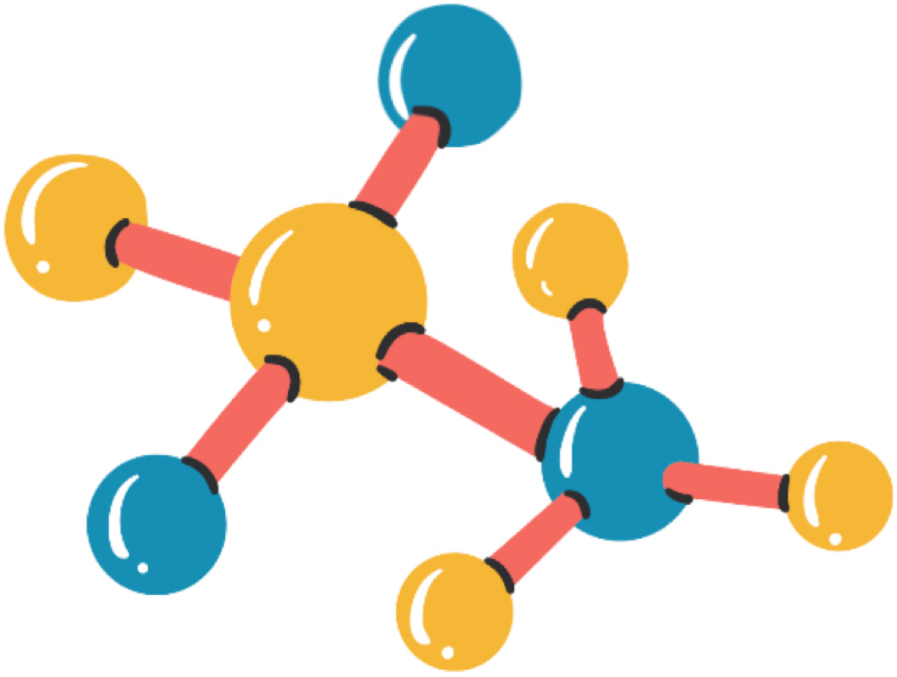
Biochemistry



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Regulation through conformational changes:

These regulatory mechanisms include:

A. Allosteric activation and inhibition.

B. Phosphorylation or other covalent modification.

C. Protein-protein interactions between regulatory & catalytic subunits or between two proteins.

D. Proteolytic cleavage.

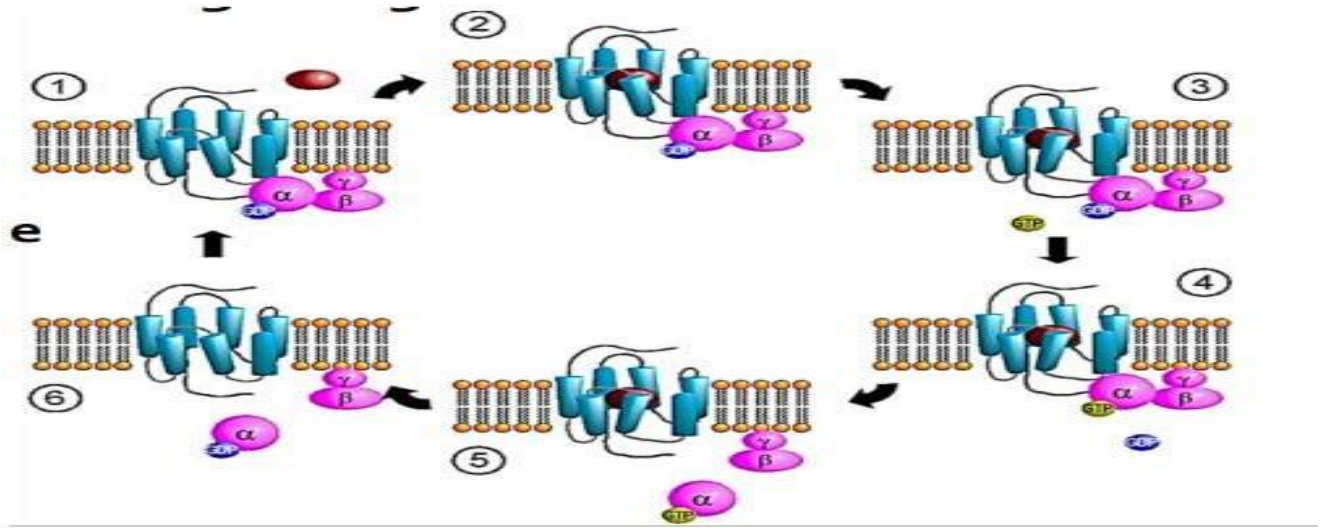
Conformational changes from protein-protein interactions:

G-protein: a family of trans-membrane proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signaling factors

G-protein is usually 3 subunits:

1- Alpha which stands by itself, and it has a fatty acid that connect it to the membrane from inside.
2- Beta and 3- Gamma: Beta is connected to Gamma and Gamma has a fatty acid that connect it to the membrane from inside.

