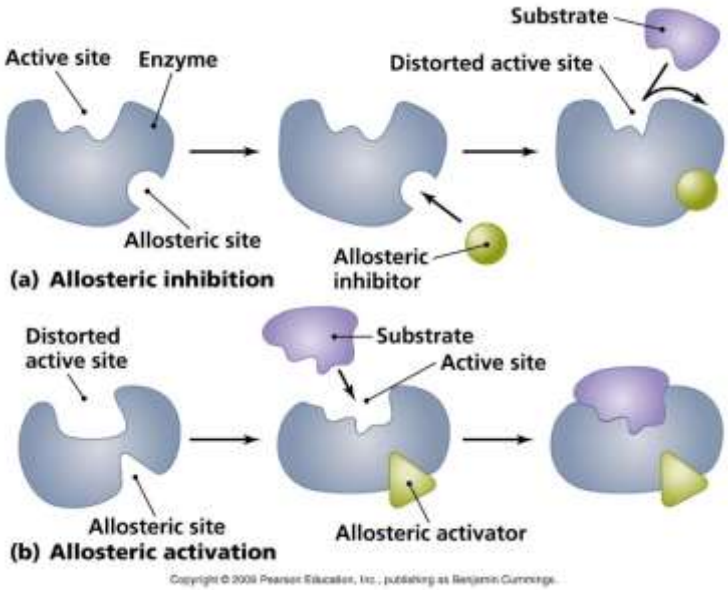
***Allosteric enzyme :** is a multi-subunit protein (more than one polypeptide/quaternary structure) , some subunits which contain active site are named catalytic subunits , and other which contain regulatory site are named regulatory subunits .

*All allosteric enzymes can change their structure by binding with modifier which is either a substrate(called homotropic) or a regulatory molecule (called heterotropic)
How they change their structure ? by triggering a conformational change via modifying non-covalent interaction.

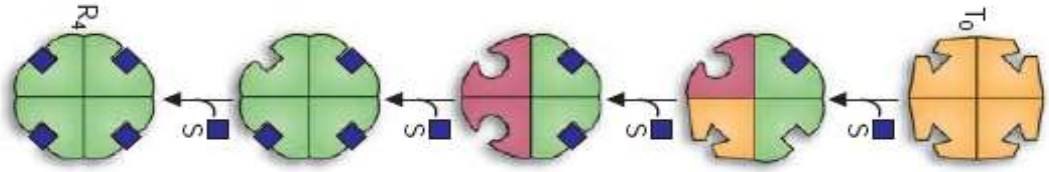
*The regulatory molecule (allosteric modifier) can be:

A) A negative allosteric modifier (inhibitor) causes the enzyme to have less activity , to have less catalytic efficiency , less affinity to substrate , R to T conformation. (negative cooperativity)

B) A positive allosteric modifier (activator) causes the enzyme to be more active , to have more catalytic efficiency , more affinity to substrate , T to R conformatio. (positive cooperativity)



Ex: The binding of the substrate at one active site causes the enzyme to become more active and binds to a second substrate at a different active site with more ease .



* Note : regulatory molecule = Allosteric modifier

regulatory site = Allosteric site

* Note : (regulatory site "Allosteric site" is physically separated from active site "Catalytic site" , each of them present in separate subunit)

Types of allosteric enzymes :

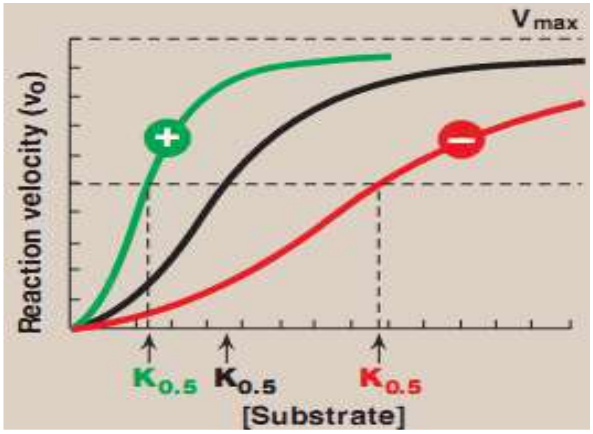
The Michaelis-Menten model can not explain the kinetic properties of allosteric enzymes , So we describe allosteric enzyme using tow pattern :

1- K system : the same Vmax and different $K_{0.5}$

A particular enzyme is said to be follower to the K system , when the maximum velocity (Vmax) is not affected while the $K_{0.5}$ is affected , if we use modifier to activate or inhibit the original enzyme.

in other word (upon using allosteric regulation , the velocity of reaction when all enzymes are saturated will not be affected , but the needed concentration of substrates to fill half of the enzymes is affected "$K_{0.5}$ will be larger in case of inhibition , and smaller in case of activation") .

- $K_{0.5}$ is used instead of Km "used for allosteric enzymes" .

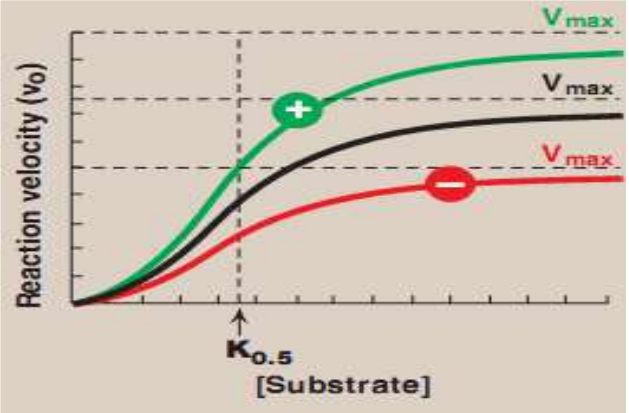


Note a sigmoidal plot (S shape) of allosteric enzyme without modifier. and near-hyperbolic plot with activators .

