The Endocrine System

BIOCHEMISTRY

☑ Sheet ☐ Slide ☐ Handout

Number:	4
Subject:	
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Price:

Salam alaikum ☺

-It is Eid but there is no escape from studying! That's the Tax of medicine so happy Eid all :P

-This sheet was written according to the records of section 1 & 2 and includes everything in the slides so there is no need to refer to them ☺

Review

As we studied in the last lecture; we have a receptors called 7-transmembrane helix receptors, all of these receptors bound to G-protein **so** we can also called them "**G-protein** coupled receptors".

G-protein and its pathway:

Is a multimeric protein that composed of three subunits:

- α :which dissociates after the activation of G- protein(when bound to GTP).
- β , γ: which stay attached together .

NOTE: - α and β γ can interact with other proteins.

- α and γ subunits have covalently attached fatty acid.

However; inactive G-protein is bounded to GDP and away from the receptor, once the hormone is bound to the receptor this will cause conformational changes and bring the G-protein close to the receptor , once the G-protein binds the receptor; the exchange of GDP to GTP will occur so G-protein become **active** .

When the G-protein becomes active one of its subunit which is α subunit will dissociate in order to (stimulate or inhibit) other proteins or enzymes.

BUT what is determine the action of α subunit either it activation or inhibition of other protein or enzyme?

1- The nature of the subunit.

There are:

- α subunits which are stimulatory in their nature(called Gs).

- α subunits which are inhibitory in their nature(called Gi).

2- The nature of the receptor also plays a role.

There are receptors which are either stimulatory in their nature like the "Adrenergic receptors" OR inhibitory in their nature like the "alpha2 receptors"

NOW what is the destination of α subunits?

The most common target for α subunits is an enzyme called **"adenylate cyclase**" which converts ATP →cAMP; this enzyme can produce 1000 cAMP per second.

TO SUM UP:

One hormone is bound to one receptor "**one to one**"→each receptor will bound to around **100** G-protein→α subunit of each G-protein will dissociate then stimulate **one** adenylate cyclase →each adenylate cyclase by itself can convert **thousand** of ATP to cAMP per second So→ for 1 hormone there will be 100,000 of cAMPs that are formed per second. "This is what's meant by **amplification** process" .

After we got the needed response we have to end the action and this happen through the **GTPase** activity within the G-protein .This enzyme can spontaneously hydrolysis the GTP into GDP slowly. Once GDP is bound this inactivate G-protein so the three subunits rebound together **again** and G-protein go away from the receptor, and there are some other methods that will be discussed later on in this sheet.

END OF THE REVEIW

As mentioned above that α subunit in the G-protein will activate **mostly** **adenylate cyclase** to form cAMP **But** are there any other target of α subunit?

-Actually yes; one of them is the **"phospholipase c"** : as the name implies this enzyme will breakdown phospholipids.

-There is another target which is the **membrane ions channels** it either opens or closes them.

Types of alpha G-protein [G α subunit]

NOTE: G-protein classes are defined based on the sequence of their **α** subunits

G_s "stimulatory" → ↑ Adenylate Cyclase
G_{olf} "olfactory " → ↑ Adenylate Cyclase
Transducin → ↑ cGMP Phosphodiesterase
G_i "inhibitory" → ↓ Adenylate Cyclase
G_o "open" → Ca2+ Channels
G_q → ↑ Phospholipase C

-Their names were determined according to their amino acids sequences

The Pathways: As we said adenylate cyclase is mostly the main target of α subunit;

[1] cAMP Cascade:

Adenylate Cyclase is the downstream of G-protein, it is a membrane protein composed of 12 helices that spanning the membrane, and has two large intracellular domains which are responsible for catalysis and conversion of ATP INTO cAMP