So, Beta-Gamma works as a dimer and Alpha works as a monomer, the three subunits are associated together.



When a hormone binds to the cell surface, the receptor will have conformational changes because it's a protein → the conformational change will affect its relationship with the G-protein (it will affect alpha subunit because alpha is the one that is attached to it directly) → the conformational change in Alpha will make it exclude the GDP (leave it), it can't phosphorylate the GDP which it has → it removes the GDP and replace it with a new GTP → once it's attached to GTP another conformational change will happen, the affinity between the Alpha & Beta-Gamma now is very low → Alpha will be released(not released exactly, it starts moving in the inner surface membrane) because it's detached from Beta-Gamma and looks for aprotein or an enzyme which it can bind to (ex. Adenylate cyclase which can convert the ATP into cAMP (Recall: the cAMP ha an effect on PKA))

So, how to end this?

→ Because Alpha subunit has an internal enzymatic activity which acts slowly (it has a slow hydrolytic activity, the Alpha subunit works as a hydrolase; it hydrolysis phosphoanhydride bond which present between the phosphate groups) → it removes one phosphate from GTP and we will have GDP again → once it's GDP; it comes back to its original shape → conformational change will happen again

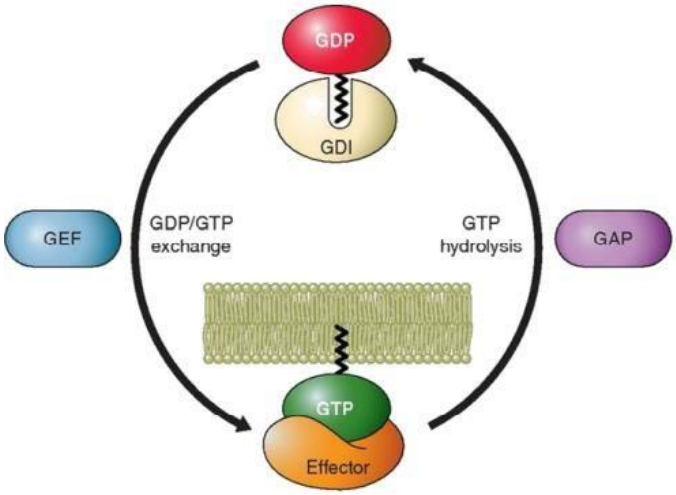
and the affinity for Beta-Gamma will return high, and Alpha will bind again to Beta-Gamma and the receptor and we are finished.

أول مرة شلنا ال GDP و حطينا بداله ال GTP, فارتبطت ال Alpha مع ال GTP و رجعتها ل GDP , وهيك رجع البروتين لشكله الأساسي.

Why did we talk about G-protein in an enzyme lecture?!

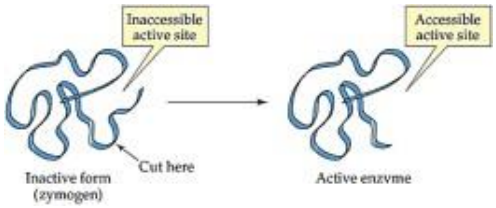
Because they work as enzymes; the Alpha subunit has an internal hydrolytic activity which works slowly.

There are other forms of G-protein which are not trimeric in their nature, we call them monomeric G-proteins (has one monomer); it behaves exactly like the Alpha subunit (exchange between the GDP and GTP) → once the GTP is attached slow hydrolases of GTP into GDP occurs → if the GTP is bond its active, if the GTP is hydrolyzed to GDP it's inactive and it gets back to its original shape.



Example: RAS protein.

Proteolytic Cleavage Zymogens (pro-or -gen)



You cut something from the enzyme irreversibly usually at the N-terminus (hydrolytic reaction); it won't come back again; you produce an inactive form of an enzyme then you cut a piece of it to become active.

Why do I synthesize an enzyme in an inactive form and then when I need it, I will convert it to an active form?

1. Place of synthesis for the enzyme is different than the place of acting, I'll send it to another place to work on, so I should synthesize it in an inactive form, once it reaches the place where it should act it will be activated by proteolytic cleavage.

2. To reduce time response; in the case of blood clotting, I won't wait for a cut to happen to produce the enzyme (I have bleeding, life threatening) so you need the enzymes to be there all the time; you produce them in an inactive form and keep them there once you need them you will only activate them.

Zymogens: enzymes that are synthesized in an inactive form and then they will be converted to their active form through proteolytic cleavage (I'll cleave part of the enzyme to open the active site).

How can I know these zymogens?

