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BIOCHEMISTRY

faculty of medicine - JU2017

Sheet ○ Slides

DONE BY

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DOCTOR

Ma'moun

Cofactors are non-protein compounds, they are divided into 3 types:

- Protein-based.
- Metals: if they are bounded **tightly** (covalently) to the enzyme we call them **metallo-proteins**
  If they are bounded **loosely** (non-covalently) to the enzyme, we call them **metal-associated proteins.**
- Small organic molecules (co-enzymes) [vitamins]:
  If they are bounded **tightly** (covalently) to the enzyme, we call them **Prosthetic group**.
  If they are bounded **loosely** (non-covalently), we call them **Co-substrates.**

Co-enzymes in general:

They are organic materials that can bind to a place close to the active site or to a part of it (in order to affect the enzyme ,they have to bind to it). They regulate the function of the enzymes those enzymes are called **conjugated enzymes**,

but Not all enzymes need co-enzymes to function, some enzymes such as chymotrypsin normally function without them (the sidechains of the active site do the job completely)

- Eg :- 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

Co-enzymes are originally from Vitamins (all water –soluble vitamins work as co-enzymes, lipid-soluble vitamins don't work as co-enzymes).

Coenzymes are divided into two types:

1) Activation-transfer coenzymes (vitamins 1/5/6/7).
2) Oxidation–reduction coenzymes (vitamins 2/3).

Water-Soluble Vitamins

Name	Coenzyme or Active Form	Primary biochemical function
Thiamin	Thiamine pyrophosphate (TPP)	Aldehyde-group transfer
Riboflavin	Flavin mononucleotide (FMN) Flavin adenine dinucleotide (FAD)	Hydrogen-Atom (electron) transfer Hydrogen-Atom (electron) transfer
Nicotinic Acid	Nicotinamide adenine dinucleotide (NAD) Nicotinamide adenine dinucleotide phosphate (NADP)	Hydrogen-Atom (electron) transfer 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

1. Activation-Transfer Coenzymes:

They usually participate directly in catalysis by transferring groups from one molecule to another and **forming covalent bond** with the substrate

There are two groups of the coenzyme:

1- **Functional group→** forms a covalent bond with the substrate.
2- **Binding group→** binds tightly to the enzyme.

(some structures could be responsible for both binding and forming the covalent bond).

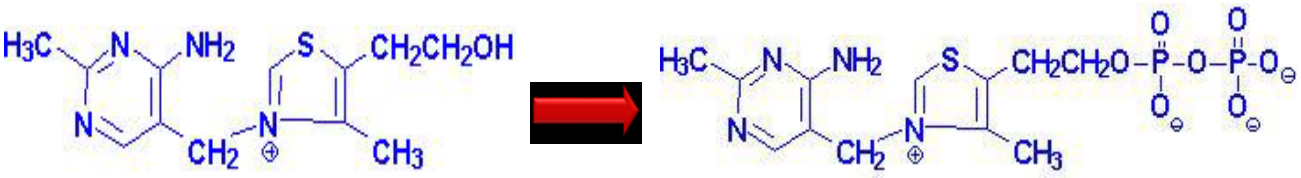
Note: It depends on the enzyme for additional specificity of substrate & additional catalytic power (the both groups increase the specificity of the enzyme and the functional group increases its catalytic power)

We will talk about 4 Co-enzymes that are considered as activation transfer co-enzymes.

1. Thiamin pyrophosphate, TPP:

The source is Thiamin (vitamin B1) (inactive form) → it binds to Pyrophosphate (2 phosphate groups PP) and converted to its active form, this happens in the brain and liver.

Function: it is involved in **decarboxylation** reactions.



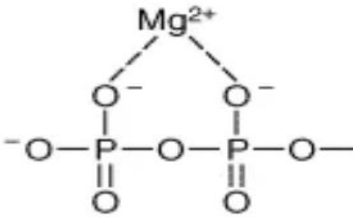
Inactive active

Recognize the whole structure of the active form (2P)

Functional group: the carbon atom (between N and S in the thiazole group (the middle ring)

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

- **Chelated magnesium** means that the mineral is bounded to a one or more negatively charged group and forms o cycle.