2- V system : the same $K_{0.5}$ and different Vmax

A particular enzyme is said to be follower to the V system , when the maximum velocity (Vmax) is affected while the $K_{0.5}$ is not affected , if we use modifier to activate or inhibit the original enzyme .

in other word (upon using allosteric regulation , the needed concentration of substrates to fill half of the enzymes is not affected , but the velocity of reaction when all enzymes are saturated will be affected "V max will be larger in case of activation , and smaller in case of inhibition") .



Note a sigmoidal plot (S shape) of allosteric enzyme without modifier and near-hyperbolic plot with activators

Note: hemoglobin is an example of allosteric protein .

- Allosteric inhibitors usually have a much stronger effect on enzyme velocity than competitive and noncompetitive inhibitors.

- Allosteric enzymes are not limited to regulation through inhibition and allosteric effectors may function as activators.

- The allosteric effector needs not bear any resemblance to substrate or product of the enzyme.

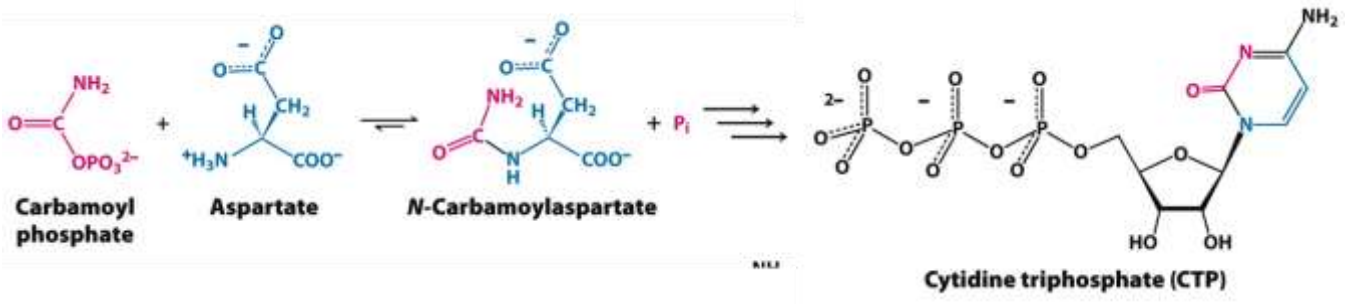
- The effect of an allosteric effector is rapid , occurring as soon as its concentration changes in the cell.

* Feedback regulation of metabolic pathways by end products or by signal molecules that coordinate multiple pathways.

*So let us study an example of allosteric enzyme which is aspartate transcarbamylase (ATCase):

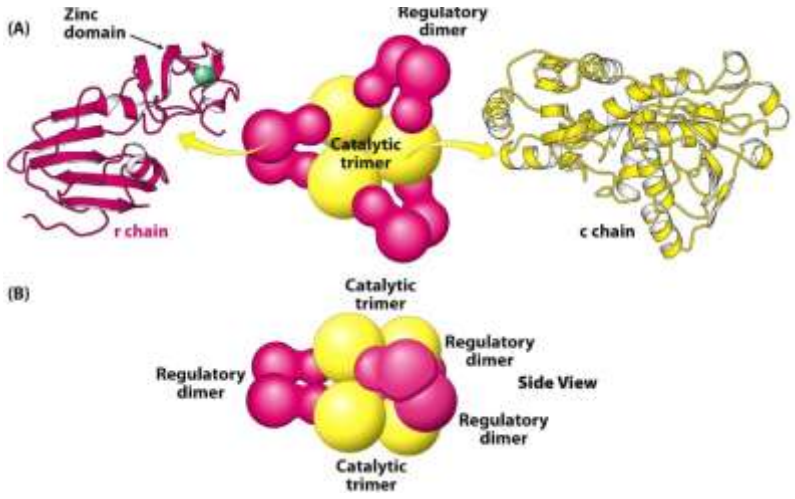
ATCase catalyzes the first step ”the rate limiting step “ in the synthesis of pyrimidine nucleotides (cytidine triphosphate (CTP)). These enzyme follow the k system .

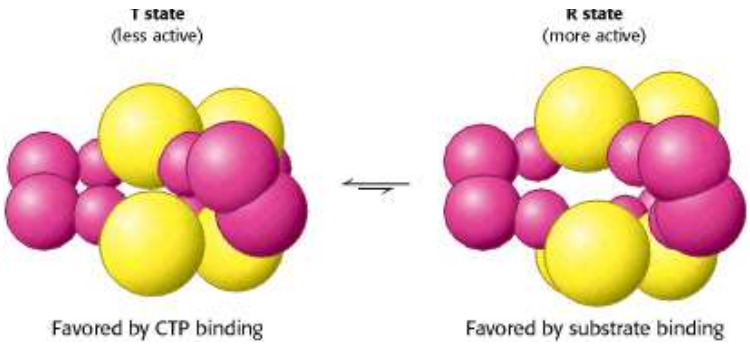
when the cell needs CTP , they stimulate this enzyme .



ATCase consists of 12 polypeptide chains: six catalytic subunits (two 4rimmers) and six regulatory subunits (three dimers) .

It exists in two forms: T state (less active) and R state (more active).





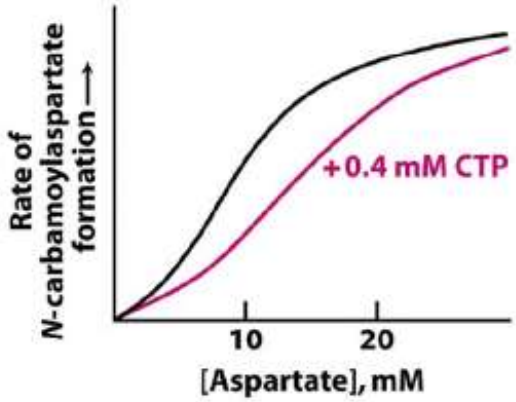
The allosteric regulation of ACTase:

1) The CTP (Pyrimidine) "the end product" acts as a negative modifier for the ACTase enzyme .

when there is a high con. of CTP inside the cell then the cell does not need more of it ,so these molecule will start binding at the regulatory subunits of ACTase enzyme , so inducing a major rearrangement of subunits positions which stabilizing the T state of enzyme , increase $K_{0.5}$ of enzyme and decrease the binding affinity for Asp (substrate) at active sites on catalytic subunits , so decrease the rate of producing CTP .

