Lecture :..19.....

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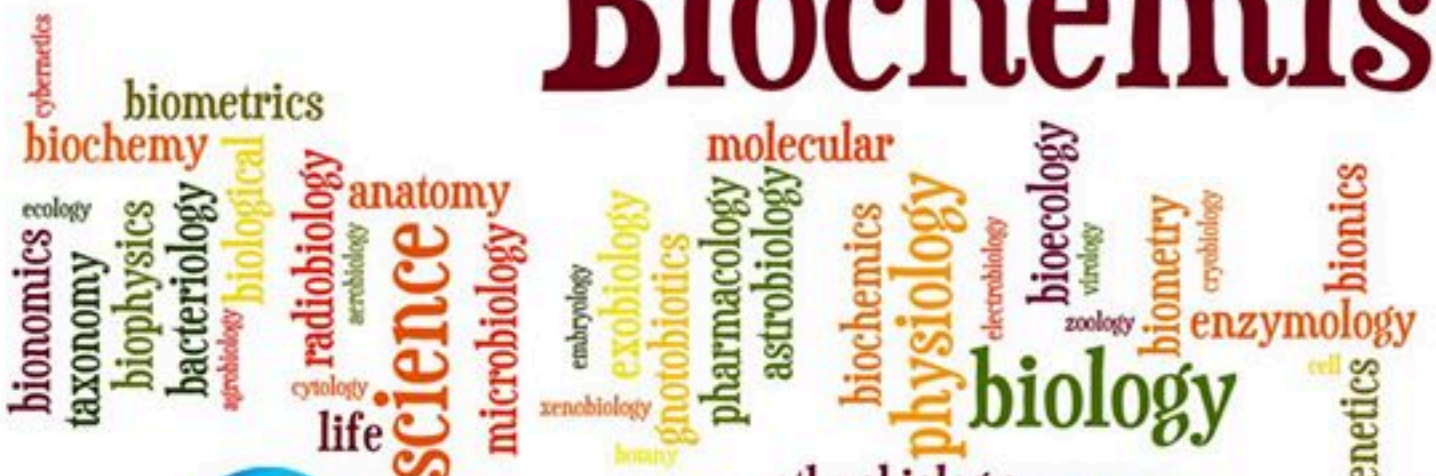
☐ Slide ■ Sheet

Medical Committee
The University of Jordan

# Biochemistry




# Enzymes 4

At the end of this sheet you should be able to:

1-Name the functional groups in catalysis.

2-know the cofactors and differentiate between activation transfer coenzymes and acid base coenzymes.

3-know the water soluble vitamins, their coenzyme (active form), and their primary biochemical function.

4-know the catalytic metals and differentiate between metaloenzymes and metal-activated enzymes.

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The last thing we talked about last lecture was energy diagram presents mechanism of action for **chymotrypsin**.

-How does **chymotrypsin** works?

Enzymes work by their own, it is only an amino acid sequence, we called it the catalytic triad, and for **chymotrypsin** this sequence **is Seriene, histidine** and **aspartic acid**.

In previous lectures we classified enzymes into:

## 1-simple:

It is only a sequence of **amino acids** without a **non-protein** part.
Example: chymotrypsin.

**2-conjugated:**

A **non-protein** part binds to it in order to be **active** and they include coenzymes, metalloenzymes and metal-activated enzymes.

-Lets talk about **simple enzymes** in details:

**Functional groups** (amino acids) in the active site include all **polar amino** acids which can be part in the **catalytic part** of the enzyme and aids to its function.

-**W**hy all amino acids that make up the catalytic part are polar?

Because they contain polar **oxygen, nitrogen and sulfur** which has a **free lone pair** of electrons which can participate in the **nucleophilic** attacks (which are done through electrons). And those are different from the **charged amino acids** (+ or -) which participate in the acid base attacks. ☺

**W**hat are the amino acids that make **covalent catalysis**?

1-seriene 2-histidine 3-lysine 4-cystein

-**W**hy do we find histidine in so many active sites (proteoactivesites)?

Because the Pka of the side chain of **histidine** is close to the physiological one (Pka=6 for histidine) so it can participate in **acid base** catalysis around physiological situations.

Note:

We talked about serien in the last lecture and we have seen that it can't participate in acid base reactions unless it was activated by histidine, however, histidine can be active by itself because its Pka is close to the physiological one.

# Conjugated enzymes:

Some enzymes need coenzymes to function

What are the structures that enzymes can bind?

**1-metals** **2-organic compounds**

**3-organometalic** compounds (**Heme** for example)

**Coenzymes:**

What are **coenzymes**?

They are organic **cofactors** that aids in the enzyme function.

**Most coenzymes** are derived from **vitamins**.

-Why are **vitamins** so important?