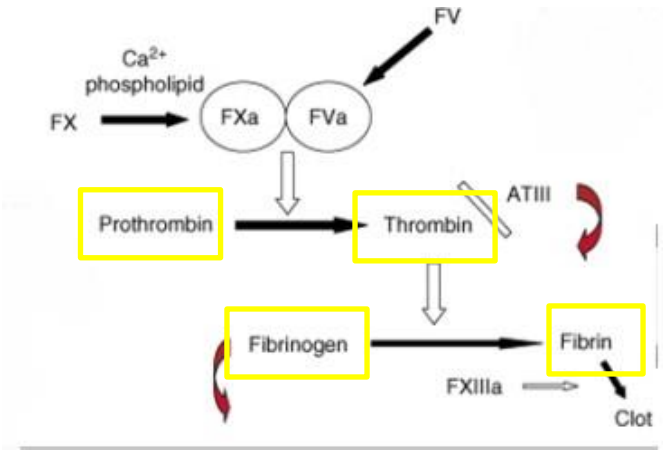
From their name, you must memorize them, the name either will be preceded by the prefix (pro) or it will be followed by the suffix (gen).

Examples:

Pepsinogen in the stomach will become pepsin, why do we need it to stay in the stomach? so once you eat you won't go and produce the enzyme you will only activate it. If you will wait to produce the enzyme you may enter a hypoglycemic shock while waiting because you're eating to absorb energy.

When you eat, the parietal cells will start secreting HCl in the stomach, and it will activate some pepsinogen and it becomes pepsin, once the pepsin is active it will activate all other molecules of pepsinogen (we call it auto catalytic activation).

- Trypsinogen becomes Trypsin.
- Chymotrypsinogen becomes Chymotrypsin.
- Pro-Carboxy peptidase a becomes Carboxy Peptidase a.
- Pro-Carboxy peptidase b beco mes Carboxy Peptidase b.
- Pro-Elastase becomes Elastase.
  - ➢ These enzymes are secreted from Pancreas (digestive enzymes).
- Blood clotting: The soluble protein fibrinogen is converted to the insoluble protein fibrin.



We finished controlling enzymes through conformational changes.

Non-Specific Regulators:

This mechanism can affect any enzyme regardless of their shape, reactants, products, conformation and structure.

These regulators affect the common things between proteins, since all proteins have peptide bonds, hydrogen bonds, hydrophobic interactions and affected by heat, pH, etc. (we are talking about anything that make the enzyme in its 3D shape).

Regulation through Changes in Amount of Enzyme:

All the ways that we discussed before were too fast in regulating enzymes, it occurred very sharply and very shortly, but this way of regulation needs time (slower), this is why we call this way of regulation an adaptive way (it needs time maybe an hour, days or months, until the body is adapted to produce a certain number of enzymes which matches body's situation.(it adapts your body according to the situation you are in, according to your age, environment, gender, living place ... etc.).

A. Regulated Enzyme Synthesis:

- Regulated by increasing or decreasing the rate of gene transcription (induction & repression) ♣ Usually slow in humans (hours to days)
- Sometimes through stabilization of the messenger RNA.

B. Regulated Protein Degradation:

- Can be degraded with a characteristic half-life within lysosomes. ♣ During fasting or infective stress: gluconeogenesis increase & synthesis of antibodies (protein degradation increases).
  ♣ Increased synthesis of ubiquitin

Effect of Temperature:

As temperature increases, the kinetic energy of molecules increases so the reaction rate increases, but it increases to a limit.

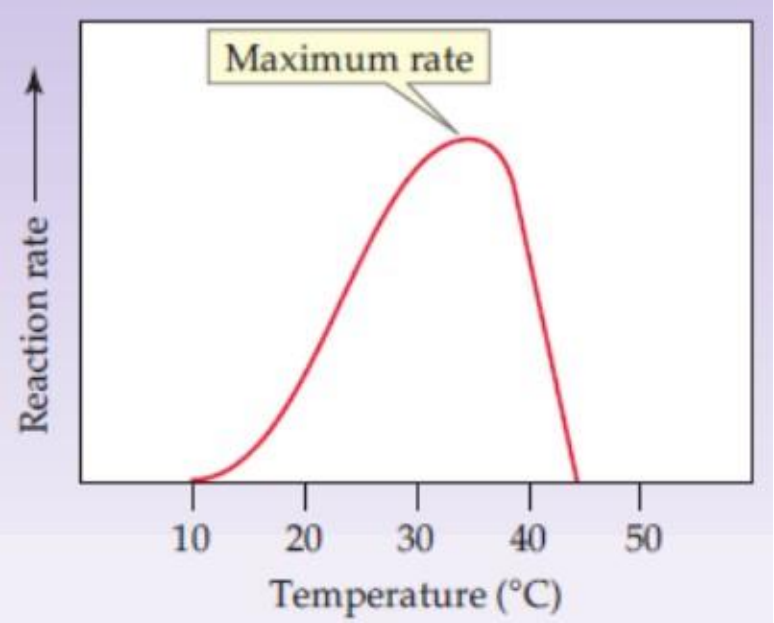
Protein denaturation: most proteins when the temperature increases above 40 C starts to denature and some of them start to denature above 50 C, that's why fever is dangerous. Also, we use very high temperatures while sterilization; to kill any mode of life.

