Lecture :..23.....

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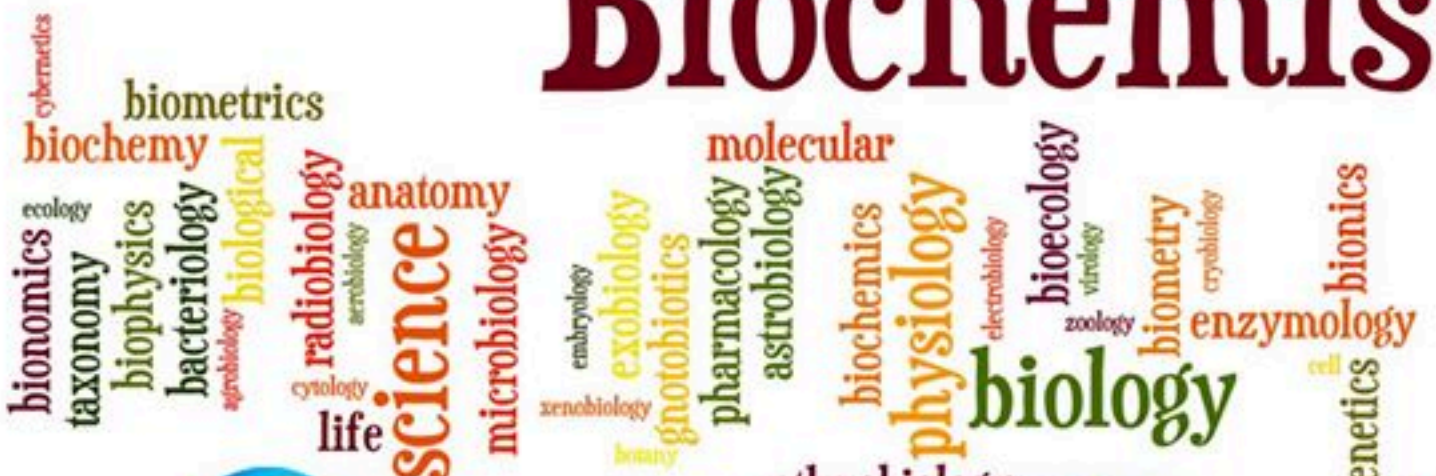
☐ Slide ■ Sheet

Medical Committee
The University of Jordan

Biochemistry



Mousa Suboh

Regulation Through Conformational Changes

These regulatory mechanisms (conformational changes) include:

1. Allosteric activation and inhibition.
2. Phoshorylation or other covalent modification.
3. Protein-protein interactions between regulatory and catalytic subunit or between two proteins.
4. Proteolytic cleavage.

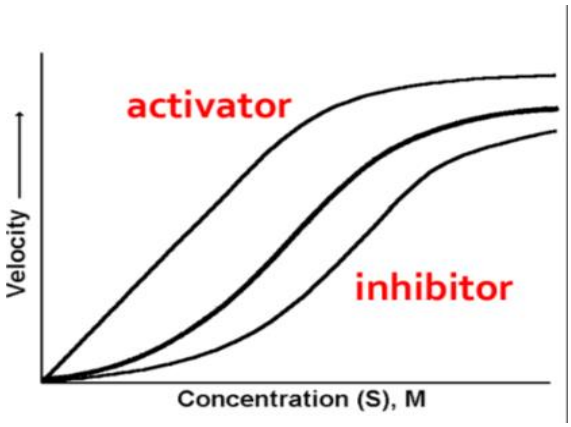
These types of regulation can rapidly change an enzyme from an inactive form to a fully active conformation.

Quick revision:

In the previous lectures we have taken two of these mechanisms:

First one: allosteric enzyme activation and inhibition as a model of regulation.

You have to know how this model works as well as to be able to understand and read the sigmoidal shape of it and how this shape is affected by inhibitors and activators.



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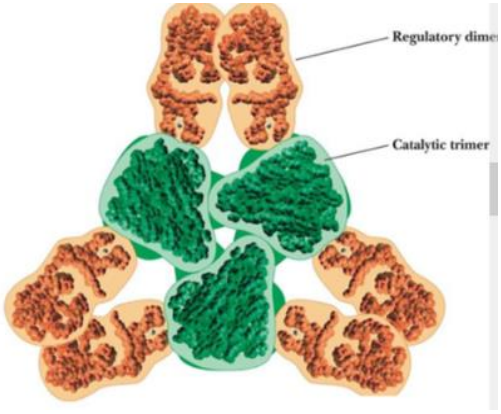
Also, we have taken an example about allosteric enzyme regulation which is ATCase (Aspartate carbamoyltransferase), you have to know the structure of ATCase which consist of six regulatory subunits (3 regulatory dimers) and six catalytic subunits (2 catalytic trimers).