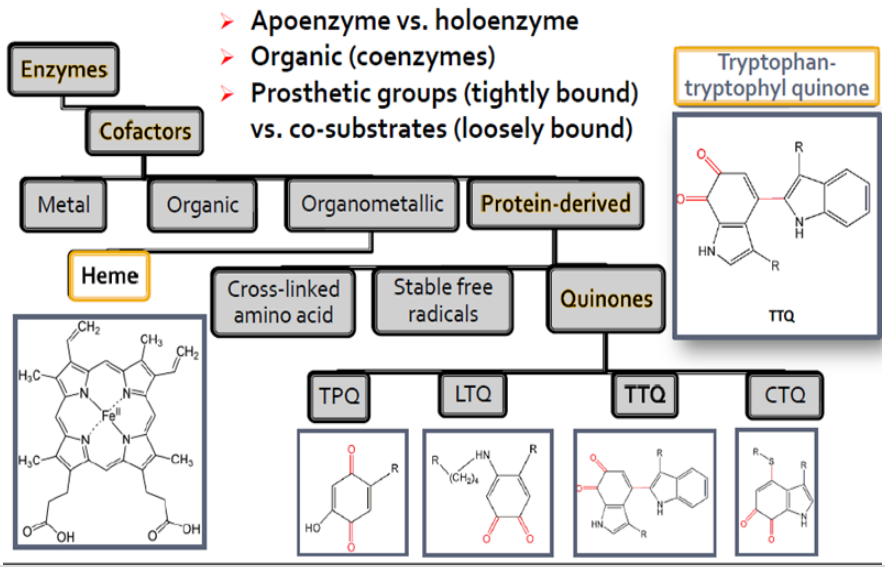
Because they aid to the **coenzyme structure** in order to function, So **enzymes** can work and the reactions in our body would occur.

Every coenzyme has a specific reaction that it catalyze.

**Coenzymes** are classified into **two** big groups which are

1-**activation transfer coenzymes**:

They activate their **substrates** by binding



them through **covalent** bond, so what they do is **covalent catalysis**.

2-**oxidation reduction coenzymes:**

They **abstract** electrons and delete their electrons so we are dealing here with electrons in the form of:

**1-free electrons.**

**2-hydrogens that contain electrons.**

**3-oxygen that contain electrons.**

So they **don't** form a covalent bond with their substrates as in the activation transfer coenzymes and they are **not** part of the covalent catalysis process.(two major differences but I only see one :p).

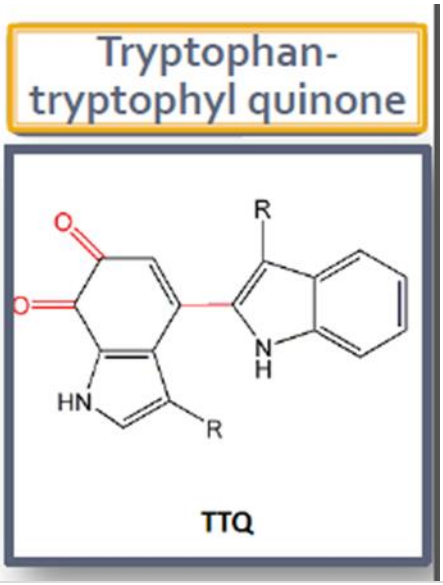
**Cofactors** in general they can be:

**1-metalic** **2-organic** **3-metalorganic**

**4-Protein derived:**

This means that there are **proteins** that are only an **amino acid sequence** and they don't need a **non-protein** part to bind it in order to be **active**, however, it **is not active** by **itself** because there should be a **modification** on the amino acids which does the catalysis. For example:

**Two Tryptophans** are close to each other and have to be **cross-linked**. After cross linking it is **not** a tryptophan but it is a whole structure that can do the **catalysis**.



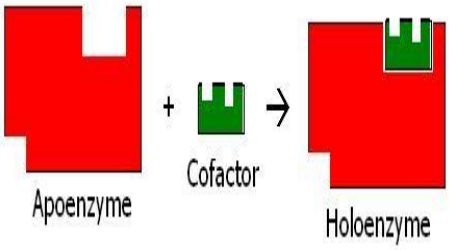
We classified conjugated enzymes into **two** major types:

**1-Holo enzymes:**

When the **non-protein** part is bound.

**2-apoenzyme:**

When the **non-protein** part isn't bound.




-When the **cofactor** is organic we call it **coenzyme**.

-when the **coenzyme** is tightly bound to the enzyme we call it **prosthetic group.**

when it is **loosely** bound we call it **cosubstrate**.

**-Metalloenzymes** vs **metal-activated enzymes**

**Metaloenzymes**

When the metal is **tightly** bound to the enzyme, And it's part of its structure, so as if the metal was removed the enzyme will be **denatured.**

**Metal-activated enzymes:**

The metal is **loosely** bound to the enzyme, and acts as an **activator** meaning that when it binds, the enzyme will be **activated** and when it unbind the enzyme will be **inactivated** but denaturation will **not** occur and the structure will be **preserved**.

