بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

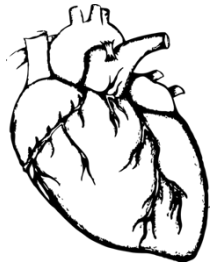
BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture 17

Vitamins & Cofactors

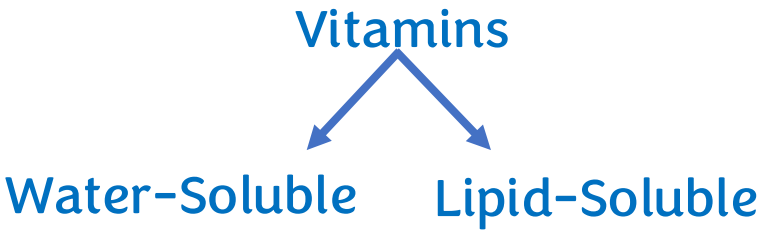
Reviewed by : Khaled Abdalla
Hamza Sarairah
Muhanad Barhoom



Color coding used in the modified:

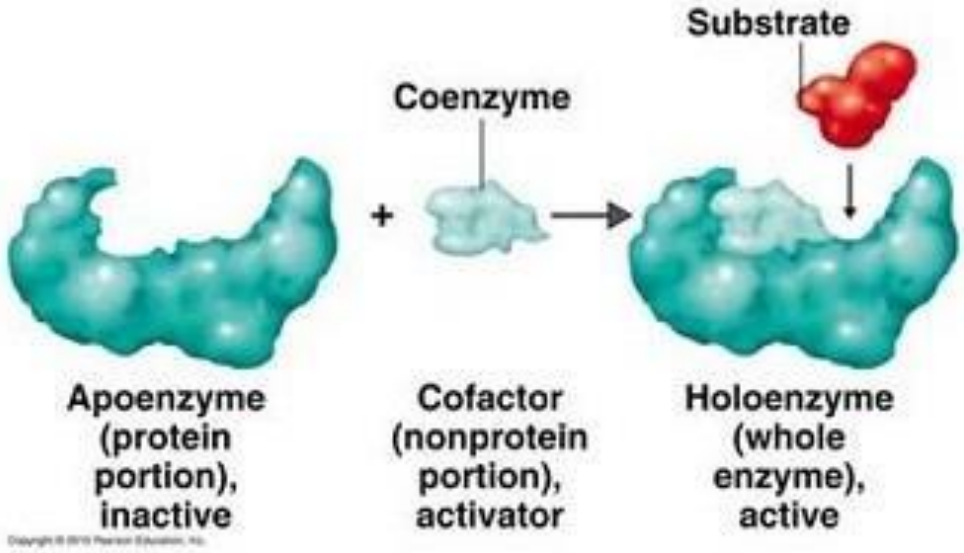
- *Black: the original slides*
- *Blue: the doctor’s explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Water-soluble vitamins



Catalytic strategies of enzymes

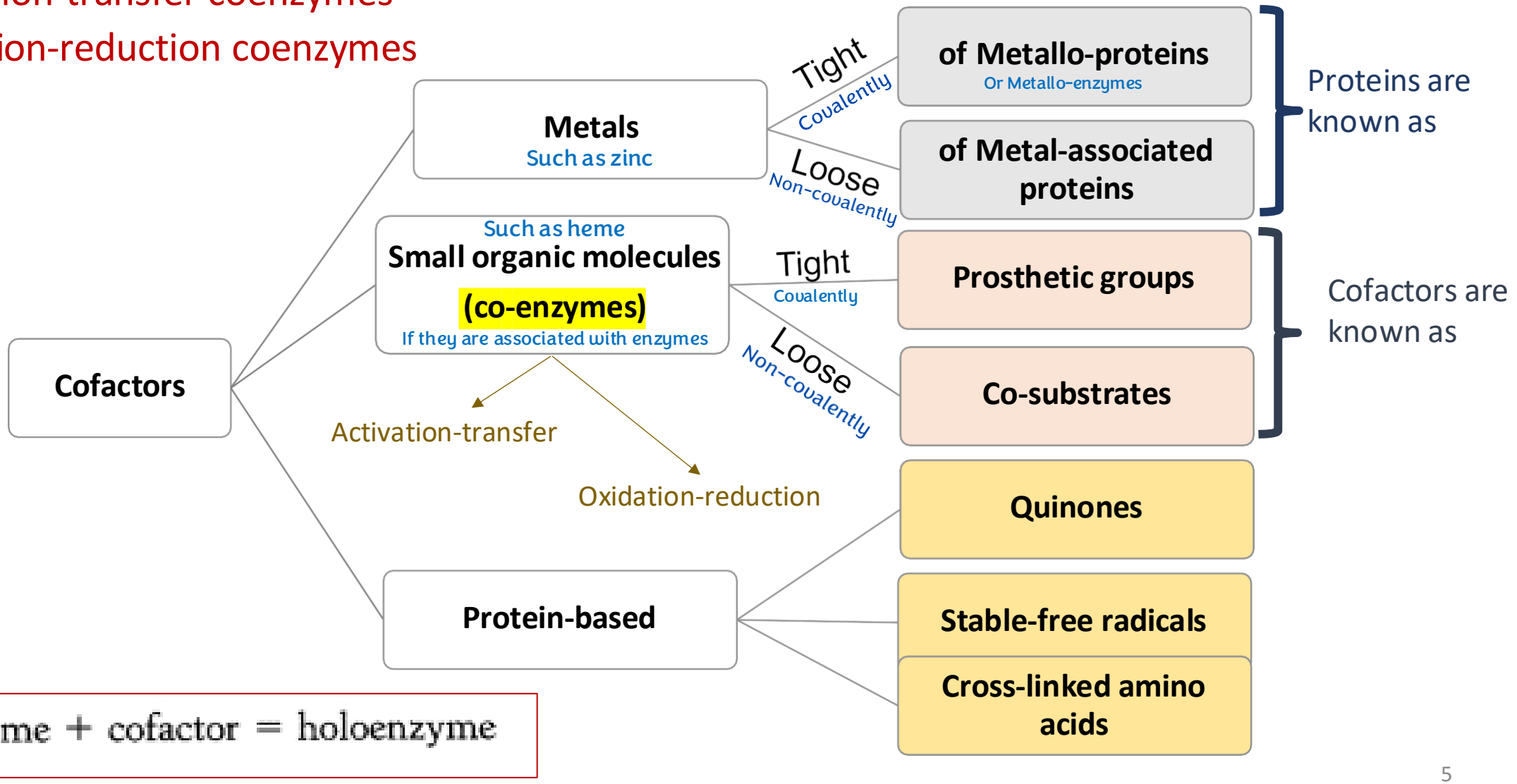
- Enzymes carry out reactions utilizing different catalytic strategies.
  - Some enzymes, such as chymotrypsin, rely on specific, reactive, polar amino acid residues within the active site to catalyze reactions.
  - Other enzymes need cofactors (nonprotein compounds that participate in the catalytic process).
    - These are called conjugated enzymes (holoenzyme vs. apoenzyme)



Classification of cofactors

Coenzymes are:

1. Activation-transfer coenzymes
2. Oxidation-reduction coenzymes



Apoenzyme + cofactor = holoenzyme

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

Do NOT memorize.

The table nicely summarizes the lecture.

Activation-transfer coenzymes

Oxidation-reduction coenzymes

Water-soluble vitamins are divided into 3 groups:

1. Vitamin B group
2. Vitamin C group
3. Lipoic Acid group

Activation-transfer coenzymes

Activation-transfer coenzymes

- The functional group of the coenzyme directly participates in catalysis.

The coenzyme itself is the one which catalyzes the reaction.

- Characteristics:
  - Two important chemical groups in the coenzyme:
    - A functional group that forms a covalent bond with the substrate.
    - A binding group that binds tightly to the enzyme.

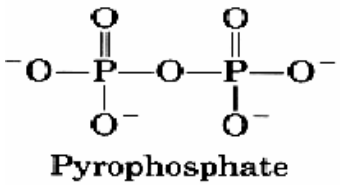
You may wonder what is the role of the enzyme since the coenzyme can catalyze the reaction by itself. Well, here is the explanation.

- The enzyme specifies the substrate & the catalytic mechanism.

If the coenzyme is the one which catalyze the reaction, the enzyme's role is to provide the proper environment and positioning for the coenzyme and the substrate. The enzyme ensures that the coenzyme is correctly aligned with the substrate, enhancing the efficiency and specificity of the reaction. Also, the enzyme optimize the surrounding conditions and facilitate the interaction between the coenzyme and the substrate.

1) Thiamin pyrophosphate, TPP

Vitamin B1

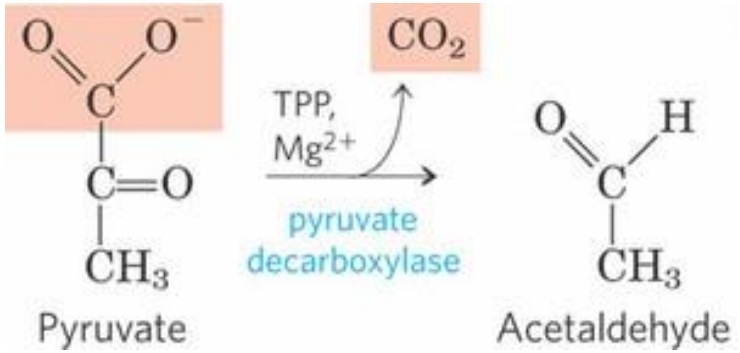


Pyrophosphate is a structure of two phosphate groups connected.

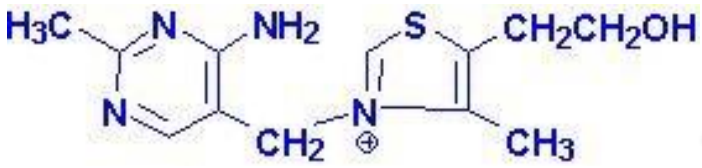
Do NOT memorize the structure of the vitamins, but you must be able to identify the vitamin & its functional and binding groups.