Side info: when the temperature in a human is above 42, almost all proteins are denatured, so the metabolism will stop and the person will eventually die.

During surgeries we attach the major vessels to a pump to supply the organ we're dealing with, metabolism shouldn't stop in our bodies otherwise tissues will die (if oxygen is not delivered to your tissues a thrombus will happen). In certain surgeries when I deal with major vessels like Aorta, we decrease the temperature of the patient so the reaction rate and metabolism will decrease.

Not only increasing temperature affects enzymes but also decreasing it.

*Effect of temperature is general, there is no optimum temperature. (no specific number).



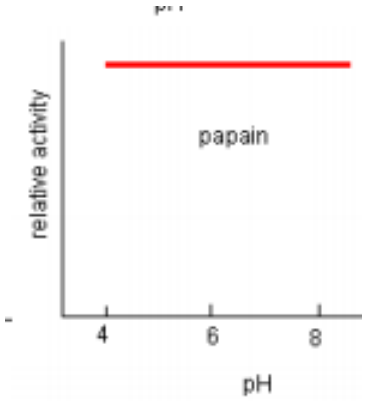
*Temperature affects all enzymes regardless of their nature, whenever you increase the temperature the reaction rate will increase, but it increases to a limit.

Effect of pH:

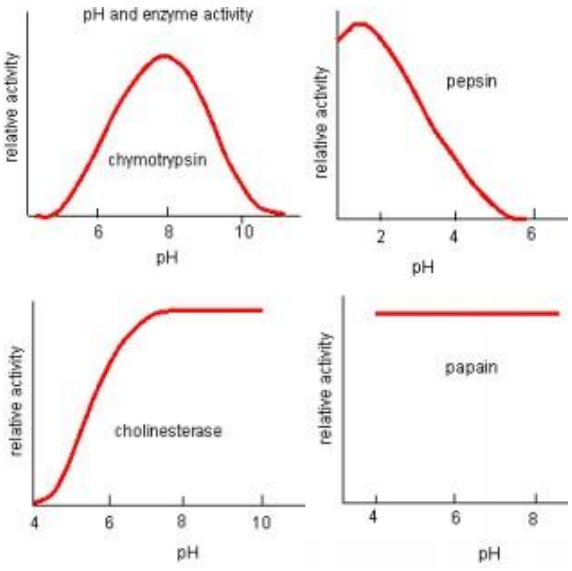
- The effect of pH is an enzyme dependent.
- Changing the pH will change the protonation state on certain amino acids.

When protonation changes, if it was negative and then lost its electron it will lose its ionic interaction between negative and positive.

If the enzyme has a very low ionic interactions or salt bridges, does changing the pH affects the catalytic activity of the enzyme? No, we call this state papain, changing the pH the catalytic activity doesn't change.



- Most enzymes have their max activity between (5-9).
- Extremes of pH denatures protein.

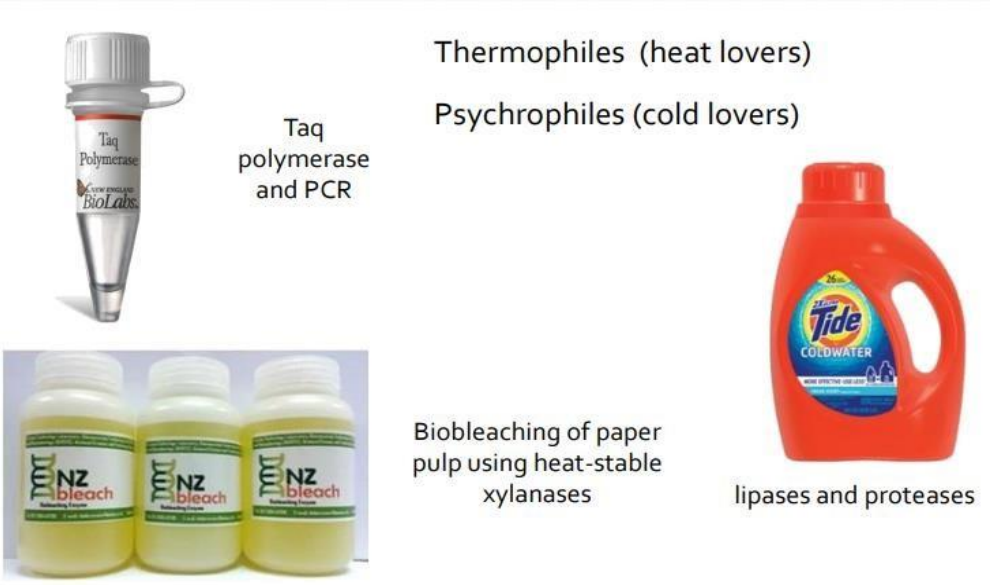


- Pepsin works when pH is around 2, when it reaches the intestine, it's inactivated because the pH increases.
- Chymotrypsin works in intestine where pH=8.(Affected by both, increasing and decreasing pH).
- Choline esterase maximum activity is around pH=6 and it keeps its activity regardless increasing pH. (Affected only by decreasing pH).