Within the structure of the **coenzyme** we have two parts:

1-The first for **catalysis** (function)

2-And the other for **binding** (with

Important note:

Coenzymes generally weather they were activation transfer or oxidation reduction, are not functioning by their selves but after they bind their enzymes they will function with 100% efficiency

The enzyme).

## Coenzymes:

# 1-activation transfer coenzymes

It makes **covalent catalysis** within the structure of the coenzyme itself.

**Characteristics**:

**1-**Two groups in the **coenzyme**:

A)one forms a **covalent bond** (function).

B)the other responsible for **binding** tightly with the enzyme.

**2**-Depends on the **enzyme** for additional **specificity** of substrate and additional catalytic power.

-We have **four** types of **activation transfer coenzymes** which are:

## 1)TPP (thiamine pyrophosphate)

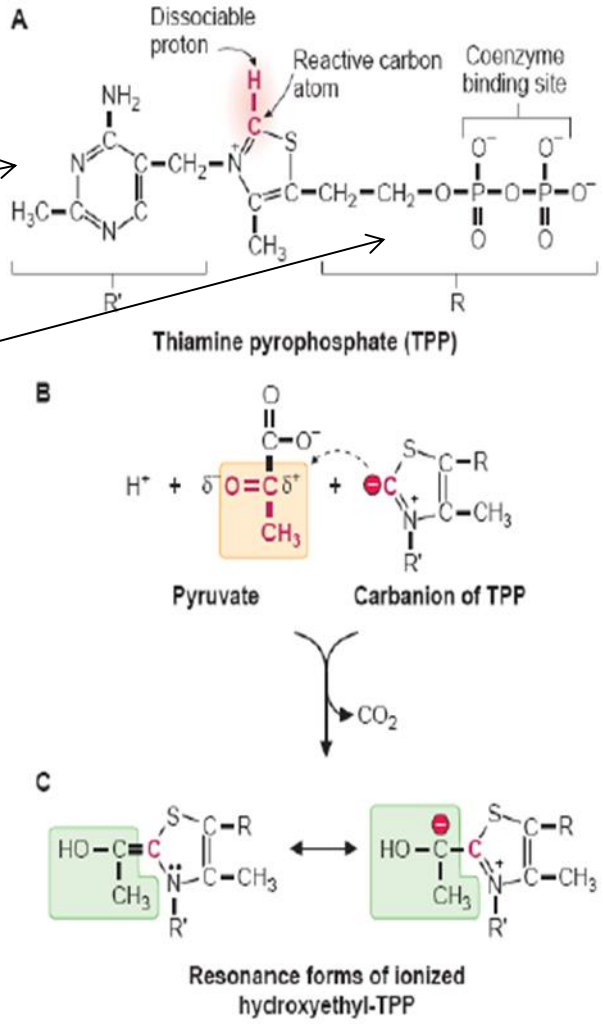
The name as we can see has **two** parts:

**1-Thiamine:**

Which is a **derivative** of vitamin **B1**.

**2-Pyrophosphate:**

Which means **Two phosphates** together.




Note:

Vitamins are classified into two different groups:

1-fat soluble: we took them before in lipids A,D,E,K and none of them can act as a coenzyme .

2-Water soluble: these are needed to build the structure of coenzymes and vitamin B1 is one of them.

Why do we need the coenzyme **TPP**?

It is responsible for **decarboxylation** reactions only (which removes a **carboxylic** group from the **substrate**).

Note:

You should always remember that every coenzyme is responsible for a specific reaction and in this example its decarboxylation reaction, and by saying a specific reaction I don't mean for one substrate but I mean the type of reaction which is decarboxylation.

When we look at the structure of **thiamine pyrophosphate** we find **two** phosphates which means that there are a lot of **negative** charges by oxygen atoms. --Why are those so important?

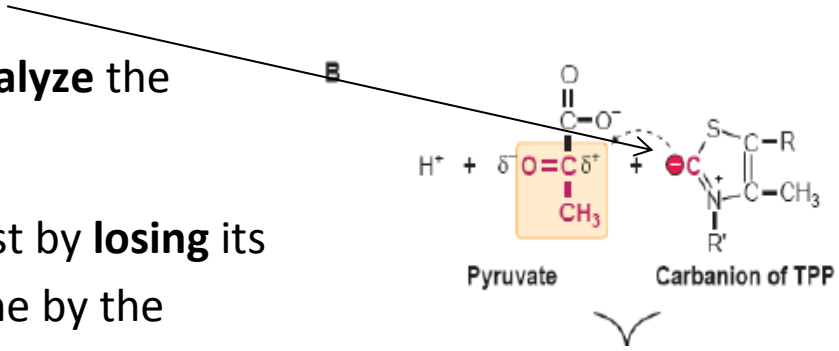
Because they help the **coenzyme** to bind with **Mg** which **preserve** the structure (when Mg is bound it will **stabilize** the whole structure).