In addition to that, you have to know the allosteric inhibitor (CTP) and the allosteric activator (ATP) for ATCase.



Second one: phosphorylation as a model of regulation.

The example that we talked about is glycogen phosphorylase.

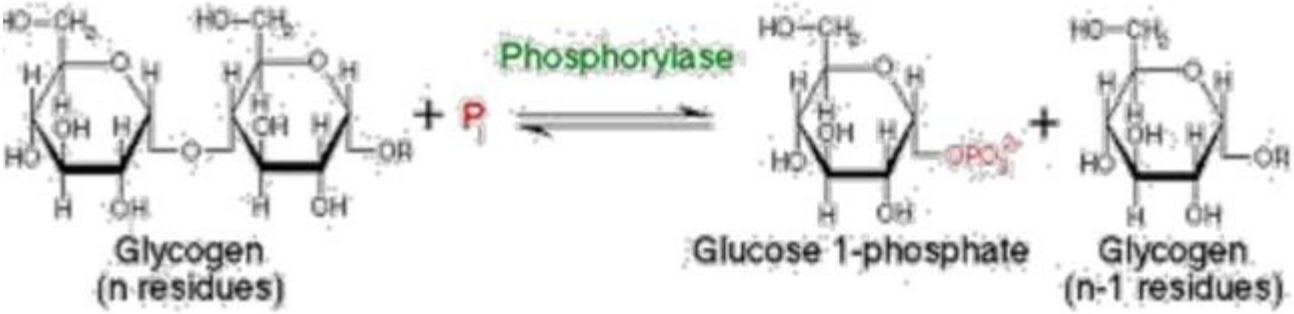
You need to know its purpose and when and why we need it.

You need to know also the activators and the inhibitors for this enzyme. It can be activated by (AMP) allosterically , why? Because of the low ATP content due to exercise so you need glucose to make ATP (High concentration of AMP). It can be activated also by phosphorylation by the glycogen phosphorylase kinase which is regulated by:

A. AMP B. Protein kinase A C. Ca++/Calmodulin

The doctor mentioned protein kinase A, how it works and its structure, which is formed from two regulatory subunits and two catalytic subunits.

This is the inactive form of protein kinase A (two regulatory and two catalytic subunits), how it becomes active? Through binding of 4 cAMP molecules as regulatory materials to the regulatory subunits which cause the dissociation of the two catalytic subunits, and now the protein kinase A is active. After becoming active it can phosphorylate other enzymes or proteins and make them active or inactive (because phosphorylation does not mean activation all the time). One of the enzyme that protein kinase A phosphorylate is glycogen phosphorylase kinase which becomes active and causes the splitting of glucose subunit form glycogen to make ATP for the body.



How cAMP can be increased? By adrenaline which is secreted in the stress situation.

Third one: protein-protein interaction as a model of regulation.

How interaction between proteins can activate or inactivate specific enzymes and how they regulate enzymatic function for G proteins?

First, what is the G protein?

G protein is specific type of protein involved in transmitting signals. There are two types of G proteins:

1. `*Small monomeric G proteins/contain one monomer.
2. *Large heterotrimeric G proteins.

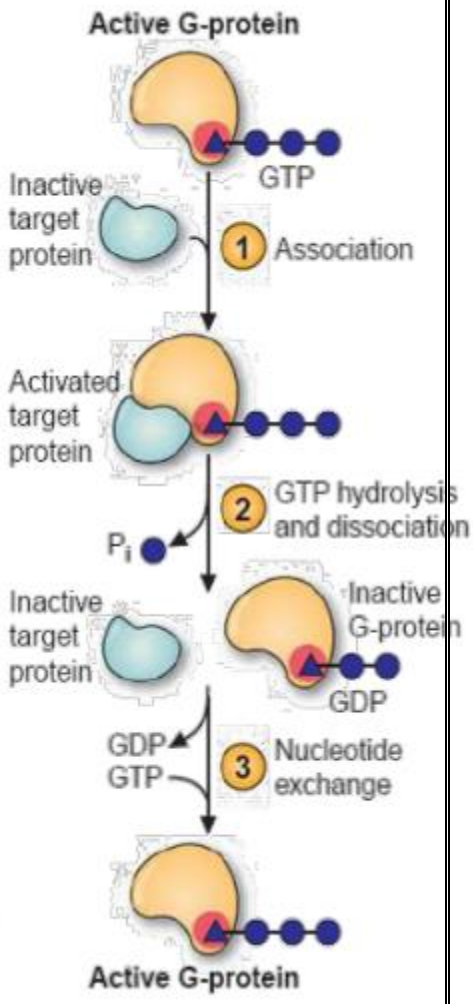
Why they are called G proteins? Because they are activated through GTP (guanosine triphosphate).

