Factors affecting enzyme activity

Like catalysts, enzymes are also affected by a number of factors that regulate enzyme action. These factors are related to the chemical nature of enzymes as enzymes are proteins, and proteins are affected by most of these factors.

1. Enzymatic effectors :

The speed of an enzymatic reaction depends on both :

1. The enzyme concentration.
2. The substrate concentration present in the reaction medium.
3. The presence or absence of effectors.

The latter play a key role *in vivo*, as they act on and adapt the functioning of enzymes to their biological environment. They are also effective in the experimental study of enzymes outside living organisms.

1.1. Temperature :

An increase in temperature increases the speed of an enzymatic reaction, but only within a very limited range, due to :

1. An increase in the number of molecules that are in an activated state.

2. An increase in the probability of combination between the enzyme and the substrate.

The factor that leads to an increase in the speed of a biological process following a temperature increase of around 10°C is called Q10 (temperature coefficient).

1. 2. pH :

pH affects the ionization state of the enzyme and that of the substrate. Consequently, the rates of enzymatic reactions are sensitive to pH variations in the range [2,11]. pH modifies :

1. The hydrogen bonds, ionic bonds, or Van der Waals forces that bind the substrate to the active site.
2. The forces binding the monomers of oligomeric enzymes.
3. The conditions for electron or proton transport within the enzyme.

<u>Enzyme behavior towards pH :</u>

1. Some enzymes are not greatly affected by pH variations, such as salivary amylase.

2. Some enzymes act within a narrow pH range, such as pepsin and trypsin.

3. Others, which catalyze reversible reactions, have a different optimum pH when acting in one direction or the other.

1. 3. Inhibitors :

The inhibition of enzyme activity by small molecules or ions acts as an essential control mechanism in biological systems (regulation of metabolic pathways by retro-inhibition).

Experimentally, enzyme inhibition can provide information on the mechanism of action of enzymes and their substrate specificity: residues essential for catalysis can be identified using specific inhibitors.

1. 4. Allosteric effectors :

- The functioning of allosteric enzymes is controlled by effectors : activators or inhibitors. These can be small molecules that are different from the substrate. They can be organic molecules or mineral ions (Ca^{++}, Zn^{++}, Ni^{++}, Mg^{++}, Mn^{++}, Co^{++}, Fe^{++} or Fe^{+++}, Mo^{4+}).
- They have a distinct binding site.
- They cause a successive change in the conformation of the subunits, producing an increase in enzyme activity (in the case of activators).
- In some cases, the substrate binds to the enzyme, forming a common complex with the activator.

2. Functional classification of enzymes :

1st class : enzymes that work without coenzymes

For these enzymes, the reaction takes place via the catalytic groups of the protein.

- These may be residues of side chains with nucleophilic properties, such as :
  - ✓ Imidazole from histidine ;
  - ✓ Carboxylate from aspartate and glutamate ;
  - ✓ Amino groups of arginine and lysine ;
  - ✓ Alcohol function of serine and threonine ;
  - ✓ Protein dipoles.
- Certain enzymes require the presence of a metal cation (cofactor) coordinated to the protein via atoms such as oxygen, nitrogen, or sulfur (metalloenzymes).

2nd class : enzymes functioning with a dissociable coenzyme

- In this case, the coenzyme acts as a second substrate.
- Certain groups of the protein may be involved.

3rd class : enzymes that contain a prosthetic group

In this case, the reaction involves a metal that changes its valence or spin state (transition metal) or through electronic movement (FMN and FAD).

3. Coenzymes :

3.1. Definition :

Coenzymes are biological molecules that are non-protein, low molecular weight, and thermostable, bound to an enzyme protein. They are different from substrates and participate in the enzymatic reaction without being modified. They are synthesized by the body or provided by food (vitamins).

3.2. Roles :

Coenzymes function as acceptors and transporters of radicals released during catalysis. They bind and transport :

- ✓ Hydrogen and electrons during oxidation-reduction reactions.
- ✓ Radicals other than hydrogen and electrons in other reactions.

3.3. Types of coenzymes :

3.3.1. Prosthetic groups (linked coenzymes) :

These coenzymes are strongly bound to the enzyme by covalent bonds that cannot be separated without denaturing the protein (they do not dissociate from the enzyme).