Note : a non-competitive inhibitor changes $K_{0.5}$

Note : heterotypically regulation (because the molecule used for regulation is different than substrate)

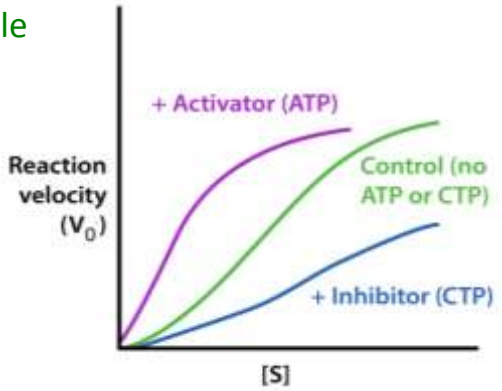


2) The ATP (purine) acts as a positive modifier for the ACTase enzyme .

when there is a high con. of ATP inside the cell , then the cell need to balance this high amount of purine by increasing rate of producing CTP , so the ATP molecule will start binding at the regulatory subunits of ACTase enzyme , so inducing a major rearrangement of subunits positions which stabilize the R state of enzyme , decrease $K_{0.5}$ of enzyme and increase the binding affinity for substrate at active sites on catalytic subunits , so increase the rate of producing CTP which is pyrimidine .

Note : heterotypically regulation (because the molecule used for regulation is different than substrate)

Note : Since this enzyme follow the K system , the Vmax will not be affected by regulation.



The enzymatic regulation via a modulators :

We will study the enzymatic regulation via a modulator by taking the phosphorylation process as an famous example.

So firstly : what is the phosphorylation cascade????

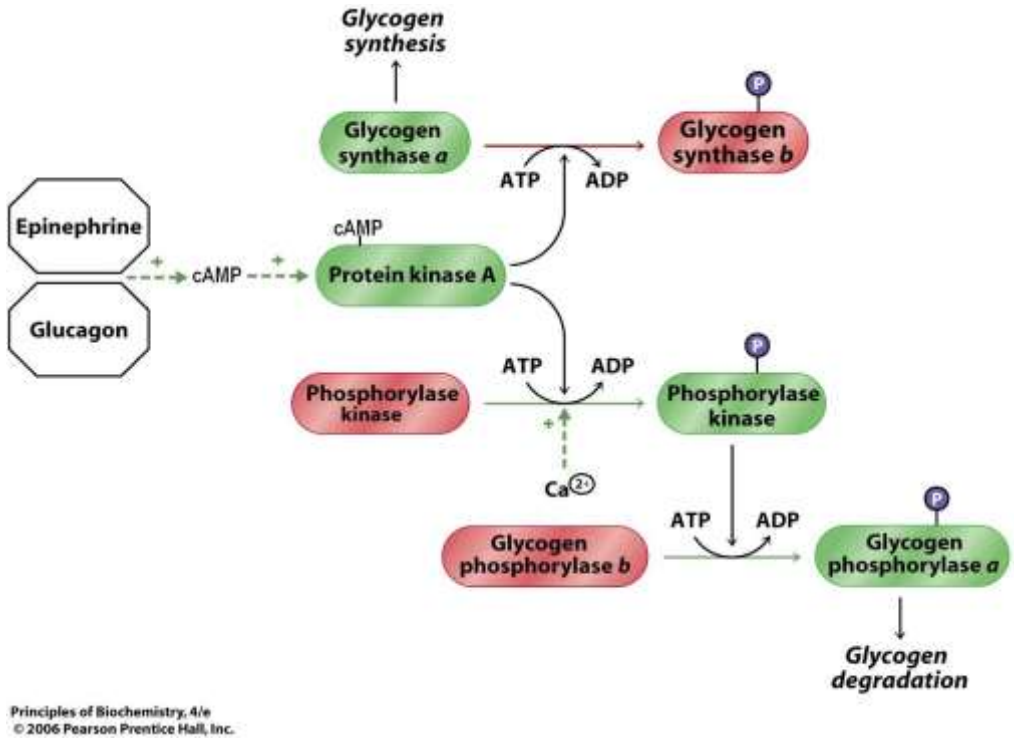
Whenever we have a high glucose in blood , the pancreas secrete insulin which activate the pathway of store glucose in liver.

Whenever we have a low glucose in blood , they are regulated by tow hormones which lead to the exact same effect but in different places of the body (epinephrine in muscle tissue , glucagon in liver tissue) , these two hormones bind to receptors on the surface of cell so induce a signal which activate adenylyl cyclase , adenylyl cyclase synthesis cAMP, increase cyclic AMP which is the regulatory modifier that bind to protein kinase A activating it .

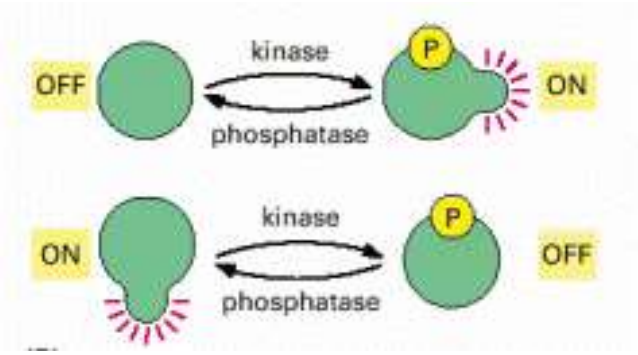
Protein kinase A phosphorylates 2 enzymes :

1) Glycogen synthase enzyme , so inhibit it lead to inhibit glycogen synthesis .

2) Phosphorylase kinase , so activate it . this Phosphorylase kinase phosphorylates glycogen Phosphorylase activating it so lead to activate glycogen degradation .



Note : The addition or removal of a phosphate group to an enzyme may activate or inactivate these enzymes .



