بسم الله الرحمن الرحيم

BioChemistry | FINAL 11

Enzymes
pt. 3

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CATALYSIS

FUNCTIONAL GROUPS IN CATALYSIS

- In enzymes: there has to be functional groups of amino acids inside the active site in order to do their job.
- **Not all enzymes rely on their active site for catalysis (chymotrypsin vs. conjugated enzymes)**
- **Conjugated: coenzymes, metal ions, & metallocoenzymes**

A. Functional Groups on Amino Acid Side Chains:

-Almost all polar amino acids(either charged or uncharged) **(nucleophilic catalysis)**

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

-Histidine: pKa, physiological pH & acid–base catalysis Histidine as a residue is found in many enzymes' active site since the pKa of its side chain (6.5-7.4) is close to the physiological pH. (residue here means that the amino acid is bound, which means that the carboxyl group and the amine group of the amino acid are bound and no longer free, which means they don't play a role in the ionization process, only the side chain does). (histidine, when it isn't a residue, has a side chain pKa of 6)

-Some enzymes are able to do their job using only their amino acid sequence, while others require the help of cofactors. Cofactors that are organic in their nature are called **coenzymes**

B. Coenzymes in Catalysis

- **Usually (but not always) synthesized from vitamins**
- **Each coenzyme is specific for a type of reaction**

- **They are either:**

* **Activation-transfer coenzymes**

* **Oxidation–reduction coenzymes**

Enzymes-cofactors

Enzyme cofactors

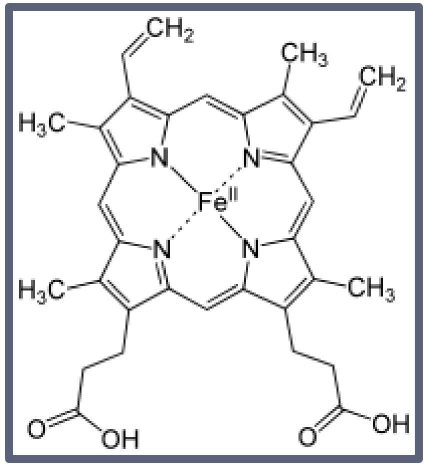
- Apoenzyme vs. holoenzyme
- Organic (coenzymes)
- Prosthetic groups (tightly bound) vs. co-substrates (loosely bound)

- **Enzymes**
  - **Cofactors**
    - Metal
    - Organic
    - Organometallic
      - **Heme**
    - **Protein-derived**
      - Cross-linked amino acid
      - Stable free radicals
      - **Quinones**
        - TPQ
        - LTQ
        - **TTQ**
        - CTQ

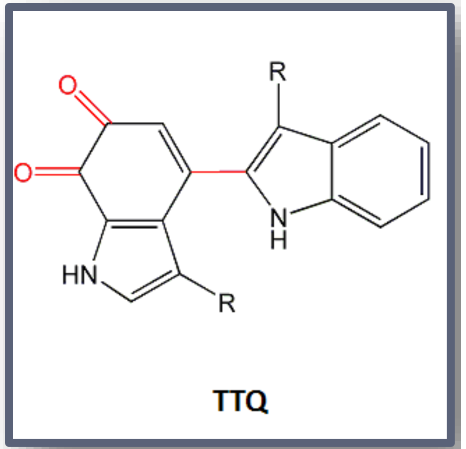
metals participate in the catalytic process

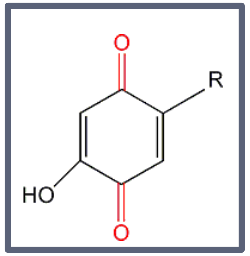
Structure is organic only

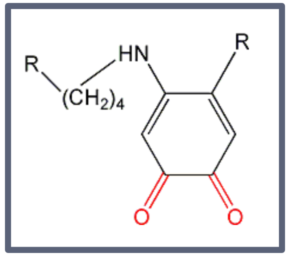
Protein derived is when protein is modified into amino acids that function as coenzymes (it's not required now)

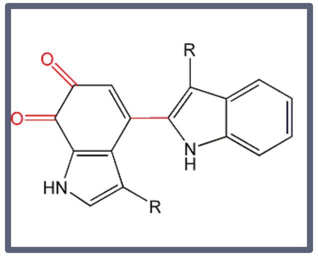


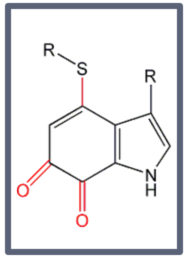
Tryptophan-tryptophyl quinone











ACTIVATION-TRANSFER COENZYMES

- Usually participate directly in catalysis by forming a covalent bond (during the catalytic process, this covalent bond will be broken down again)

- Characteristics:
  - Two groups in the coenzyme:
    - Forms a covalent bond (functional group) (responsible for the catalytic process)
    - Binds tightly to the enzyme (binding group)
  - Dependence on the enzyme for additional specificity of substrate & additional catalytic power

- Coenzymes are organic materials that are derived from vitamins.
(**vitamins themselves don’t act as coenzymes**, since they need to be modified, apart from vitamin C which does not require modification in order to function as coenzyme)

For each coenzyme, we shall know the original vitamin, the functional form of this coenzyme (the modification that occurred, and which enzymes it helps and which reactions it participates in)

- Properties of Vitamins are organic molecules that we can’t synthesize in our bodies and they are needed in small amounts (healthy food is sufficient, since enzymes are already found in small amounts).

