24

BIOCHEMISTRY

Faculty of medicine – JU2018

◉ Sheet ○ Slides

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Enzymes in disease diagnosis

Enzymes can be used as biomarkers; when a tissue gets damaged, cells open up and release their contents into the blood. That's why; high levels of these biomarkers in blood indicates tissue damage that can be caused by, for example, being beat up, therefore, athletes after doing sports have high amount of skeletal muscle proteins in blood as a result of muscle damage (or cell rupture). Another example could be the viral infection in liver cells which get damaged and die. So, enzymes and proteins get released into the blood.

*Normally, these biomarkers (enzymes) exist in small amounts; because cells release them through a process called "cell shedding". **However, the presence of enzymes in serum indicates tissue or cellular damage.**

*The measurement enzyme amount in serum is of **diagnostic significance**.

We are looking for certain enzymes like:

1) The amino transferases: alanine transaminase (ALT) and aspartate aminotransferase, (AST). **(Amino acid ↔ Keto acid).**

2) Lactate dehydrogenase, LDH.

3) Creatine kinase (CK) (also called creatine phosphokinase, CPK).

*In order for an enzyme to be considered a biomarker, it has to fulfill number of criteria:

1) It has to be doable (easy to be done).
2) Reproducible (if I do the test now or tomorrow, I'll get the same results).
3) It has to be acceptable (I shouldn't harm the individual in order to do the test).
4) Cheap.
5) Sensitive and specific; that I have **to be able** to detect a biomarker in order to tell if a person has a disease or not, it should not be an enzyme that is present in a very low concentration in blood. Also, the increase / decrease in its concentration should give me a **STRONG** indication for a certain pathological condition (unlike myoglobin; this will be discussed below).
   -What makes an enzyme specific (to an organ/ tissue /disease) is the presence of isozymes. (Recall that they're tissue-specific).

ASL and ALT:

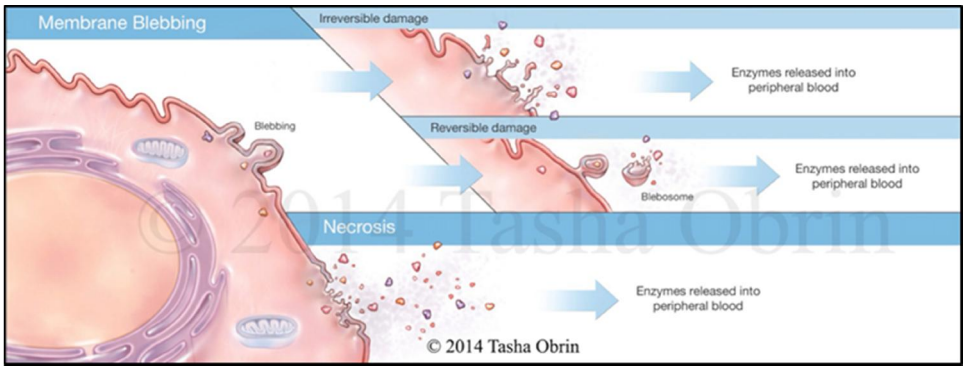
• Typical Liver enzymes.

• ALT is predominantly in hepatocytes (ALT is more specific for liver).

• If both AST & ALT are released (increased conc. in blood) it could be liver damage.

• The **ratio** of **ALT \ AST** is diagnostic:

-If the conc. of **ALT** is **high**, and the conc. of **AST** is **high** in blood and the ratio **< 1**, the diagnosis is liver disease / damage **(Not a result of a viral origin).** If the ratio **> 1** the diagnosis is liver damage (**a result of viral origin "viral hepatitis").**



Protein profile in myocardial infarction

***Myocardial infarction: heart muscle cells damage/ heart attacks.**

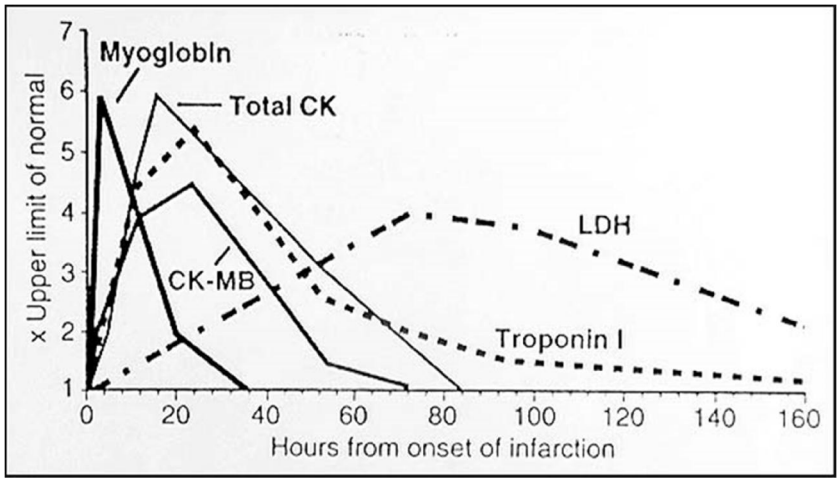
When someone feels a chest pain and rushes to the emergency room to check up, the doctors look for certain biomarkers, which are **increased** as a result of cardiac muscle damage, such as;

- **Myoglobin**:

*It's the first enzyme that comes up during a heart attack.

