



Enzymes IV

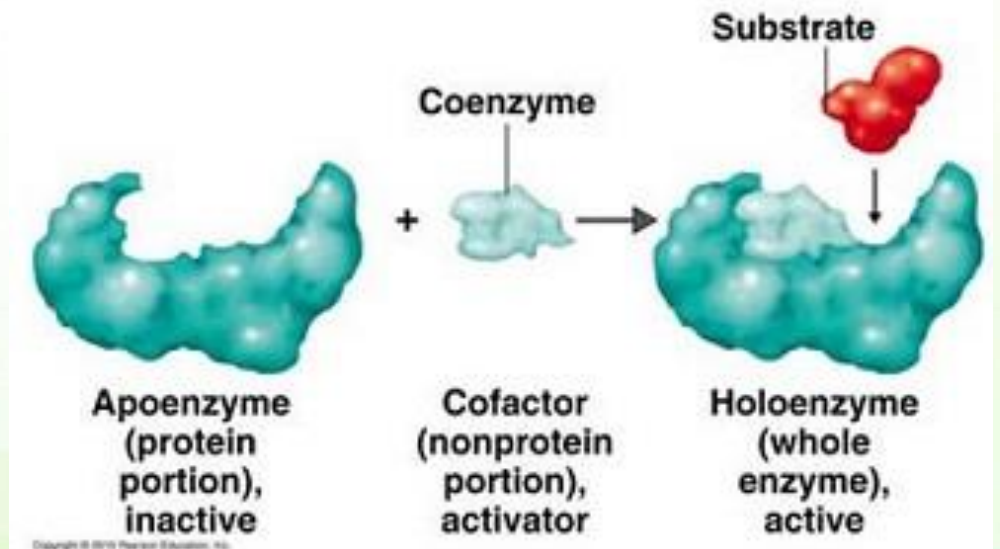
Cofactors

Summer semester, 2023

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 need cofactors (nonprotein compounds that participate in the catalytic process).
 - Conjugated enzymes (Holoenzyme vs. apoenzyme)

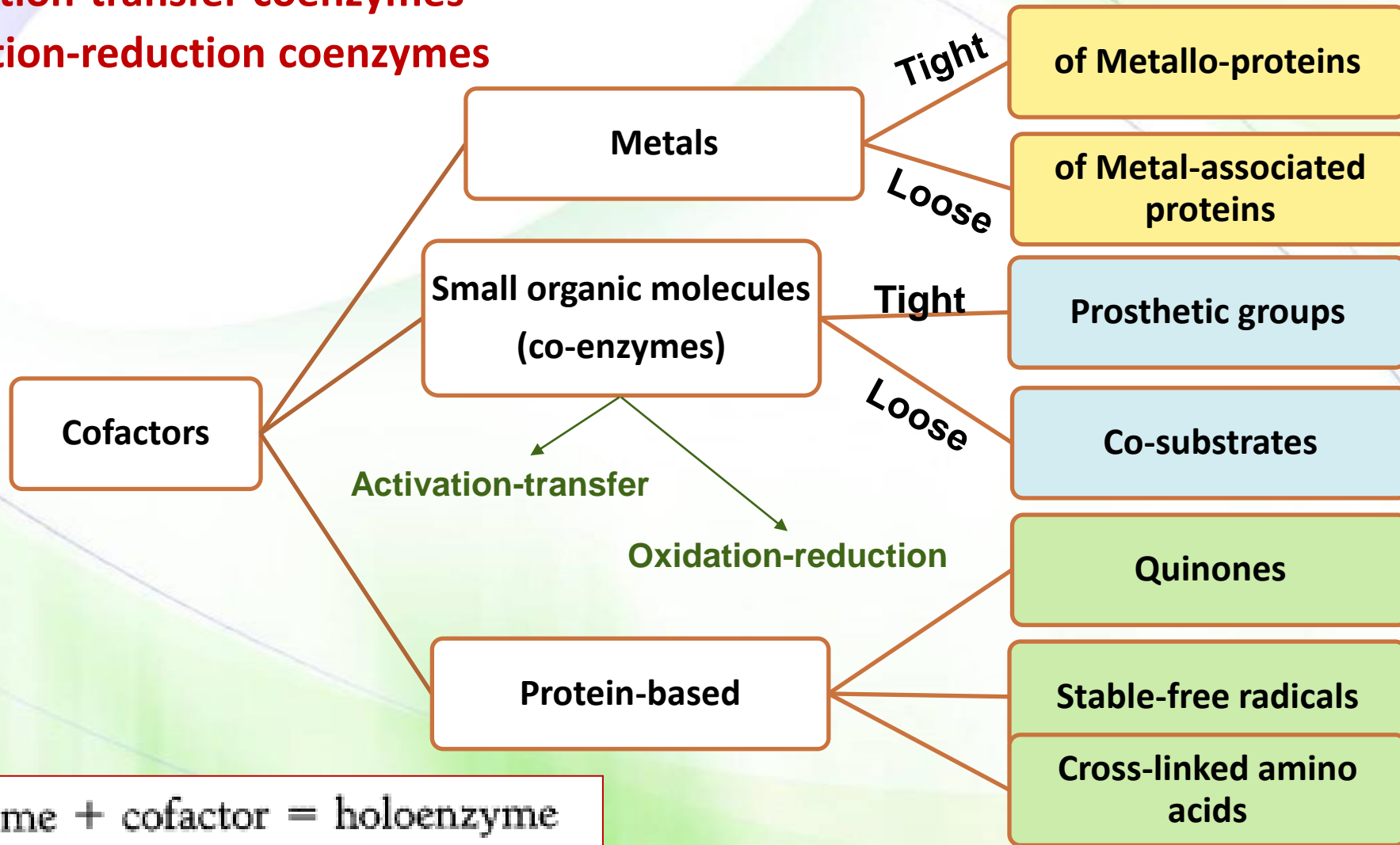


Classification of cofactors



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

ACTIVATION-TRANSFER COENZYMES



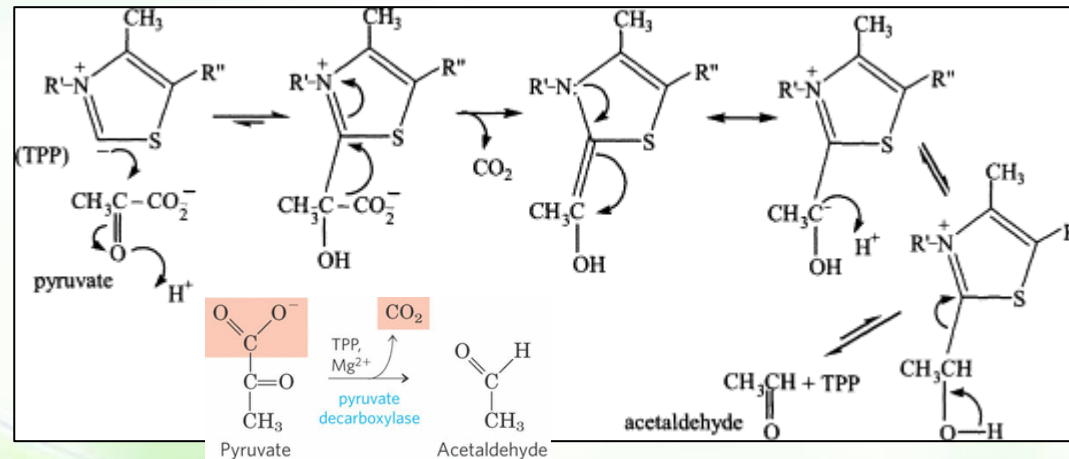
- The functional group of the coenzyme directly participates in catalysis.
- Characteristics:
 - Two groups in the coenzyme:
 - A functional group that forms a covalent bond with substrate.
 - A binding group that binds tightly to the enzyme.
 - Dependence on the enzyme for additional specificity of substrate & additional catalytic power

Thiamin pyrophosphate, TPP

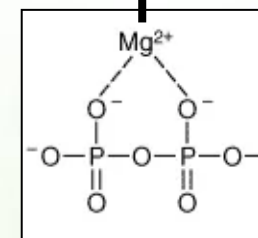


- Thiamin (vitamin B1) is converted to its active form, thiamin pyrophosphate, TPP, in the brain & liver.
- It is involved in **decarboxylation** reactions.
- The pyrophosphate provides negatively charged oxygen atoms and chelates Mg^{2+} that is tightly bound to the enzyme.

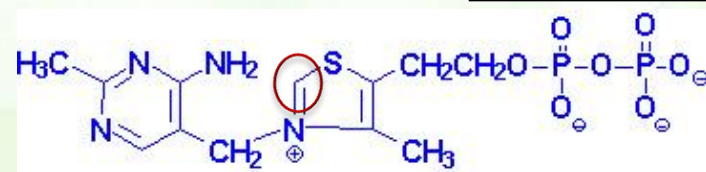
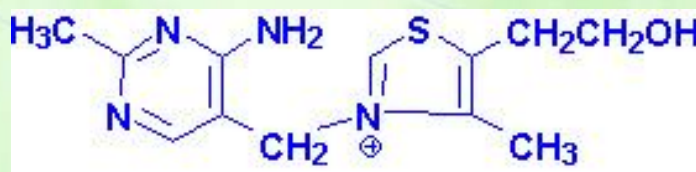
The reactive thiamin carbon binds to the keto group of substrates releasing CO_2 .



