Doctor 022

Biochemistry

Sheet no. 24

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Enzyme 4: Cofactors

Catalytic strategies of enzymes:

Sometimes enzymes can't carry out reactions without the help of small molecules, those small molecules can be metals organic molecules etc.

Those small molecules are known as **Cofactors**, they can either facilitate or participate in the reaction.

Enzymes carry out reactions utilizing different catalytic strategies.

-Some enzymes, such as chymotrypsin, rely on amino acid(has reactant R group) residues within the active site. (So, they don't depend on cofactors).

-Almost all polar amino acids participate in nucleophilic catalysis.

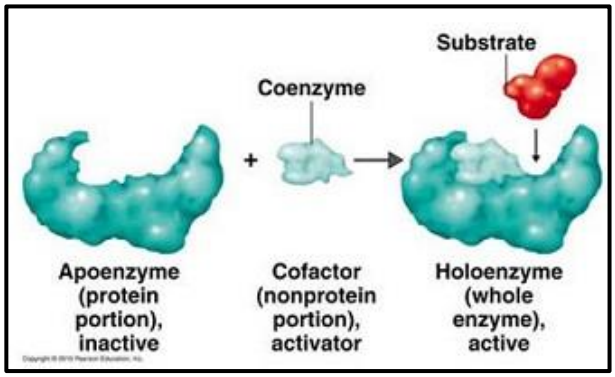
-Ser, Cys, Lys, & His can participate in covalent catalysis

-Histidine: because its Pka is near the physiological pH & acid–base catalysis.

Recall: individual histidine's pka=6 but when it is a part of the polypeptide its PH is near the physiological PH is around 7.4.

-Other enzymes need cofactors (**nonprotein** compounds that participate in the catalytic process).

-Additional info (coenzymes are organic cofactors so, all coenzymes are cofactors but not all cofactors are coenzymes. For example, metal ions are cofactors but not coenzymes)



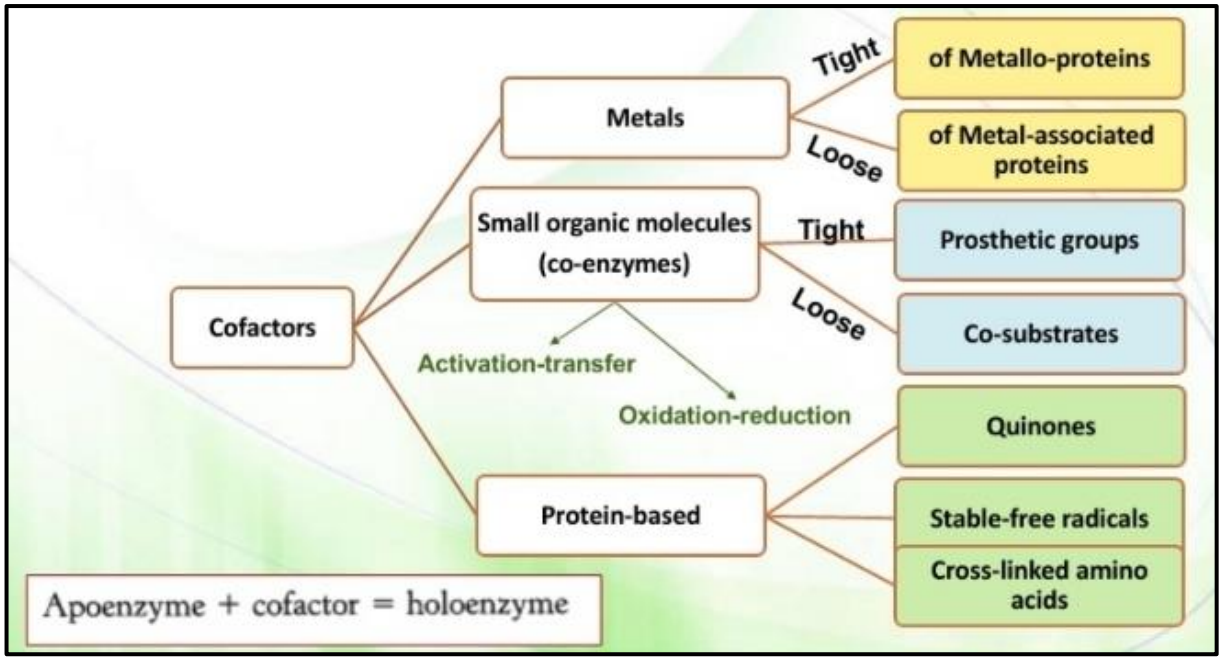
These enzymes are called Conjugated enzymes (Holoenzyme contains a nonprotein group vs apoenzyme doesn't contain a nonprotein group)

Classification of cofactors

Coenzymes are involved in two types of reactions:(1) activation-transfer coenzymes (transfer groups), (2). oxidation-reduction coenzymes

Figure Notes:

-protein-based cofactors are "read only", they aren't required for exam.



-When metals are tightly bound to proteins (**covalently** bonded), the proteins are called **Metallo-proteins**, however when they are loosely bound (by **non-covalent** bonds, electrostatic interactions mainly); the proteins are called **Metalo-associated** protein.

-When organic molecules are tightly bound to the protein (**covalent** bond), the co-enzymes are called **prosthetic groups**, however when they are loosely bound (**non-covalent** bonds); they are called **co-substrates**.

-note that in metals the naming was for the protein, but in organic molecules the naming (or classification) was for the group itself.

Ex: Hemoglobin→ heme group→ covalently bounded→ prosthetic group.

Water-Soluble Vitamins

You should know the names and the active forms of the Vitamins, but the functions are only nice to read it.

We are speaking here about water soluble vitamins, not lipid soluble ones (vitamins A/K/E/D).

The vitamins are not used directly, they must be modified first to the active form.

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. they participate in the reaction, not facilitators, they themselves move groups.

Characteristics:

Two groups in the coenzyme's structure:

A **functional group** that forms a covalent bond with substrate، participate in the reaction.

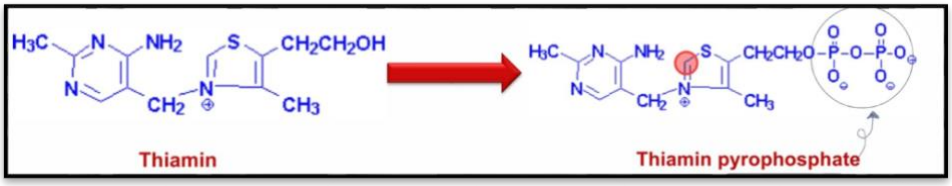
A **binding group** that binds tightly to the enzyme, it binds to the same enzyme and becomes part of the active site.

Dependence on the enzyme for additional specificity of substrate & additional catalytic power

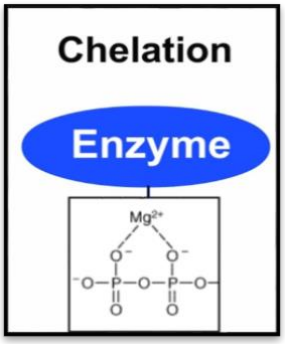
-Now, we will talk about some ACTIVATION-TRANSFER COENZYMES, what you must know about those vitamins is (Their name, active form, type and mechanism of their reactions, functional group and the binding group) Structures aren't required.

1.Thiamine pyrophosphate, TPP

Thiamin (vitamin B1) is converted to its active form, thiamin pyrophosphate (pyrophosphate means 2 phosphate groups attached together) TPP, in the brain & liver.



It is involved in decarboxylation **reactions.** (The molecule will lose 1 carbon in form of CO_2)

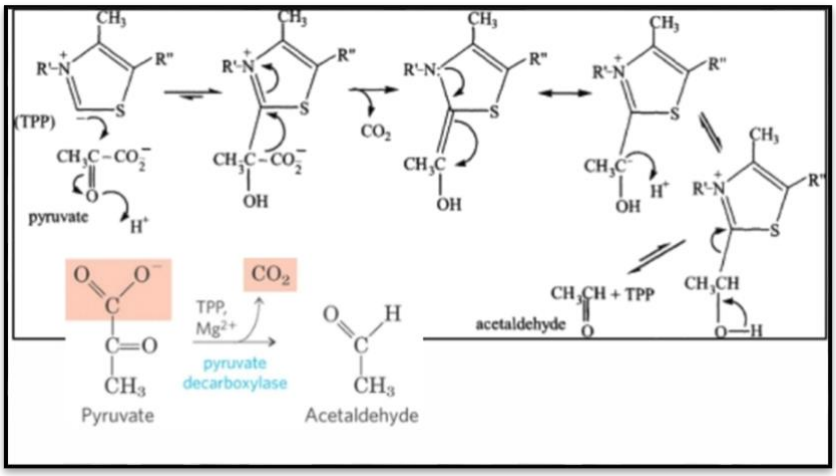


What is the purpose of thiamin pyrophosphate? The pyrophosphate provides negatively charged oxygen atoms and **chelates Mg^{+2}** that is tightly bound to the enzyme. What does chelation mean?

Check the figure, 2 oxygen atoms linked to **Mg^{+2}** and the **Mg^{+2}** is covalently linked with the enzyme, so the pyrophosphate is a part of the active site of the enzyme, linked via **Mg^{+2}**.

One of the important reactions (decarboxylation reactions) that TPP is involved in is the conversion of pyruvate (3 carbon molecule) to acetaldehyde (2 carbon molecule).

Binding group is pyrophosphate, the **functional group** is the carbon that falls between the sulfur and nitrogen, this carbon will link to the pyruvate then there will be electron rearrangement, where the pyruvate loses CO_2, Check the figures.

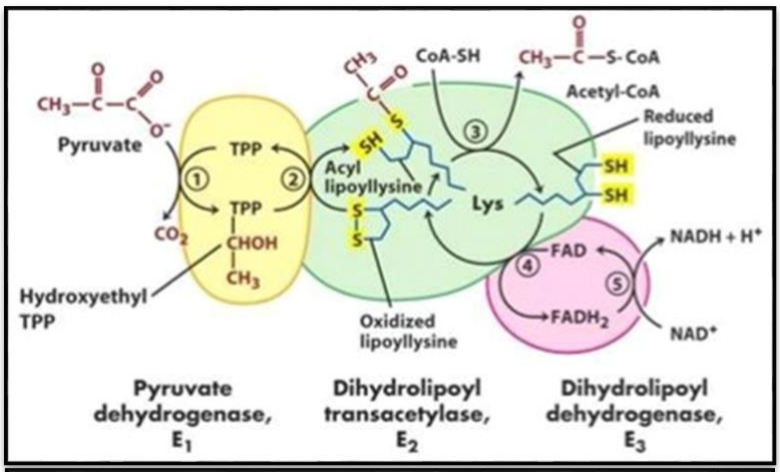


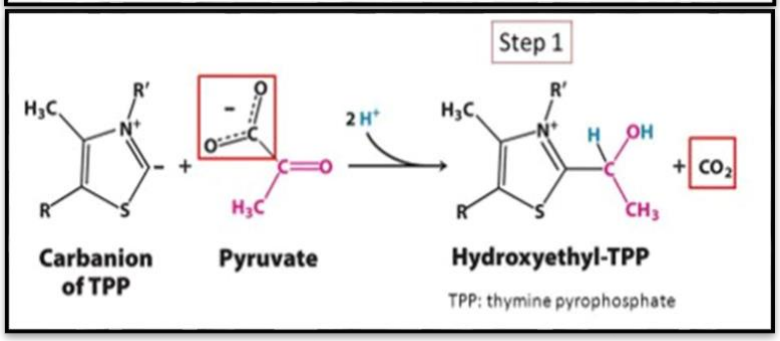
Additional note:
Chelation is a chemical process where a large organic molecule, called a chelating agent or chelator, forms a complex with a metal ion by forming multiple coordination bonds.

pyruvate dehydrogenase complex

Decarboxylation of pyruvate into acetyl CoA is one of the important reactions catalysed by Pyruvate dehydrogenase complex.

A complex refers to a cellular strategy aimed at accelerating reactions. Instead of individual enzymes catalyzing their reactions in isolation, multiple enzymes can collaborate by forming a complex, thereby enhancing their catalytic efficiency. In our scenario (Pyruvate dehydrogenase complex), a group of three enzymes functions together in a coordinated manner to catalyze their respective reactions.





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

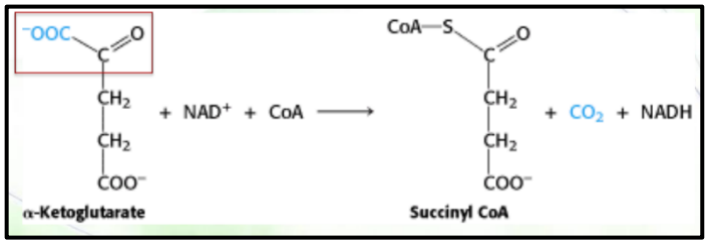
Decarboxylation of pyruvate into acetyl CoA by the pyruvate dehydrogenase complex (forming CO2 from pyruvate oxidation)

-Additional note: TPP is involved in the first step in this complex (E1).

α-ketoglutarate dehydrogenase

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

The α-ketoglutarate dehydrogenase (α-KGDH) is an enzyme complex, similar to the pyruvate dehydrogenase complex. It is part of the citric acid cycle (also known as the Krebs cycle or TCA cycle) and plays a crucial role



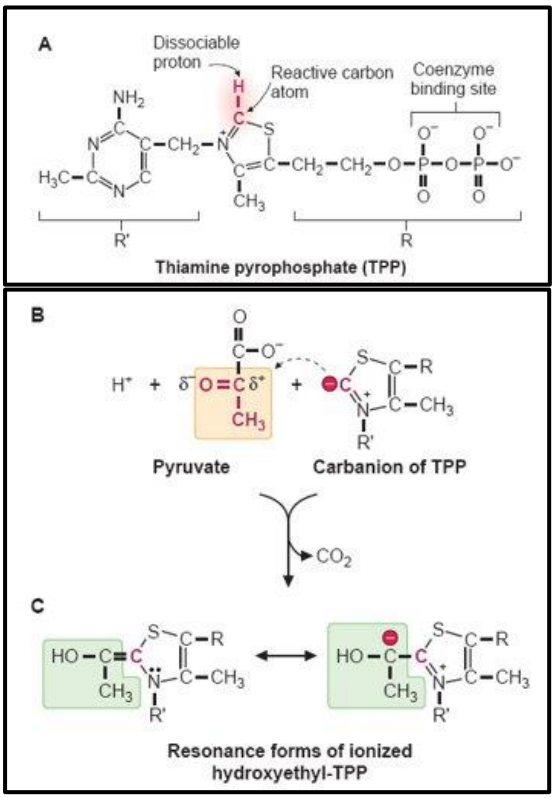
in the decarboxylation of α-ketoglutarate (a TCA cycle intermediate) into succinyl-CoA (removal of CO2).

Glutarate: a molecule that has five carbons.

-additional information: TPP serves as a cofactor for the α-ketoglutarate dehydrogenase enzyme (E1), the first step in the complex.

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.



The functional group (carbon between sulfur and nitrogen) will form an interaction with pyruvate leading to the rearrangement of electrons, release of CO2 and conversion of pyruvate into a Two-carbon molecule.

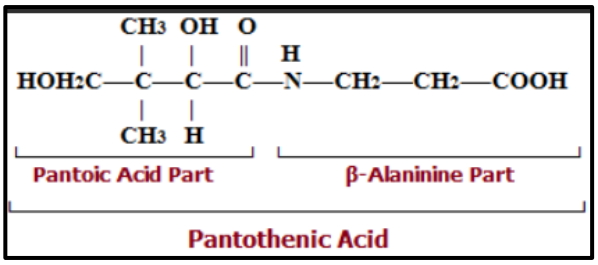
2.Coenzyme A (CoA)

It is a large molecule.

Source: pantothenate (B5): made of al β-alanine and pantoic acid.

-which of the following is an amino acid involved in B5 formation? β-alanine

Metabolism of carbohydrate, fats, and proteins where it attacks carbonyl groups & forms acyl thioesters (the "A").



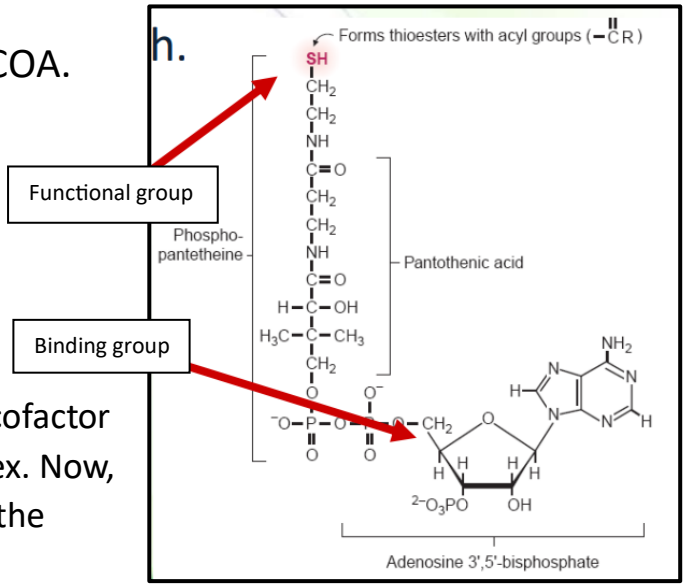
A molecule with CoA conjugated to it is energy-rich like acytel COA.

Functional group: sulfhydryl group (nucleophile), a terminal thiol group (sulfhydryl group) that binds to the substrate (like acetyl, giving us acetyl COA), This bond is a high energy bond.

Binding group: adenosine 3' ,5'- bisphosphate (tight & reversible), This group, resembles a nucleotide with an additional phosphate at the C3 position

(comprises a pentose, a phosphate group, and an adenosine nitrogenous base). It forms a covalent bond with the enzyme, exhibiting both strong and reversible binding characteristics.

example of enzyme that require COA.



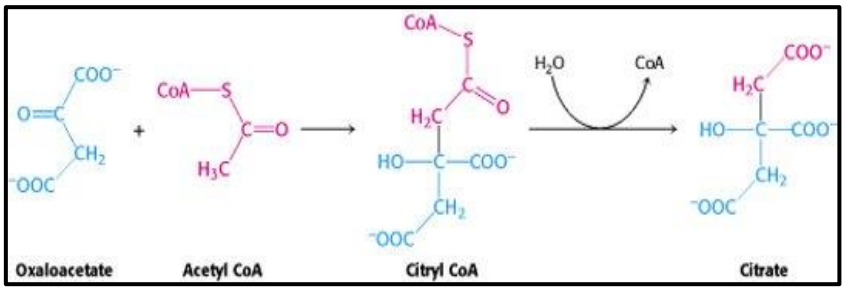
1.Conversion of pyruvate into acetyl CoA by the pyruvate dehydrogenase complex. (At the end of glycolysis)

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

-Previously, our focus was on the TPP cofactor in the pyruvate dehydrogenase complex. Now, in addition to TPP, we'll be addressing the involvement of the CoA cofactor in the complex.

-Additional info: In the pyruvate dehydrogenase complex, the CoA (coenzyme A) cofactor is involved in the second step. This step is catalyzed by the enzyme dihydrolipoamide acetyltransferase (E2).

2.Condensation of acetyl CoA and oxaloacetate into citrate by citrate synthase (a transferase), inside the mitochondria.



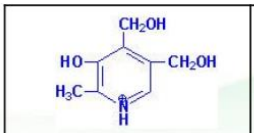
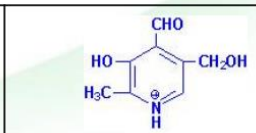
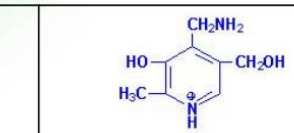
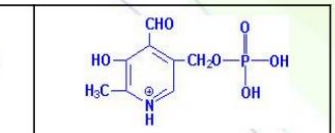
-So basically, these two reactions are interconnected. At the end of glycolysis, the **conversion of pyruvate to acetyl CoA** takes place, enabling its entry into the Krebs cycle. This transformation is facilitated by the pyruvate dehydrogenase complex, involving the assistance of both TPP and CoA. Following this, within the mitochondria, **acetyl CoA combines with oxaloacetate to give citrate** by the enzyme citrate synthase, a process that is aided by CoA.

3.Pyridoxal phosphate (vitamin B6)

Sources: pyridoxal, pyridoxamine and pyridoxine.

Metabolism of amino acids via reversible transamination reactions.

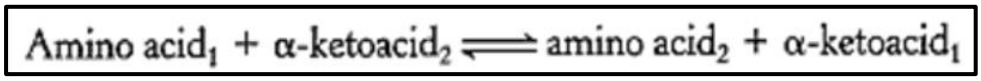
Recall that Transamination is a reversable biochemical reaction involving the transfer of an amino group (NH2) from one amino acid to a keto acid, resulting in the formation of a new amino acid and a new keto acid.

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

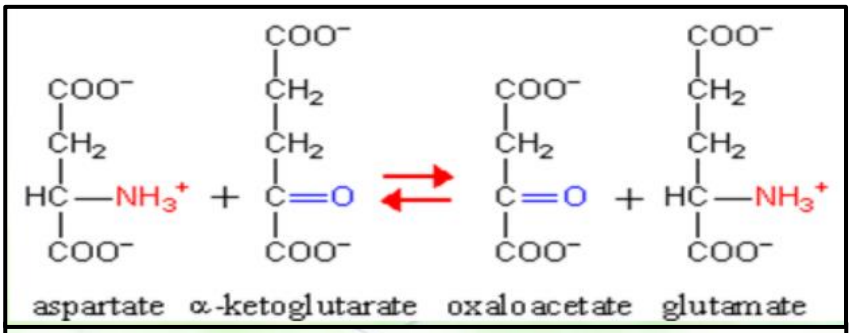
All of these are forms of vitamin B6, but only pyridoxal phosphate is the active from

The main reaction that vitamin B6 participates in is Transamination.

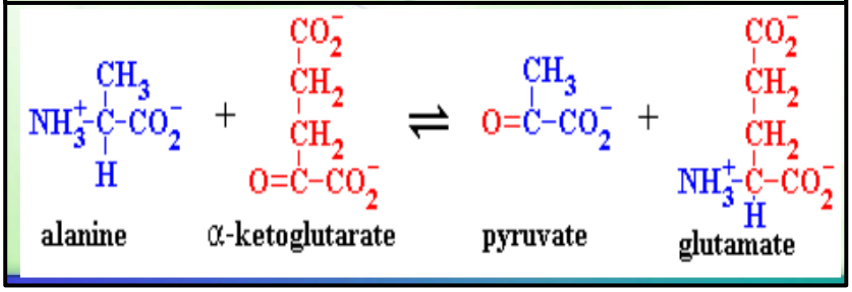
this is the main formula for the reaction:



-This is an example of transamination, the two amino acids here are aspartate and glutamate, while the ketoacid here is oxaloacetate.



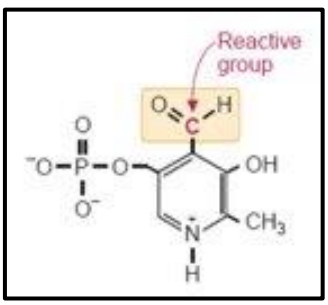
-another example is alanine and glutamate, when the ketoacid is pyruvate.



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.



Here the aldehyde group is both the binding and the reactive group.

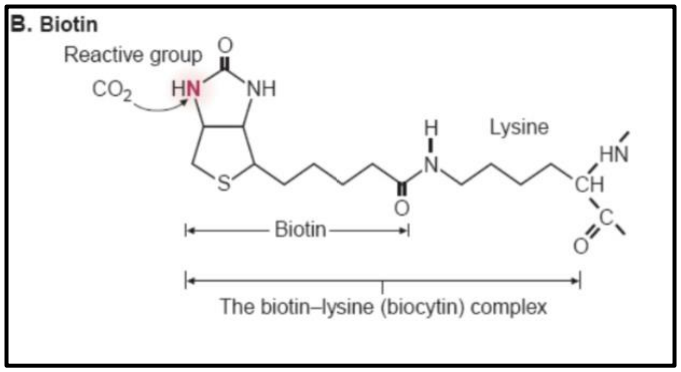
-additional info: the aldehyde group in vitamin B6 (pyridoxal phosphate) acts as the binding group, the aldehyde group in B6 is responsible for forming a Schiff base linkage with the amino group of the amino acid substrate. This binding is crucial for anchoring the amino acid to the enzyme's active site, positioning it for the subsequent chemical transformation.

Upon the entry of an amino acid into the active site, the amino group forms a covalent linkage with the aldehyde present. As a result, an electron rearrangement occurs, leading to the departure of the amino acid from the active site as a ketoacid, devoid of its amino group. Simultaneously, the amino group becomes attached to a vitamin molecule.

Following this, a distinct ketoacid (distinct from the one generated in the initial reaction) binds to the active site. It undergoes a reverse reaction within the site, leading to its exit as an amino acid.

-structures are not important.

4.Biotin (vitamin B7)



It is required for carboxylation reactions.

covalently bound to Lys.

Sources: food & intestinal bacteria

Deficiencies are seen after long antibiotic therapies (because that kills the intestinal bacteria) or excessive consumption of raw eggs (egg white protein, avidin, has a high affinity for biotin). Egg white is referred to as avidin which binds to biotin making it unavailable for working as a co-factor.

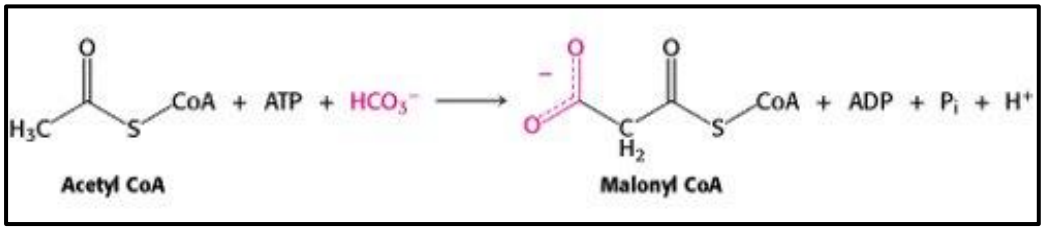
Examples of enzymes: (Enzymes that use it)

1.Pyruvate carboxylase

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

Basically, the **pyruvate** is carboxylated (co2 added to it) and converted to **oxaloacetate**.

2.Acetyl CoA carboxylase (fatty acid synthesis)



Here **acetyl CoA** is carboxylated to **Malonyl CoA**, an important reaction in the synthesis of lipids.

Oxidation—Reduction coenzymes

A number of coenzymes work within oxidoreductases.

Unlike ACTIVATION-TRANSFER COENZYMES, Oxidation—Reduction coenzymes don't bind to the substrate and only bind to the enzyme itself.

Each coenzyme has a unique functional group that accepts and donates electrons and is specific for the form of electrons it transfers (hydride ions(H-), hydrogen atoms, oxygen).

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 (vitamin E&C)

Again: Dependence on the enzyme for additional specificity of substrate & additional catalytic power.

coenzymes can't work individually; they need enzymes to work because enzymes provide a favourable environment for the coenzyme to work and they give specificity for the coenzyme and they are also important for the proximity between the coenzyme and the substrate.

1.FAD and FMN

The precursor is riboflavin (vitamin B2).

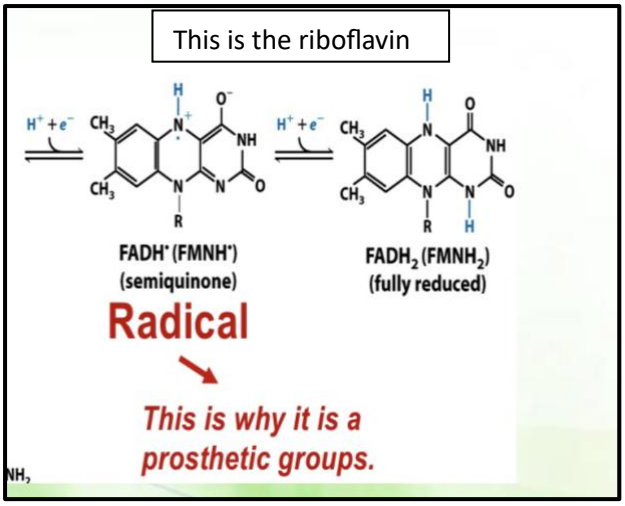
Both are prosthetic groups of flavoproteins.

FMN = Flavin Mononucleotide

FAD = Flavin Adenine Dinucleotide

FAD is composed of FMN and an adenine nucleotide.

FAD accepts electrons in the form of hydrogen atoms donated separately and sequentially.



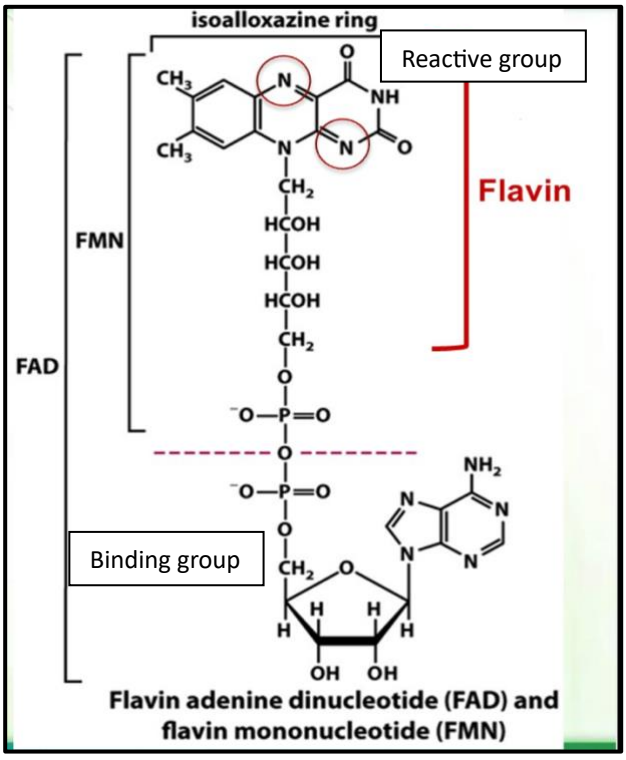
The phosphate and the adenosine nucleotide are the binding groups.

The two circled nitrogen groups in the ring are the reactive groups, they accept electrons.

Both adenine and flavin contain nitrogen atoms in their rings.

They are covalently bonded to the active site, that's why they are considered as prosthetic groups.

What is the importance of this covalent bonding?



As previously discussed, FAD accepts electrons in a sequential manner, resulting in a time gap between the acceptance of the first and second hydrogen atoms. Consequently, when the first hydrogen atom attaches, FAD generates a radical referred to as FADH+. This radical is highly reactive and possesses potential harm to the cell. To prevent detrimental reactions with other hydrogen atoms, FAD becomes covalently bonded to the enzyme, which restricts its ability to damage the cell. It patiently awaits the binding of the next hydrogen atom after the formation of FADH2 takes place, eliminating the presence of the damaging radicals.

They are involved in reactions resulting in the formation of double bonds or disulfide bonds. (because it accepts the hydrogens)

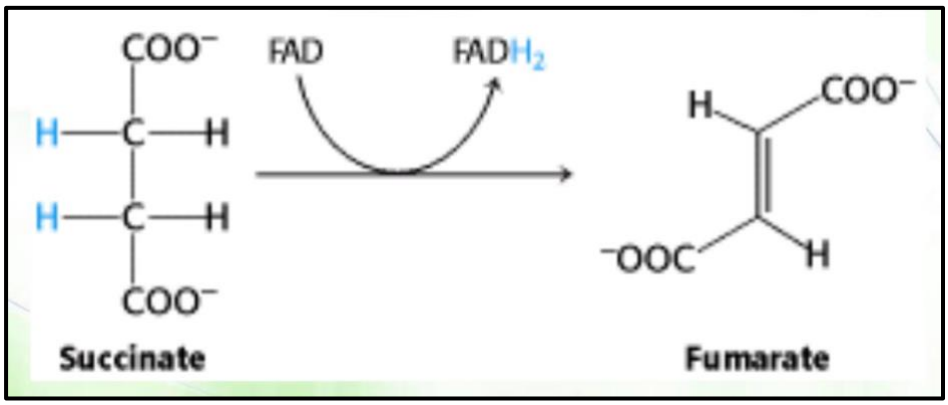
Succinate dehydrogenase

Oxidation of succinate into fumarate by succinate dehydrogenase

Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate.

During this process, FAD (Flavin Adenine Dinucleotide) is reduced to FADH2

A Double bond is formed in the product.



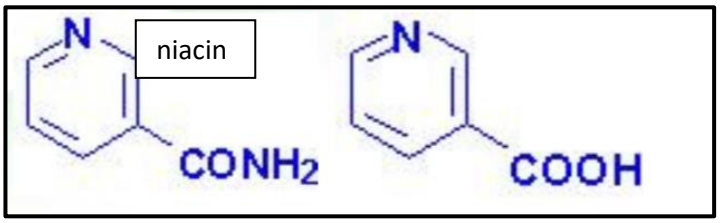
2.NAD+ and NADP+

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

These are cosubstrates for numerous dehydrogenases.

They function as co-substrates for dehydrogenase enzymes.

These co-substrates originate from niacin, a derivative of vitamin B3.



Due to their inability to form covalent bonds with enzymes, they are designated as co-substrates, and they are not prosthetic groups (they don't bind covalently).

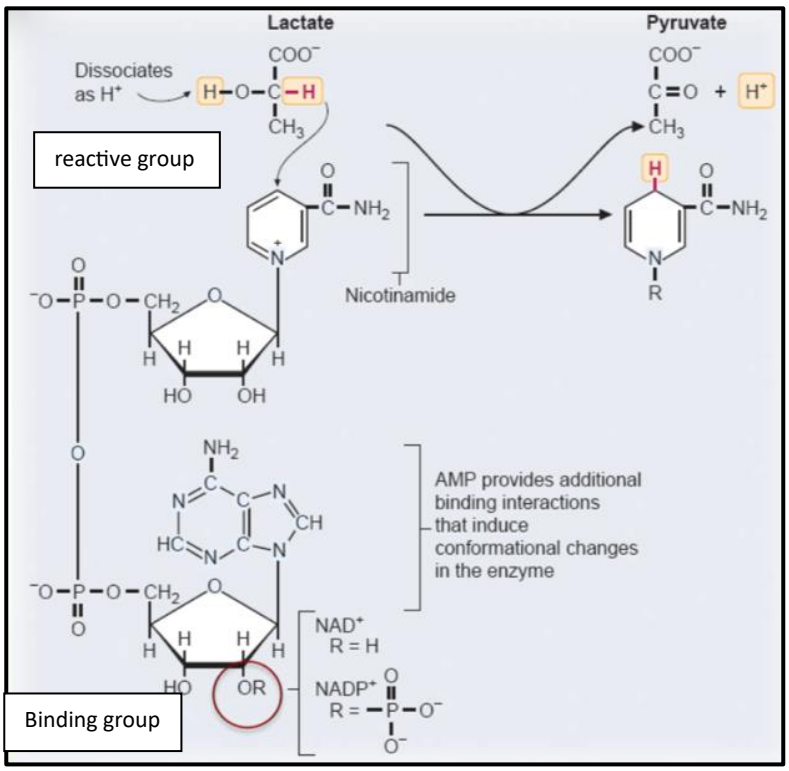
The mechanism of action

The functional group (C opposite to N) accepts a hydride ion from the substrate, dissociates, & a keto group (CO) is formed, the nitrogen in the ring helps to increase the electrophilicity.

In the context of NAD+ and NADP+, the niacin ring functions as the **reactive group**, while the nucleotide segment of the molecule serves as the **binding group** that engages and attaches to the enzyme.

At carbon C2, an R group is present. If this R group is a hydrogen (H), the resulting molecule is NAD+. Conversely, if the R group takes the form of a phosphate group, the resulting molecule is NADP+.

The phosphate doesn't participate in the reaction, but it rather determines which enzyme will NAD+ or NADP+ bind to .



The ADP portion of the molecule binds tightly.

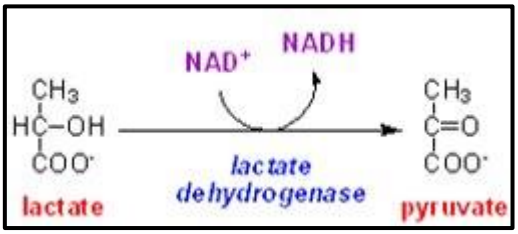
They are generally involved in the oxidation of alcohols and aldehydes.

The substrate undergoes oxidation, initiating with the detachment of a hydrogen atom from the substrate, which then associates with the ring structure as a hydride ion. Following this, the second hydrogen atom dissociates as a hydrogen ion, leading to the creation of a double bond within the substrate as a keto group, exemplified in our case by the conversion of lactate into pyruvate.

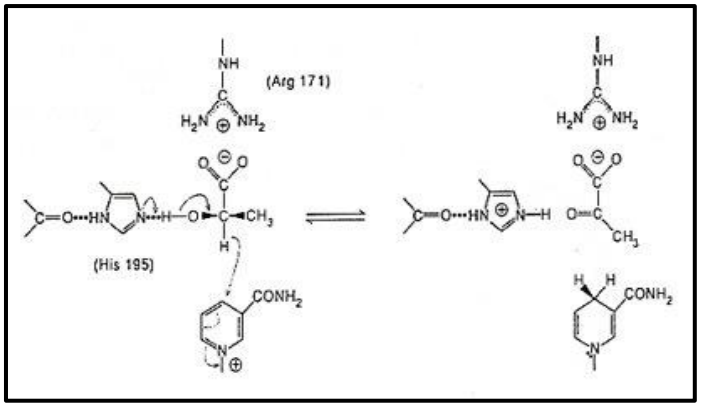
NAD+ is usually involved in catabolism reactions while NADP+ is involved in anabolism reactions, this specificity is due to the presence or absence of the phosphate group.

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.



Additional info: The enzyme's histidine residue facilitates interaction between the proton of lactate's hydroxyl group and the active site, aiding NAD+ in extracting the hydrogen with its electrons, transferring a hydride ion. This prompts lactate oxidation and the formation of a keto group (-C=O). The histidine stabilizes the transition state, enabling proton and electron transfer.



The process involves replacing the hydroxyl group with a keto group, ultimately yielding pyruvate. Specific enzyme details and structural considerations influence the reaction's intricacies, highlighting the role of histidine and NAD+ in this conversion.

3.Vitamin C

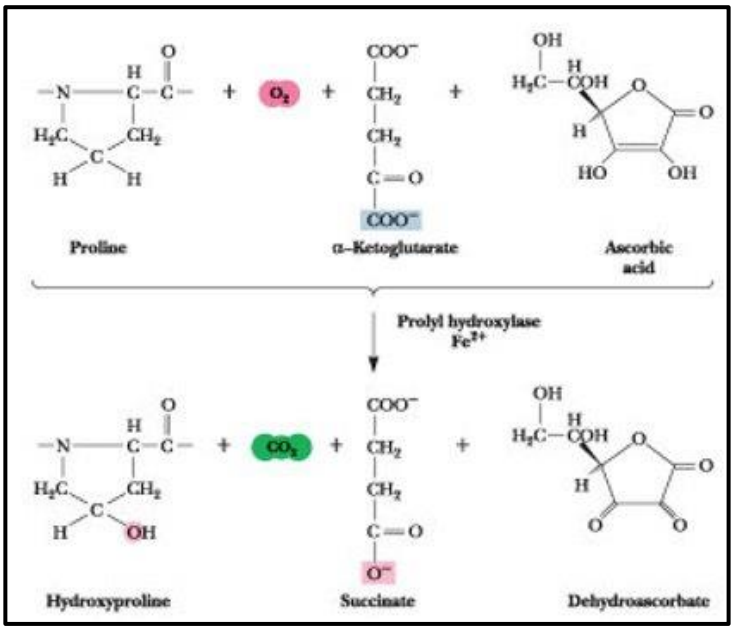
Ascorbic acid.

Example: prolyl hydroxylase.

synthesizes 4-hydroxyproline (collagen).

An antioxidant.

It serves as a cofactor for the enzyme prolyl hydroxylase in the synthesis of collagen as we discussed in the fibrous protein lecture.



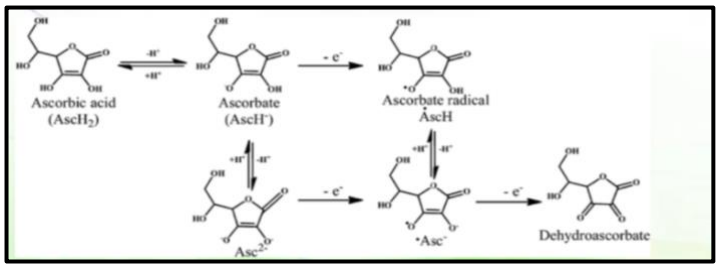
It can also function as an antioxidant, meaning that it can remove radicals and reactive oxygen species.

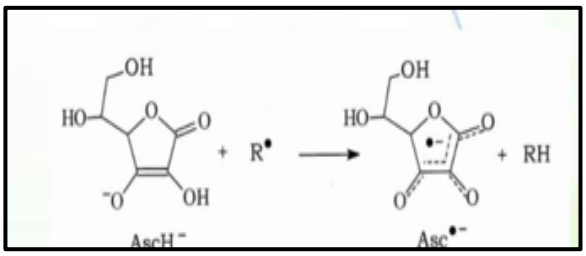
Ascorbate, the antioxidant

Reactive oxygen species oxidize (take electrons from) ascorbate into a radical itself, which is then oxidized into dehydroascorbate.
This helps us to get rid of the reactive oxygen.

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

In the scenario of fatty acid oxidation, when a fatty acid undergoes oxidation, electron transfer occurs along the hydrocarbon chain and then from one fatty acid to the other potentially leading to detrimental effects on the plasma membrane.
However, a different outcome arises when electrons are directed towards an antioxidant, such as vitamin C. In this case, the electron's dispersion throughout the ring structure of vitamin C is facilitated by resonance. This resonance-driven electron distribution yields a relatively stable molecule, effectively protecting to the cell from potential harm.





Metals

They act as electrophiles (Can be reduced).
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.

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

They can work as facilitators or participants.

Advantages

They carry positive charges and, hence, can form relatively strong yet kinetically labile (likely to be changed) bonds.

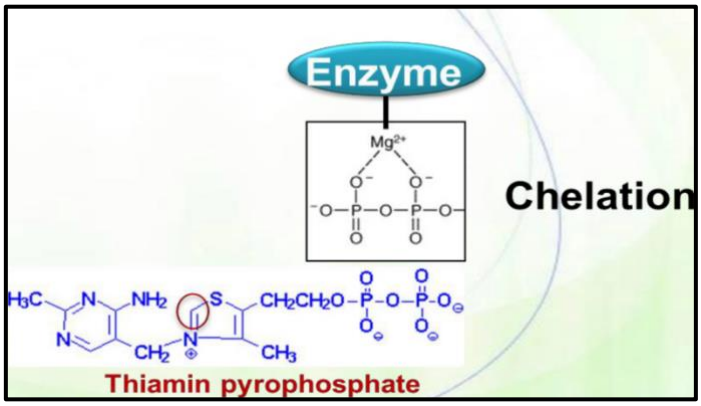
They are stable in more than one oxidation state (Example: iron can be Fe^{+2} or Fe^{+3}).

They can bind multiple ligands enabling them to participate in binding substrates or coenzymes to enzymes (They can form multiple covalent bonds).

Example on these functions:

Mg2+ connects the negatively charged phosphate groups of thiamine pyrophosphate to basic amino acids in the enzyme (We discussed it earlier).

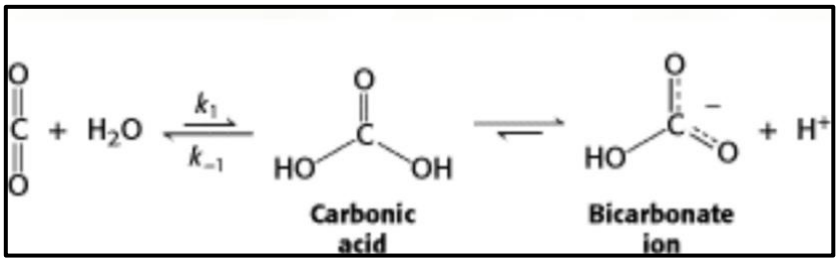
The phosphate groups of ATP are usually bound to enzymes through Mg2+ chelation.



Carbonic anhydrases

Carbonic anhydrase is the enzyme that facilitates the formation of carbonic acid.

Although CO2 hydration and HCO3- dehydration occur spontaneously, almost all organisms contain carbonic anhydrases, because they carry out rapid processes such as respiration.



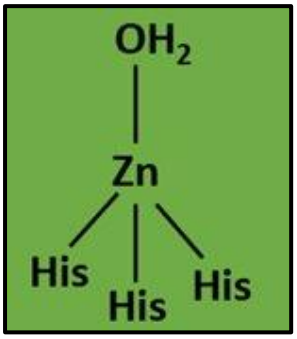
So, even though this reaction can happen on its own, we still require an enzyme to make it happen faster because it is extremely important.

Mutations in carbonic anhydrases have been found to cause osteoporosis (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.

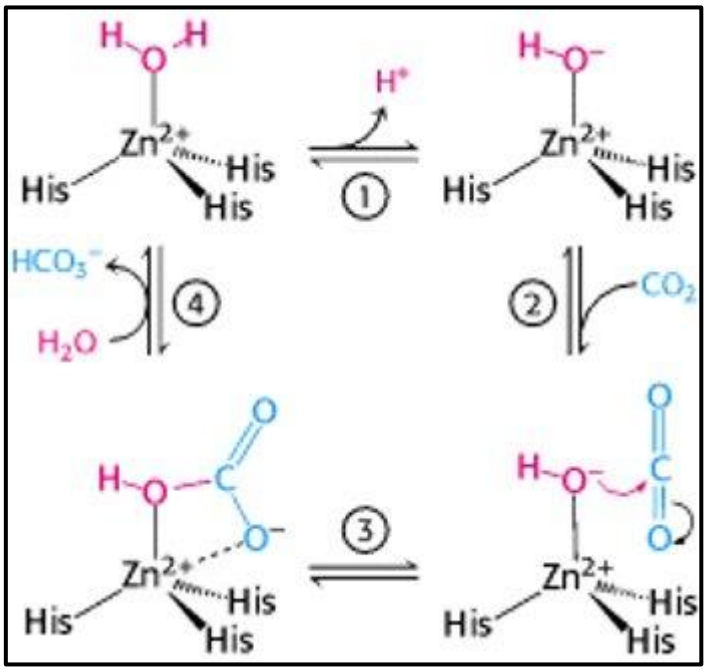


So, the Zn^{+2} ion will form covalent bonds within the active site, attaching to three imidazole rings and also

creating a covalent linkage to the substrate, which happens to be water. (3 bonds with the active site and one bond with the substrate)

Mechanism of action

Zinc facilitates the release of a proton from H2O generating a hydroxide ion.



The CO2 substrate binds to the enzyme's active site and is positioned to react with the hydroxide ion.

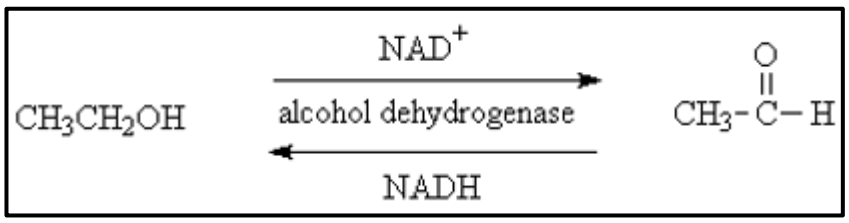
The hydroxide ion attacks CO2 converting it into a bicarbonate ion.

The catalytic site is regenerated with the release of the bicarbonate ion and the binding of another H2O.

So basically, Zinc Initially assists in proton release from water, generating a hydroxide ion. Carbon dioxide binds to the enzyme's active site and reacts with the hydroxide ion. This reaction transforms CO2 into a bicarbonate ion.

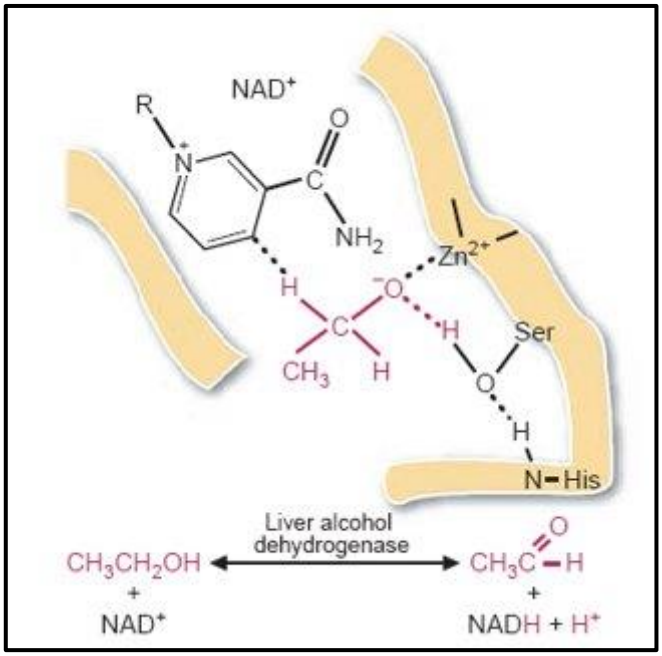
Subsequently, the enzyme's active site is regenerated as the bicarbonate ion is released, making way for a new cycle.

Catalytic Metals



Some metals do not participate in enzyme catalysis directly but facilitate a reaction.

Like in the enzyme “alcohol dehydrogenase”, this reaction involves the reduction of NAD^+ and the oxidation of the alcohol.



If you look at the figure, ethanol (the alcohol) doesn’t bind to either the active site of the enzyme or to NAD^+, so how does it bind?

Histidine pulls the electrons from serine which in turn pulls the electrons from ethanol oxidizing it into an acetaldehyde.

Then those electrons will be donated to NAD^+ in the form of hydride ion.

During those intermediates, the charges are stabilized by the zinc.

So, even though the zinc doesn’t participate in the reaction, it facilitates it by stabilizing the charges.

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+.