23

BIOCHEMISTRY

faculty of medicine - JU2017

Sheet ○ Slides

Done By

Tala Saleh

Contributed in the Scientific Correction

Ahmad Attari

Contributed in the Grammatical Correction

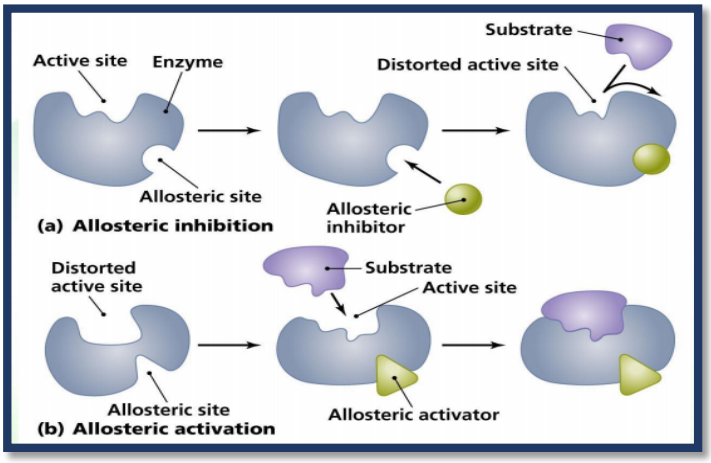
Minna Mushtaha

Doctor

Mamoun Ahram

In the previous lecture, we discussed the mechanisms of regulating enzymes through inhibitors. Now, we will start this lecture by discussing regulation through conformational changes.

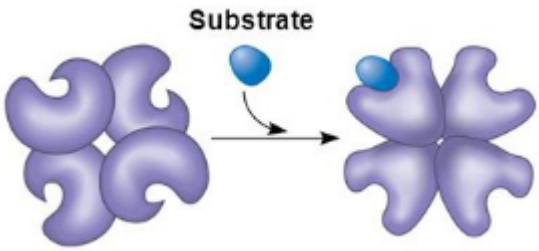
Regulation Through Conformational Changes



It occurs by **changing the conformation** of an enzyme in a certain way to rapidly affect the **activity of the enzyme** from an **inactive form to a fully active** form, or vice versa. These regulatory mechanisms include:

1) **Allosteric activation and inhibition**
2) **Phosphorylation or other covalent modifications**
3) **Protein - Protein interactions between regulatory & catalytic subunits or between two proteins**
4) **Proteolytic cleavage**

Allosteric Regulation



Allosteric enzymes are multi-subunit enzymes with **catalytic** *(active site)* and **regulatory subunits**. The binding of a **regulatory molecule** *(Allosteric modifier*) triggers **conformational changes** by **modifying** non-covalent interactions; this results in an apparent change in the **binding affinity** at a **different** binding site.
Note: *All Allosteric proteins have a* ***quaternary structure****.*

Allosteric Modifiers

They are molecules that **bind non-covalently** to the **Allosteric site** *(a physically separate site from the active site).* Types of modifiers:

1) **Negative Allosteric Modifier** *(inhibitor):* Causes the enzyme to be **less** active *by making the active site* ***less*** *fitting for the substrate.*

2) **Positive Allosteric Modifier** *(activator):* Causes the enzyme to be **more** active *by making the active site* ***more*** *fitting for the substrate.*

Modifiers can also be:

1) **Homotropic:** The modifier is the **substrate** itself.

