Enzymes
Part II: Enzymes-cofactors

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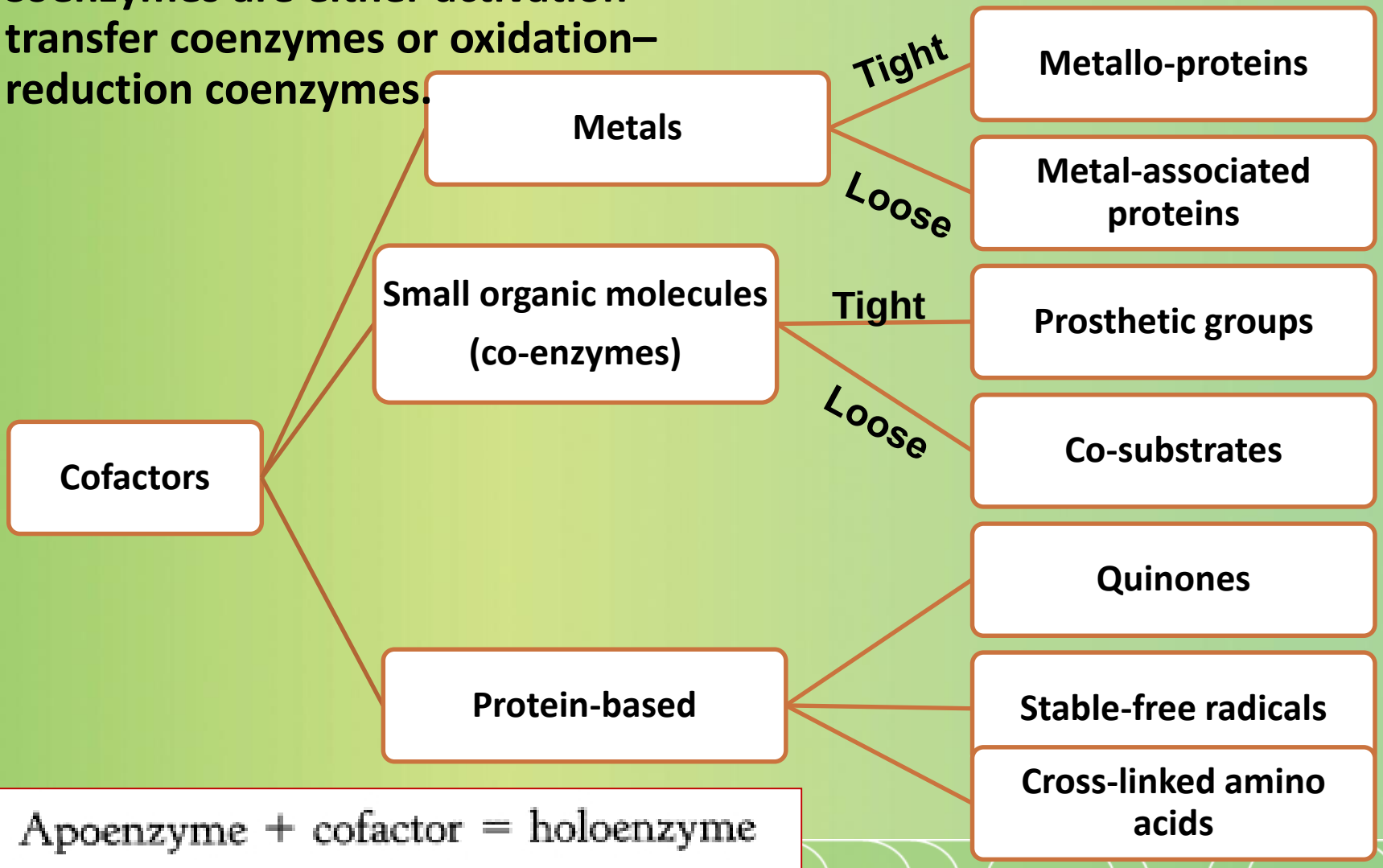
Catalytic strategies of enzymes

- **Enzymes carry out reactions utilizing different catalytic strategies.**
  - Some enzymes, such as chymotrypsin, rely on amino acid residues within the active site.
    - Almost all polar amino acids participate in nucleophilic catalysis.
    - Ser, Cys, Lys, & His can participate in covalent catalysis
    - Histidine: pKa, physiological pH & acid–base catalysis
  - Other enzymes increase their repertoire by employing cofactors (nonprotein compounds that participate in the catalytic process).
    - Conjugated enzymes

Classification of cofactors

Coenzymes are either activation-transfer coenzymes or 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

ACTIVATION-TRANSFER COENZYMES

- **They usually participate directly in catalysis by forming a covalent bond.**

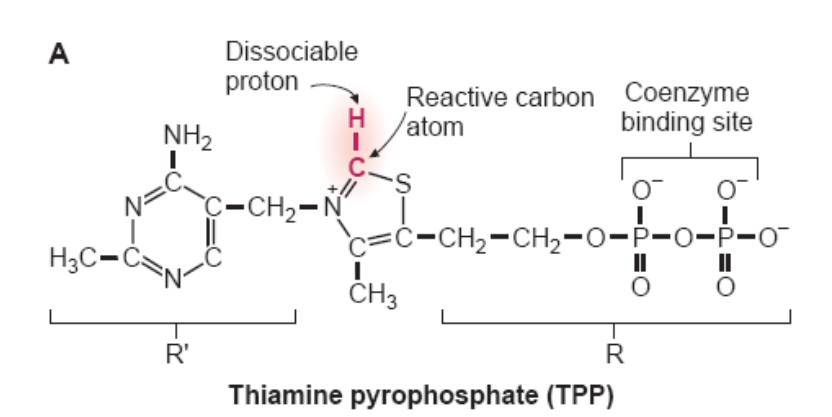
- **Characteristics:**
  - **Two groups in the coenzyme:**
    - **Forms a covalent bond with substrate (functional group)**
    - **Binds tightly to the enzyme (binding group)**
  - **Dependence on the enzyme for additional specificity of substrate & additional catalytic power**

Thiamin pyrophosphate, TPP

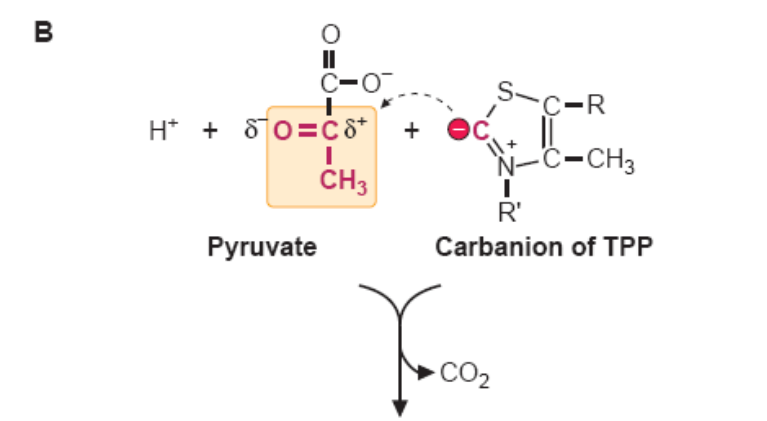
- Thiamin (vitamin B1) is rapidly converted to its active form, thiamin pyrophosphate, TPP, in the brain & liver.
- It is involved in decarboxylation reactions.
- The pyrophosphate provides negatively charged oxygen atoms and chelates Mg2+ that is tightly bound to the enzyme.

Mechanism of action

- The functional group is the reactive carbon atom, which forms a covalent bond with a substrate keto group while cleaving the adjacent carbon–carbon bond.