SMALL MONOMERIC G PROTEIN:

We have to know these two things before we start explaining protein-protein interaction as a model of regulation:

- G protein + GTP >>>> active and can bind other proteins.
- G protein + GDP >>>> inactive and cannot bind other proteins.

When the small monomeric G protein binds GTP, it becomes active and after that it can bind other proteins and activate them (transfers these proteins from the inactive state to the active state). These activated proteins (by G proteins) can now do a function, during that time (functioning of the activated protein) GTP will be exposed to slow hydrolysis (breaking down certain bonds by water).



Note: hydrolysis of (ATP/GTP/CTP) is accomplished by adding water molecule

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between phosphates so this causes the splitting of one phosphate, two phosphates..... from the whole molecule. (because they are made through condensation RXN)

So GTP is exposed to slow hydrolysis which will cause the exit of one phosphate group from the GTP and GTP is converted to GDP. Once GTP is converted to GDP there will be conformational changes in the G protein which will cause the splitting of G protein from the target protein and the target protein becomes inactive.

After that we can have the active form of the G protein again by converting GDP to GTP (adding another phosphate group to GDP to convert it to GTP) and it can activate another proteins. So we regulate the action of these proteins by the conformational changes between G proteins and the target protein.

This process (protein-protein interaction) can be regulated by accessory proteins, such as:

GAPs (GTPase-activating proteins).

Increase the rate of GTP hydrolysis (converting GTP>>>GDP) which will cause the dissociation of the target protein from G protein. (Inhibits the process)

GEFs (guanine nucleotide exchange factors).

Increase the rate of GTP exchange for bound GDP so they are activating G protein.

Remember >>> G protein + GDP >>>>> inactive.

G protein + GTP >>>> active

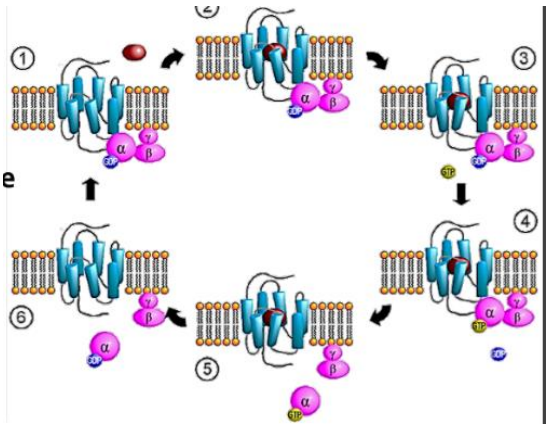
GDIs (GDP dissociation inhibitors).

They prevent GDP dissociation, so they prevent the replacement for GPD with GTP and as a result they keep G protein in the inactive state.

LARGE REGULATORY G PROTEINS

This type of G protein consist of two parts, first part is in the membrane which is affected by hormones and the second part is in the inner surface of the cell membrane we have three subunits (alpha/beta/gamma).

- G protein + GDP >>>> inactive + all complex act as one subunit
- G protein + GTP>>> active + a-subunit dissociate from the whole complex.



When the GTP replaces the GDP which is connected to a-subunit, there will be conformational change causes the affinity of a-subunit to decrease for the other subunits (beta/gama) and this causes the

dissociation of a-subunit, after that, the dissociation of a-subunit will activate or deactivate a specific protein.

Note: ai-subunit ›››› inhibitory a-subunit.

as-subunit ›››› stimulatory a-subunit.

So the dissociation of a-subunit does not mean all the time activation, it either causes activation (as-subunit) or inhibition (ai-subunit).

When the a-subunit which was connecting with GTP bind with GDP (replacements GTP with GDP) the affinity of a-subunit increases for the other subunits (beta/gama) and it will bind with whole complex.

Fourth one: proteolytic cleavage as a model of regulation.

What does it mean? Breaking down part of the enzyme (protein) through proteolytic process, this means that you are breaking down peptide bond.