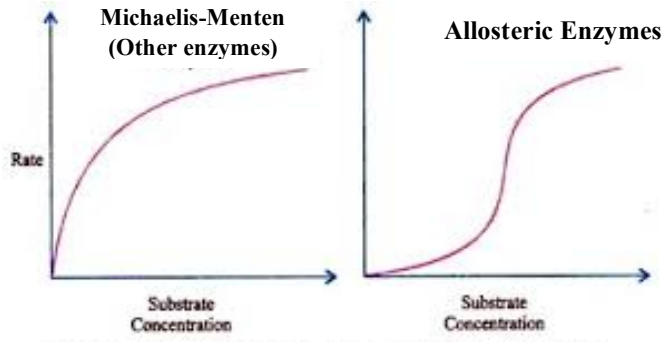
   *The binding of the substrate causes the enzyme to become **more active** and causes the binding to a second substrate at a **different** active site to be easier. This is known as **Positive Cooperativity**.*

   Example: O_2 is a homotropic allosteric modulator of hemoglobin.

2) **Heterotropic:** The modifier is **different** from the substrate.

Does the Michaelis-Menten model explain the kinetic properties of Allosteric enzymes?

No, because Allosteric enzymes **do not show a hyperbolic plot** since they have **multiple** active sites.



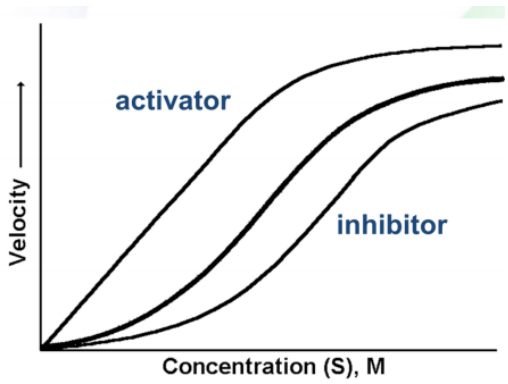
These multiple active sites exhibit the property of **Cooperativity**, where the binding of one active site affects the affinity of **other active** sites on the enzyme; these affected active sites result in a **sigmoidal plot** that has both **V_{max}** and **K_{50}.**

*(K_{50} is the **same concept** of K_M in **other** enzymes; where 50% of the effectors are bound to the Allosteric enzyme).*

Adding an Inhibitor to the Allosteric Enzyme

→ **Disables** the conversion of the subunits to the **active form**
→ **Lowers** the reactions' rates
→ **Does not change** V_{max}
→ **Increases** K_{50}, *which means that we need more substrate concentration to reach K_{50}*
→ **Lowers** affinity

*The plot shifts to the **right** and becomes **more sigmoidal**.*



Adding an Activator to the Allosteric Enzyme

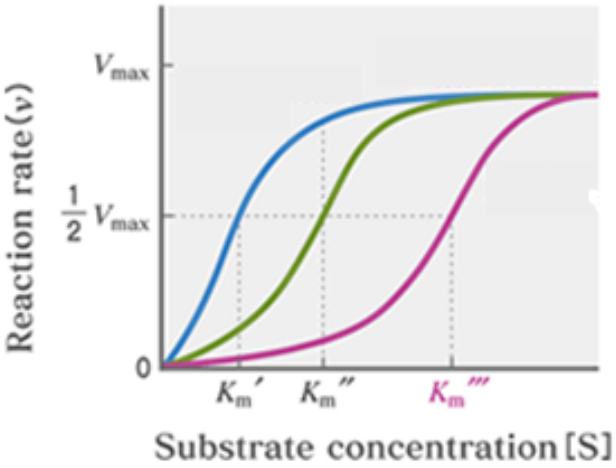
→ **Increases** the conversion of the subunits to the **active form**
→ **Does not change** V_{max}
→ **Decreases** K_{50}, *which means that we need less substrate concentration to reach* K_{50}
→ **Increases** affinity

*The plot shifts to the **left** and becomes close to a **hyperbolic plot.***

Note: *Vmax **does not change** in **allosteric inhibition**, unlike in **incompetitive inhibition**.*

*Why? Because increasing the [S] induces **conformational changes** in the enzyme, **the affinity of the enzyme** towards the **inhibitor decreases** until it is almost **negligible**. Therefore, we reach the **normal** V_{max}.*

The curve (only substrate) in the previous graph (in the middle) is hypothetical



Question: If only **activation** occurred, which of the following curves would be the substrate alone?