- Thiamin (vitamin B1) is converted to its active form, thiamin pyrophosphate (TPP).
- It is involved in **decarboxylation** reactions.
- The negatively charged oxygen atoms of the pyrophosphate tightly link TPP to the enzyme via Mg^{2+} through chelation.
- The reactive thiamin carbon binds to the substrates releasing CO_2

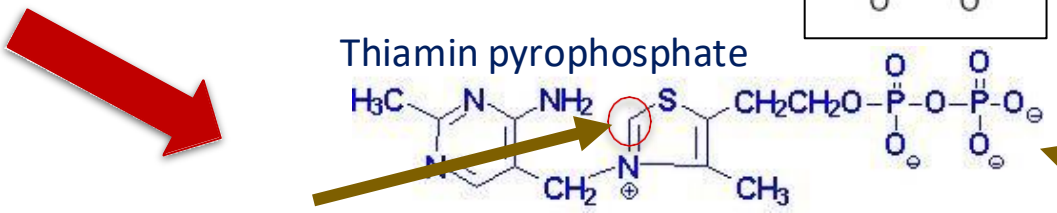
Releases energy.



Chelation: a chemical reaction where a metal ion is linked to non-metal groups by two or more bonds within the same molecule (the same case in iron with heme).



Thiamin

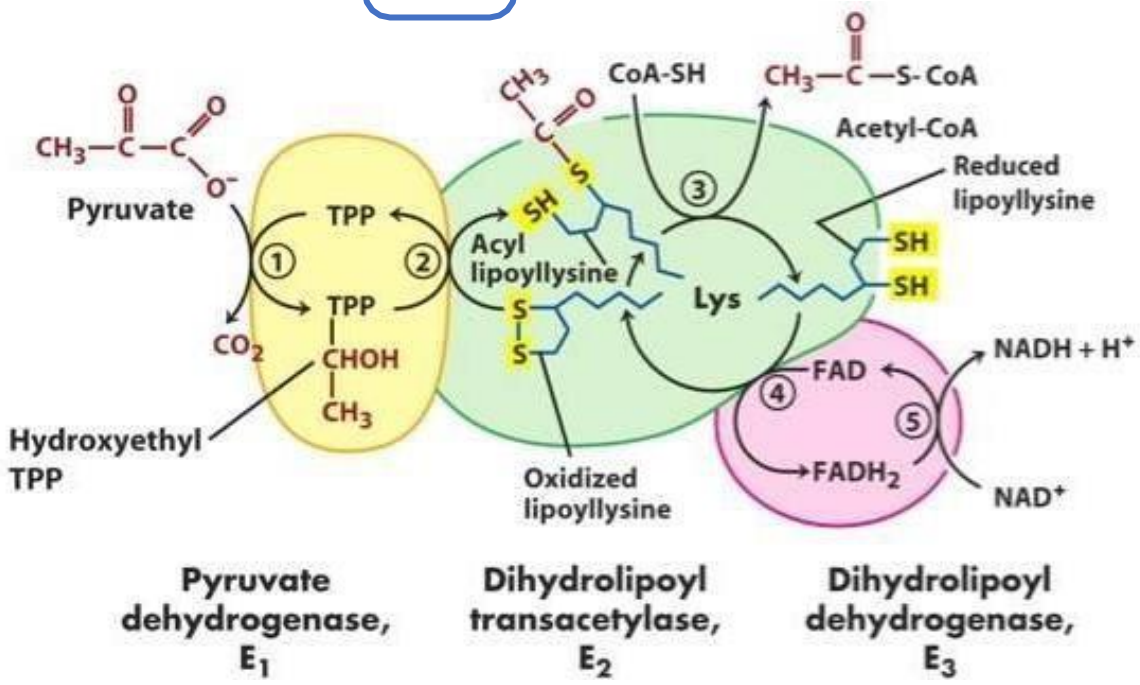


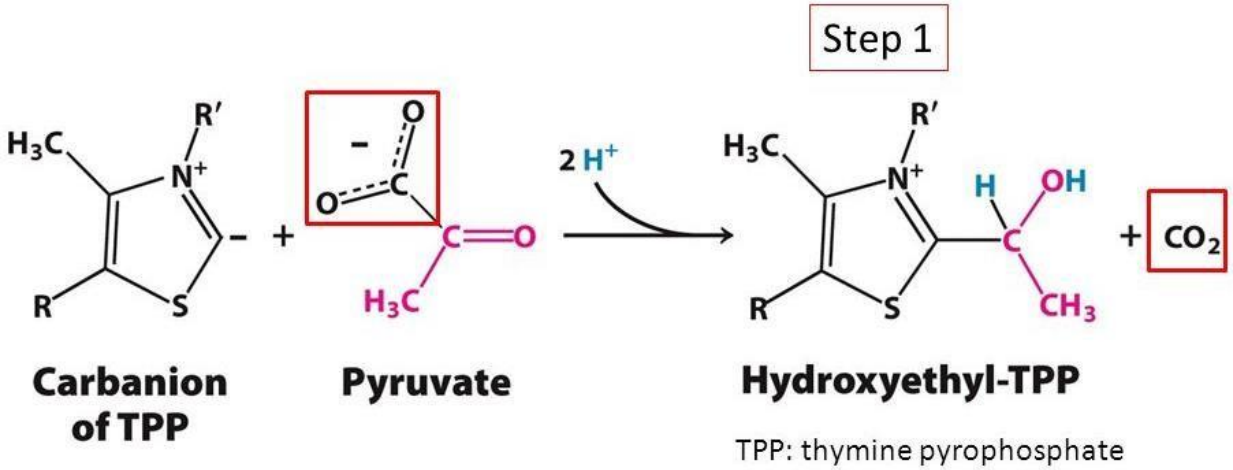
Pyruvate dehydrogenase complex

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

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

  A reaction which needs TPP.



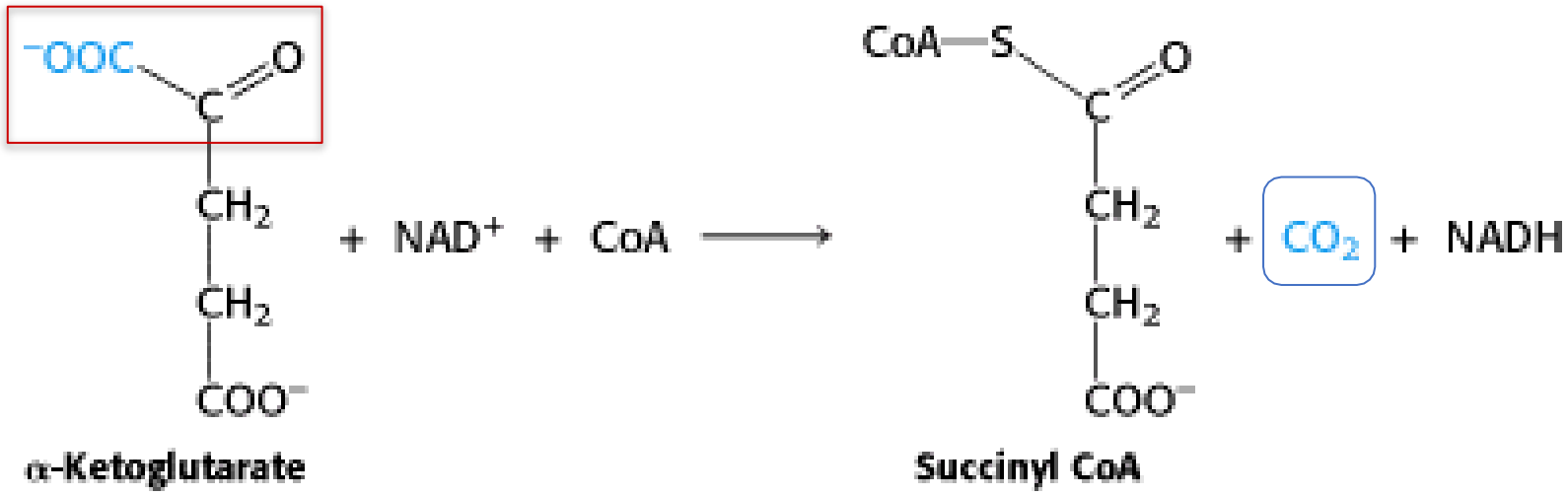


TPP: thymine pyrophosphate

α-ketoglutarate dehydrogenase

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

Another reaction which needs TPP.



α-Ketoglutarate

Succinyl CoA

This reaction is one of Krebs cycle's reactions.

Binding α-ketoglutarate with CoA requires energy, which is provided by the decarboxylation reaction catalysed by TPP (thiamine pyrophosphate).

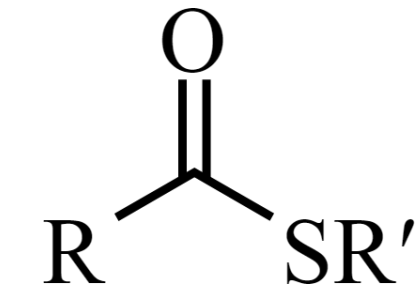
2) Coenzyme A (CoA)

Vitamin B5

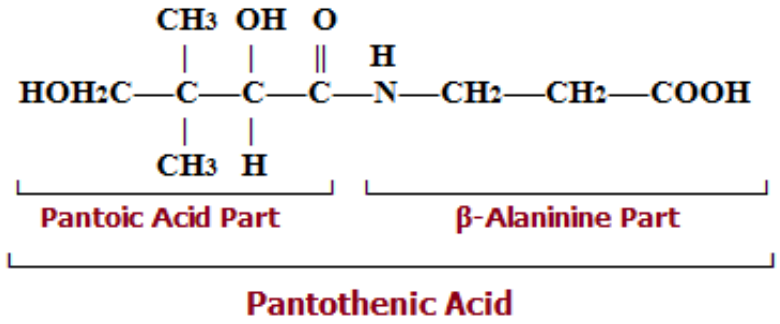
- Source: pantothenate (vitamin B5): made of β-alanine and pantoic acid.
- Function: metabolism of carbohydrates, fats, and proteins where it attacks carbonyl groups & forms acyl thioesters (the “A”).
- A molecule with a conjugated CoA is energy-rich.

