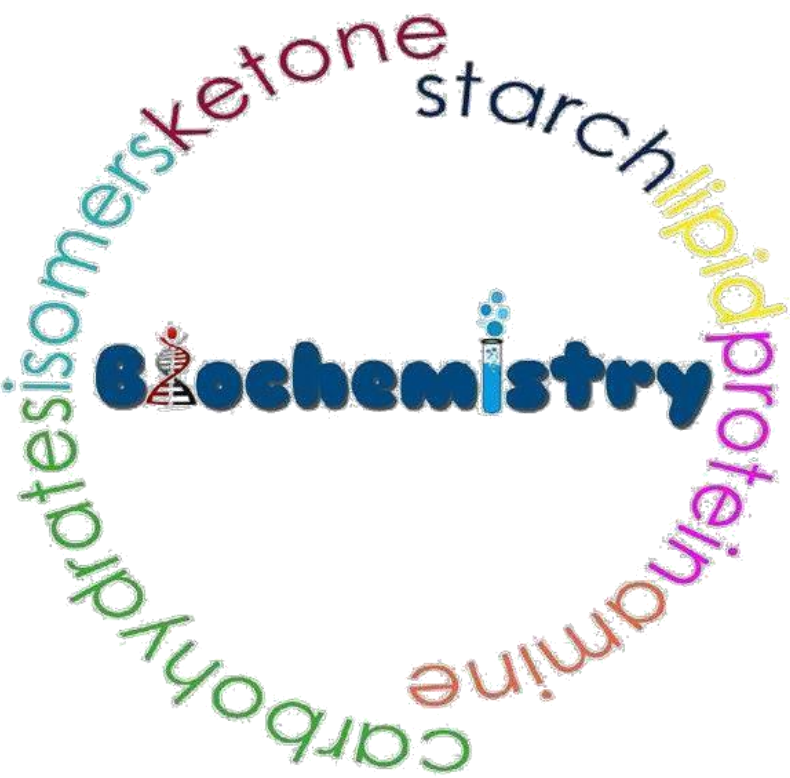


◉ Sheet ○ Slides

Subject :	cofactors
Done by :	Hammam Oqaili and Mohammed Oqaili
Corrected by :	Tawfiq barqawi
Number :	13

This sheet contains :

Dr Nafith and Dr Mamoun records

Almost all information in slides for Doctors

Some notes from text book (Marks Basic medical Biochemistry)

Recap:

Enzymes can be classified according to their **structure** into: **simple** enzymes and **conjugated** enzymes.

1) Simple (apoenzymes): as their name indicates, they contain only an amino acid sequence packed and folded into a 3D structure which contains an active site responsible for catalysis reactions.

In the active site of simple enzymes, we can find:

A. Hydrophobic amino acids: which are responsible for hydrophobic interactions and help in orientation of the substrate in the best way.
B. Polar amino acids: which help in catalysis of reactions. We commonly find histidine. Why?

   Because its imidazole ring has a pKa near the physiological pH so it can accept or donate proton.

2) Conjugated (holoenzymes): enzymes that are composed of an amino acid sequence with a non-protein compound (cofactor) that participates in the catalytic process and makes the enzyme active.

Classification of cofactors:

1) Metals: they may be tightly bound to the enzyme (metallo-proteins) or loosely bound (metal-associated proteins). We will discuss them at the end.
2) Small organic molecules (co-enzymes): may be tightly bound to the enzyme (prosthetic group) or loosely bound (co-substrates).
3) Organometallic: a combination of an organic molecule with a metal (the most common example is the Heme group).

4) Protein-based (protein derivative): meaning that not all cofactors are external but we may have some cofactors from the enzyme itself. These type of cofactors have three types: **quinones**, **stable-free radicals** and **cross-linked amino acids**. We will discuss them briefly:

    a) Quinones: structures mainly composed of an organic ring each connected to two oxygen atoms with a double bond.

    b) Stable-free radicals: they occur when the amino acid is changed into a radical after breaking it.

    c) Cross-linked amino acids: they occur when two amino acids from the enzyme are cross linked to each other.

For example:

- Tryptophan cross linked to another tryptophan.
- Tyrosine cross linked to lysine.
- Tyrosine cross linked to tryptophan.

End of recap.

Co-enzymes:

Co-enzymes are organic molecules that are derived from **vitamins** to make the enzyme active, and they cannot function without enzymes. The source of co-enzymes is vitamins. So what are vitamins?

Vitamins are small organic molecules which our body cannot synthesize so we need to take them in small amounts.

We can classify co-enzymes into two groups:

1. Activation transfer co-enzymes.

2. Oxidation reduction co-enzymes: present in reactions that involve transfer of electrons between molecules.

The main difference between the two classes is the type of binding between the co-enzyme and substrate as follows:

1. Activation transfer co-enzymes: bind covalently to substrates.

2. Oxidation reduction co-enzymes: bind non-covalently to substrates.

1- Activation transfer co-enzymes:

Activation transfer co-enzymes form covalent bonds with substrates (this is the main characteristic of it) and these covalent bonds are needed in the catalytic process. They are composed of **two** parts: a **functional** group (catalytic) which forms covalent bonds with substrates and a **binding** group which binds tightly to the enzyme itself.

Now, we will discuss coenzymes one by one. After that, you should be able to answer these questions and other specific information about each type.

- What is the name of vitamin that the coenzyme comes from?

- What is the active form of the coenzyme?

- What is the difference between a vitamin and the active form of a coenzyme?

- What is the binding group and catalytic group of coenzyme?

- What is the type of reaction that the coenzyme catalyses?

Think about this question :

Although coenzymes look as though they should be able to catalize reaction by themselves, they have almost no catalytic power without binding to enzymes. Why?