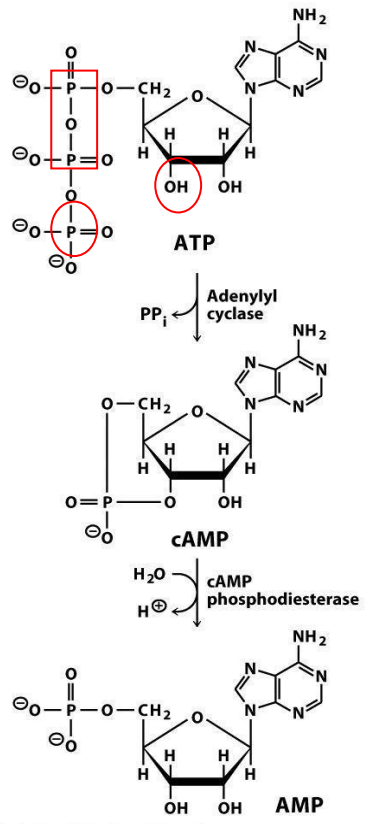
How this conversion occurs:

ATP molecules have three phosphate groups.

The oxygen on carbon number 3 of the ribose will attack the first phosphate ;and the other 2 phosphate groups will released as form of " **PPi**" "pyrophosphate.

****We can imagine that we form a cycle between phosphate and oxygen.**

****breaking the bond that is formed between oxygen and phosphate in cAMP will transfer it into AMP which can be returned into ATP again .**



-The effects of cAMP on our body are wide and usually its activation.

For example: increasing the secretion of acids by gastric mucosa.

Clinical case:

People who suffer from gastric ulcer in their stomach (due to high acid secretion),they must not take anything that increase increases cAMP in mucosa cells because cAMP will increase acid secretion so this will worsen their condition .

For that reason these patient should avoid drinking coffee because :It inhibits "phosphodiesterase enzyme" which breaks down the cAMP to AMP. So if we inhibit this enzyme , there will be an increase in cAMP thus more releasing of acids within mucosa cells →their case becomes worse .

Note that As we know coffee makes us awake due to caffeine acting as a blocker of adenosine receptors. It will bind to adenosine receptors instead of adenosine substrate and this prevents the initiation of the sleep process.

Normally : to initiate sleep adenosine molecules bound to their receptors in the brain.

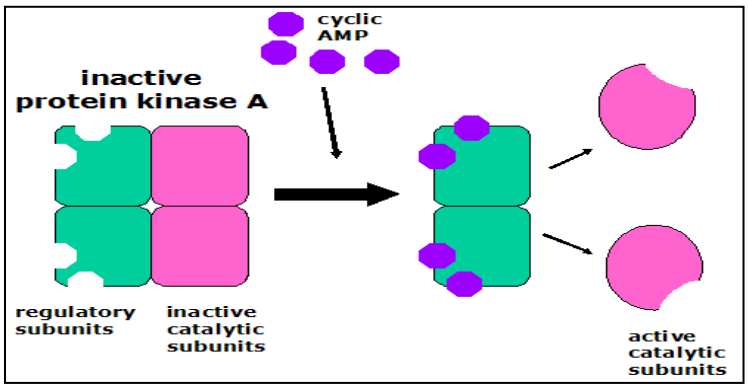
→Now after the formation of cAMP it will go to their target, and the main target is "**protein kinase A**" this protein is a tetrameric protein (have 4subunit (2 regulatory, 2 catalytic)).

Once cAMP bind to this protein its subunits will dissociate from each other, now this enzyme is in active form.9

As name implies:

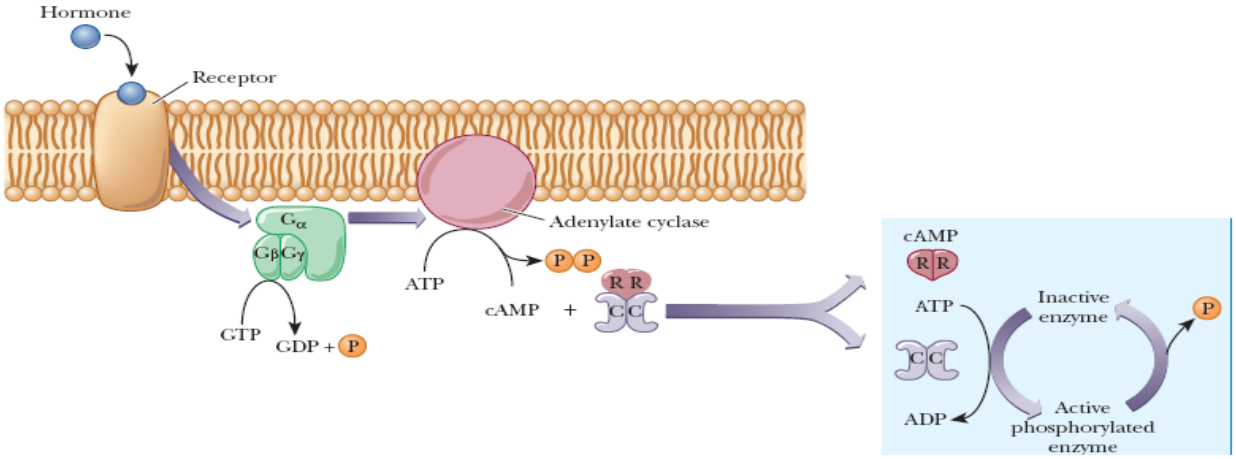
(Kinase=phosphorylation)

(**A**=related to its substrate which c**A**MP)



-note that phosphorylation occurs on serine or threonine".

NOTE: Phosphorylation doesn't always mean activation; it sometimes it leads to inhibition as in glycogen synthase enzyme.



<u>Signal-as a whole- must switch off (termination)and this occurs by :</u>

1- Hydrolysis of cAMP by phosphodiesterase enzyme

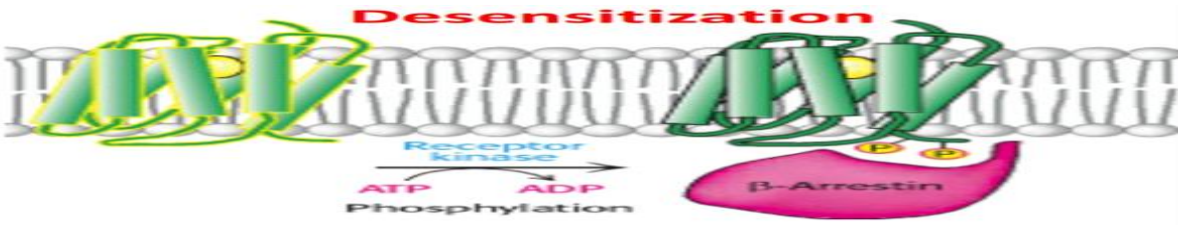
2- Inhibition of the activated G-protein by the action of GTPase within α subunits which transform GTP→GDP

3-Decrease the hormone concentration: dissociation of hormone from the receptor.

4- Phosphorylation of the "hormone bound-receptor" followed by binding to **β-Arrestin**:

How does **β-Arrestin** work ?

(After the hormone **(yellow)** binds to the receptor (7TM) there is something inside the cell called **receptor kinase** that comes and phosphorylates the receptor itself , Once the receptor is phosphorylated (phosphate is added to serine or threonine) now beta arrestin can bind to the receptor and as the name implies it arrests "stop" the action of the receptor. What β-arrestin actually does is binding to the receptor and preventing the G protein from binding to the receptor so G-protein will not be active).