3.3.2. True coenzymes (free coenzymes/cosubstrates) :

These coenzymes form non-covalent bonds with the enzyme and are easily dissociated from the protein part of the enzyme. This bond is renewed with each reaction performed.

3.4. Classification of coenzymes :

Coenzymes are classified into two main categories :

- ✓ Oxidation-reduction (Redox) coenzymes
- ✓ Atom group transfer coenzymes

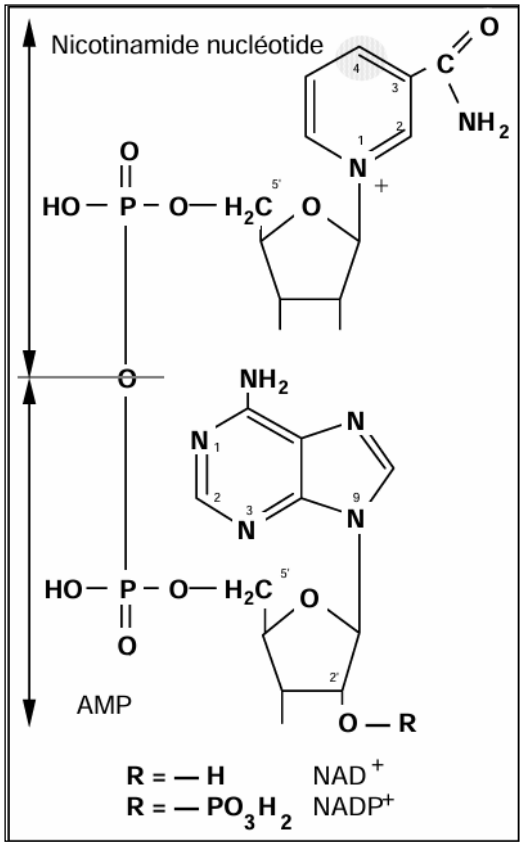
3.4.1. Oxidation-reduction coenzymes :

They participate in redox reactions by transporting hydrogen atoms in the form of electrons and protons or electrons alone. They are found in all cellular redox reactions and in organized electron transport sequences such as respiration and photosynthesis.

a. Nicotinic (or pyrimidic) coenzymes :

Designates all hydrogen carriers derived from niacin (vitamin B3 or PP). The two most common types are :

- **NAD^+** : Nicotinamide Adenine Dinucleotide.
- **$NADP^+$** : Nicotinamide Adenine Dinucleotide Phosphate.



Some are found in a free state in mitochondria, chloroplasts, and cytosol.

They reversibly bind a hydride ion (H^-) : the reducing substrate loses two hydrogen atoms, and the co-substrate NAD(P) is reduced to NAD(P)H,H^+ during an initial reaction catalyzed by a dehydrogenase :

$$NAD^+(P) + 2e^- + 2H^+ \longleftrightarrow NAD(P)H,H^+$$

b. Flavinic coenzymes :

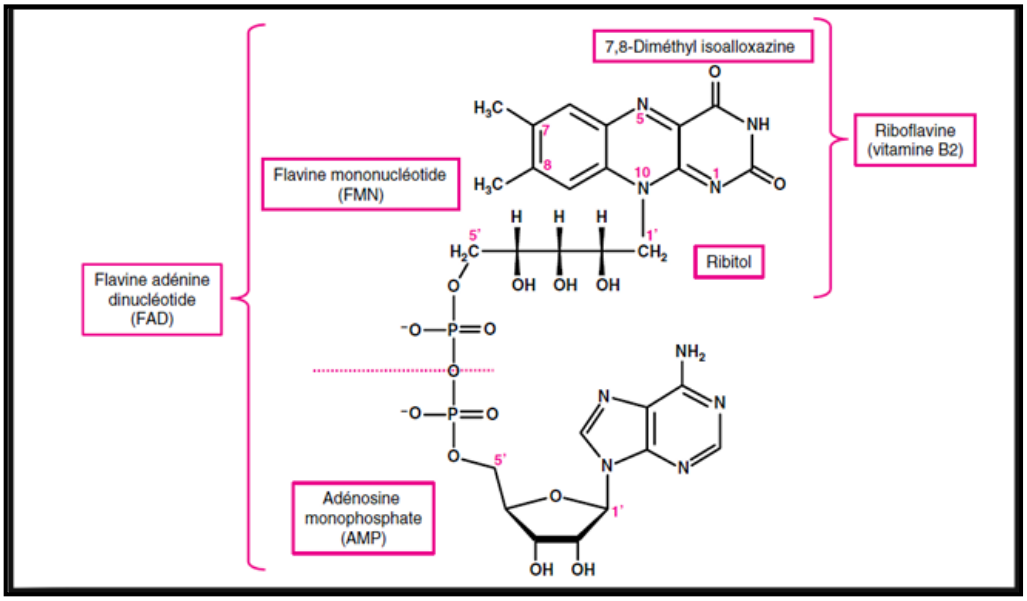
They are derived from vitamin B2 or riboflavin.