*Since **activation** occurred, the plot will be shifted to the **left**. Therefore, the normal state will be the farthest to **right** (red curve) and any other curves are just varying activation states. (We cannot say the one on the right is inhibited since inhibition did not occur; only activation did.)*

Question: If **inhibition** occurred, which of the following curves would be the substrate alone?

*Since **inhibition** occurred, the plot will be shifted to the **right**. Therefore, the normal state will be the farthest to **left** (blue curve) and any other curves are just varying inhibition states. (We cannot say the one on the left is activated since activation did not occur; only inhibition did)*

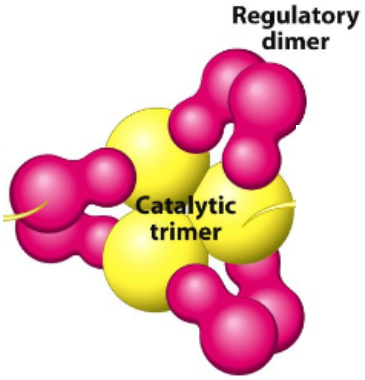
Aspartate Transcarbamoylase (ATCase) Reaction

ATCase is **a highly regulated enzyme** that catalyzes the **first step in pyrimidine biosynthesis**. ATCase **controls the rate of pyrimidine biosynthesis** by changing its **catalytic velocity** in response to cellular levels of **both pyrimidines and purines concentrations.**

ATCase structure:

ATCase consists of two units *(12 polypeptides in total):*

1) **Catalytic unit: 6** subunits organized into **2 trimers.**
2) **Regulatory unit: 6** subunits organized into **3 dimers.**



ATCase exists in two forms:

1) **T state *(taut):* less** active
2) **R state *(relaxed):* more** active

The end-products of the reactions' pathway roles:

1) **CTP: Cytidine triphosphate, a pyrimidine.**

High Concentration of CTP **inhibits ATCase** activity (feedback inhibition).

→ **Decreases** the affinity for Asp *(substrate)* / **Increases** K_{50}
→ **Decreases** enzymatic activity

*Binding of **CTP** to the regulatory subunits results in an equilibrium shift towards the **T state** (Inhibits ATCase).*

2) **ATP: The end-product of a parallel pathway, a purine.**

High concentrations of ATP **activate ATCase** because ATP is a source for nucleotides synthesis.

*Binding of **ATP** to the regulatory subunits results in an equilibrium shift towards the **R state** (Activates ATCase).*

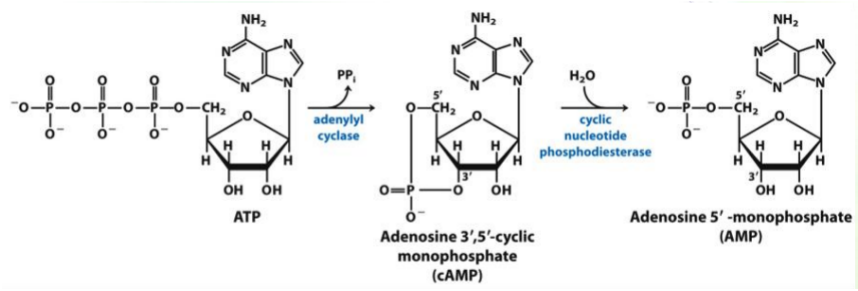
Notes:

- **Allosteric inhibitors** usually have a **much stronger** effect on an enzyme's velocity than **competitive and noncompetitive inhibitors.**
- Allosteric enzymes **do not** only regulate through **inhibition**, but also through **activation**, since Allosteric regulators *(effectors)* may also **function as activators.**
- The Allosteric effector **does not necessarily** need to **resemble** the **substrate** or the **product** of the enzyme.