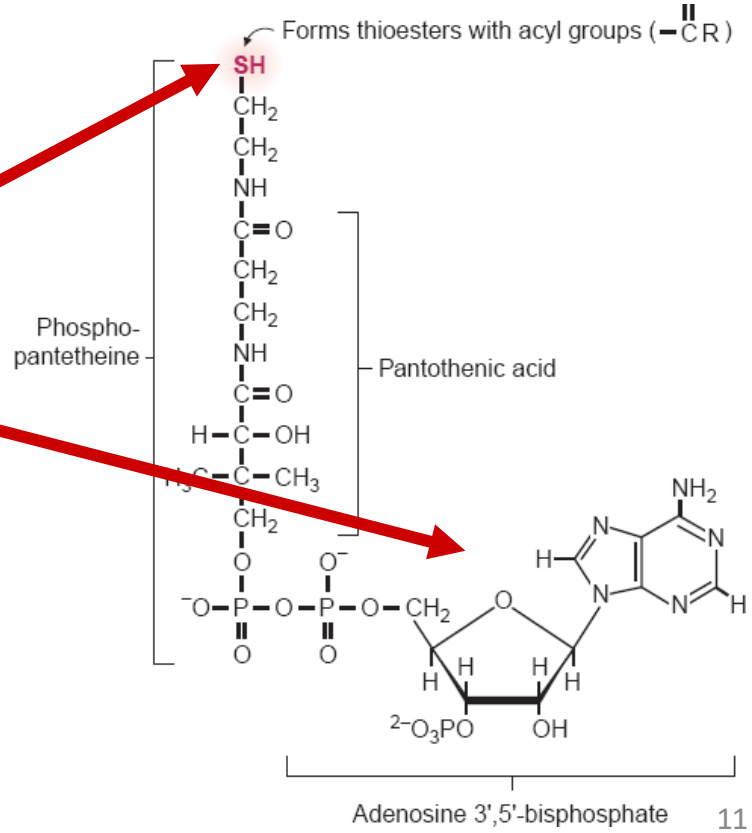
Functional group: sulfhydryl group (nucleophile)

Binding group: adenosine 3’,5’- bisphosphate (the nucleotide), the binding is tight & reversible



Acyl thioester





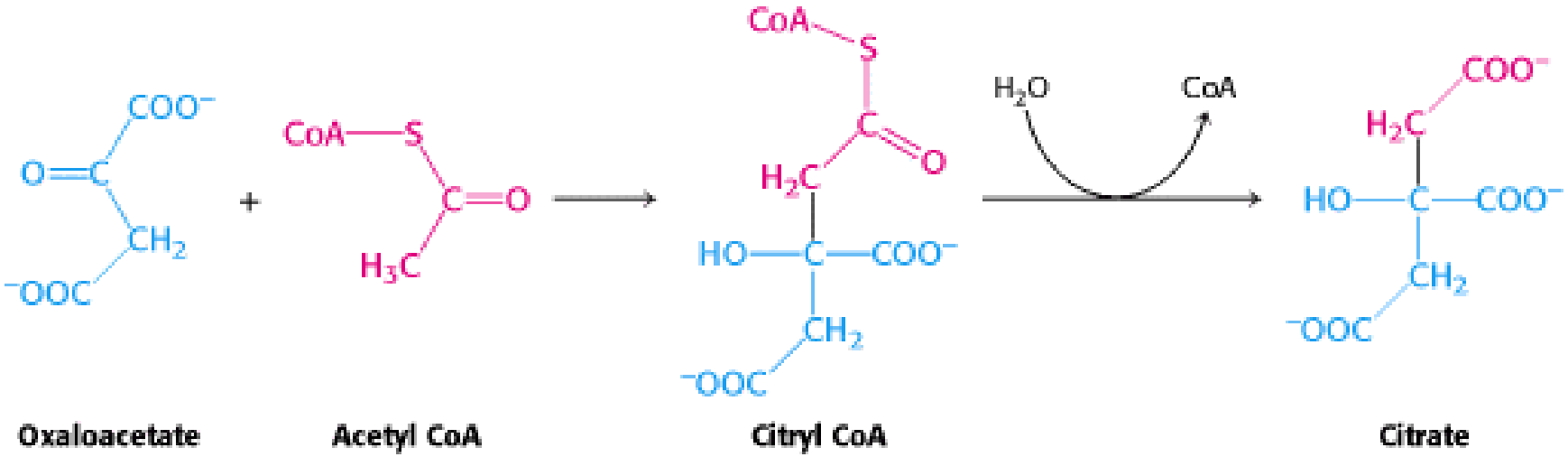
Examples of enzymes that need CoA

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

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

Acetyl CoA is a high-energy molecule because of the high-energy bond linking the acetyl group to CoA. This bond makes it easier for the acetyl group to be transferred and attached to other molecules, such as oxaloacetate.

- Condensation of acetyl CoA and oxaloacetate into citrate by citrate synthase (a transferase) In Krebs Cycle

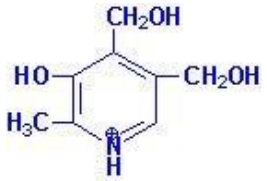
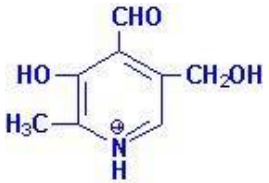
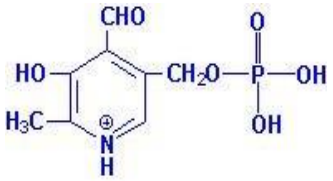


3) Pyridoxal phosphate (PLP)

Vitamin B6

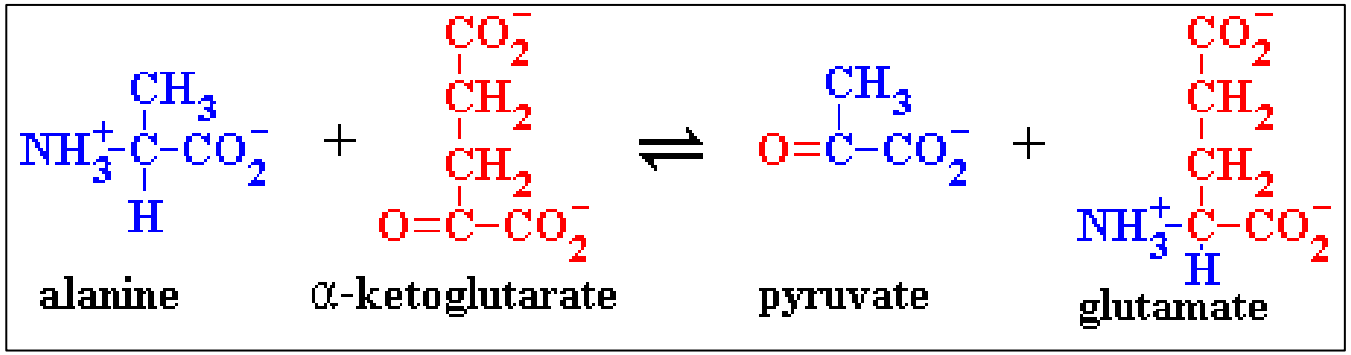
- Sources: pyridoxal, pyridoxamine and pyridoxine
- Metabolism of amino acids via reversible **transamination** reactions

Transamination is the process of converting keto acids into amino acids and vice versa.
Transaminases are the enzymes that catalyse this reaction.

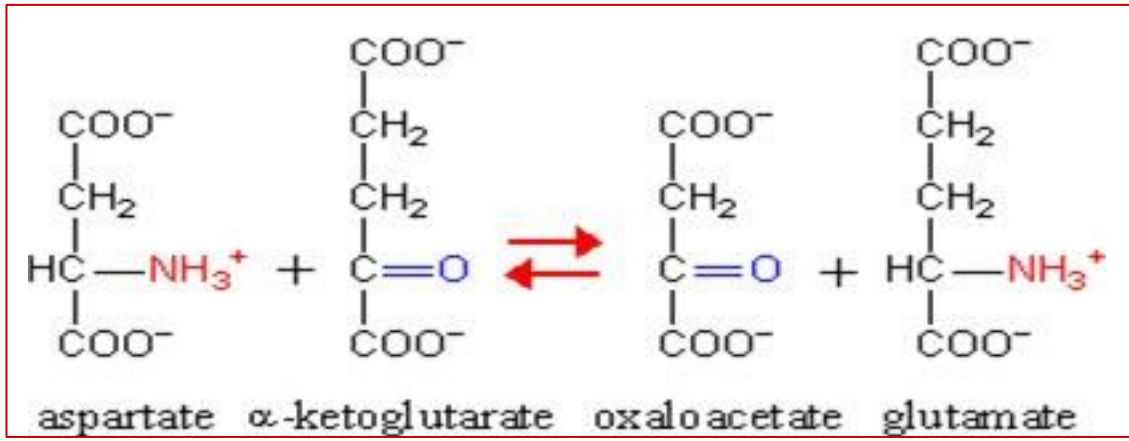
			
Pyridoxine	**Pyridoxal**	**Pyridoxamine**	**Pyridoxal phosphate**

Pyridoxal Phosphate is the functional group responsible for the transamination reactions