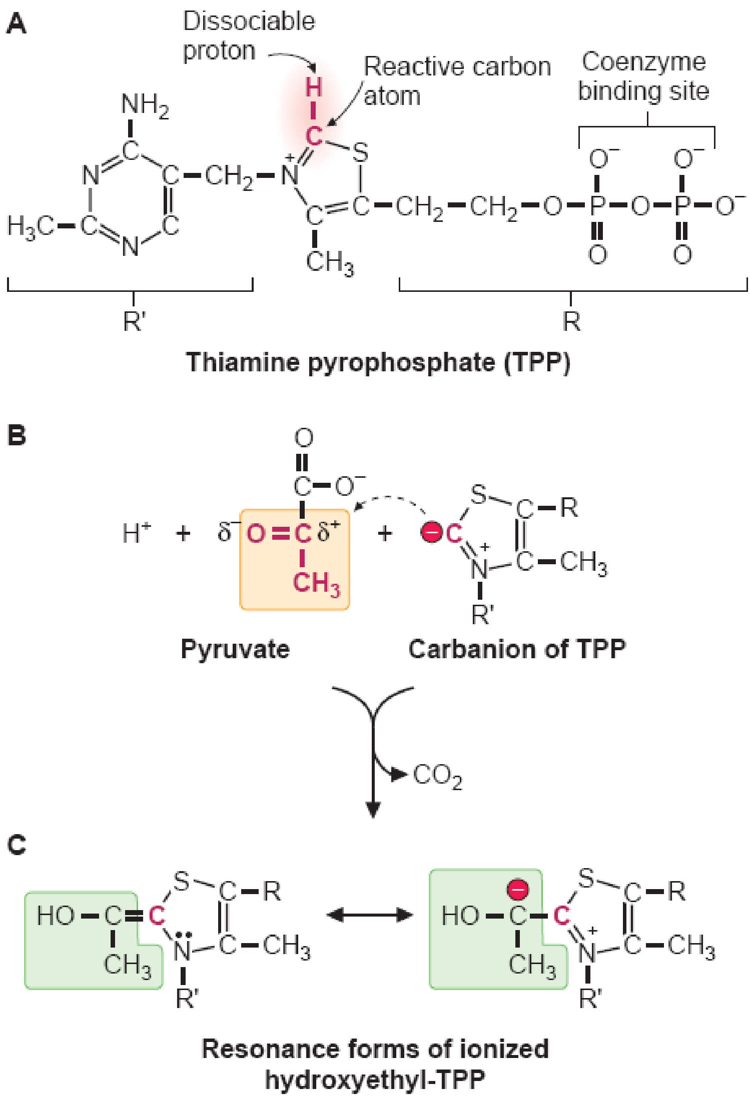
- **There are 13 discovered vitamins** which are classified into 2 types:

-**water soluble:** (9 types: B1,B2,B3,B5,B6,B7,B9,B12,C) (function as coenzymes)
-**lipid soluble:** (4 types: A,D,E,K) (don’t function as coenzymes except vitamin K)
Vitamins B9 and B12 will be discussed @ *next semester* ☺

ACTIVATION-TRANSFER COENZYMES

1 - TPP

- Thiamine pyrophosphate [modified form of thiamine(vitamin B1)]
- Source: thiamine (B1) (original vitamin)
- Decarboxylation reactions (all decarboxylation reactions require TPP to be bound to the enzyme)
- Pyrophosphate (2 phosphate groups connected to each other, these 2 phosphates are responsible for binding):
  - Provides negatively charged oxygen atoms
  - Chelate Mg^{2+} (tight binding)
- Thiamine ring is responsible for catalysis
- Functional group (reactive carbon atom)
- Reactive thiamine carbon forms a covalent bond with a substrate keto group while cleaving the adjacent carbon– carbon bond
- Carboxylic carbon is found terminally so the adjacent carbon will get bound which weakens the bond between this carbon and the carboxylic carbon, so the carboxylic group will leave the molecule as CO2



Thiamine pyrophosphate (TPP)

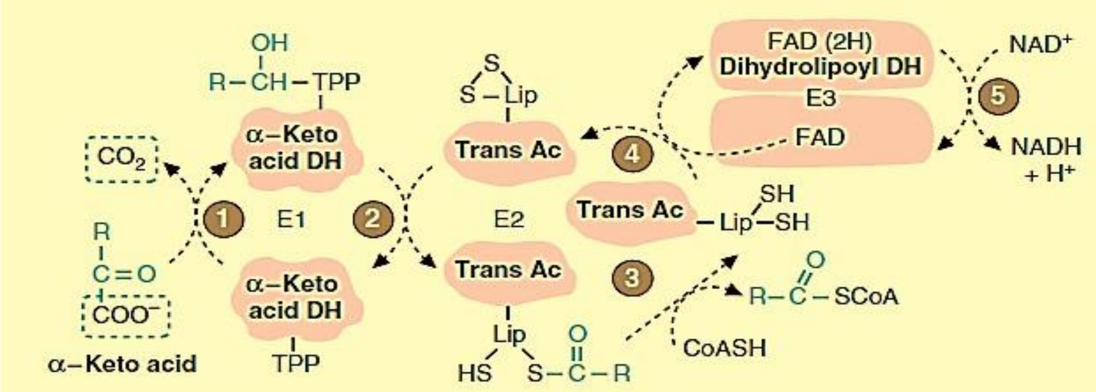
Pyruvate

Carbanion of TPP

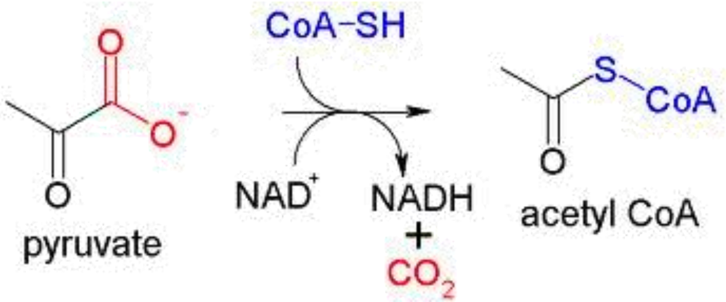
Resonance forms of ionized hydroxyethyl-TPP