What are the enzymes that we need to break part of them to make them active? We called them zymogens

Zymogens are the enzymes secreted in the inactive form and then will be activated, but why we need that (secret the enzyme in the inactive state and then convert it to the active state)?

1. For energy conservation (this is the main reason for protein regulation). You don't need it now; you will activate it when it is needed.

2. Because sometimes the body synthesize the enzyme in one place and it's required in another place, you don't need it to be active at place of synthesis and this what happens mostly with the digestive enzymes that are synthesized in pancreas and are active in the intestine.

To know that the enzyme is synthesized in the inactive form and then converted to the active form we use this way of naming:

The name of the enzyme begins with the prefix (pro-), or the name of the enzyme ends with the suffix (-gen) these indicate that the enzyme is in the inactive state, for example:

- Pro-thrombin (inactive state) is converted into thrombin (active state) during the process of blood clotting, after that the thrombin exert its function to convert fibrinogen(soluble) to fibrin (insoluble) which causes blood clotting.
- Pro-carboxypeptidase A and Pro-carboxypeptidase B are digestive enzymes secreted from the pancreas and function in the intestine.
- Chymotrypsinogen is converted to chymotrypsin by protyolytic process through the protease Trypsin ; during this process you remove two dipeptide from the molecule.
- Pepsinogen which is converted to pepsin by the acid (HCL) after that by the pepsin itself. It undergoes autocatalysis to convert more pepsinogen into pepsin.
- Trypsinogen is converted to Trypsin through a proteolytic process by Trypsin itself and other materials in the intestine. (autocatalytic activation)

Regulation through changes in amount of enzymes

We can regulate the amount of the enzyme by two ways:

1. Control the enzyme synthesis (increases it or decreases it).

2. Protein (enzyme) degradation.

What is the difference between these two methods and all the previous methods of regulation?

The speed, these two methods are very slow in comparison with the previous methods because in order to control the synthesis or the degradation of an enzyme we need hours, days and sometimes weeks. On the other hand, the previous methods of regulation are instant and control the enzyme right away (activation or inhibition).

If these two methods are very slow in comparing with the other methods, why do we need them?

When you are exposed to a condition or situation like illness or extreme changes in the environment you need higher or less protein(enzyme) concentration , these slow methods will control protein levels.(adaptation process)

The process of protein synthesis can be controlled not only by gene regulation that depends on synthesizing more protein (translation) by making more mRNA (transcription) but also by stabilizing the mRNA molecule. mRNA molecule is short lived, it makes one protein and then it will be gone, so when we stabilize mRNA it will make more

proteins than usual and this is a faster way to regulate synthesis of proteins (enzymes).

Protein degradation happens in two ways:

1. lysosomes pathway: which is specialized in proteins that come from outside the cell, these proteins undergo endocytosis and then they fuse with the lysosome to degrade.
2. Proteosomes pathway which is specialized in proteins found within the cell, this pathway is regulated by the binding of protein called ubiquitin, so when you need to increase the degradation process you can increase the synthesis of protein ubiquitin and then this protein will bind the proteins that will be degraded.

Non-specific regulators of all enzymes function

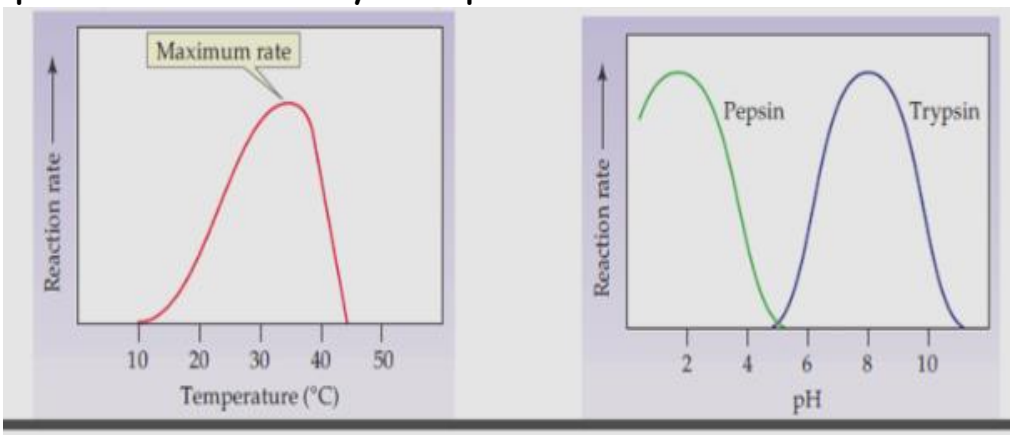
All the process that was mentioned before are specific for certain protein or groups of proteins.