Thiamine pyrophosphate (TPP)

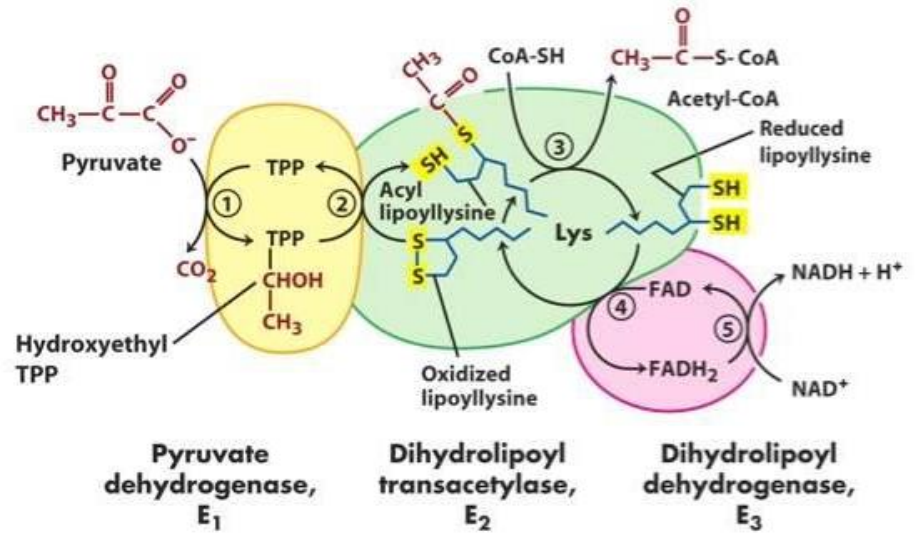


Resonance forms of ionized hydroxyethyl-TPP

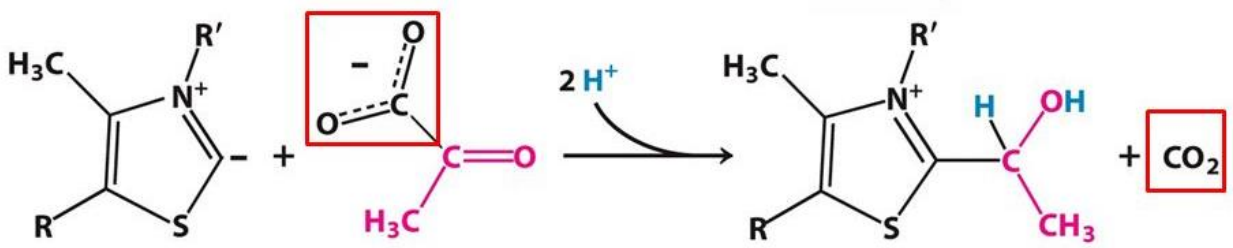
Pyruvate dehydrogenase complex

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + CO_2 + \text{NADH}$$

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



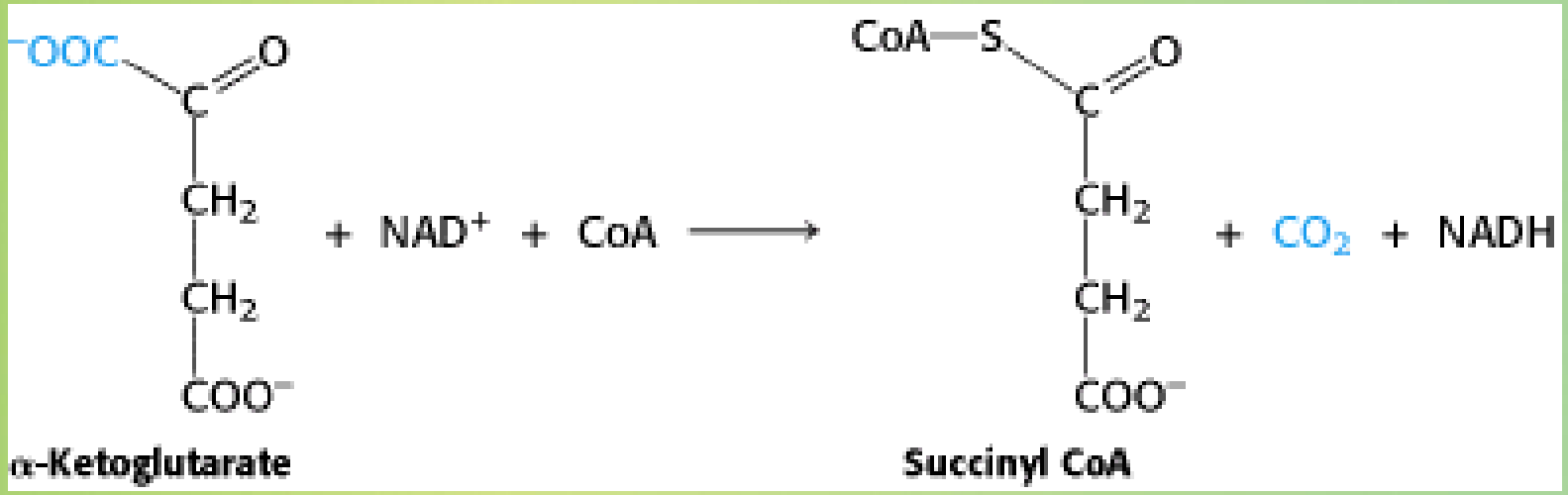
Step 1



TPP: thymine pyrophosphate

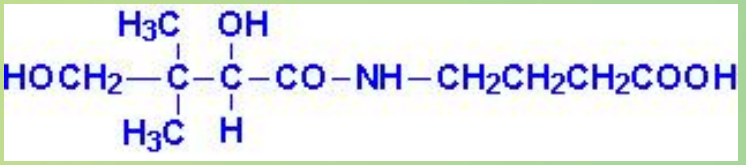
α-ketoglutarate dehydrogenase

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



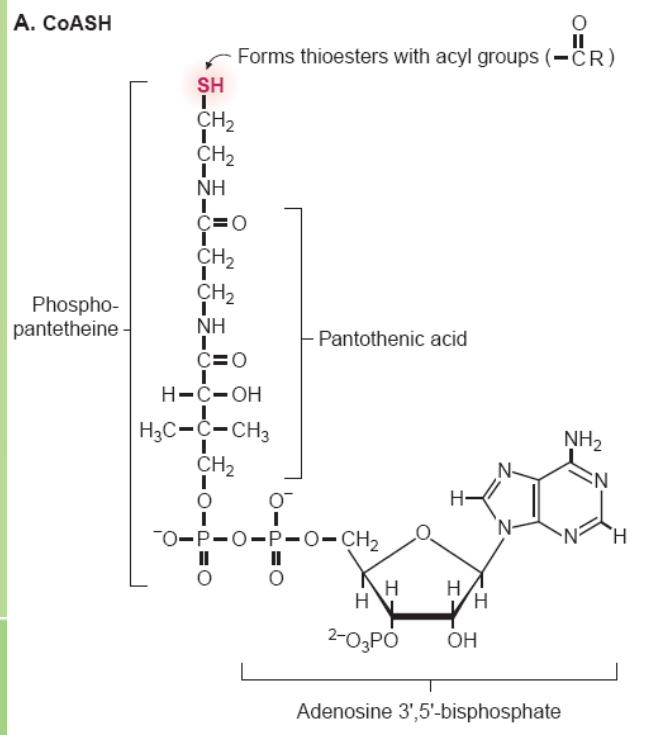
Coenzyme A (CoA)

- Source: pantothenate (B5), which is a precursor of alanine and pantoic acid.



- It is important for the metabolism of carbohydrate, fats and proteins where it attacks carbonyl groups & forms acyl thioesters (the "A").