The doctor said we don't have to memories this cascade of event but we have to understand the idea .

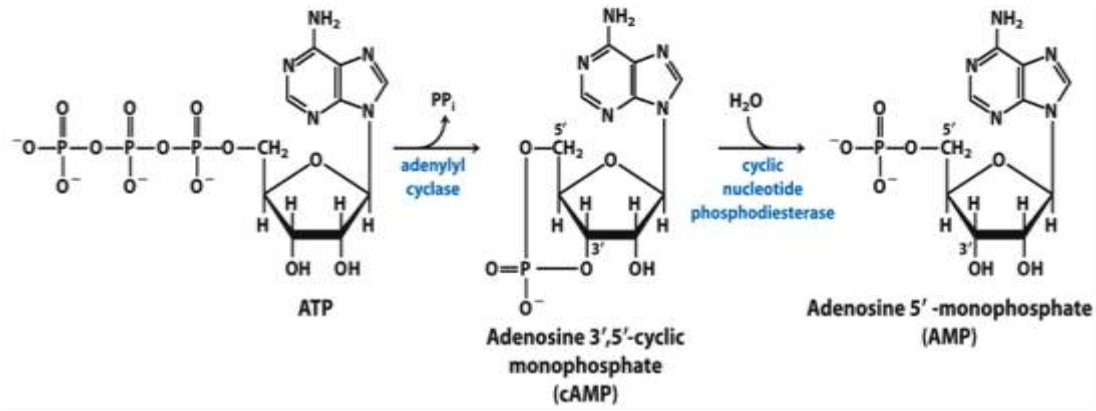
cAMP:

1* is structurally modified AMP synthesized by adenylyl cyclase enzyme .

2* Cells terminate it by phosphodiesterase enzyme .

3*Can have dramatic effects on enzymes .

4* Acts as a secondary messenger so activate protein kinase A (PKA) .



Again we don't have to memories the reaction.

What is the structure of protein kinase A ?

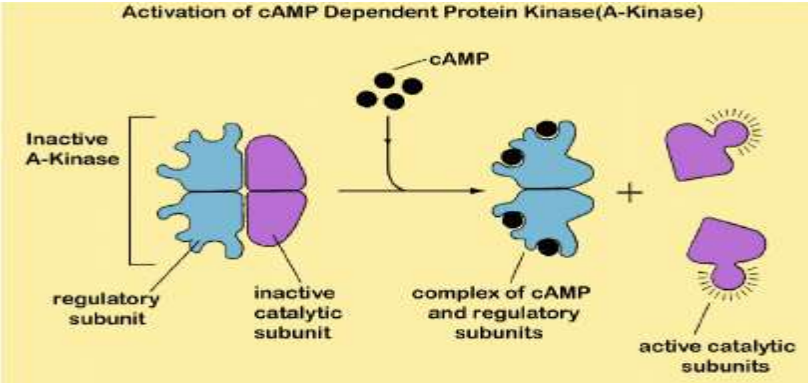
It is a serine/threonine protein kinase , phosphorylates several enzymes that regulate different metabolic pathways .

serine/threonine protein kinase mean the ability of protein to add phosphoryl group in serine or threonine amino acids)

When inactive composed of four subunits : two regulatory subunits (R) bind to two catalytic subunits (C) inhibiting it .

The two regulatory subunits have a high affinity to cAMP , so when there is high con. of cAMP , four molecules of cAMP bind to the two regulatory subunits leads to the dissociation of R2C2 into an R2 subunit and two active and free C subunits .
The active C subunits go and phosphorylate other proteins including the 2 enzymes that we talk about them in previous page .

Note : when the protein kinase A is activating , it loses his quaternary structure .

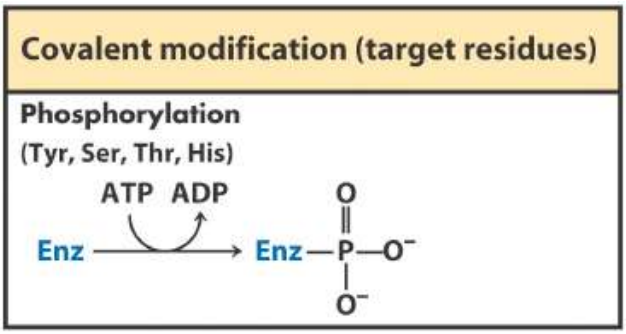


Another mechanism of regulation of enzymes is known as covalent modification of enzymes which is rapid and transient ….. **include :**

1) Phosphorylation (the covalent addition of a phosphate group to one of its amino acid side chains) .

In more details , which amino acids we covalently added a phosphate group ??

Usually serine , threonine , and tyrosine .



Note 1: ATP mostly is the phosphoryl donor in these reactions , which are catalyzed by protein **kinases** , And they belong to **transferase** class .

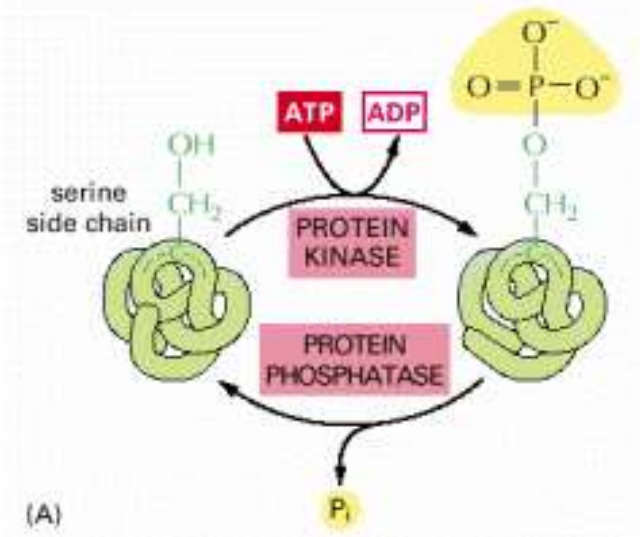
Note2: The removal of phosphoryl groups (dephosphorylation) by hydrolysis is catalyzed by protein **phosphatases** , And they belong to **hydrolases** class .