We have two ways of regulation applied to all enzymes (non-specific) and they are: temperature(T) and pH.

How (T) affect enzyme (what is the relation between T and enzyme function)?

As you increase T the enzyme activity will be increased, why? Because when we increase T we increase the kinetic energy for the active site and for the substrate (more movement), so more collisions will occur and that will increase the percentage of the

productive collusions which causes reactions. So the more you increase T the more you increase the enzyme activity to a certain point which presents the enzyme optimal T.



(the T at which enzyme reach its maximal activity), after this point, as you increase T the activity for the enzyme will drop and the protein will start the process of denaturation which causes the protein to lose its shape and therefore its function.

Most enzymes denature at 50*C.

Human enzymes are most active at 37*C, but if we increase the T above 37*C this will increase their activity until they reach denaturation.

(Denaturation affect H-bonds/covalent bonds .... but does not affect peptide pond ... so T affect 2*/3*/4* structure for the protein but does not affect the 1* structure).

All our human metabolic processes are controlled by enzymes.

The idea behind controlling metabolic pathways by T is that it is used in hospitals during surgery ,for example doctors during cardiac

surgeries ,they connect a blood pump to the aorta so it will pump the blood into the aorta and the aorta will send this blood to all over the body and after that they can work on the heart without affecting the homeostasis, but when they want to work on the aorta itself how is the blood going to reach the whole body? In this condition they use another technique which is putting ice around the patient to make his body temperature very low in order to make metabolic processes very slow or stop them for a short period of time (around 40 min) and then after the surgery the patient get back to his normal state.

<u>How pH affects protein and what is the relationship (formula) between pH and protein activity?</u>

Change in the pH will lead to changes in the charges of amino acids that form the protein. As a result, it will affect the bonds by affecting electrostatic attractions/salt bridges/ionic interactions and this will lead to change in the protein overall shape and the protein loses its function.

The difference between pH and T:

Each enzyme has a specific optimal pH to work on, increasing or decreasing (not only increasing) the PH from the optimal one will cause protein denaturation, most enzymes in our body work around physiological pH if they were within cells or compartments.

Enzymes that work in large open spaces in the body work on the PH of that space, such as stomach and intestine.

EX. pH of the stomach is around 2 and the optimal PH for pepsin is also around 2 (pepsin is an enzyme found in the stomach)

Optimal pH for Trypsin is around 8 (usually it's between 5 and 9)

For example if we have an enzyme has an optimal pH around 7, so from 6 to 7 as you increase the pH the activity increases and from 7 to 8 as you increase PH the activity decreases.....And if the pH becomes below 6 or above 8 the enzyme (protein) denature .

There are groups of enzymes that can tolerate extremely high or low T and PH , they are called extremozymes, they are found in living organisms (such as bacteria) that live in very cold or hot environment.

Extremophiles that live in cold temperatures are called Pshycrophiles. Like Bacteria that live in the bottom of seas and oceans where temperature is very low.

Extremephiles that live in hot temperatures are called Thermophiles. Like Bacteria that can live in very hot geysers which reach boiling point temperature (100*C).

So we can take these enzymes which work in very extreme T or PH and use it for commercial use, for example:

- PCR (polymerase chain reaction): Which is a very important method in the genetics field, by PCR you can take a small piece of DNA and know the whole sequence of this DNA.For example, you can take a lock of hair from someone and after that you can know the sequence of the DNA, how is that? By having small part of the DNA

we can do polymerisation (converting the small piece into large piece) and this process (polymerisation) depends on the presence of enzyme called taq polymerase that work on high T and we can bring this enzyme from bacteria which live in very high T, this enzyme can tolerate T from 94 to 100*C .

- Bleaching of paper by xylanases, enzyme that works under very low pH.
- Tide (cleaning material) work on cold water, contain enzyme that can work in cold T such as lipases and proteases which breakdown fat, lipids and proteins that are found on clothes.

Principles of pathway regulation

All of these mechanisms of regulation that we have taken are controlled by principles that determine when do I need or need not enzyme regulation.

- **Counter regulation of opposing pathway:**

What is the meaning of biochemical pathway? It means a chain (series) of biochemical reactions.

All biochemical reactions in our body, mostly they are present in pathways.

We have this biochemical pathway (glycolysis) which converts glucose to pyruvate through a series of reactions.

What is the purpose of biochemical pathway?

It is synthesis or degradation of certain molecules.