Referring to the previous point:

1-The part responsible for **binding** is the **pyrophosphate**.

2-The part responsible for **catalysis** is the **ring** (to be specific it is the carbon **within** the ring).

-How can this carbon **catalyze** the reaction?



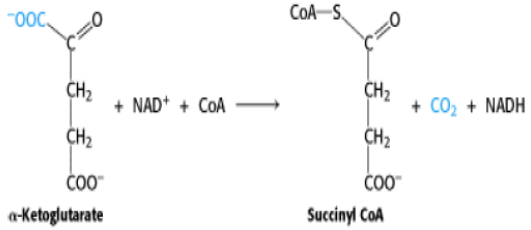

It should be **activated** first by **losing** its **Hydrogen** and that is done by the close **histidine** that can **abstract** the hydrogen. Now we have the **activated** carbon that will **attack** the **carbonyl ketone** group (on C 2) so the bonds around this carbon will be **weaken** (between **c1** and **c2**) and C1 will **leave** as a **carboxylic group** and this is why we call it **decarboxylation** reaction.

**Thiamine** is rapidly converted to its active form **thiamine pyrophosphate (TPP)** in the **brain** and **liver**.

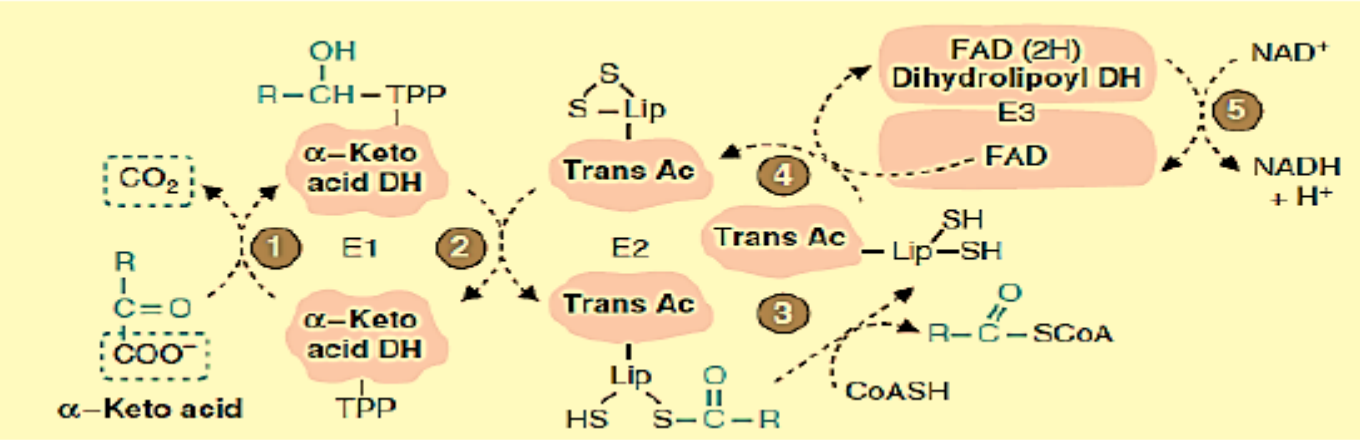
-where Can we find **TPP**?

We can find it bound to enzymes responsible for decarboxylation reactions such as:

**1- α-ketoglutarate dehydrogenase (in Kribs cycle)**:

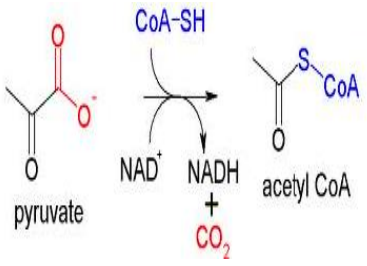



**Kribs cycle** begins with **4-carbons** and it takes another two carbons so now we have a compound with **6 carbons** and we need to get it back to its **original state** (4-carbon). So the enzyme **α-ketoglutarate dehydrogenase** (which has a TPP) will release two carboxylic groups in two separate reactions restoring back the 4-carbons molecule out of the 6-carbons molecule after two cycles releasing two (CO2)s out.




2-**Pyruvate dehydrogenase (during glycolysis):**

During **glycolysis Glucose** will be converted to **two** pyruvate molecules (**3 carbons each**). after that we need to convert **pyruvate** to **Co-A** (2 carbons) and this is done through **weakening** bonds around carbon (**2**) (so the bond between **C1 and C2** will be weaker) and C1 will leave as a **carboxylic group** (CO2) and this is why we call it a **decarboxylation reaction**. The enzyme pyruvate dehydrogenase uses the coenzyme TPP



to remove a carboxylic group from **pyruvate** to convert it to **Acetyl Co-A**.

Now lets talk about the second activation transfer coenzyme.