*It's not a specific enzyme meaning that it's not a good indicator because it's also present in skeletal muscles which might be damaged due to physical activity.

A person felt chest pain and rushed running to the hospital, in that case myoglobin would not be a good indicator of myocardial infarction because the increase in myoglobin might be due to skeletal muscle damage while running.



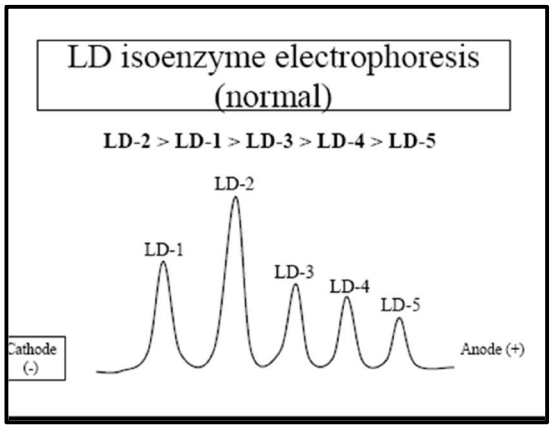
-LDH

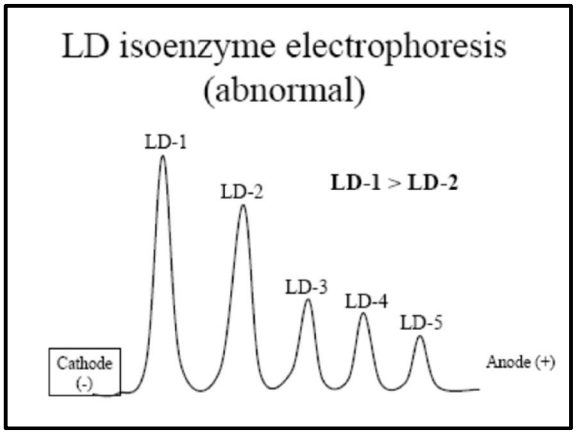
*Recall that LDH1 and LDH2 mainly exist in myocardial tissue, LDH5 mainly exists in liver.

A comparison of serum levels of **LDH1/LDH2 ratio** is diagnostic for myocardial infarction (heart attacks);

Normally, this ratio is **less than 1** (LDH2>LDH1). Following an acute **myocardial infarction**, the LDH ratio will be **more than 1** (LDH1>LDH2); because of the increased release of **LDH1**, which is highly present in **heart muscles.**

LDH is specific, but it can't be detected early; only after a few days from the onset of the myocardial infarction.





-If someone has suffered from a chest pain 3 days ago, we should look at LDH concentration because other enzymes go up and immediately -within two to three days- they go down.

-CPK (creatine phosphokinase, also called creatine kinase (CK))

Found primarily in heart and skeletal **muscle** as well as in the brain. It helps in the generation of energy (ATP).

This enzyme exists in many tissues and has different isozymes;

1) CPK-**BB** (CPK**1**).

2) CPK- M**B** (CPK**2**).

3) CPK-MM (CPK3).

M stands for muscle

B stands for brain

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

Notice that in skeletal muscle; most of the CPK is muscle specific MM (about 99%), while in cardiac muscle there are both MM (80%) and MB (20%). So, if we have an increase in MM isozyme and a **significance** increase in MB, then there is cardiac muscle damage. On the other hand, if we have an increase in MM only, then there is skeletal muscle damage.

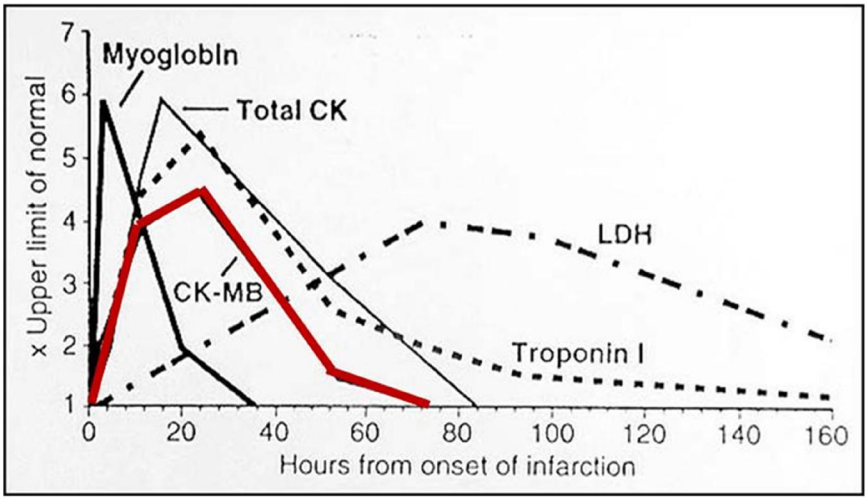
CPK is a better marker than LDH, because it peaks after 24 hours from the onset of the myocardial infarction (before LDH), so you can do early diagnosis.