There are two types of pathways: one of them is synthesis pathway(anabolism) that synthesize proteins or carbohydrates for example and the other is breakdown (metabolism) pathway, these two pathways share certain steps but they should be different also in certain steps (means different enzymes),and this is what we call it counter regulation of opposing pathways.

Why do not we have the same enzyme to regulate or to control both of these pathways (synthesis/breakdown)?

Because you mix them together and this will affect the function of the pathway, for example, you have two streets and the cars pass through those two streets in opposite direction ,it is better to split the two street with a barrier in order to prevent the cars from hitting each other, same principle apply to biochemical pathway, you have to split between synthesis pathway and degradation pathway by having enzymes in the synthesis pathway that are not found in the degradation pathway and also the opposite of that.

The pathways must be different at least in a single enzyme.

- **Tissue isozymes of regulatory proteins.**

It is always logical to have different copies of the enzyme which will act differently in different tissue.

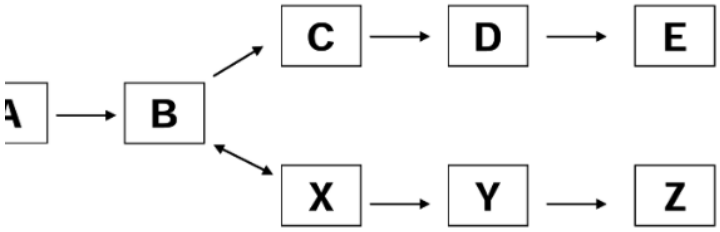
- **Regulation at rate limiting step.**

It is always logical to control the rate determining step (the slowest step in a reaction) why? Because this step will determine the speed of the entire reaction and if you try to affect another step rather than rate determining step it won't affect the rate (speed) of the pathway..

Rate limiting step has high Km which means low affinity and high amount of the substrate to achieve the control of it.

- **Committed step:**

You are walking in a pathway, (A) gives you (B) and from (B) you have two different pathways (B>>>C / B>>>X) giving you two different product (E/Z).



Why we call it committed step? Because B can give you either E or Z, so the conversion from B to C makes B committed to give you E and the conversion from B to X makes B committed to give you Z. (Commit means: be responsible for)

So it is always logical to control the committed step of a pathway, why? Because once you regulate it, you will have different pathways giving you different products.

Usually in a pathway the rate determining step is also the committed step, but it is not a must.

The committed step can be reversible or irreversible depending on the rxn. (mostly reversible)

- **Feedback regulation:**

It is always logical that when a certain material becomes excess as a product it must be regulated (increase or decrease the activity) for that enzyme that make it.

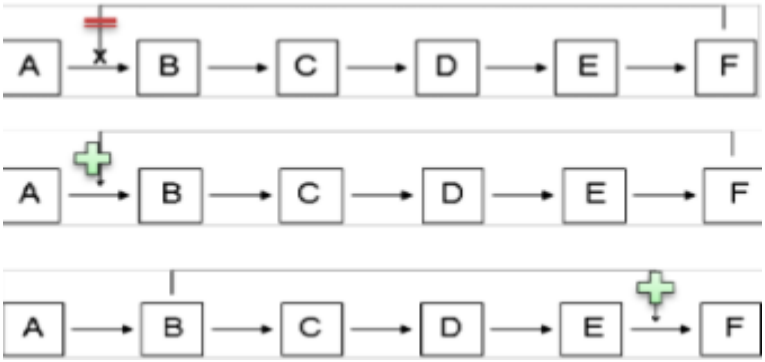
In our body we have two different ways for feedback regulation:

First one , we call it the feed-forward regulation, in this way if you increase the concentration of the substance at the beginning of the pathway this will cause the activation and the excitement of all the following steps. This is found in pathways that produce toxic compounds and waste.

For example, urea cycle which exclude urea from nitrogenous compounds and then urea is converted into urine. When the nitrogenous compounds increase in the body it will cause activation of the urea cycle to exclude more and more urine (the substrate increase the activity of the pathway /feed-forward regulation). When you reach the required level, substances in the pathways will cause inhibition.

Second one, we call it feedback regulation (positive or negative),in this way the final product of certain pathway affects certain steps in that pathway (increase or decrease the activity) ,so when you

have pathway increasing the last product and this product causes ether activation or deactivation we call it feedback regulation (activation >> positive / inhibition >>> negative).



In the negative feedback regulation or feedback inhibition the last product will inhibit the first enzyme in the pathway (rate determining enzyme or committed enzyme). The product isn't needed anymore.