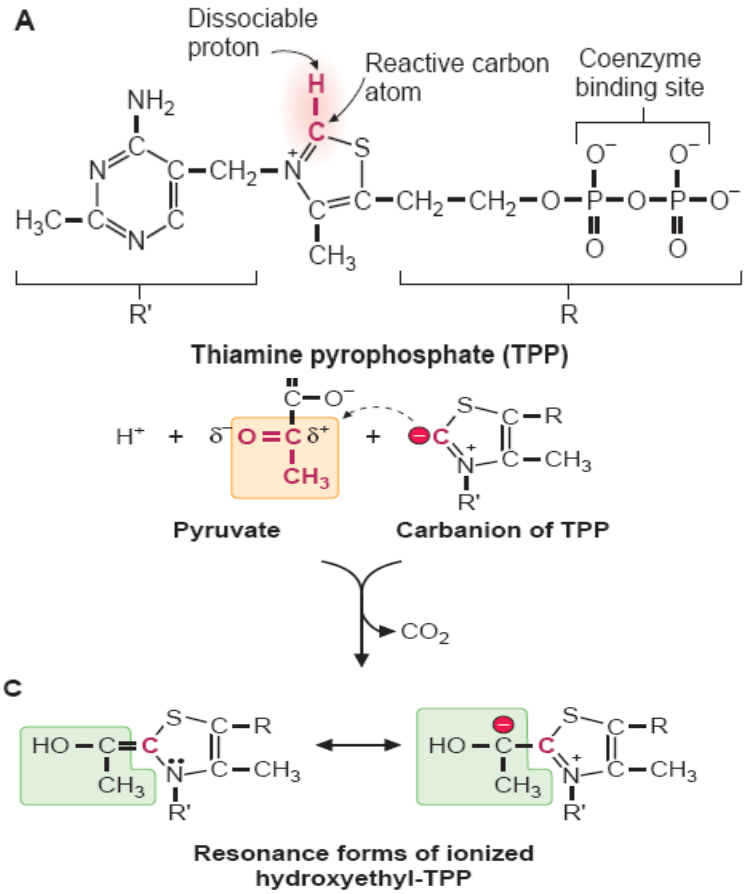


Reactions TTP is related to:

- Pyruvate dehydrogenase complex
  This complex contributes in oxidation-reduction reactions. The pyruvate dehydrogenase complex has three enzymes, one of them is Decarboxylase, so it should have TTP.

The pyruvate dehydrogenase complex has three enzymes:
1-decarboxylase
2- hydrogenase
3- transacetylase.

Mechanism of action

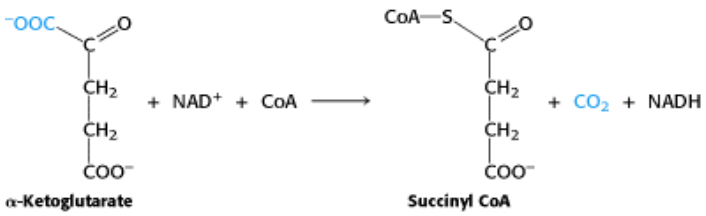


The functional group is the reactive carbon atom that forms a covalent bond with a substrate's keto group while cleaving the adjacent carbon– carbon bond.

DETAILED EXPLANATION

Functional group has a reactive carbon atom that loses a hydrogen atom making it unstable. The reactive carbon (of the thiazole ring) reacts with the keto group of the substrate (note that it is bounded to another carbon), which causes the bond between the carboxyl group and the carbon in the substrate to weaken breaking of the carboxyl group from the rest of the substrate , then the remaining R group leaves the enzyme.

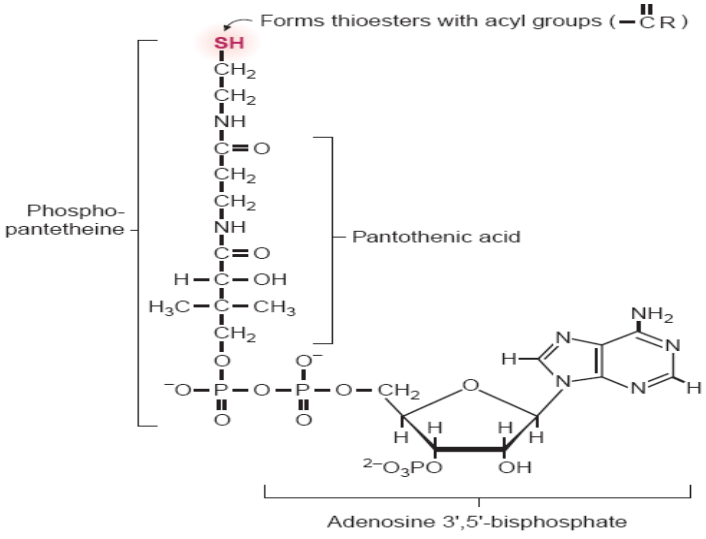
- Alpha keto dehydrogenase complex. (α-ketoglutarate dehydrogenase) converts α-ketoglutarate into succinyl CoA by decarboxylation.



2. Coenzyme A (CoA)

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

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.