Exteremozymes

Enzymes that work under harsh conditions (extreme pH, very high or low temperatures).

They were discovered from the living organisms that live in hot springs.



Abzymes- cutting edge science

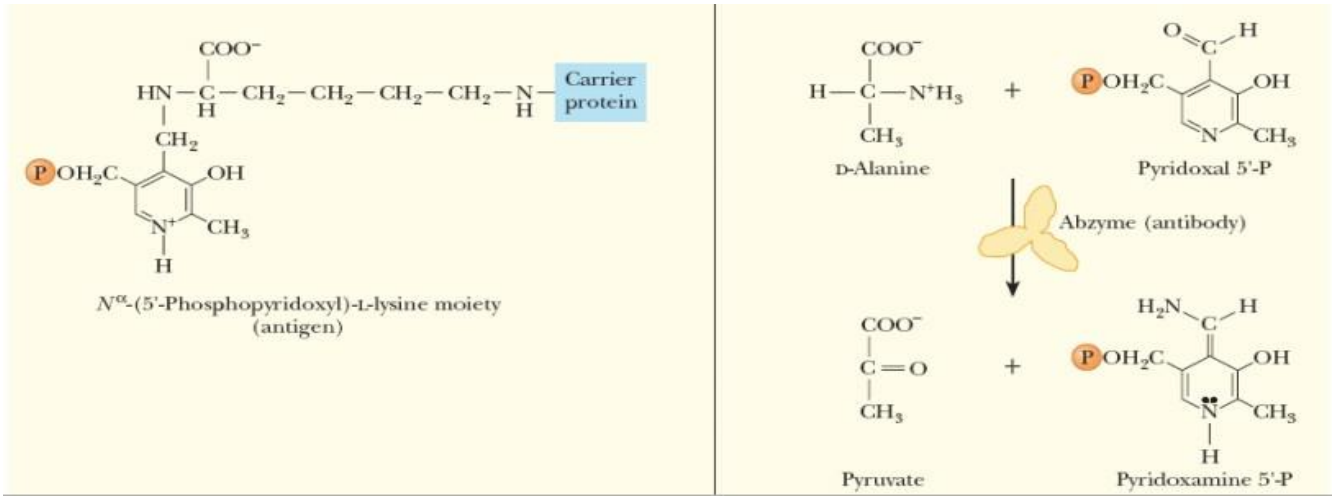
Abzyems = anti body + enzyme, abzymes are anti-bodies which acts like enzymes, and they work more effectively compared to enzymes.

We said before that we can't synthesize a transition state (a substrate in a transition state), but the idea here that we synthesize a material which looks like transition state (analog, but not an inhibitor) and inject it in an animal, so the animal will synthesize antibodies against it (because its foreign).

And as we know that antibodies are proteins and the molecule is like a transition state, so the binding between them will looks like the active site.

→ Then we extract the antibodies from the animal to use them, now antibodies have a catalytic activity (they don't have it normally) because they were synthesized against molecules which look like the transition state, and it will have higher affinity of binding to substrate (because it's very specific to an antigen that we are delivering to it (the transition state)) than enzyme.

- We call these catalytic antibodies → Abzymes.
- Remember that it is produced in animals.



Ribozymes

When we defined the enzymes, we said that they are protein enzymes with exception to ribozymes (they are RNA molecules which work like enzymes).

The catalytic efficiency of catalytic RNAs is less than that of protein enzymes but can greatly be enhanced when they are bound to protein subunits.

Note: they don't catalyze any reaction; instead, they catalyze a reactions which are specific for RNA molecules.

They catalyze splicing reactions and are involved in protein synthesis, examples: telomerase & RNase P.

Regulation of metabolic pathways

Single reactions rarely occur in your body, instead, they occur in series of biochemical reactions where every reaction is leading to other reaction till we achieve the final product, these series are called metabolic pathways.

• There are different types of metabolic pathways:

Linear pathways: every reaction is leading to the next reaction till we achieve the final product, and every reaction is catalyzed by a different enzyme.

Cyclic pathways (for example Krebs cycle and urea cycle): it's the same as linear but in the end, we regenerate the first molecule that we started with.

Spiral pathways: same as linear ones but each step is catalyzed by the same group of enzymes.