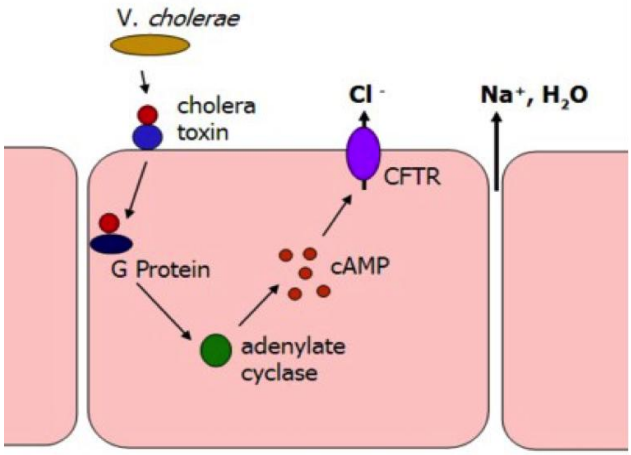


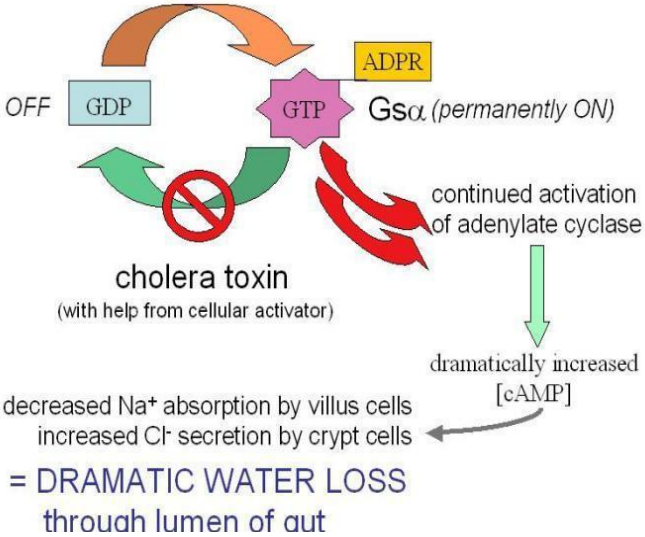
What would happen if there is any defect in the signal transduction of cAMP cascade ?

Cholera:

A disease that is characterized by severe diarrhea (severe Dehydration). It is caused by bacteria called vibrio cholera that secretes toxins which bound to the (7-TM receptor) that found in the intestinal epithelial cells.

This binding process is very strong ; this binding will stimulate more GTP protein and according to that→ more α subunit dissociate →more cAMPs .The action of it is to open channels for Na ions to move out, and when Na ions leak out from intestinal cells they will attract water with them. So person will suffer from diarrhea. It can be severe diarrhea and if not controlled it can end up with death.





Another disease is the **"whooping cough "**; same mechanism as cholera toxins.

[2] PIP2 cascade:

In this pathway G- protein activates another type of enzymes which is the **phospholipase C** .

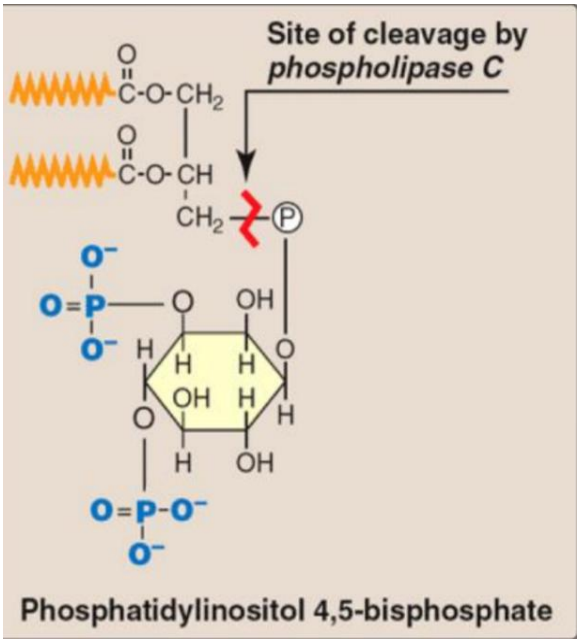
As the name implies phospholipase enzyme breaks down phospholipid and the phospholipid of the lipase c is called **Phosphatidylinositol 4,5-bisphosphate (PIP2)** which is composed of glycerol molecule binding two fatty acid molecules and inositol ring attached with the glycerol molecule by phosphate group .

NOTE: this pathway is used by many hormones (e.g. ADH)

-PIP2 can be broken on the phosphodiester bond and so gives two molecules:

1. Inositol triphosphate (IP3):

A molecule with 3 phosphates,And a ring with oxygens thus →it's very hydrophilic.



