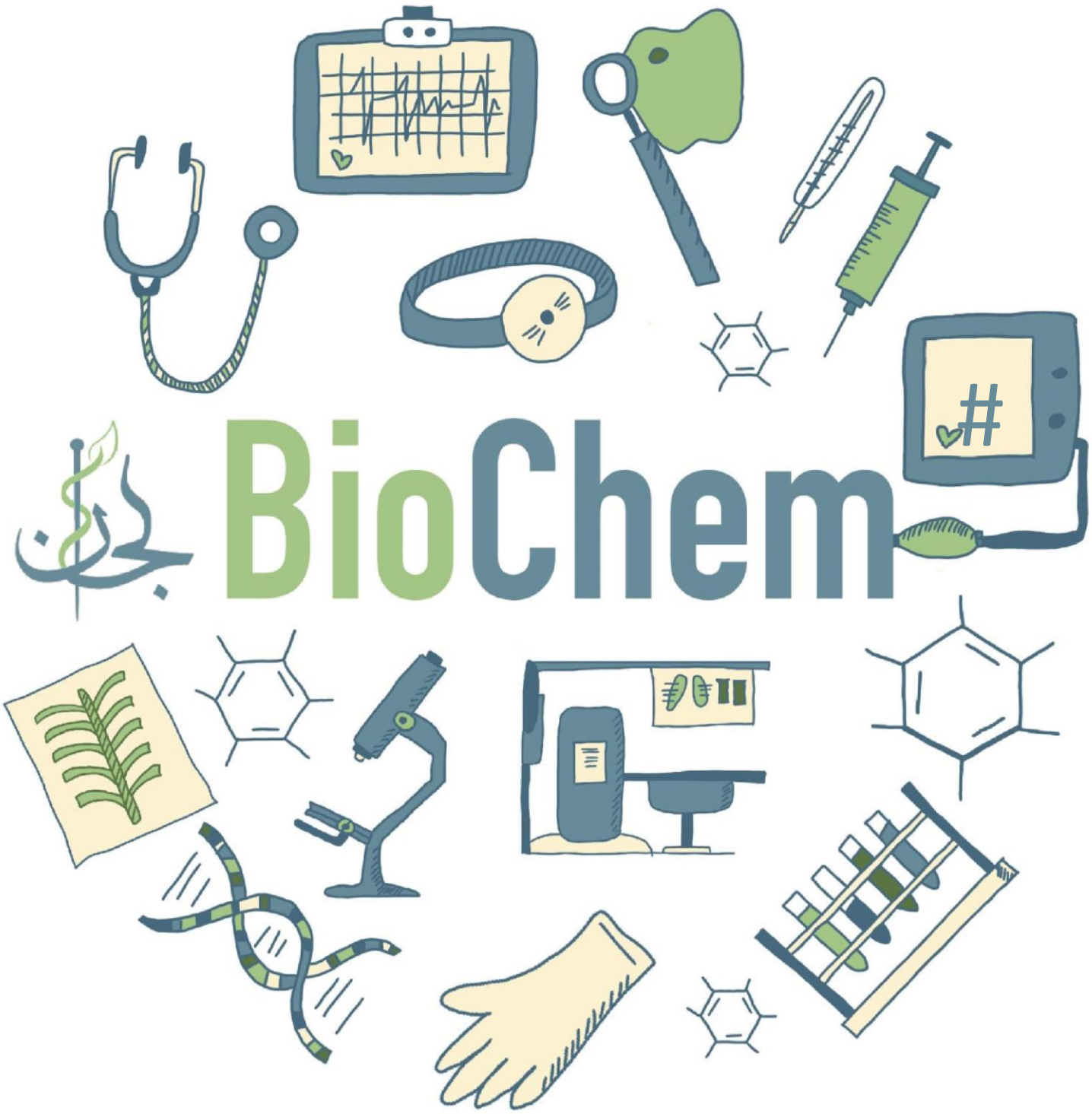

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Enzymes - cofactors

Cofactor: non-protein compounds that participate in the catalytic process, without it the enzyme will be very weak.
Just for knowledge: there are some protein-derived cofactors.
Enzymes are: **Conjugated** (if they can or need to be bounded to cofactor)
Simple (if they just need the help of amino acids sequence in active site)
Holoenzyme: bounded to its cofactor/ **Apoenzyme**: not bound to its cofactor.
Types of cofactors:

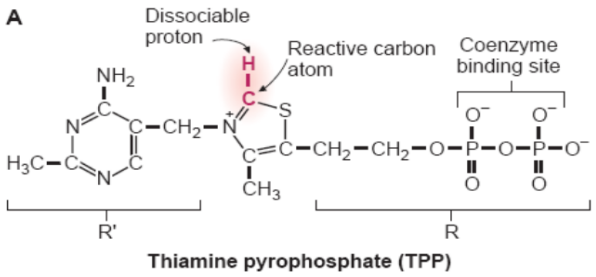
1. Coenzymes→ organic compound many of them are derived from vitamins.
2. Metal ions → Mg^{+2}, Fe^{+2}…
3. Metalloenzymes→ combination of organic and metallic structures.

Coenzymes: **Prosthetic** groups (tightly bound) vs. **Co-substrates** (loosely bound).
Note: In tightly bound state if the coenzymes are being removed the enzyme collapsed(degraded).
Things that help the enzyme to do its function: cofactor & amino acid side chain in the active site {polar and non-polar amino acids}
You will not find active site without polar amino acid (why?)
Because polar amino acids do the catalysis, non-polar amino acids help in binding only.
Justify: Histidine (polar amino acid) is almost in the active site?
The pKa of the R group is too close to the physiological pH (it can donate and accept a proton at neutral PH and participate in acid-base catalysis).
Note: each coenzyme is specific for a type of reaction.
Types of coenzymes: a) Activation transfer coenzymes
b) Oxidation reduction coenzymes: help in Oxidation reduction reactions

A) Activation transfer coenzymes: activate their substrate then they initiate covalent bond with them and substrate.
Depending on structure: 1- **binding group** (Binds tightly to the enzyme)
2- **functional group** (Forms a covalent bond with a portion of substrate/ catalysis).
***Coenzymes depend on the enzyme for the maximum activity (additional specificity of substrate &additional catalytic power), **why** they designed in this way? **Way of regulation**.
Coenzymes usually derived from vitamins (we know 13 until now).
Vitamins: a) lipid soluble (4): A, D, E& K (ADEK) →don't work as coenzymes
b) Water soluble (9): C, B (1, 2, 3, 5, 6, 7, 9& 12), we need them to work as coenzymes (most of them will be modified before working)
(However, vitamin K helps in adding carboxylic group to glutamate residue on clotting factors)

1- Thiamine pyrophosphate (TPP): consists of 2 structures
Pyrophosphate (2 phosphates linked together (negative charges)), thiamine (vitamin B1)
This coenzyme participates in **Decarboxylation reactions**

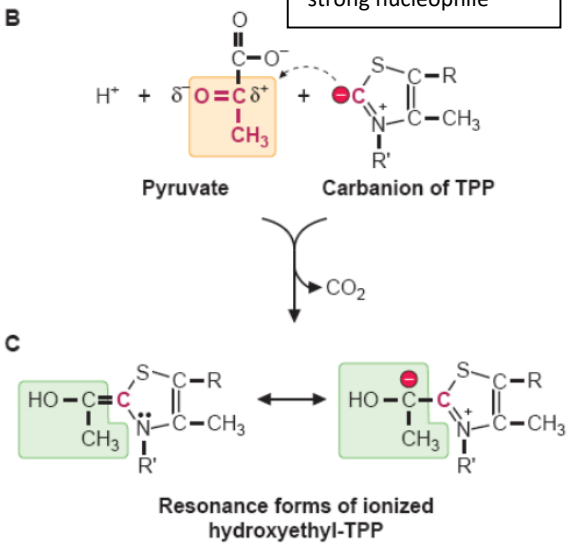
Pyrophosphates provide negatively charged oxygen atoms that tightly bind to the active site, usually via Mg^{+2}) **Binding group**.
In Picture A the carbon (reactive carbon) that next to the nitrogen loses the hydrogen by the action of side chains of amino acids in the active site like histidine.