A significant amount of CPK-MB is released after myocardial infarction leading to an increased **CPK-MB / total CPK ratio**.

*Note that the better cardiac marker is CPK-MB (CPK2), because CPK-MM (CPK3) is highly present in skeletal muscle cells.

CPK-MB is **not** a good marker if the patient suffered chest pain **more than 2 days** ago as CPK-MB will decrease **within 2 days**.

CPK-MB would be a good idea if the patient felt chest pain more than 2 day ago and is **not feeling well** upon his doctor's visit; therefore, CPK-MB is a good biomarker when a **second** event happens (re-infarction).



CPK is good for:

1) EARLY DIAGNOSIS, it peaks after 24 hours.

2) RE-INFARCTION DETECTION, it decreases within two days.

- يعني الشخص بعده موجوع، وبدنا نعرف إذا صار معه re-infarction ولا من المرّة الأولى، منفحص الـCPK-MB إذا **عالي** معناته صار معه re- infarction لأنه الـCPK-MB المفروض ينخفض خلال يومين !

-Troponin

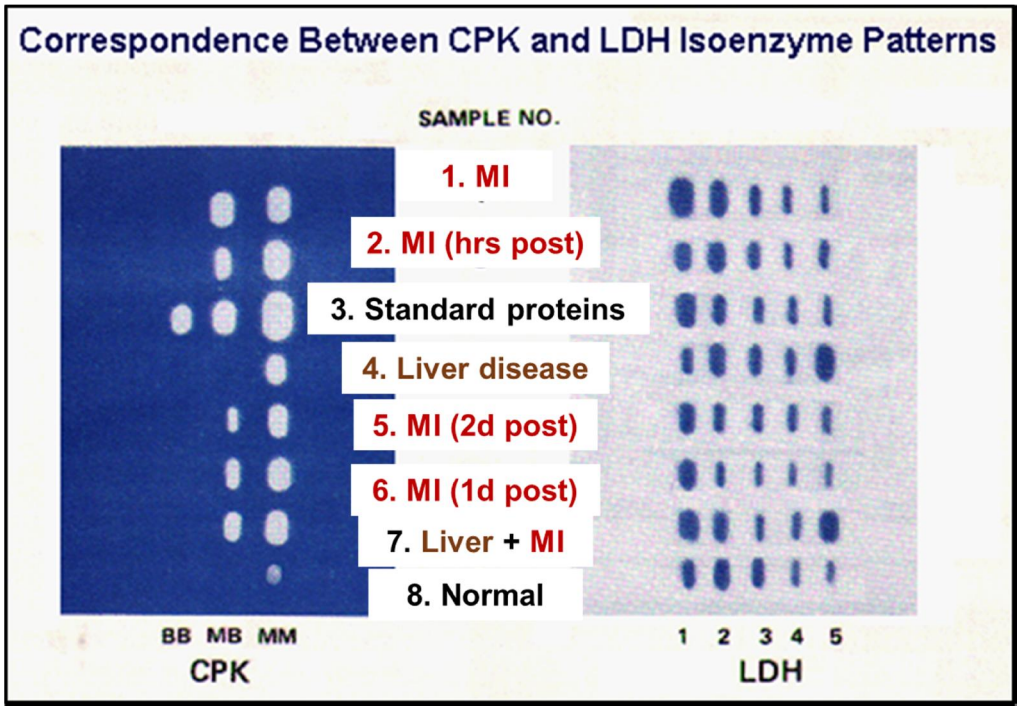
-Troponin levels rise within **four to six** hours after the beginning of chest pain or heart damage, and stay elevated for at least **one week**.

-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.

- It's not an enzyme, it's an actin binding protein which helps in muscle contraction. It has isoforms (NOT isozymes because they are not enzymes), one of which is specific for cardiac tissue.

-It's *the gold standard.*

Example:



*We separate isozymes according to charge or size.

-Sample# 1 MI (myocardial infarction) patient. The specimen was collected at a time when the activity of both LDH and CK were elevated. Note the **LDH flip (LDH1>LDH2)** and the **high relative activity of the MB isozyme.**

-Sample# 2 MI patient who experienced chest pain **only several hours** previously. Total **CK** is significantly elevated. Note that LDH2>≈LDH1.

-Sample #3 represents results for standard protein (a sample that contains equal amounts of all proteins; it is like a ruler).

-Sample# 4 a patient with liver disease.

-Sample# 5 MI patient (2 days post MI) **LDH flip is evident**, but **lower CK levels than sample 6 (below)**.