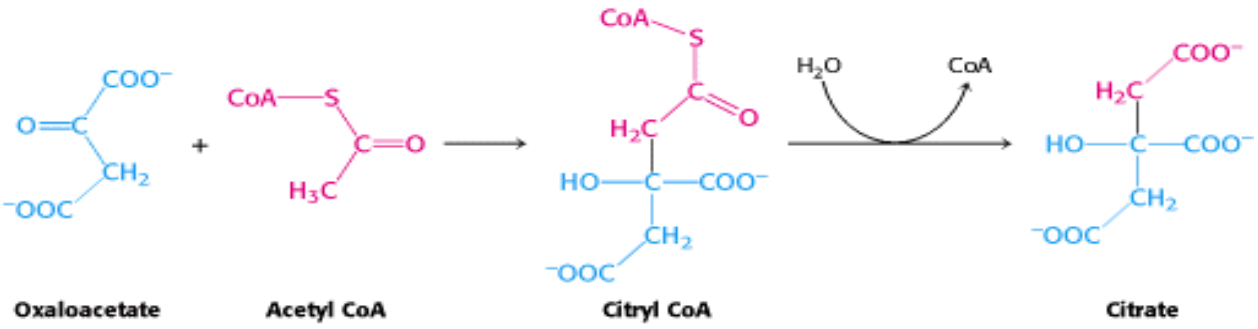
- Co-enzymes should not change in anyway during the reaction, but with some co-enzymes that we have studied, they change during the reaction like: NAD+ changes to NADH. CoA attaches to carbons (so it is technically changed). Why are they are still considered as co-enzymes? Because in subsequent reactions, they will return to their original structure. (**DR NAFED!!!)**

Examples of enzymes:

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

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

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



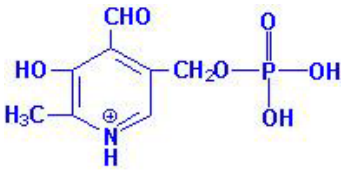
3. Pyridoxal phosphate (vitamin B6) →(ACTIVE)

- **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:** the rest of the molecule.

Pyridoxine

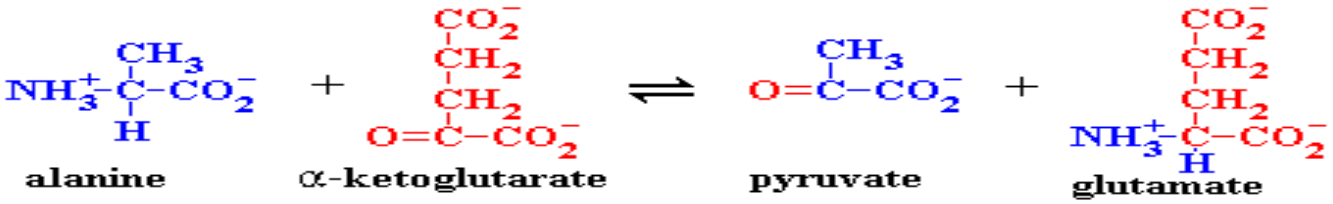
Pyridoxal

Pyridoxamine



pyridoxal phosphate

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



- When alfa-ketoglutarate (intermediate of the citric acid cycle) binds with alanine→
  this reaction makes a connection between the metabolism of carbohydrates and the metabolism of proteins.

Examples:

Amino acids	alanine	glutamate	aspartate
↓transaminases↓			
Keto acids	pyruvate	Alfa-ketoglutarate	oxaloacetate

- Aspartate aminotransferase

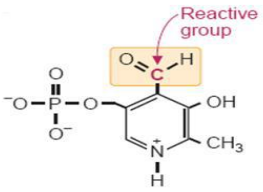
$$\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

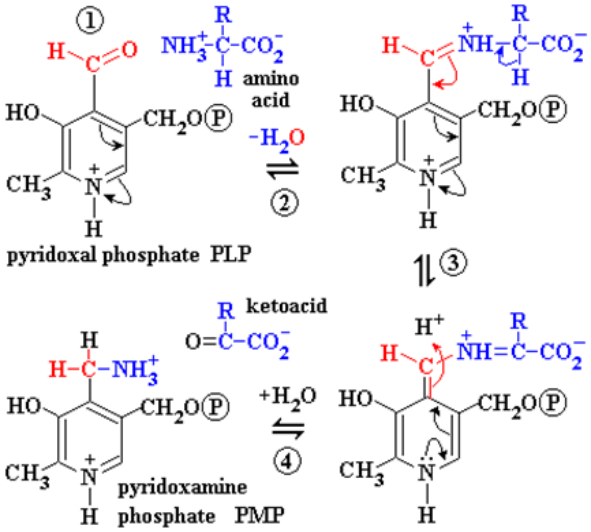
- Alanine aminotransferase

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

Mechanism of action:

- The reactive aldehyde forms a covalent bond with the amino groups, then the ring nitrogen withdraws electrons from bound amino acid (cleavage of bond).
- Binding and functional groups are within the ring.