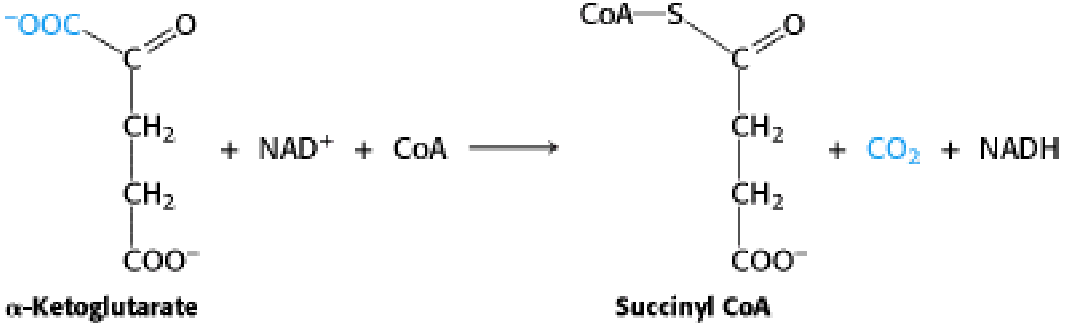
Thiamin (vitamin B1)

- Thiamin (vitamin B1) is rapidly converted to its active form, thiamin pyrophosphate, TPP, in the brain & liver
- Required by pyruvate dehydrogenase & α-ketoglutarate dehydrogenase



This is a big complex, at the level of enzyme 1, it is connected to TPP in order to have a decarboxylation reaction which release CO2.





- **Thiamine** as a vitamin could have a deficiency. Alpha ketoglutarate dehydrogenase, or pyruvate dehydrogenase, Alpha Ketoacid Dehydrogenase are 3 different complexes found inside the body that function differently, but all produce similar product in the end because they use the same mechanism
  (all use TPP mechanism for decarboxylation reaction).
- In glycolysis, glucose is converted into pyruvate (3 carbons). Pyruvate dehydrogenase then converts pyruvate into acetyl-CoA (2 carbons) by removing the carboxylic group from pyruvate.
- In Krebs cycle, 2 CO2 molecules are released through the same mechanism
- **Pyruvic acid** (حمض الحصرم) is found a lot in grapes

ACTIVATION-TRANSFER COENZYMES

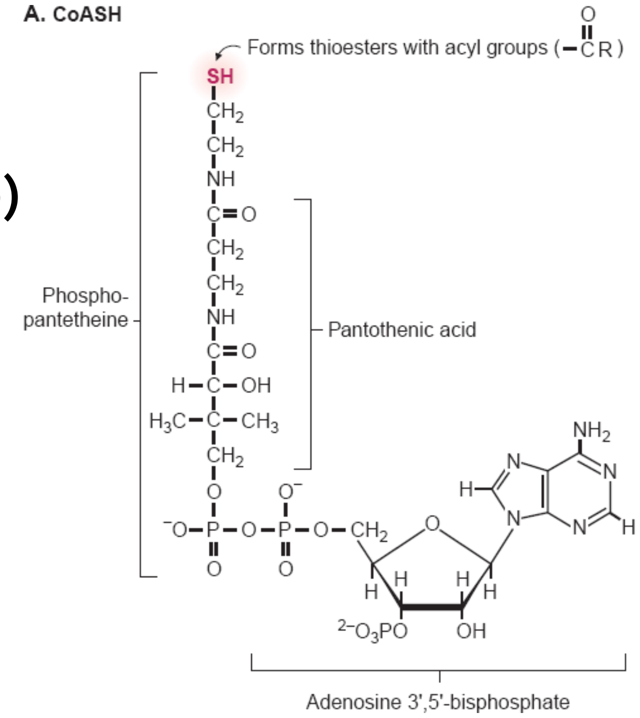
2 - Coenzyme A (CoA)

- Source: pantothenate (B5) (original vitamin)

- The modified form (Coenzyme A) is made from vitamin B5 connected to adenosine diphosphate as well as modified cysteine.

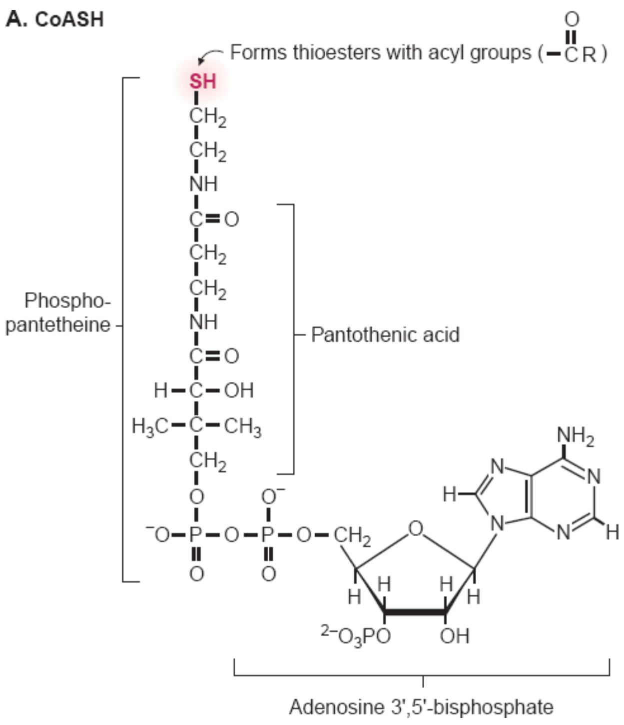
- Binding group: adenosine 3′,5′-bisphosphate (tight & reversible)
- Functional group: sulfhydryl group (nucleophile)
  - Attacks carbonyl groups & forms acyl thioesters (the “A”)