First coenzyme: Thiamin pyrophosphate (TPP)

Name of vitamin: vitamin B1 (Thiamin).

Active form: Thiamin pyrophosphate.

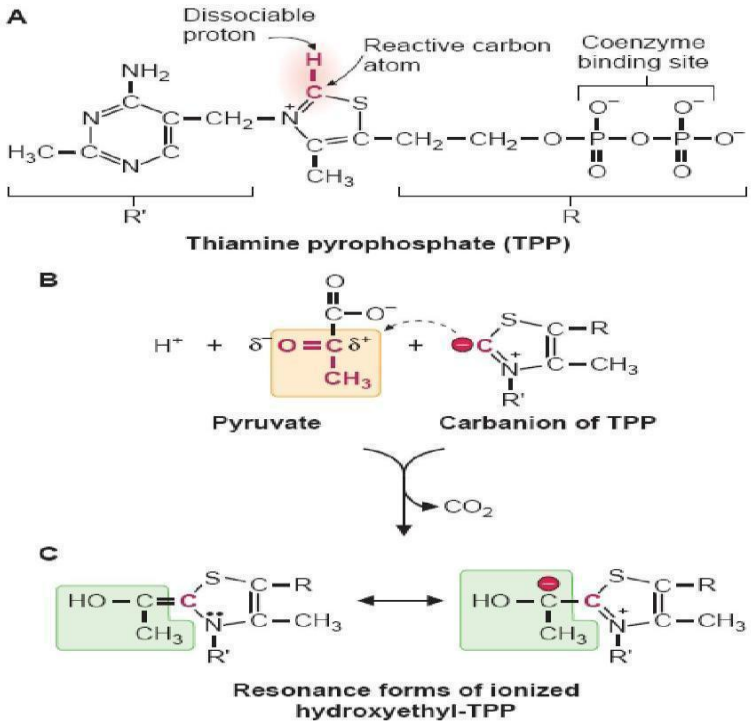
Binding group: pyrophosphate (negatively charged).

Catalytic group: the carbon that is present in the thiamin ring (between S and N).

Type of reaction: Decarboxylation reaction.

Difference between vitamin and active form: pyrophosphate group (2 inorganic phosphates).

Mechanism of action: near the reactive carbon there is a histidine amino acid (doesn't appear in the figure) which binds to a dissociable hydrogen on the reactive carbon (make thiamin a better nucleophilic attacking group). When the reactive carbon loses its hydrogen to histidine, carbon becomes negatively charged (more reactive) so it attacks the keto group and cleaves the adjacent carbon-carbon bond. (this mechanism involves transfer of a proton so we can consider it an acid-base catalysis).



Notes:

1. When you see CO2 as product of a reaction you must know that the reactant contains a carboxyl group (before the reaction occurs) and the reaction is a decarboxylation reaction.

2. Substrates of decarboxylases must contain keto groups.

TPP co-enzyme is present in many enzymes such as:

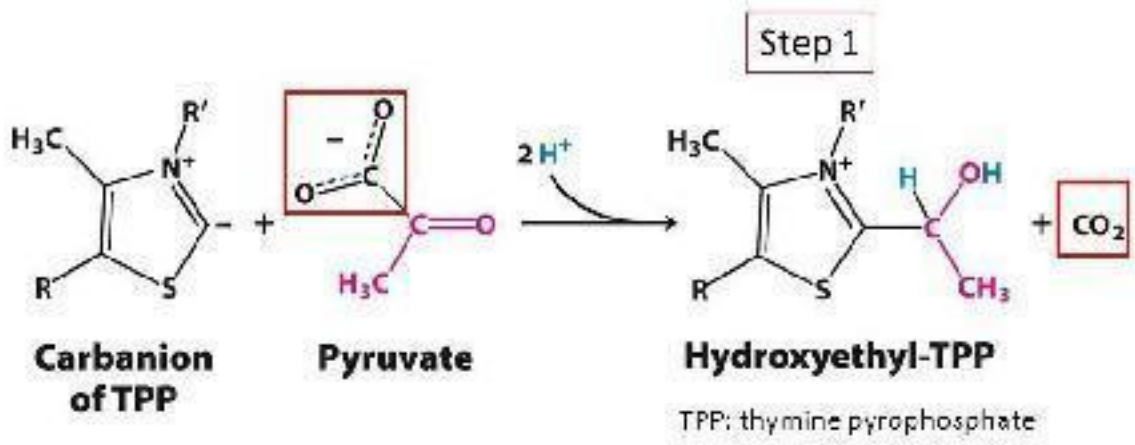
a) **Pyruvate dehydrogenase complex**:

Pyruvate is a keto acid composed of 3 carbons. Amino acids contain an amino group attached to the alpha carbon, while keto acids contain a carbonyl group instead.

The pyruvate dehydrogenase complex will catalyse the conversion of pyruvate into acetyl COA.

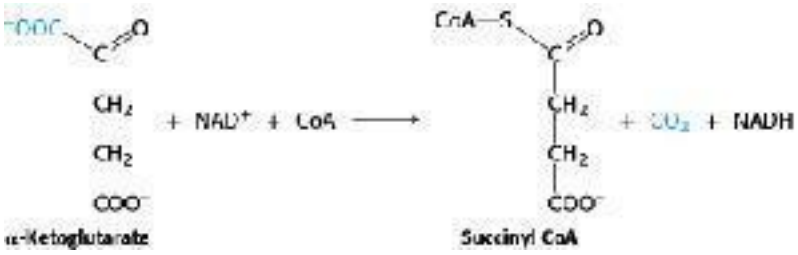
This complex isn't a single enzyme. It has three enzymatic activities divided among three enzymes. These enzymes are not present as three polypeptides but they are composed of 60 polypeptides. The complex has 60 active sites but it only has 3 main enzymatic functions.