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



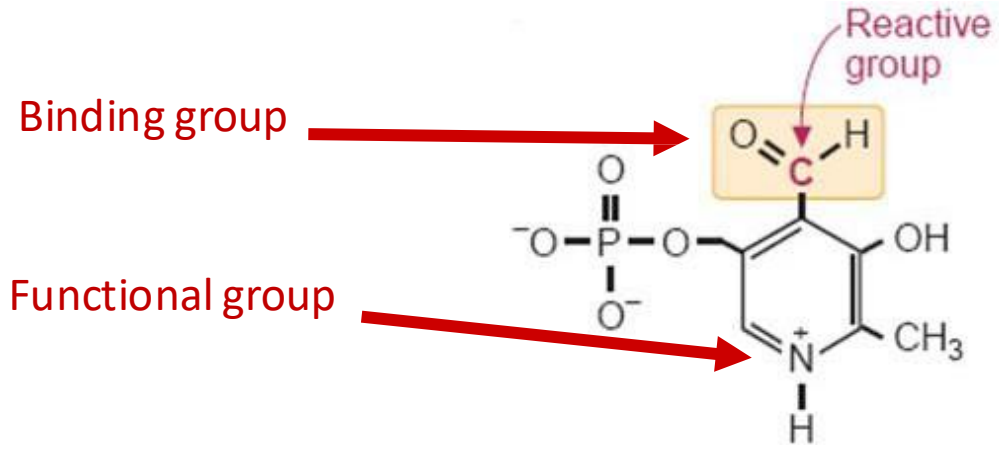
Important reaction

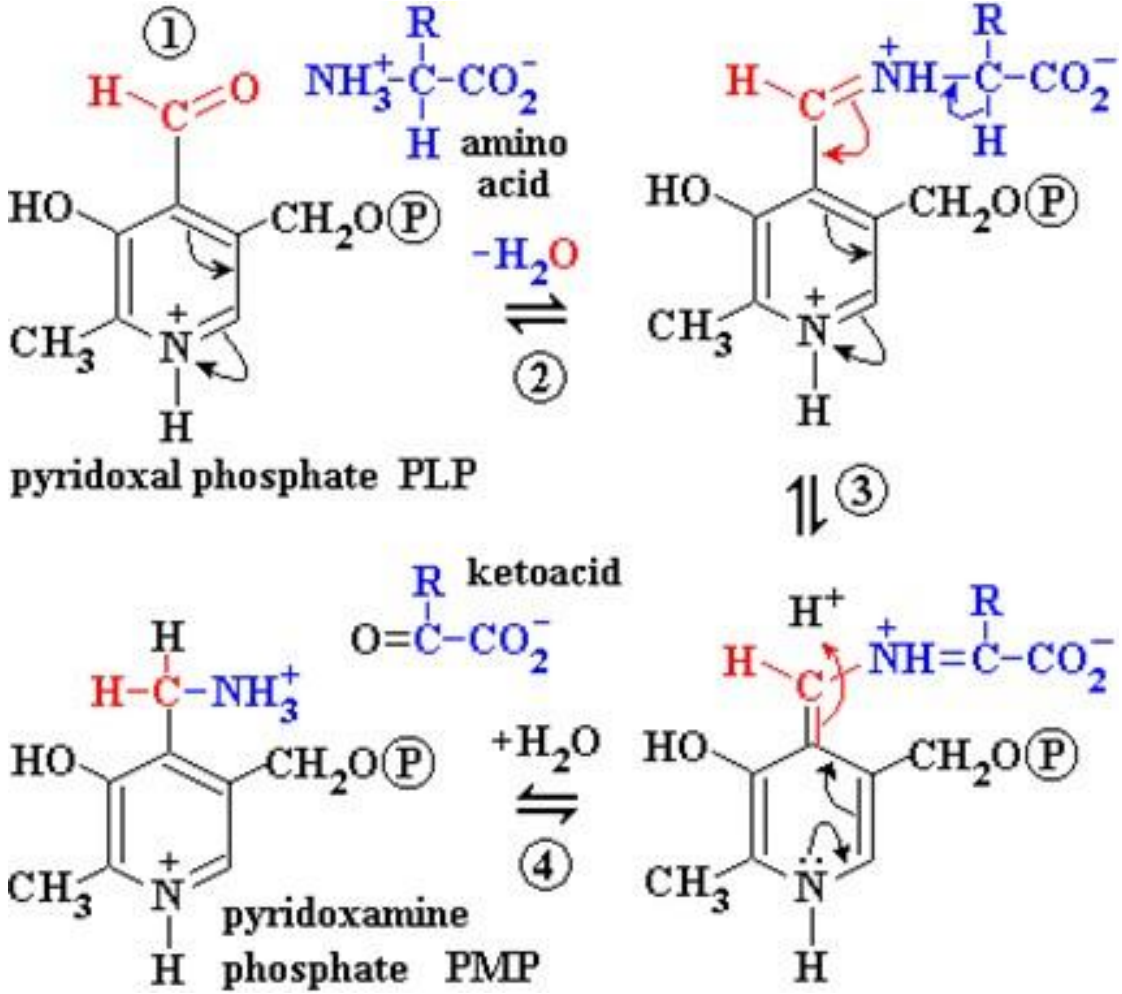


Mechanism of action

The Mechanism is not required.
Rest assured that the questions will be basic and not too detailed (what's vitamin B6, what does it do, what is the functional group)

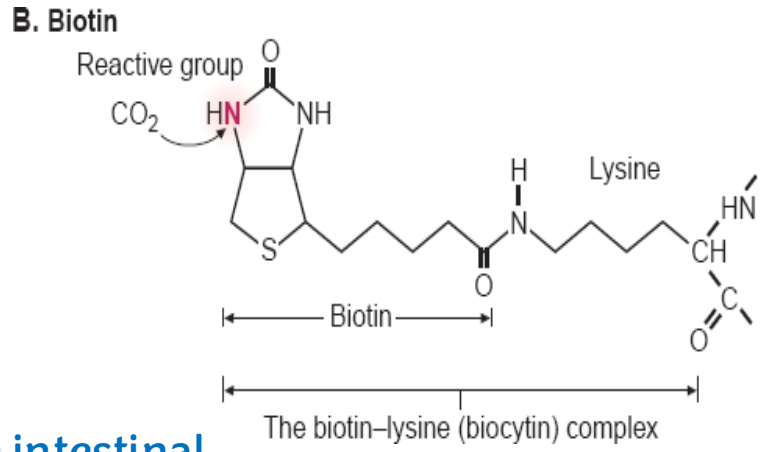
- The aldehyde of PLP covalently bonds with the amino group, the ring nitrogen withdraws its electrons, and the amino group is sequestered releasing a keto acid.
- A keto acid then enters the active site, and the amino group is given to it in a reverse reaction.
  - Binding and functional groups are within the ring.





4) Biotin

Vitamin B7



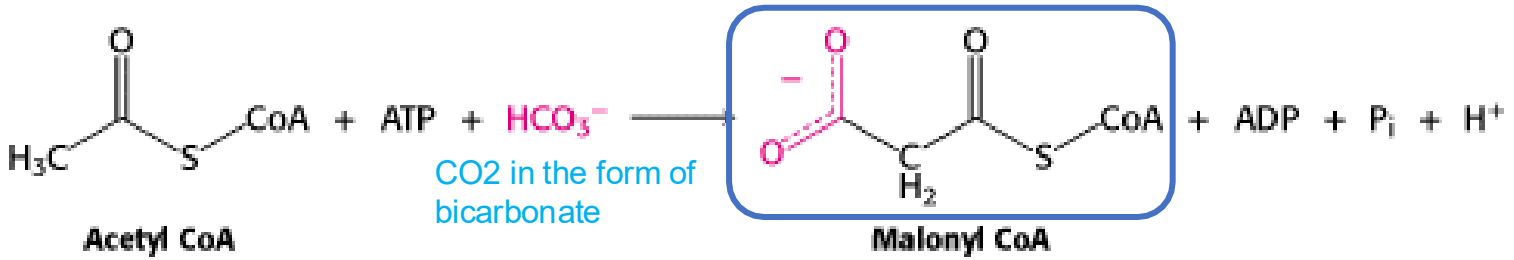
- It is required for **carboxylation** reactions.
  - covalently bound to the enzyme through lysine residue found in the active site
- Source: food & intestinal bacteria.

It's rare for someone to develop biotin deficiency, since its source is the intestinal bacteria (which we all have). However, here are some reasons of biotin deficiency:

1. Long antibiotic therapies
2. Excessive consumption of raw eggs (egg white protein, avidin, has a high affinity for biotin).

- Examples of enzymes:
  - Pyruvate carboxylase
  - Acetyl CoA carboxylase (fatty acid synthesis)

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



Intermediate of fatty acid synthesis.

Oxidation-reduction coenzymes

Helps substrates accept or donate electrons in different forms

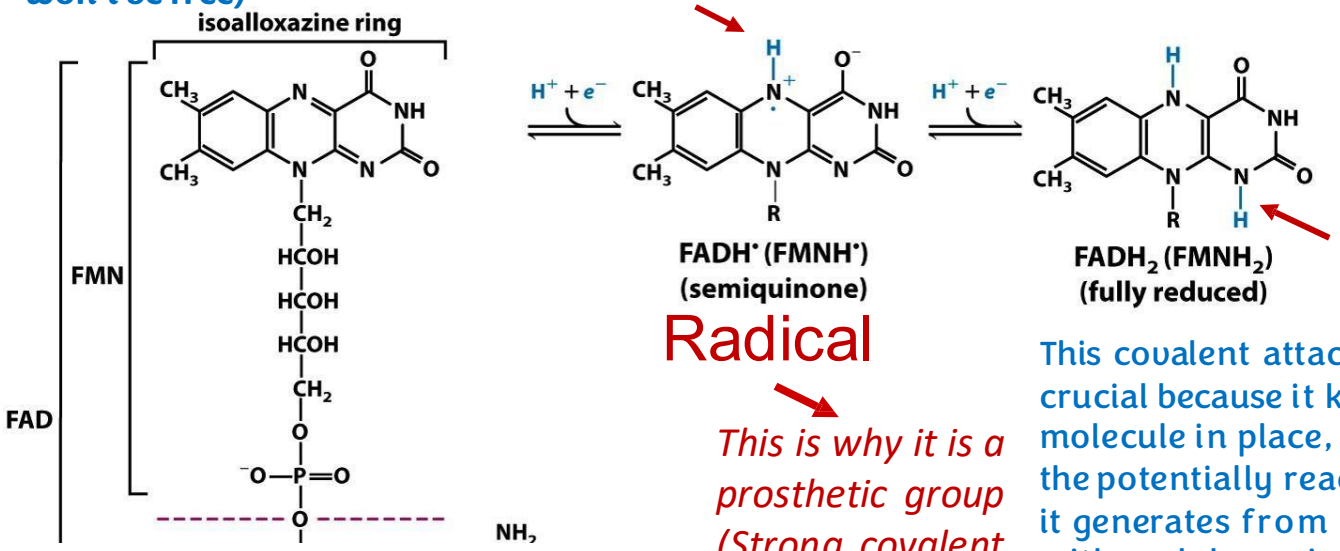
Oxidation-reduction coenzymes

- A number of coenzymes work within oxidoreductases.
- Each coenzyme has a unique functional group that accepts and donates electrons in the form of:
  1. Hydride ions
  2. Hydrogen atoms
  3. Oxygen.
- The coenzymes may bind the enzymes but not the substrates.
- Most common: NAD^+ (niacin, vitamin B3) & FAD^+ (riboflavin, vitamin B2)
- Other enzymes use metals to transfer single electrons to O_2
  - Vitamins E & C
- Again: Dependence on the enzyme for substrate specificity and catalytic power

1) FAD and FMN

- The precursor is riboflavin (vitamin B2).
- Both are prosthetic groups of flavoproteins.
- FAD accepts electrons in the form of hydrogen atoms donated sequentially. (why?)
- They are involved in reactions resulting in the formation of double bonds or disulfide bonds.