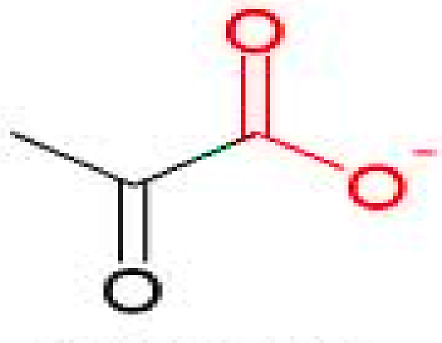
- How it is different from usual? (regeneration & acyl-CoA derivative)
- Like some others (NAD^+), why do they call them coenzymes?
  - Common to so many reactions
  - The original form is regenerated by subsequent reactions
  - Synthesized from vitamins
  - The amount in the cell is nearly constant



Coenzyme A: (Acetyl-CoA, Propionyl-CoA, Tiglyl-CoA, Malonyl-CoA), all have the following structure, what is special about them is having the thiol group (originally from cysteine) which is the catalytic part of Coenzyme A, while adenosine is responsible for binding to the enzymes. In order for the CoA to react, the hydrogen leaves and the sulfur binds to the carbons of interest. CoA is responsible for the transfer of highly reactive carbons.

- For example: When pyruvate dehydrogenase acts on pyruvate, it removes a terminal carboxyl group leaving behind a terminal carbonyl group, which is unstable, that is why CoA binds to it to stabilize it and transfer it through solutions. The formed bond between the sulfur and the carbon is a highly energetic bond (such as between phosphorus and oxygen, or phosphorus and carbon). That means when CoA detaches from carbon, high energy is released, which can be stored elsewhere

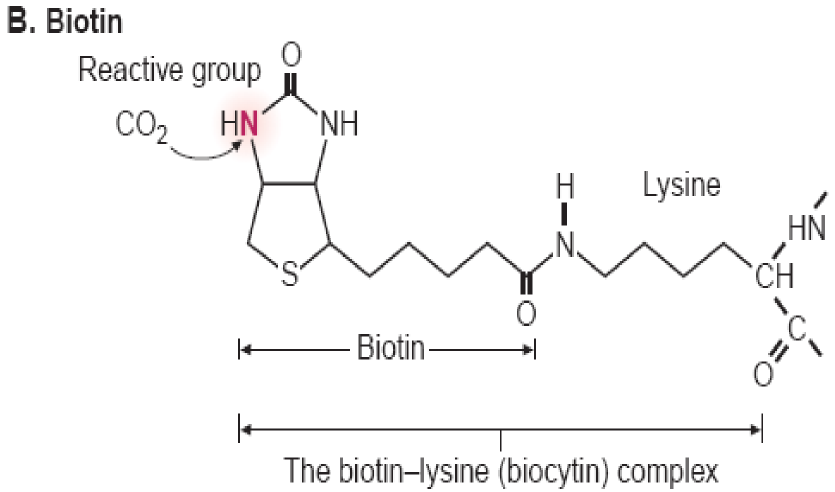




pyruvate

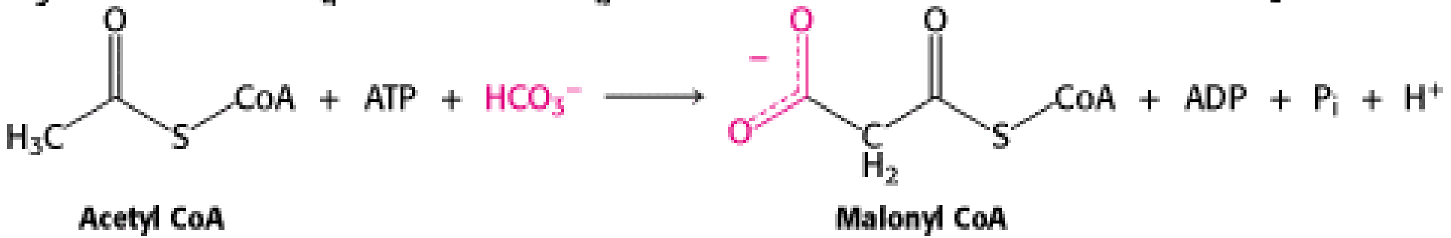
ACTIVATION-TRANSFER COENZYMES

3 – Biotin (B7)