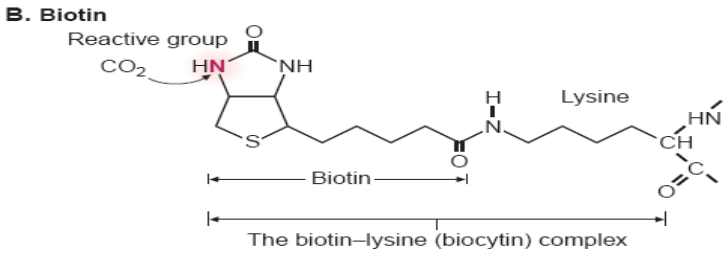


4. Biotin (vitamin B7)

- **Source:** food and intestinal bacteria
- **Function:** carboxylation (covalently bound to **LYS**)
- **Functional group:** NH of the ring
- **Binding group:** the rest of the molecule.

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) or excessive consumption of raw eggs (egg white protein, avidin, has high affinity for biotin.

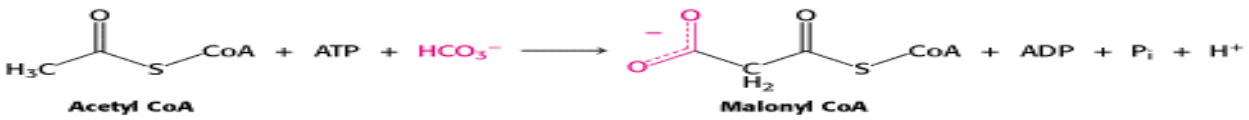
- its active structure consists of its main structure bounded covalently to Lysine, creating a structure called **(Biocytin)**



Examples:

- Pyruvate carboxylase
- Acetyl CoA carboxylase (fatty acid synthesis)

$$\text{Pyruvate} + CO_2 + ATP + H_2O \rightleftharpoons \text{oxaloacetate} + ADP + P_i + 2\,H^+$$



- malonyl is the starting point in fatty acid synthesis

2.OXIDATION–REDUCTION COENZYMES

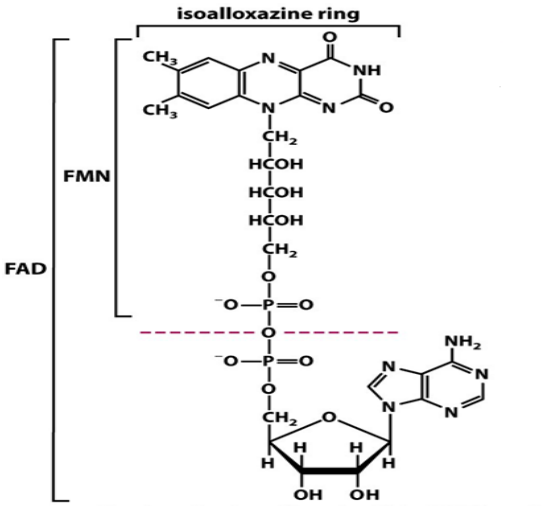
- A number of coenzymes that work within oxidoreductases.
- Each coenzyme has a unique functional group that accepts and donates electrons and is specific for the form of electrons it transfers (e.g., hydride ions, hydrogen atoms, oxygen).
- they do not form covalent bonds with the substrate, in fact, a portion of the coenzyme binds the enzyme.
- Most common: NAD$^+$ (niacin, B3) & FAD (riboflavin, B2)
- Others: such as Vitamins E & C work with metals to transfer single electrons to O_2 to protect the body from reactive oxygen species. They can be regenerated.

Reminder: coenzymes do NOT bind to the substrate, they interact with the enzyme helping in extracting or donating electrons to the substrate

Reminder:
The coenzyme is mainly responsible for catalysis
The enzyme is mainly responsible for determining substrate specificity and contributing to the catalytic power

