Subject :	**Enzyme regulation, and Enzymes in Medical Diagnosis**
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Number :	**18**

Regulation through conformational changes

In the previous lecture we talked about conformational changes in Allosteric enzymes and phosphorylation as a way of regulation through conformational change from covalent modification. In this sheet we will talk about conformational change from protein-protein interaction.

G protein:Trans-membrane protein with 3 subunits : alpha (α), beta (β) ,gamma (γ)

Regulation through G protein :

In the normal state a subunit is bound to GDP andβ- γ subunits inhibit the a subunit by binding to it. When a neurotransmitter or hormone binds to the receptor associated with the G protein then an exchange between GTP and GDP (Gα binds to GTP) causing Gα to be activated by the dissociation of Gα and Gβγ subunits, Gα could be inhibitory (Gi) or stimulatory (Gs) . Gα then binds to the target causing its effect (activation or inhibition) but it is important to keep in mind that Gα subunits are said to possess an internal clock because they are GTPase that hydrolyze their own bound GTP to GDP and phosphate After the hydrolyzing conformational change occurs to Gα Gα and the target protein dissociate from each other and the Gβγ subunits return to binding to Gα (back to its inactive state).

Note: the process of hydrolyzing GTP occurs slowly (Gα slow intrinsic GTPase activity).

Monomeric G protein is small regulatory molecules which has only one subunit that functions by the same concept of multimeric protein. Ex. RAS protein.

	Monomeric G protein	Multimeric G protein
the cause of activation	GTP binding	Separating the subunits from each other

Proteolytic cleavage

Such proenzymes which are precursor proteins (precursor of proteases) that must undergo irreversible cleavage (peptide bond break down) to become fully functional termed as zymogens. To denote the inactive zymogen form of an enzyme the name is modified by the addition of the suffix "ogen" or the prefix "pro".

*zymogen in the prematurely state (the inactive state) cannot hydrolyze proteins because of the additional piece on their active site usually towards the N-terminal side, during synthesis of zymogen.

Chymotrypsinogen ⟶ stored for a direct use in vesicle within pancreatic cells until secreted to the intestinal lumen to be used (it moves from one place to another).

Zymogen ⟶ cleaved by other proteases ⟶ become active protease (active zymogen).

Fibrinogen and prothrombin are involved in blood clotting,they are cleaved to the active form (fibrin,thrombin).

Non-specific regulators affecting all enzymes and needs time (slow)

A brief concept: the rate of the reaction is proportional to the amount of enzyme present.

- Protein synthesis or degradation as a way of Regulation of enzymes.

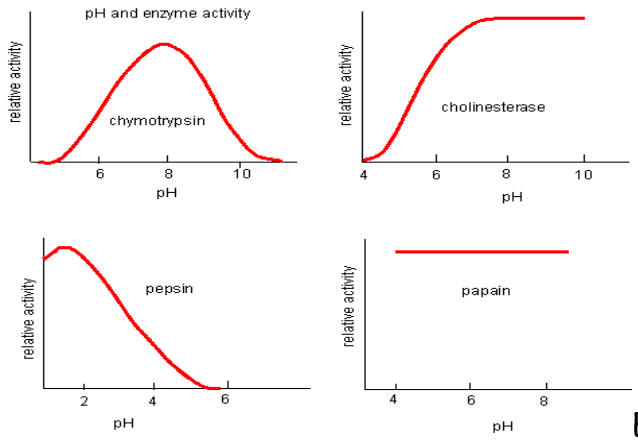
Protein synthesis begins with the process of gene transcription.

DNA ⟶ RNA ⟶ the primary amino acid sequence of the protein.

*the rate of enzyme synthesis or degradation is an adapted way to the environment or the disease state the organism has (gluconeogenesis increases and synthesis of antibodies ⟶ protein degradation increases). Regulation can also occur by increasing or decreasing the rate of gene transcription sometimes through stabilization of mRNA.

Effect of temperature: temperature affects the kinetic energy of all enzymes which increase the effective collisions per unit of time and the velocity will increase until an optimum temperature is reached ⟶ if we increase the temperature above the optimal temperature, then denaturation of the enzyme occurs.