Regulation Through Modulators

Small molecule modulators can have **dramatic effects** on the **enzymes**.

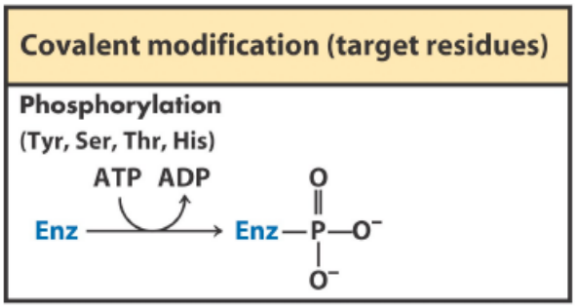
Example: cAMP *(structurally modified AMP)* activates **protein kinase A**.



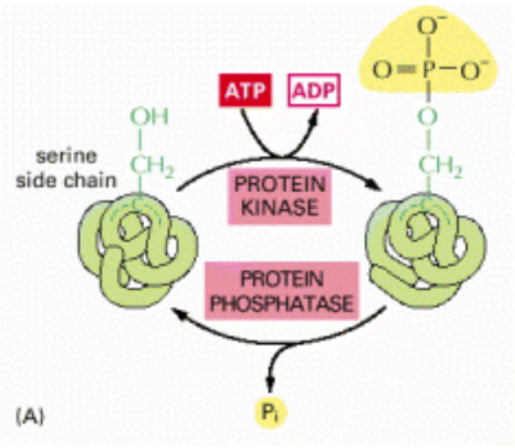
Till now, we talked about regulation through allosteric behavior; another way for regulation is:

Reversible Covalent Modification

Covalent Modification *(Phosphorylation)*



A **common** mechanism in which an enzyme molecule **accepts a phosphate** group *(covalent addition)* into one of its amino acids' **side chains**, changing its activity. Usually a **serine**, **threonine** or **tyrosine** residue *(amino acids that contain hydroxide)*. **ATP** is *mostly* the **phosphoryl donor** in these reactions, which are catalyzed by **protein kinases.**



Dephosphorylation by hydrolysis is catalyzed by **protein phosphatases**.

Note: *Dephosphorylation is **not the reversal** of phosphorylation.*

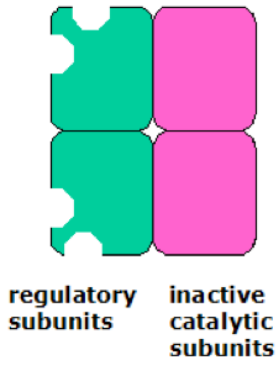
Note: *The **addition/removal** of the phosphate group from an enzyme may **activate** or **inactivate** it.*

Note: *Kinases are **transferases**, and phosphatases are **hydrolases***

Why is phosphorylation effective *(most common)*?

- **Rapid** and **transient** regulation of an enzyme's activity *(reversible)*.
- Adds **two negative** charges *(from the phosphate group)*.
  → **New electrostatic** interactions and accordingly **changing conformation**.
- Can form **three or more hydrogen bonds,** altering substrate **binding** and **catalytic activity**.
- Can take place in *only* **less** than a second or over a span of hours.
- Often causes **highly amplified effects**.