There are two types :

- ✓ Flavin mononucleotide (FMN), which is membrane-bound and involved in electron transport in the respiratory chain ;
- ✓ Flavin adenine nucleotide (FAD), which is cytosolic.

Flavinic coenzymes are linked to their apoenzyme by a covalent bond. Together, they form flavoproteins. Coenzymes are the prosthetic groups of these enzymes.

The reactive part of FAD and FMN that participates in oxidation-reduction is the dimethylisoalloxazine ring of riboflavin.

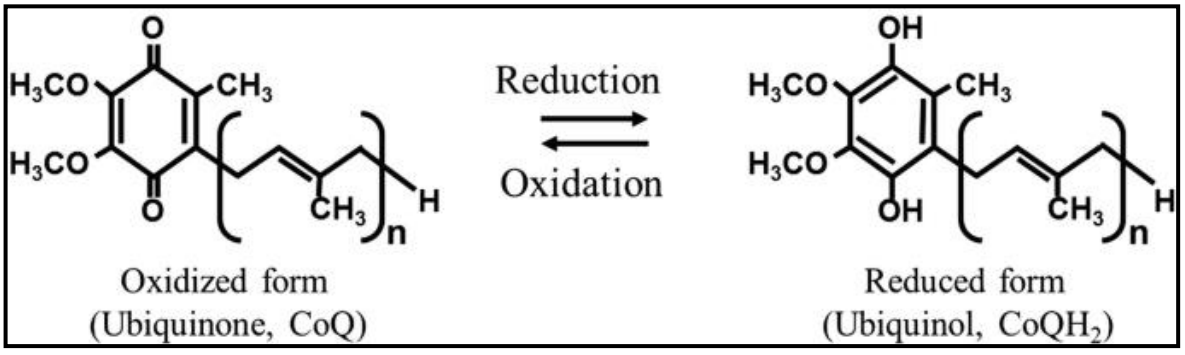


c. Quinone coenzymes (ubiquinone and plastoquinone) :

Ubiquinone, also known as coenzyme Q10, is a liposoluble coenzyme that transports hydrogen from organic substrates to oxygen in the mitochondrial respiratory chain. It is not a vitamin and can be synthesized by all cells. It is not attached to a protein and can circulate freely in the phospholipid layer of the inner mitochondrial membrane.

Several quinone coenzymes are currently known. They differ in the length of their side chain, which consists of n polymerized isoprene radicals. The number n is equal to 10 in ubiquinone, hence the name coenzyme Q10.

Quinones have two quinone functions which, through reduction, are transformed into dihydroquinone by accepting 2 electrons and 2 protons released by the substrate.

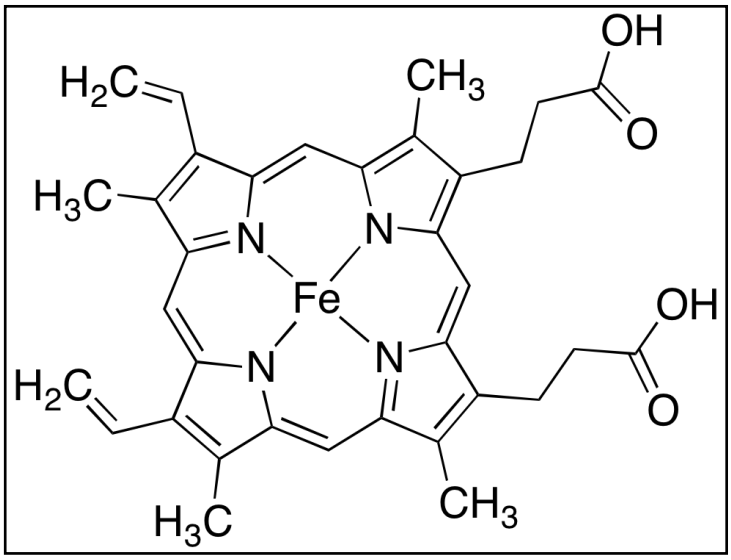


d. Heme coenzymes (metalloporphyrins) :

Metalloporphyrins are prosthetic groups linked to their respective apoenzymes by covalent bonds. These coenzymes result from the union, in the form of a complex, of an iron atom and a porphyrin (derived by substitution of the tetrapyrrole ring). They are found in cytochromes and peroxidases.