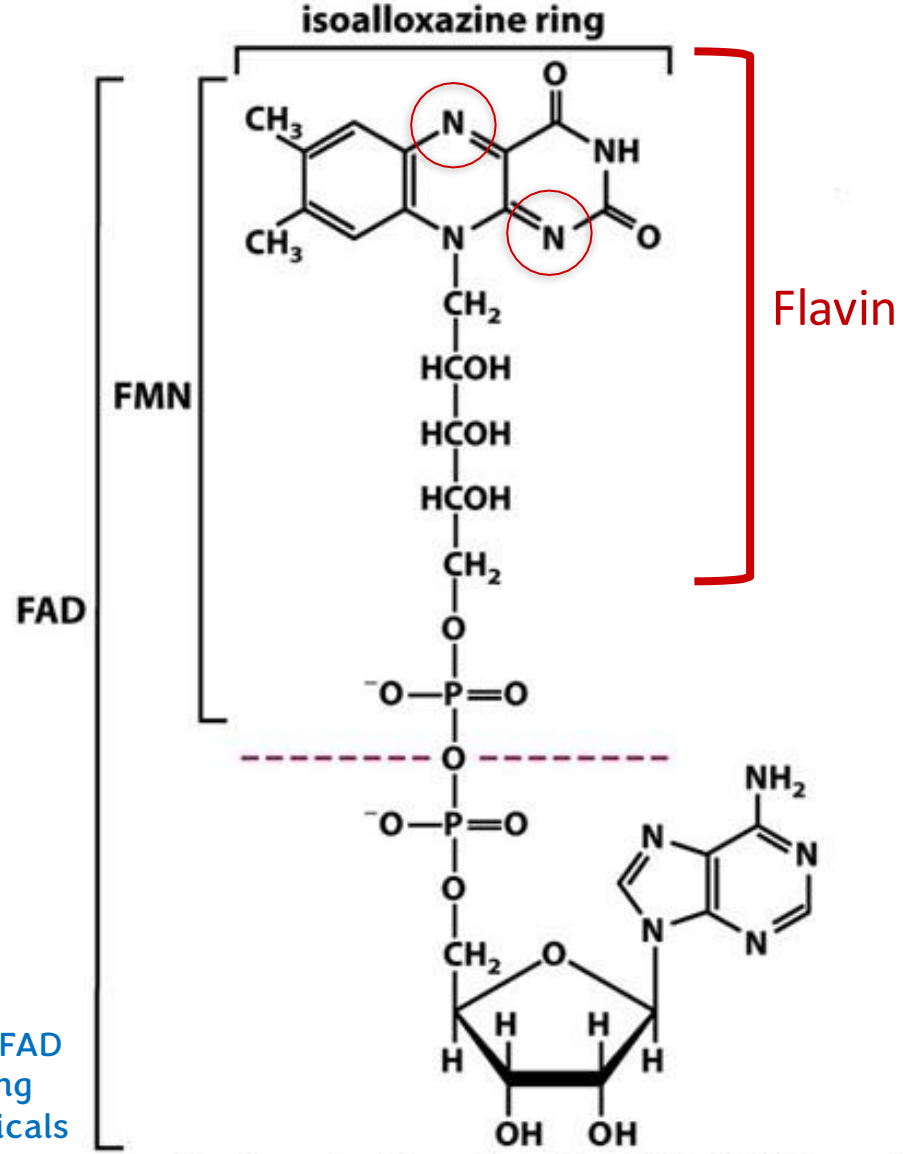
When This group accepts the first electron it becomes radical and if this molecule attacked any other molecule (DNA for example) it will damage it causing mutations and if it attacked the fatty acids of the membrane the cell dies, so the cell protects it self by anchoring this molecule through **covalent bonds→(the radical intermediate won't be free)**



Radical

This is why it is a prosthetic group (Strong covalent interaction).

This covalent attachment is crucial because it keeps the FAD molecule in place, preventing the potentially reactive radicals it generates from interacting with and damaging other molecules in the cell

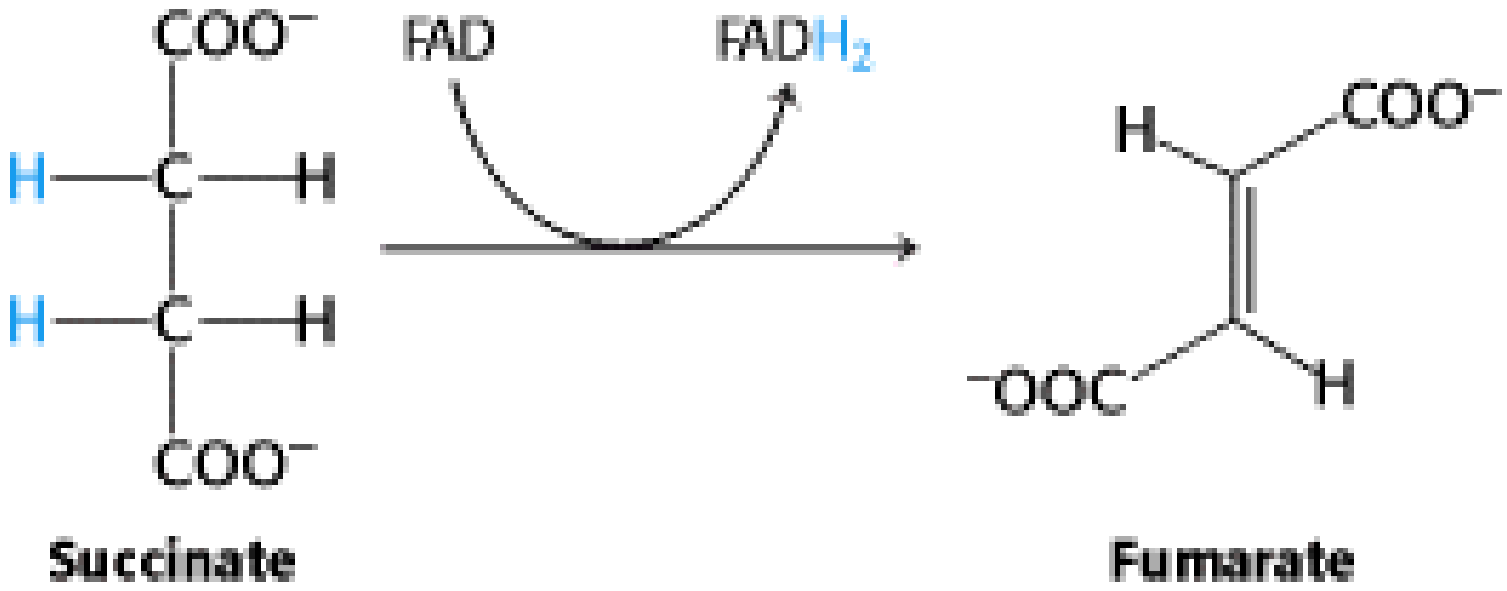


Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

An enzyme that needs FAD.

Succinate dehydrogenase

- Oxidation of succinate into fumarate by succinate dehydrogenase



2) NAD^+ and $NADP^+$

- Precursor of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) is niacin (vitamin B3).

Niacins

- These are cosubstrates for numerous dehydrogenases.

Binds non-covalently to the enzyme.

Also FAD is a coenzyme of the dehydrogenase but the difference between NAD and FAD is the binding to the enzyme one is covalently bound the other binds loosely.

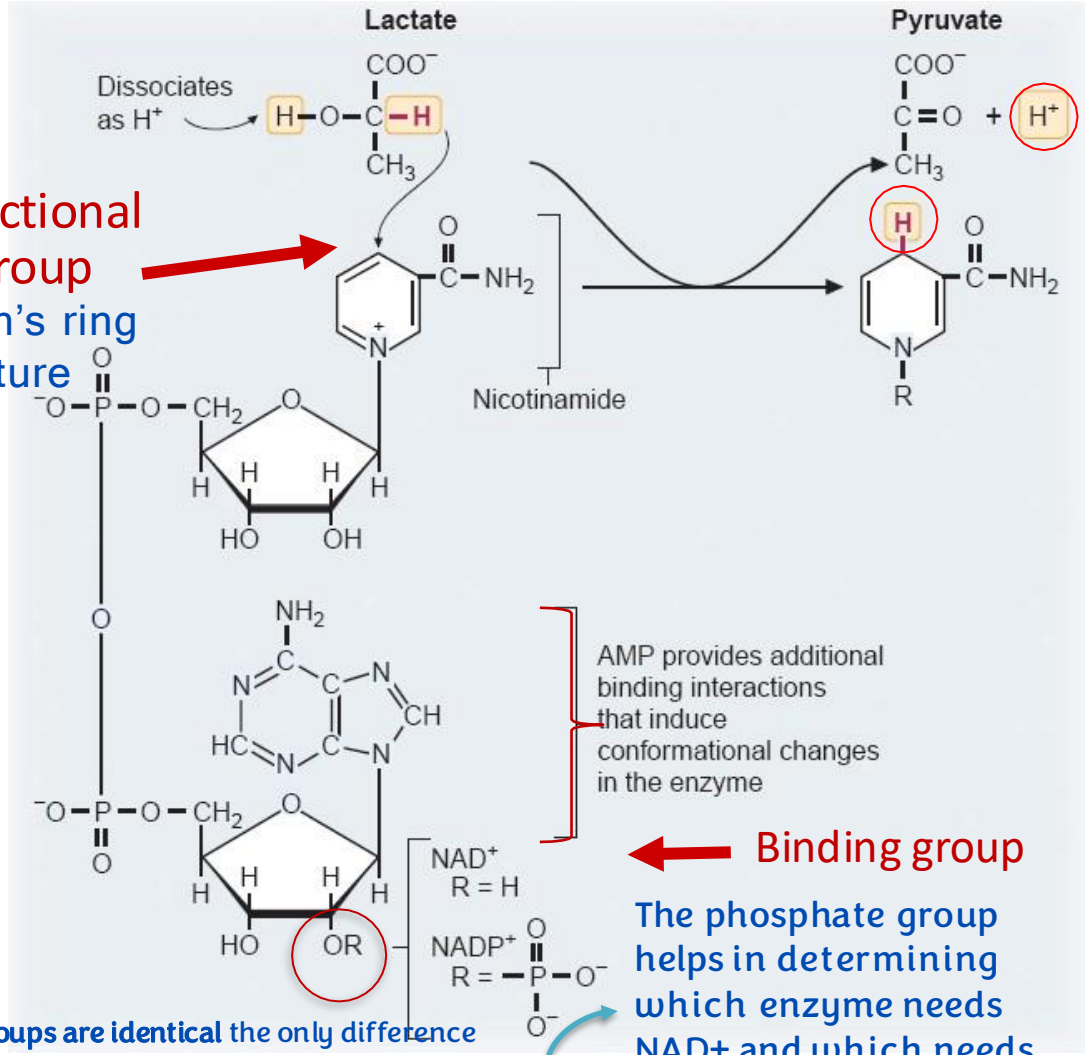
Mechanism of action

- The functional group (C opposite to N) accepts a hydride ion from the substrate, dissociates, and a keto group (CO) is formed.
- The ADP portion of the molecule binds tightly to the enzyme.
- They are generally involved in the oxidation of alcohols and aldehydes.