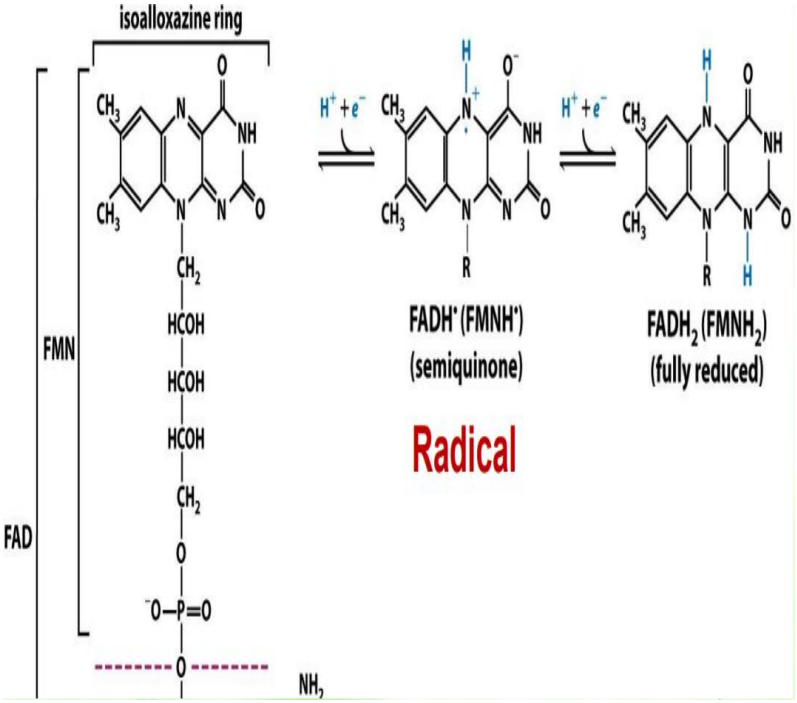
FAD and FMN

- The precursor is **Riboflavin** (aka. vitamin B2).
- Both are prosthetic groups of flavoproteins.
- **FAD** is a redox cofactor, more specifically a prosthetic group of a protein, involved in several important enzymatic reactions in metabolism.
- **FAD** is a dinucleotide whereas **FMN** is a mononucleotide, FMN is included in FAD
- They can accept 2 protons (and 2 electrons) sequentially



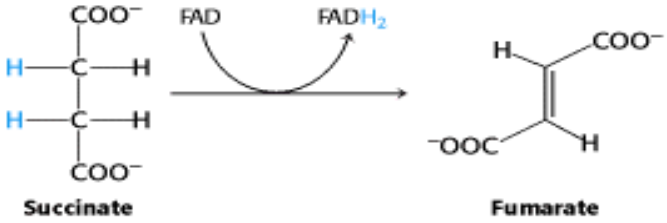
Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

- A radical **(FADH⁺)** is formed when **FAD** is transferred into **$FADH_2$** (same applies for FMN to $FMNH_2$). Generally, radicals are dangerous and can damage many molecules in the cell (such as fatty acids). But for this case FAD and FMN are **prosthetic groups** (bound covalently to the active site), which means they're not free and they can't attack other molecules.



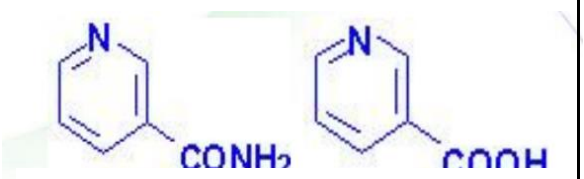
Succinate dehydrogenase

- Oxidation of succinate into fumarate by **succinate dehydrogenase**
- This enzyme is found in the inner membrane of the mitochondria. The $FADH_2$ produced is needed in the electron transport chain.



NAD+ and NADP+

- They're electron carriers that participate in oxidation-reduction reactions and can accept electrons in the form of hydride ion.
- Precursor of **NAD^+** and **$NADP^+$** is niacin (vitamin B3).
- NAD^+ consists of a nicotinic ring, adenosine and dinucleotide. NADP+ is different from NAD+ only by a phosphate group instead of a hydrogen atom.
- The difference between NAD^+ and $NADP^+$ is a phosphate group
- The phosphate group has nothing to do with the function of the coenzyme, instead it determines to what enzymes they bind

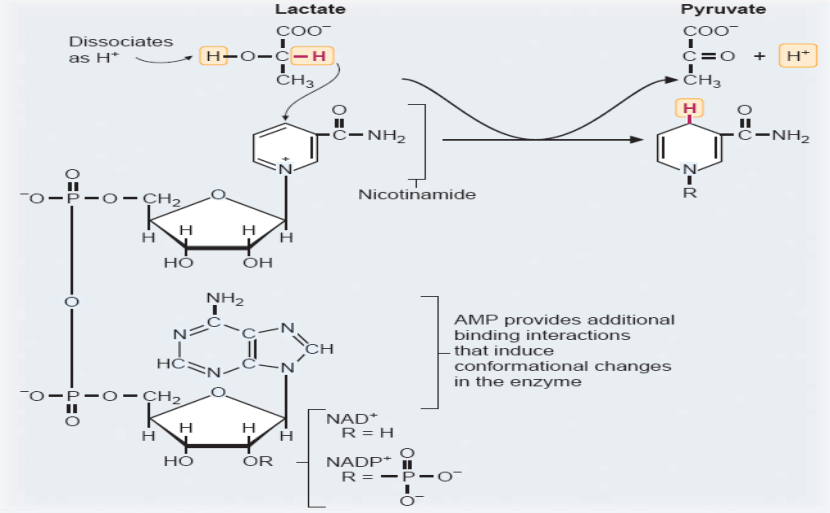


This is both, the reactive group of NAD^+ and $NADP^+$ and also a precursor of them (vitamin B3)

- NAD^+ participates in **catabolism**. $NADP^+$ participates in **anabolism**
- Both of them do NOT bind covalently to the enzyme (they're not prosthetic groups, they're co-substrates)
- The cofactor accepts a hydride ion from lactate producing NADH or NADPH, a proton dissociates