Cytochromes

Chromoproteins are present in all cells. The ferroprotoporphyrin prosthetic group is the same. Only the apoenzymes that are firmly attached to them by covalent bonds are different, giving them different redox potentials.



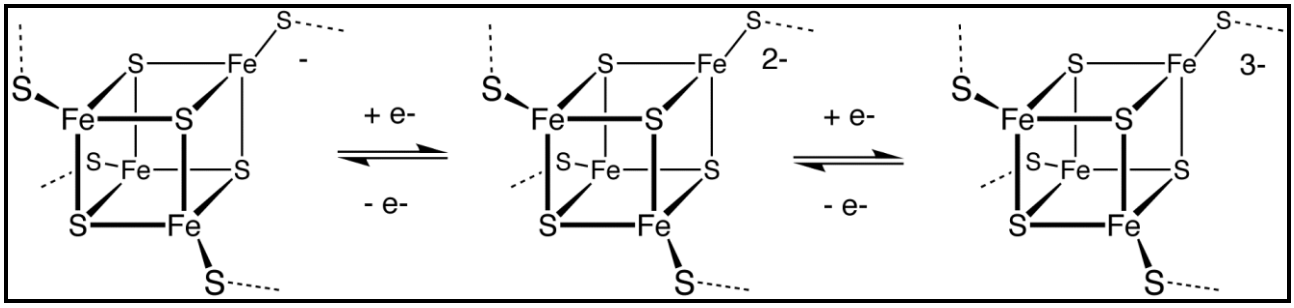
All cytochromes function on the same principle. It is the iron in the prosthetic group that transports electrons through the reversible conversion of ferric iron to ferrous iron :

$$Cyt\ –Fe^{3+} + e^- \longleftrightarrow Cyt\ –Fe^{2+}$$

e. Iron-sulfur proteins :

They are involved in electron transport sequences in both the respiratory chain and photosynthesis. The iron is non-heme and the sulfur is in the form of sulfide.

Electron transport still occurs through a change in the valence of iron, which reversibly changes from ferric iron to ferrous iron.



3.4.2. Atom group transfer coenzymes :

3.4.2.1. Coenzymes involved in the transport of one-carbon radicals :

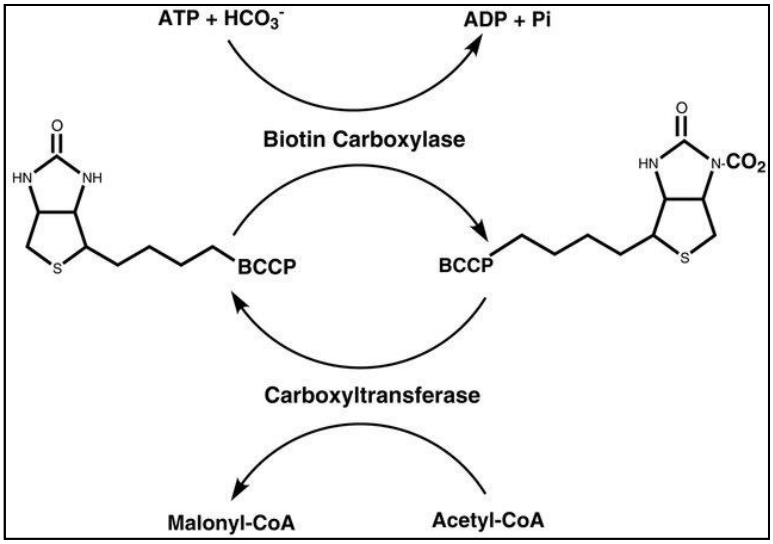
The mono-carbon radicals that can be transported by their specific coenzymes are : CO_2, $-CH_3$, -CHO, $-CH_2OH$.

a. CO_2 transport coenzymes :

CO_2 is transported in carboxylation and decarboxylation reactions. Two coenzymes serve as CO_2 transporters : biotin for small molecules and vitamin K for proteins.