Alcohol→aldehyde
Aldehyde →carboxylic acid

Upon oxidation

Remember, we have talked about to lactate dehydrogenes in the previous lectures. In the heart, this enzyme converts lactate into byruvate And it plays a significant role in diagnosis of cardiac diseases like myocardial infarction



Extra explanation of NAD+'s function:

Lactate has two hydrogen atoms attached to its C2 carbon. During the reaction, NAD+ accepts one of these hydrogen atoms as a hydride ion (it accepts the electron in the form of H–), which includes one proton and two electrons. The second hydrogen atom is released as a proton (H+), which then dissociates into the surrounding environment. This process results in the oxidation of lactate to pyruvate and the reduction of NAD+ to NADH.

NAD &NADP+ genuinely do the same thing there **functional groups are identical** the only difference between them is the phosphate group in the binding region So they differ in the enzyme they bind to

- Usually NAD works in catabolism rxns(breaking up molecules)
- And NADP+ usually works in a abolish rxns (building up molecules) like FA Synthesis

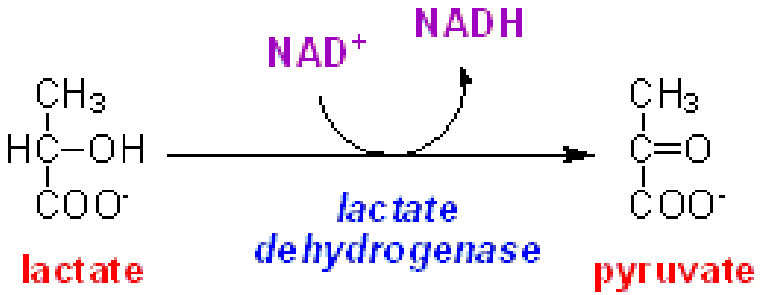
Lactate dehydrogenase

An enzyme that needs NAD+

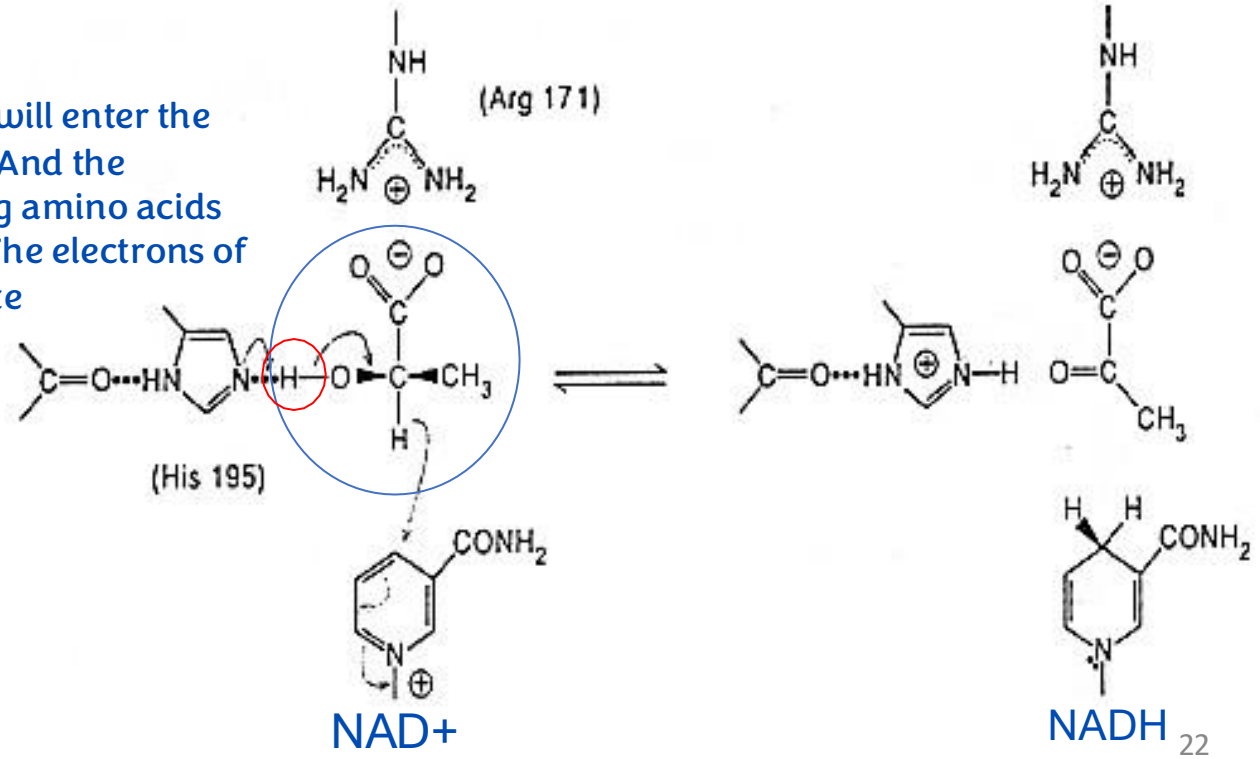
The active site facilitates the reaction by:

- The enzyme's histidine **(which is in the active site)** binds the proton of (-OH) on lactate making it easier for NAD^+ to pull off the other hydrogen with both electrons (a hydride). **Then, the H+ that is bound to the Histidine will be released.**
- A keto group (-C=O) is formed.

There is no binding between NAD and substrate the coenzyme only attracts the hyrogen atom



The lactate will enter the active sites And the surrounding amino acids rearrange.The electrons of the substrate

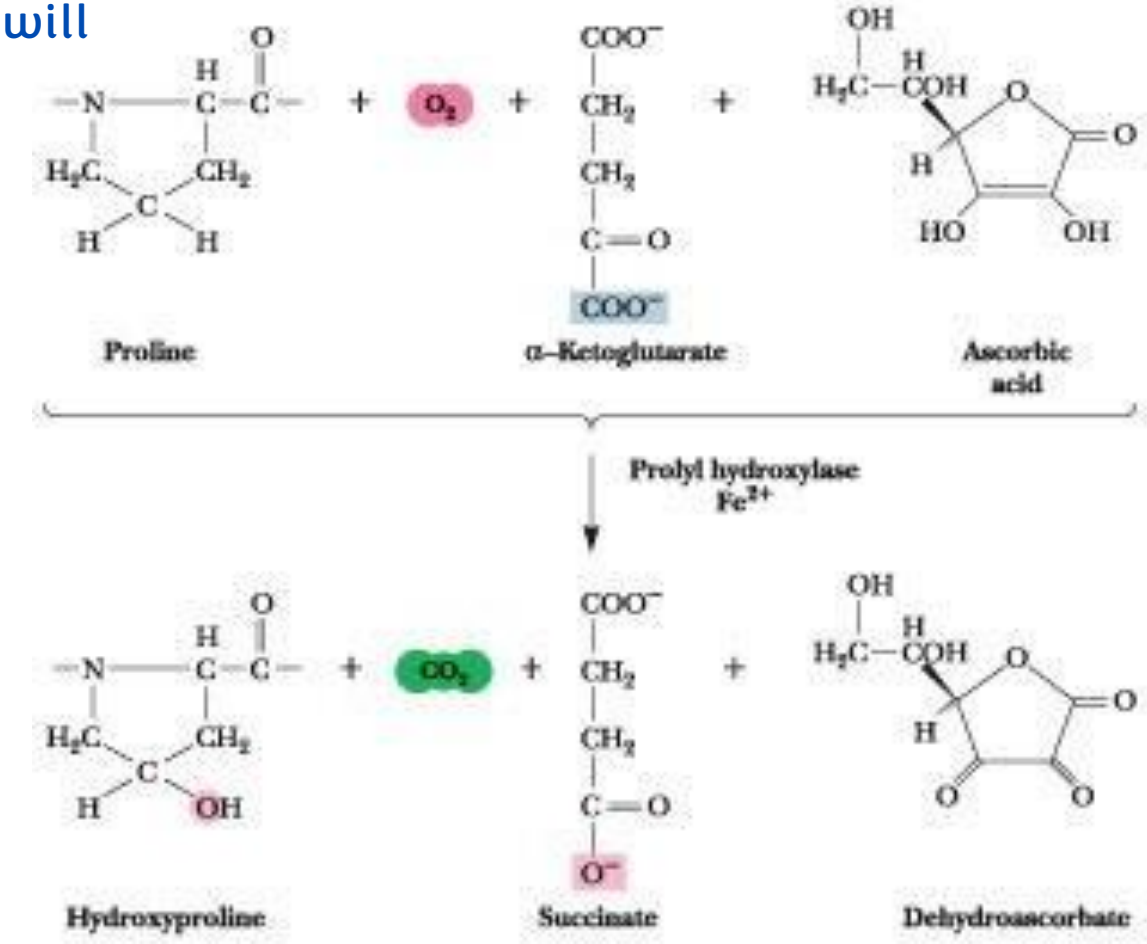


3) Vitamin C

It's essential for the hydroxylation process of proline forming hydroxy-proline which is important for stabilizing collagen structure lack of vitamin-c will cause scurvy.