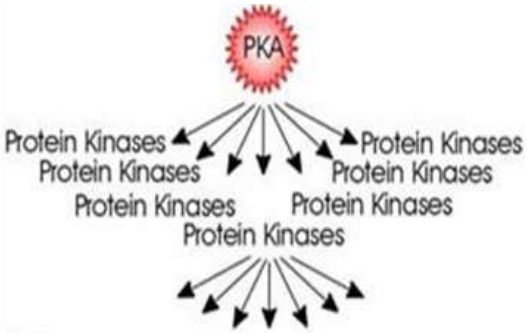
Why is the phosphorylation an effective mode of regulation???

1- The phosphate group has a multiple negative charges , so when added to enzyme they produce a huge change on non-covalent interaction (electrostatic interactions and/or hydrogen bonds) altering substrate binding and catalytic activity , so a rapid and huge altering on structure of enzyme .

2- Phosphorylation often causes highly amplified effects in less than a second or over a span of hours .

Amplified mean that one molecule of PKA can phosphorylates several phosphorylase kinases , each of them can phosphorylates several glycogen phosphorylase .



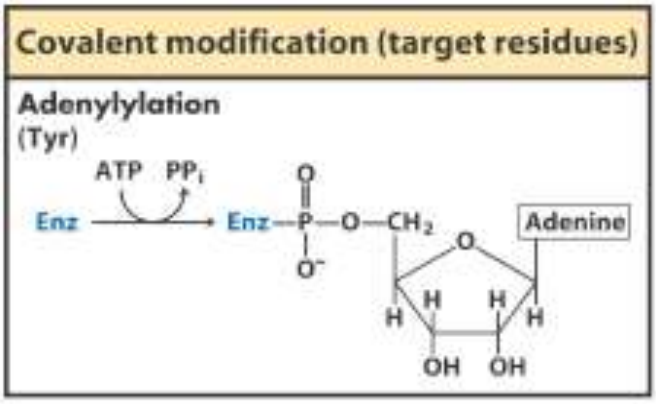


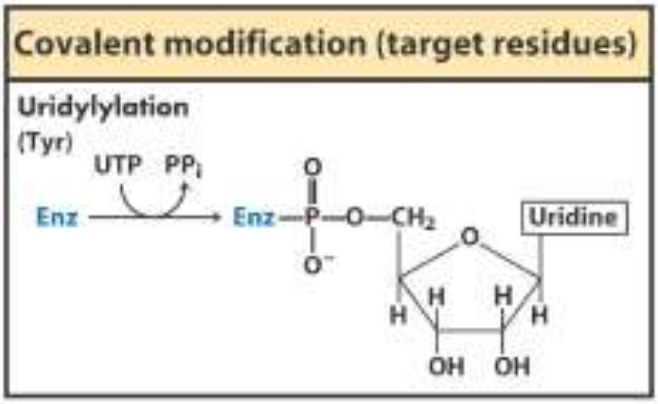
2) Adenylylation (addition of adenylyl group) .

- AMP is transferred from ATP to (Tyr) residues through <u>phosphodiester linkage</u> .

- The addition of bulky AMP **inhibits** cytosolic enzymes .

3) Uridylylation (addition of uridylyl group) .

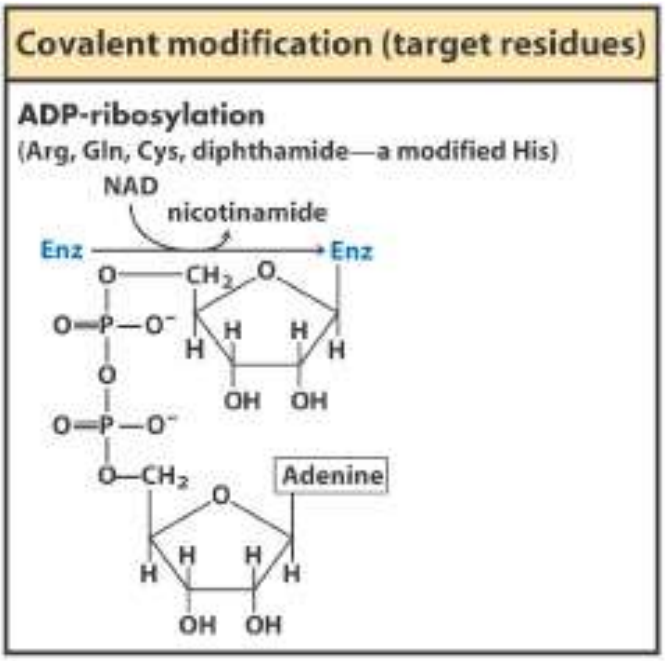


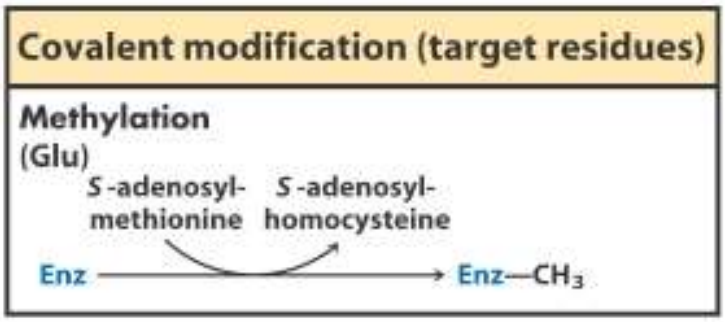


4) ADP – ribosylation (addition of adenosine diphosphate ribosyl group) which inactivates enzymes .

5) Methylation of carboxylate side chains masking **negative** charges .