NOTE:

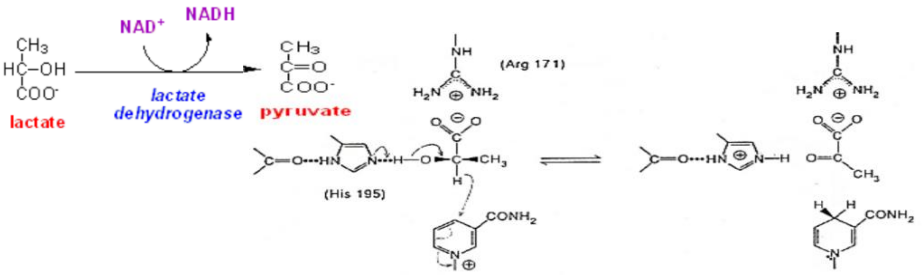
- FAD accepts 2 protons sequentially, it forms a radical, that's why it is prosthetic
- NAD^+ accepts a single hydride, it doesn't form a radical, so it doesn't have to be prosthetic.



Lactate dehydrogenase

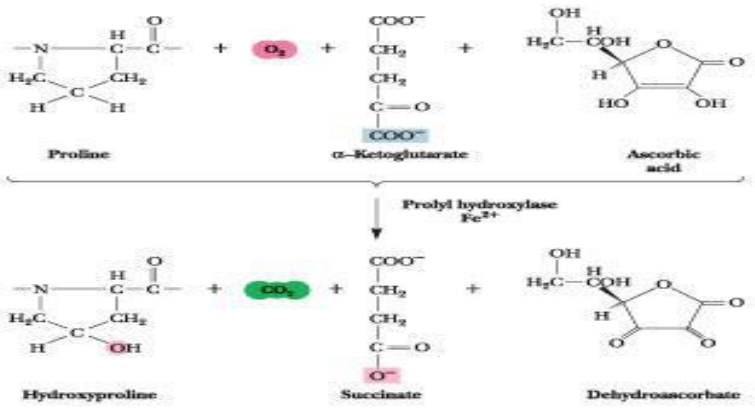
Mechanism:

- Lactate dehydrogenase's **histidine** binds the **proton** of (-OH) on lactate making it easier for NAD^+ to pull off the other hydrogen with both electrons (a hydride).
- A keto group (CO) is formed.



Vitamin C

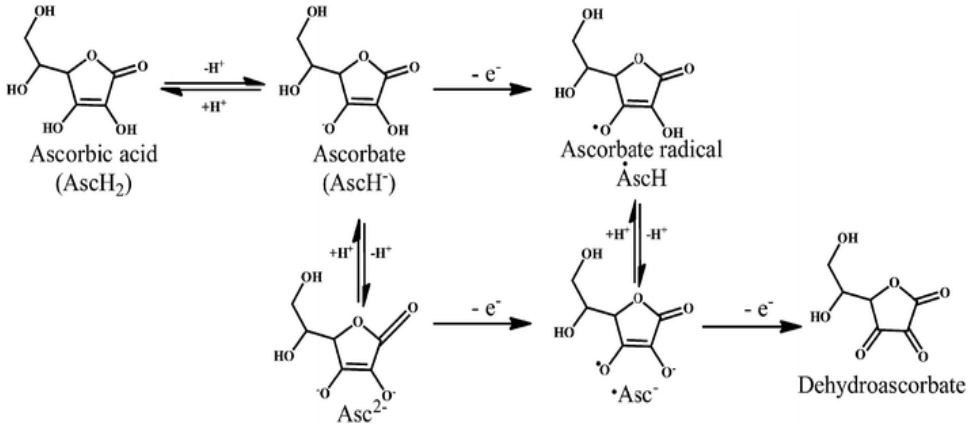
- Ascorbic acid, it's Important for reactions including prolyl hydroxylase to synthesize 4-hydroxyproline
- It works as an anti-oxidant too