-Sample# 6 MI patient (the 1st day post MI); CK level **is elevated** with a **high relative MB** isoenzyme activity and the **LDH flip is evident**.

-Sample# 7 MI patient with passive liver congestion or the patient was involved in an accident as a consequence of the MI, and suffered a crushing muscle injury.

-Sample #8 results are from a normal specimen.

An exception to enzymes: Ribozymes (This is extra to types of enzymes)

-Ribozymes are enzymes made of both protein and RNA (the ratio of protein is higher).

-Catalysis is performed by RNA; a protein isn't the functional part.

-The protein part of the Ribozyme supports the structure of the RNA, and enhances the binding of the substrate to the RNA.

- The catalytic efficiency of RNA is less than protein enzymes, but it can be enhanced and stabilized by the presence of protein subunits.

*Examples:

i. Before translation, RNA processing occurs and this includes RNA splicing reactions which are catalyzed by Ribozymes.

ii. During translation, the formation of peptide bonds in the ribosome is catalyzed by Ribozymes.

**Cofactors:

Sometimes enzymes cannot function by themselves and they need help, and help can be provided by non- protein groups.

Chymotrypsin-for example- relies on amino acid residues only, but other enzymes need help from non-protein groups (conjugated enzymes).

-Catalytic strategies of enzymes

Enzymes carry out reactions utilizing different catalytic strategies.

1) Some enzymes, such as **chymotrypsin**, rely on amino acid residues within the active site.

-Almost all **polar** amino acids participate in nucleophilic catalysis. Ser, Cys, Lys & His can participate in **covalent** catalysis. Histidine: pKa, physiological pH & **acid-base catalysis**.

2) Other enzymes increase their repertoire by employing cofactors (nonprotein compounds that participate in the catalytic process). Cofactors can **participate** in the reaction (**and are released as a product**) or can **facilitate** the reaction (during the **transition state**).

In this case; enzymes are called conjugated enzymes (Holoenzymes).

Classification of cofactors

We can classify cofactors according to:

1) Chemical structure
2) How they're bound to proteins

-Three types:

***Protein-based.**

***Metals:**

(1) Tightly and covalently associated with enzyme (the enzyme ITSELF is called Metallo-**enzyme**).

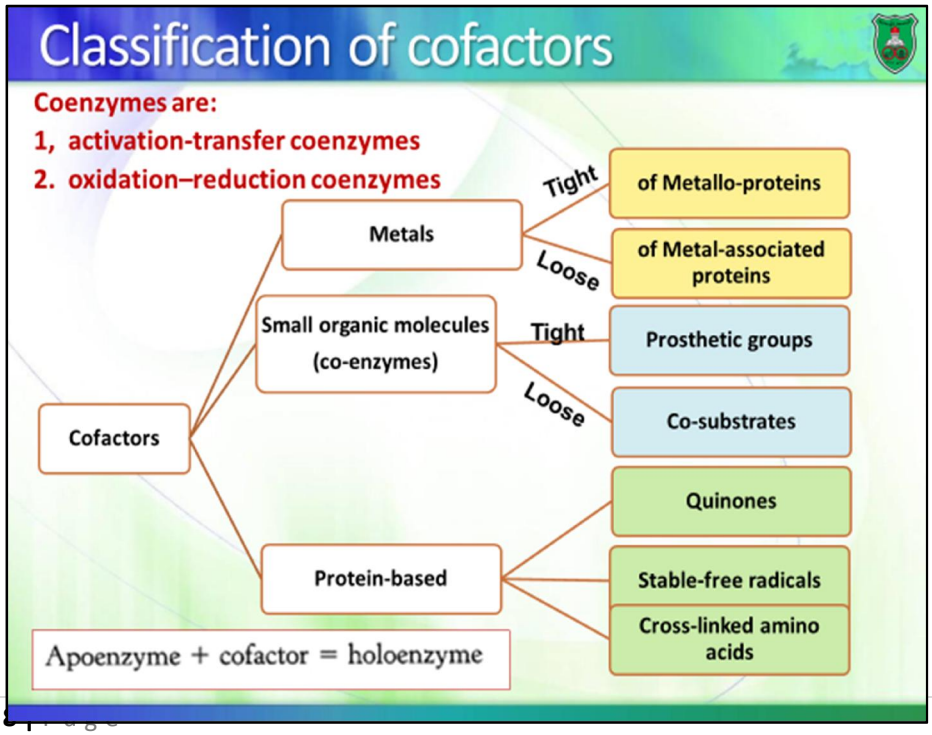
(2) Loosely associated with enzyme (Metal-associated **enzymes**).

***Small organic molecules (co-enzymes) [vitamins]:**

(1)Tightly associated to enzymes (prosthetic groups).

(2)Loosely associated to enzymes (Co-substrates →they participate in the reaction and are changed by the end of it).