When glucose goes through glycolysis to make pyruvate, it goes over ten step reaction pathways! So, to cut glucose into two three carbon unit molecules named pyruvate it has to go through 10 steps.

- Now we have moved from enzymatic reaction to the metabolic pathways, and how they are controlled and regulated.

It does not make sense to control each step (enzyme) in a pathway in the same level of regulation and restriction, this doesn't mean that enzymes aren't regulated in pathways, they are regulated but not in a high manner because that needs a very large amount of energy.

So, we are looking at how the body is dealing with our metabolic pathways.

Principles of Pathway Regulation

1) Counter regulation of opposing pathways.

Cells for example build up molecules in cytosol and degrade them in mitochondria and this decreases mistakes possibility because some of opposing pathways require the same enzyme that turns the substrate to product and turns the product to substrate.

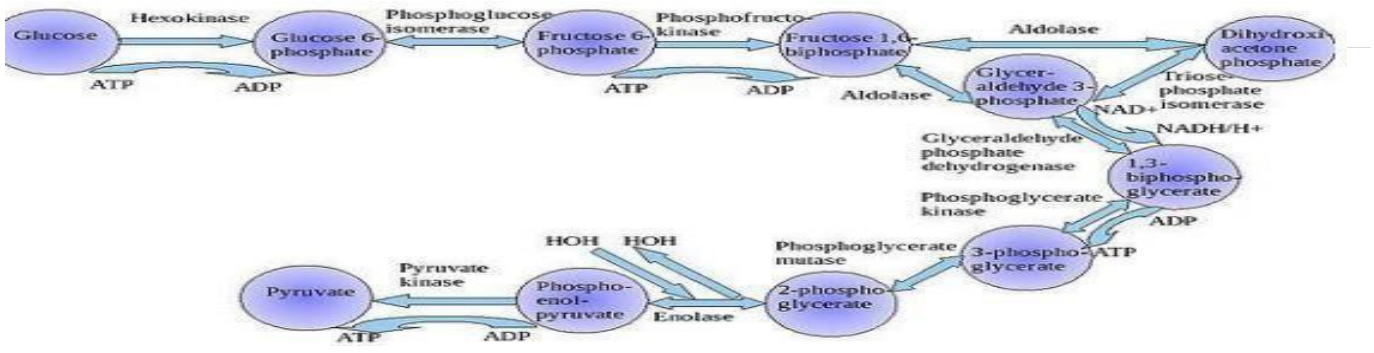
2) Isozymes.

Isozymes made a huge contribution to the regulation of metabolic pathways and that's by expressing each isozyme in the tissue that it works inside.

3) Regulation at the rate-limiting step.

- Each pathway has a rate limiting step at which they are regulated in a very high manner using high amount of energy, for clarification, The regulation happens on the enzyme that is responsible of the rate limiting step.
- It is the slowest step in the pathway so when it occurs everything after it will be done faster (i.e., if there is ten men holding each other's hands, and they are trying to run as fast as possible, their speed will be determined by the slowest one of them even if Usain Bolt is with them) & is usually not readily reversible.
- The enzyme of the rate limiting step has high K_m value towards its substrate.

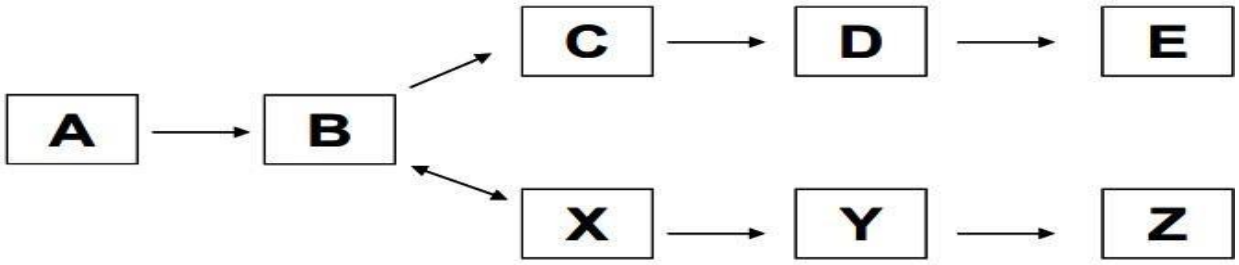
Usually, it's the first committed step in a pathway.



4) The committed step.

- A committed step in a metabolic pathway is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return.

For example, when glucose is phosphorylated inside the cell after that it may build glycogen, or it can enter glycolysis, or it can be degraded into lipids. So, it can go in different pathways, each one of them has its own committed step that when glucose goes through it, it will be committed to complete the pathway of that committed step.



In this example, the committed step for making product E is (B → C), not (A → B).

The committed step for making product Z is (X→ Y), not (B→X) because it's a reversible step and if X is turned to B, it may be converted to C and it will go the second pathway.