**2)Coenzyme A (Co-A):**

It has three parts which are :

1-**adenosine**

2-**pantothenate(pantothenic acid)**

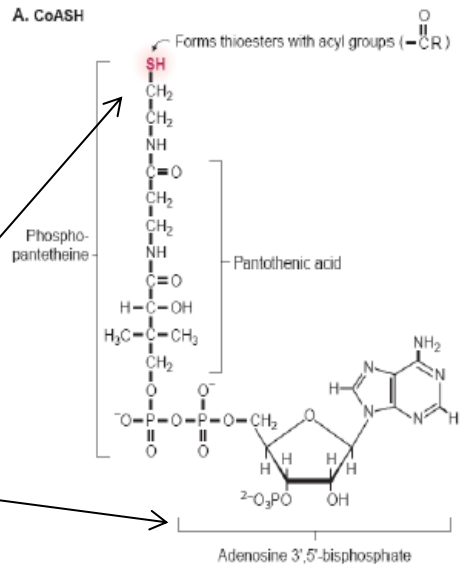
3-**modified cysteine**: and we need it to provide the **reactive sulfur group**.

-What is the **functional** group in **Co-A**?

It is the **sulfhydryl** group provided by **cysteine**.

How does the functional group (sulfhydryl) work?

It **attacks carbonyl groups** and forms **acyl thioesters** and the rest of the structure is for **binding** (more negative charges, calate metals) to **stabilize** the structure between the **enzyme** and the **coenzyme.**




So the two parts are:

1-**binding group**: **adenosine 3',5'-bisphosphate** (**tight and revesible**)

2-**funtional group**: **sulfhydryl** group derived from **cysteine** (and is part of pantothenate).

(:p أحلى من أسهم الدكتور أمجد)

-Why is it called **Co-A**?

We have **two** parts for the name (Co + A)

1-**Co**: because it **helps** the enzyme (from the word corporate).

2-A: it is coming from the **Acyl group** (three carbons or more) because it is considered as the **universal acyl carrier**. So Co-A binds a molecule with three carbons or more (**Acyl**) and **transfer** it to another place.

**Source**: from **Pantothenate** which is **vitamin B5.**

**Co-enzyme A** and some other Co-enzymes such as **$NAD^+$** and **FAD** enter different reactions with a structure (**$NAD^+$** for example) and exit with another structure (**NADH**) so it is **not** the same material, and we mentioned that enzymes should **not** be **consumed** during the reaction and this **doesn't** imply to those. So why do we consider them as **Coenzymes**?

1-They are **common** to so many reactions.

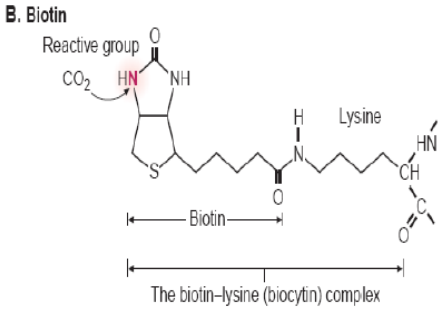
2-The **original** form is **regenerated** by subsequent reactions (NADH $\longrightarrow$ $NAD^+$ in the electron transport chain for example).

3-synthesized from **vitamins**.

4-The amount in the cell is nearly **constant** and they are needed in **small** amounts.

The third type of activation transfer Coenzyme is:

## 3) Biotin (Vitamin B7)




It is needed for **carboxylation** reactions which is the addition of a **carboxylic** group to the substrate, so it does the **opposite** of **thiamine Pyrophosphate** (**TPP**), so Biotin acts as a kinase for carboxylation reactions.

-How does **Biotin** work (**Mechanism**)?

It **adds** a **carboxyl group** to an existing **Acyl** group, so it adds a carbon to a molecule with three carbons or more (**Acyl group**) increasing the length of its chain by one.

Note

Vitamins are species dependent.

For example vitamin C for humans is ascorbic acid and its different from vitamin C for dogs which is something else.

-**Biotin** is found **covalently** bound to **Lysein** which aids to the **Biotin's stability** and that complex (Biotin + Lysein) is called **Biolysein**.

-Can we generate **vitamins** in our body?

Referring to the definition of vitamins No we can't.
**Vitamin**: is a molecule that **can't** be synthesized in our body, and is needed in **constant** and **little** amount and we get it from external sources.

-Where can we get vitamins from?

1-from **diet** (food) **mostly.**

2-**intestinal bacteria** which can **synthesize** vitamins by their own.

-**Deficiency** in Vitamins is rare to occur because intestinal bacteria are synthesizing **Vitamins** all the time.

Causes of Vitamin deficiency:

1-It occurs with long antibiotic treatment (supplements we take when we have bacterial infection) which may kill the intestinal Bactria that synthesize vitamins.