The first step in this reaction is the decarboxylation of pyruvate:



b) **α-ketoglutarate dehydrogenase:**

Function: Decarboxylation of α-ketoglutarate into succinyl CoA.



Second coenzyme: Coenzyme A (CoA)

Name of vitamin : vitamin B5 (pantothenate (B5), which is composed of alanine and pantoic acid (fatty acid).

Active form: COA

Binding group : the adenosine-3,5-bisphosphate binds to the enzyme (tight and reversible binding).

Catalytic group : sulfhydryl group (nucleophile) of the cysteine amino acid binds to the acyl group because it is terminal.

First Function: As we mentioned before, pyruvate is converted to acetyl CoA but gradually (pyruvate --> acetate --> acetyl CoA -->), acetyl CoA then enters the Krebs cycle. So, pyruvate is converted to acetate (a 2 carbon molecule while we call others with higher number of carbons acyl) and since carbons of acetate are very reactive they cannot remain for a long time without binding to another molecule. But how will this complex enter the Krebs cycle?

The molecule that acetate binds to is CoA, producing acetyl CoA. After binding together, this complex enters the Krebs cycle. Therefore, CoA acts as an acyl transfer molecule (first function).

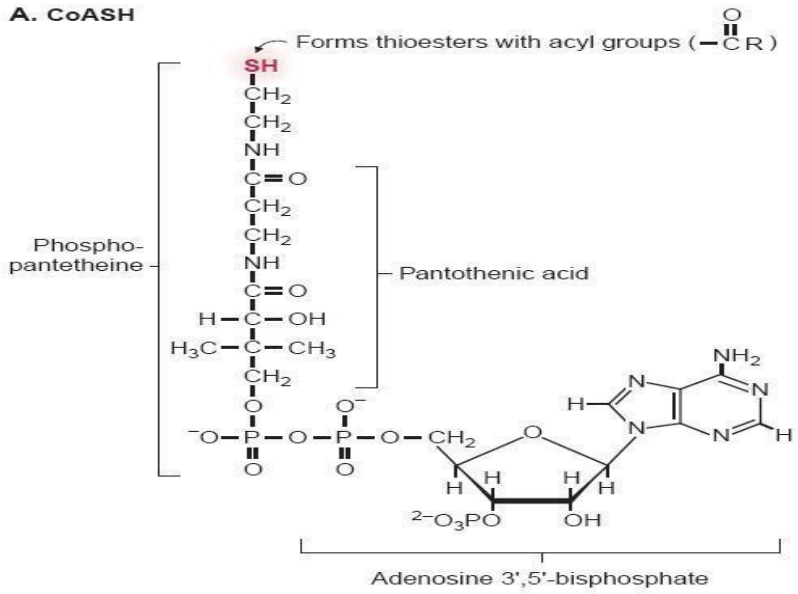
- What structure allows CoA to do this function?

CoA is composed of: an adenosine-3,5-bisphosphate (nucleotide) molecule connected to another molecule (mostly hydrocarbon) called pantothenic acid and connected to cysteine amino acid.

Second Function: CoA is related to energy as follows: when we break the sulfur-carbon bond (the carbon of the acyl group), a large amount of energy is released. So, when CoA separate from the acyl group, high amount of energy will be released and this energy will be used to run later reactions.

Note: when this reaction ends CoA will not remain in its original shape, but that doesn't fit the concept enzymes, so why do we still consider them as coenzymes?

Because they will enter other reactions and after these reactions CoA will restore its original shape and lose the added structures.



Third coenzyme: Pyridoxal phosphate (PLP)

Name of vitamin : Vitamin B6 (pyridoxine) (it is not the active form within the body).

Active form : PLP (Pyridoxal phosphate)

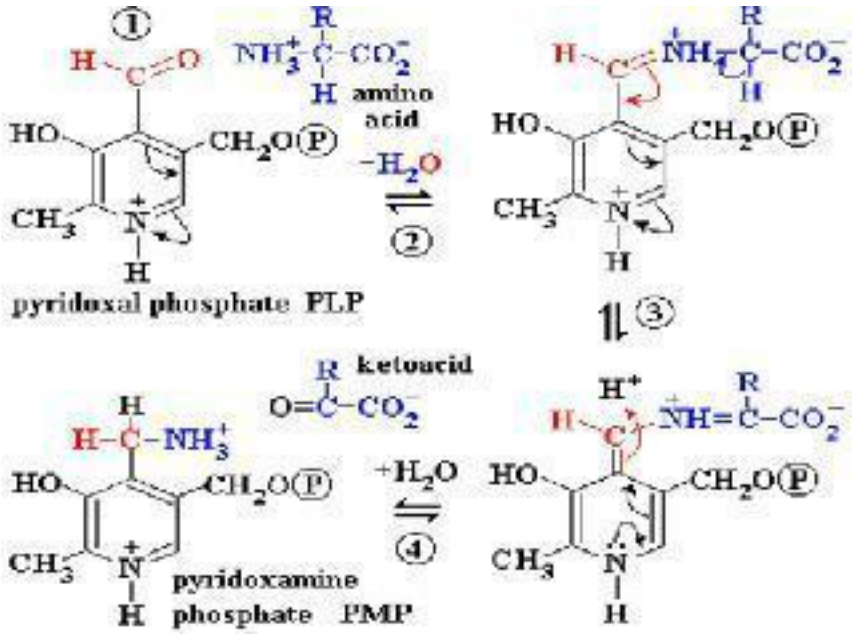
Binding group: phosphate group Catalytic group: aldehyde group

Function : responsible for the metabolism of amino acids via reversible transamination reactions. PLP is always used in the transaminase class of enzymes which convert amino acids to keto acids. When we remove an amino group from an amino acid the amino acid will be converted to a keto acid by formation of a double bond between oxygen and the alpha carbon (which lost its amino group). The amino group attaches to the co-enzyme (PLP) and remains attached until a keto acid binds to it and that keto acid will be converted to an amino acid.