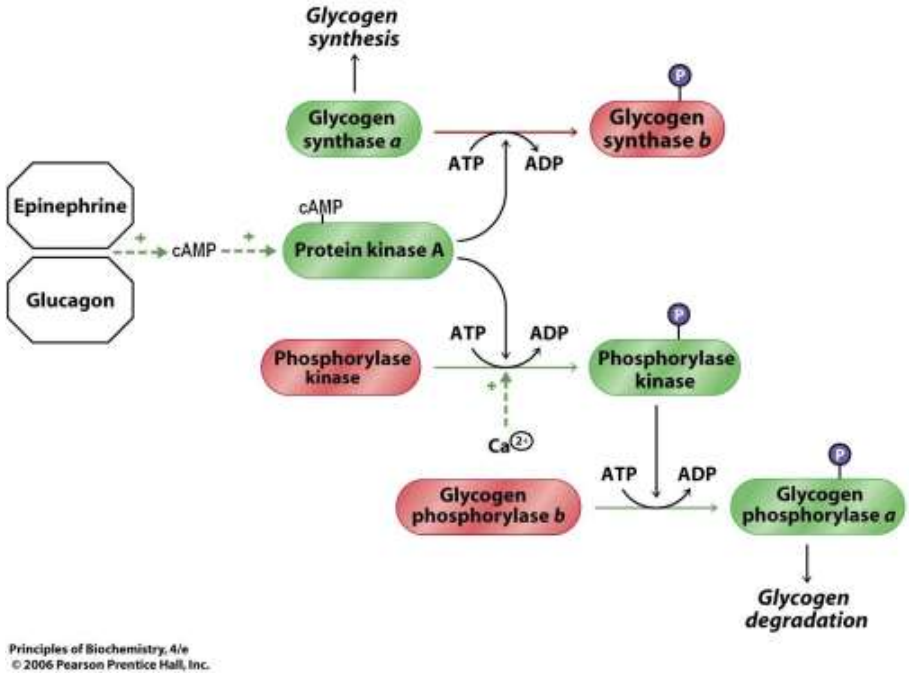
6) Acetylation (from acetyl Co) to "Lysine" residues masking **positive** charges .





Remember phosphorylation cascade :

cAMP activates Protein kinase A that phosphorylates multiple enzymes regulating Glycogen synthesis or Glycogen degradation .



Now let us talk about **Glycogen phosphorylase** :

Its function : stimulate glycogen degradation by adding phosphate group in order to release glucose from glycogen .so glucose is removed from glycogen in form of **glucose – 1 – phosphate** .

$$\text{glycogen}_{(\text{n residues})} + P_i \longrightarrow \text{glycogen}_{(\text{n–1 residues})} + \text{glucose 1-P}$$

This enzyme exists in 4 forms :

1) **T** state (**inactive** form of enzyme) .

2) **R** state (**active** form of enzyme) .

3) dephosphorylated (when enzyme is not phosphorylated its known as **phosphorylase b**)

4) phosphorylated (when of enzyme is phosphorylated its known as **phosphorylase a**)

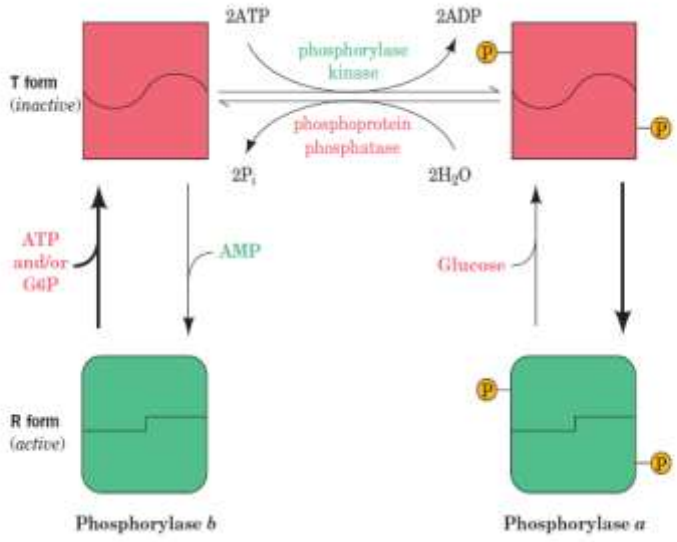
- Both phosphorylase b and phosphorylase a exist as equilibria between an active R state and inactive T state .

- Phosphorylase b is usually inactive because the equilibrium favors the T state .

- Phosphorylase a is usually active because the equilibrium favors the R state .

- **The transition of phosphorylase b between the T and the R state is controlled by :**
1) The energy charge (ATP and AMP) of the muscle cell .

2) Concentration of glucose -6-phosphate .



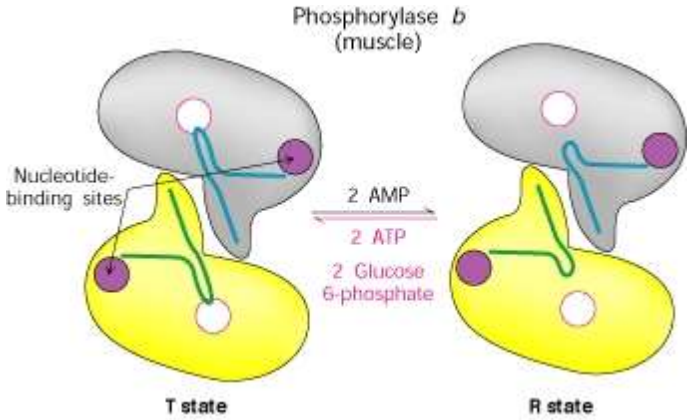
THE MECANISM OF TRANSITION FROM ONE FORM TO ANOTHER ON GLYCOGEN PHOSPORYLASE :

1 when you have high level of ATP or Glucose – 6 – phosphate , the enzyme becomes more in the T state , why ? in order to not waste energy and not to waste glucose from glycogen "there is sufficient energy".