- Known as Ascorbic acid or ascorbate as its conjugate base.
- Example: prolyl hydroxylase
  - synthesizes 4-hydroxyproline (collagen)
- An antioxidant

Vitamin-C is essential for reducing the oxidizing effects of inflammatory molecules **antioxidant.**



Ascorbate, the anti-oxidant

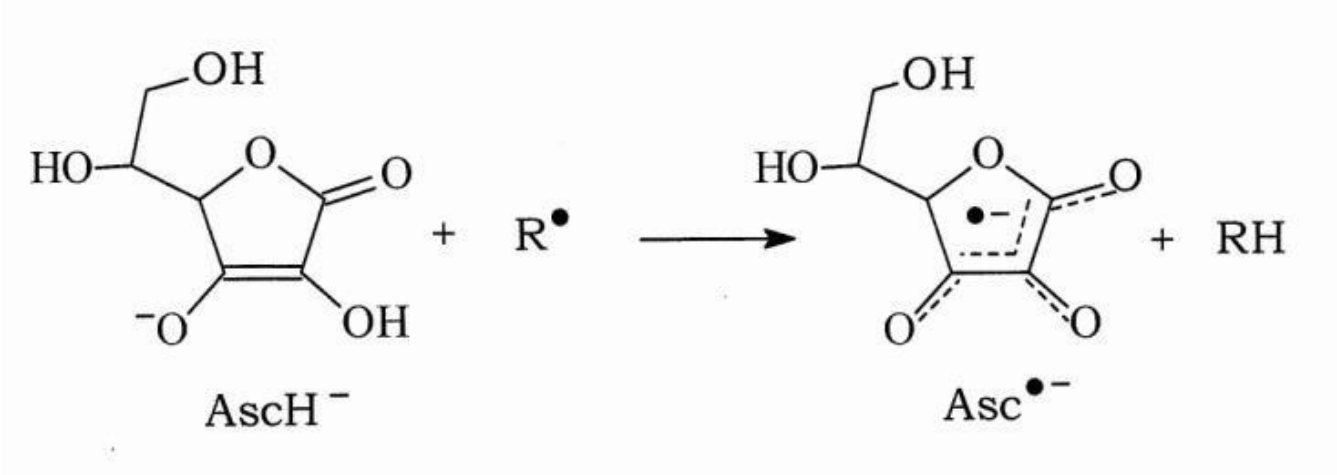
Ascorbate reduces radicals by giving them an electron.

Ascorbate can become a **stable radical** after donating an electron because its ring structure stabilizes the unpaired electron through **resonance** (the e^- will keep rotating in the ring so it's harmless).

- Reactive oxygen species oxidize ascorbate into a radical, which is then oxidized.

Radicals are very reactive, if it reacts with a fatty acid for example, it will damage the plasma membrane.

- The oxidized forms of ascorbate are relatively stable, unreactive, and do not cause cellular damage.
- The ring structure of vitamin C (and other anti-oxidants) is preferable due to formation of resonance.



Metals

Recall that they may be:

- covalently bound to enzymes (in metalloproteins).
- non-covalently bound to enzyme (in metal associated proteins).

Metal	Enzyme
Zn^{2+}	Carbonic anhydrase Carboxypeptidase
Mg^{2+}	Hexokinase
Se	Glutathione peroxidase
Mn2+	Superoxide dismutase

The last 2 metals are not required.

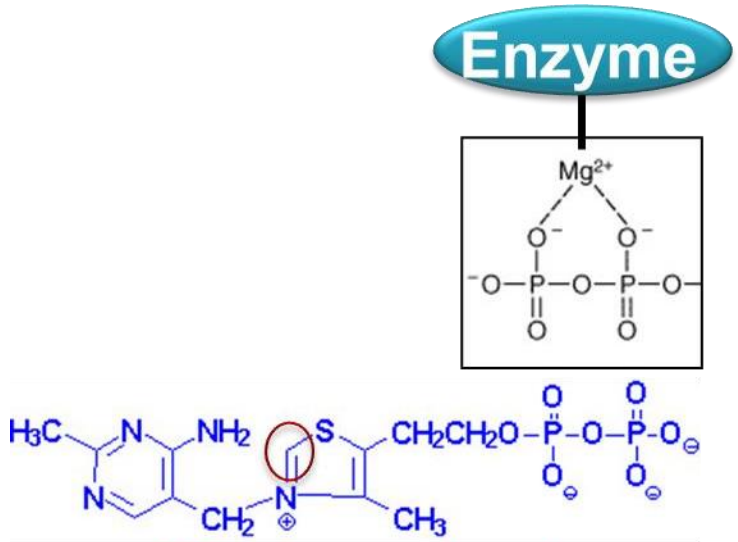
- They act as electrophiles.
- They assist in binding of the substrate, or they stabilize developing intermediates anions in the reaction.
- They can also accept and donate electrons in oxidation-reduction reactions.

Advantages

The for phosphate groups of vitamin B1 will bind to the Mg+2 and this will do chelation to the enzyme(bindinggroup)

- They carry positive charges and, hence, can form relatively strong yet kinetically labile (*likely to be changed*) bonds.
- They are stable in more than one oxidation state.
- They can bind multiple ligands enabling them to participate in binding substrates or coenzymes to enzymes.

- Mg^{2+} connects the negatively charged phosphate groups of thiamine pyrophosphate to basic amino acids in the enzyme.
- The phosphate groups of ATP are usually bound to enzymes through Mg^{2+} chelation.

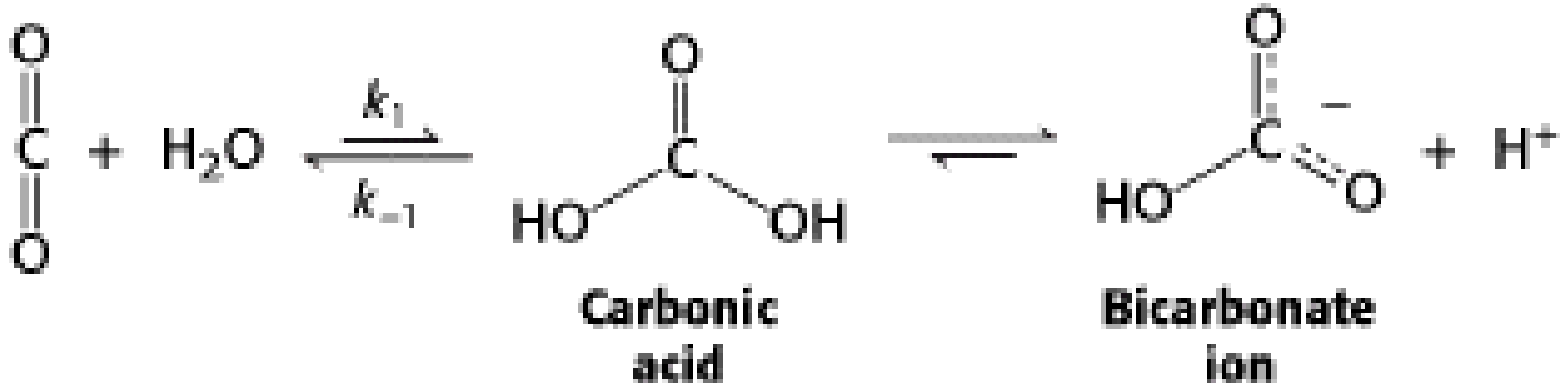


Carbonic anhydrases

An enzyme which needs zinc.

- Although CO_2 hydration and HCO_3^- dehydration occur spontaneously, almost all organisms contain carbonic anhydrases, because they carry out rapid processes such as respiration.

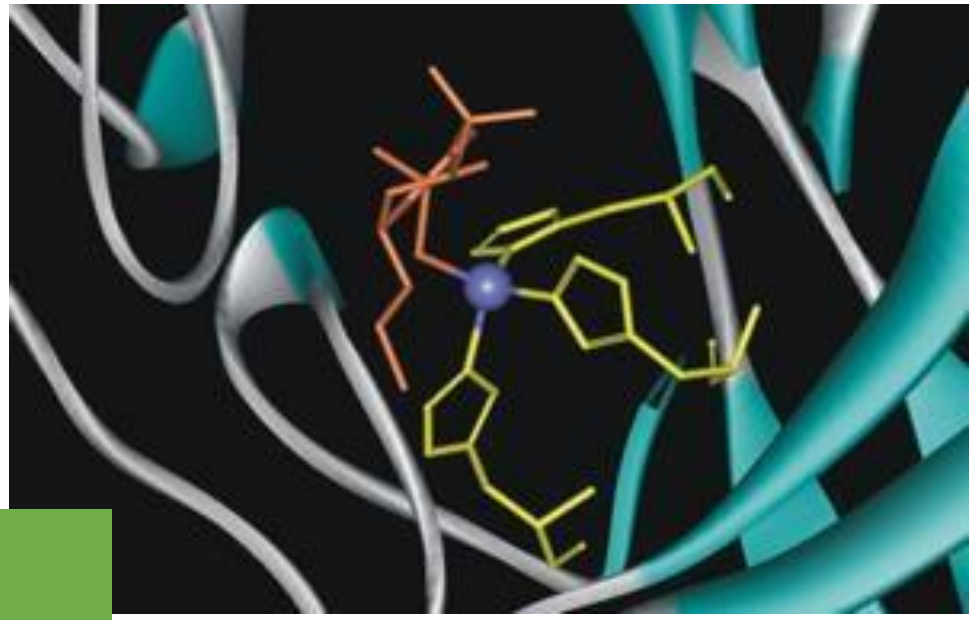
Deficiency of carbonic anhydrases could cause mental retardation.

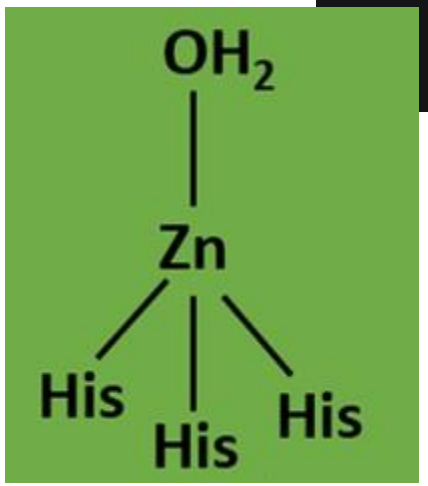


Zinc binding to the enzyme

It is found in the active site.

- Zinc is found only in the +2 state in biological systems.
- In carbonic anhydrase, a zinc atom is bound covalently to three imidazole rings of three histidine residues in the active site of the enzyme, and a fourth site is occupied by a water molecule also the bond between Zn and H2O is covalent.