- Binding group: adenosine 3',5'-bisphosphate (tight & reversible)
- Functional group: sulfhydryl group (nucleophile)

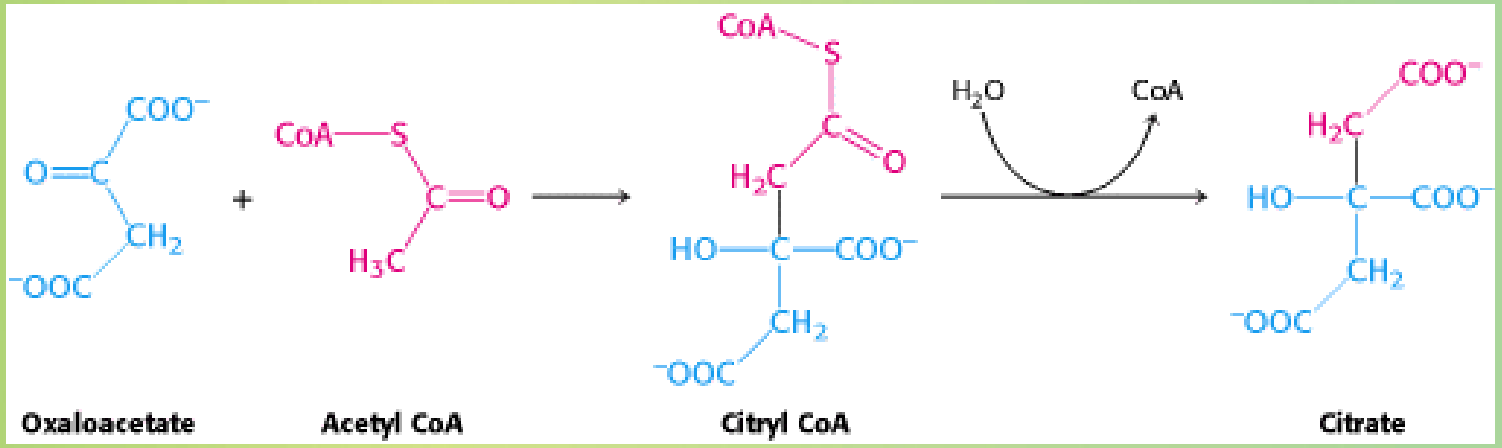


Examples of enzymes

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

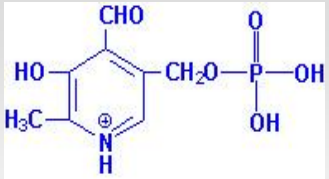
$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH}$$

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

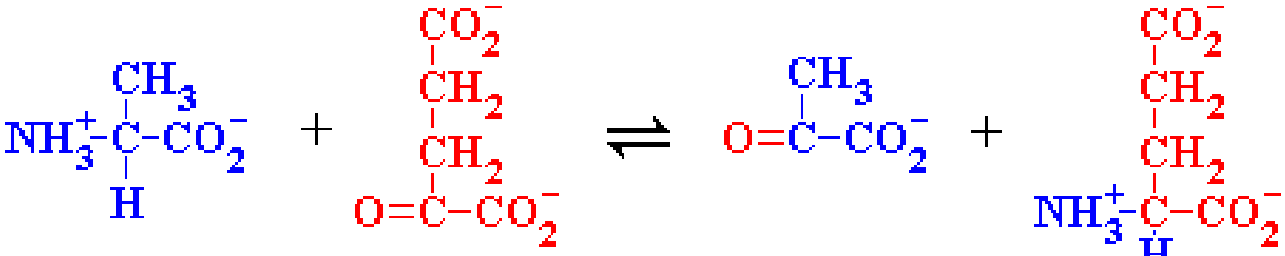


Pyridoxal phosphate (vitamin B6)

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

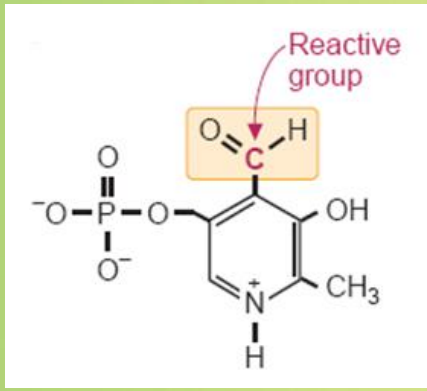
			
Pyridoxine	**Pyridoxal**	**Pyridoxamine**	**pyridoxal phosphate**

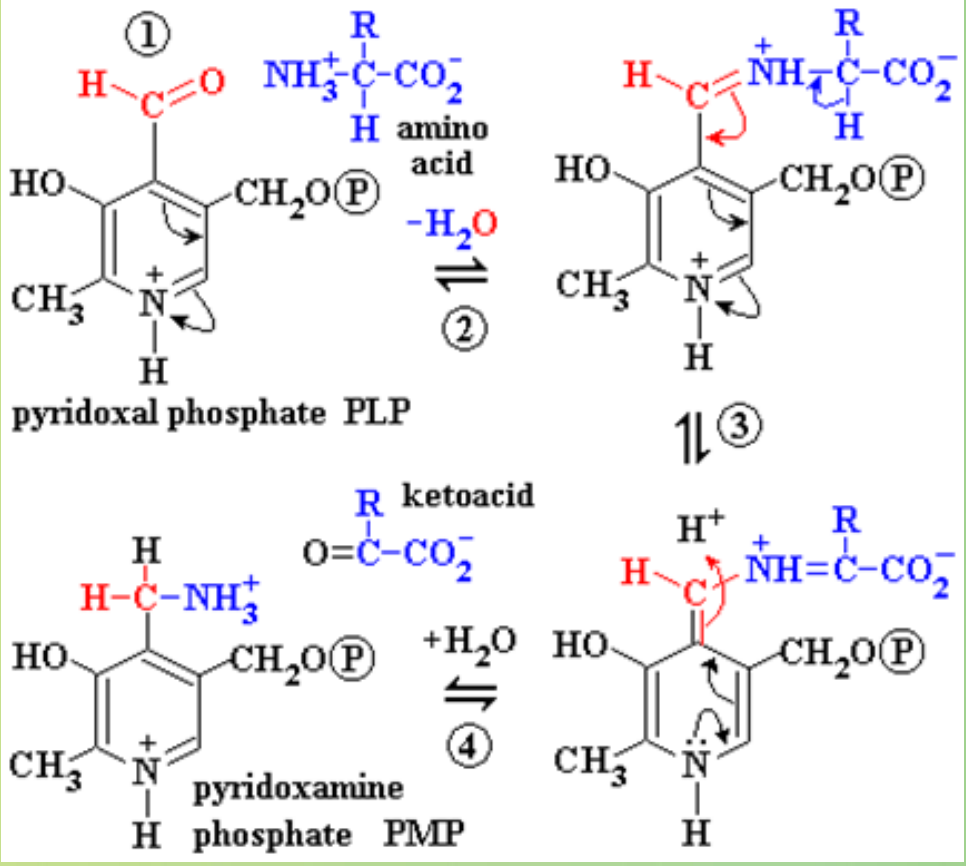
$$\text{Amino acid}_1 + \alpha\text{-ketoacid}_2 \rightleftharpoons \text{amino acid}_2 + \alpha\text{-ketoacid}_1$$



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.