2. Diacylglycerol (DAG) :

glycerol back bone with 2 acyl groups (acyl : any number of carbon units) ... the DAG molecule is an amphipathic molecule(because of the presence of the hydrophobic character in the side of the diacyle molecules .. whereas the remnant carbon attaches with hydroxyl group so hydrophilic .)

●Now **where** is the whole **PIP3** molecule found?

-It is found in the membrane

●So what happens in this cascade exactly?

-First, the hormone will bind the receptor and activate it ⟶ The receptor will activate G-protein ⟶ Releasing of the alpha subunit will occur ⟶ the alpha subunit will bind the **phospholipase c** which is an enzyme attached to the membrane ⟶ The enzyme will start breaking down the **PIP3** into IP3 & DAG

NOTE: any enzyme activated by the G-protein should be attached to the membrane; because the G protein is already attached to the membrane as long as the hormone is binding.

●BOTH of **IP3** & **DAG** give signal BUT which is the actual second messenger and **Why** ?

-IP3 is the main second messenger; because it's hydrophilic so it is easy to move in the cytosol and reach hundreds of molecules and enzymes in order to change their action... since the receptors are embedded in the membrane; we need to have second messengers that can reach intracellularly.

#**Before** explaining the pathway of each molecule lets highlight the **phospholipase c** enzyme:

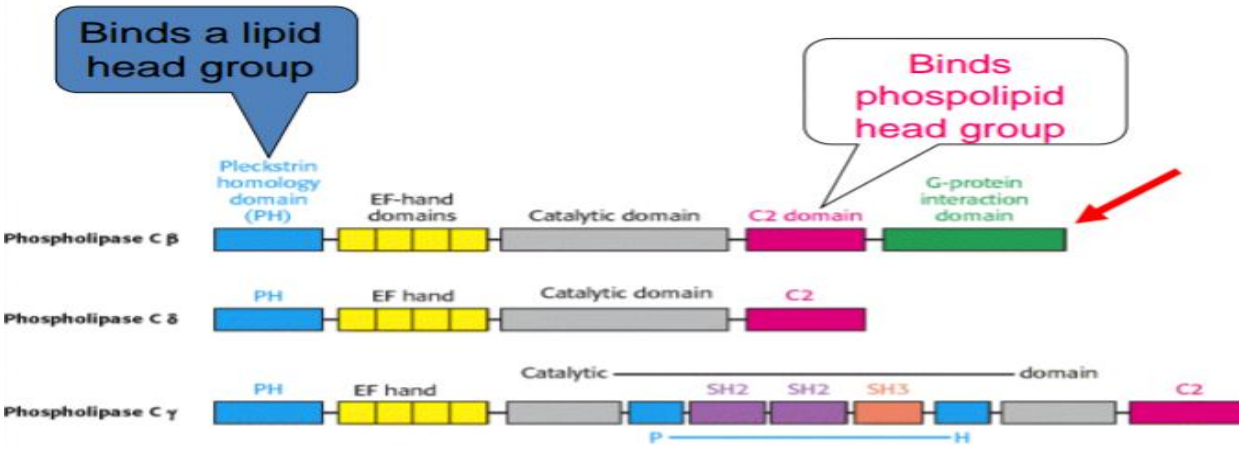
▪Phospholipase c has many isoforms not all of them are target for G protein.

▪Beta isoform is the only one where the G protein can bind so it is the only isoform that can interact with G protein.

▪In each isomer there are domains which their function is to stabilize the enzyme into the membrane; those domains are:

- C2 domain: binds phospholipid head group.

-PH domain: binds lipid head group.



Let's go back to the IP3 &DAG pathways:

1] IP3 pathway:

▪when the IP3 is released; it will move to its target which is the calcium channels.Calcium channels are present in the membrane of the sER(mainly) as well as the plasma membrane.