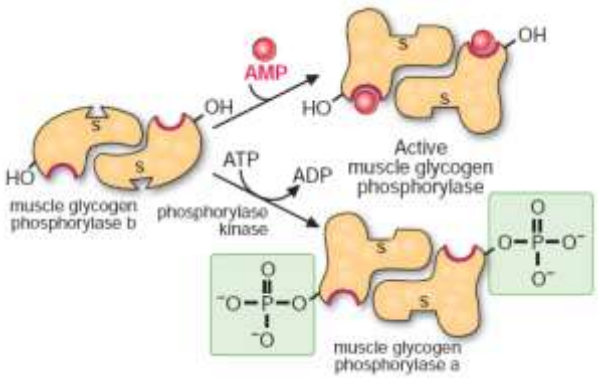
Note : ATP acts as a negative allosteric effector by competing with AMP and so favors the T state (inactive) .

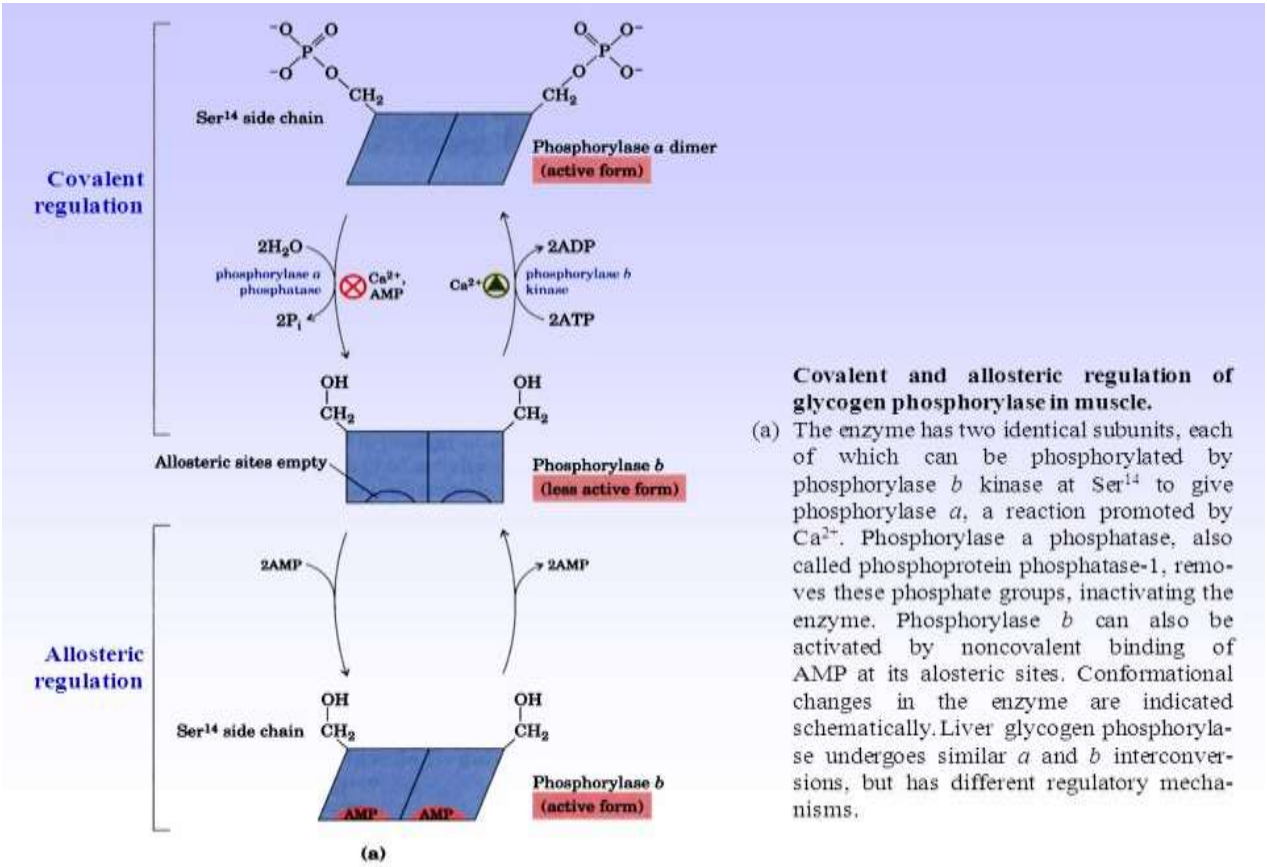
Note : Glucose – 6 – phosphate also favors the T state of phosphorylase b , an example of feedback inhibition .

2 When you have high level of AMP "energy is lacking and low in muscle cells" the AMP binds on binding site of phosphorylase b and stabilizes the conformation of phosphorylase b in the R state (active) .



So we have 2 ways of regulation , either by phosphorylation or by binding to a modifier (AMP , glucose-6-phosphate)





Covalent and allosteric regulation of glycogen phosphorylase in muscle.
(a) The enzyme has two identical subunits, each of which can be phosphorylated by phosphorylase *b* kinase at Ser^{14} to give phosphorylase *a*, a reaction promoted by Ca^{2+}. Phosphorylase a phosphatase, also called phosphoprotein phosphatase-1, removes these phosphate groups, inactivating the enzyme. Phosphorylase *b* can also be activated by noncovalent binding of AMP at its alosteric sites. Conformational changes in the enzyme are indicated schematically. Liver glycogen phosphorylase undergoes similar *a* and *b* interconversions, but has different regulatory mechanisms.

Regulation - Large regulatory molecules :

1) G protein :is a trimers protein bound to 7 trans membrane receptor known as GPCRs . Made of three subunits (α , β , γ)