Examples

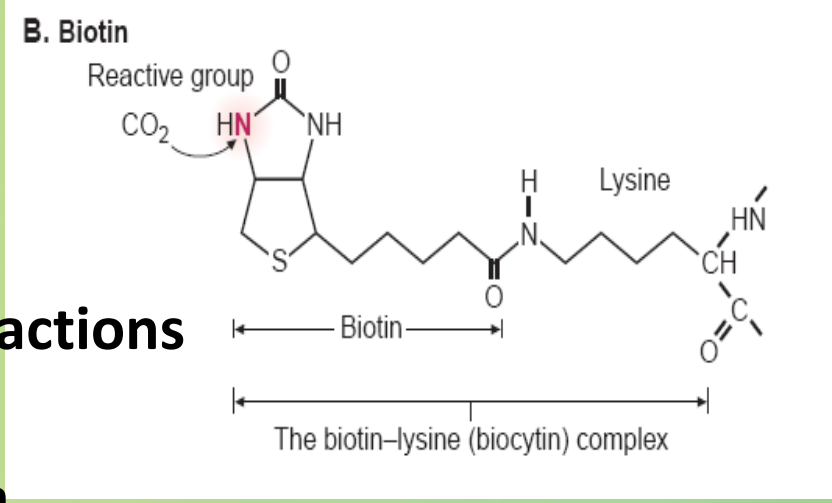
- **Aspartate aminotransferase**

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

- **Alanine aminotransferase**

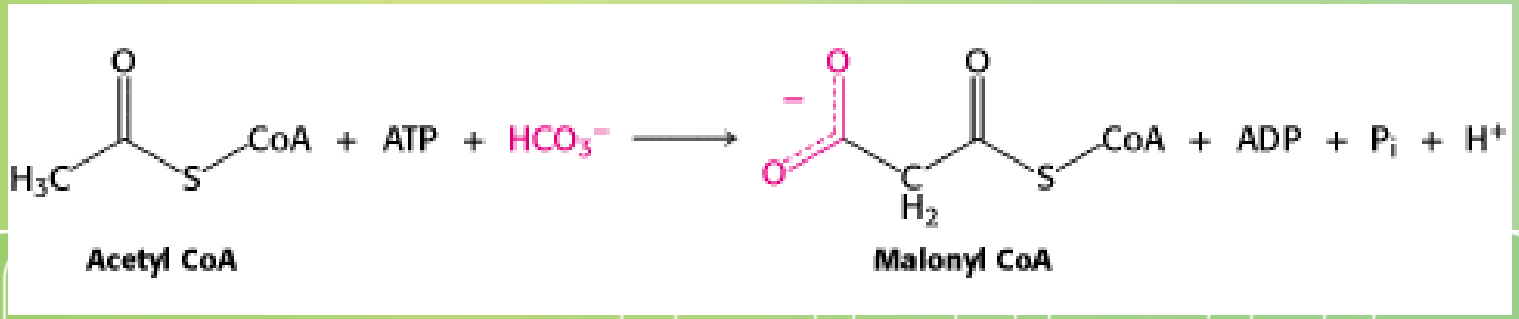
$$\text{Alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate}$$

Biotin (vitamin B7)



- It is required for carboxylation reactions
  - (covalently bound to Lys)
- Source: food & intestinal bacteria
- Deficiencies are generally seen after long antibiotic therapies or excessive consumption of raw eggs (egg white protein, avidin, high affinity for biotin)
- Examples of enzymes
  - Pyruvate carboxylase
  - Acetyl CoA carboxylase (fatty acid synthesis)

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

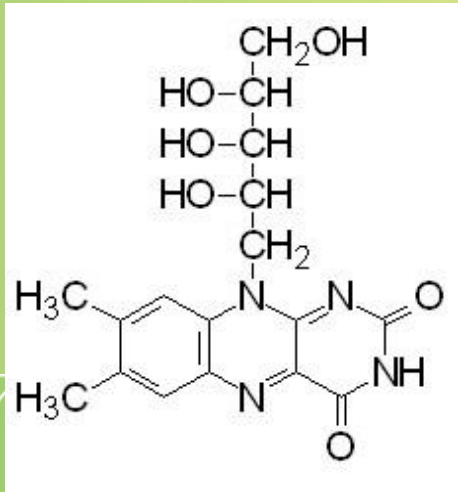


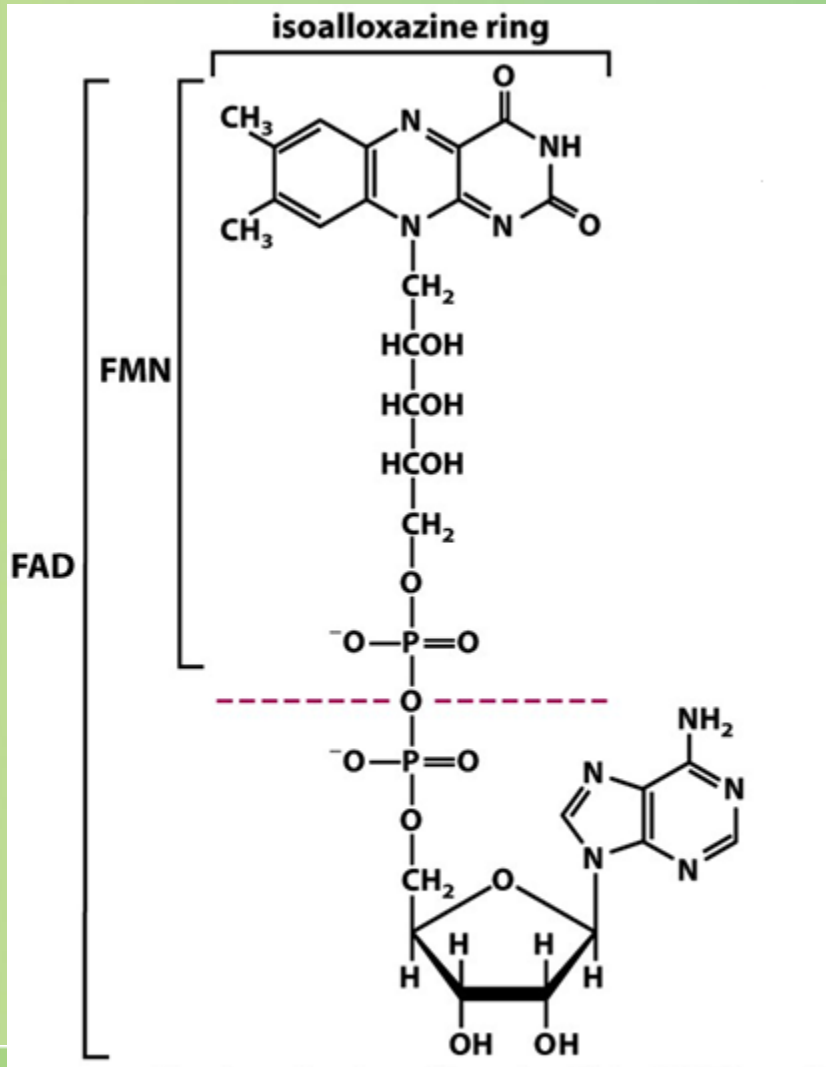
OXIDATION–REDUCTION COENZYMES

- A large number of coenzymes work in enzymes categorized as 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).
- These do not form covalent bonds with the substrate, a portion of the coenzyme binds the enzyme.
- Most common: NAD+ (niacin, B3) & FAD (riboflavin, B2)
- Others: work with metals to transfer single electrons to O2 (Vitamins E & C)
  - Again: Dependence on the enzyme for additional specificity of substrate & additional catalytic power

FAD and FMN

- The precursor is riboflavin (vitamin B2).
- Both are prosthetic groups that are important for redox reactions.
- Proteins that require FMN or FAD as cofactors are called flavoproteins.