The **calcium concentration** is divided as:

[In the SER] or [extracellular] > [cytosol] (the cell has very law amount of Ca ions in its cytosol).

▪When IP3 binds the calcium channels the will open and accordingly the calcium will exit from the high concentration toward the low concentration and from outside to the inside of the cell but the **main** is come from the S**ER** because it has much more calcium channels compare to those in the plasma

membrane .. Around 80 % of the proteins in the ER membrane are calcium channels.

How many IP3 molecule should be bound to the calcium channel to make it active ¿

-The calcium channel is composed of 4 subunit means there are 4 binding sites, if at least 3 **IP3** molecules bind that will be enough to activate the channel and open it widely.

NOTE: the binding of the IP3 to the Ca channels is **cooperative binding** which means it's very hard to bind the first molecule but the second molecule will bind easier and the third is much easier to bind.(after the first molecule binding within a short period of time you will have a full opening of the channel and release of the Calcium) . **((Sigmoidal shape plot))**

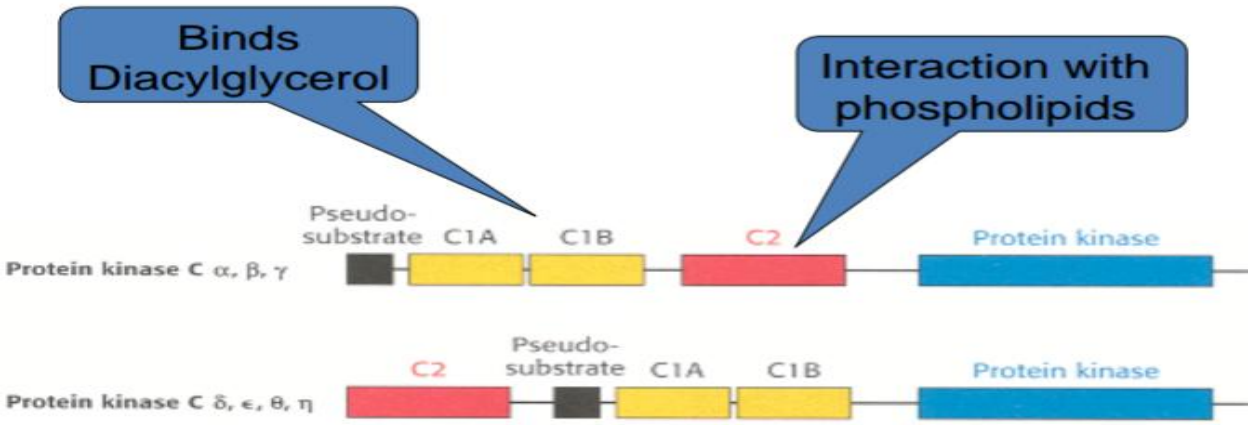
▪**Now** the calcium is in the cytosol... it must bind to something what is it?

- It can bind to either **calcium binding proteins(calmodulin)** OR to **the protein kinase c.**

▪By the time the calcium binds to the protein kinase c; it will partially activate the kinase and then the function of the **DAG** comes.

2] DAG pathway : this pathway is mainly **dependent** on the **IP3** pathway , as when the calcium binds to the **protein kinase c** ;it will let it go to the **DAG** molecule to bind to it.once bound it will become fully active and able to do its function which is **phosphorylation**.

Protein kinase c as phospholipase c has many **isoforms** each one composed of many domains such as:



-**C1 domain**: This bound to DAG.

-**C2 domain:** which interact with the phospholipid of the membrane.

-Catalytic domain.

- Ca binding domain.