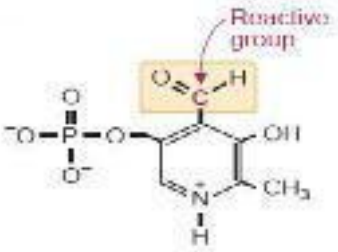
Mechanism of action:

The reactive aldehyde forms a covalent bond with the amino groups, then the ring's nitrogen withdraws electrons from the bound amino acid (cleavage of bond).

Figure showing mechanism of action :



Note: binding and functional groups are within the ring.



The general formula for transaminase action is:

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

We should know at least three amino acids and their corresponding keto acids:

1. Alanine amino acid and its corresponding keto acid is pyruvate.

2. Aspartate (aspartic acid) amino acid and its corresponding keto acid is Oxaloacetate

3. Glutamate (glutamic acid) amino acid and its corresponding keto acid is alpha ketoglutarate.

Fourth coenzyme: Biotin (B7)

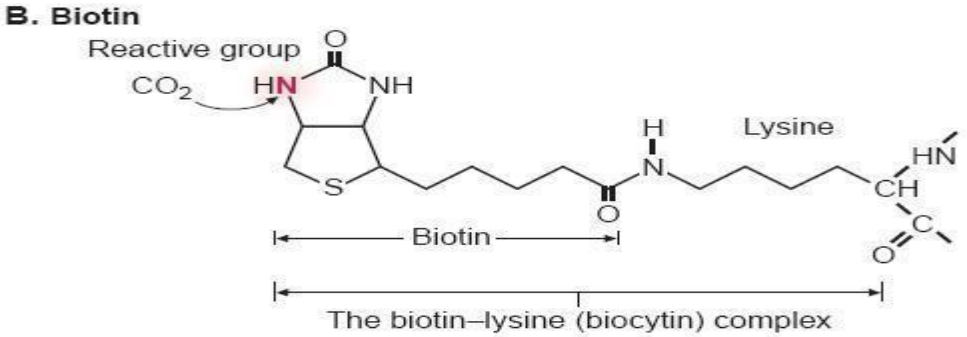
Name of vitamin : biotin (it is not the active form).

Active form is the **Biocytin** molecule which is a complex of biotin and the amino acid lysine connected with a covalent bond.

Note: lysine is an amino acid present in ***the active site*** and binds to biotin converting it to the active form, biocytin.

The functional group is the N atom that covalently binds a CO2 group in an energy requiring reaction. This nitrogen is found in the biotin's ring.

Biotin is the only activation transferase co-enzyme that does not use a phosphate group as the binding group (it doesn't have a phosphate group).



Function: biotin acts as a carboxylation molecule (adds a CO2 to the substrate). Whenever you hear a carboxylase enzyme you should know that this enzyme contains biotin as a part of it.

Because carboxylases cause the addition of a CO2 molecule to the substrate making it a larger molecule, therefore they fit under the ligases family of enzymes.

Source of biotin: food and intestinal bacteria (normflora).

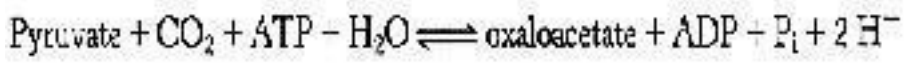
Deficiency of biotin can be happen because of:

1. Long antibiotic therapies which cause bacteria to die.

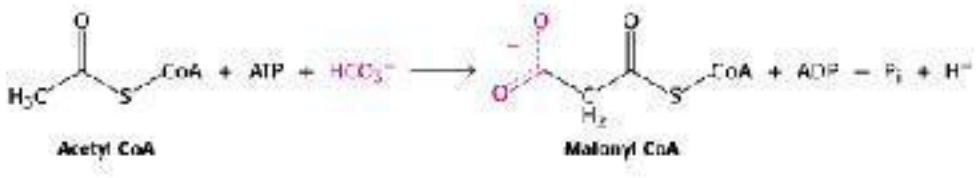
2. Excessive consumption of raw eggs. Egg white protein, **avidin**, has high affinity for biotin. It combines with it hindering its absorption.

Some examples on Biotin function:

1. Pyruvate carboxylase



2. Acetyl CoA carboxylase (fatty acid synthesis)



2- Oxidation reduction co-enzymes:

As we have mentioned before, co-enzymes are derived from water soluble vitamins (vitamin C and vitamin B group). Until now we have discussed vitamins B1, B5, B6 and B7 and now we will discuss vitamins B2 and B3.

Each coenzyme has a unique functional group that accepts and donates electrons and is specific for the form of electrons it transfers (e.g., hydride ions, hydrogen atoms, oxygen).

These do not form covalent bonds with the substrate, a portion of the coenzyme binds with the enzyme (this is the main difference between them and activation transfer coenzymes).

Most common: NAD+ (niacin, B3) and FAD (riboflavin, B2).

They depend on the enzyme for additional specificity for the substrate and additional catalytic power like activation transfer coenzymes (this is the answer for the question asked (back to page 5) previously).

Note: this subclass of co-enzyme works in the dehydrogenase class of enzymes.

First coenzyme: Vitamin B2 (FAD) :

Name of vitamin: vitamin B2 (riboflavin).