- **Biotin** (original vitamin) **is required for carboxylation reactions (covalently bound to Lys in the active site)**
- The active form after modifications is called biocytin.
- Carboxylation reactions is considered to be in the ligases class
- **Source: food & intestinal bacteria** →rarely will there be deficiency in biotin because even if we don't get it from food there is another source which is intestinal bacteria
- However, **Deficiencies are generally seen** if the following occurs:
  - **Long antibiotic therapies** →This kills normal flora which is found in the intestines, it is responsible for producing biotin
  - **Excessive consumption of raw eggs** (egg white protein, avidin,→has high affinity for biotin)
  - People who consume raw eggs, they will get the protein avidin which goes into intestines and binds to biotin. So biotin won't get absorbed, but when we cook eggs, this protein will get denatured, so it no longer has affinity to biotin
- **Pyruvate carboxylase**
- **Acetyl CoA carboxylase (fatty acid synthesis)**

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

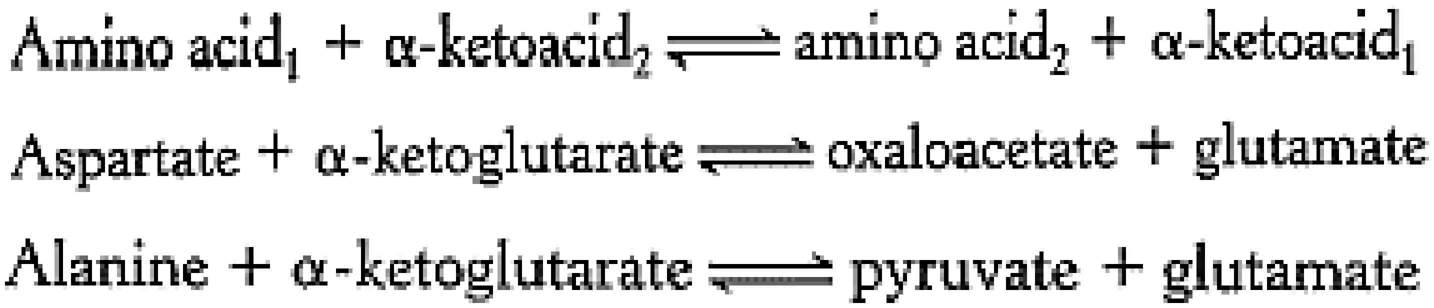


- There are 2 types of transferase enzymes:

-Kinase: it transfers phosphate group

-Aminotransferase (transaminase): it transfers amino groups.

- We get these amino groups from amino acids. When an amino acids releases its amino group, a keto group forms instead of the amino group forming keto acid. This amino group transfers to a keto acid forming an amino acid.



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

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

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

- We are requited to know 3 amino acids and their corresponding keto acids.

-Alanine (amino acid) (3 carbons)←→pyruvic acid (keto acid)

-Aspartic acid (amino acid) (4 carbons)←→oxaloacetate (keto acid)

-Glutamic acid (amino acid) (5 carbons)←→alpha ketoglutarate (keto acid)

(i.e. alanine loses amino group it becomes pyruvic acid,When aspartic acid loses its amino group,it becomes oxaloacetate and so on)

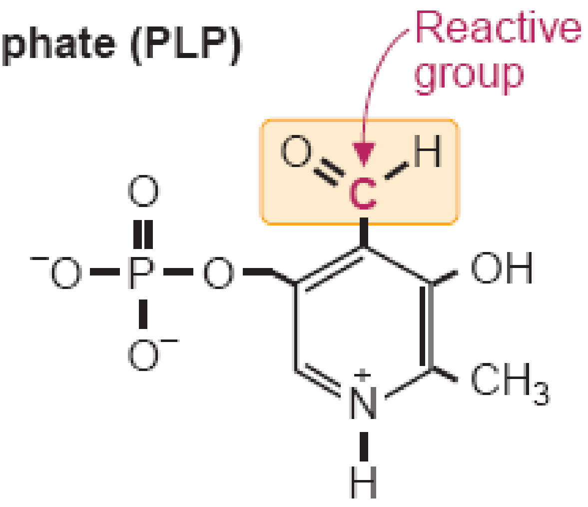
Oxaloacetate and alpha ketoglutarate are important molecules in Krebs cycle

ACTIVATION-TRANSFER COENZYMES

4 - PLP

- **Synthesis: Pyridoxine (B6)** **(Original vitamin)**
- It gets modified into pyridoxal phosphate(PLP)
- (vitamin B6 converted into aldehyde and added phosphate)
- The binding group is phosphate group
- The catalytic group is the ring found in the pyridoxine
- **Functions in the metabolism of amino acids (transaminases) (Responsible for converting amino acid into keto acid and converting keto acid into amino acid)**
- **Reversible reactions**
- **Mechanism:**
  - **Reactive aldehyde forms a covalent bond with the amino groups**
  - **Ring nitrogen withdraws electrons from bound amino acid (cleavage of bond)**

C. Pyridoxal phosphate (PLP)



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

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

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

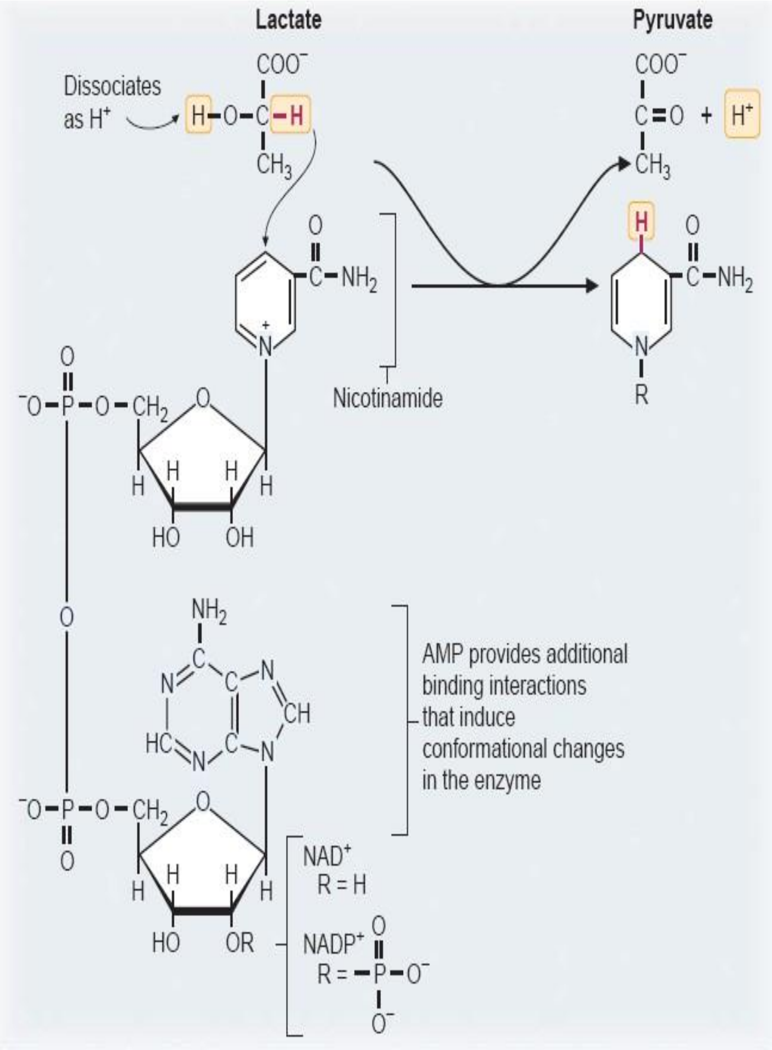
OXIDATION–REDUCTION COENZYMES

- **A large number of coenzymes**
- **Do not form covalent bonds with the substrate**
- **Most common: NAD$^+$ (niacin, B3) & FAD (riboflavin, B2)**
- **Riboflavin** (original vitamin) **gets modified into Flavin Mononucleotide(FMN) and Flavin Adenine Dinucleotide (FAD)**
- **Niacin (Nicotenic acid)** (original vitamin) **gets modified into Nicotinamide Adenine Dinucleotide (NAD+)**
- **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**

OXIDATION–REDUCTION COENZYMES

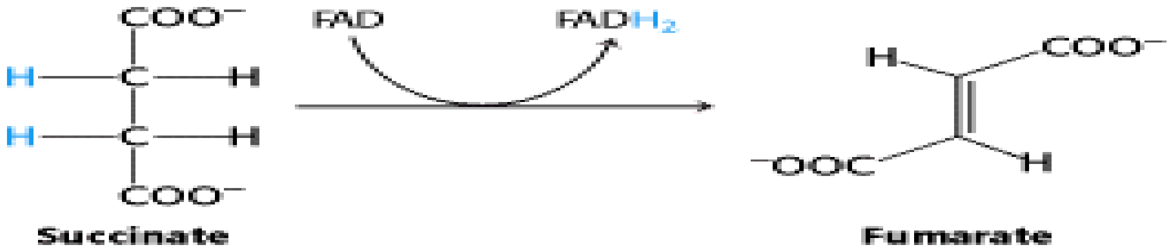
1 – NAD$^+$

- In NAD, there are 2 nucleotides (Nicotinamide and adenine)
- NAD+ functions as an electronic carrier, where electrons are added to the nicotinic ring.
- When NAD+ gets converted into NADH, it binds to one hydride ion (H-) which contains 2 electrons
- NAD+ is responsible for oxidation-reduction reactions
- NADP+ is similar in structure to NAD+, the difference is an additional phosphate group attached to the oxygen of carbon no.2 in ribose. NAD+ has hydrogen there.
- There is no difference between NAD+ and NADP+ in terms of function (Both carry 2 electrons on their Nicotinamide ring), however there is a difference between them for regulation purposes (i.e. difference in binding)
- Functional group (C opposite to N)
- Accepts a hydride ion
- The H$^+$ from substrate dissociates, & a keto group (CO) is formed
- (ADP) portion of the molecule binds tightly
- The role of enzymes' Histidine

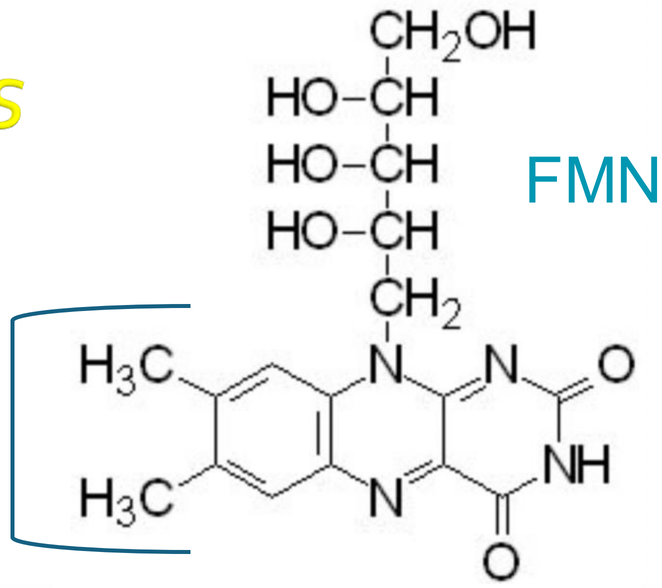


OXIDATION–REDUCTION COENZYMES
2 – FAD & FMN

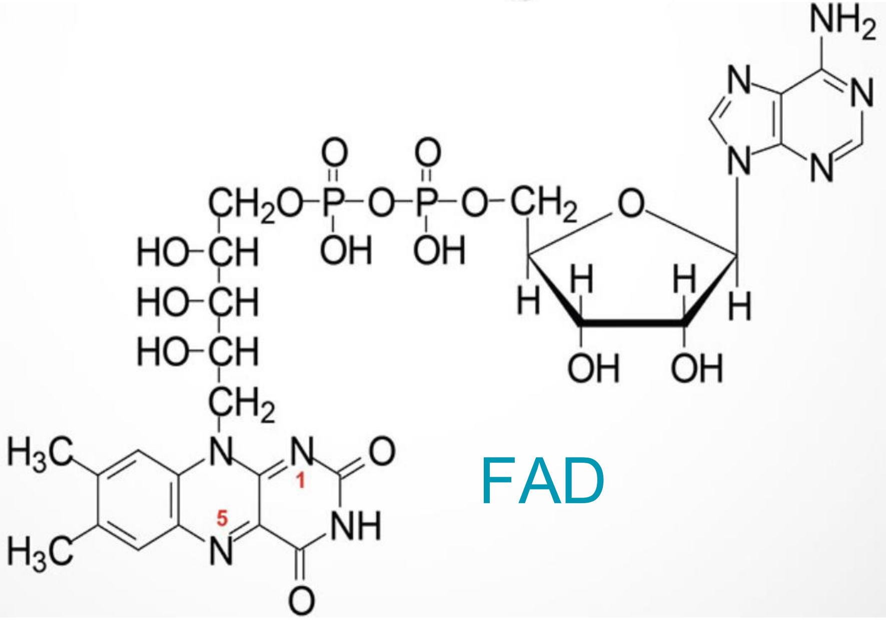
- Source: Riboflavin (B2)
- FMNH$_2$ and FADH$_2$
- Flavoproteins
- FAD has 2 nucleotides (flavin and adenine)
- FMN (Flavin mononucleotide) has 1 nucleotide (without adenosine)
- FAD and FMN both work as electron carriers (oxidation-reduction coenzymes). The 2 nitrogens found in the flavin ring are the ones who carry electrons in the form of hydrogen. FAD and FMN don't differ in function (they both receive 2 hydrogen ions becoming FADH2 and FMNH2 so they both get 2 electrons(one electron on each nitrogen)) however there is a difference between them for regulation purposes (i.e. difference in binding)
- FAD and FMN are prosthetic groups (tightly bound)
- FAD and FMN both receive their electrons from 2 different sources, hence they both might attach to only one electron, at this stage, it is considered a free radical, so they both might be harmful. (Unlike NAD+ which doesn't go through the one electron state.)
- This means that FAD and FMN are never found freely in the solution which means we can never measure its concentration. Instead, they are found inside proteins/enzymes where they can do their function. NAD can be found freely inside the solution (it binds to enzyme, does its function, then return to the solution.
- Succinate dehydrogenase
- Pyruvate dehydrogenase complex