Enzyme



Chelation

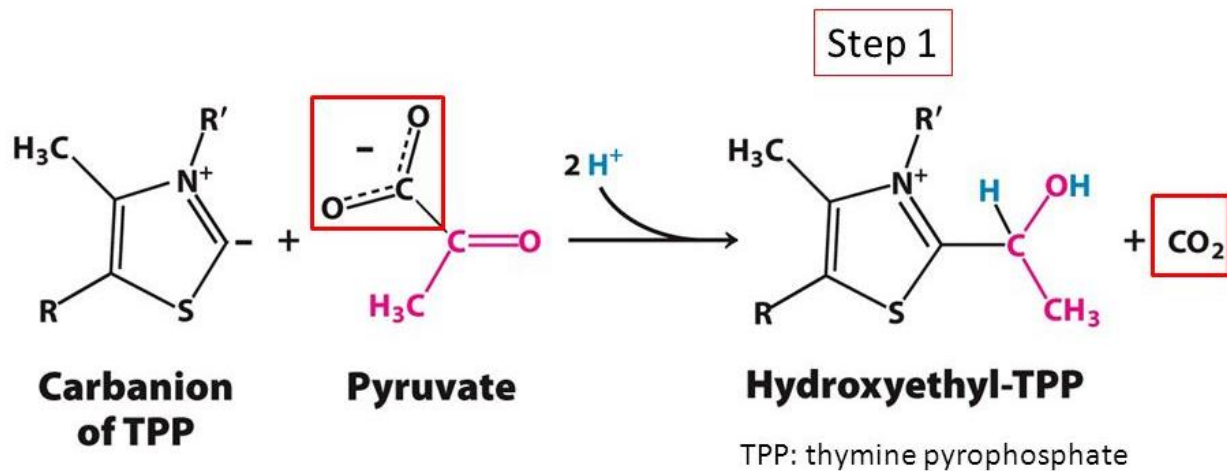
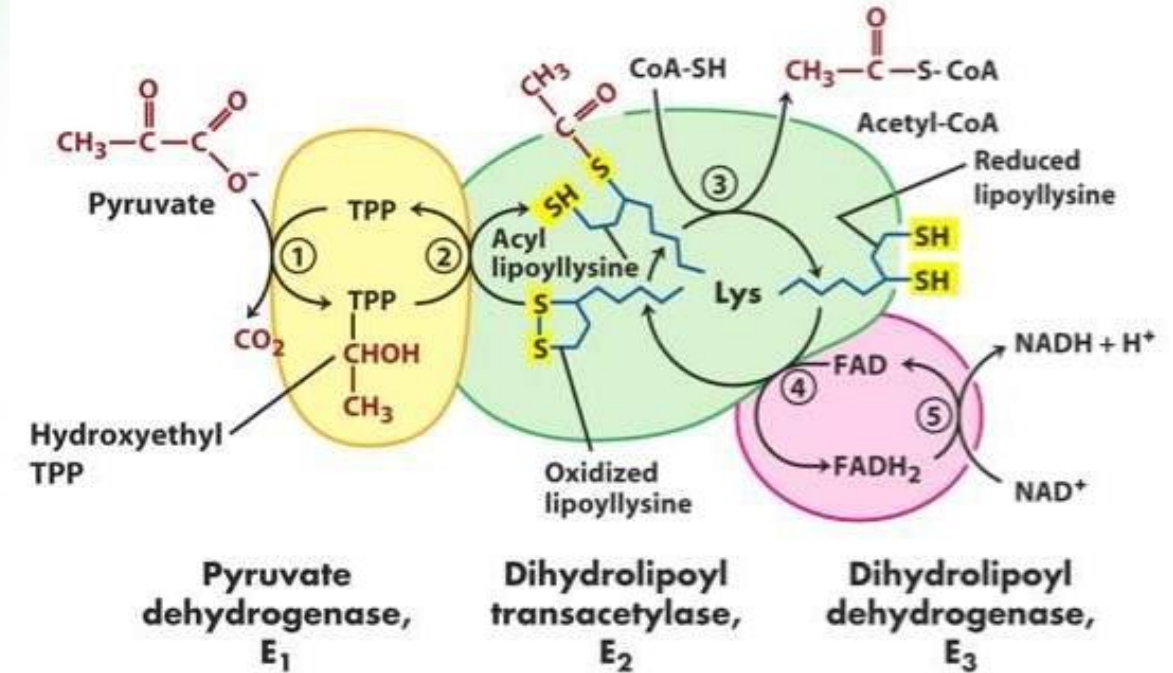


Thiamin pyrophosphate

Pyruvate dehydrogenase complex



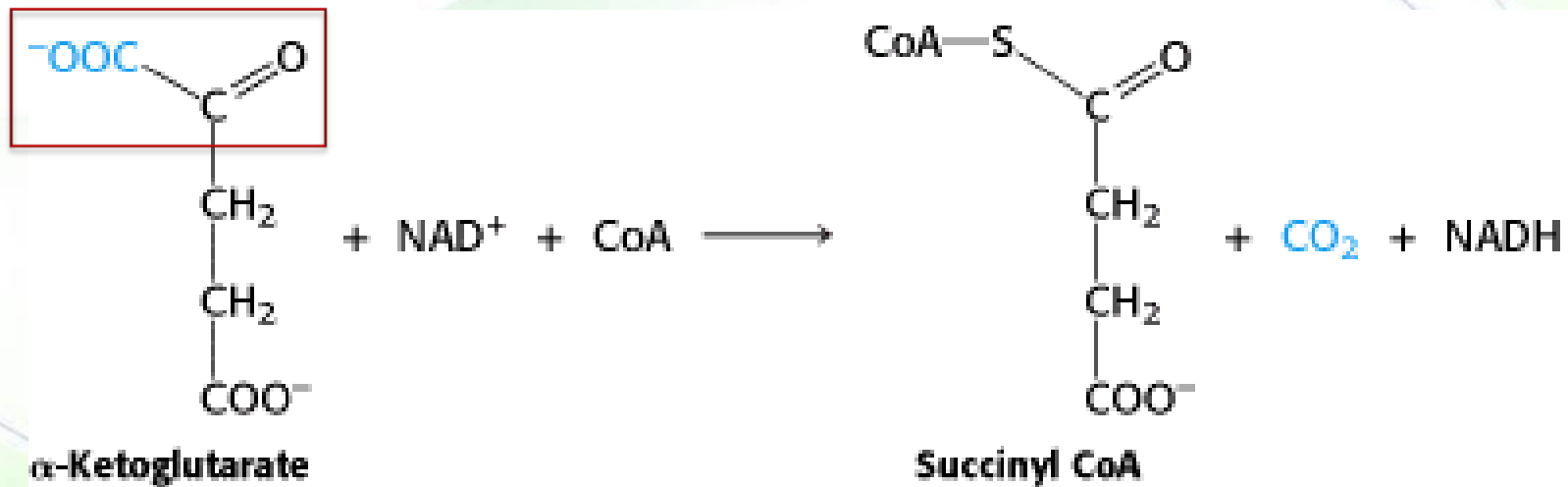
- Decarboxylation of pyruvate into acetyl CoA by the pyruvate dehydrogenase complex