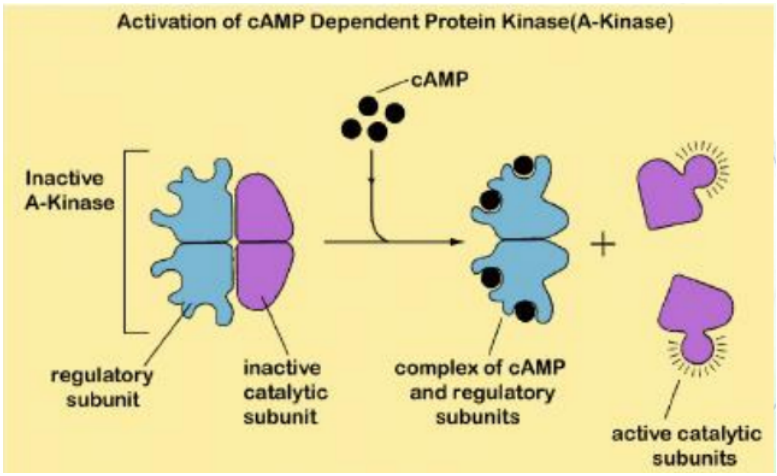
Protein Kinase A Structure



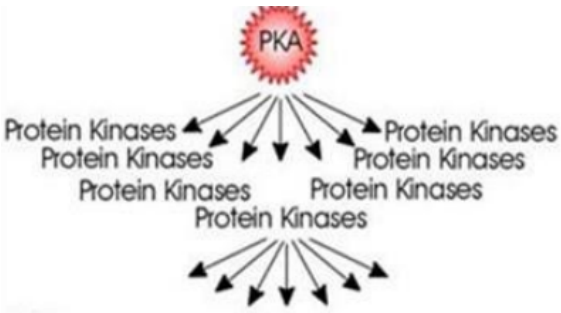
When **inactive**, PKA is a **tetramer** consisting of:

- **Two regulatory** *(R)* subunits with **high** affinity for **cAMP**.
  Each regulatory subunit binds to two cAMP molecules (4 cAMPs total).
- **Two catalytic** *(C)* subunits.

The binding of two **cAMPs** to each **regulatory subunit** leads to the **dissociation** of the R2C2 → R2 + two active C subunits.



Protein Kinase A *(serine/threonine protein kinase)* **phophorylates** many enzymes that regulate **different metabolic pathways**.

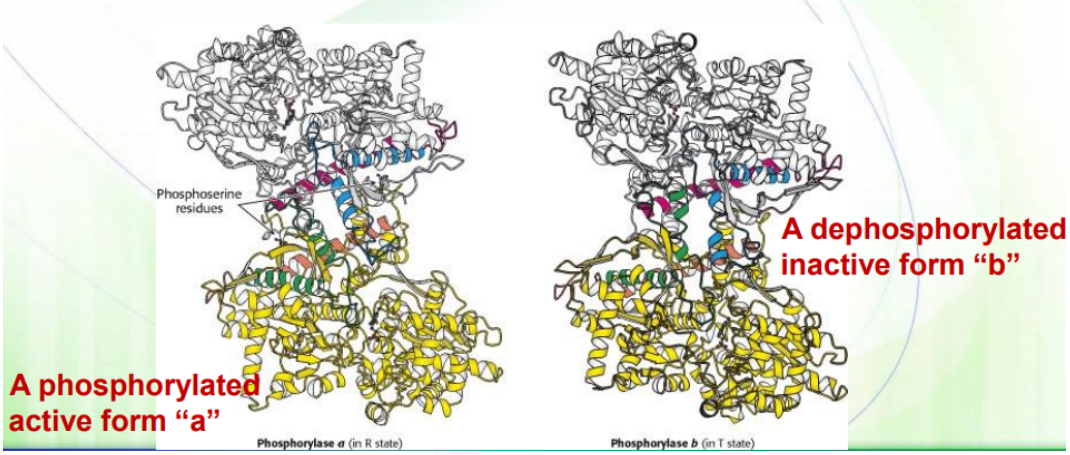


Note: *The effect of PKA is* ***amplified.***

Example of an enzyme regulated by PKA:

Glycogen phosphorylase kinase (GP)

GP catalyzes the **removal of glucose molecules** from glycogen. The enzyme exists in **two** forms:

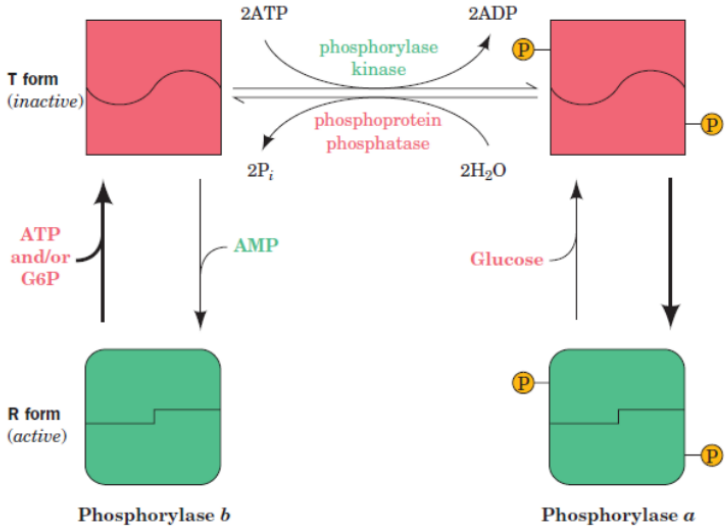


1) **Phosphorylase B (inactive state).**

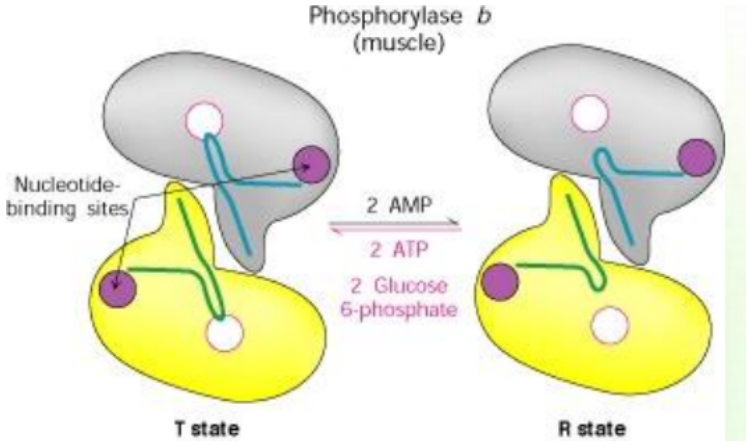
It shifts between the R and T state in equilibrium, and it favors ***the T state.***

2) **Phosphorylase A (active state).**

It shifts between the R and T state in equilibrium, and it favors ***the R state****.*



Note: *Allosteric Enzymes are influenced by* ***substrate concentration****. For example, at* ***high concentrations of substrate / activator****, more enzymes are found in the* ***R state****. The* ***T state*** *is favored when there is an* ***insufficient amount of substrate*** *to bind to the enzyme or when an* ***inhibitor is present****. In other words, the T and R state equilibrium* ***depends on the concentration*** *of the substrate.*



The transition of **phosphorylase B** between the **T and the R state** is **controlled** by the **energy charge** *(ATP and AMP)* of the muscle cell. *How?*

- **High concentrations of AMP** → Phosphorylase B is **active,** and AMP binds to a nucleotide-binding site, stabilizing the conformation into the **R state**.

- **ATP acts as a negative allosteric** effector by **competing** with **AMP** → Phosphorylase B is **inactive,** favoring the **T state**.

Note: Glucose 6-phosphate also favors the **T state** *(feedback inhibition)*.

Phosphorylation Cascade

*Note how a single hormone can **activate** a kinase while **inhibiting** the other.*