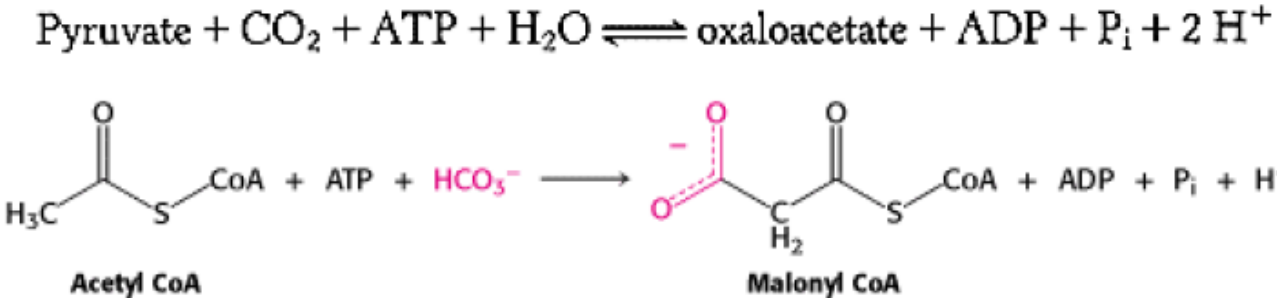
2-It can also occur with **excessive** consumption of **raw egg** which has the egg white protein **Avidin** that **have very high affinity** for **Biotin** so it binds to it and causes its deficiency.

-Where can we find **biotin**?

In any enzyme that adds a carboxylic group through carboxylation reactions such as:

**1-pyruvate carboxylase** ( we talked about it when we mentioned ligases, converts pyruvate to oxaloacetate).

**2-acytyl Co-A carboxylase** used in fatty acids synthesis.




The fourth example of activation transfer Coenzymes is:

## 4)pyridoxal phosphate (PLP)

What is the source of **PLP**?

In order to get the **two Ps**, we also need **Pyridoxine, pyridoxamine, pyridoxal**, and these structures represent **vitamin B6** and if you add two phosphates you get the **PLP**.

-Why do we need the PLP?

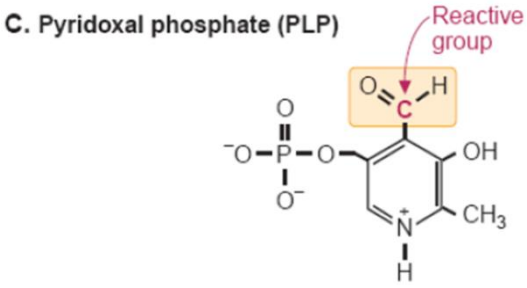
It is required for all **transamination** reactions (ALT + AST) catalyzed by **transaminases** so it functions in the **metabolism of amino acids.**

-What is the mechanism of action for **PLP**?

Note:

Transamination reactions are **reversible**

The structure of PLP contains a **reactive aldehyde group** which can **attack** the **nitrogen** in the **amino group** through its **reactive carbon** and forms a **covalent bond** with it, and then the **nitrogen** in the ring **withdraws** electrons from **bound amino acid** (cleavage of bond).




-What does **trans aminases** do?

They convert the **amino acids** into **Keto acids** and the **keto acids** into **amino acids**, and that's done through the **reactive aldehyde group** that attacks the amino group in the amino acid causing the **cleavage** of bond **facilitated** by:

**1-the nitrogen.**

**2-the ring which can abstract the hydrogen.**

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

$$\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

$$\text{Alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}$$

## 2-Oxidation reduction coenzyme

They **don't** work through covalent catalysis like activation transfer coenzymes.

We will be talking about **two** of them:

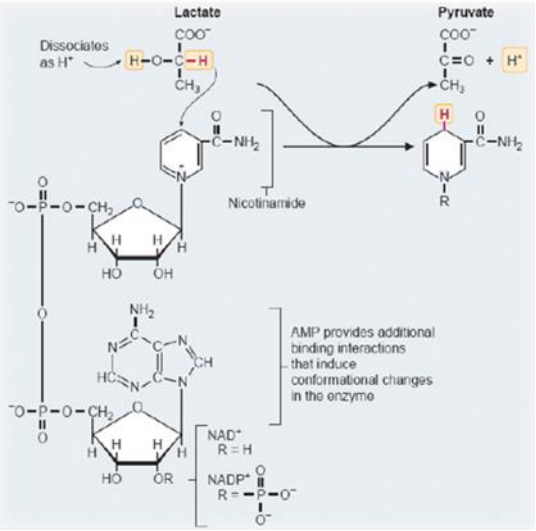
1-$NAD^+$(Nicotinamides)

2-FAD (flavins)

3-others work with metals to transfer single electrons to $O_2$ (vitamin E and C which work as antioxidants that can withdraw electrons to deal with free radicals).

- Depends on the **enzyme** for additional **specificity** of substrate and additional **catalytic power**.

**1) $NAD^+$:**




Functional group: **N opposite to C**

What does $NAD^+$ stand for?

**Nicotineamide adenine dinucleotide.**

So it has two nucleotides which are:

**1-nictotinic acid. 2-adenine.**

-$NAD^+$ is divided into two parts:

1-**Adenosine** with phosphate responsible for **binding** (with the enzyme).