α -ketoglutarate dehydrogenase



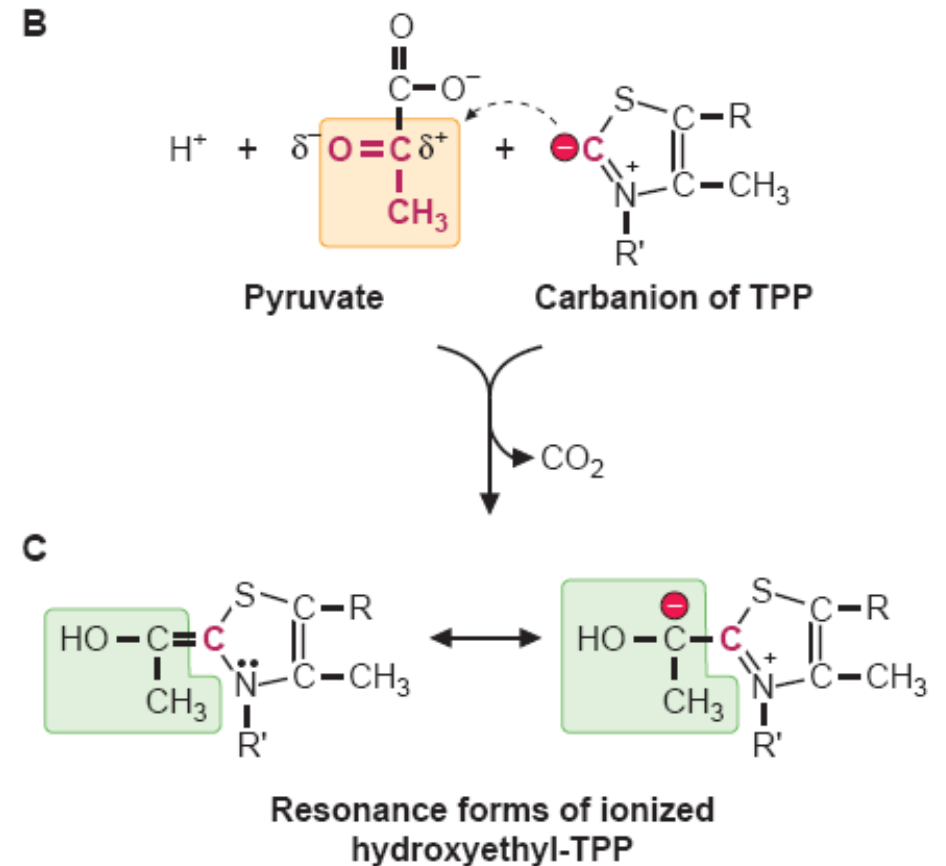
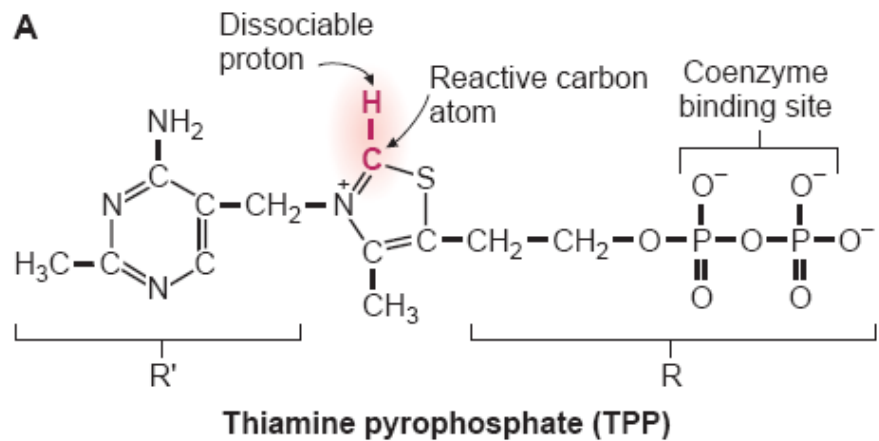
- Decarboxylation of α -ketoglutarate into succinyl CoA by α -ketoglutarate dehydrogenase



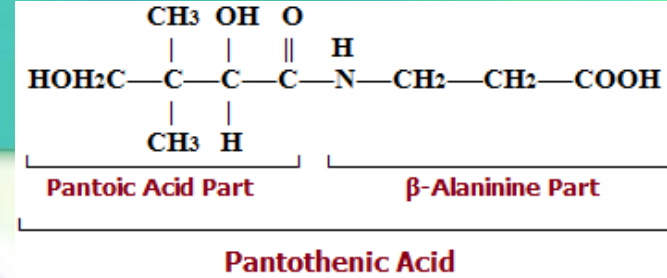
Mechanism of action



- 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.

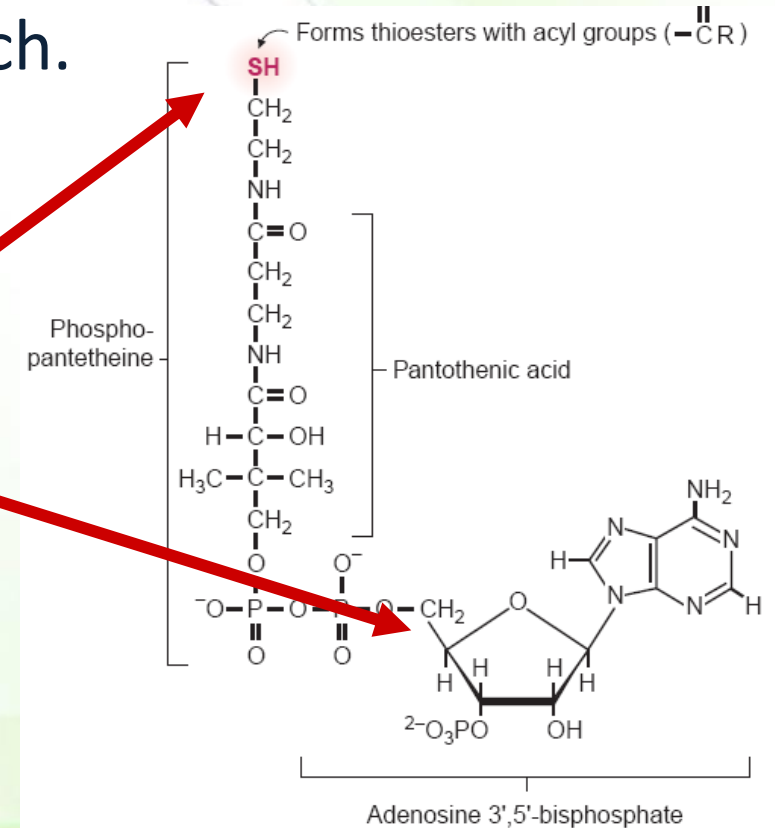


Coenzyme A (CoA)



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

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



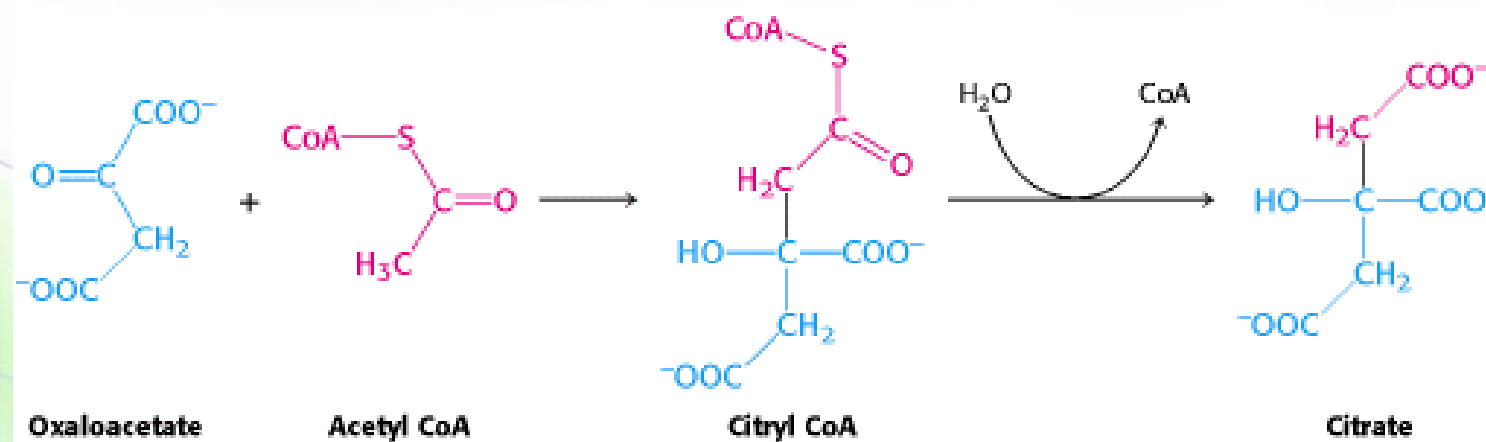
Examples of enzymes



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



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

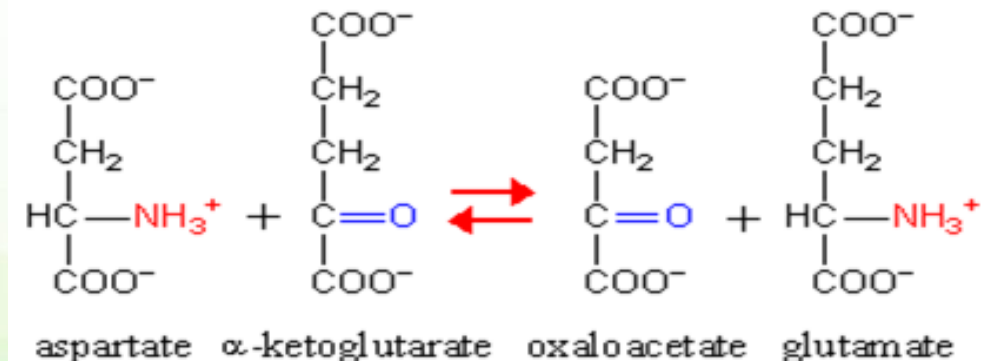
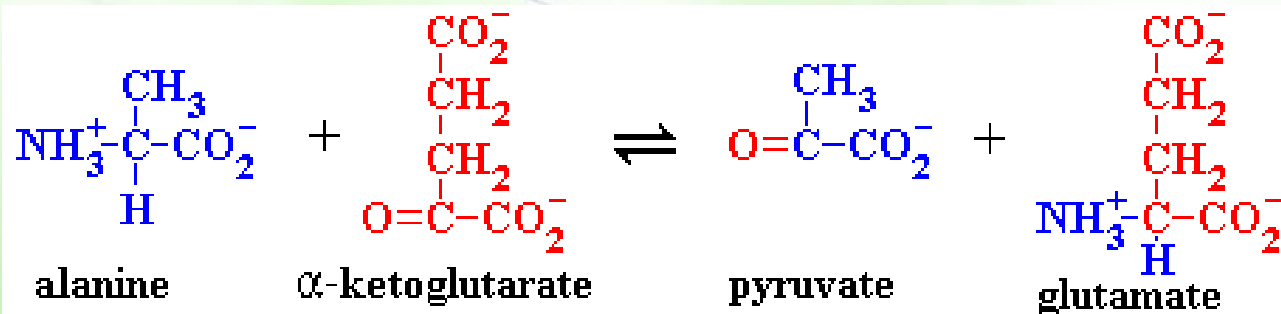


Pyridoxal phosphate (vitamin B6)



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

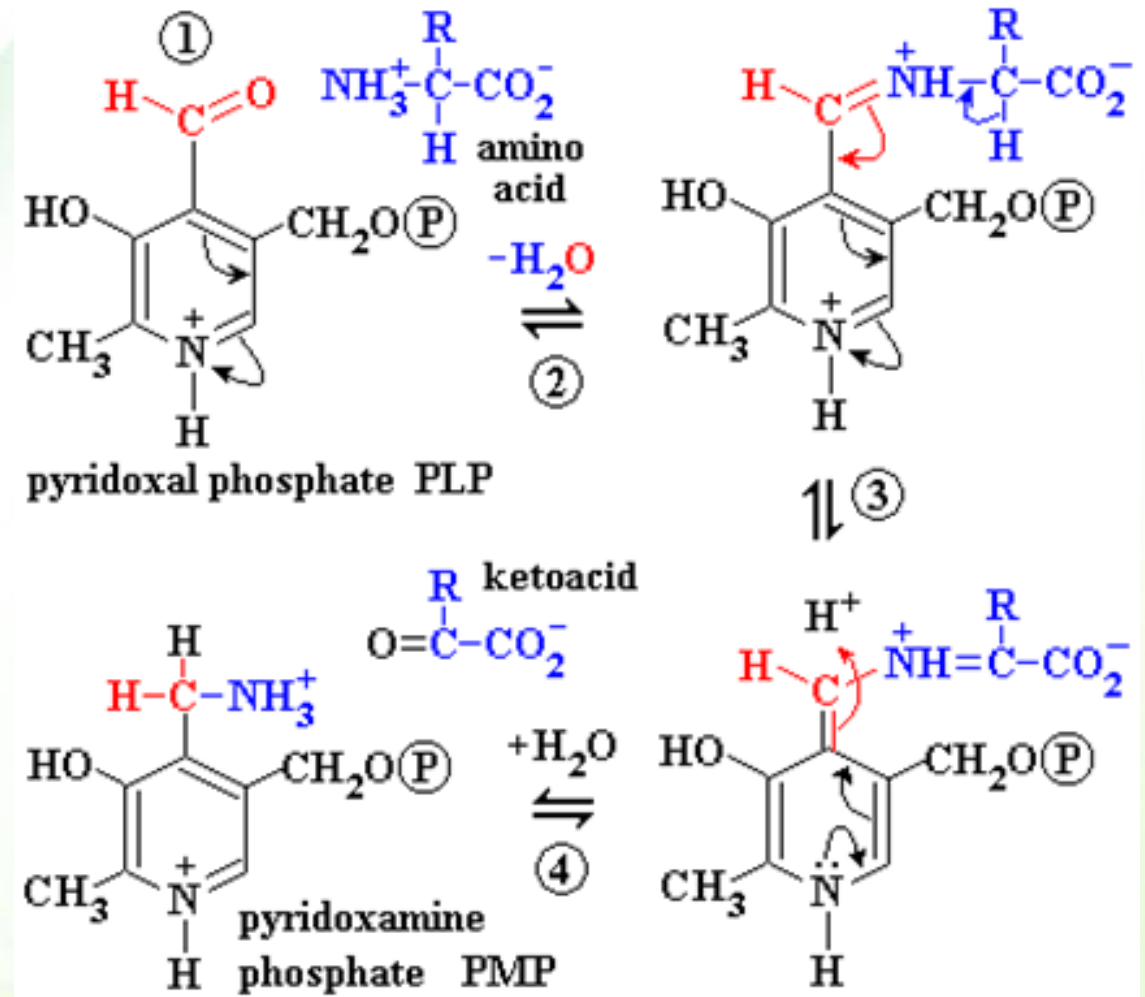
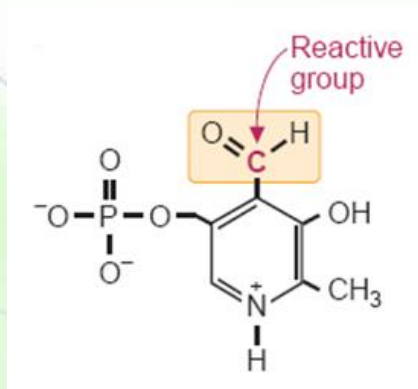
Pyridoxine	Pyridoxal	Pyridoxamine	pyridoxal phosphate



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.



Biotin (vitamin B7)



- It is required for **carboxylation** reactions.

- covalently bound to Lys

- Source: food & intestinal bacteria

- Deficiencies are seen after long antibiotic therapies or excessive consumption of raw eggs (egg white protein, avidin, has a high affinity for biotin).

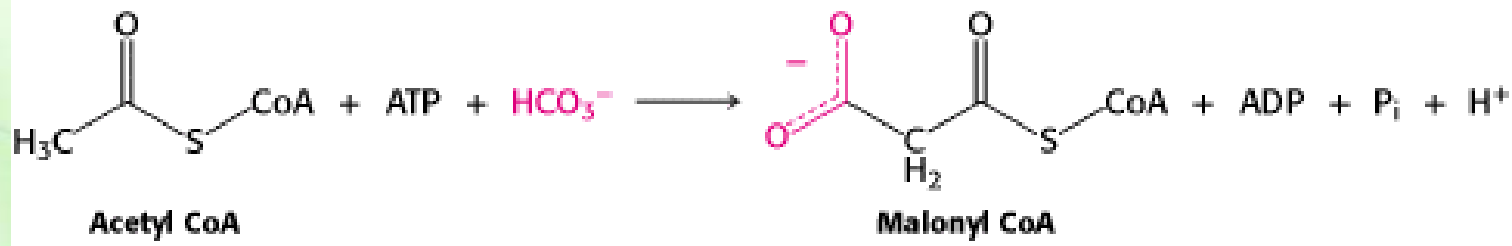
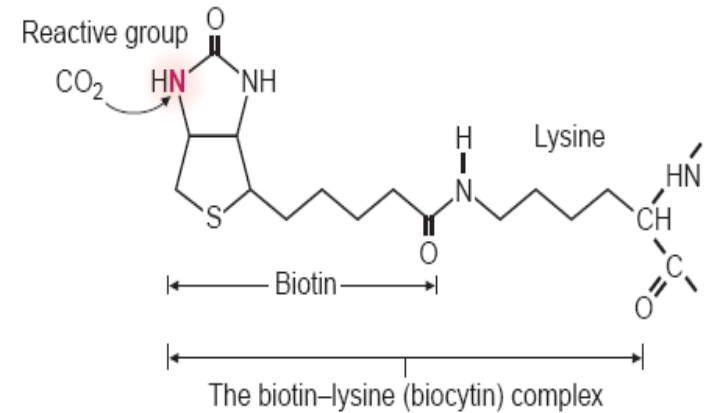
- Examples of enzymes:

- Pyruvate carboxylase



- Acetyl CoA carboxylase (fatty acid synthesis)

B. Biotin



OXIDATION–REDUCTION COENZYMES

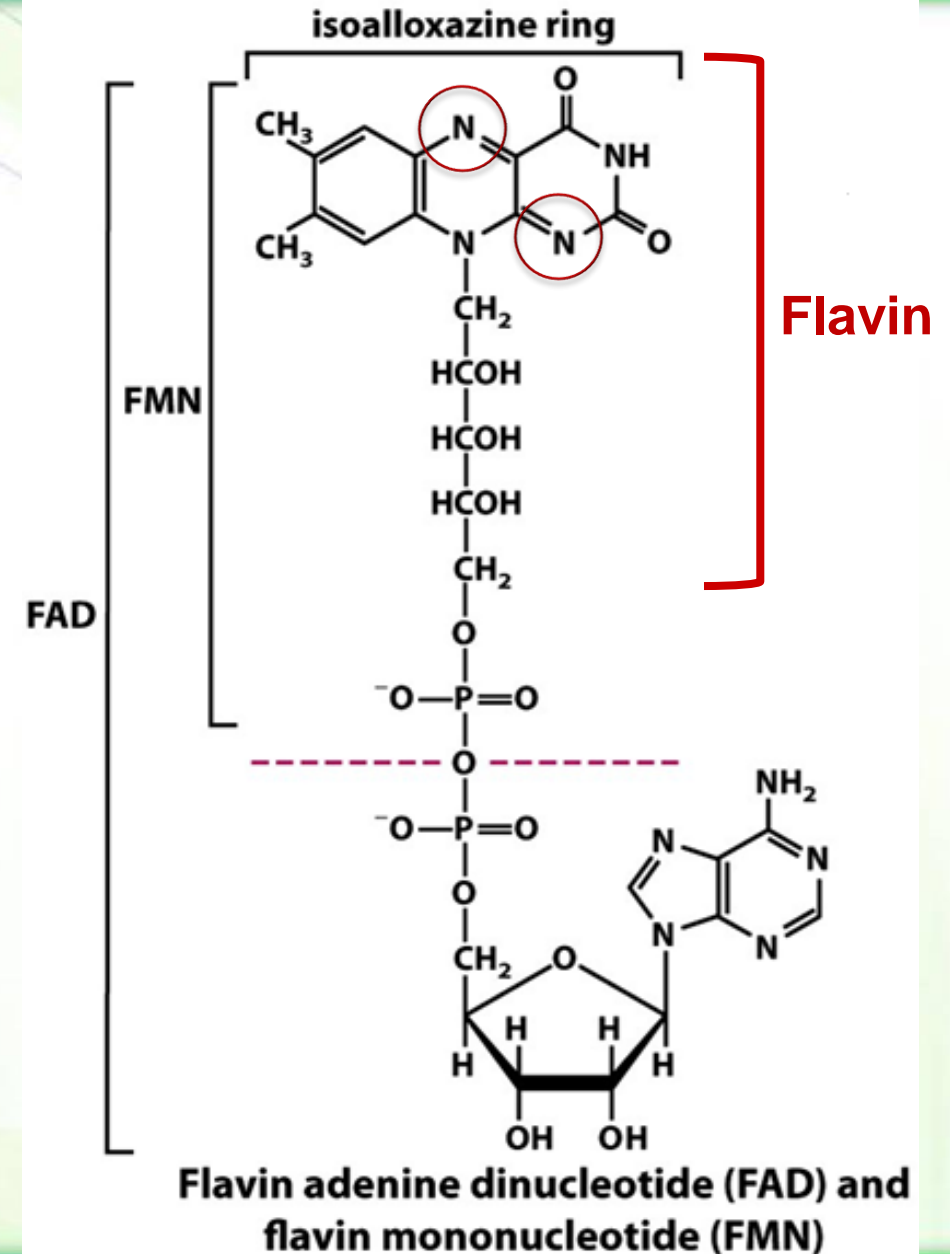
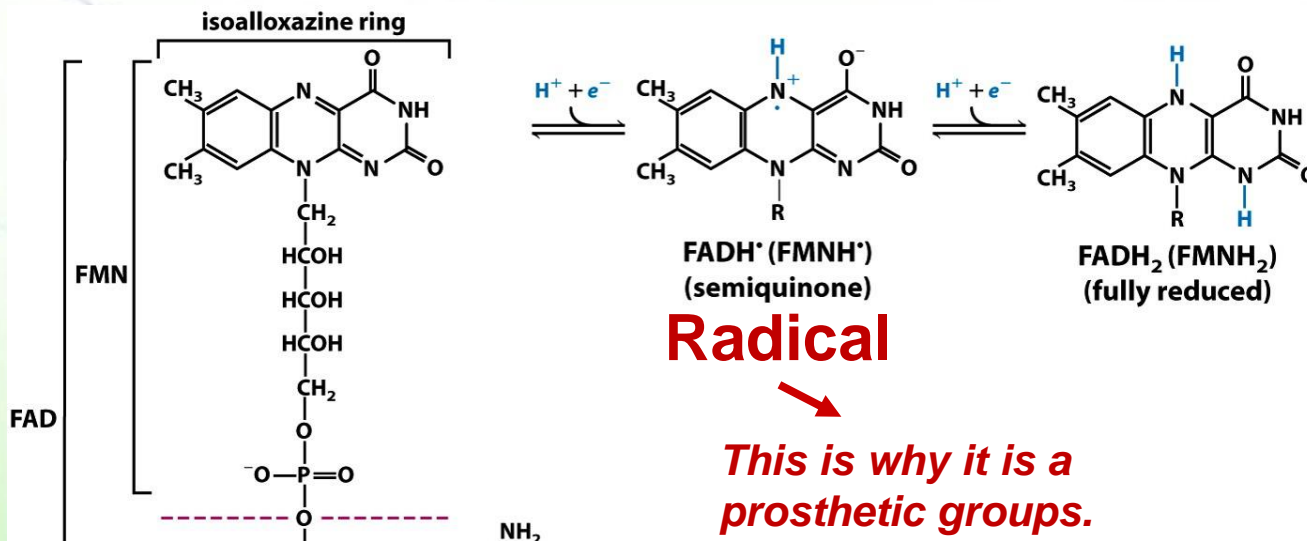


- A number of coenzymes work within oxidoreductases.
- Each coenzyme has a unique functional group that accepts and donates electrons and is specific for the form of electrons it transfers (e.g., hydride ions, hydrogen atoms, oxygen).
- **These do not form covalent bonds with the substrate**, a portion of the coenzyme binds the enzyme.
- Most common: NAD^+ (niacin, B3) & FAD (riboflavin, B2)
- Others: work with metals to transfer single electrons to O_2 (Vitamins E & C)
 - Again: Dependence on the enzyme for additional specificity of substrate & additional catalytic power

FAD and FMN



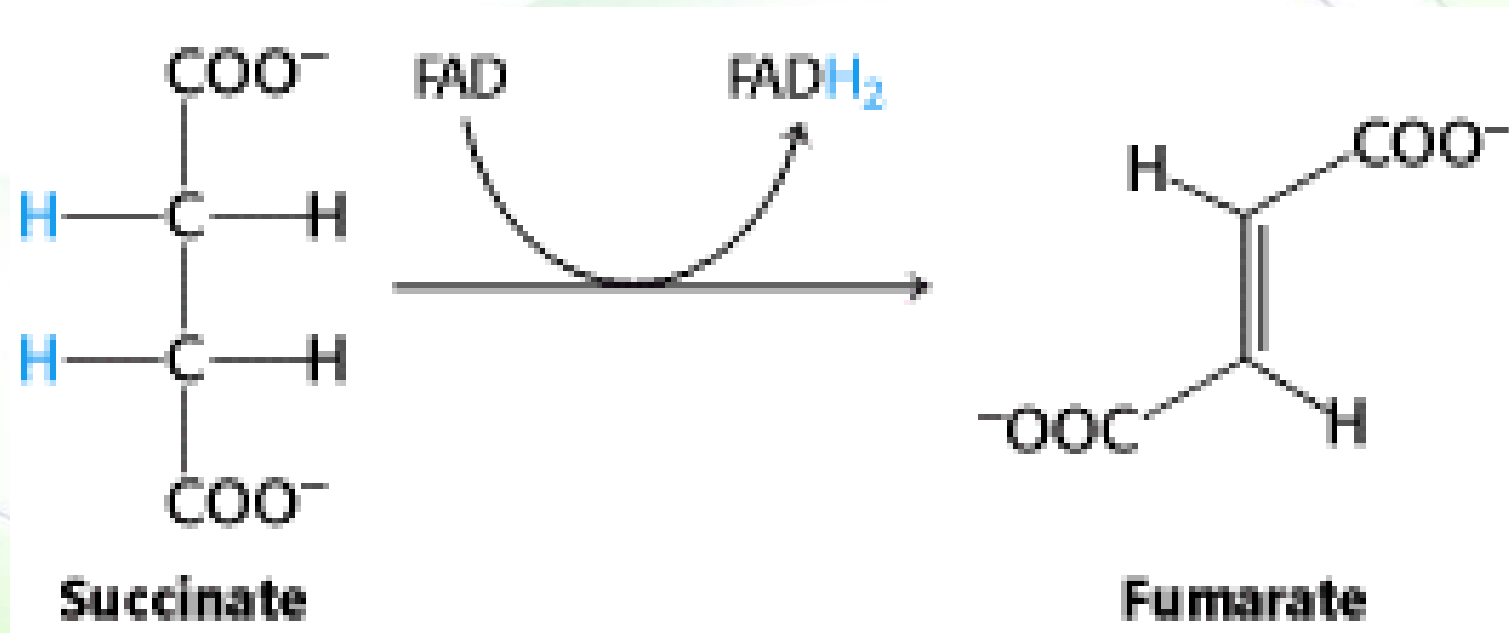
- The precursor is riboflavin (vitamin B2).
- Both are prosthetic groups of flavoproteins.
- FAD accepts electrons in the form of **hydrogen atoms** donated separately and **sequentially**.
- They are involved in reactions resulting in the formation of double bonds or disulfide bonds.



Succinate dehydrogenase



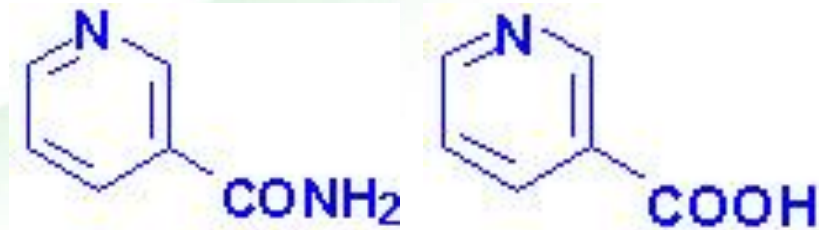
- Oxidation of succinate into fumarate by succinate dehydrogenase



NAD⁺ and NADP⁺



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

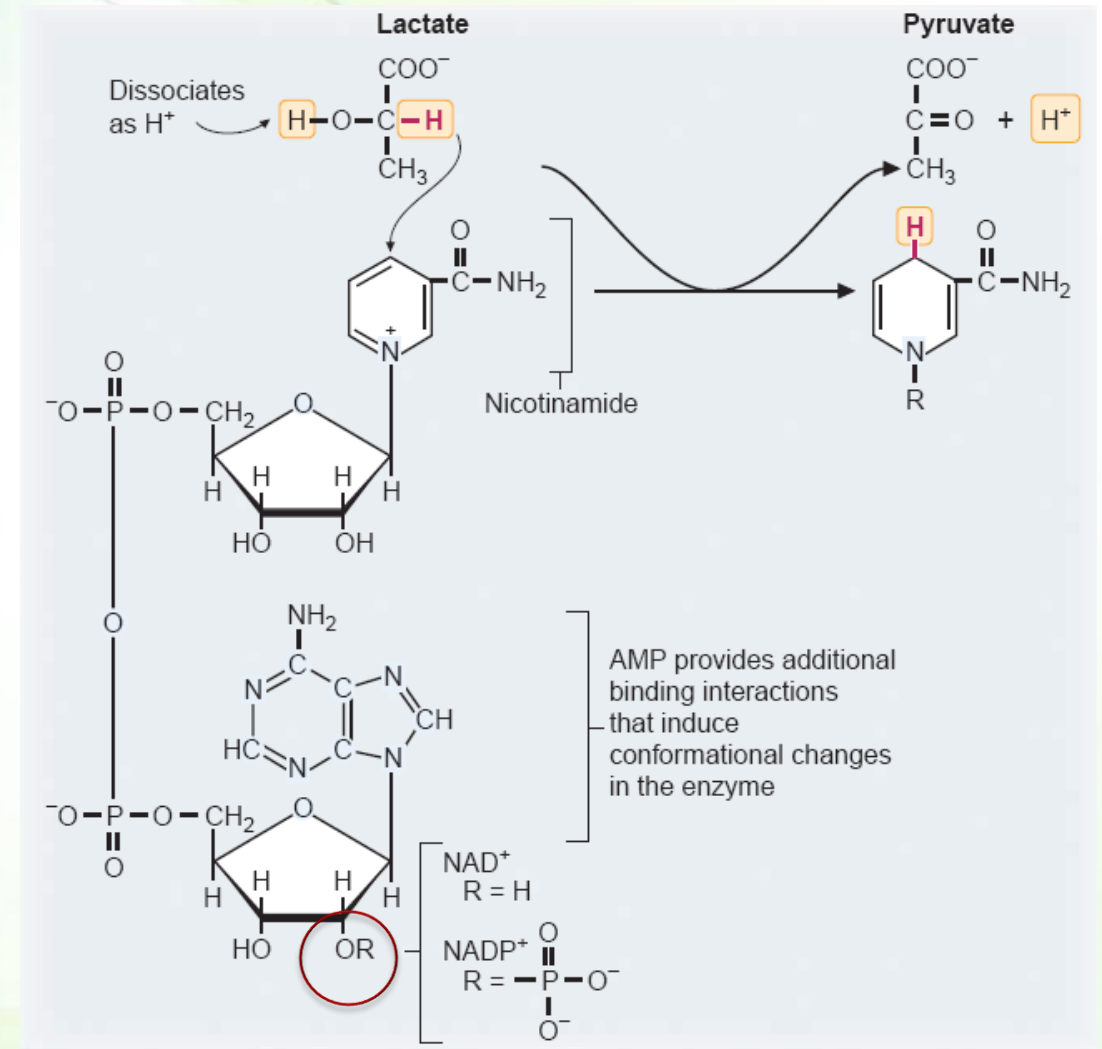


- These are cosubstrates for numerous dehydrogenases.

Mechanism of action



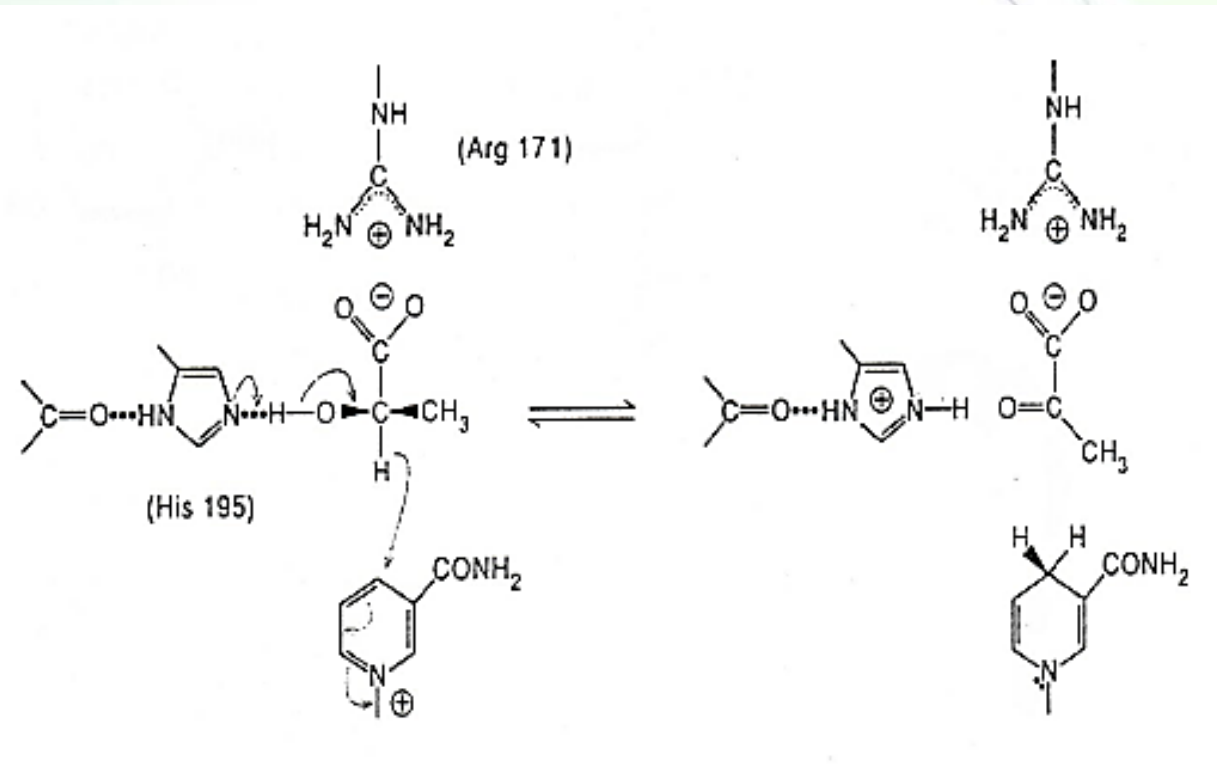
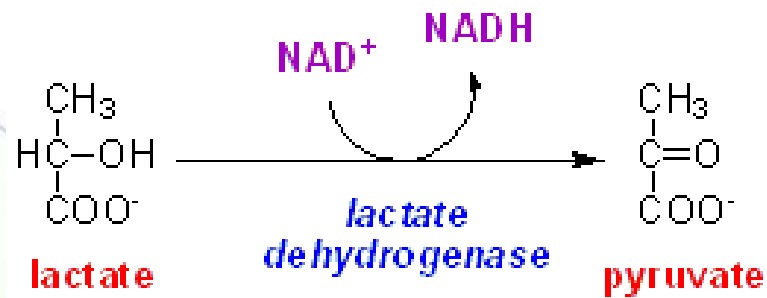
- The functional group (C opposite to N) accepts a hydride ion from the substrate, dissociates, & a keto group (CO) is formed.
- The ADP portion of the molecule binds tightly.
- They are generally involved in the oxidation of alcohols and aldehydes.



Lactate dehydrogenase



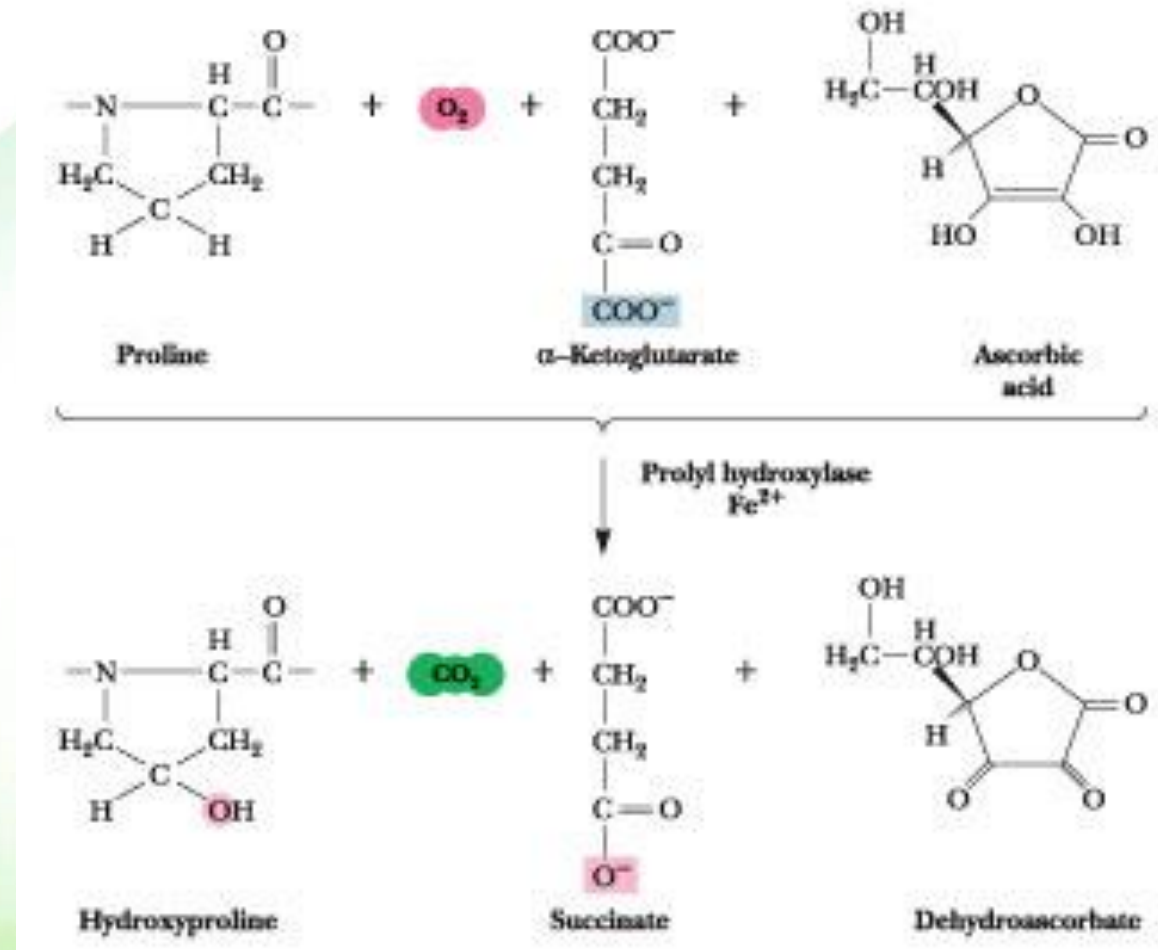
- The enzyme's histidine binds the proton of (-OH) on lactate making it easier for NAD^+ to pull off the other hydrogen with both electrons (a hydride).
- A keto group (-C=O) is formed.



Vitamin C



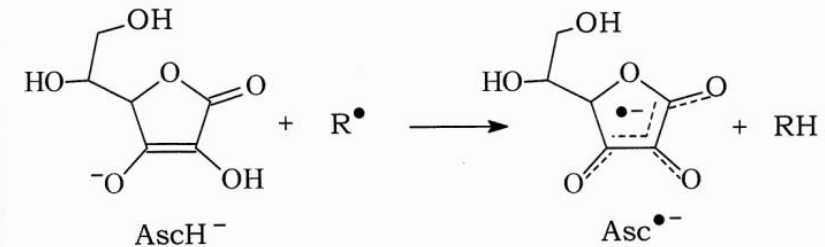
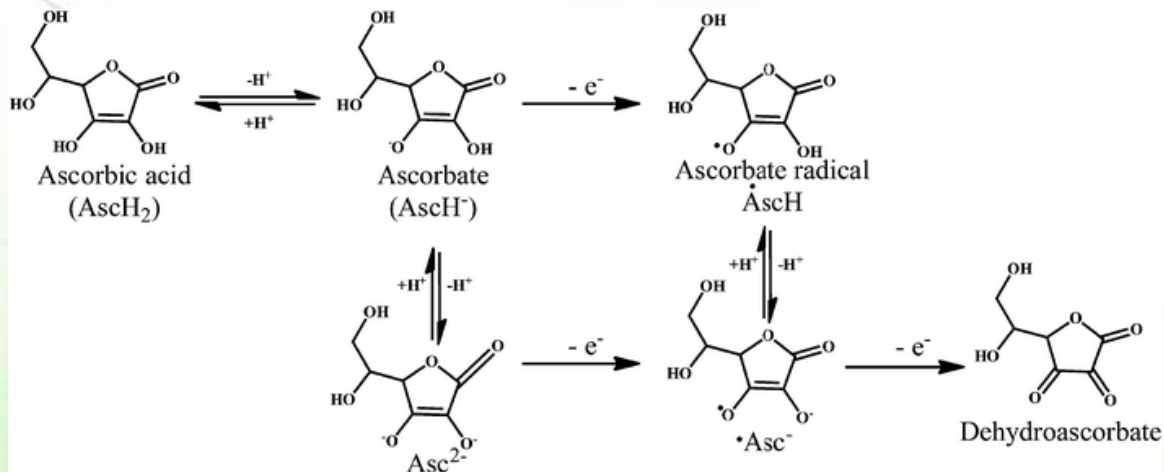
- Ascorbic acid
- Example: prolyl hydroxylase
 - synthesizes 4-hydroxyproline (collagen)
- An antioxidant



Ascorbate, the anti-oxidant



- 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.



Metals



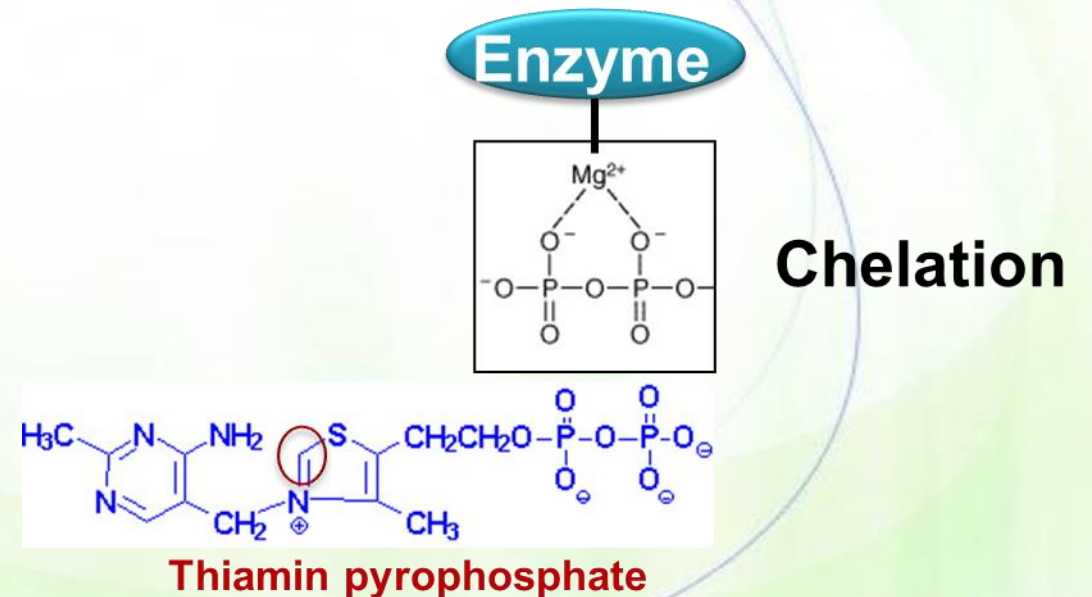
Metal	Enzyme
Zn ²⁺	Carbonic anhydrase Carboxypeptidase
Mg ²⁺	Hexokinase
Se	Glutathione peroxidase
Mn ²⁺	Superoxide dismutase

- 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



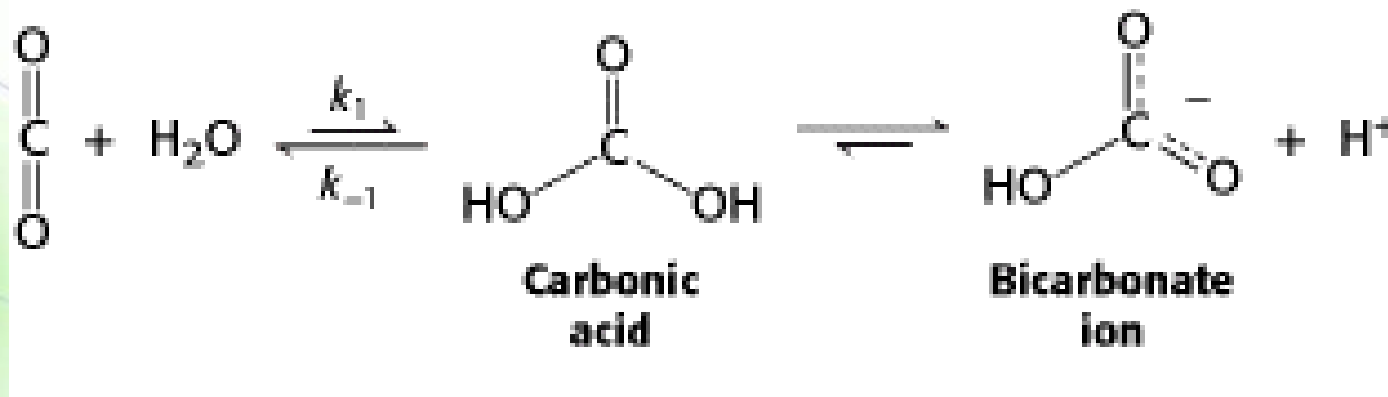
- They carry positive charges and, hence, can form relatively strong yet kinetically labile (likely to be changed) bonds.
- They are stable in more than one oxidation state.
- They can bind multiple ligands enabling them to participate in binding substrates or coenzymes to enzymes.
- Mg^{2+} connects 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



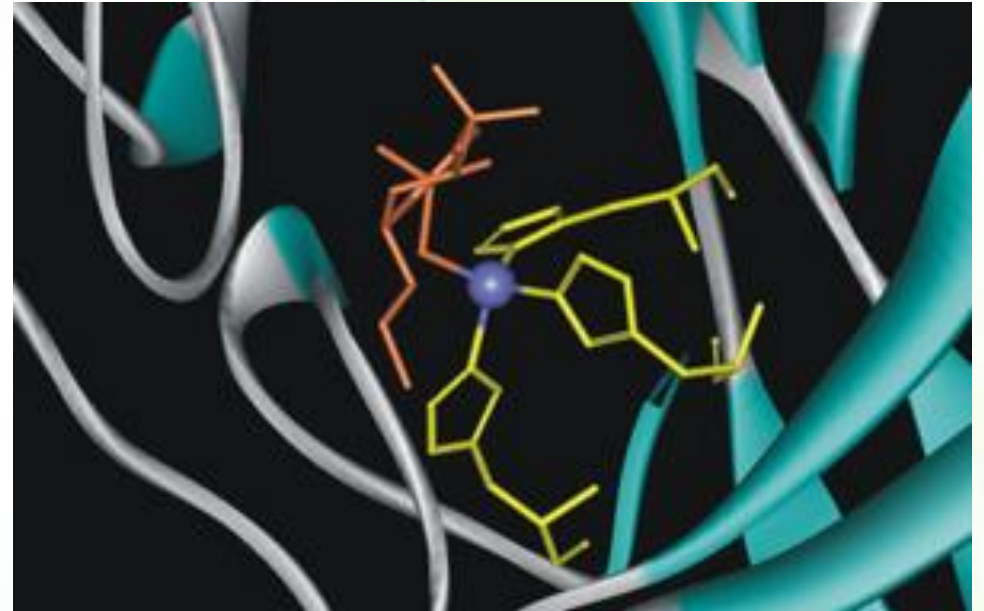
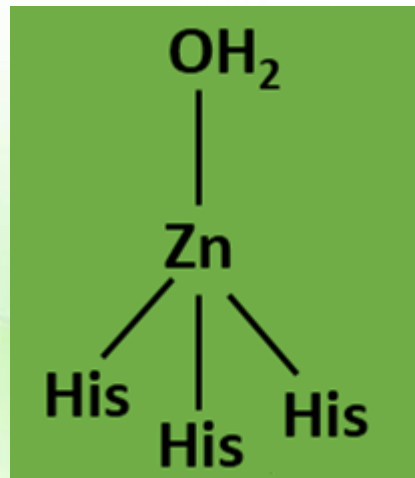
- Although CO_2 hydration and HCO_3^- dehydration occur spontaneously, almost all organisms contain carbonic anhydrases, because they carry out rapid processes such as respiration.
- Mutations in carbonic anhydrases have been found to cause osteopetrosis (excessive formation of dense bones accompanied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



Zn binding to the enzyme



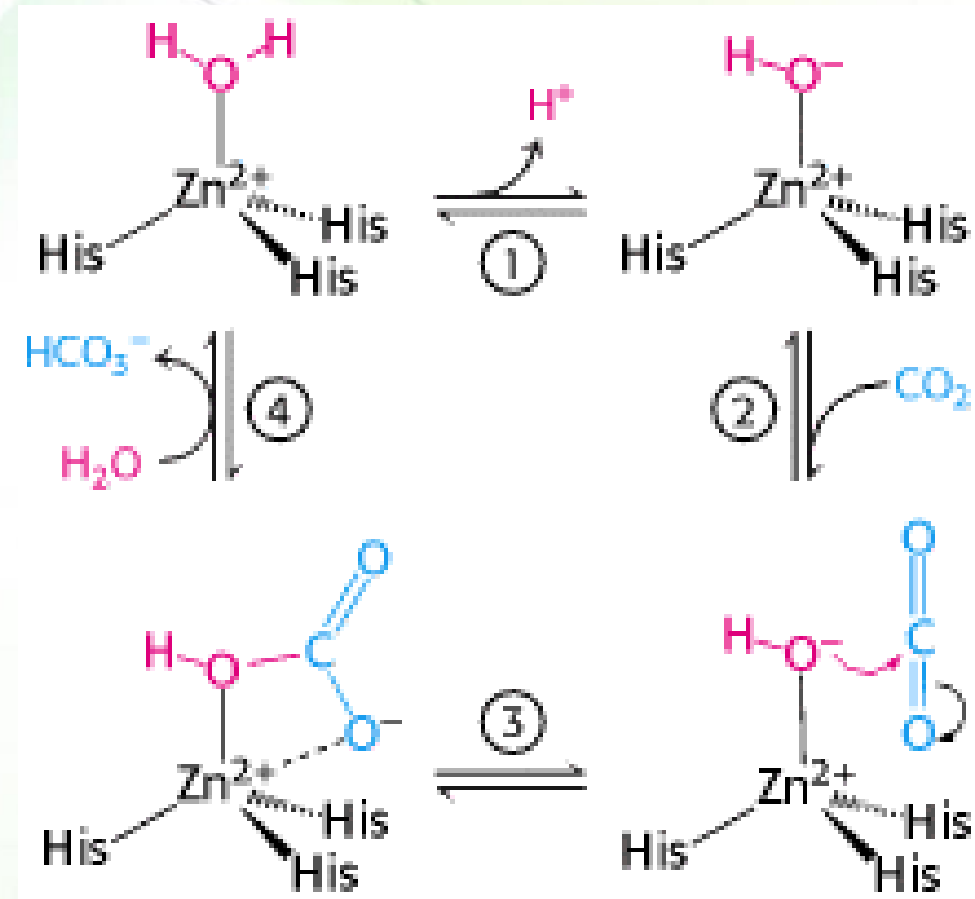
- Zinc is found only in the +2 state in biological systems.
- In carbonic anhydrase, a zinc atom is bound to three imidazole rings of three histidine residues and an additional site is occupied by a water molecule.



Mechanism of action



- Zinc facilitates the release of a proton from H_2O generating a hydroxide ion.
- The CO_2 substrate binds to the enzyme's active site and is positioned to react with the hydroxide ion.
- The hydroxide ion attacks CO_2 converting it into a bicarbonate ion.
- The catalytic site is regenerated with the release of the bicarbonate ion and the binding of another H_2O .



Catalytic Metals



- Some metals do not participate in enzyme catalysis directly but facilitate a reaction.
- The histidine of alcohol dehydrogenase pulls a proton off the active site's serine
- The serine pulls off the proton of the substrate's OH^- group, leaving the oxygen with a negative charge.
- The charge is stabilized by zinc.
- A hydride is then transferred to NAD^+ .