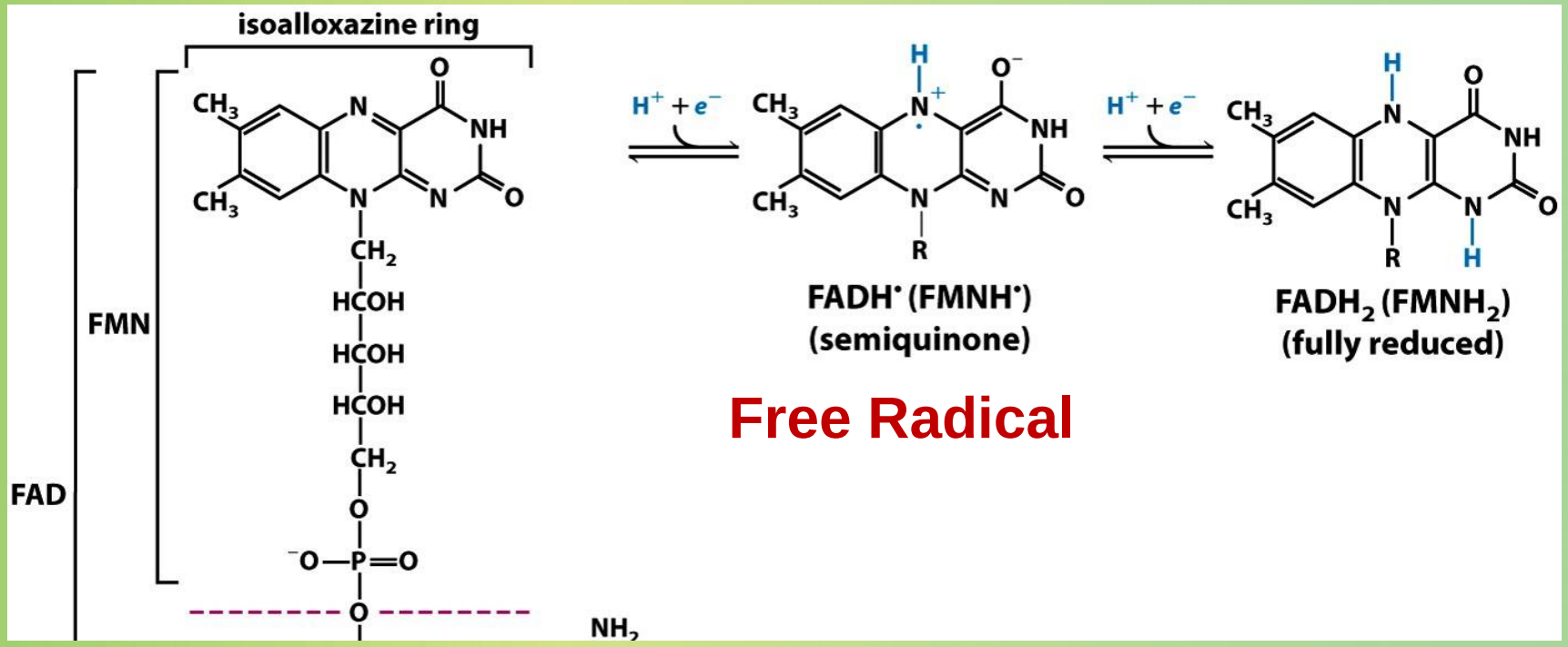




Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

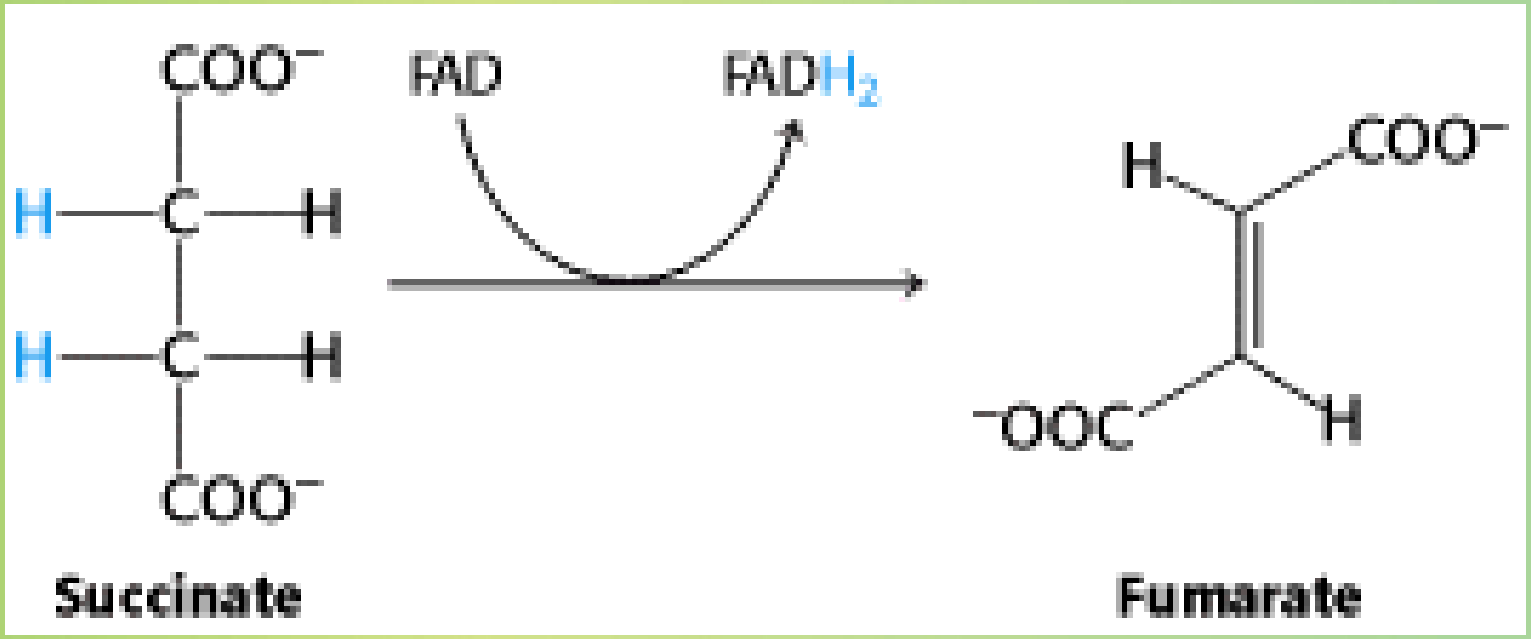
Why is it a prosthetic group?

- Protection mechanism
- Transfer of electrons is sequential.



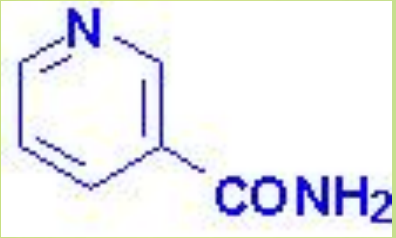
Succinate dehydrogenase

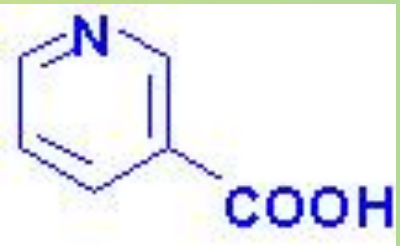
- **Oxidation of succinate into fumarate by succinate dehydrogenase**