Effect of PH: is different to different enzymes (the effect is enzyme dependant) the enzyme with high amounts of ionic interactions ⟶ denaturation occurs



Each enzyme has an optimum ph

Chymotrypsin ⟶ ph 8

pepsin ⟶ ph 2

Papain ⟶ isn't affected by ph

Cholinesterase ⟶ works at ph > 5-6 (the activity decrease when the ph is lower than 6)

Extremozymes: are enzymes which works under extreme conditions (very high or low temperature. very high or low ph values)

*regulatory enzymes work around (37- 40 °C) close to physiological ones, and between (5-9) ph values.

*extremozymes are found naturally within organisms that live in that conditions (some bacteria live in very hot water).

ABzymes:

AB: AntiBody

*they are catalytic antibodies (antibody which woks as enzyme) made as antibodies against analogs of the transition state (they have an arrangement of amino acids that are similar to the active site of the enzyme in the transitional state).

How we make them?

We make transition state analogs which can be active(they don't inhibit the enzyme) then inject these analogs into an animal which will make antibodies for these analogs (These antibodies mimic the structure of the transitional state analog) then we extract these antibodies from the animal and purify them.

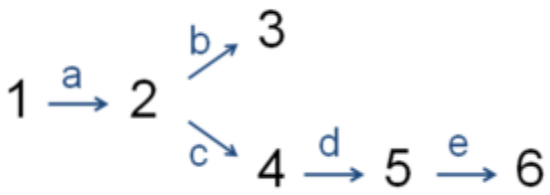
*the antibodies have the ability to bind the transitional state and catalyze the reaction by break down of the substrate.

Ribozymes: (enzyme that is not a protein (the only exception)), some RNA molecule are capable of catalyzing the reactions.
Ex. Telomerase enzyme which covers the deficiency during DNA replication ,Rnase B
*ribozymes are much less efficient compared to protein enzymes but if they were coupled to proteins their deficiency will improve.
*ribozymes are involved in RNA synthesis and protein synthesis.

Regulation of metabolic pathways

The different means of regulating enzymes activity described previously are used to control metabolic pathway in which a series of sequential reactions occur.
1-Regulating metabolic pathway through separation (compartmentalization) of degradation from anabolic (synthesis) pathway.

*A pathway also may have a branch point, regulation occurs at the rate limiting step (km is high for that enzyme and require high amount of energy, and it is usually irreversible).
"Changes in the rate limiting step influence flux through the rest of the pathway."
*Usually the rate limiting step is the first committed step in the pathway.



The committed step for production of 6 is (the step 2 4 (the step catalyzed by enzyme c)) which is the first irreversible step in the pathway.

Feedback regulation → Inhibition
Feedback regulation → Stimulation (positive feedback regulation)

*the end product of a pathway controls its own rate of synthesis by regulating the rate limiting step (affecting the enzyme that catalyzes it).

Feed forward regulation :

When the first step in the pathway runs, its product (intermediate in the pathway) activates the downstream enzymes in the pathway to prepare these enzymes catalysis to quickly take up the intermediates and process it .
Ex. The pathway of toxins and poisons to avoid accumulating of these toxins and poisons in the body.

Enzyme complexing: some associated enzymes (complex enzyme collected together), so the reaction becomes fast with less energy lost.
Examples of enzymes that exist as a complex:
1-pyruvate dehydrogenase complex contains 3 enzymes: the first one is decarboxylase, the second one is transacylase "transfer acyl to CoA", and the third one is dehydrogenase.
Remember: pyruvate dehydrogenase "complex" converts pyruvate to acetyl CoA
2-aketoglutarate in krebs cycle.
3- aketo acid dehydrogenase.

Enzymes in medical diagnosis:
The idea behind using enzymes in diagnosis is to measure the serum levels of numerous enzymes (due to isoforms of enzymes ex. Lactate dehydrogenase which has 5 isozymes with different levels of each in different tissues).
*when a tissue is damaged this results in releasing of intracellular components into the blood then the normal level of enzymes in the blood changes relating to the levels of the enzymes in different tissues in the body,once they are damaged.
You have to know these enzymes:
1-Alanin transaminase ALT
2- Aspartate aminotransferase AST

These are the typical liver enzymes. ALT is particularly diagnostic of liver involvement as this enzyme is found predominantly in hepatocytes. When the serum sample shows a high measurement of ALT or AST (or the ALT/AST ratio >1), this indicates that there is a viral damage or disease in the liver (viral hepatitis). Normally in liver disease or damage that is not of viral origin the ratio of ALT/AST is less than 1.
3-lactate dehydrogenase LDH
4- creatin kinase CK (also called creatin phoshokinase CPK))
CPK is found primarily in heart and skeletal muscle as well as the brain. Therefore, measurement of serum CPK levels is a good diagnostic for injury to these tissues. Like LDH, there are tissue-specific isozymes of CPK:

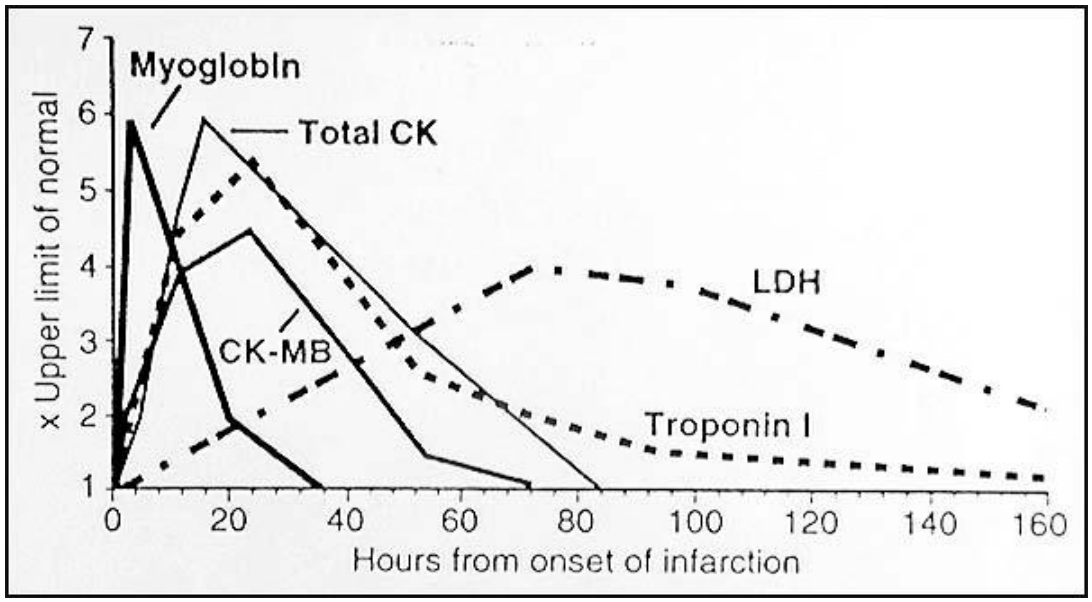
CPK3 (CPK-MM) is the predominant isozyme in muscle.
CPK2 (CPK-MB) accounts for about 35% of the CPK activity in cardiac muscle, but less than 5% in skeletal muscle.
CPK1 (CPK-BB) is the characteristic isozyme in brain and is in significant amounts in smooth muscle.

Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB **20% MB** **80% MM**	97% BB 3% MB 0%MM

5- Alkaline phosphatase(increasing levels of it in the serum sample ► we suspect that the patient may have bone tumor)

Proteins involved in myocardial infarction:*
1-CK-MB it is getting to the normal state after 2 days
2-LDH-1 : the ration (LDH-1/LDH-2) normally in the blood is less than 1 but in myocardial infarction it is more than 1
3-troponins in MI
Troponin levels rise within four to six hours after the beginning of chest pain or heart damage, and stay elevated for at least one week.
This long elevation allows detection of a myocardial infarction that occurred days earlier, but prevents detection of a second infarction if it occurred only days after the first.

4-myoglobin



Study the diagram well»

Remember:

CPK : found in the heart, brain and the skeletal muscles.
CPK-MM ⟶ found in skeletal muscles
CPK-MB ⟶ found in cardiac muscle (abnormal levels in the blood could be myocardial infarction)
CPK-BB ⟶ found in the brain (abnormal levels in the blood could be tumor in the brain)

*Note: According to the tissue which the patient complains about, you should be concerned about the measurements of the tissue-related enzymes in the serum sample you took from the patient, then you choose the enzyme that is still in the period in which the enzyme levels are abnormal corresponding to the patient pain (you ask the patient when he felt pain in this tissue).
For example if a patient comes to you complaining about chest pain which they had a weak ago, then you should see the level of troponin.
*Note: Myoglobin and troponin are not enzymes but have a high diagnostic values.

THE END
GOOD LUCK EVERYONE ☺
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