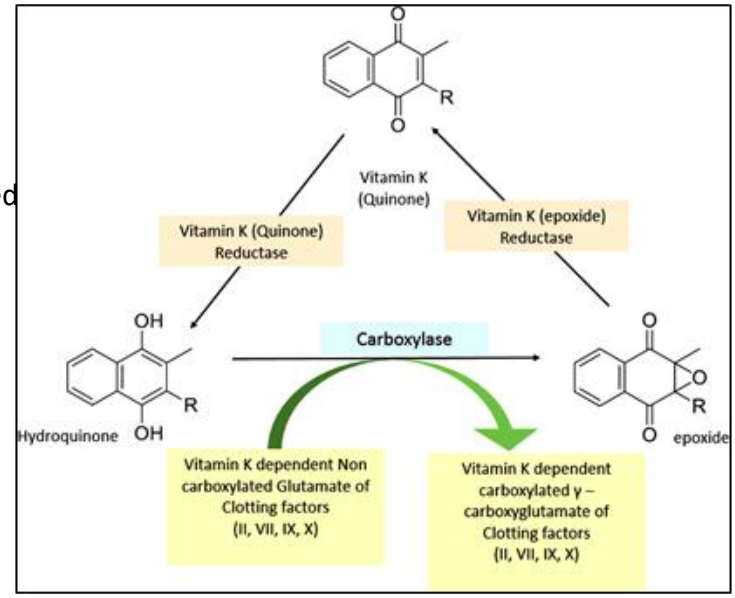
Biotin (vitamin H/vitamin B7) :

This coenzyme binds to the apoenzyme via a covalent amide bond formed between the acid function of the biotin lateral chain (COOH) and the amine function (NH2) of the lysine residues of the apoenzyme.



Vitamin K :

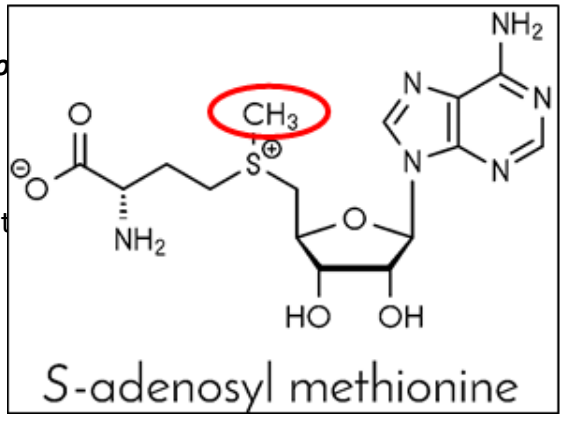
These vitamins are involved synthesis of carboxylase coenzymes.



b. Coenzymes that transport radicals o

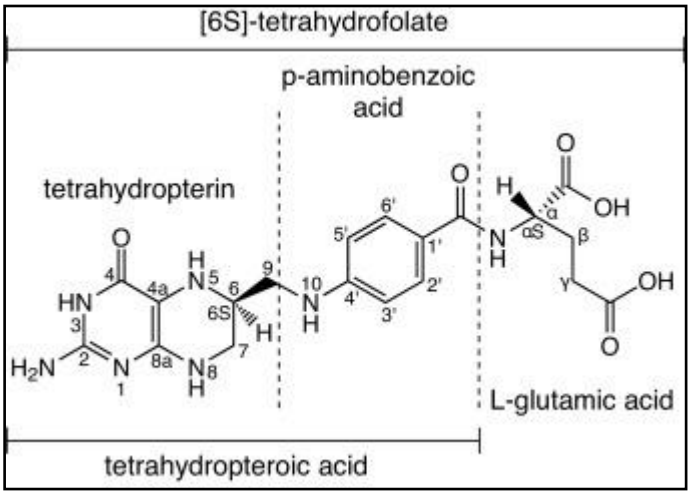
S-Adenosyl Methionine :

It is a methyl donor in reactions cat by the methyltransferase group.



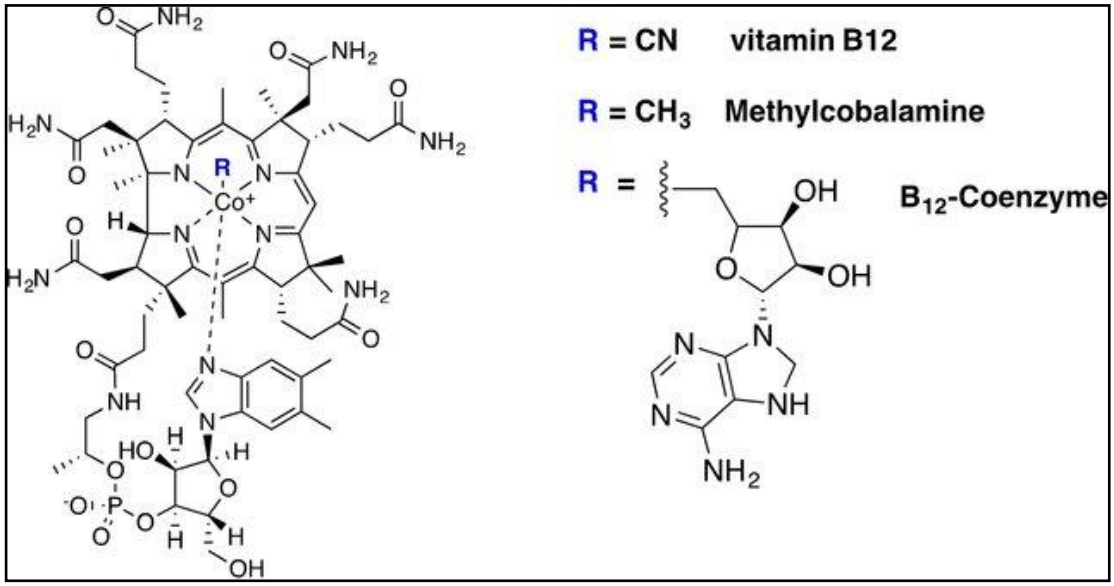
Tetrahydrofolic acid (THF) :

This coenzyme is derived from folic acid, or vitamin B9. It enables the transfer of methyl radicals (CH_3) via nitrogen N5 and formyl groups (CHO) via nitrogen N10.



Methylcobalamin :

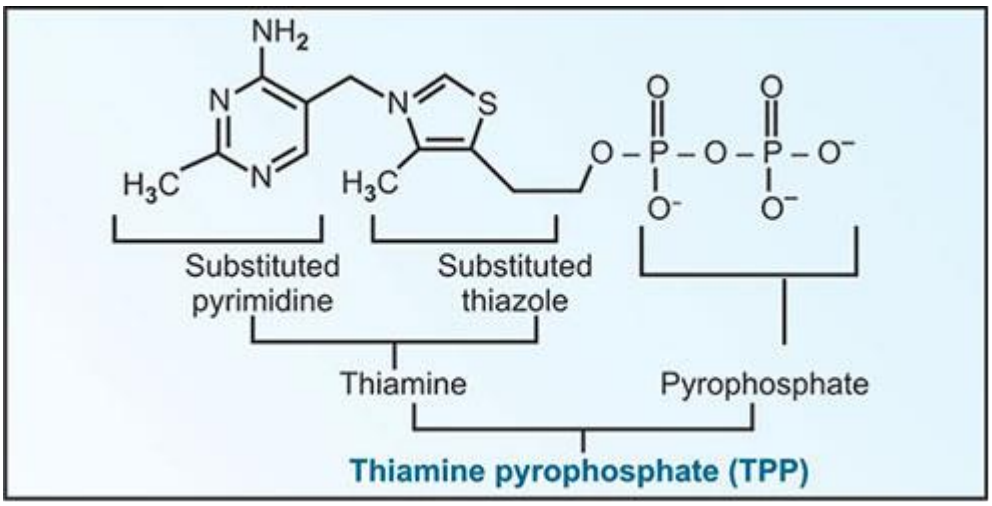
It is derived from cobalamin (vitamin B12). Methylcobalamin helps transfer methyl groups, particularly in the conversion of folate necessary for DNA synthesis.