❖ Keep this in your mind: usually in metabolic pathways the committed step is the same as the rate limiting step (usually NOT always), so if you want a specific product, you will go to the committed step of it and regulate it.

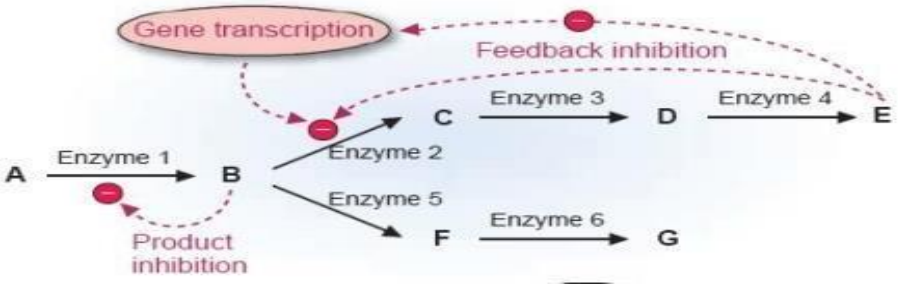
5) Feedback regulation.

- ❖ It is always logical to have feedback regulation because this makes a huge difference in energy consumption.
- ❖ This type of regulation is much slower to respond to changing conditions than allosteric regulation.
- ❖ There are three types of feedback regulation:

1) Negative feedback regulation (feedback inhibition): ones the final product of the pathway increases up the physiological limits, it will come and inhibit the first enzyme in the pathway, and this always makes sense.

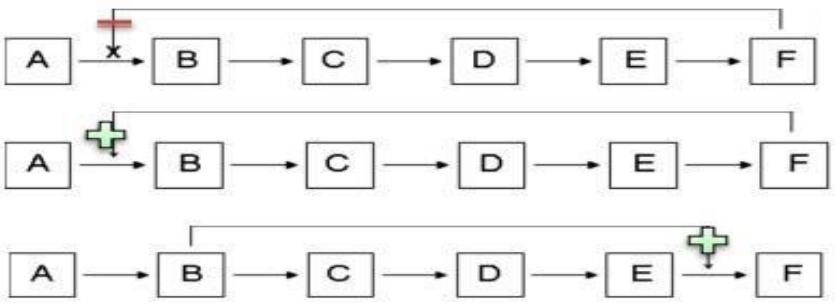
2) Positive feedback regulation: the final product will come back and increase the activity of the first enzyme in the pathway, and this makes sense for some cases.

3) Feed-forward regulation: one of the intermediate products



goes and activates an enzyme ahead of it, and thismakes sense because we want enzymes that convert toxic products to non-toxic ones to be ready all the time, so ones the toxic product is made, these enzymes convert it to non-toxic product immediately.

(In the example below, we want the enzyme that catalyzes E→F to be ready to work before E is produced)



6) Enzyme compartmentalization.

It is a way of regulating metabolic pathways by synthesizing certain enzyme which are required for certain processes and then localizing them in certain compartment where the need of them in that compartment, this is done by signaling because nothing in your body understand like you.

وقفة تدبرية: تدبر قوله سبحانه: "وَفِي الْأَرْضِ آيَاتٌ لِلْمُوقِنِينَ * وَفِي أَنْفُسِكُمْ أَفَلَا تُبْصِرُونَ"

In other words, its a mechanism by which rate of reactions become faster, reducing area of diffusion. In this way, enzymes are sequestered inside compartments where access to their substrates is limited.

- For example, enzymes of Krebs cycle are synthesized in the nucleus by mRNA then expressed in cytosol by ribosomes then it will be transferred to the mitochondria to work inside it, so by making compartmentalization you will decrease the time needed for any material to find the enzyme needed for it.
- Lysosomes: proteins get transported to lysozymes, and hydrolytic enzymes are also expressed inside it, this compartmentalization also happens in Golgi apparatus,mitochondria, etc.

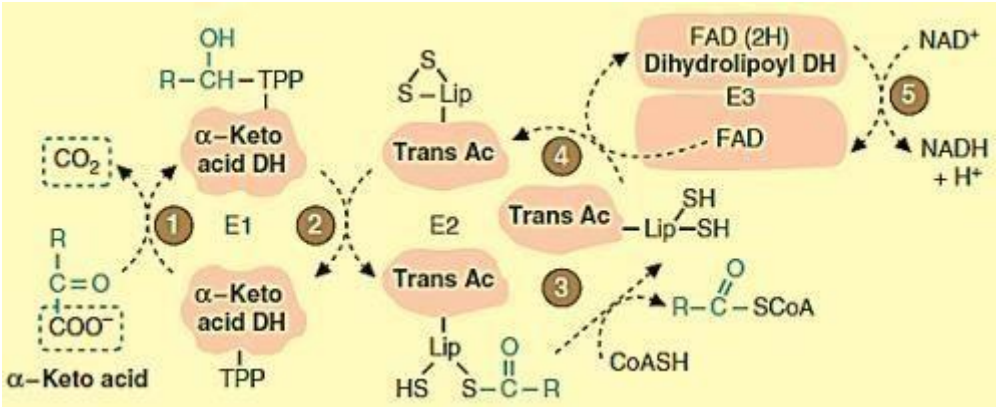
7) Enzyme complexing.