NAD+ and NADP+

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

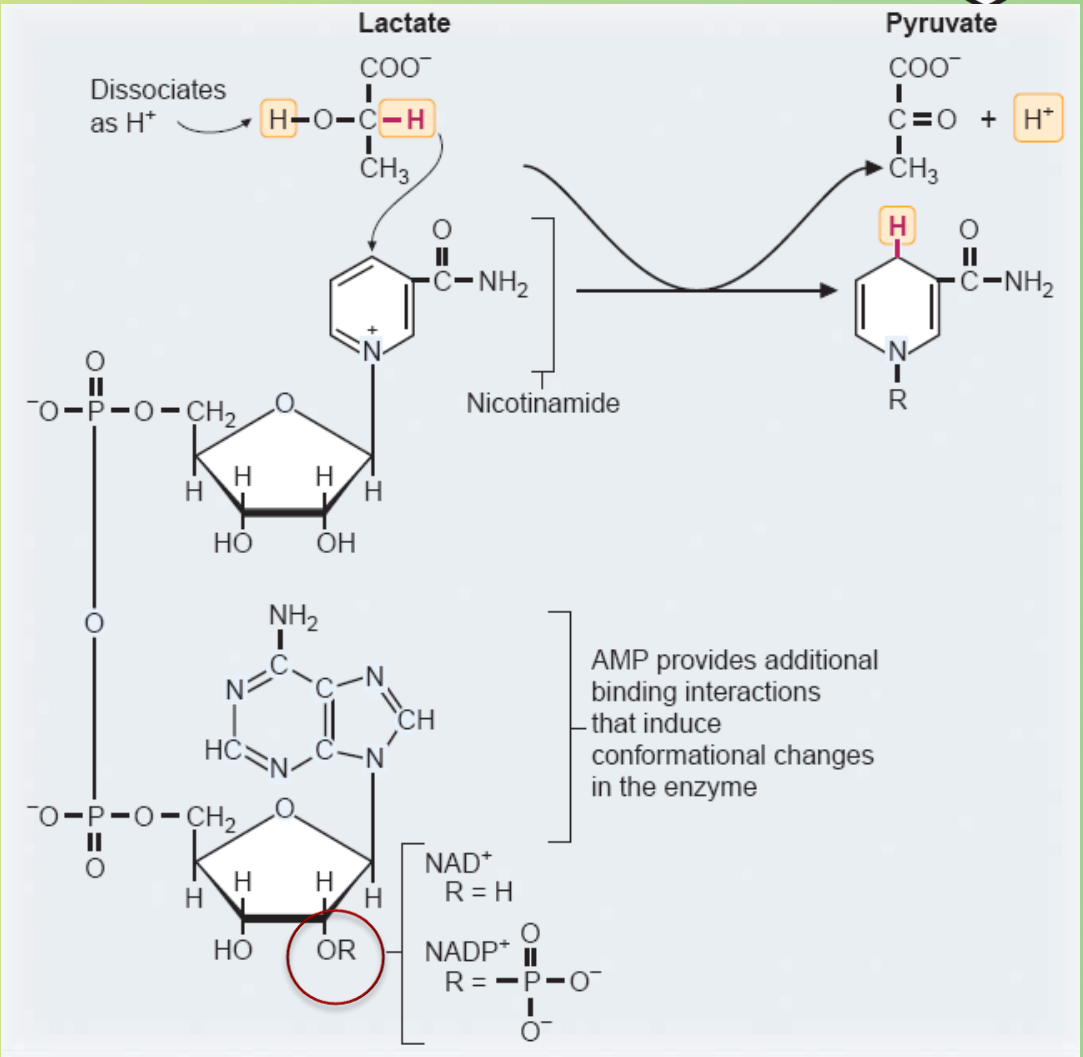




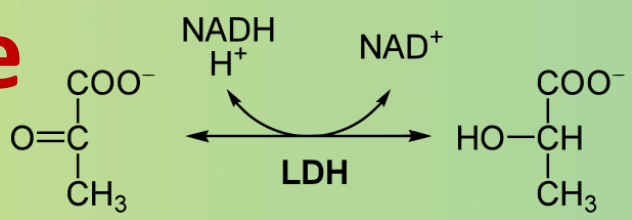
- These are cosubstrates for numerous dehydrogenases.

Mechanism of action

- The functional group (C opposite to N). The cofactor accepts a hydride ion from the substrate, dissociates, & a keto group (CO) is formed.
- The ADP portion of the molecule binds tightly.