Positive feedback regulation or feedback activation has the same function of forward-feed regulation but in different technique one uses the final product (feedback) and the other uses the substrate (forward-feed).

The positive feedback regulation and the forward-feed regulation both of them has relationship with toxic compound, both of them lead to increase the activity of the pathway to prevent intermediate toxins from accumulating in the body.

- **Enzyme compartmentalization:**

It is necessary that the enzymes that make certain process in certain place to put them in one compartment. For example, if you need to break down fatty acids so instead of making the required enzyme search the entire cell to find its substrate, we define a certain pathway for fatty acids to go through and we keep the enzyme in small space to preserve time effort and energy that are needed for the enzyme to catch its substrate.

Examples about enzyme compartmentalization:

- All energy metabolisms happen in the mitochondria except glycolysis that occur in the cytosol.
- Fatty acid degradation happens in the mitochondria while fatty acid synthesis happens in the cytosol.
- Degradation of extracellular proteins takes place inside ompartments found within the cells called lysosomes.

So there is a distinction in the process of (synthesis/degradation) inside the cell and that's what we call it compartmentalization.

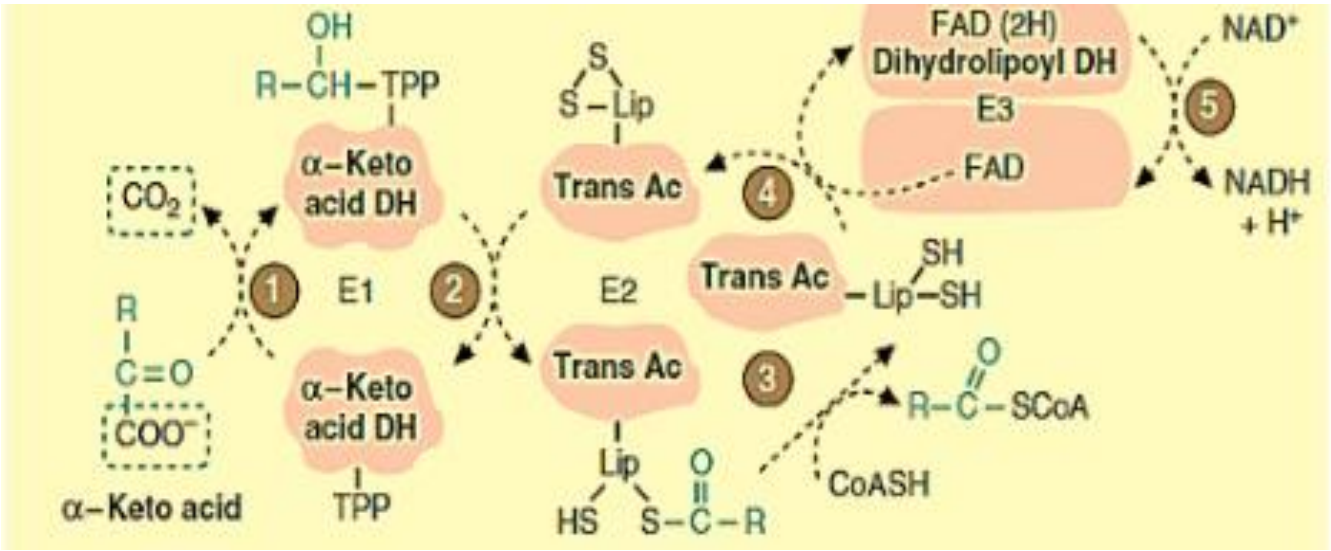
- **Enzymes complexing:**

For example if you have something and you can give it only to the one who sits beside you and when he take that thing from you he converts it to something else and then can give it only to the one who sits beside him .... so it is always logical to put you in one group and that's happens in the body ... you may have the product for the first enzyme only used to affect another enzyme and the product from

the second enzyme is only used to affect another , so I put them in one large complex called (multi-enzyme complex).

Inside the complex we have variable numbers of enzymes depending on the function that our body needs.

For example we have the multi-complex enzyme (a-ketoglutaric dehydrogenase or pyruvate dehydreogenase) that converts pyurvate into actyl coA…. this complex contain three enzymes (E1/E2/E3) E1 is (thiamine pyropshosphate) that convert 3 carbon pyruvate into acetate (through immediate decarboxylation reaction it forms acetate because of thiamine)after that E2 (transacetase) transfer the acyl group from acetate to coenzyme A forming aceytyl coA as a final product , we have another enzyme E3 (dehyrogenase enzyme) to convert the complex enzyme to its original state.