Remember when we talk about heme in myoglobin we said it is a prosthetic group why? Because it is tightly bound *covalently* to the protein. Also, FADH2 is a prosthetic group. BUT NADH is a co-substrate



Classification according to the reaction that they help:

• Activation-Transfer coenzymes: Help in transferring groups (adding or taking out).

• Oxidation-Reduction coenzymes: Donating & accepting electrons.

#Activation-Transfer coenzymes

-Participate directly in catalysis by forming **a covalent bond.**

- Characteristics:

▪ Two groups in the coenzyme. a functional group that forms **a covalent bond** with substrate, and a binding group **that binds tightly to the enzyme**. Both could be the same site but usually they are not.

▪ **Coenzymes depend enzymes** for additional specificity of substrate & catalytic power.

- What characterizes these cofactors is that they form covalent bonds with portions of the substrates.

-The binding group mediates the interaction between the cofactor and the enzyme.

-If we add a cofactor to a substrate, no reaction will take place, because enzymes are needed to provide cofactors with specificity and catalytic power.

*In general these cofactors are derived from vitamins.

-Vitamins: organic molecules that the body cannot synthesize and we have to get them from food in small quantities.

-Our body convert vitamins to their active forms (as cofactors).

A-Thiamin pyrophosphate, TPP:

Thiamin (vitamin B1) is converted (it must be modified to become active) to its active form, TPP, in the **brain & liver**, by the addition of 2 phosphate groups that help in the binding of **cofactors with enzyme**.

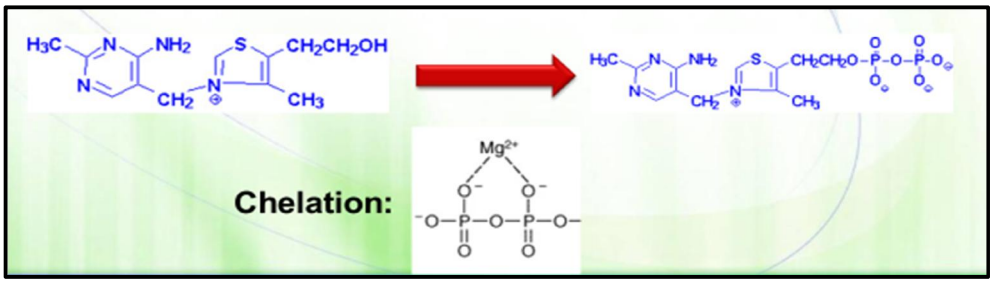
TPP is involved in decarboxylation reactions.

The pyrophosphate provides negatively charged oxygen atoms and chelates **Mg2+** that **is tightly bound to the enzyme. (Meaning that chelation is needed for the binding between the enzyme and the binding group).**

The phosphate groups participate in chelation reactions.

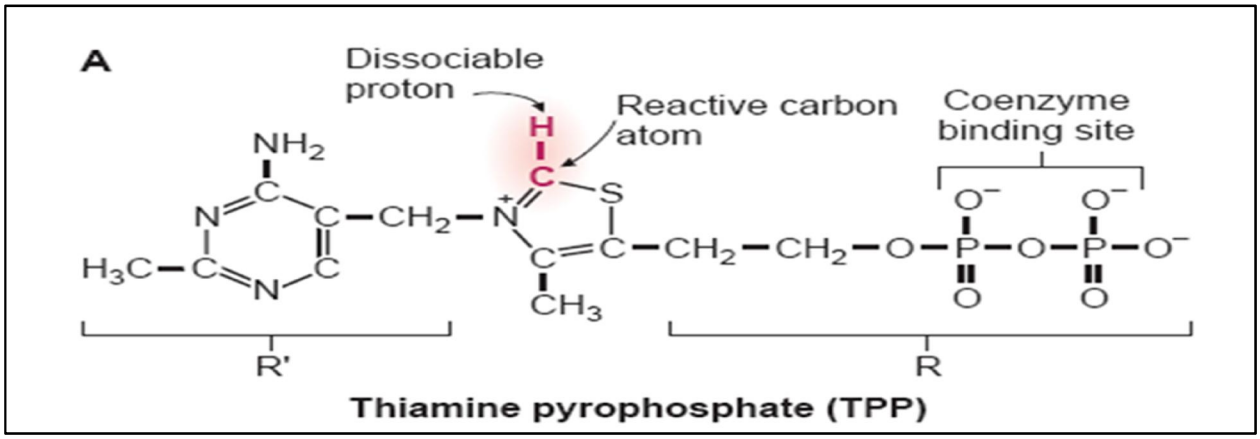
Pyro phosphate: two phosphate groups.

Chelation is when a mineral bind to one or more negatively charged groups and forms a cycle.



-Functional group: the reactive carbon atom that forms a **covalent** bond with a substrate's keto group while cleaving the adjacent carbon–carbon bond.

-Binding group: the pyrophosphate (it provides negatively charged oxygen atoms and chelates Mg2+ that is tightly bound to the enzyme).