If you needed a reactant to make you a product and this product is not used by any enzyme except by a certain enzyme, so let the first enzyme to bind to the second enzyme in a complex and the second bind to the third and so on. So whenever you hear a name of enzyme followed by the name complex expect to find more than one enzyme gathered together where each enzyme doing a separate function.

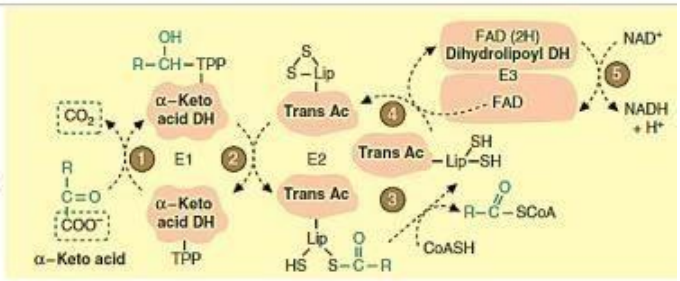
- In our example, there are three enzymes, its pyruvate dehydrogenase which converts pyruvate into Acetyl coA, Acetate is two carbon unit and pyruvate is three carbon unit, so there is a shortage of one C which gets out as CO_2, so the first enzyme does the decarboxylation, the second does the trans acylation, the third one does the dehydrogenation, and this is why we called it pyruvatedehydrogenase complex. (Taking the name from the last step and adding the word complex so we don't say just pyruvate dehydrogenase).

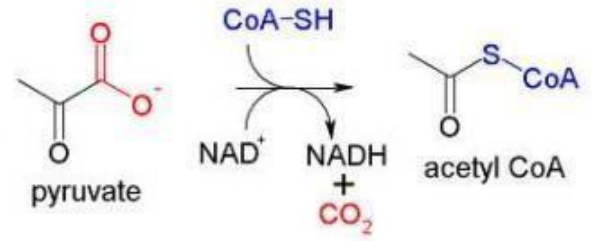
The doctor explained the figure bellow as follows: -

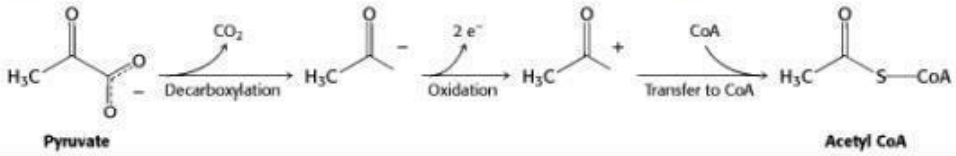
- In the first reaction, by the enzyme number one which worksas decarboxylase, CO_2 gets out from pyruvate, and you will be left with a two-carbon unit having C double bond O (carbonyl group) at its terminal which is not stable.
- The second enzyme takes this unstable product and with the help of the coenzyme lipoic acid it gives you the productAcetyl coA, so it makes the carbonyl group which reactive tobe stabilized by binding coA (we said previously that coA works as trans acyl carrier group, it transfers the acyl group that you want from one place to another because its reactive) and the product of the reaction is acetyl coA.
- The process in the second reaction gives you more H, so theenzyme must get rid of them, thus these hydrogens will be taken out by the third enzyme to the FAD molecule so it becomes $FADH_2$ and this is not the original form of the enzyme, the two hydrogens will be taken by NAD^+ and the product of the reaction will be NADH, so the third enzyme (E3 in the picture) works as a dehydrogen



- *7. Enzyme complexing (A multienzyme complex)*
- Complexing various enzymes that share one process
- Product of enzyme A pass directly to enzyme B
- Pyruvate dehydrogenase (mitochondria) 3 enzymes: decarboxylation, oxidation, & transfer of the resultant acyl group to CoA







قال رسول الله ﷺ : " مَن جلسَ في مجلِسٍ فَكَثرَ فيهِ لغطُهُ ، فقالَ قبلَ أن يقومَ من مجلسِهِ ذلِكَ : سُبحانَكَ اللَّهمَّ وبحمدِكَ ، أشهدُ أن لا إلَهَ إلَّا أنتَ أستغفرُكَ وأتوبُ إليكَ ، إلَّا غُفِرَ لَهُ ما كانَ في مجلِسِهِ ذلِكَ " (رواه أبو داوود والترمذي).

تم بحمد الله