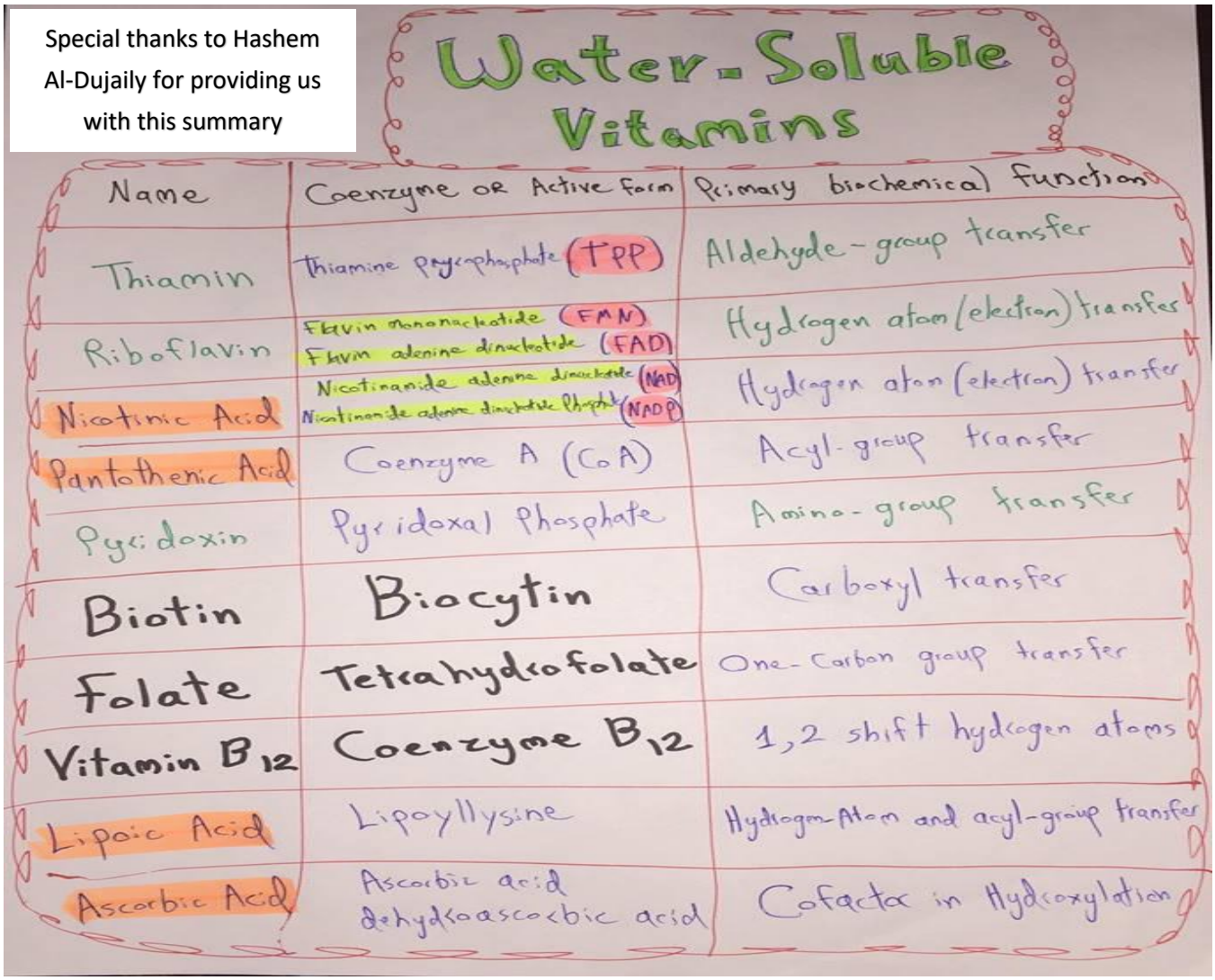
Ascorbate

- Reactive oxygen species oxidize (take electrons from) ascorbate into a **radical** itself, which is then oxidized into dehydroascorbate.
- The oxidized forms of ascorbate are relatively stable, unreactive, and do not cause cellular damage. The structure of vitamin C (and other anti-oxidants) is preferable due to formation of resonance.

Ascorbate radical (and other anti-oxidants) doesn't damage the cell because it's a ring structure that's stabilized by resonance, in contrast to ROS that are chain structures which have no resonance and as a result can be harmful to the cell



Special thanks to Hashem Al-Dujaily for providing us with this summary



CATALYTIC METALS

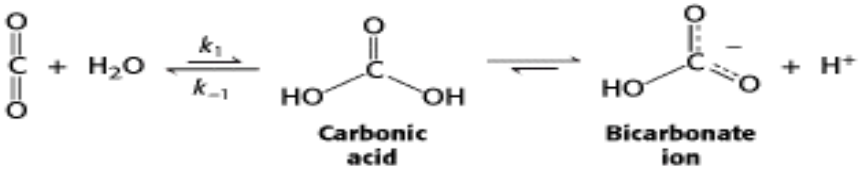
Metal	Enzyme
Zn^{2+}	Carbonic anhydrase Carboxypeptidase
Mg^{2+}	Hexokinase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase

- A catalytic metal is an enzyme with a metal attached to it.
- They act as **electrophiles**. They can also accept and donate electrons in oxidation–reduction reactions. They assist in binding of the substrate, or they stabilize developing anions in the reaction.
- They're stable in more than one oxidation state (recall: ferrous and ferric states of iron with hemoglobin)
- They carry positive charges and, hence, can form relatively strong yet kinetically labile (likely to be changed) bonds.
- They can bind multiple ligands in their coordination sphere enabling them to participate in binding substrates or coenzymes to enzymes.