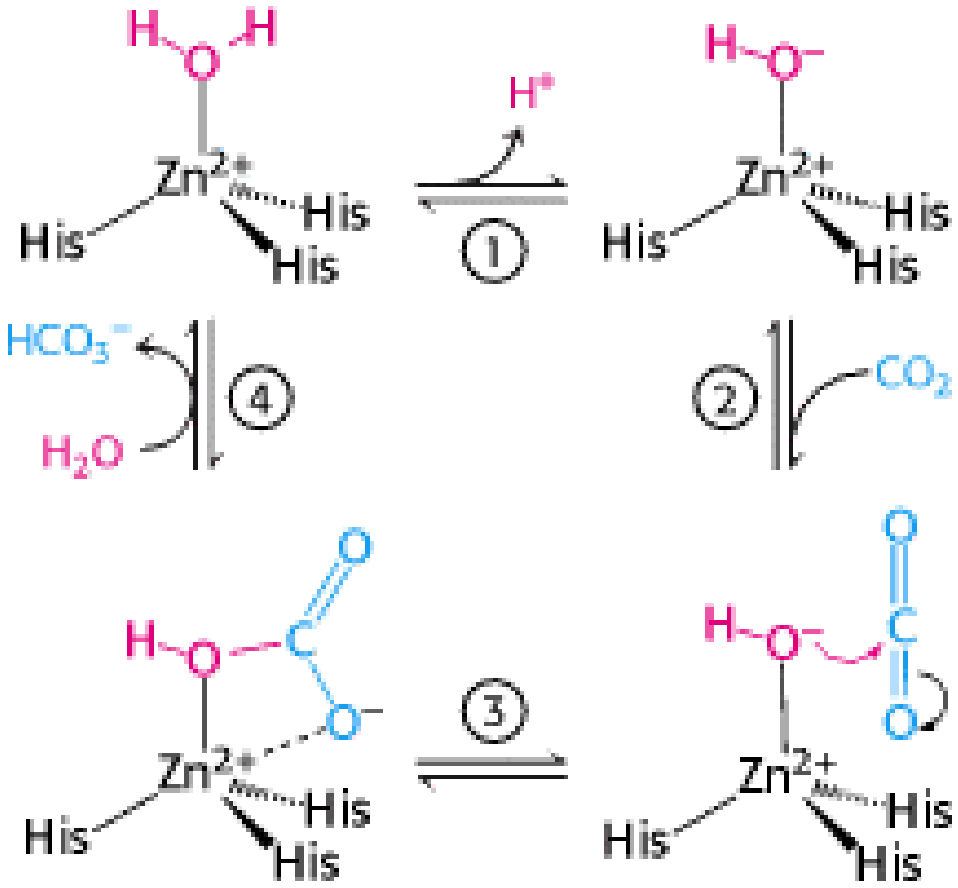


Mechanism of action

The zinc in this enzyme is the component that did the rxn

Focus on the slide's notes and on the mechanism of the reaction.

- Zinc facilitates the release of a proton from H_2O generating a **hydroxide ion**.
- The CO_2 binds to the enzyme's active site and reacts with the hydroxide ion.
- generating a **bicarbonate ion**.
- The catalytic site is regenerated with the release of the bicarbonate ion and the binding of another H_2O.

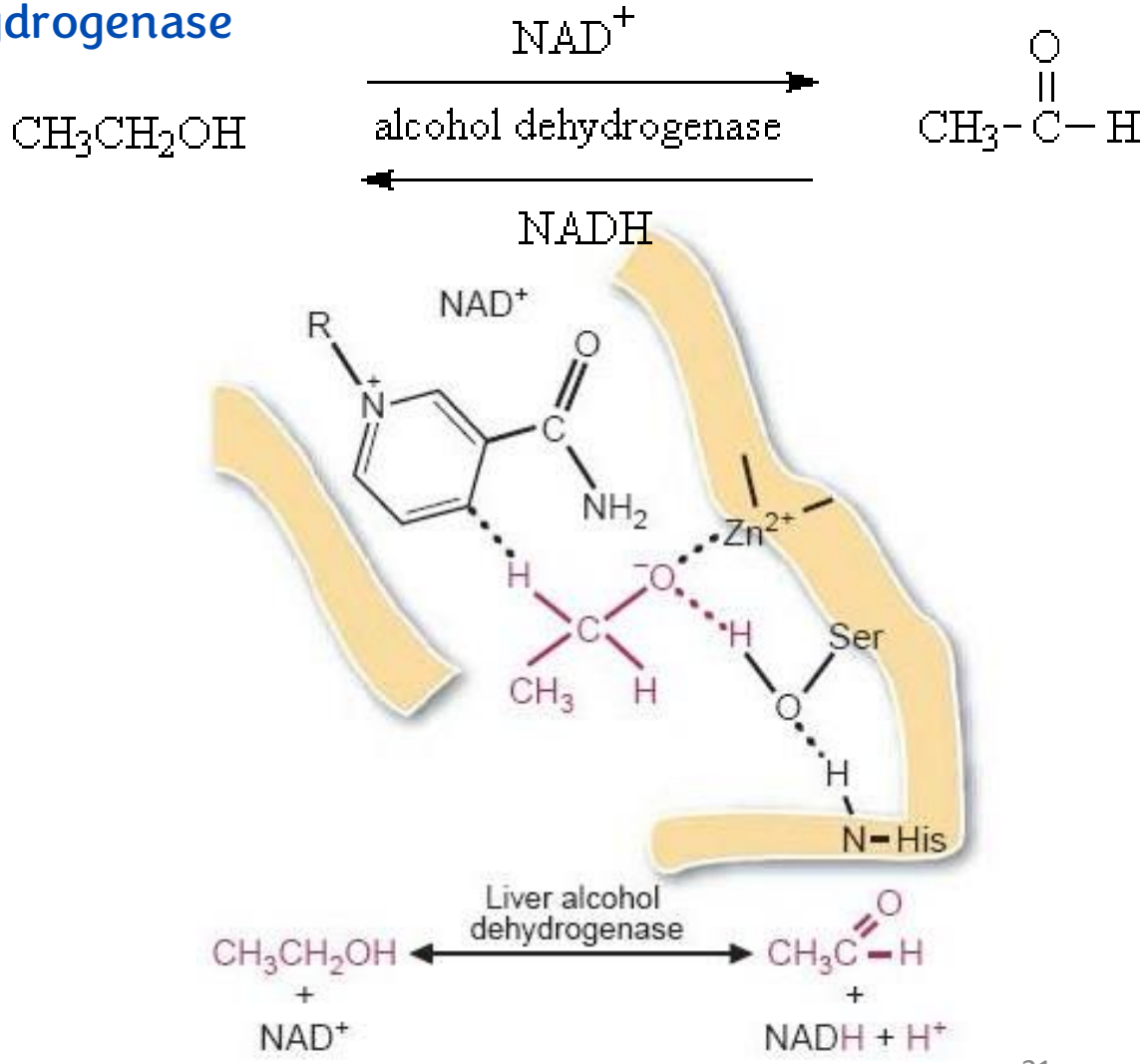


Catalytic Metals

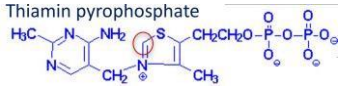
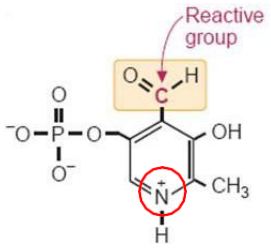
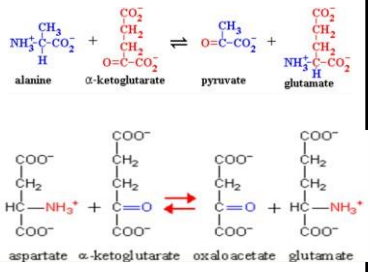
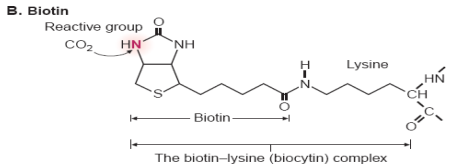
Remember: alcohol dehydrogenase is an oxidoreductase that works in the presence of vitamin B3 (niacin) the zinc participates in the rxn but it doesn't induces the formation of final product (it stablizes the negative intermediate),,the surrounding Amino acids are the ones that Catalyzes the rxn

Another enzyme which depends on metals is alcohol dehydrogenase which converts alcohols to aldehydes.

- Some metals **do not participate** in enzyme catalysis directly but **facilitate** a reaction.
- The **histidine and serine** residues of alcohol dehydrogenase pull a proton off the alcohol and gives electron to NAD which leaves as NADH leaving the oxygen with a negative charge.
- **The charge is stabilized by zinc.**
- An aldehyde is formed and released.
- The proton is transferred as a hydride to NAD^+ forming NADH.



Activation-transfer coenzymes

Coenzyme Name (The active form)	Inactive Form	Functional Group	Binding Group	Reaction	Examples
Thiamin Pyrophosphate (TPP)	Thiamine (Vitamin B1)		Pyrophosphate (Chelation via Mg +2)	Decarboxylation	1. Pyruvate dehydrogenase complex 2. α-ketoglutarate dehydrogenase
Coenzyme A (CoA)	Pantothenate (Vitamin B5) Made of β-alanine and pantoic acid	Sulfhydryl group	Adenosine 3',5'-bisphosphate (the nucleotide)	Metabolism of carbohydrates, fats and proteins Attacks carbonyl groups & forms acyl thioesters (the "A").	1. Pyruvate dehydrogenase 2. Citrate synthase
Pyridoxal phosphate (PLP)	Vitamin B6 Sources: Pyridoxal, Pyridoxine and Pyridoxamine		Aldehyde	Transamination	
Biotin	Vitamin B7 Sources: Food & intestinal bacteria			Carboxylation Covalently bind to Lys	1. Pyruvate carboxylase 2. Acetyl CoA carboxylase

Oxidation-reduction coenzymes

Coenzyme Name (The active form)	Inactive Form	Functional Group	Binding Group	Reaction	Examples
FAD & FMN	Riboflavin (Vitamin B2)	—————	—————	Oxidation-reduction	Succinate dehydrogenase
NAD+ & NADP+	Niacin (Vitamin B3)	C opposite to N in nicotinamide	NAD+ -> R=H NADP+ -> $R=PO3^{-2}$	Dehydrogenation Oxidation of alcohols & aldehydes	Lactate dehydrogenase
Ascorbic acid Vitamin C	—————	—————	—————	Hydroxylation (E.g. Reducing oxidizing effects)	Prolyl hydroxylase

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 22	"It is oxidase"	Removed
V1 → V2			

Quiz Time !

Additional Resources:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks' Basic Medical Biochemistry (6th edition)

رسالة من الفريق العلمي: