Enzymes IV

Vitamins and cofactors

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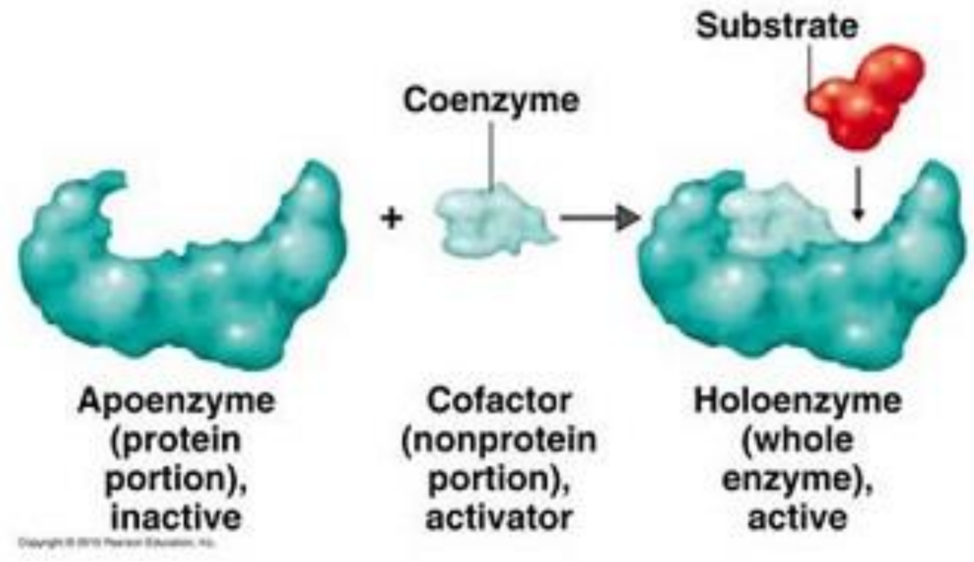
Water-soluble vitamins

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)

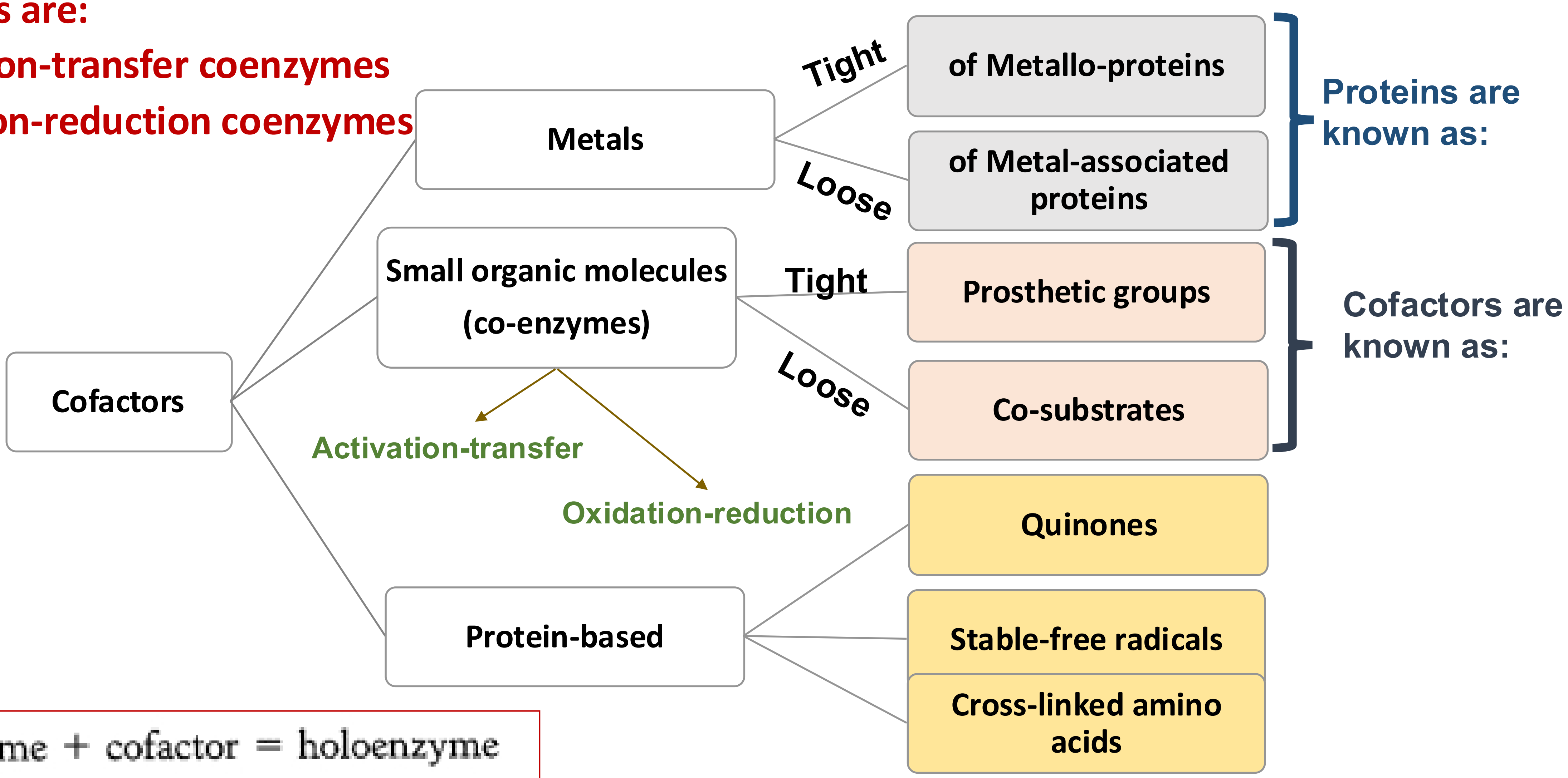


Classification of cofactors

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

Activation-transfer coenzymes
Oxidation-reduction coenzymes

Activation-transfer coenzymes

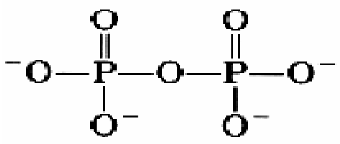
Activation-transfer coenzymes

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

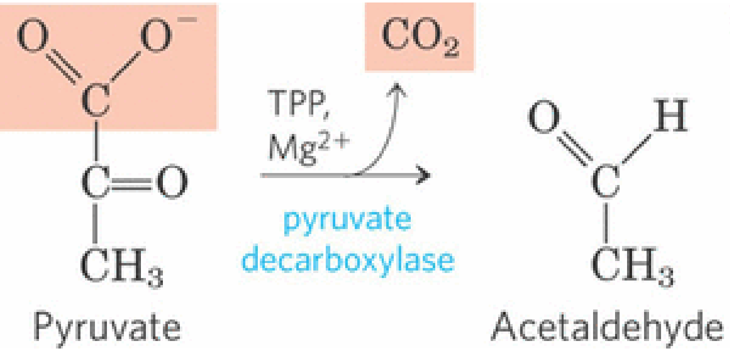
- Characteristics:
  - Two chemical groups in the coenzyme:
    - A functional group that forms a covalent bond with the substrate.
    - A binding group that binds tightly to the enzyme.
  - The enzyme specifies the substrate & the catalytic mechanism.

Thiamine pyrophosphate, TPP

Vitamin B1

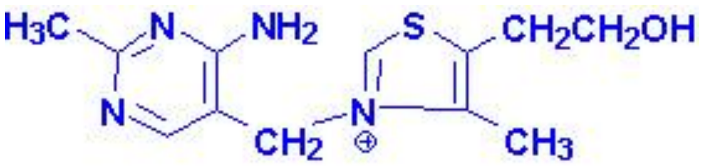


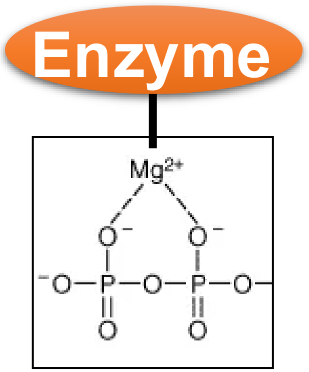
- Thiamine (vitamin B1) is converted to its active form, thiamine pyrophosphate (TPP).
- It is involved in **decarboxylation** reactions.
- The negatively charged oxygen atoms of the pyrophosphate tightly link TPP to the enzyme via Mg^{2+} through chelation.
- The reactive thiamin carbon binds to the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



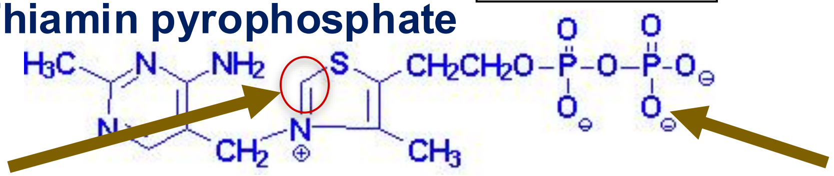
Chelation: a chemical reaction where a metal ion is linked to non-metal groups by two or more bonds within the same molecule

Thiamin





Thiamin pyrophosphate



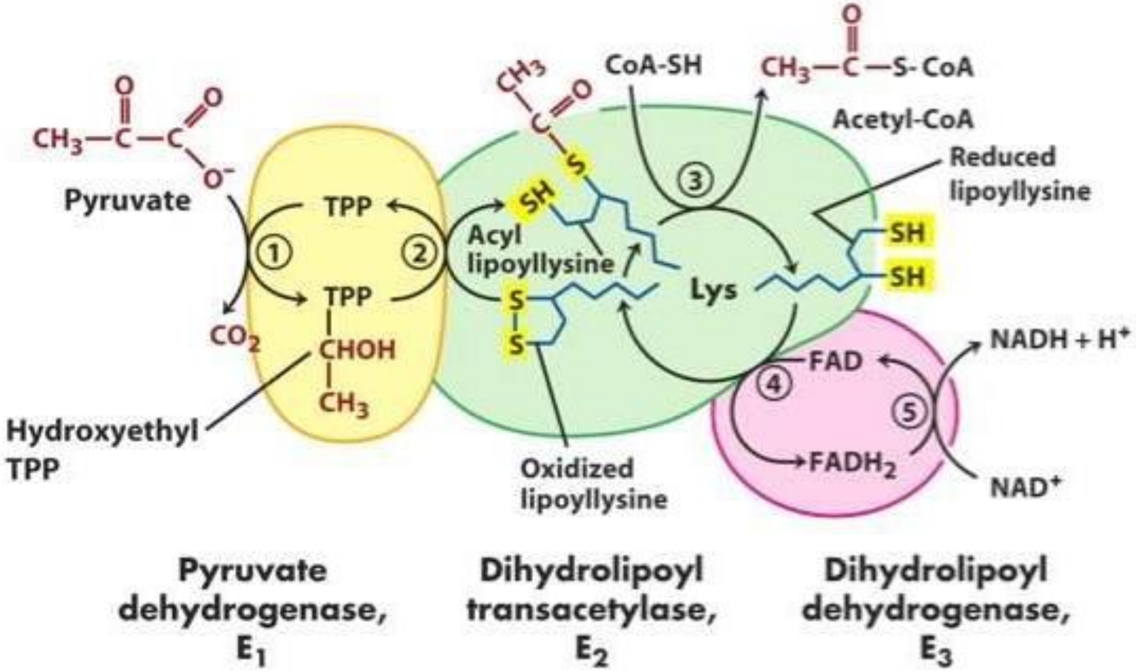
Reactive group

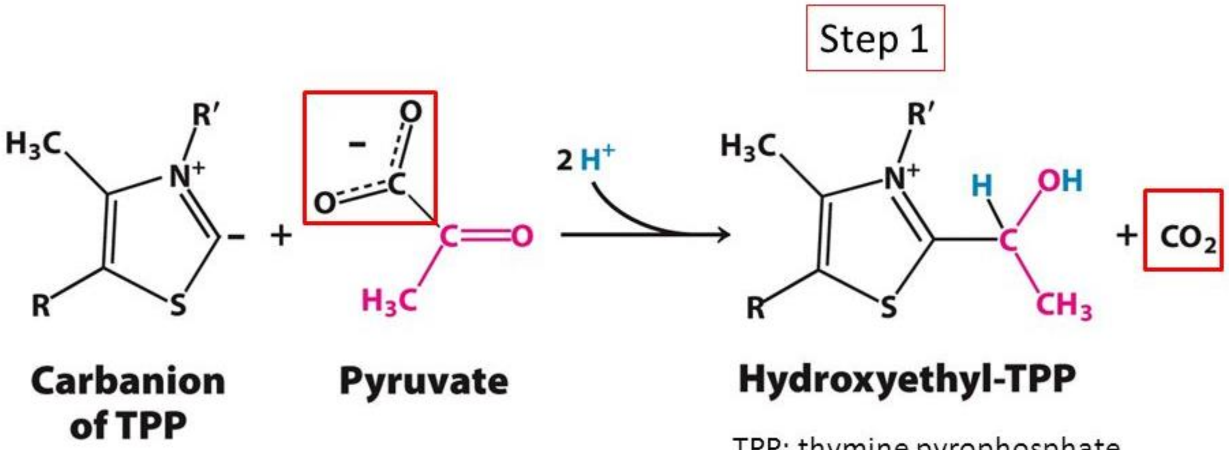
Binding group

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

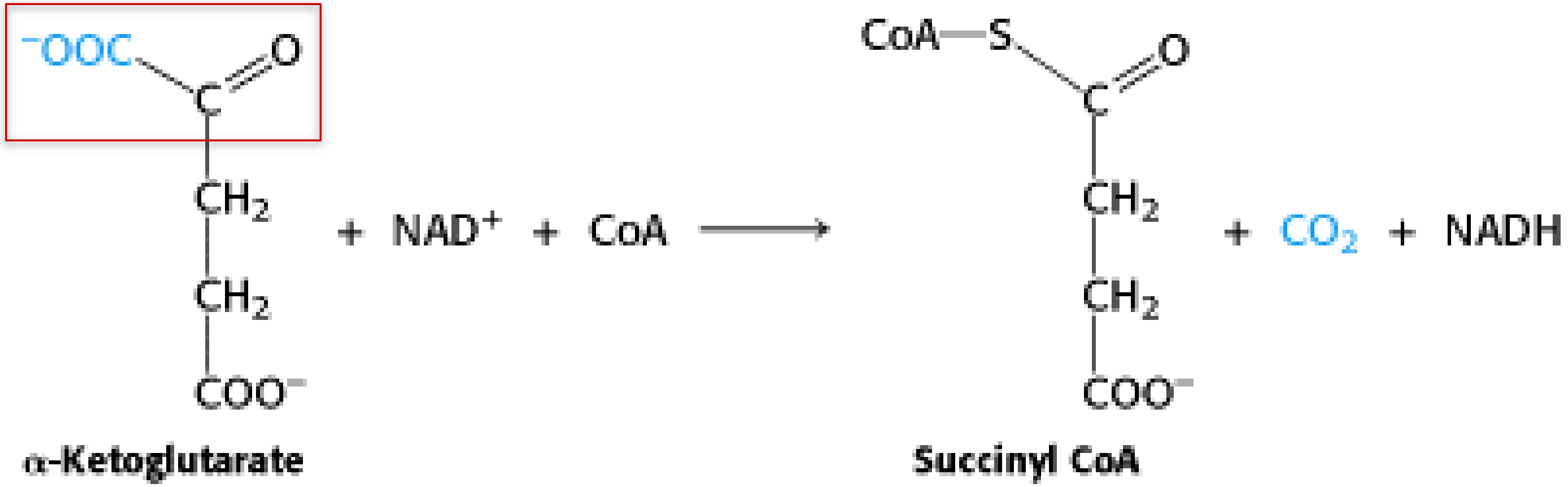




TPP: thymine pyrophosphate

α-ketoglutarate dehydrogenase

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



Coenzyme A (CoA)

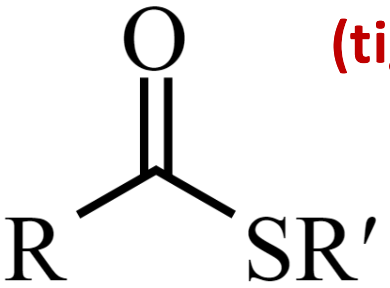
Vitamin B5

- Source: pantothenate (vitamin B5): made of al 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.

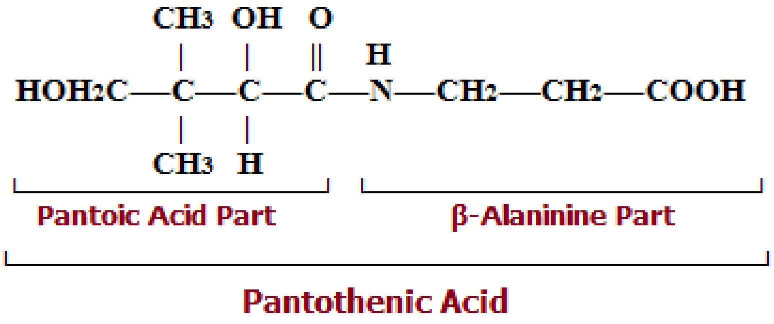
Functional group: sulfhydryl group (nucleophile)

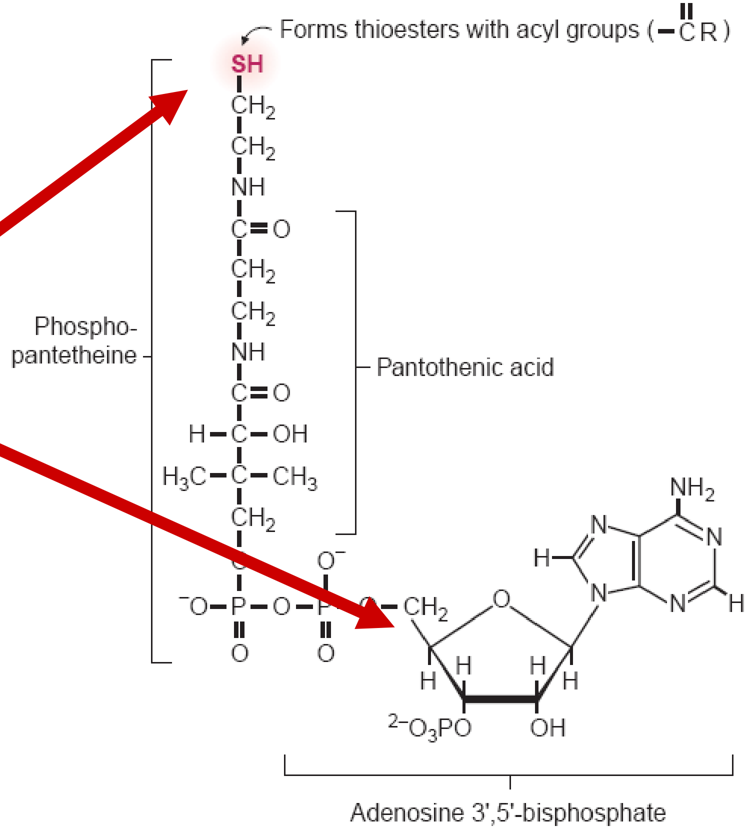
Binding group: adenosine 3’,5’-bisphosphate

(tight & reversible)



Acyl thioester



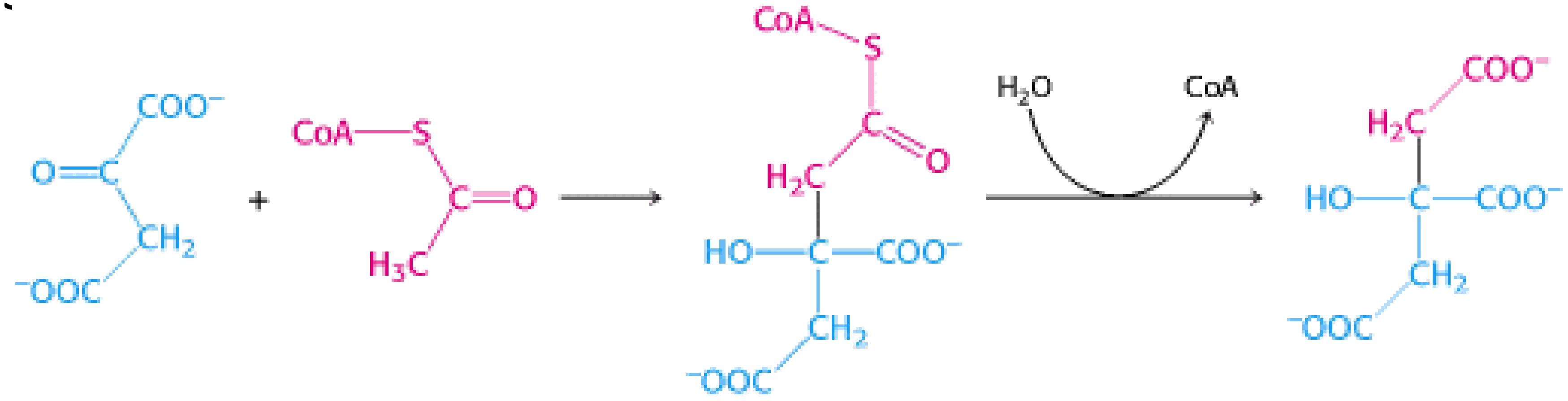


Examples of enzymes

- 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 (a transferase)

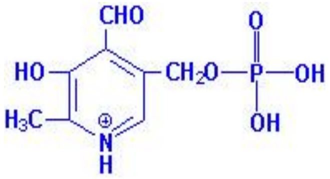


Oxaloacetate   Acetyl CoA   Citryl CoA   Citrate

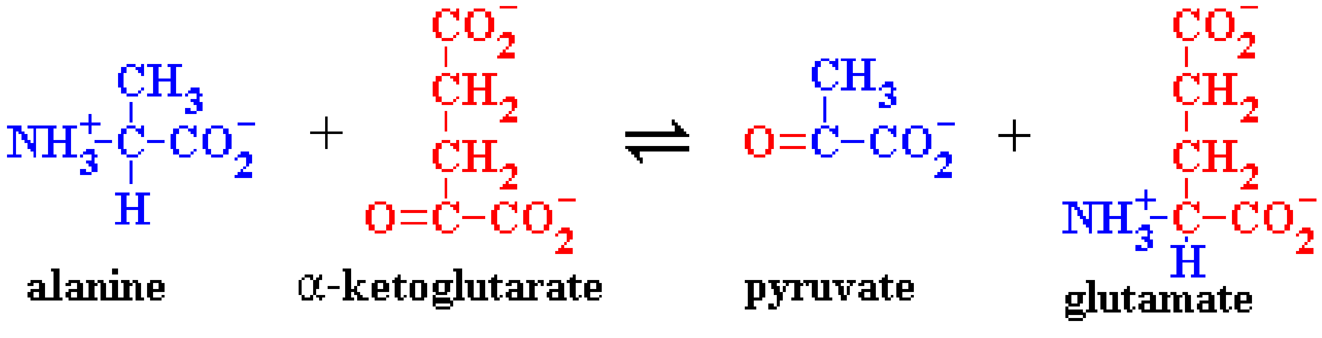
Pyridoxal phosphate

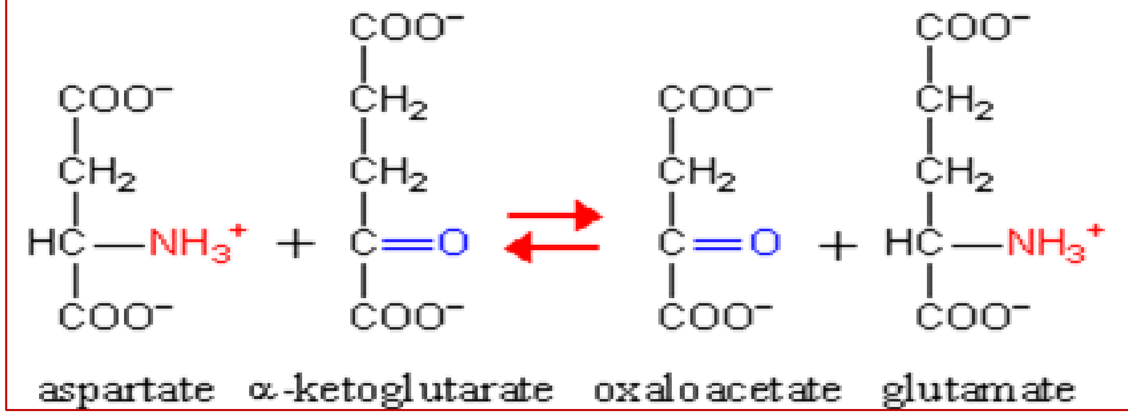
Vitamin B6

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

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

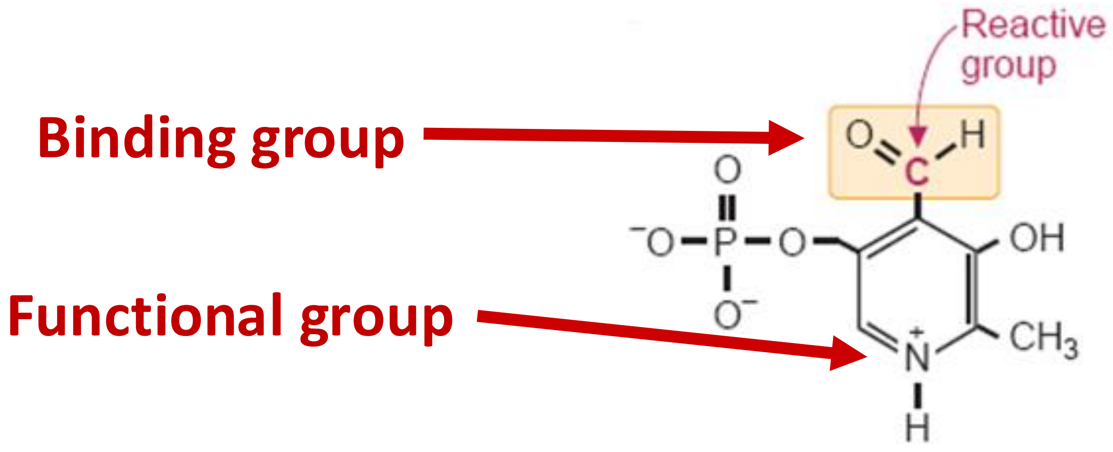
$$\text{Amino acid}_1 + \alpha\text{-ketoacid}_2 \rightleftharpoons \text{amino acid}_2 + \alpha\text{-ketoacid}_1$$

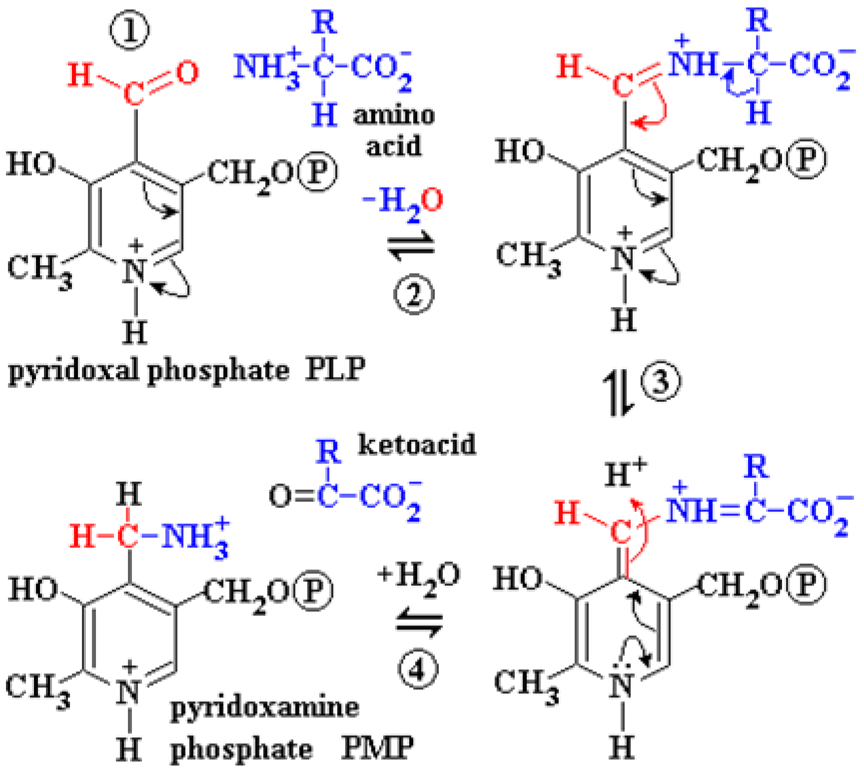




Mechanism of action

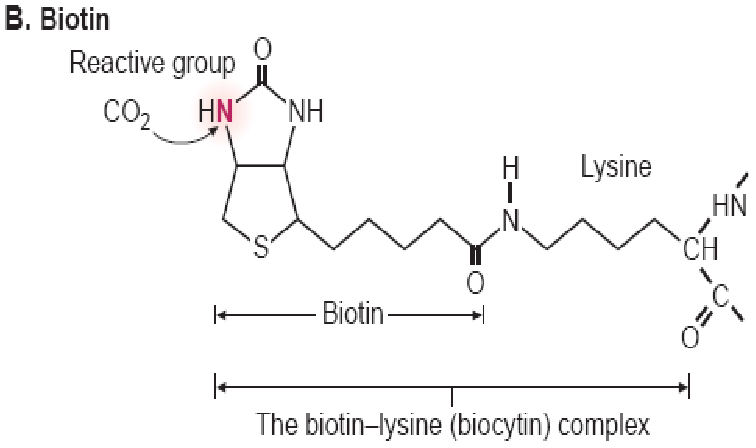
- The aldehyde of PLP covalently bonds with the amino group, the ring nitrogen withdraws its electrons, and the amino group is sequestered releasing a keto acid.
- A keto acid then enters the active site and the amino group is given to in a reverse reaction.
    - Binding and functional groups are within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





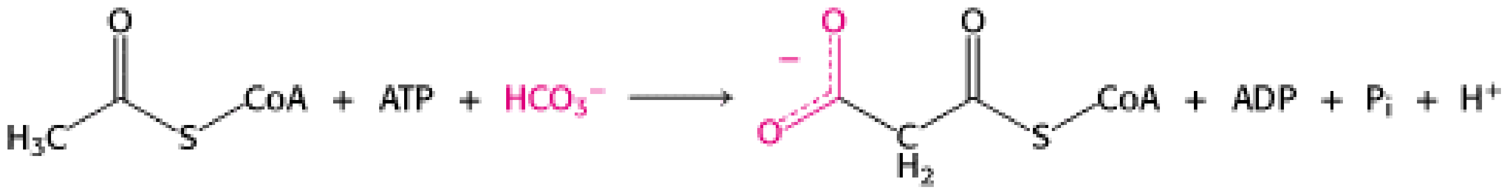
Biotin

Vitamin B7



- It is required for **carboxylation** reactions.
  - covalently bound to the enzyme through 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)

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



Acetyl CoA **Malonyl CoA**

Oxidation-reduction coenzymes

Oxidation-reduction coenzymes

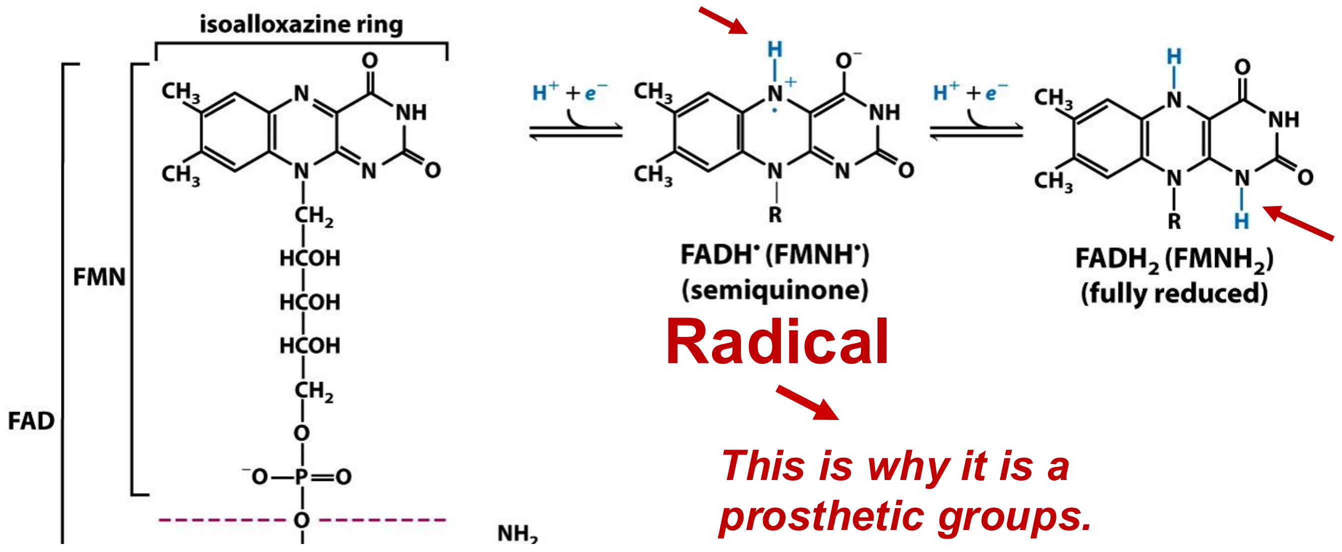
H^{+} hydrogen ion

$H{:}^{-}$ hydride ion

- A number of coenzymes work within oxidoreductases.
- Each coenzyme has a unique functional group that accepts and donates electrons in the form of hydride ions, hydrogen atoms, or 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

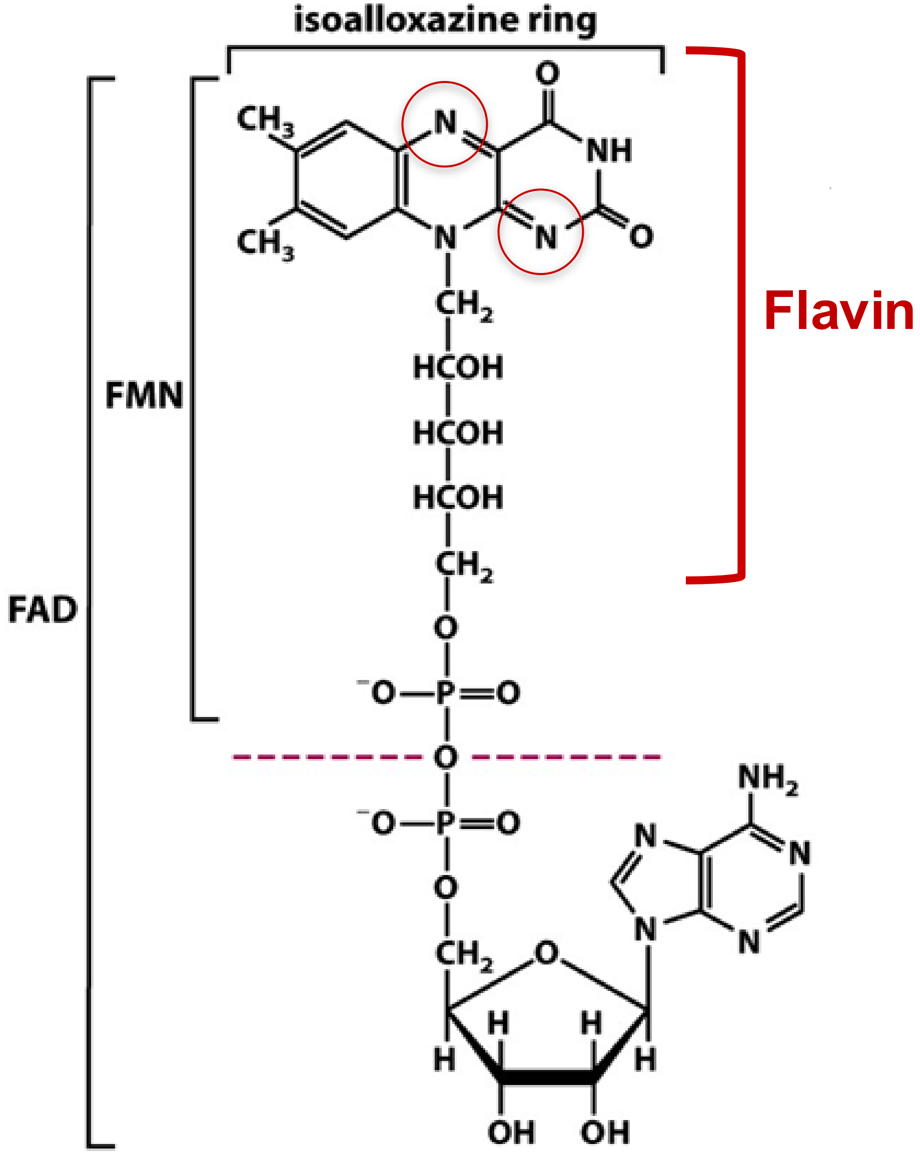
FAD and FMN

- 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.



Radical

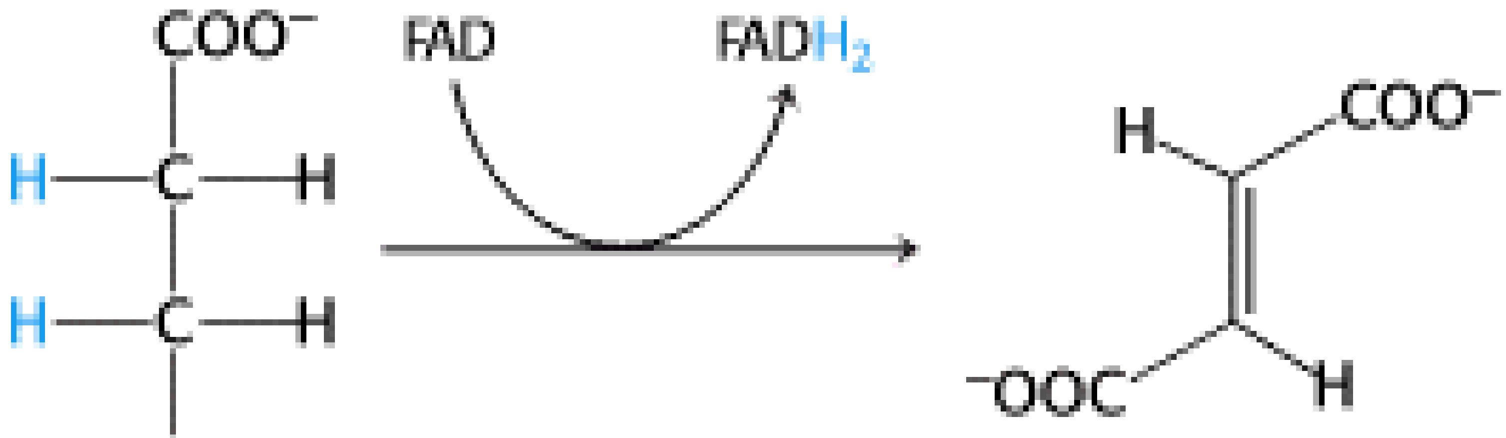
This is why it is a prosthetic groups.



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

Succinate dehydrogenase

- Oxidation of succinate into fumarate by succinate dehydrogenase

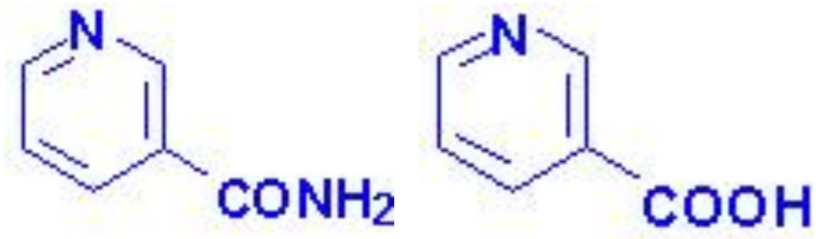


Succinate

Fumarate

NAD$^+$ and NADP$^+$

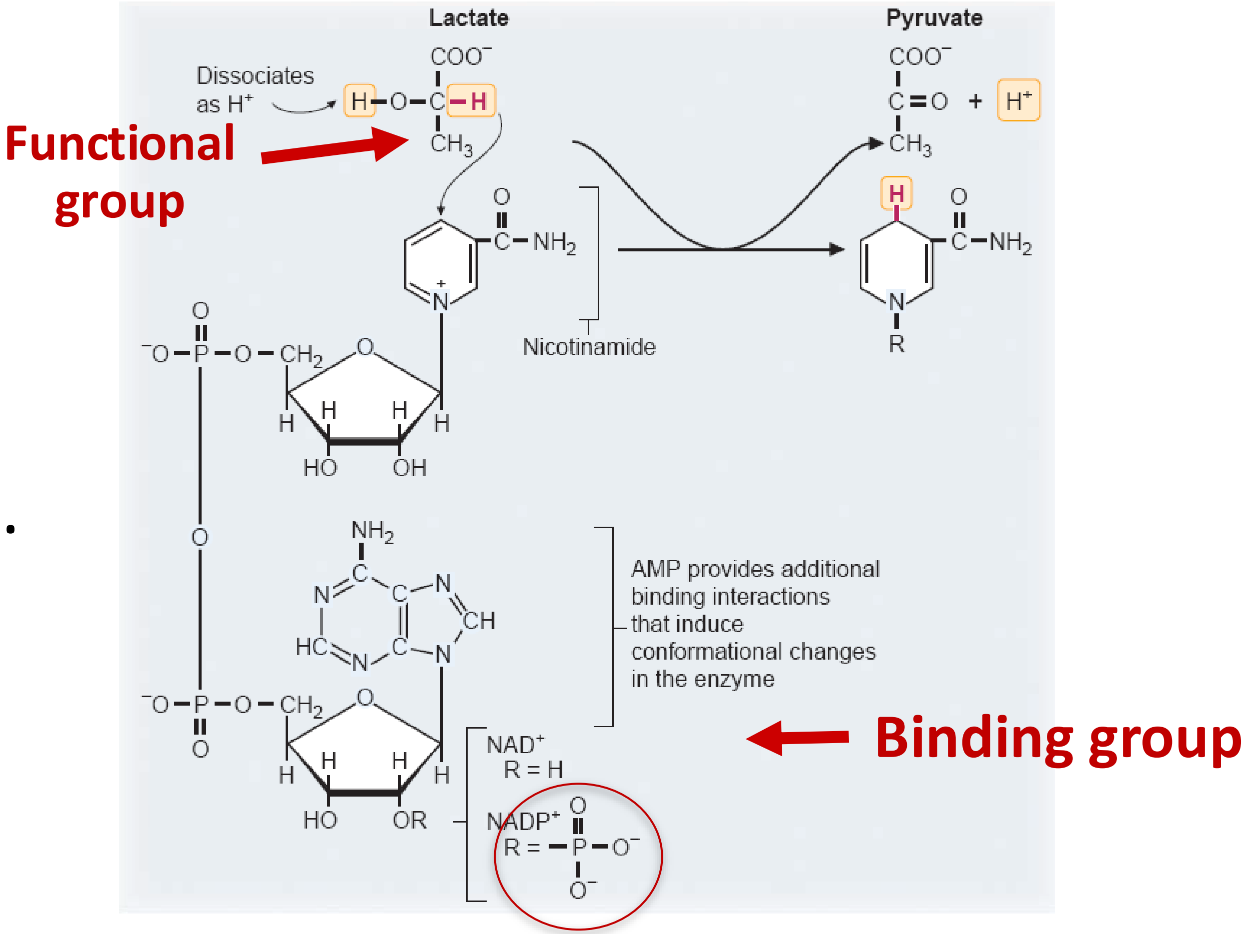
- Precursor of nicotinamide adenine dinucleotide (NAD$^+$) and nicotinamide adenine dinucleotide phosphate (NADP$^+$) is niacin (vitamin B3).



- These are cosubstrates for numerous dehydrogenases.

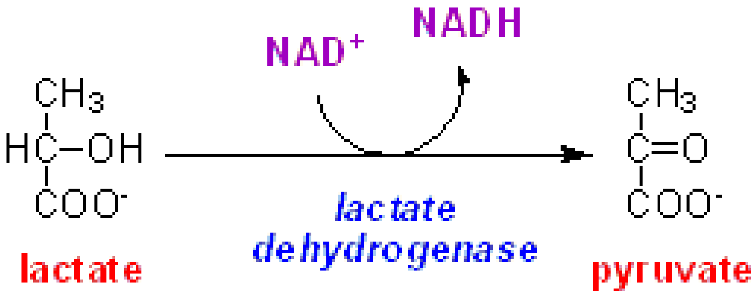
Mechanism of action

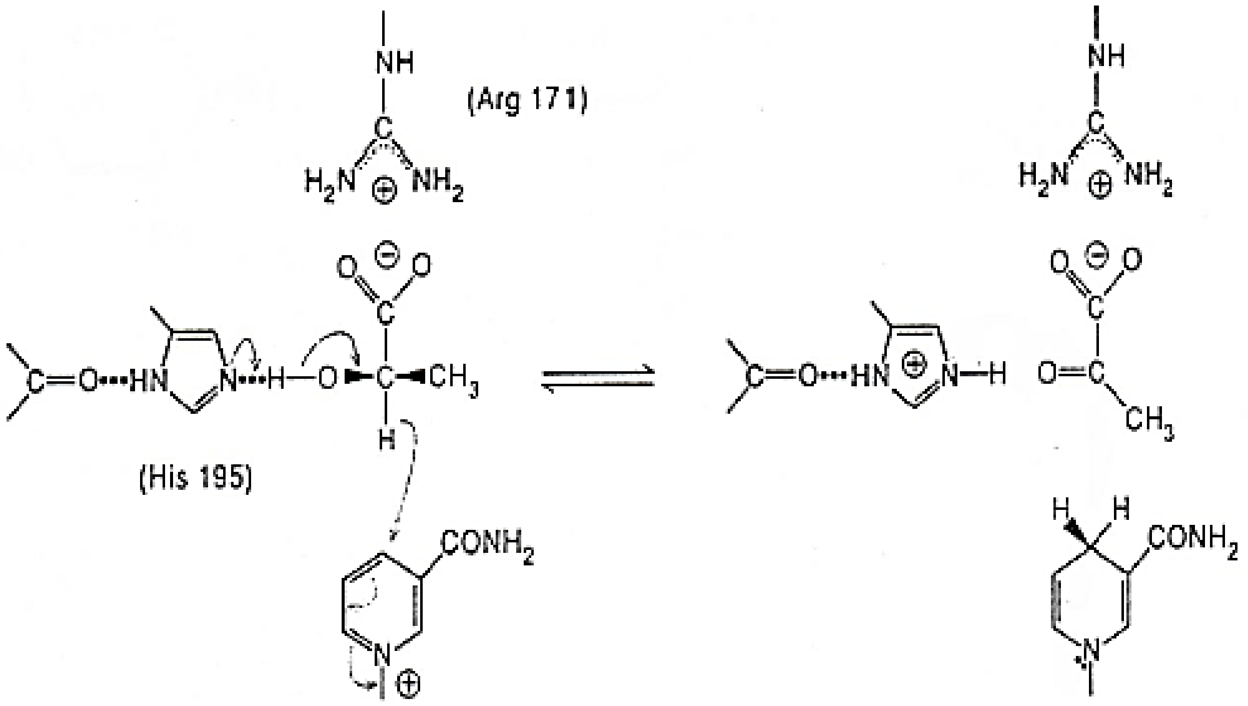
- The functional group (C opposite to N) accepts a hydride ion from the substrate, dissociates, and a keto group (CO) is formed.
- The ADP portion of the molecule binds tightly to the enzyme.
- They are generally involved in the oxidation of alcohols and aldehydes.



Lactate dehydrogenase

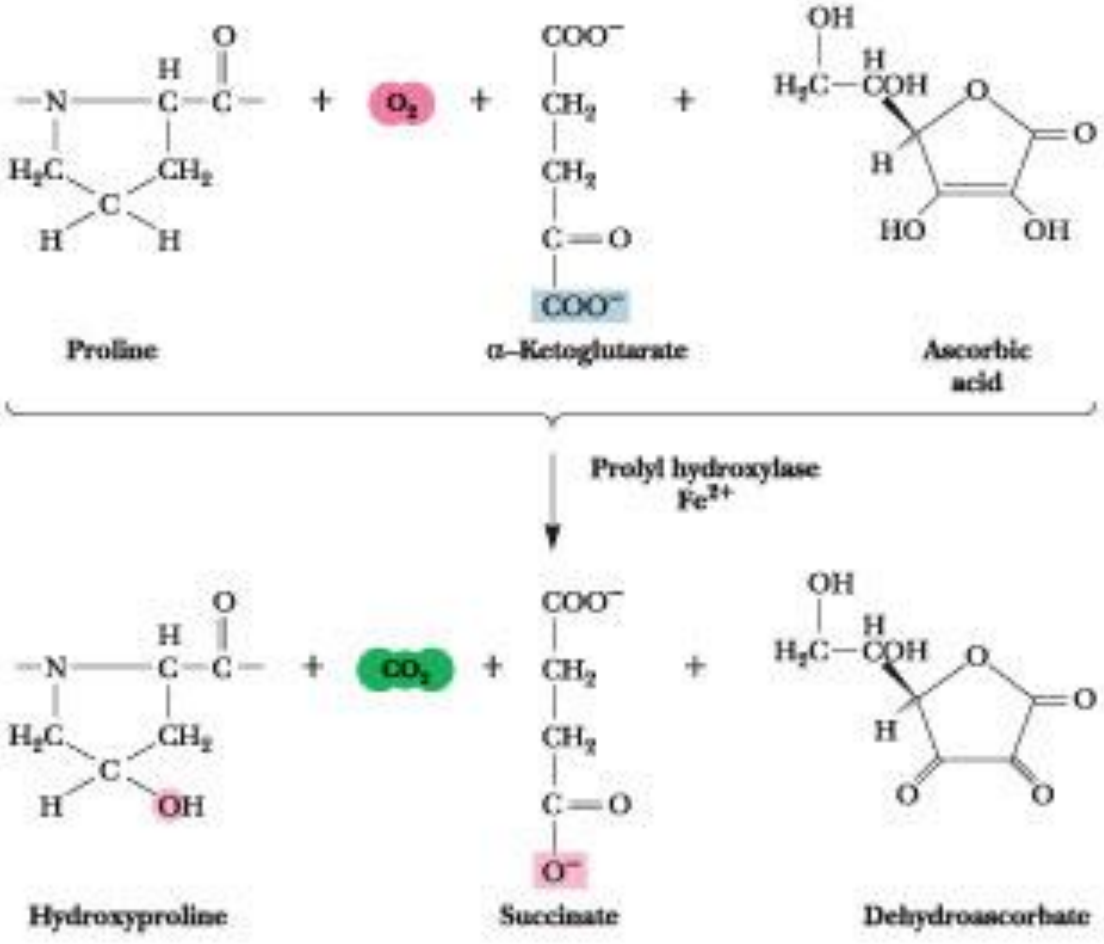
- The enzyme's histidine binds the proton of (-OH) on lactate making it easier for NAD^+ to pull off the other hydrogen with both electrons (a hydride).
- A keto group (-C=O) is formed.





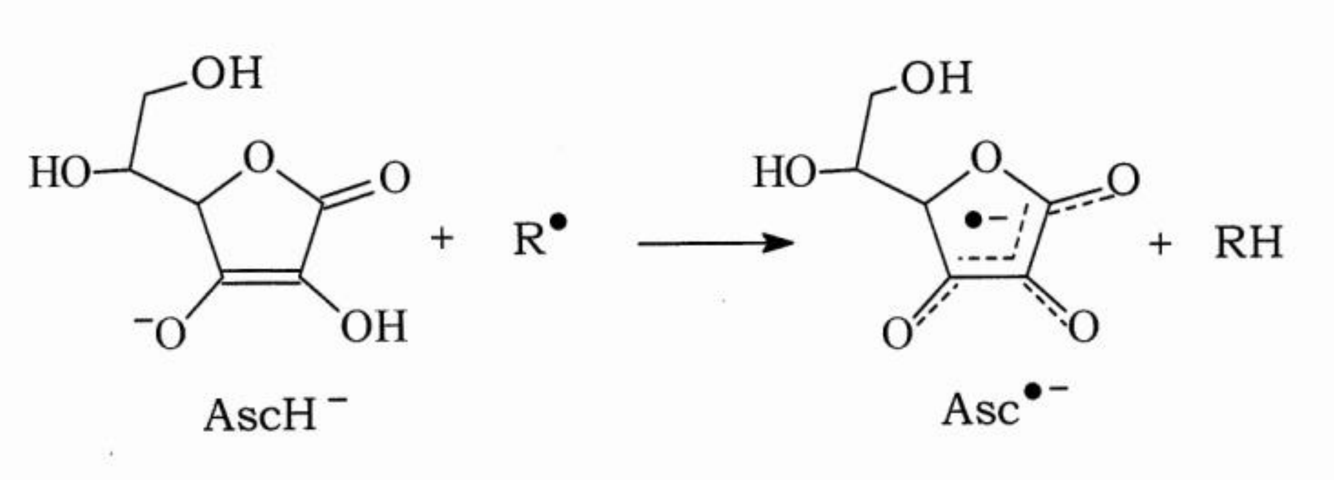
Vitamin C

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



Ascorbate, the anti-oxidant

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

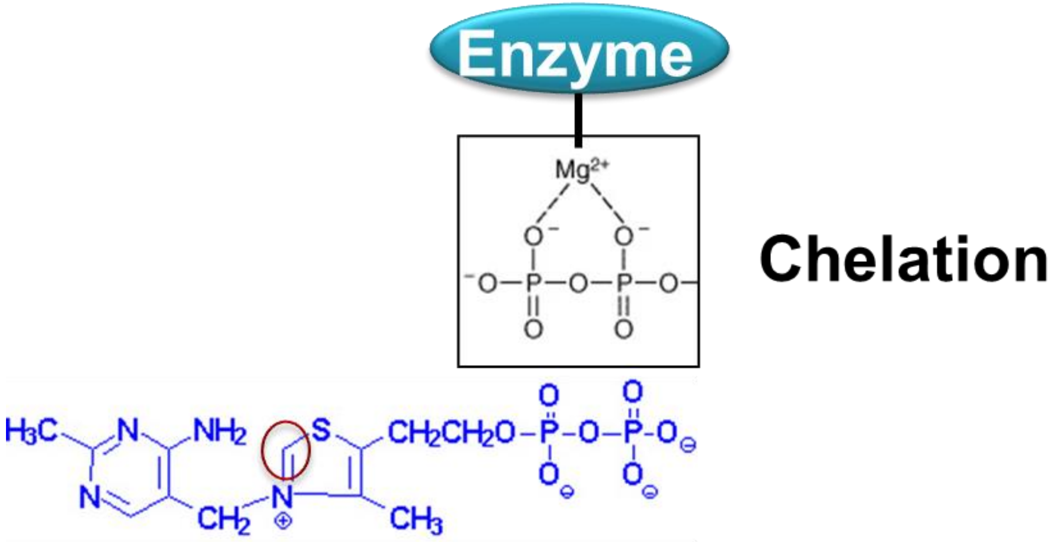
Metal	Enzyme
Zn^{2+}	Carbonic anhydrase Carboxypeptidase
Mg^{2+}	Hexokinase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase

- 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.

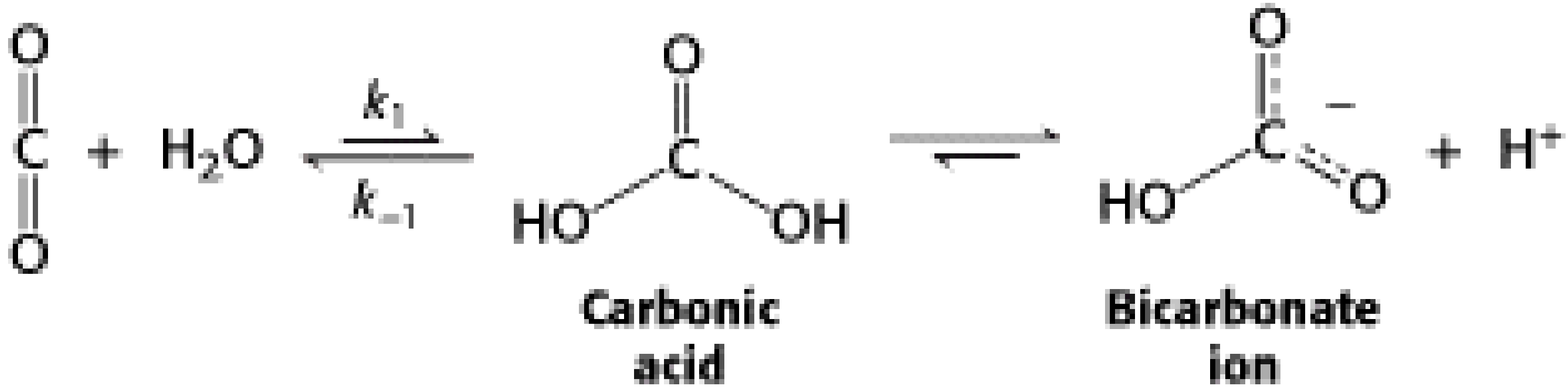
- Mg^{2+} connects the negatively charged phosphate groups of thiamine pyrophosphate to basic amino acids in the enzyme.
- The phosphate groups of ATP are usually bound to enzymes through Mg^{2+} chelation.



Thiamin pyrophosphate

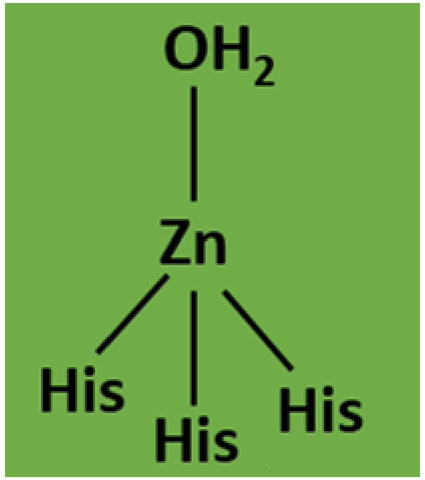
Carbonic anhydrases

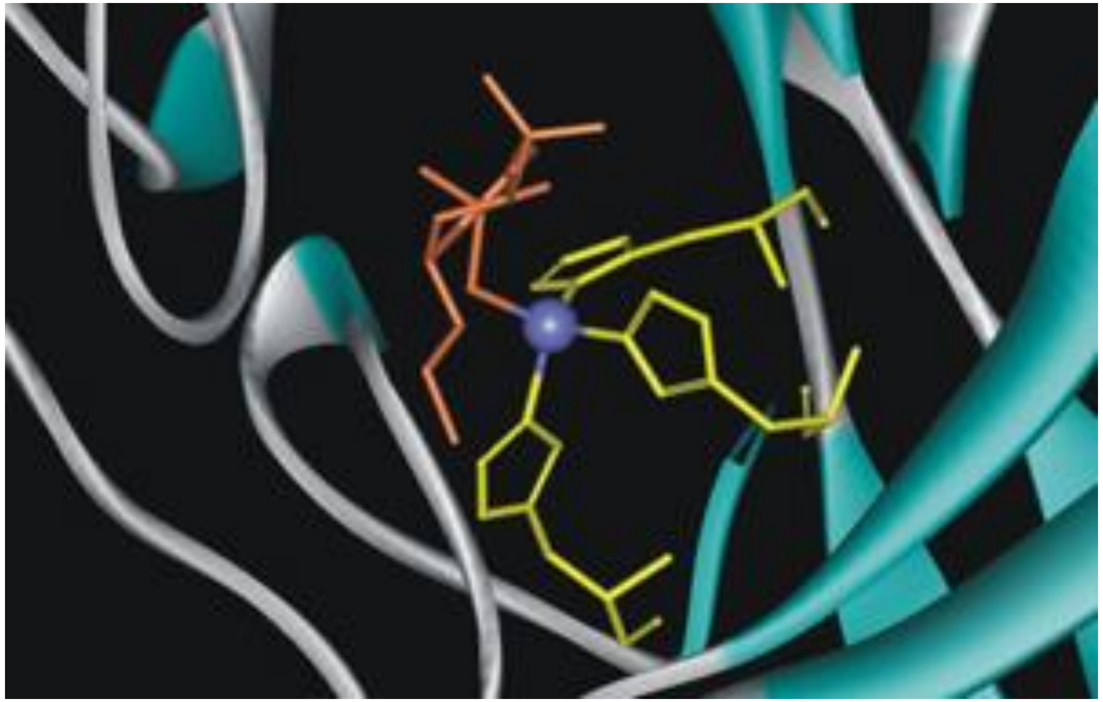
- Although CO_2 hydration and HCO_3^- dehydration occur spontaneously, almost all organisms contain carbonic anhydrases, because they carry out rapid processes such as respiration.



Zinc binding to the enzyme

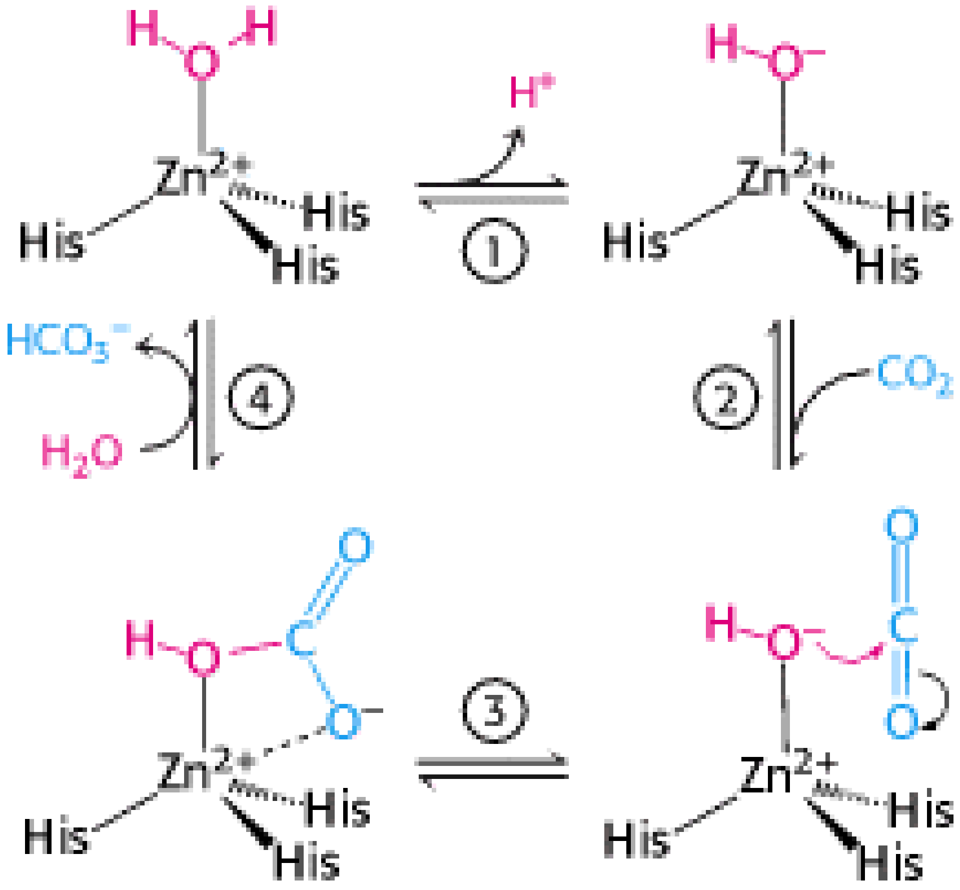
- Zinc is found only in the +2 state in biological systems.
- In carbonic anhydrase, a zinc atom is bound to three imidazole rings of three histidine residues, and a fourth site is occupied by a water molecule.





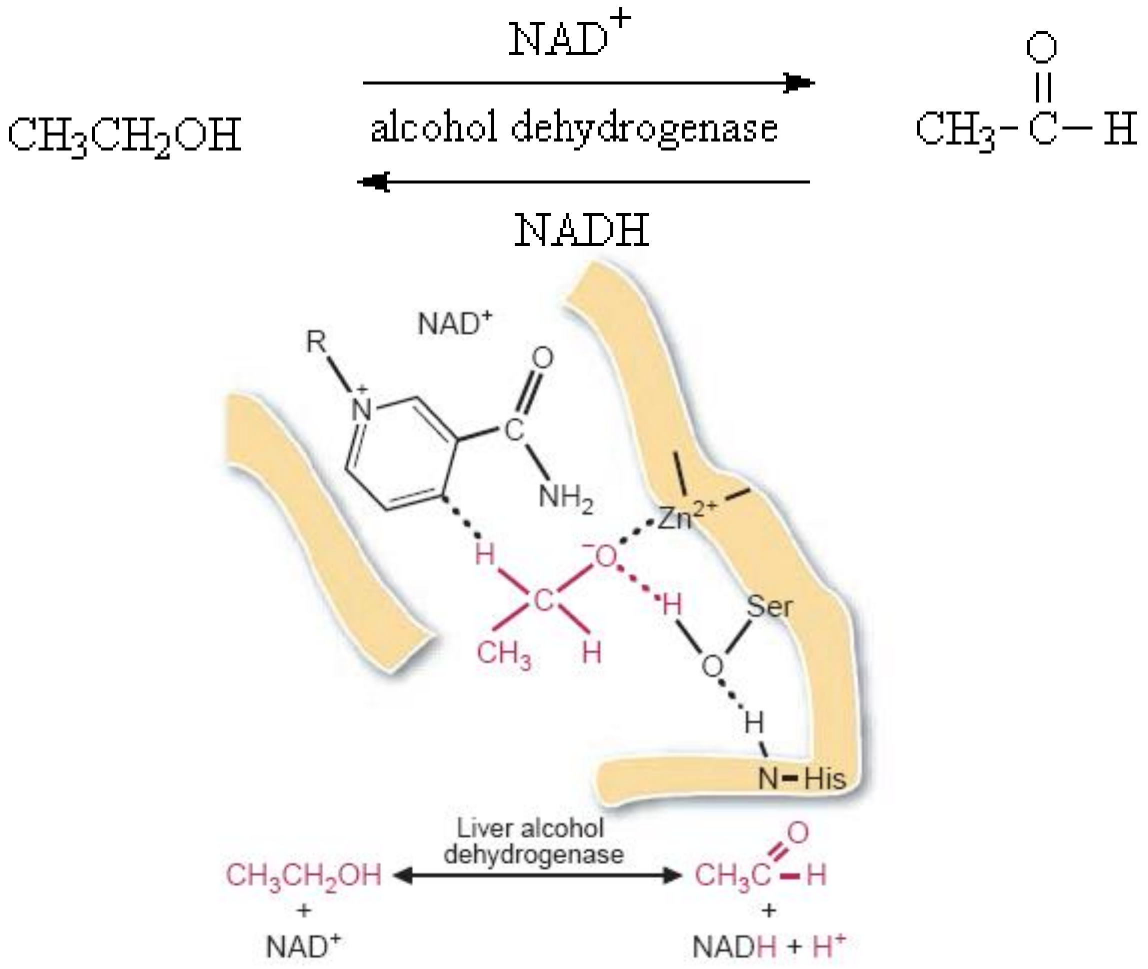
Mechanism of action

- Zinc facilitates the release of a proton from H_2O generating a hydroxide ion.
- The CO_2 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

- 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 leaving the oxygen with a negative charge.
- The charge is stabilized by the zinc.
- An aldehyde is formed and released.
- The proton is transferred as a hydride to NAD^+ forming NADH.



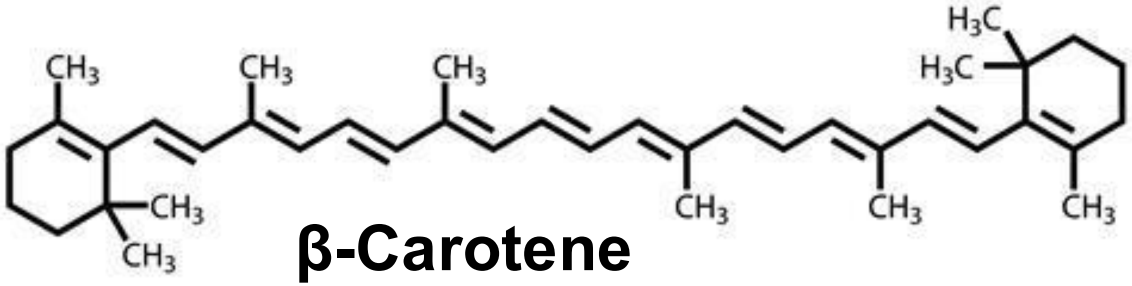
Lipid-soluble vitamins

Lipid-soluble vitamins

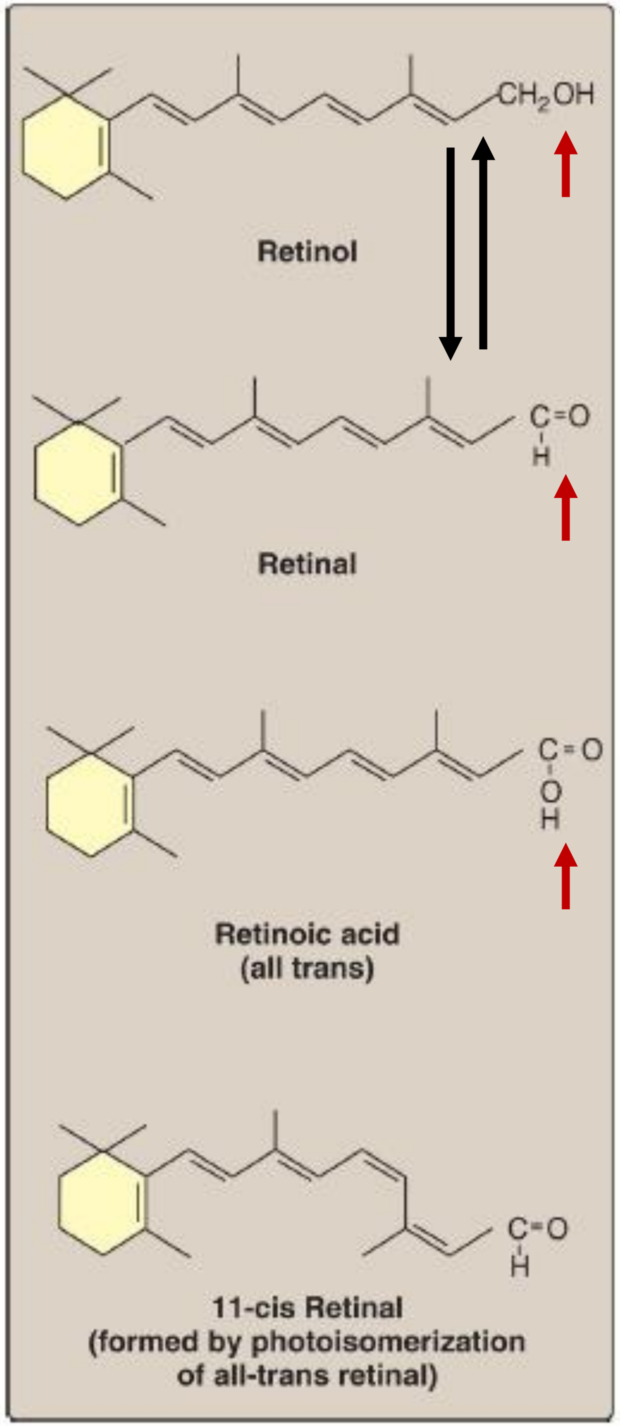
Vitamin	Main function	Deficiency
A	Roles in vision, growth, reproduction	Night blindness, cornea damage
D	Regulation of Ca^{+2} & phosphate metabolism	Rickets (children), Osteomalacia (adults)
E	Antioxidant	RBCs fragility
K	Blood coagulation	Subdermal hemorrhaging

All dietary fat-soluble vitamins are carried in chylomicrons.

Vitamin A



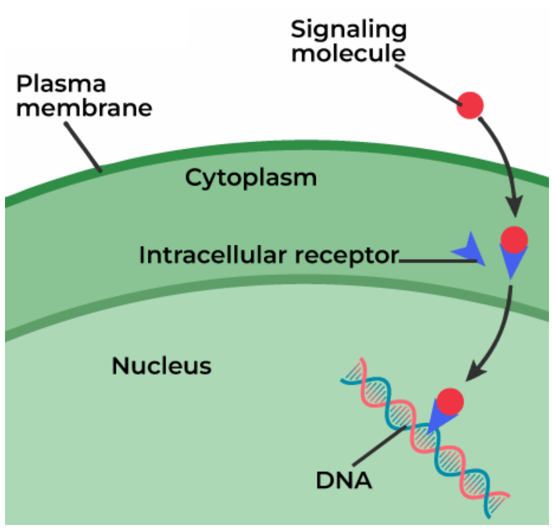
- The retinoids are forms of vitamin A.
- They are derived from β-Carotene, which is cleaved in the intestines to yield 2 molecules of retinal.
- Retinal and retinol are inter-convertible.
- Retinoic acid mediates most of the actions of the retinoids, except for vision and spermatogenesis.

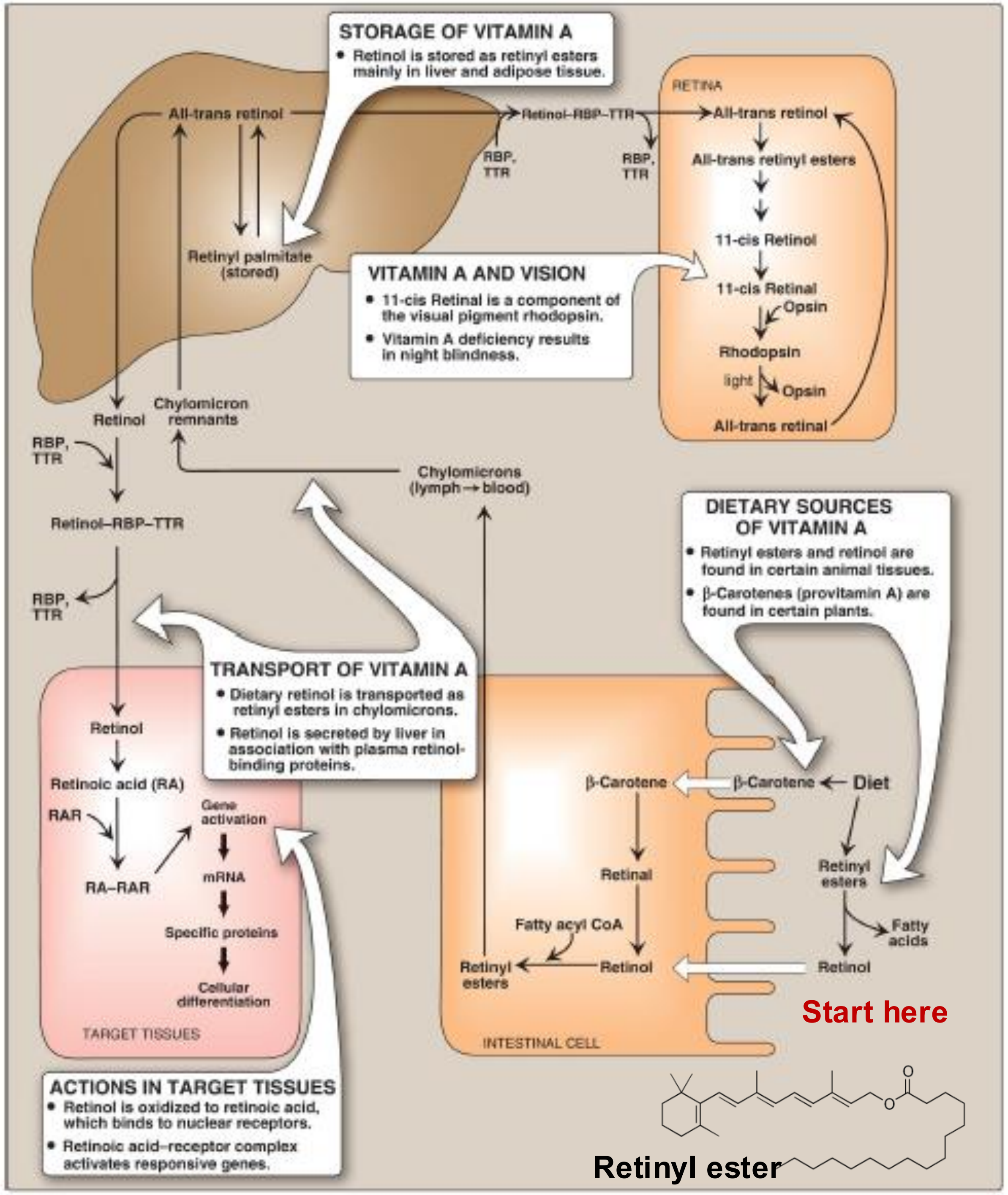


Absorption & transport

- Intestinal cells absorb β-Carotene and retinol (from retinyl esters), then re-esterified with a fatty acid.
- Retinal esters are transported to the liver via chylomicrons for storage.
- Retinol is released and binds the plasma retinol-binding protein (RBP) complexed with transthyretin for transport into target cells.
- In the retina, retinol is oxidized to retinal, which is involved in vision.

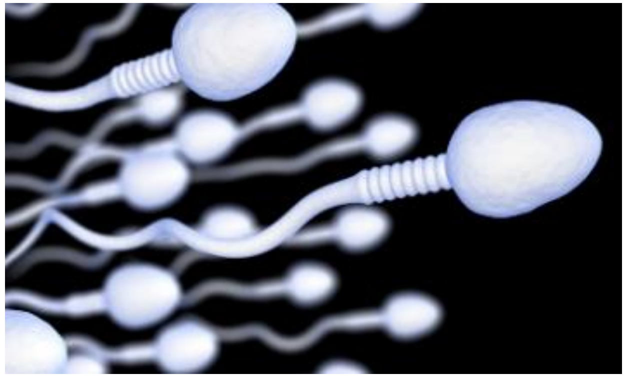
- **In target cells, retinol is oxidized to retinoic acid, which binds to intracellular receptors and the complex binds to DNA elements regulating transcription.**

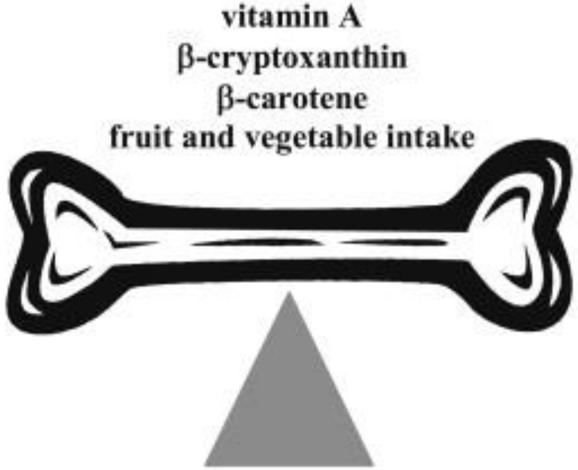




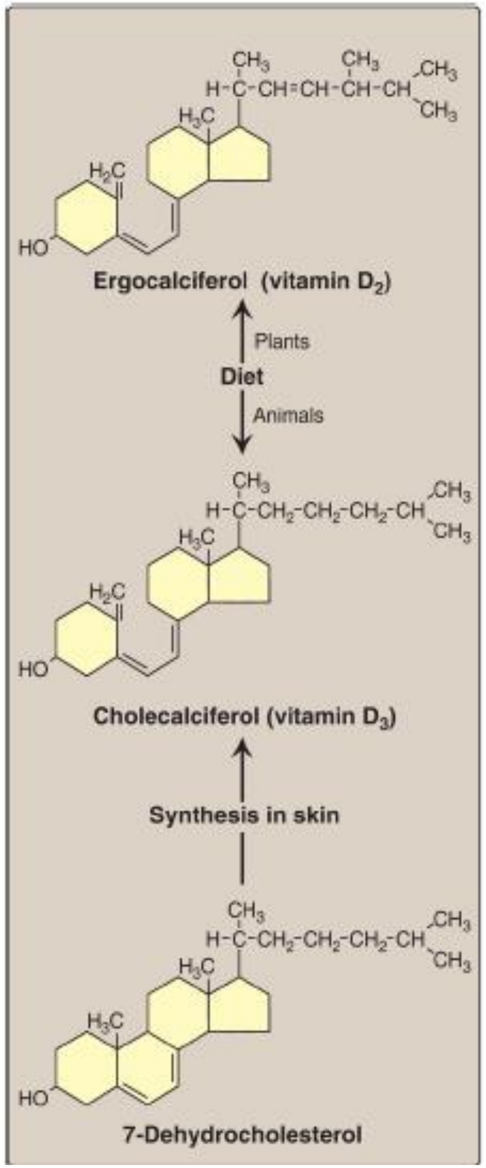
Functions of vitamin A

- Reproduction: Retinol and retinal (not retinoic acid) are essential for spermatogenesis.
- Growth (retinoic acid): Vitamin A is important for the growth and bone development of children.
- Animals given vitamin A only as retinoic acid from birth are blind and sterile.

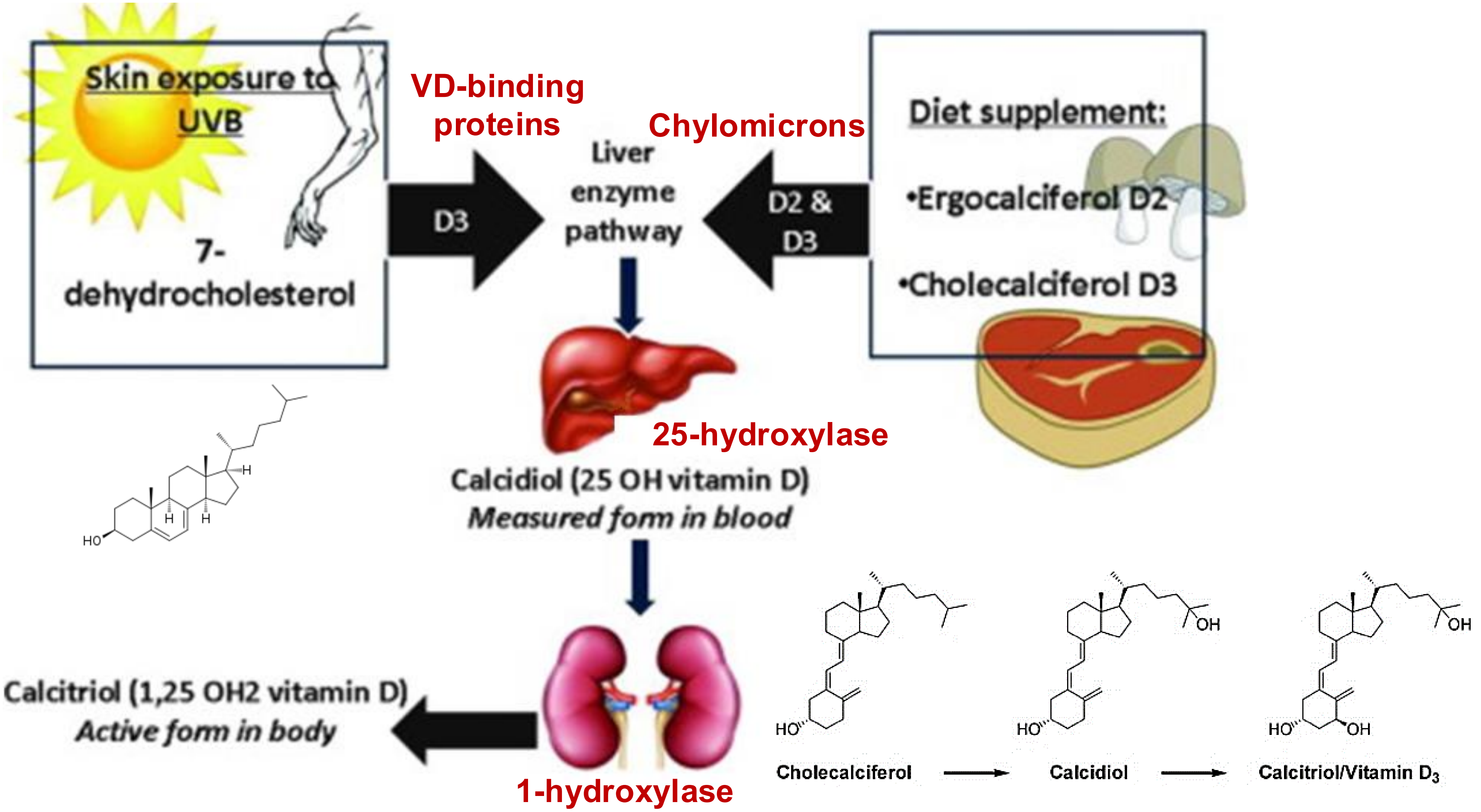




Synthesis of vitamin D

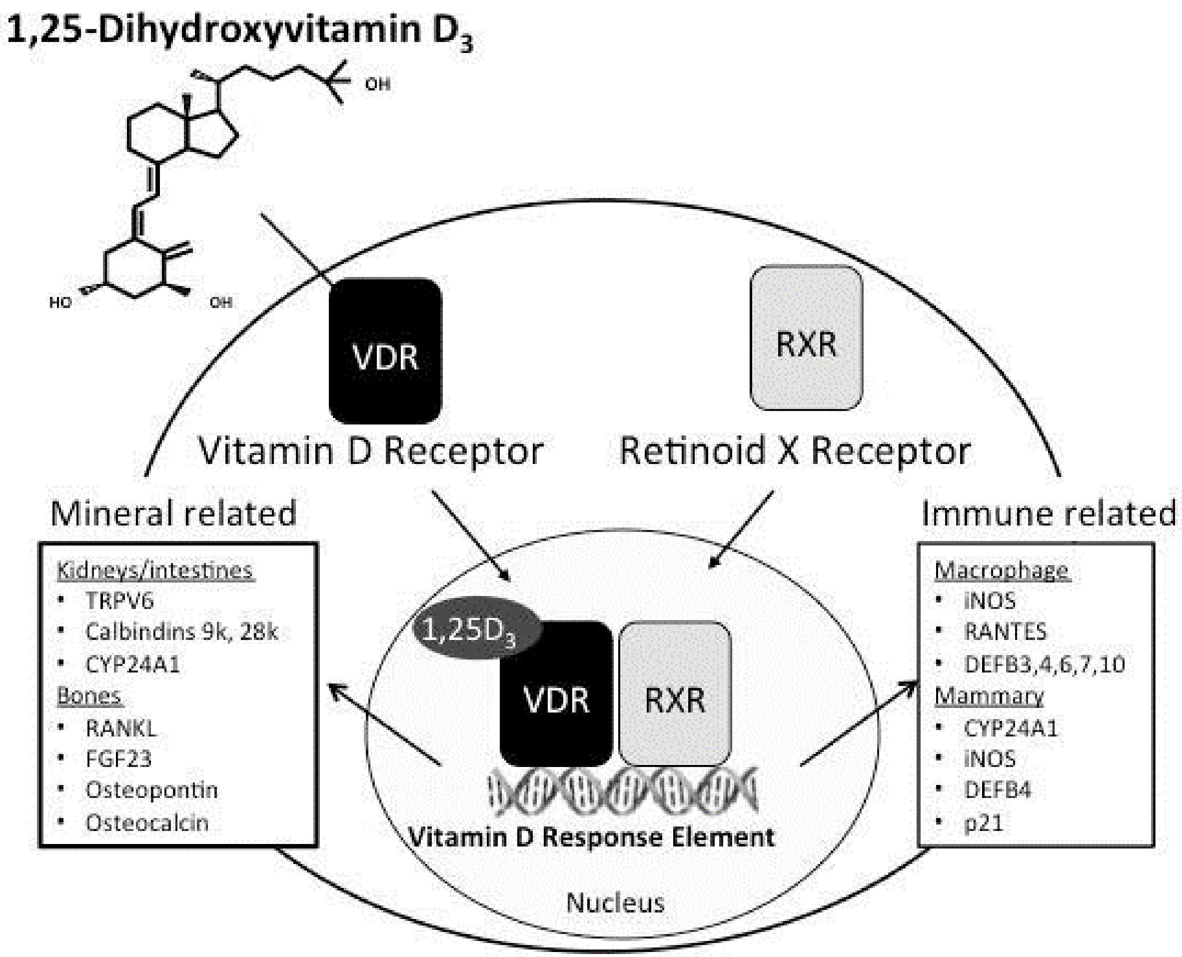


It is a sterol with hormone-like functions.



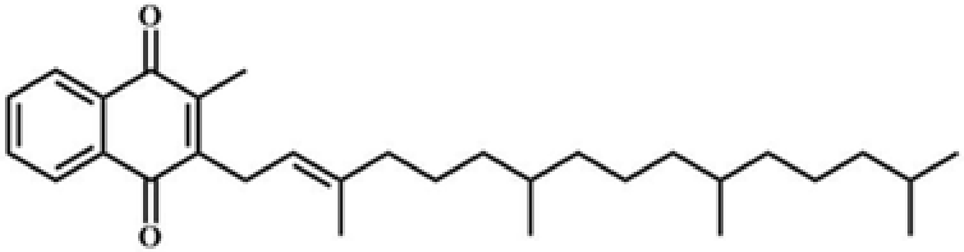
Mechanism of action of vitamin D

- The active molecule: 1,25-dihydroxycholecalciferol (or calcitriol) binds to an intracellular vitamin D receptor, interacts with the DNA of target cells, and regulates gene transcription.
- Functions: regulating the serum levels of calcium and phosphorus.

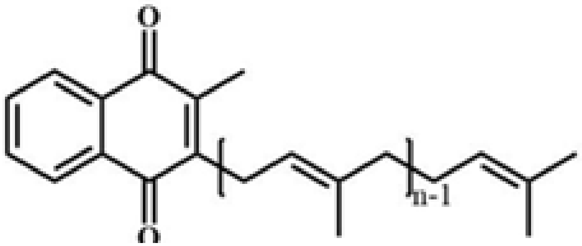


Forms of vitamin K

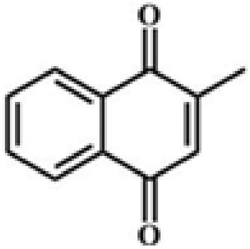
- Vitamin K exists in several active forms:
  - As phylloquinone (or vitamin K1) in plants
  - As menaquinone (or vitamin K2) in intestinal bacteria
  - A synthetic form of vitamin K, menadione, can be converted to K2.



Vitamin K_1
(phylloquinone)



Vitamin K_2
(menaquinone)



Vitamin K_3
(menadione)

- **It can be synthesized by the gut microbiota.**
- **It is important for blood coagulation through the carboxylation of glutamate residues in clotting proteins.**

Vitamin E

- There are different forms of vitamin E, but α-tocopherol is the most active form.
- The primary function is as an antioxidant.