Ribozymes

At the beginning when we defined enzymes, we said that all enzymes are proteins except ribozymes, so what are ribozymes?

Ribozymes are RNA molecules capable to catalyze certain reactions, for example, they control the process of splicing RNAs and some of them control their own self-splicing process.

Catalytic efficiency of RNAs is less than that for proteins, why? Because the protein enzymes are larger in size comparing with RNAs so the number of bonds which make the active site are more in number and stronger and the regulation of the protein enzymes isbetter.

The catalytic efficiency for RNAs is greatly found when they are connected to other proteins.

Enzyme in medical diagnosis

The principle of enzymes that are used in medical diagnosis is that these enzymes are found in certain tissues in the body (there are enzyme for the brain,heart,liver,bone....) so when the cells in these tissues die this will cause increase in the concentration of these enzymes (that was found in these tissue before) in the blood , so when you take a blood sample for test you will recognize the damged tissue from the high level of enzymes in the blood.

According to that you have to know each tissue and its associated enzymes:

You have to memorise this table, and know each enzyme and the organ or the tissue which is damaged.

Creatine kinase (CK) (brain, heart, skeletal muscle)

Aspartate transaminase (AST)	Damage to heart or liver
Alanine transaminase (ALT)	Damage to heart or liver
Lactate dehydrogenase (LH)	Damage to heart, liver, or red blood cells
Alkaline phosphatase (ALP)	Damage to bone and liver cells

Enzymes in relation with medical diagnoses have isozymes , such as:

- LDH has five copies each found in a certain tissue / so after you detect LDH you have to know which isozyme in order to know the damaged tissue for example type 1 indicate a problem in the heart, type 5 in the muscles, type 3 in the liver, type 4 for renal injuries .
- CK/have isozymes in the brain, liver and the heart so if you have detected the brain isozyme you know that there is a problem in the brain and so on.

Brain tumour or cancer and heart attacks causes the concentration of the brain and heart enzymes to increase in the blood because both of them causes the degradation of cells and tissues.

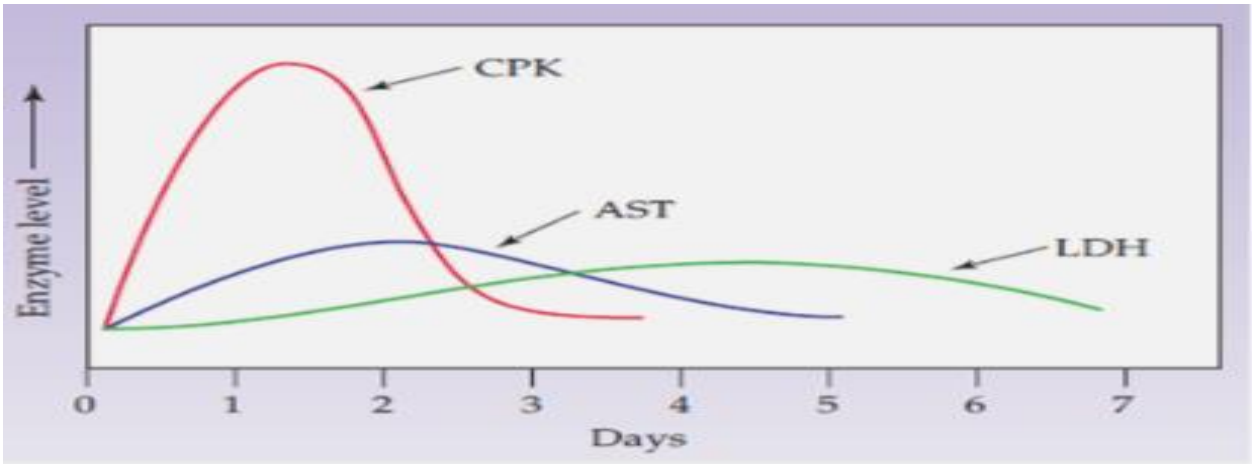
ALT and AST:

They are liver enzymes, but you have to know that ALT is more specific for liver disease than AST.

AST>>>found in the heart and liver.

ALT>>>found in the heart and liver, but the concentration of this enzyme in the liver is much higher than the heart.

This figure show enzyme level after few days for a person has heart attack:



The fastest enzyme that I can use it to know that this person has heart attack is CPK , AST is slower and LDH is more specific but it is very slow. So the enzymes have different timings.

Note: Please refer to the slides for further necessary information

Best wishes ^.^

Done by: Mo2nes Badaineh