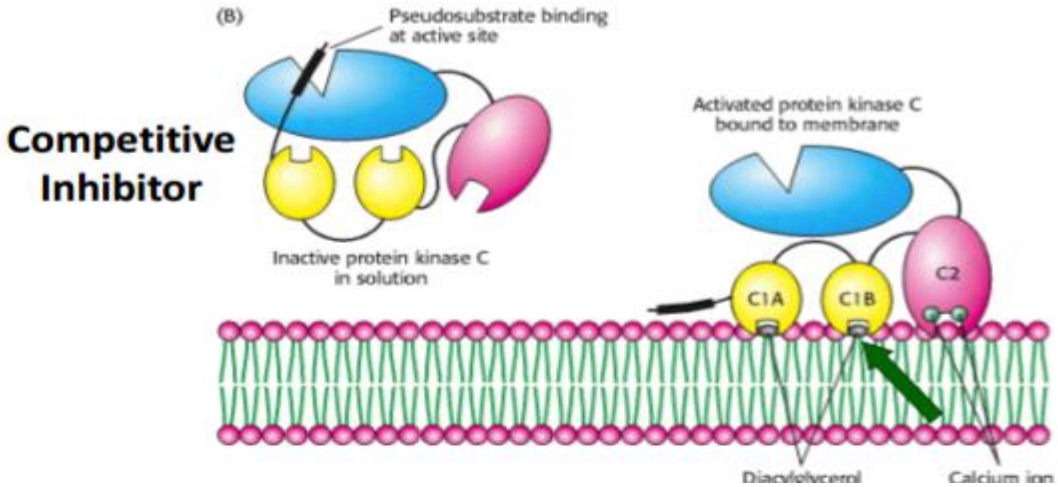
-pseudosubstrate domain : this is a domain with a sequence of amino acids that looks like the substrate but it is not a substrate ..It mimics the substrate in order to block the active site **so** this pseudosubstrate works like a **competitive inhibitor**.(it differs from the sequence of the substrate by few amino acids)

Look at the figure bellow , the first sequence is for the pseudosubstrate and the second is for the substrate .. they look like each other with small difference that the substrate in its seq contains **serine** and **threonine** to be phosphorylated, whereas the sequence of the pseudosubstrate contains **alanine**(hydrophobic amino acid) which cannot be phosphorylated and works as a cover on the active site.

The pseudosubstrate sequence → A-R-K-G-A-L-R-Q-K

The substrate sequence → X-R-X-X-(S,T)-Hyd-R-X

Let's know exactly how the **protein kinase c** becomes active : when the calcium binds in its binding site of the protein kinase c it will bring the **C1 domain** binding the **DAG** of the plasma membrane and will pull the **pseudosubstrate domain** and **expose** the active site and protein kinase C will function.



After reaching the needed response the signals have to be **terminated** → the **IP3** will be degraded by the action of **phosphatase** enzyme which will pull out one by one phosphate group from the IP3 molecule . BY the time the first phosphate is pulled out the molecule will be **inactive**.

-Sometimes the phosphatases aren't available to degrade the IP3 **so** there is another way to achieve the termination which is by **adding new phosphate (by kinases)** ,in this case we will have tetra phosphate binding the inositol ring and the molecule won't

be active because in **both** cases we change the main structure which is inositol 1,4,5-trisphosphate ; and any change in the composition of the phosphate groups on the ring or increase or decrease in their numbers will **inactivate** the molecule . In the case of increasing; if the phosphatase presents then the dephosphorylation will take place on the 1st , 4th or 5th phosphate but **not** the extra one(this will prevent being active again).

NOTE: some patients who suffer from depression and psychological disorders can use **lithium ion** which inhibits phosphatases, so IP3 will stay active, and the patient becomes more active . so its an antidepressant drug.

This pathway of signal transduction is dependent on the calcium but why calcium ¿¡ what specific about it ¿

▪ The concentration gradient (the difference of concentration among the cytosol and ER) of the Ca is very high equals around 10,000 folds(so any small change in conc. In cytosol will have a big effect).

▪Ca has positive charge makes it able to interact with the negatively charged amino acids of the proteins and enzymes.

▪Ca can bond (6-8) bonds with water, polar amino acids and negatively charged amino acids means it is stable and strong and by its many bonds can induce conformational changes and change the activity.

▪Ca is a bulky molecule when it comes to bind the protein; it induce conformational changes as to fit.

Pardon us for any mistakes ☺

وكل عام و أنتم بخير