Flavin ring(rings containing nitrogen)→

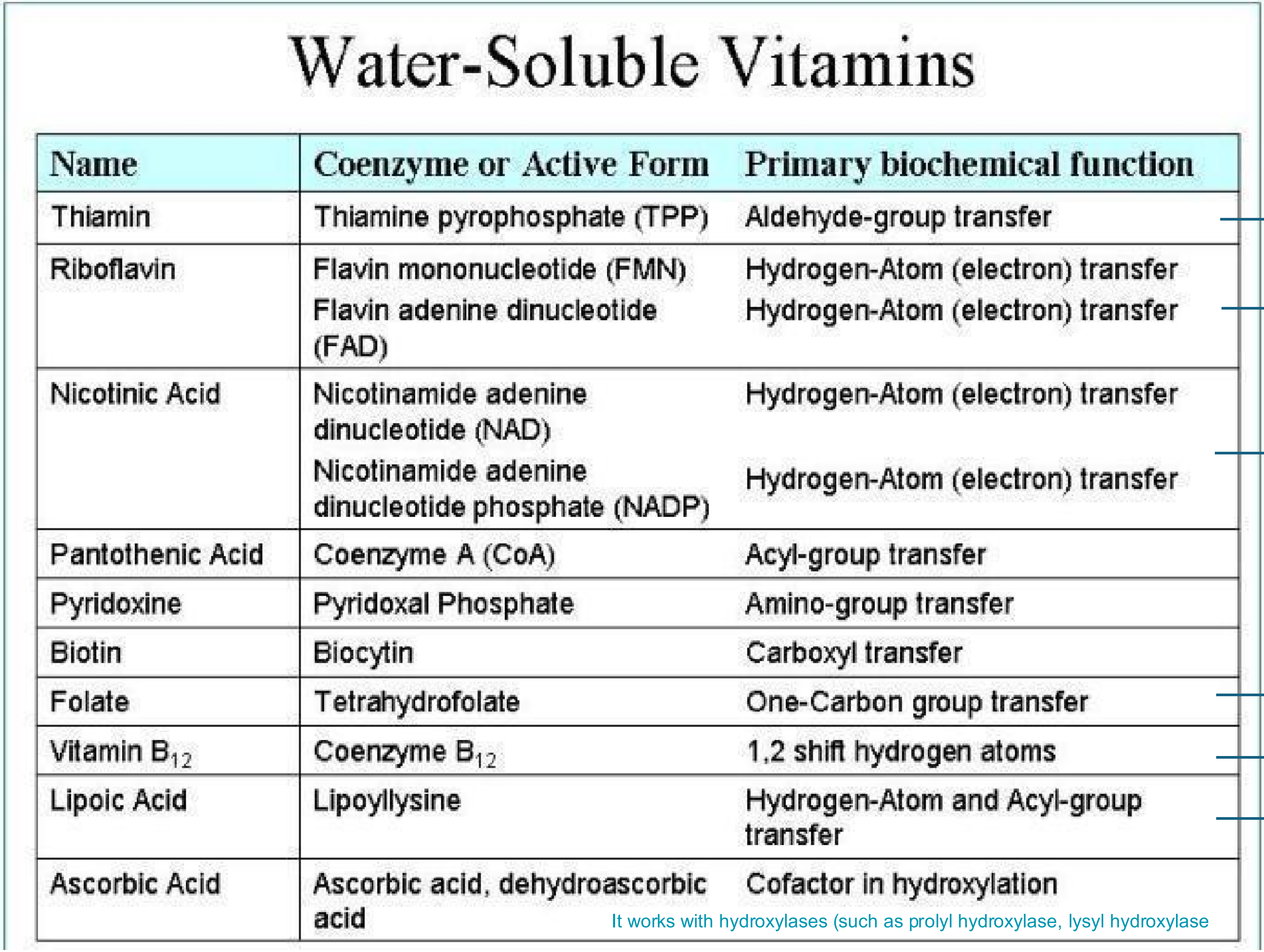


FMN



FAD

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

Works in decarboxylation reactions

Works in oxidation-reduction reactions

Works in oxidation-reduction reactions

Will be discussed next semester

Will be discussed next semester

Will be discussed next semester

It works with hydroxylases (such as prolyl hydroxylase, lysyl hydroxylase

Catalytic Metals

- **Metals can be tightly bound (metalloenzymes) or loosely bound (metal-activated enzymes)**
- In metalloenzymes (tightly bound enzymes), removing the metal will denature the enzyme, so the metal is here for both structural and catalytic purposes
- While in metal-activated enzymes (loosely bound enzymes), removing the metal → the structure of the enzyme is preserved
- **Acting as electrophiles**
- **Metal-activated enzymes; the metal either required or enhances activity (Mg^{2+}, Mn^{2+}, Ca^{2+}, & K^{+})**
- **Phosphofructokinase & TPP; (Mg^{2+}) is required to coordinate the phosphate groups on the ATP for a successful reaction (chelation)**

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

Memorize this table, make sure u know the metal and its corresponding enzyme

Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP

Catalytic Metals

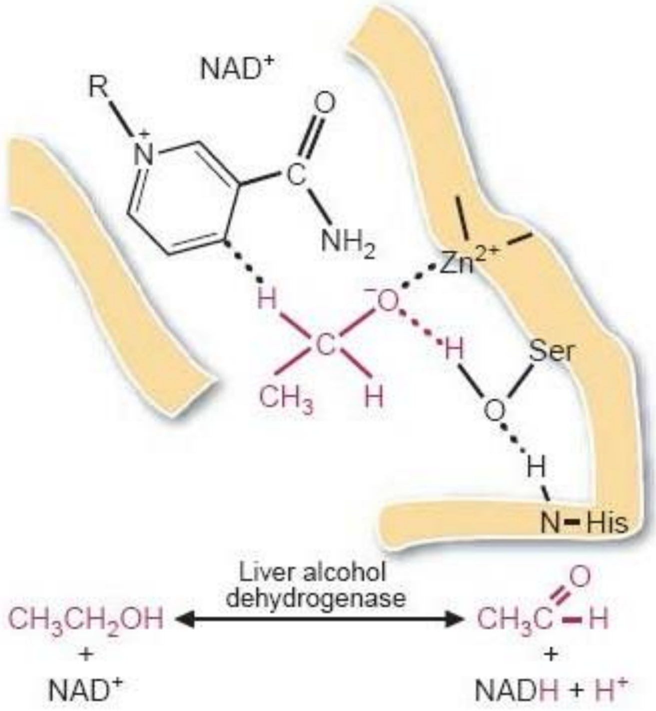
- Examples of catalytic metals that can be found in enzymes are: calcium, iron, zinc, copper
- **Alcohol dehydrogenase (ADH)**: it performs dehydrogenation of alcohols (oxidation of alcohols). Any dehydrogenation reaction has NAD+ as coenzyme (unless otherwise specified)
- During dehydrogenation of ethanol, serine will pull the hydrogen from the alcohol causing the oxygen to be more negatively charged. Thus the zinc will stabilize this oxygen causing the hydrogen to detach. This will cause the hydrogen bonded to the carbon to be more loose and will detach along with its 2 electrons to the NAD+ forming NADH. Then the carbon and oxygen will form a double bonds between them forming aldehyde

- Activated serine (pulls a proton off –OH)

- Oxyanion is stabilized by zinc

- Transfer of a hydride ion to NAD^+

- Zinc in ADH as His in lactate dehydrogenase



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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
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Additional Resources:

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نحن بحاجة للطّبيب المُخلص
نحن بحاجة للطّبيب الصّالح
نحن بحاجة للطّبيب المُؤنس
حتّى تصبح الفئة الفاسدة من الأطبّاء
هي القلّة لا تغلب الكثرة..

- الأستاذ قصيّ العسيلي، بتصرُّف