Active form: FAD (flavin adenine dinucleotide) and FMN (flavinmononucleotid).

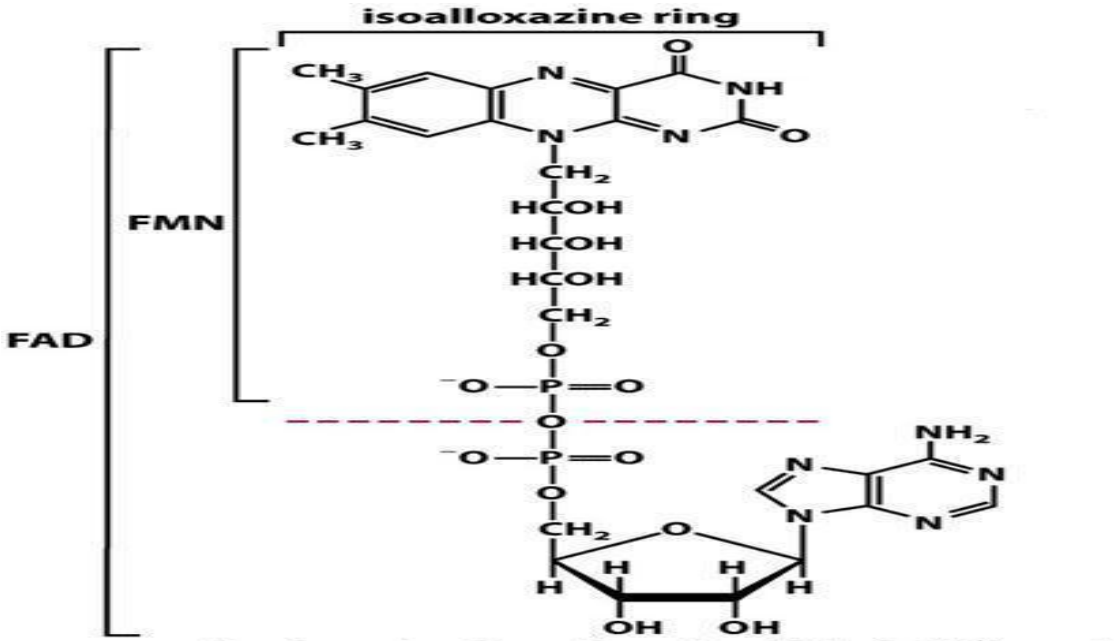
The functional group in FAD and FMN → the flavin ring.

The binding group in FAD and FMN → the phosphate group.

- The substrate binds to the Flavin ring.

Structure of FAD: it's composed of a flavin ring (attached to phosphate) connected to adenosine mono phosphate (composed of adenine connected with ribose and phosphate group).

Structure of FMN: it's composed of a flavin ring (attached to phosphate) without adenosine mono phosphate attached to it.



Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

Note: Proteins that require FMN or FAD as cofactors are called flavoproteins .

Hydrogen is present in three forms in nature:

1. Hydrogen atom (one proton and one electron).
2. Hydride ion (one proton and two electrons).
3. Proton (one proton only).

The FAD accepts two hydrogen atoms. One on each nitrogen atom in the flavin ring and it will be converted to $FADH_2$. FAD accept the hydrogens in the form of hydrogen atoms (one electron and one proton).

The taking of the two hydrogen atoms occur in two steps (transfer of electrons is sequential) as follows:

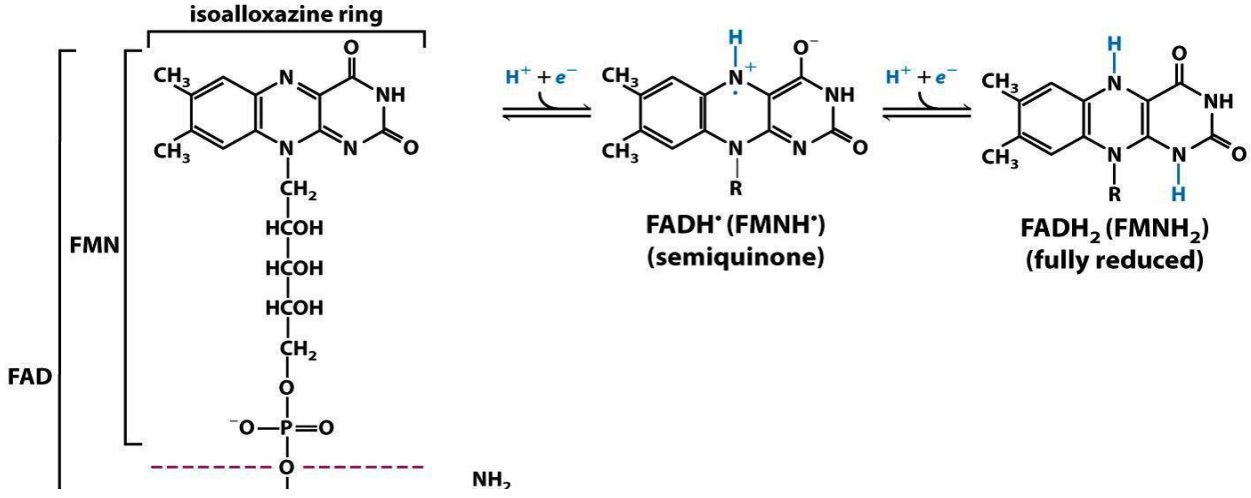
FAD → FADH → $FADH_2$

FAD enters the free radical state during its transform into $FADH_2$ and this has a functional implication. What is it?

Because of this characteristic we will never find FAD in its free form in nature or solution. It will be always bound to a protein or an enzyme. Why?