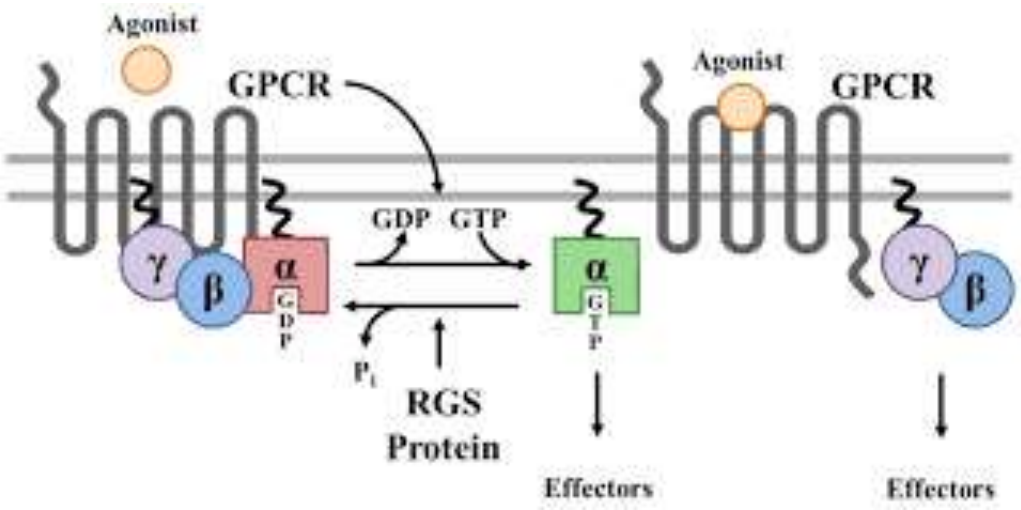
α : is the active subunit "NOT enzyme" .

β and γ : is regulatory subunits .

Ligand binds to the receptor (GPCR) this cause change in the structure of the receptor , the α subunit is released and binds to GTP instead of GDP , then the α subunit can binds to effectors and other proteins including enzymes .

Note :When they bind on guanosine triphosphate (GTP), they are <u>on</u> , and when they bind guanosine diphosphate (GDP) , they are <u>off</u> .

Note : The α subunit can be stimulatory or inhibitory .



2) Monomeric G proteins:

When GTP is bound , the conformation of the Monomeric G protein allows it to bind target proteins , which are then activated or inhibited .

The G protein hydrolyzes a phosphate from GTP to form GDP, which changes the G protein conformation and causes it to dissociate from the target protein .

GDP is exchanged for GTP, which reactivates the G protein .

It is cycle between exchange and hydrolysis .

These proteins have enzymatic activity even though they aren't enzymes , because this enzymatic activity affects in the protein itself not other proteins , same thing with Gα .

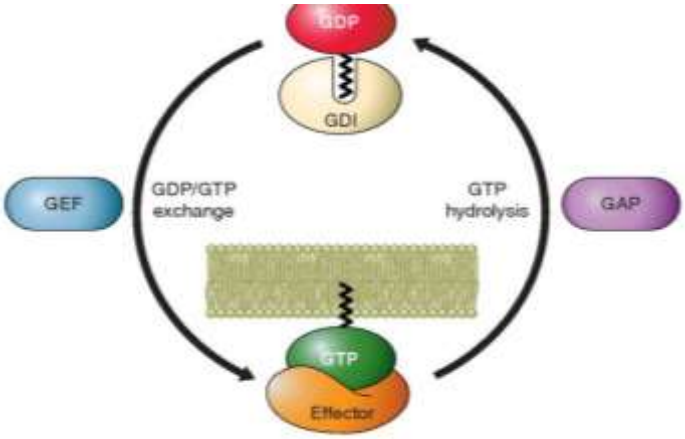
The activity of many G proteins is regulated by :

1) GAPs [GTPase-activating proteins] : they hydrolysis GTP to GDP .

2) GEFs [guanine nucleotide exchange factors] : they bind to monomeric G protein and they release GDP from each protein allows to GTP to bind to these proteins .

3) GDIs [GDP dissociation inhibitors] : they bind to Monomer G protein or Trimer G protein and prevent release GDP from the protein so it is stay inactive .

Note: GAPs and GDIs are inhibitors , GEFs are activator .

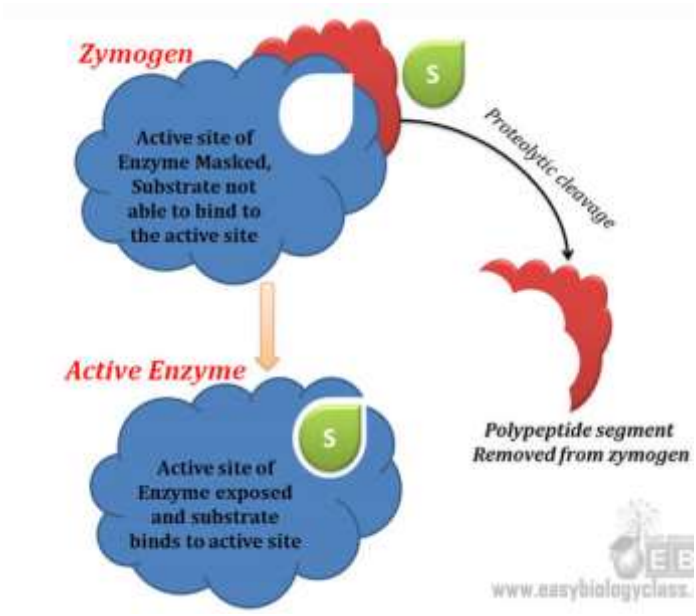


Irreversible covalent modification (proteolytic activation) :

The cell synthesis some enzymes in inactive form called **(Zymogens or proenzyme)** which is inactive precursors of enzymes **.**

Why is zymogen inactive ??? it does have a polypeptide part of it covers the active site prevents the substrate to bind on active site . so Zymogens require proteolytic cleavage which mean irreversibly removing part of the polypeptide chain (this removed part usually known as the pro region present at the N-terminus) .

As a result of cleavage the proenzyme is converted to functional enzyme which has a non covered active site so substrate can bind to it .



Examples: digestive enzymes such as chymotrypsin , trypsin , elastase and pepsin that get activated when food is ingested .

These enzymes are found as zymogens , why ? because it is digestive enzymes , it can digest anything (food or tissues) and we don't need to be activated inside the tissue like pepsin in stomach , so it is wonderful protective mechanism .

when we eat food , there is a signal transmit from our mouth to pancreas then it releases enzymes to intestine as zymogens then it get activated by proteolytic cleavage .

Note : Trypsinogen (zymogen) is activated via removal of the first six amino acids at the N-terminus .