2-**nicotinic acid** responsible for **catlysis** (function).

-What are the **forms** of hydrogen are there in life?

1-$\mathbf{H^+}$:has **no** electrons(proton).

2-$\mathbf{H^-}$: has **two** electrons (Hydride ion).

3-**H**:has only **one** electron (hydrogen atom).

-How many **electrons** can $NAD^+$ **accept**?

**Two** electrons at once (At the same time) by receiving $\mathbf{H^-}$ (hydride ion) which means that it **doesn't** undergo an intermediat state of one electron, so ethere it looses the two electrons or accepts them.

-Why can't we have an intermediat with one electron only?

Because having a molecule with one free electron only makes it a **free radical** intermediate which is harmful to our body. That's why it can only bind **two electrons at once** so its **safe** and can't harm the body.

So in the solution (cytoplasm) you can find **$NAD^+$** or **NADH** only.

-The catalitic part of **$NAD^+$** is a carbon in the ring that $H^-$ adds to, so it **takes electrons**.

-Where can we **find** them?

Since it takes up a hydrogen we can find it in **most dehydrogenases** which donates electrons (in the form of hydride ion) to $NAD^+$ converting it to NADH.

-But how can the $NAD^+$ take the hydrogen from the substrate?

The carbon should be **activated** through the **loss of proton ($H^+$)**, and this is achieved by the **histidine** located near the substrate because it **takes the hydrogen** and the carbon **becomes reactive**, and now it can donate the **$H^-$** to the ring and there will be a **double bond formation** between the carbon and the oxygen resulting in **NADH** formation.

Important Note:

Both $NADP^+$ and $NAD^+$ Do the same function

We can see that we took two hydrogens which are:

1-One in the form of **$H^+$** ,**bound to histidine.**

2-And the other one is in the form of **$H^-$ ,bound to the reactive carbon in the ring**

**-**What is the difference between **$NAD^+$** and **$NADP^+$**?

You look at the **R** group if it has a **P** atom its **$NADP^+$**, if it doesn't have a phosphate atom and has H atom instead it is called **$NAD^+$**.

The second acid base coenzyme is

**2)Flavin adenine dineocleotide (FAD):**

As the name implys we have two nucleotides:

1-flavine (flavin ring): responsible for **catalysis**.

2-Adenin (Adenosine) responsible for **binding** with the enzyme and it accepts **two hydrogens** in the form of **hydrogen atoms Hs** (each one of them has only one electron)

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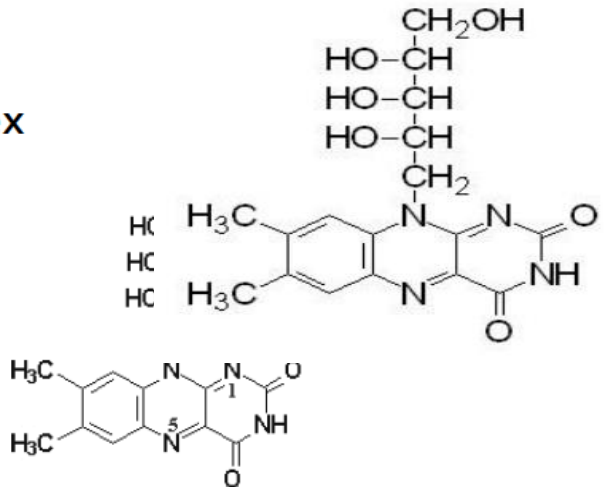


-**W**here does these electrons bind?

One of them binds to the first **nitrogen** and the other binds to the other **nitrogen.**

-Because FAD can accept **two** electrons in the form of **two** hydrogens, it can be in the form of **free radical state** where it has one hydrogen (and one electron) bound and the other is not yet bound.

**FAD+H** $\longrightarrow$ **FADH+H** $\longrightarrow$ **$FADH_2$** Or(**FMNH then $FMNH_2$**).

-Since FAD and FMN can be in the form of free radical, how can we protect the body from their resk?

They are always bound to their enzymes (proteins) and **can't** be found in the solution in the free state (you can't find **flavin** coenzyme swimming in the solution) and can't move from one enzyme to another so its bound to its enzyme.

Keep this in mind for the next semester….

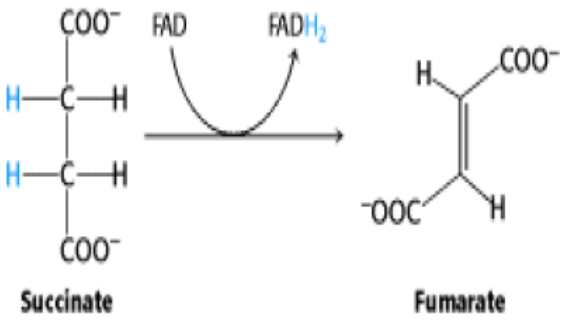
-What is FMN?