Because it has the ability to make a free radical during its reactions and if this molecule was free it will cause damage to nearby organelles or molecules because it is very reactive. But because it is always within the enzyme when this molecule is being converted to a free radical it will not cause damage because it is inside the enzyme .

Example of enzyme that FAD works as coenzyme with it is :
succinate dehydrogenase which converts succinate to fumarate .



Second coenzyme: Vitamin B3 (NAD+)

Name of vitamin: vitamin B3 (niacin/ nicotinic acid).

Active form: NAD+ (nicotinamide adenine dinucleotide) and NADP+ (nicotinamide adenine dinucleotide phosphate) .

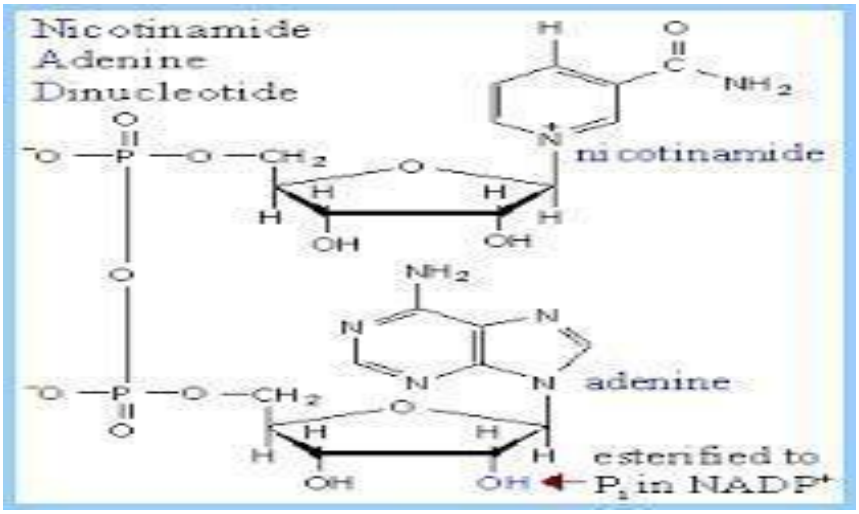
What is the functional group in NAD+ and NADP+?

- The nicotinic ring (more specifically the carbon opposite to the nitrogen in the ring).

What is the binding group in NAD+ and NADP+? ..... The phosphate group.

Structure of NAD+: it's composed of a nicotinic ring (the functional group) that is connected to adenosine mono phosphate (adenine + ribose + phosphate).

Structure of NADP+: same as NAD+ expect that it contains an extra phosphate group connected to the ribose of adenosine mono phosphate while NAD+ has a hydrogen atom in its place.



Mechanism: the cofactor accepts a hydride ion from the substrate, dissociates, and a keto group (CO) is formed.

Note: NAD+ accepts two electrons to become NADH. It accepts one hydrogen with two electrons (in the form of a hydride ion) and the remaining proton is released into the solution.

$$NAD^{+} + 2e^{-} + 2H^{+} \rightarrow NADH + H^{+}$$

We mentioned that the only difference between NAD^+ and $NADP^+$ is the phosphate group. Does that affect their function?

- No, because their function is to accept a hydrogen atom and that occurs on the nicotinic ring which is found in both of them.

What role does the phosphate group play then?

- Better regulation and more specificity

Note: NAD^+ accepts just one hydrogen and in one step only which is different from FAD, and because of that it doesn't enter the one electron state (free radical state) therefore we can find it in nature and free in the solution (not always bound to an enzyme).

Note: both FAD and NAD^+ are composed of dinucleotide and one of these nucleotides is the adenosine mono phosphate (adenine +ribose+phosphate) which is found in both of them. What is the other nucleotide?

- In FAD → the Flavin ring.
- In NAD^+ → the nicotinic ring.

This is the major difference between these two coenzymes.

What is the type of binding between FAD and the enzyme and between NAD^+ and the enzyme?

- Between FAD and the enzyme → tight binding therefore FAD is a prosthetic group. This is important for redox reactions.
- Between NAD^+ and the enzyme → loose binding therefore NAD^+ is a co-substrate.

After you reach this state, you may notice that the two subclasses of coenzymes have a ring structure as their functional group. **Why**?

- Ring structures cause the rearrangement of electrons without releasing them to outside (the electrons move inside the ring). This ability prevents damaging of organelles and molecules by these electrons. But if the functional group was in the open chain state, the electrons will move until they reach the end of the chain then they will bind to another molecule and damage it.

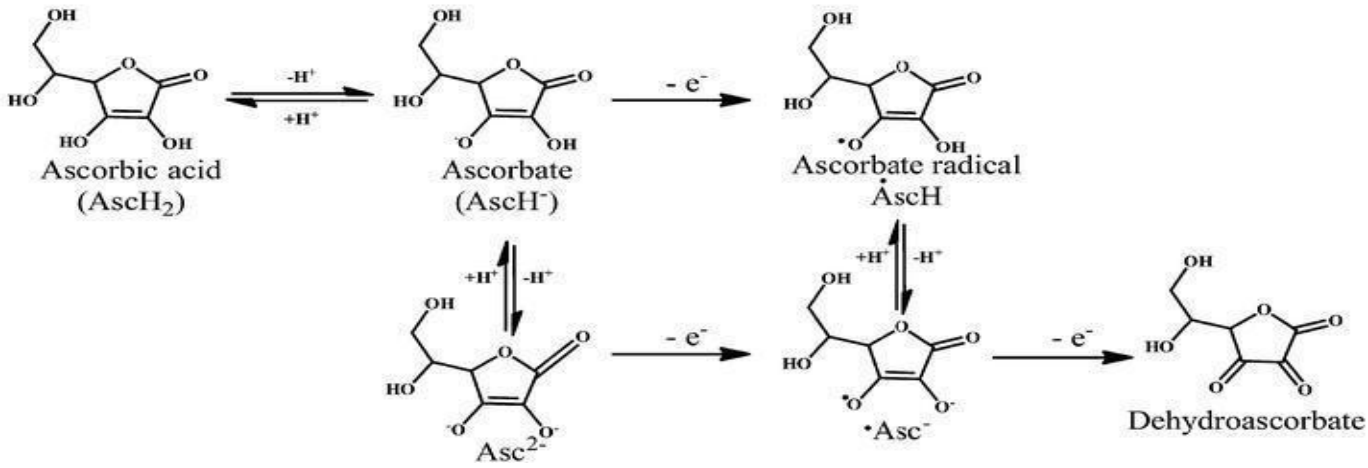
Vitamin C as co-enzyme:

Vitamin C has two functional roles in the body : synthesizing 4- hydroxy proline AND antioxidant

Synthesizing 4- hydroxyl proline in collagen : we talk about this in previous lectures in protein structure . NOW you should know that Vitamin C is coenzyme for prolyl hydroxylase .

Antioxidant : it has this property because it is energetically favorable oxidation

. what is the mechanism ? reactive oxygen species take electrons from ascorbate converting the ascorbate into a radical , which oxidized to dehydroascorbate . the reactive oxygen species are reduced to water , while the oxidized form of ascorbate are relatively stable and unreactive and doesn't cause cellular damage.



We finished coenzymes and we will move to another type of cofactors ---> **Metals**

You should try to memorize this table (in the exam they ask about it)	
Metal	**Enzyme**
Zn+2	Carbonic anhydrase and carboxypeptidase
Mg+2	Hexokinase
Se	Glutathione peroxidase
Mn+2	Superoxide dismutase

some properties of Metals :

- They carry positive charges .
- They act as electrophiles .
- They assist in binding of substrate or they stabilize developing anions in the reaction .
- They can accept or donate protons in oxidation reduction reaction.
- They are stable in more than one oxidation.
- **They can bind multiple ligands in there coordination sphere enabling them to participate in binding substrate or coenzyme to enzyme . (** for example : Mg+2 play a role in the binding of negatively charged phosphate group of Thiamin PyroPhosphate to anionic or basic amino acids in the enzyme . also , ATP are usually bound to enzyme through Mg+2 **)**

Carbonic anhydrase : this is common enzyme present in all organisms , convert CO_2 to HCO_3^- . This reaction in the body occur spontaneously in the absence of catalysts but organisms use this enzyme because they are often coupled to rapid processes such as respiration .

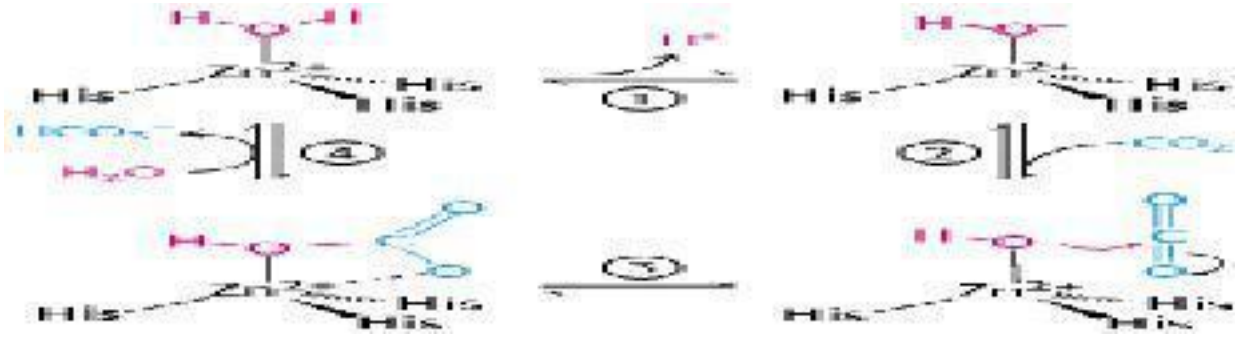
Mutations in carbonic anhydrase have been found to cause :

1) Osteopetrosis : excessive formation of dense bones accompanied by anemia .
2) Mental retardation

In carbonic anhydrase there is a Zink atom (found only in +2 state in biological system) which bound ***covalently*** to three imidizole rings of three histidiene residues and an additional site for water molecule .

what is the mechanism ?

1) Zinc facilitates the release of **proton from water molecule** generating hydroxide ion.
2) The substrate (CO_2) binds to the active site of the enzyme and positioned to react with hydroxide ion .
3) Hydroxide ion attacks the CO_2 converting it to bicarbonate ion .
4) The catalytic site is regenerated with the release of bicarbonate ion and the binding of another water molecule .



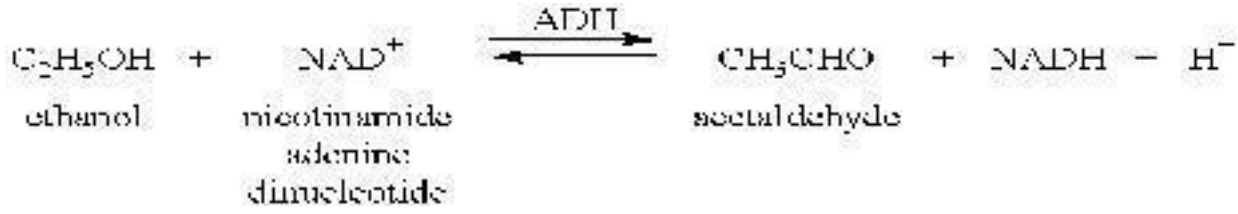
Catalytic Metals :

Theses metals bind ***covalently*** to the substrate itself to stabilize the negative charge on it . An example is ***Alcohol dehydrogenase*** which convert ethanol to acetaldehyde . alcohol dehydrogenase has an active site contain :

histidiene , serine and zinc . *the mechanism of reaction :*

1) Histidiene pulls an H off the active site serine
2) Serine pulls H off of the substrate (ethanol) hydroxyl group .
3) The oxygen in the hydroxyl group become negatively charged , so Zn bind to it for stabilizing (the reaction start when this binding occur) .