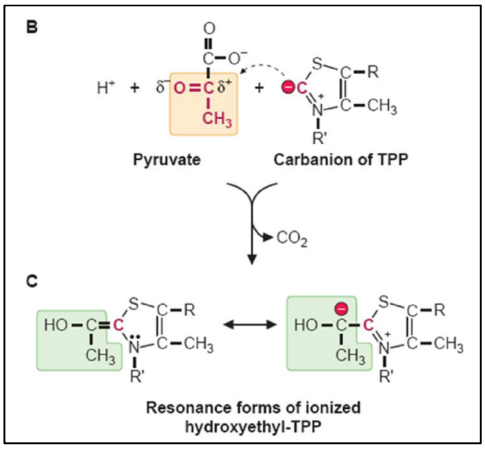
Importance of Thiamine:

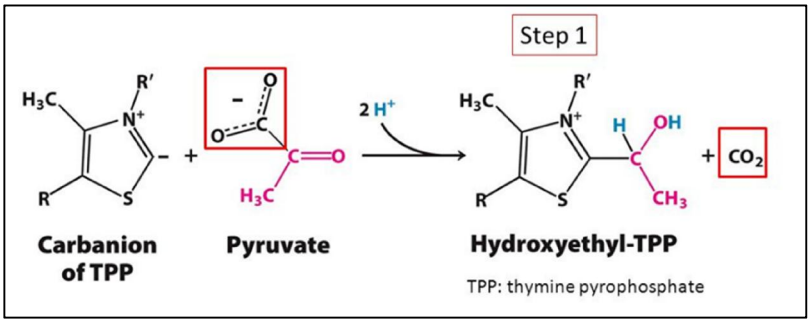
• It is required for a group of enzymes:

1- Pyruvate dehydrogenase complex:

✓ TPP is involved in **decarboxylation (release of CO2) of pyruvate into acetyl CoA** by the pyruvate dehydrogenase complex (contains 16 polypeptide chains and it catalyzes 3 reactions).

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH}$$

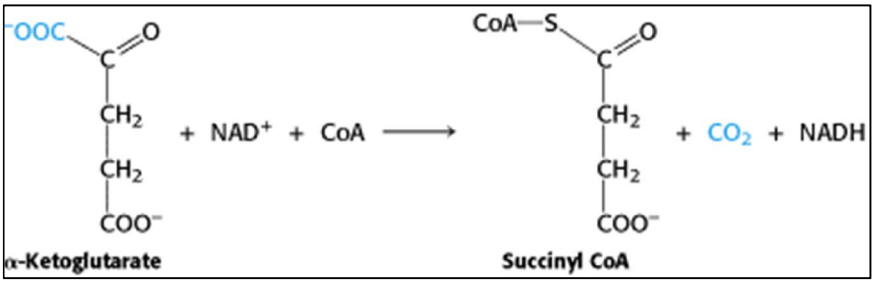




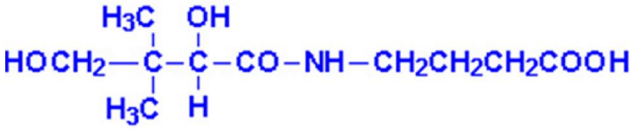
2-α-ketoglutarate dehydrogenase:

-Decarboxylation of **α-ketoglutarate into succinyl CoA** by α-ketoglutarate dehydrogenase.

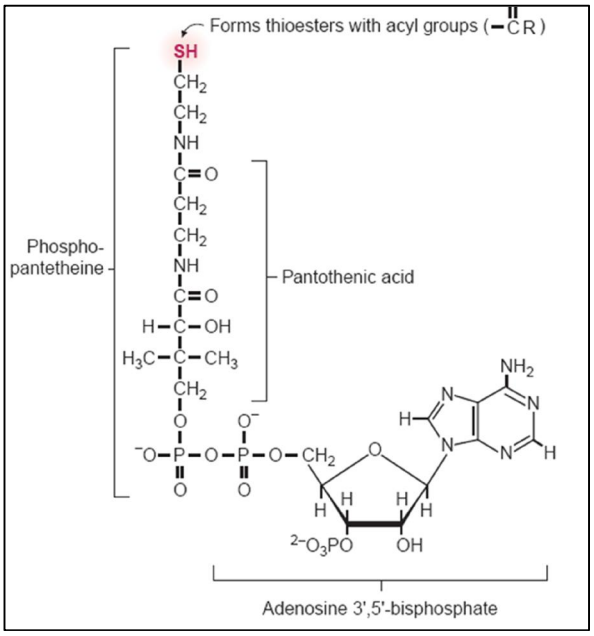
Remember that dehydrogenases belong to oxidoreductases (dehydrogenases transfer electrons with the help of NAD+/NADH and FADH/ FADH2).



B-Coenzyme A (CoA): a large organic molecule



-The Source is pantothenate (B5): made of alanine and pantoic acid.