**Flavin mononucleotide** which has only **one** flavin ring.

-In FMN the two parts are:

1-**flavin** for **catalysis.**

2-**the rest** for **binding** with the enzyme.



Succinate Fumarate

Examples of enzymes that contain (FAD):

**1-succinate dehydrogenase.**

**2-pyruvate dehydrogenase complex.**

**3-α-ketoglutarate dehydrogenase.**

Examples on enzymes that contain **FMN**:

-complex enzymes in the electron transport chain (**aldehydrogenase**).

Note:

Usually the negative charges from phosphates contribute to binding with metals which aids to stabilizing the structure.

**-Water soluble vitamins:**

**1-Thiamine.B1**

**2-Riboflavin B2**

**3-nicotinic acid B3**

**4-pyridoxine B6**

**5-pantothonic acid B5**

**6-folate B12**

**7-Lipoic acid C**

**8-Ascorbic acid C**

**There is no vitamin B4 or B8 or B11, they are 8 only.**

## Water-Soluble Vitamins

| Name | Coenzyme or Active Form | Primary biochemical function |
|---|---|---|
| Thiamin | Thiamine pyrophosphate (TPP) | Aldehyde-group transfer |
| Riboflavin | Flavin mononucleotide (FMN)<br>Flavin adenine dinucleotide (FAD) | Hydrogen-Atom (electron) transfer<br>Hydrogen-Atom (electron) transfer |
| Nicotinic Acid | Nicotinamide adenine dinucleotide (NAD)<br>Nicotinamide adenine dinucleotide phosphate (NADP) | Hydrogen-Atom (electron) transfer<br>Hydrogen-Atom (electron) transfer |
| Pantothenic Acid | Coenzyme A (CoA) | Acyl-group transfer |
| Pyridoxine | Pyridoxal Phosphate | Amino-group transfer |
| Biotin | Biocytin | Carboxyl transfer |
| Folate | Tetrahydrofolate | One-Carbon group transfer |
| Vitamin $B_{12}$ | Coenzyme $B_{12}$ | 1,2 shift hydrogen atoms |
| Lipoic Acid | Lipoyllysine | Hydrogen-Atom and Acyl-group transfer |
| Ascorbic Acid | Ascorbic acid, dehydroascorbic acid | Cofactor in hydroxylation |

**-Catalitic metals:**

Metals which are responsible for catalysis are ether:

**1-metals activated enzymes:**

which are **loosely** bound to the enzyme and it can **bind** and **dissociate**, **without denaturating** the enzyme when dissociated

**2-metaloenzymes:**

Which is part of the structure of the enzyme and is attached to it during **manufacturing** of them, so the loss of metals in this case will cause **denaturation** of the enzyme (loss of structure and function).

**-Metal activated enzymes:**

| Metal | Enzyme |
|---|---|
| $Zn^{2+}$ | Carbonic anhydrase |
| $Zn^{2+}$ | Carboxypeptidase |
| $Mg^{2+}$ | Hexokinase |
| Se | Glutathione peroxidase |
| $Mn^{2+}$ | Superoxide dismutase |

- Phosphofructokinase & TPP; ($Mg^{2+}$) is required to coordinate the phosphate groups on the ATP for a successful reaction (chelation)

-In **TPP** we have two **Ps** to bind Mg for:

1-**stabilise** the structure

2-**coordinate** the phosphate group on the ATP for successful reaction (chelation)

-There is another example in the book which is **Alcohol dehydrogenase** : as the name implys we have alcohols and you want to **remove hydrogens** from it.

-How can you remove Hydrogens from alcohols through those enzymes?

You Have **serien** (serien 3abd ennor, Ha3) group and **alcohol** (OH) , **serien** will **withdraw** the hydrogen present in the hydroxylic group, so oxygen now has one **negative charge** and this compound is called **oaxnion intermediat** (not sure about the name), and **zink** satbilizes this intermediat (without it the enzyme won't work) by forming a bond as a result the other bonds will be weak abd the hydrogen atom will be broken and this is the purpose of the enzyme (**dehydrogenation**).

**-Metaloenzymes**:Enzymes are part of the structure (when the enzyme is manufactured there will be an inclusion of metal in the strauctur e)

And if you remove the metal their will be **denaturation** and those metals do one of **two** functions :

1- **Catalysis**

2-**preserving the structure** of the enzyme

-Examples include:

**1-liver alcohol dehydrogenase** (**a dimer**, two subunits) which has **<u>two zink atoms in each monomer</u>** , each one of them for a specific function:

**A)one for structural maintainance.**

**B) the other is catalitic**

Note: since that molecule is a dimer, it **has 4 zink atoms.**

**Carbonic anhydrase** : a **zink** atom is essentially always bound to **four or more** atoms.

الشيت الشامل للطالب الهامل p:

You are special ☺