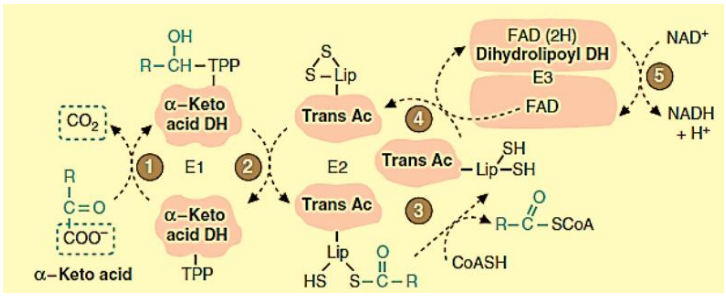
Thiamine pyrophosphate (TPP)

Reactive thiamine carbon (**catalysis/functional group)** forms a covalent bond with a substrate keto group while cleaving the adjacent carbon–carbon bond.
(The highly reactive carbon (red color) attacks the carbon in the carbonyl group so it will dissociate a CO_2).
Every decarboxylase uses the coenzyme TPP
Such as: **pyruvate dehydrogenase** (is the precursor that give Acetyl Co-A) & **α-ketoglutarate dehydrogenase** (with Isocitrate dehydrogenase complex both are in Krebs cycle)
(3 enzymes the 1st is decarboxylase)

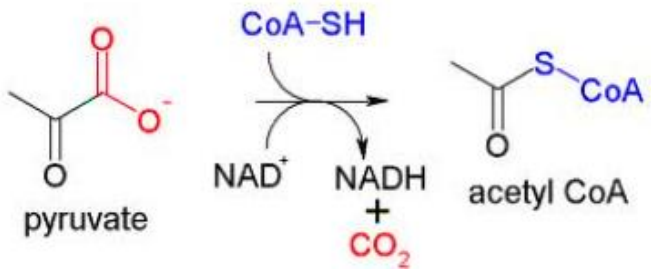
The red carbon is called carbanion and it is a strong nucleophile



α-ketoglutarate dehydrogenase complex:



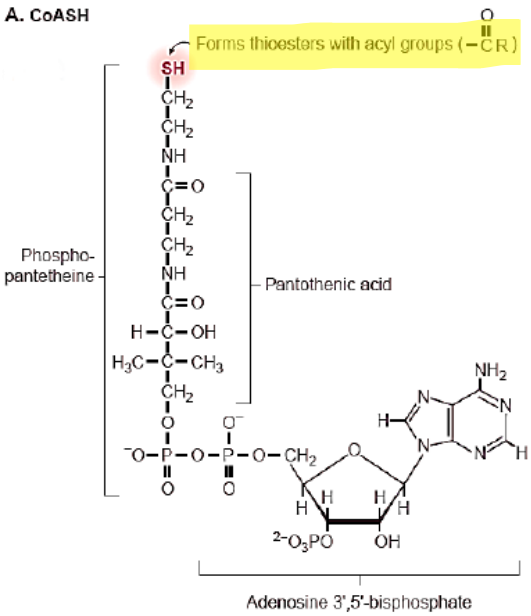
pyruvate dehydrogenase complex:



This is why one of the products of Krebs cycle is CO_2 (the same thing with **pyruvate dehydrogenase complex** the 1st enzyme is decarboxylase).

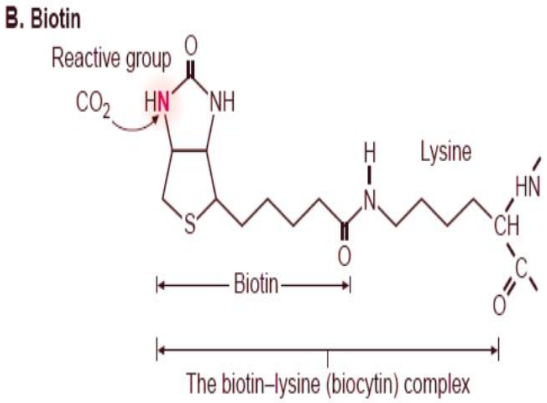
What do you expect to change in blood sample if you have deficiency in vitamin B1?
High concentration of pyruvate. (Pyruvate dehydrogenase won't act very well so there will be more pyruvate that are not decarboxylated and stay as they are).
Just to think: the pH is very controlled, if you have deficiency in vitamin B1 the previous mentioned enzymes won't act very well so pCO2 in the blood will decreased which will cause pH to increase. In the same time more α-ketoglutarate (keto-acids) will cause the pH to decrease.
What cells synthesize TPP?
- Thiamin (vitamin B1) is rapidly converted to its active form, thiamin pyrophosphate, TPP, in the brain & liver.