Mg^{2+}

- Hexokinase catalyzes the formation of Glucose-6-phosphate from ATP and Glucose. The phosphate groups of ATPs are usually bound to enzymes through Mg^{+2} **chelation**.
- Mg^{+2} also connects the negatively charged phosphate groups of thiamine pyrophosphate to basic amino acids in the enzyme.

Carbonic anhydrases



- Although CO_2 hydration and HCO_3^- dehydration occur spontaneously in the absence of catalysts, almost all organisms **contain** carbonic anhydrases, because they are often coupled to rapid processes such as respiration.
- Mutations in carbonic anhydrases have been found to cause osteopetrosis (excessive formation of dense bones accompanied by anemia) and mental retardation

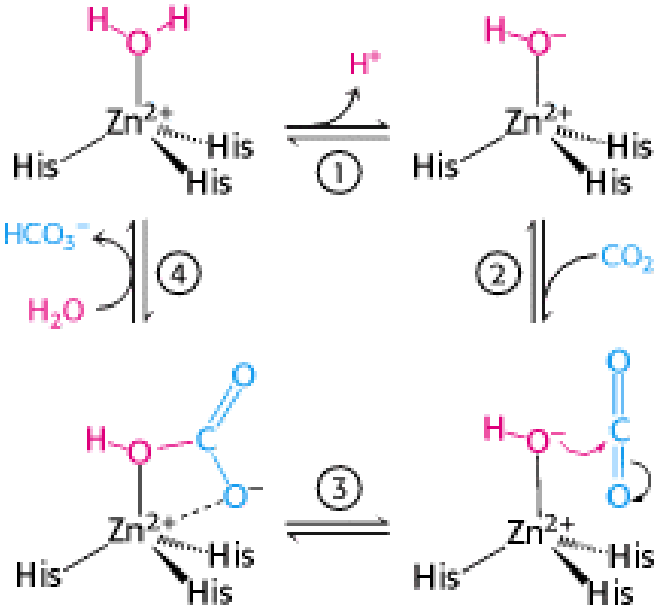
Zn binding to the enzyme

- In carbonic anhydrase, a zinc atom assists un catalyzing the reaction by binding to three imidazole rings of **three histidine residues** and an additional site is occupied by **a water molecule**.

Zinc is found only in the +2 state in biological systems.

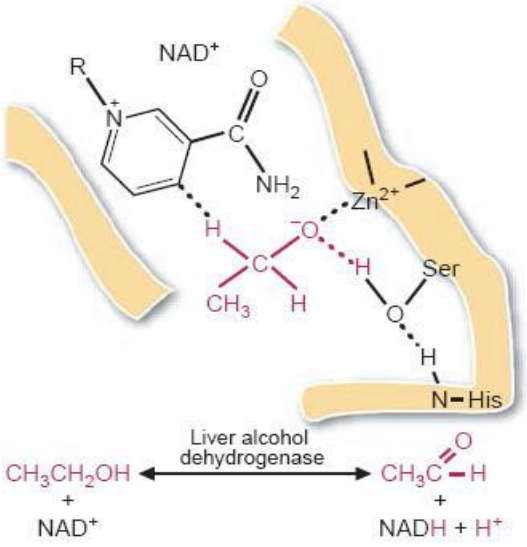
Mechanism of the reaction:

- When H_2O binds to Zinc in carbonic anhydrase, a proton dissociates, and a hydroxide ion is formed which allows the entry of CO_2 to the active site. The Zn here puts the substrate into the right orientation, so the CO_2 and H_2O are close and proximal to each other. The hydroxide ion attacks CO_2 converting it into bicarbonate ion.
- The catalytic site is regenerated with the release of the bicarbonate ion and the binding of another H_2O.



Zn and alcohol dehydrogenase

- Alcohol dehydrogenase catalyzes oxidation of alcohol into acetal aldehyde
- Zinc in the active site of alcohol dehydrogenase stabilizes the interaction between the alcohol and the active site
- we have an alcohol molecule and NAD+ (will accept electrons in the form of hydride ion).
- The histidine of ADH pulls a proton off the active site's serine, which pulls the proton off of the substrate's OH group, leaving the oxygen with a negative charge that is stabilized by zinc, and a hydride is the transferred to NAD+.
- Zinc in alcohol dehydrogenase is as Histidine in lactate dehydrogenase.



- And there are 2 types of catalytic enzymes with different functions, some metals are tightly bounded which means they're integral to the structure so if we removed the metal, the enzyme will be denatured
- The other type is loosely bound to the enzyme (enters and leaves without ruining the enzyme). For example: we have Phosphofructokinase and TPP, Mg^{+2} is required to coordinate the phosphate group on ATP to ensure a successful reaction.

Good luck future doctors