4) This facilitate the hydride ion to transfer into NAD+ .

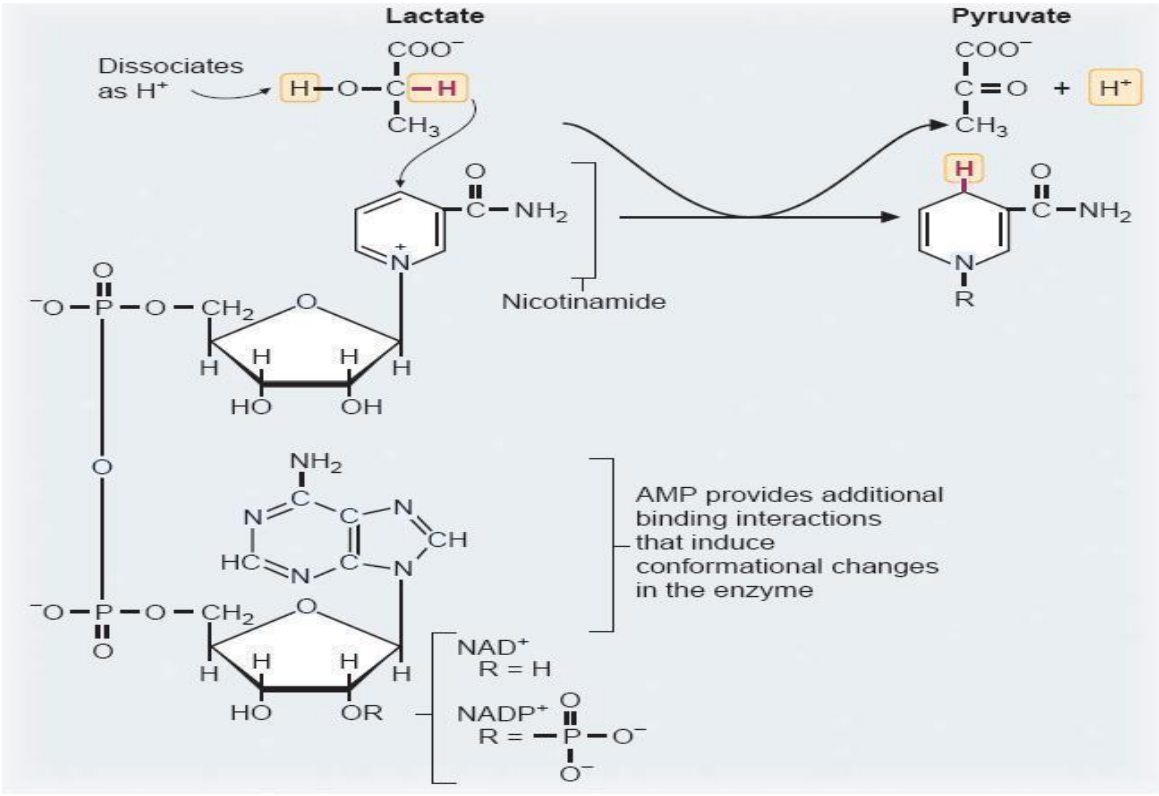


Another dehydrogenase (not metal) is known as : ***Lactate dehydrogenase*** important to know here : this enzyme **converts lactate to pyruvate and the coenzyme is NAD+ .**

Mechanism of action :

1) Histidiene in the active site bind to proton of hydroxyl group on lactate .
2) This make it easier for NAD+ to pull off the other hydrogen atom with both electrons (hydride ion) .
3) Keto group is formed .

Note : Zn in alcohol dehydrogenase as His in lactate dehydrogenase



This is the END and we will leave you with two high level questions . Try to solve them:

Read these statements about cofactors and answer the question below :

1) The portion of coenzyme that bind to substrate is called " functional group " .
2) Adenosine-3,5- bisphosphate has no functional role in coenzyme A .
3) The reactive group of biocytin is the nitrogen and the binding group is the phosphate.
4) Reactive carbon in the thiamin pyrophosphate contain dissociable hydrogen atom .
5) Zink in carbonic anhydrase act as His in lactate dehydrogenase .
6) The functional group of NAD+ is the carbon that opposite the positively charged nitrogen in adenosine ring .
7) Coenzymes don't consume in reaction (they regenerate through the reaction and can bind to another substrate .

Choose correct from this :

A) 1 and 3 is true but 5 is false
B) 1 and 2 is true but 7 is false
C) 4 and 5 is false but 3 is true
D) 1 and 2 and 3 is true
E) 5 and 6 and 7 is false

A patient was born with a mutation in an enzyme that severely affect its ability to bind an activation transfer coenzyme . which of the following is most likely to occur ?

A) The enzyme will be unable to bind to substrate of the reaction .
B) The enzyme will be unable to form transition state .
C) The enzyme will normally use a different activation transfer coenzyme .
D) The enzyme will normally substitute the functional group of an active site amino acid residue for coenzyme .
E) The reaction may be carried out by the free coenzyme .