PKA inhibits Glycogen synthase

→ Inhibits glycogen synthesis

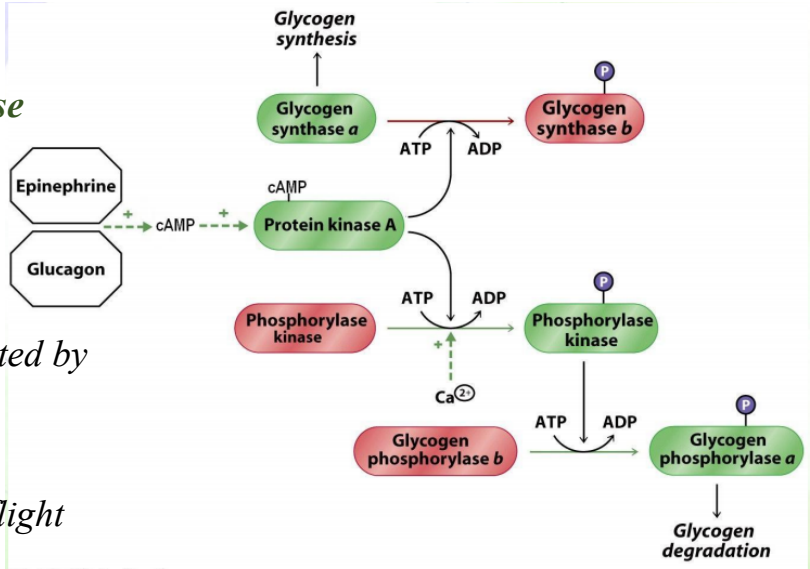
PKA activates Phosphorylase kinase

→ Phosphorylates glycogen phosphorylase B to A

→ Promotes glycogen degradation

Note: Glucagon is a hormone secreted by the pancreas (glucose level sensor), affecting the tissues.

Epinephrine is secreted in fight or flight actions, affecting the muscles.

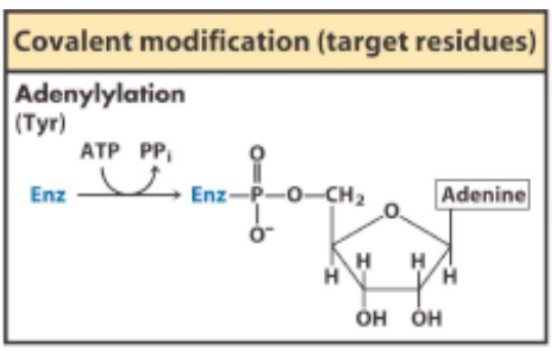


Other Covalent Modifications Besides Phosphorylation

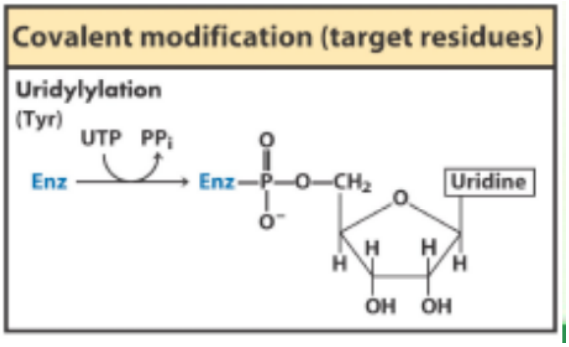
1) **Adenylylation** ***(addition of adenylyl group).***