-Function: metabolism of carbohydrate, fats, and proteins where it attacks carbonyl groups & forms acyl thioesters (the "A").

- Functional group: sulfhydryl (thiol) group (nucleophile).

- Binding group: adenosine 3',5'-bisphosphate (nucleotide).

The CoA consists of adenosine, Pantothenic acid and cysteine, the bond between the **functional group and the substrate has high amounts of energy so breaking it will release this energy (a molecule that is attached to CoA is an energy-rich molecule).**

Importance of CoA:

-It's required for a group of enzymes:

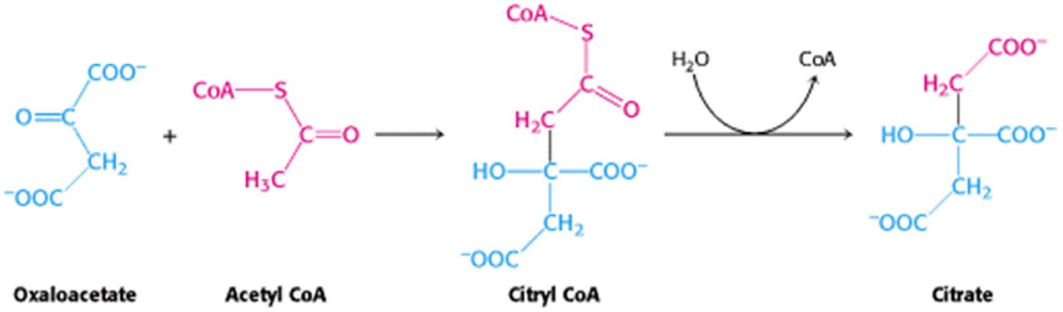
1- Pyruvate dehydrogenase complex:

-Conversion of pyruvate into acetyl CoA by pyruvate dehydrogenase complex.

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH}$$

2-Citrate synthase:

-Condensation of acetyl CoA and oxaloacetate into citrate by citrate synthase.



C. Pyridoxal <u>phosphate→ (ACTIVE)</u> (derived from vitamin B6 that is called pyridoxine)

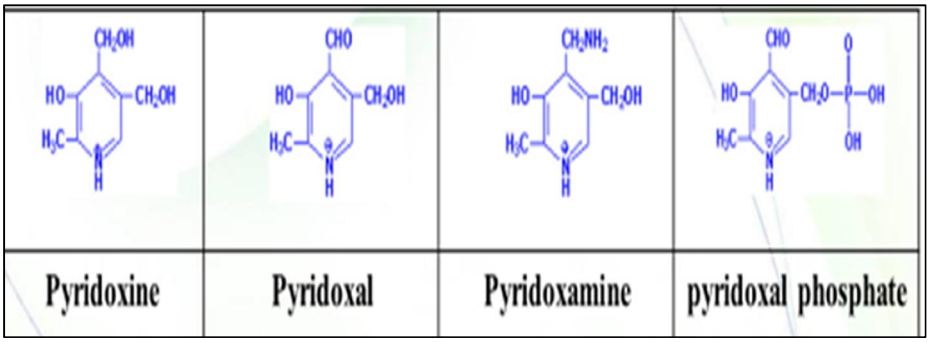
-Sources: pyridoxal, pyridoxamine and pyridoxine → **(INACTIVE)**

- Function: Metabolism of amino acids via **reversible transamination** reactions, which are responsible for transferring an amino group (from an amino acid) and place it on a keto acid to produce an amino acid.

- Functional group: the aldehyde group (CHO).

- Binding group: also, the aldehyde group (CHO).

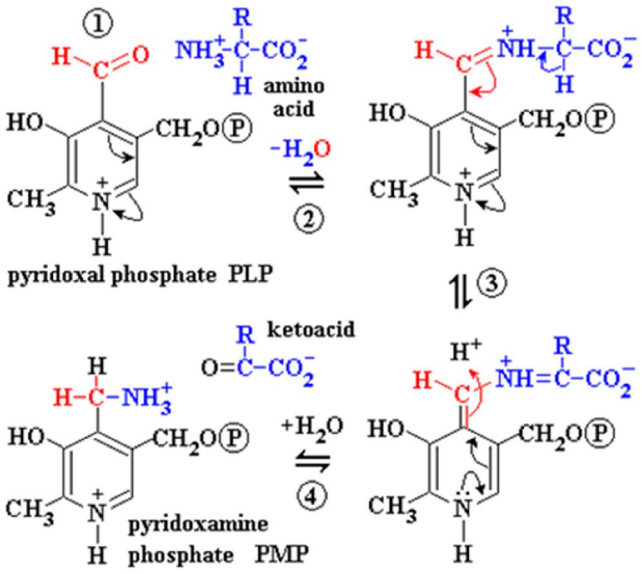
-Pyridoxal phosphate, pyridoxal, and pyridoxamine are all derived from pyridoxine. However, the functional / active one is pyridoxal phosphate.