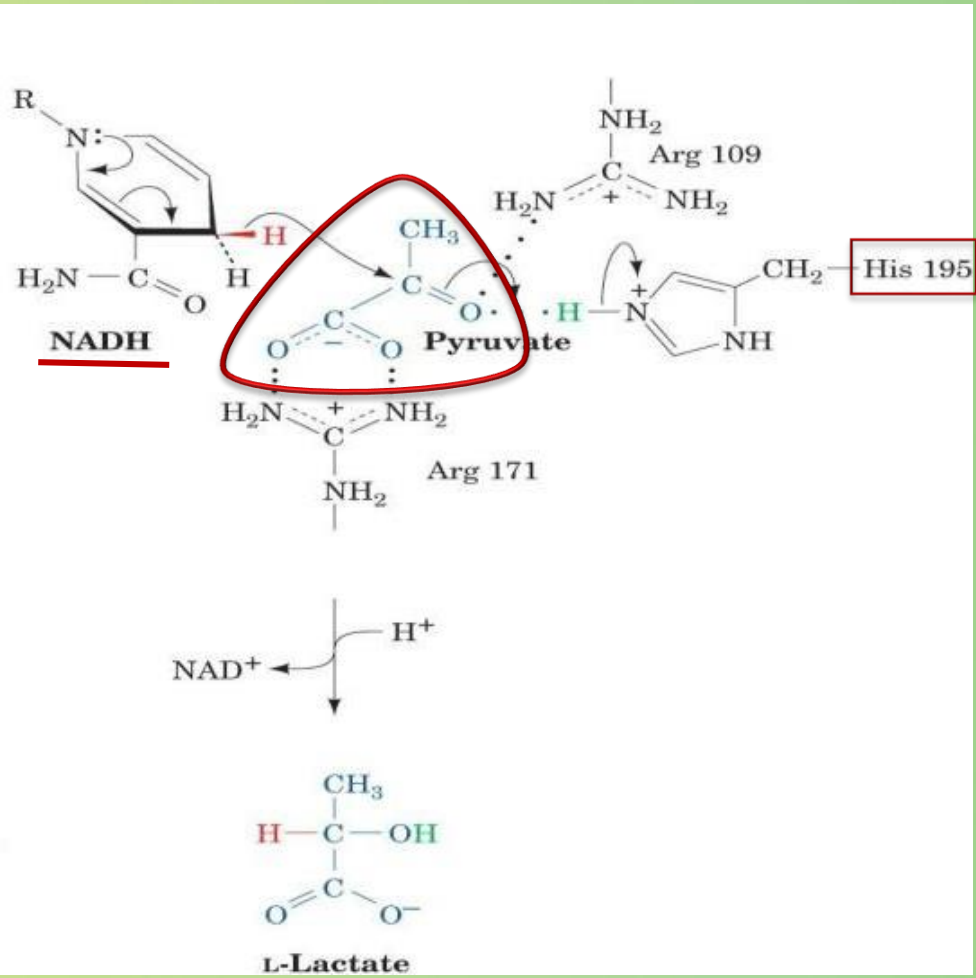


Lactate dehydrogenase



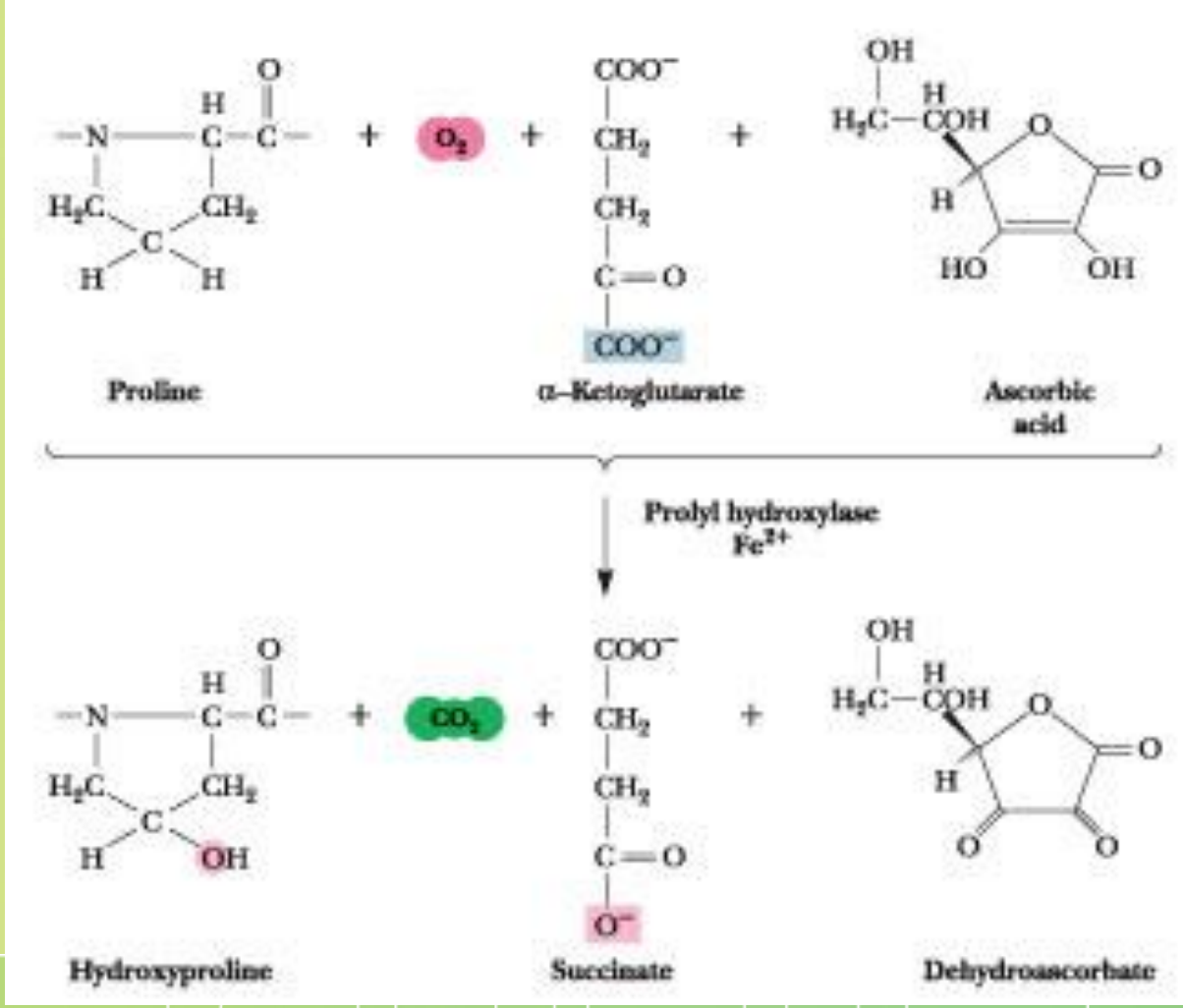
- The enzyme's histidine binds the proton of (-OH) on lactate making it easier for NAD+ to pull off the other hydrogen with both electrons (a hydride).
- A keto group (CO) is formed.

- *Shown is the reverse reaction.*

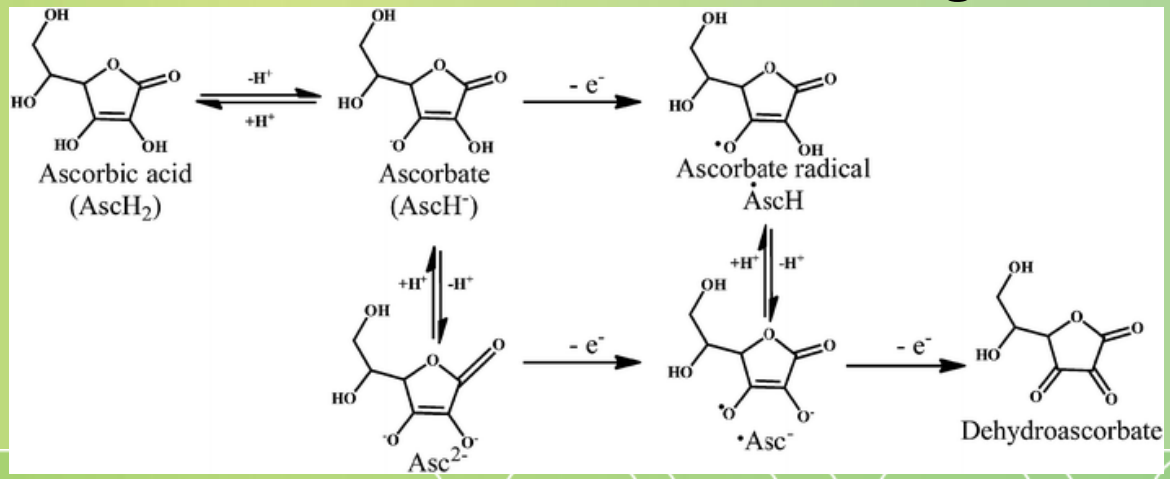


Vitamin C

- Ascorbic acid
- Example: prolyl hydroxylase
  - synthesizes 4-hydroxyproline (collagen)
- An antioxidant



- Ascorbate acts as an antioxidant by being available for energetically favorable oxidation.
- Reactive oxygen species oxidize (take electrons from) ascorbate into a radical itself, which is oxidized into dehydroascorbate.
- The reactive oxygen species are reduced to water, while the oxidized forms of ascorbate are relatively stable and unreactive, and do not cause cellular damage.



Metals

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

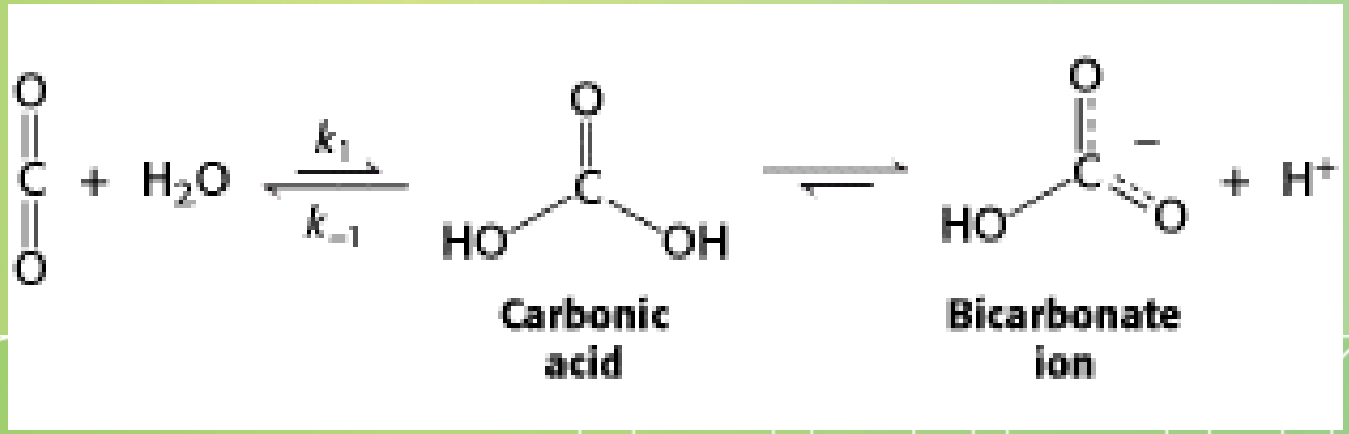
- **They act as electrophiles.**
- **They assist in binding of the substrate, or they stabilize developing anions in the reaction.**
- **They can also accept and donate electrons in oxidation–reduction reactions.**

Advantages

- They carry positive charges and, hence, can form relatively strong yet kinetically labile bonds.
- They are stable in more than one oxidation.
- They can bind multiple ligands in their coordination sphere enabling them to participate in binding substrates or coenzymes to enzymes.
  - For example, Mg^{+2} plays a role in the binding of the negatively charged phosphate groups of thiamine pyrophosphate to anionic or basic amino acids in the enzyme.
  - The phophate groups of ATP are usually bound to enzymes through Mg^{+2} chelation.