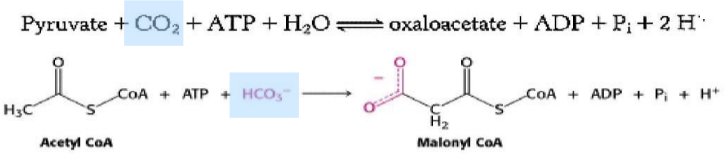
2- Coenzyme-A: is synthesized from the vitamin pantothenate (B5).
adenosine 3', 5' bisphosphate →**binding group** (tight & reversible) + pantothenic acid (vitamin B5) bound to modified cysteine.
The functional (catalysis) group: sulfhydryl group (nucleophile) at the end of the molecule it's always reactive and it attacks to the carbonyl groups & forms acyl thioesters.
Function: acyl (any number of carbon atoms) carrier group.
Coenzymes have nearly constant concentration in the body& in cycle of reactions the coenzyme comes back as it was in the first.



How this coenzyme is different from usual?
Reversible; they are regenerated during the reaction, and they are transformed during the reaction into products that dissociate from the enzyme at the end of the reaction (e.g., CoA is converted to an acyl-CoA derivative, and like [NAD+] that is reduced to NADH.
These dissociating coenzymes are classified as coenzymes and not substrates(why?)
- Common to so many reactions
- The original form is regenerated by subsequent reactions
- Synthesized from vitamins
- The amount in the cell is nearly constant

3- Biotin: (vitamin B7) in the active site it binds covalently with lysine and becomes **biocytin**.
We use it in carboxylation reactions.
(The opposite function of TPP)
Reactive (functional) group: nitrogen covalently bound to lysine in energy requiring reaction.





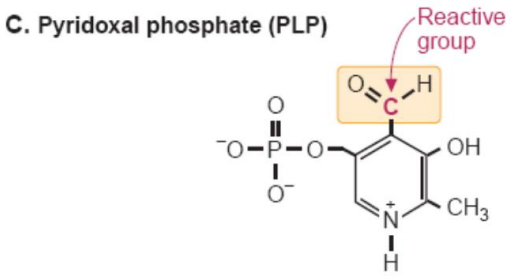
CO2 and HCO_3^- have the same effect in this reaction

It's synthesized in our bodies (not in the cells) but in the bacteria (normal flora)
Because of that it's hard to get deficiency in biotin unless you do:

- Long antibiotic therapies because that kill the intestinal bacteria.
- Excessive consumption of raw eggs (egg white protein, avidin, high affinity for biotin)

Enzymes that use it: Pyruvate **carboxylase**& Acetyl CoA **carboxylase** (fatty acid synthesis)
With carboxylases you need energy.

4- PLP: (Synthesis: Pyridoxine B6) it's modified in our bodies in which phosphate attach to it, so it'll be pyridoxal phosphate.



Phosphate group is binding group

Carbonyl group is the **catalysis group**

*It works with transaminases.

Mechanism: Reactive aldehyde forms a covalent bond with the amino groups, Ring nitrogen withdraws electrons from bound amino acid (cleavage of bond)

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

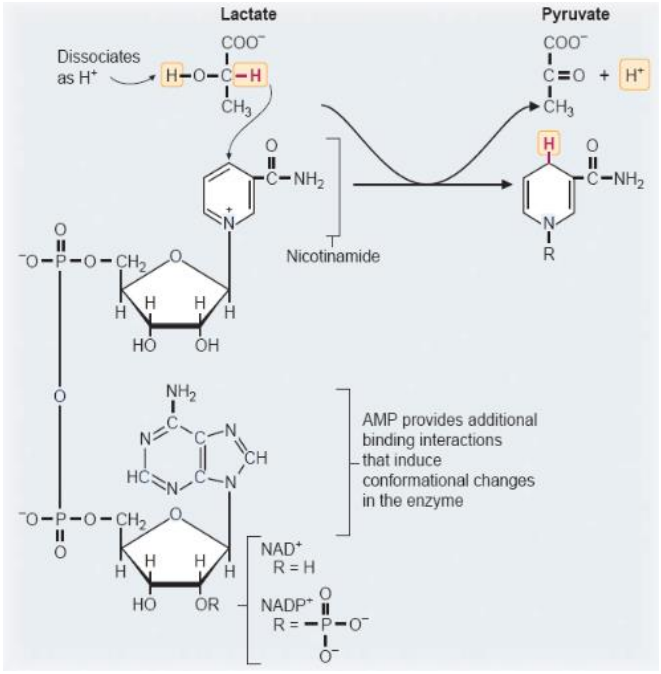
$$\text{Aspartate} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{oxaloacetate} + \text{glutamate}$$

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

B) Oxidation reduction coenzymes:

This type of coenzymes do not form covalent bond with substrate, and it depend on the enzyme for additional specificity of substrate and additional catalytic power.

1- NAD+: (Nicotinamide adenine dinucleotide), as its name states it has 2 nucleotides.



$NADP^+$= NAD^+ with phosphate group

(Look to the picture to understand)

NAD^+ take out 2 electrons in the form of hydride ions (H^-).

It comes with dehydrogenases

Nicotine ring takes H- from the substrate so the other H (which is +) can dissociate

Histidine can contribute and take the H+, so the mission of nicotine ring becomes easier.

By using it we can convert alcohol into aldehyde or ketone (depending on the type of alcohol (primary or secondary respectively)

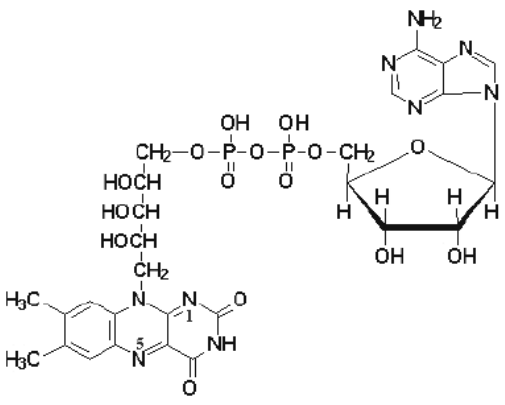
Why do I need both NAD^+& $NADP^+$?

For regulation purposes, NAD^+ (energy metabolism), $NADP^+$ (with toxic materials& building)

Catalysis group: nicotinic ring (niacin, vitamin B3)

Binding group: ADP portion.

2- FAD& FMN: (Flavin adenine dinucleotide), adenine & flavin both have N in their rings. Flavin structure have 2 free (N) which can participate in the reactions. Source: **Riboflavin (B2)**



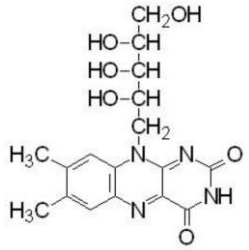
Catalysis group: flavin (I can add electrons on its N)

Binding group: ADP partition.

Both nitrogen atoms can accept 1 electron in the form of H atom.

FAD→FADH→$FADH_2$ (step by step not like NAD^+)

FMN= flavin mononucleotide. Can be $FMNH_2$
We need both FAD& FMN for regulation purposes.
Enzymes that uses them:
Succinate dehydrogenase& Pyruvate dehydrogenase complex



Just flavin (FMN)

Note: FADH& FMNH state is free radical& toxic in the cell, they're always hidden in the cell (to be sure that they are not free to go and hit other structures), but NAD^+ is free in the cell

Water-Soluble Vitamins

Name	Coenzyme or Active Form	Primary biochemical function
Thiamin	Thiamine pyrophosphate (TPP)	Aldehyde-group transfer
Riboflavin	Flavin mononucleotide (FMN)	Hydrogen-Atom (electron) transfer
	Flavin adenine dinucleotide (FAD)	Hydrogen-Atom (electron) transfer
Nicotinic Acid	Nicotinamide adenine dinucleotide (NAD)	Hydrogen-Atom (electron) transfer
	Nicotinamide adenine dinucleotide phosphate (NADP)	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

= in the next semester.

Catalytic metals:

Metals in the enzymes can be: a) tightly bound: metalloenzymes

b) Loosely bound: (metal-activated enzymes)

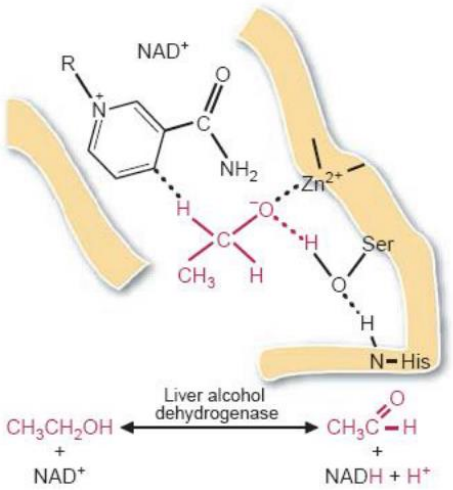
Metal	Enzyme
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	Hexokinase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase

For example: I) Alcohol dehydrogenase (ADH)

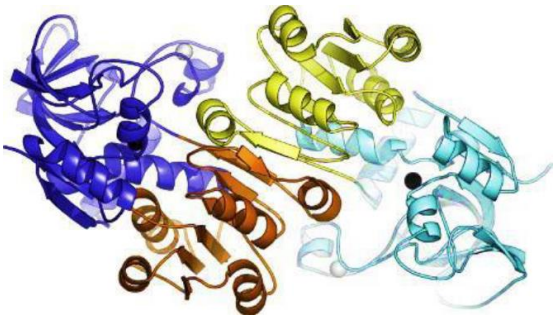
Uses alcohol as a substrate (ethanol), uses NAD+ as coenzyme (default)

Histidine takes H from serine, serine becomes reactive and takes H from alcohol, OH group becomes O^- so it will attract to Zn^{+2} (has a role in stabilizing the substrate and bind with it), so the weakest bond will be broken (C-H) and H is taken by NAD^+ to be NADH.

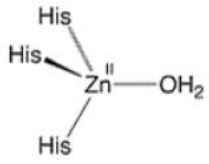
II) Phosphofructokinase & TPP; (Mg^{+2}) is required to coordinate the phosphate groups on the ATP for a successful reaction (chelation)



Metalloenzyme: removal of the metal causes denaturation. These metal ions may contribute either to the structure or the catalytic mechanism.



Liver alcohol dehydrogenase (dimer); 2 Zn^{+2} in each monomer; one for structural maintenance (joins the two subunits), the other is catalytic



$[NNN]Zn^{II}–OH_2$

Carbonic Anhydrase

Carbonic anhydrase; A zinc atom is essentially always bound to four or more groups.

SHORT QUIZ

1. An organic substance bound to an enzyme and essential for the activity of enzyme is called:

(A) Holoenzyme
(B) Apoenzyme
(C) Coenzyme
(D) Isoenzyme

2. Combination of apoenzyme and coenzyme produces:

(A) Prosthetic group
(B) Holoenzyme
(C) Enzyme substrate complex
(D) Enzyme product complex

3. Vitamins can act as coenzymes that participate in catalysis by providing functional groups. Therefore, vitamin deficiencies reflect the loss of specific enzyme activities that depend on those coenzymes. Coenzymes are best described by which one of the following?

A. In humans, they are always synthesized from vitamins. B. They are proteins.
B. They participate in only one reaction, like enzymes.
C. They are complex, non-protein organic molecules.
D. They are all carbohydrates

Q1	Q2	Q3
C	B	C

دعواتكم لنا بظهر الغيب