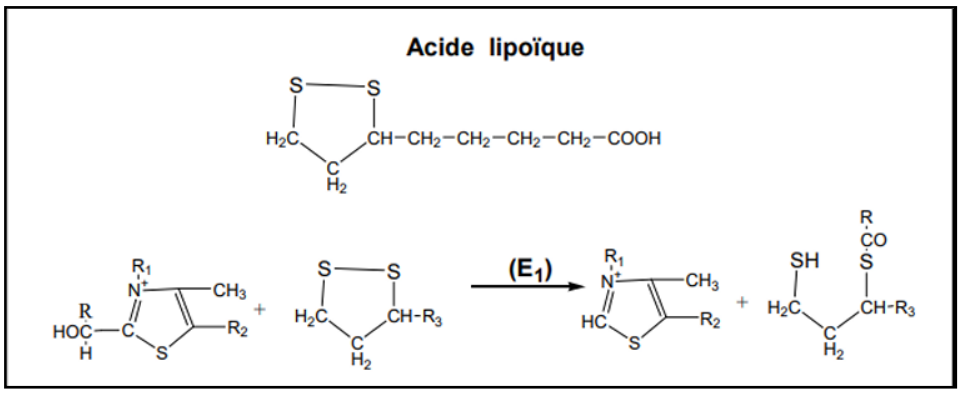
3.4.2.2. Coenzymes that transport radicals with two or more carbons :

Thiamine pyrophosphate (TPP) :

It is derived from thiamine (vitamin B1).
It forms a rosthetic complex with apoproteins. It acts as a coenzyme for enzymes that release R-CO- (acyl) radicals from more complex carbon molecules and transfer them to other coenzymes or substrates.



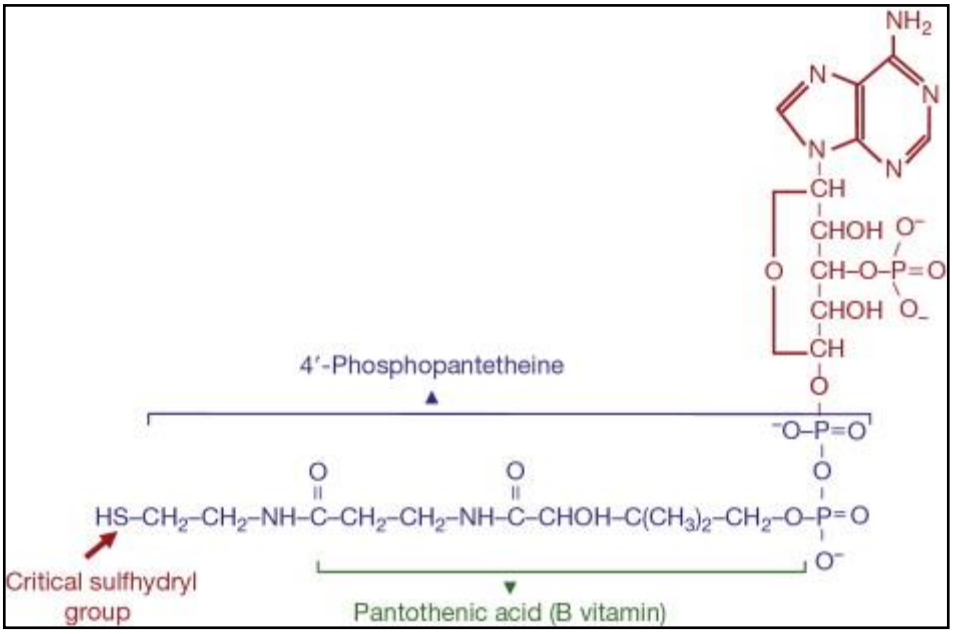
Lipoic acid :



Lipoic acid is a fatty acid consisting of 8 carbon atoms with a disulfide bridge formed between carbons 6 and 8. This coenzyme is strongly bound to the apoenzyme, and the holoenzyme formed following its binding is called lipoamide. It acts as an acceptor of alkyl radicals released by thiamine pyrophosphate.

Coenzyme A (HSCoA) :

Is a coenzyme involved in the transfer of acyl groups in numerous metabolic pathways (Krebs cycle and beta-oxidation). It binds the acyl radical to the thiol function.



3.4.2.3. Coenzymes of aminotransferases :

Pyridoxal phosphate (PLP) :

It is a prosthetic group linked to the apoenzyme by covalent bonds. Its structure is derived from pyridoxine or vitamin B6. It is involved in transamination reactions in both the degradation and synthesis of amino acids.