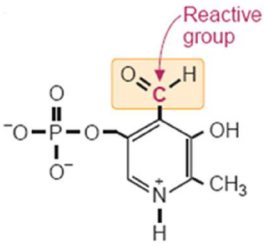
* As mentioned above, Pyridoxal phosphate is important in the metabolism of amino acids via **reversible transamination** reactions.

$$\text{Amino acid}_1 + \alpha\text{-ketoacid}_2 \rightleftharpoons \text{amino acid}_2 + \alpha\text{-ketoacid}_1$$

-The reactive aldehyde forms **a covalent bond** with the **amino group (in the substrate)**, and then the positively charged **ring nitrogen** withdraws electrons from <u>bound amino acid</u> (cleavage of bond).

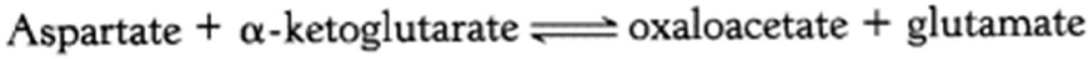


-Binding and functional groups are within the ring.

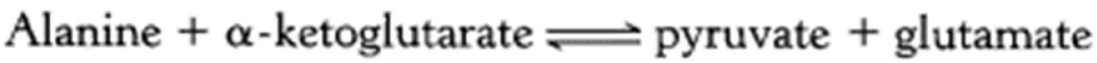


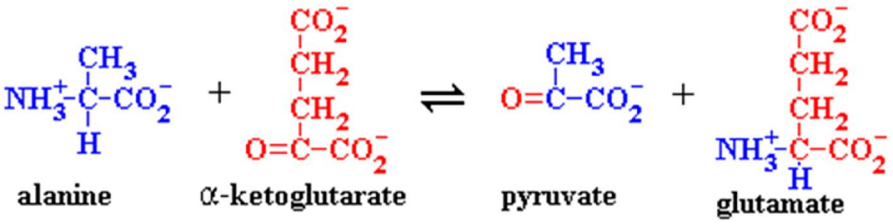
-*Examples:

1)Aspartate aminotransferase:

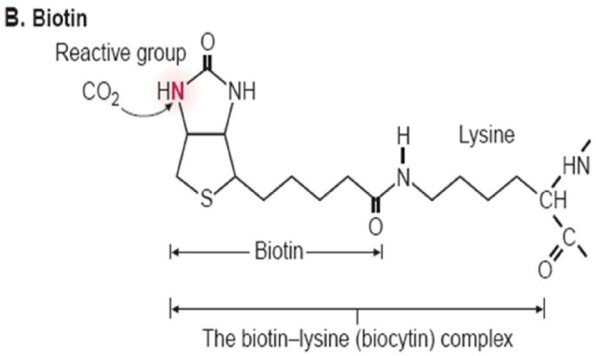


2) Alanine aminotransferase





D. Biotin (vitamin B7)



-Source: food and intestinal bacteria.

- Function: **carboxylation**.

-Functional group: **NH in the ring**; a nitrogen atom that covalently binds to CO2 in an energy-requiring reaction.

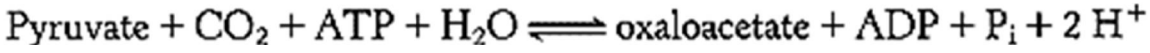
-Binding group: **the rest of the molecule**;
it doesn't contain a phosphate group, it is covalently bonded to a **lysine** in enzymes called carboxylases.

-Deficiencies in biotin are **very hard** to be found, but it is seen after long antibiotic therapies (antibiotics will kill the normal bacteria that is found in the body releasing this coenzyme), or excessive consumption of raw eggs (egg white protein, avidin, has high affinity for biotin).

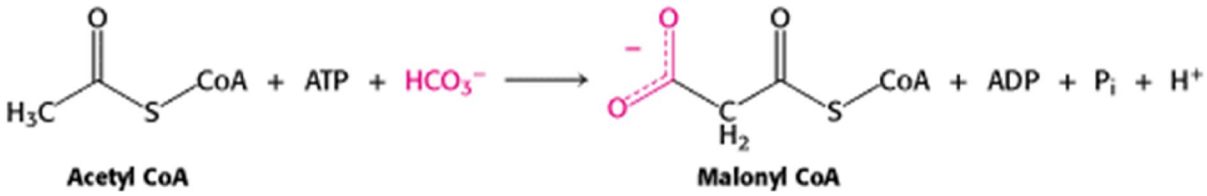
-Its active structure consists of its main structure bounded covalently to Lysine, creating a structure called (Biocytin).**

* It is required for certain enzymes, examples:

1. Pyruvate **carboxylase**.



2. Acetyl CoA **carboxylase** (fatty acid synthesis; in which **malonyl** is the starting point).



Acetyl CoA **Malonyl CoA**

Allah ma3akom.