AMP (from ATP) is transferred to a **Tyr s**

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

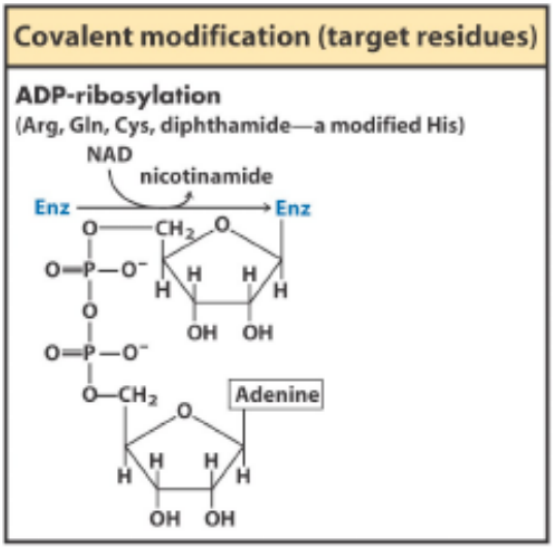


2) Uridylylation *(addition of uridylyl group).*



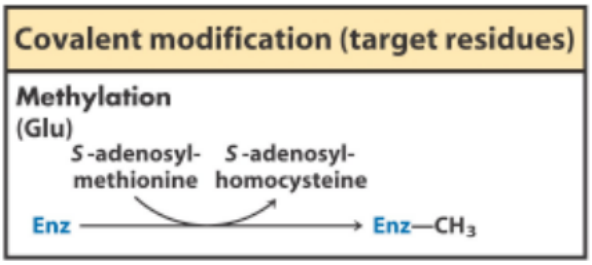
3) ADP-ribosylation.

Addition of an adenosine diphosphate ribosyl group **inactivates** key cellular enzymes.



4) Methylation.

Masks the negative charge of **carboxylate side chains** and adds **hydrophobicity.**



5) Acetylation.

Acetyl Co → Lysine

Masks **positive charges.**

6) Conformational changes due to protein-protein interaction :

G -proteins: They are trans-membrane proteins that cause changes inside the cell. They receive signals from **hormones**, **neurotransmitters**, and other **signaling factors**.

When they are bound to (**GTP**) → **On**

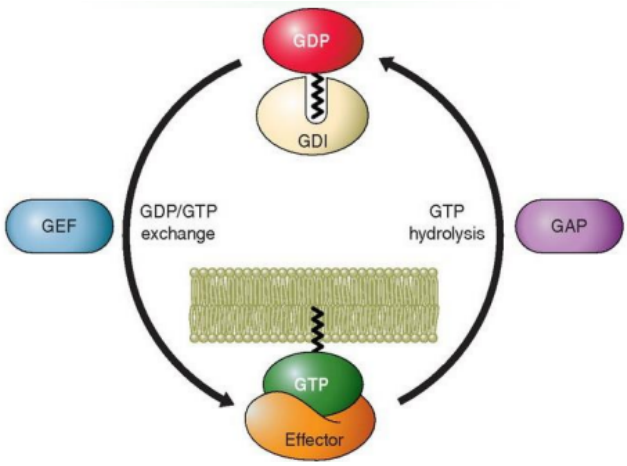
When they are bound to (**GDP**) → **Off**

- **G-protein:** a transmembrane **heterotrimer protein** made up of **α**, **β** and **γ** subunits.
- The **α subunit** is the active subunit. It can be **stimulatory** or **inhibitory.**
- **β** and **γ** subunits are the regulatory subunits
- **G-proteins** belong to a larger group of enzymes: **GTPases**.
- They are **always coupled** with **GPCRs**.
- They are the target of **25%** of drugs.

Monomeric G-proteins

Monomeric G proteins have the **same mechanisms** as the **trimeric** G-proteins. They differ only in the fact that they are **small**, with **one subunit** that works as the **alpha subunit.** They also **do not** target **adenylyl cyclase** like the trimeric G-proteins do.

1) When the monomeric G-protein is bound to **GDP,** it is **inactive**.
2) In order to **activate** the monomeric G-protein, a **guanine exchange factor** *(GEF)* is needed.
3) GEF **exchanges** GDP with a GTP, making the monomeric G-protein **active**.



The activity of many G-proteins is regulated by:

1. ***GAPs [GTPase-activating proteins]:*** *Stimulate GTP hydrolysis (decrease the activity of G-proteins)*
2. ***GEFs [guanine nucleotide exchange factors]:*** *Stimulate the exchange of GDP/GTP (increase the activity of G-proteins)*

   Note: *GTP → GDP **(Hydrolysis),** but GDP → GTP **(Exchange)***
3. ***GDIs [GDP dissociation inhibitors]:*** *Keep GDP associated (decrease the activity of G-proteins)*

Good Luck ☺