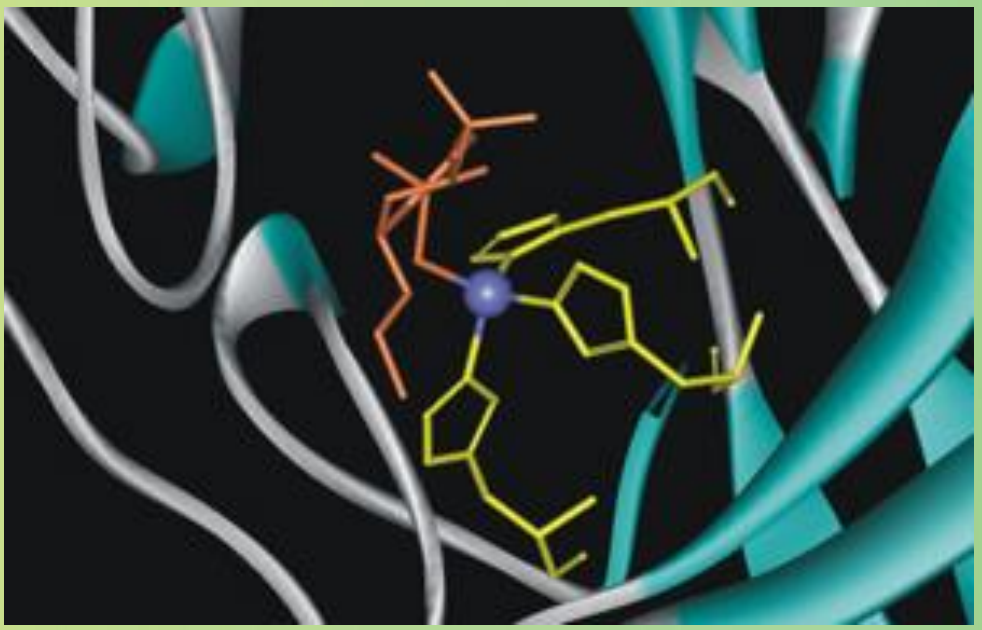
Carbonic anhydrases

- CO_2 hydration and HCO_3^-dehydration occur spontaneously at reasonable rates in the absence of catalysts, yet 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.



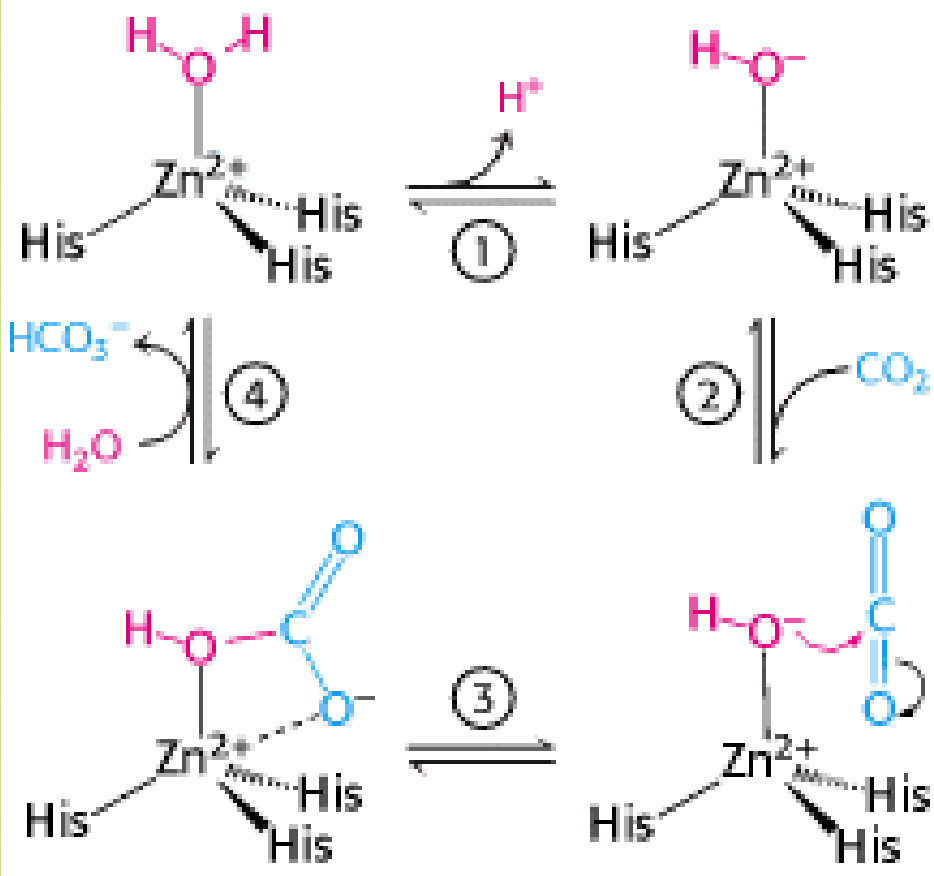
Zn binding to the enzyme

- Zinc is found only in the +2 state in biological systems.
- In carbonic anhydrase, a zinc atom is bound to three imidazole rings of three histidine residues and an additional site is occupied by a water molecule.



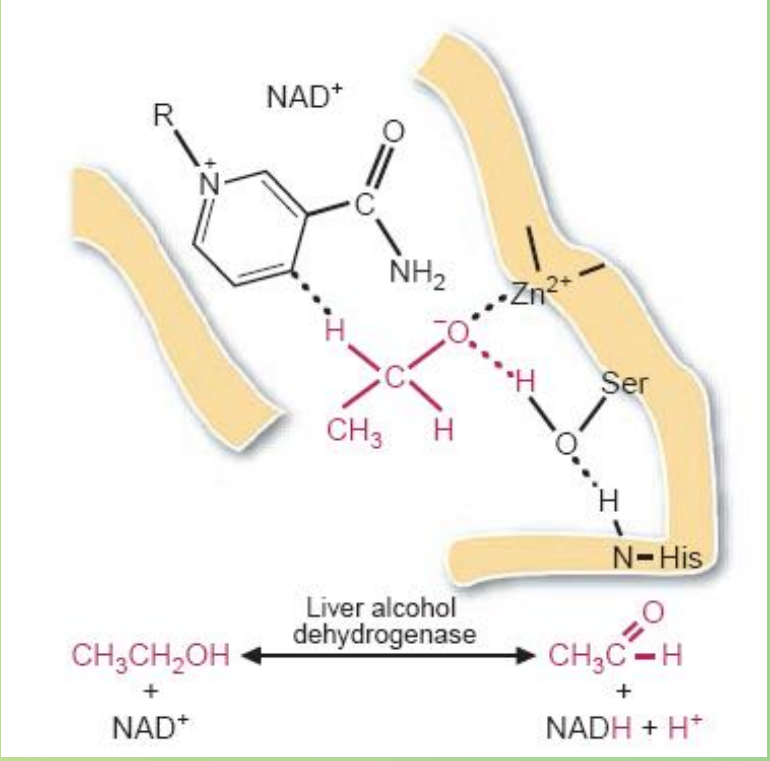
Mechanism of action

- Zinc facilitates the release of a proton from a water molecule generating a hydroxide ion.
- The CO2 substrate binds to the enzyme's active site and is positioned to react with the hydroxide ion.
- The hydroxide ion attacks the carbon dioxide, converting it into bicarbonate ion.
- The catalytic site is regenerated with the release of the bicarbonate ion and the binding of another molecule of water.



Catalytic Metals

- Some metals can bind anionic substrates or intermediates of the reaction to alter their charge distribution, thereby contributing to catalytic power.
- The histidine pulls an H off the active site serine, which pulls the H off of the substrate –OH group, leaving the oxygen with a negative charge that is stabilized by zinc, and a hydride is the transferred to NAD+.
- Zinc in ADH as His in lactate dehydrogenase.



$$C_2H_5OH + NAD^+ \underset{}{\overset{ADH}{\rightleftharpoons}} CH_3CHO + NADH + H^+$$

ethanol — nicotinamide adenine dinucleotide — acetaldehyde