

# Enzymes-cofactors

# FUNCTIONAL GROUPS IN CATALYSIS

- Do all enzymes rely on their active site only? (chymotrypsin vs. conjugated)
- Conjugated: coenzymes, metal ions, & metallocoenzymes
  - Apoenzyme vs. holoenzyme
  - Organic (coenzymes): Prosthetic groups (tightly bound) vs. co-substrates (loosely bound)
- A. Functional Groups on Amino Acid Side Chains:
  - Almost all polar amino acids (nucleophilic catalysis)
  - Ser, Cys, Lys, & His can participate in covalent catalysis
  - Histidine: pKa, physiological pH & acid–base catalysis
- 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

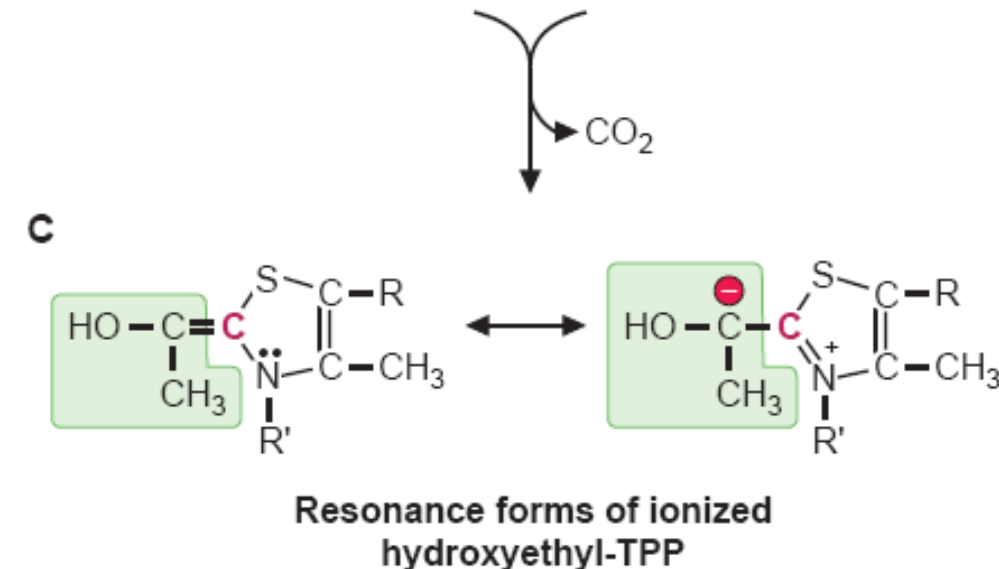
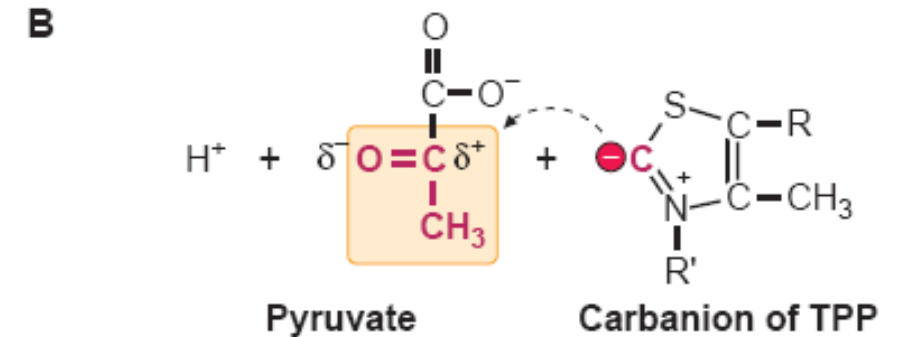
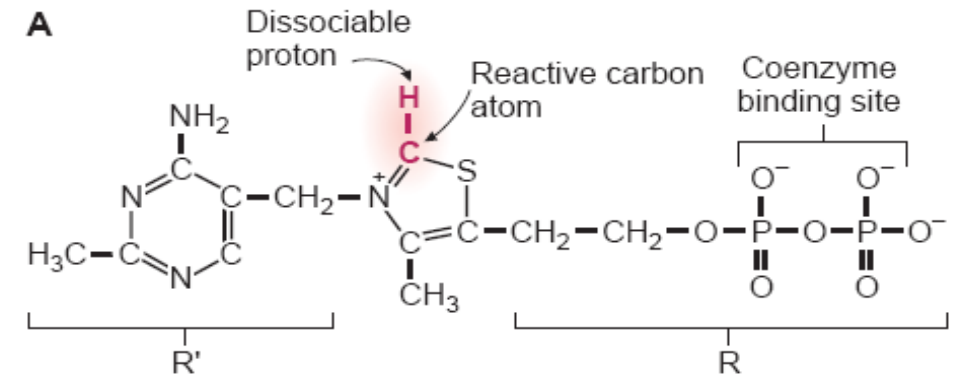
# ***ACTIVATION-TRANSFER COENZYMES***

- Usually participate directly in catalysis by forming a covalent bond
- Characteristics:
  - Two groups in the coenzyme:
    - Forms a covalent bond (functional group)
    - Binds tightly to the enzyme (binding group)
  - Dependence on the enzyme for additional specificity of substrate & additional catalytic power

# ACTIVATION-TRANSFER

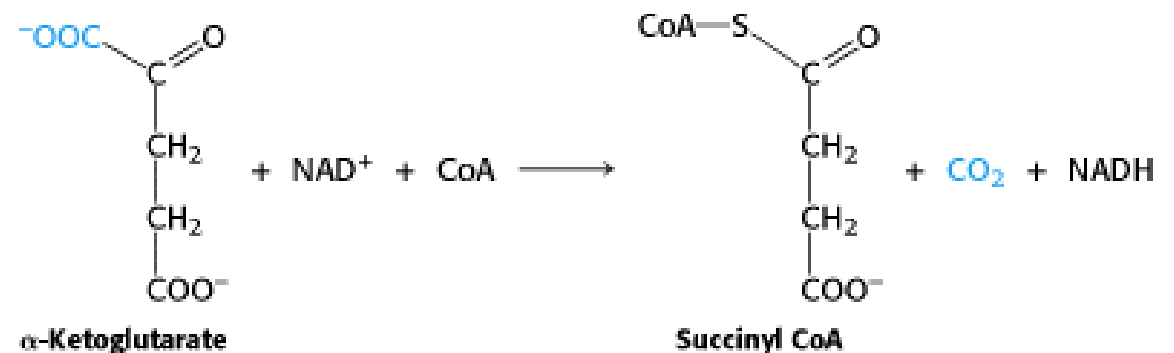
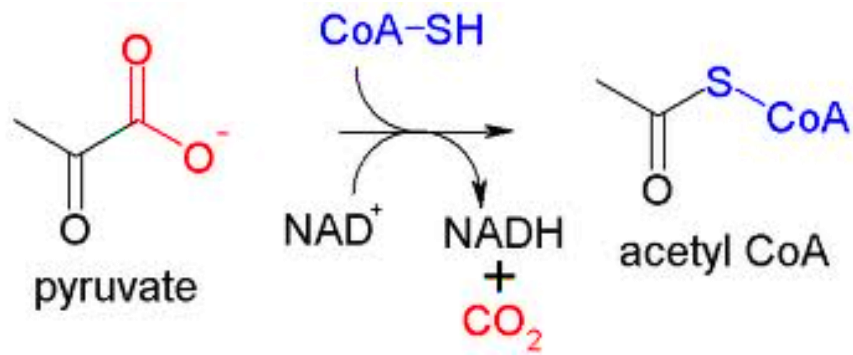
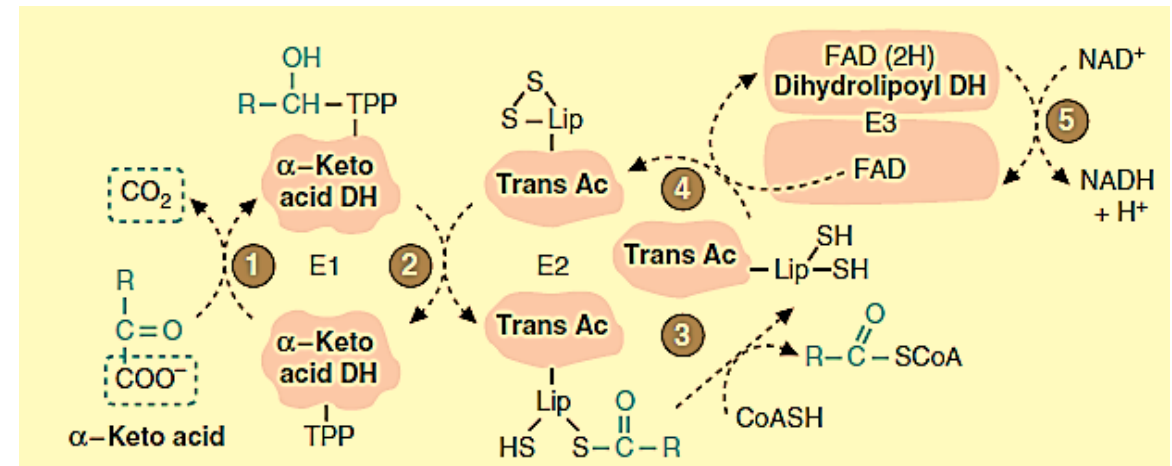
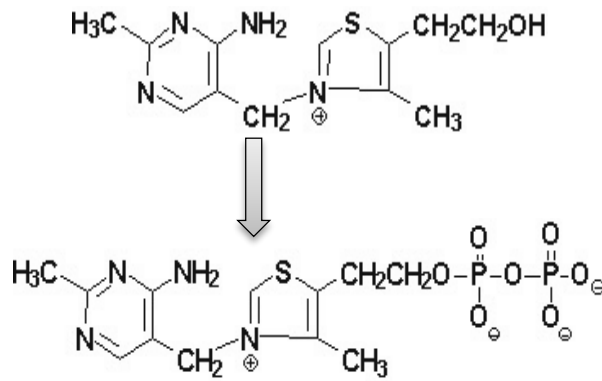
## COENZYMES 1 - TPP

- Thiamine pyrophosphate; Source: thiamine (B<sub>1</sub>); **Decarboxylation reactions**
- Pyrophosphate:
  - Provides negatively charged oxygen atoms
  - Chelate  $\text{Mg}^{2+}$  (tight binding)
- Functional group (reactive carbon atom)
- Reactive thiamine carbon forms a covalent bond with a substrate keto group while cleaving the adjacent carbon-carbon bond



# Thiamin (vitamin B<sub>1</sub>)

- Thiamin (vitamin B<sub>1</sub>) is rapidly converted to its active form, thiamin pyrophosphate, TPP, in the brain & liver
- Required by pyruvate dehydrogenase & α-ketoglutarate dehydrogenase

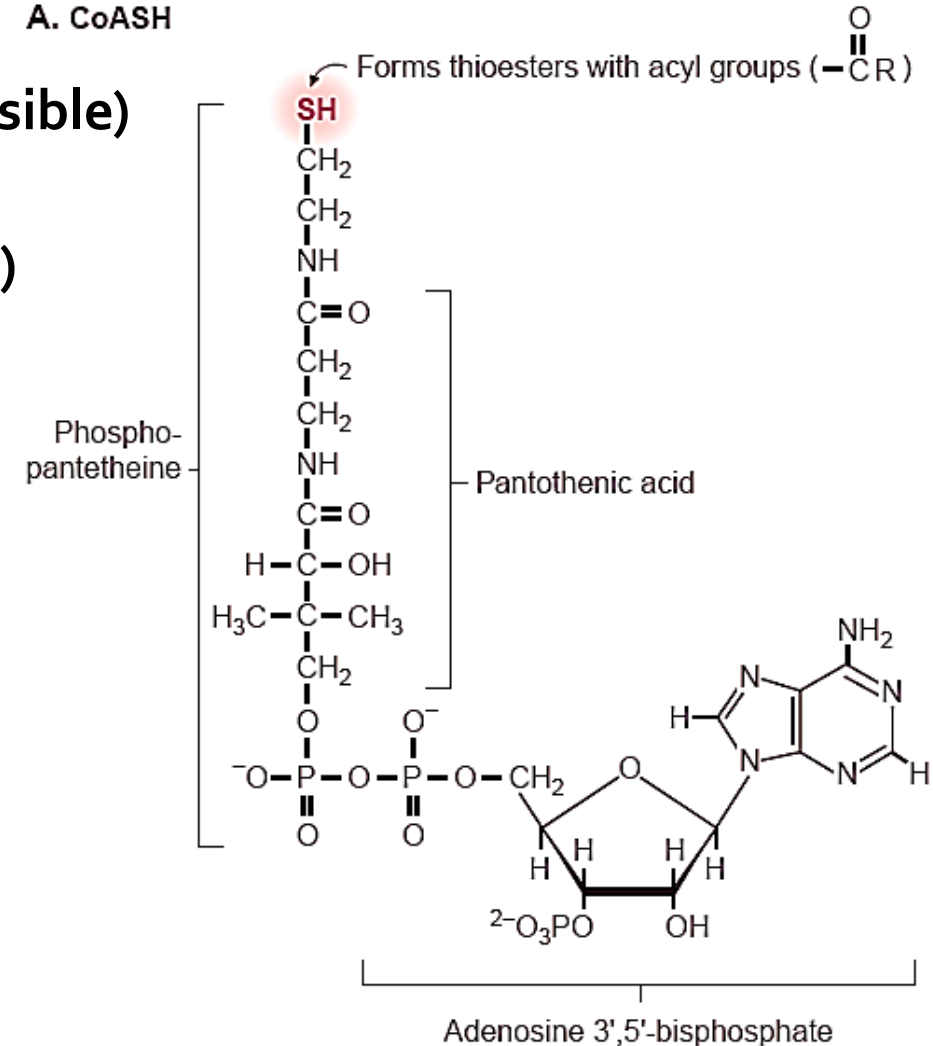


# ACTIVATION-TRANSFER COENZYMES

## 2 - Coenzyme A (CoA)

- Source: pantothenate (B<sub>5</sub>)
- 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<sup>+</sup>), 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

A. CoASH



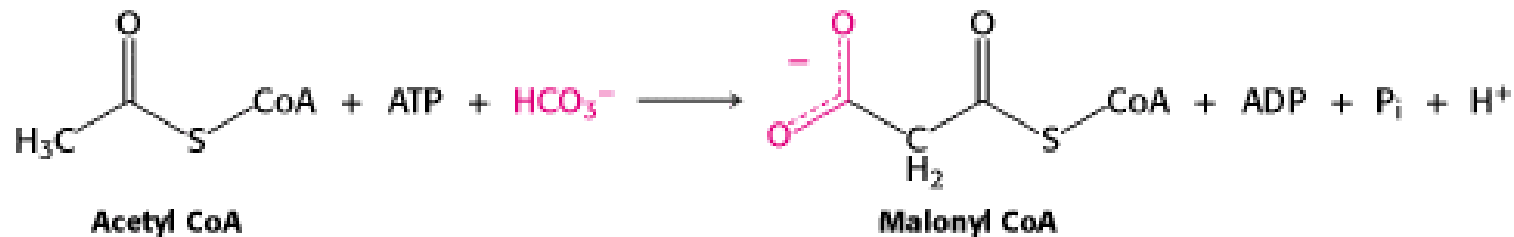
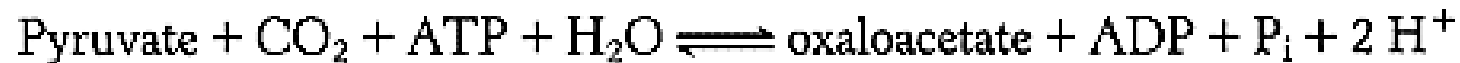
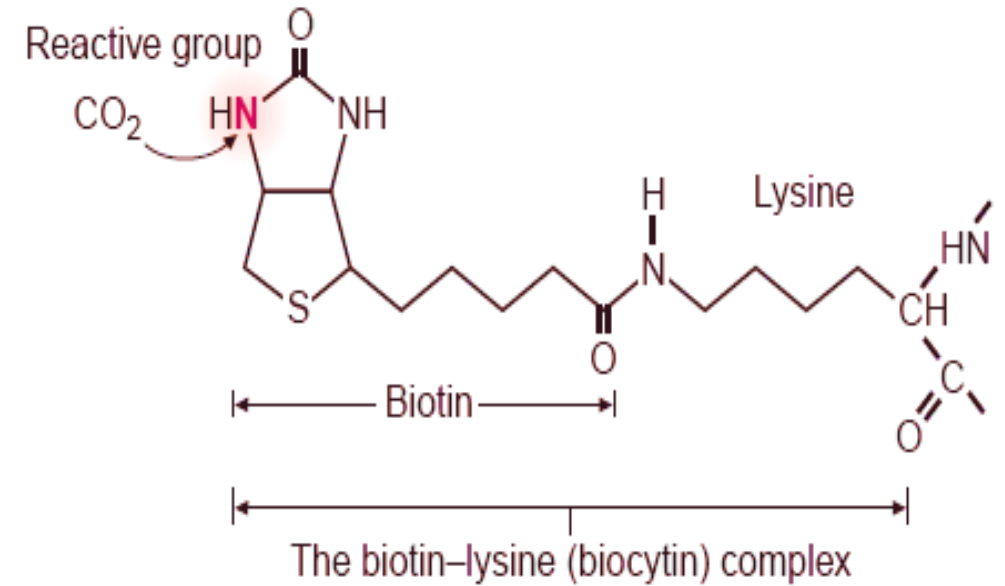
# ACTIVATION-TRANSFER

## COENZYMES

### 3 – Biotin (B7)

- Biotin is required for **carboxylation** reactions (**covalently bound to Lys**)
- **Source: food & intestinal bacteria**
- Deficiencies are generally seen
  - Long antibiotic therapies
  - **Excessive consumption of raw eggs** (egg white protein, avidin, high affinity for biotin)
- Pyruvate carboxylase
- Acetyl CoA carboxylase (fatty acid synthesis)

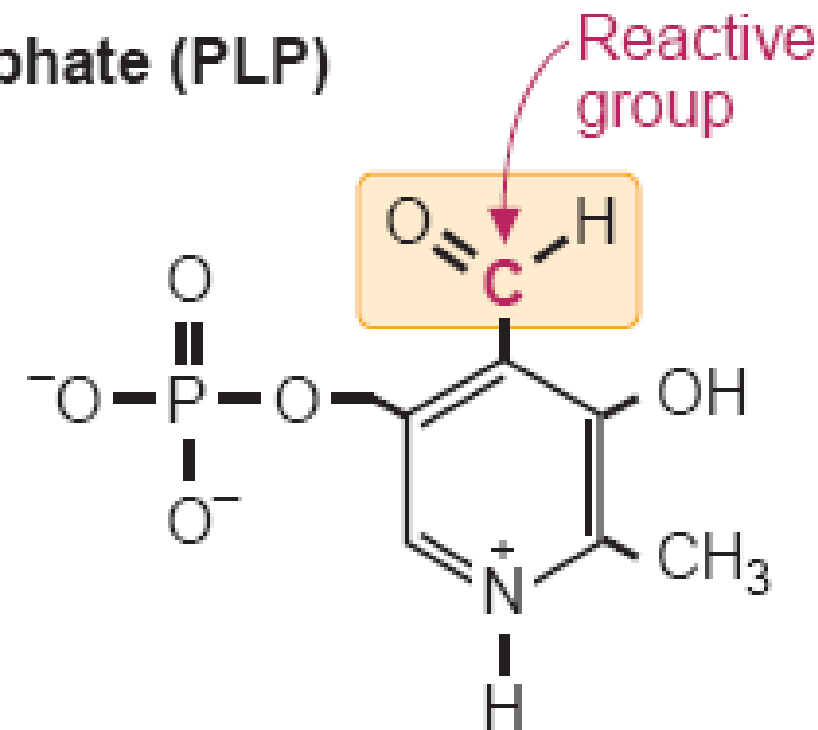
#### B. Biotin



# ACTIVATION-TRANSFER COENZYMES 4 - PLP

## C. Pyridoxal phosphate (PLP)

- Synthesis: Pyridoxine (B6)
- Functions in the metabolism of amino acids (**transaminases**)
- Reversible reactions
- Mechanism:
  - Reactive aldehyde forms a covalent bond with the amino groups
  - Ring nitrogen withdraws electrons from bound amino acid (cleavage of bond)



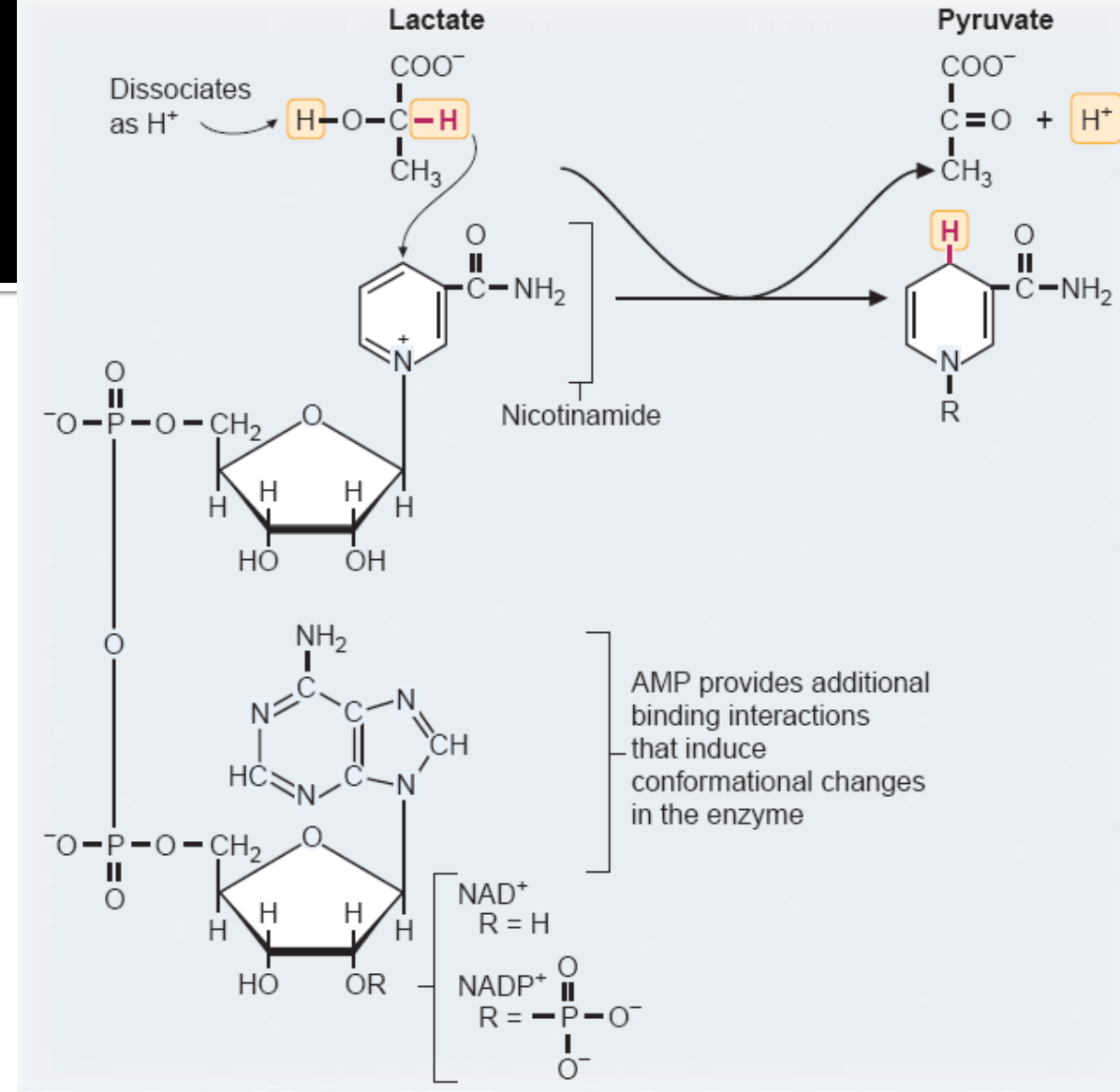


# ***OXIDATION–REDUCTION COENZYMES***

- A large number of coenzymes
- Do not form covalent bonds with the substrate
- Most common: **NAD<sup>+</sup> (niacin, B<sub>3</sub>) & FAD (riboflavin, B<sub>2</sub>)**
- Others: work with metals to transfer single electrons to O<sub>2</sub> (Vitamins E & C)
- Again: Dependence on the enzyme for additional specificity of substrate & additional catalytic power

# OXIDATION-REDUCTION COENZYMES 1 – NAD<sup>+</sup>

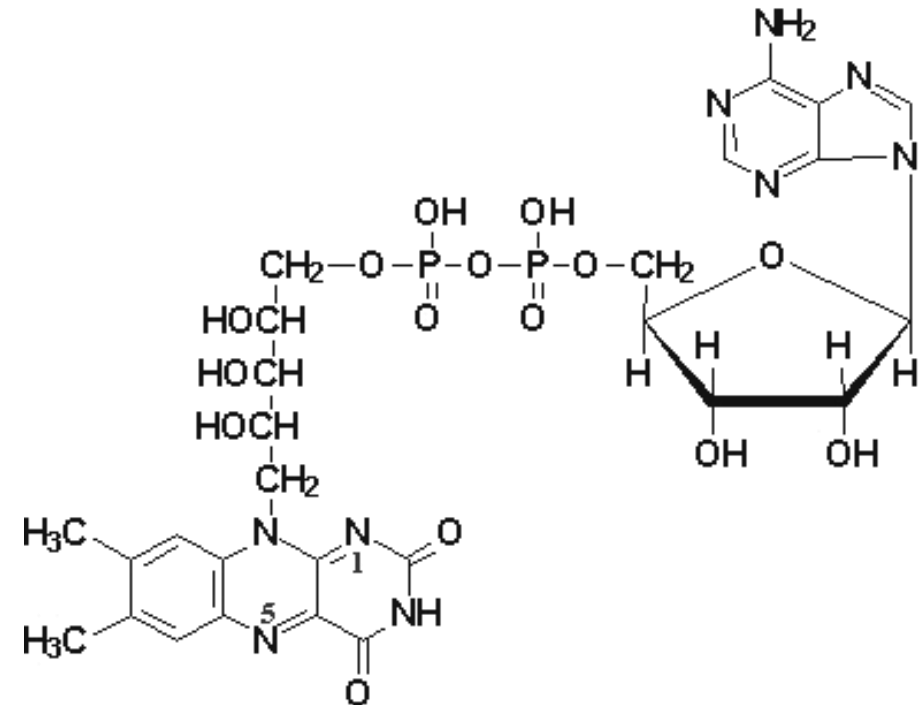
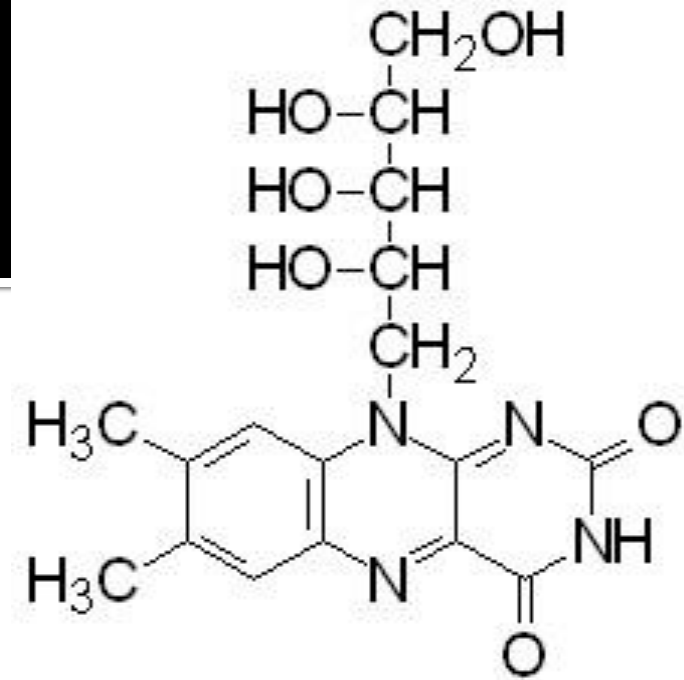
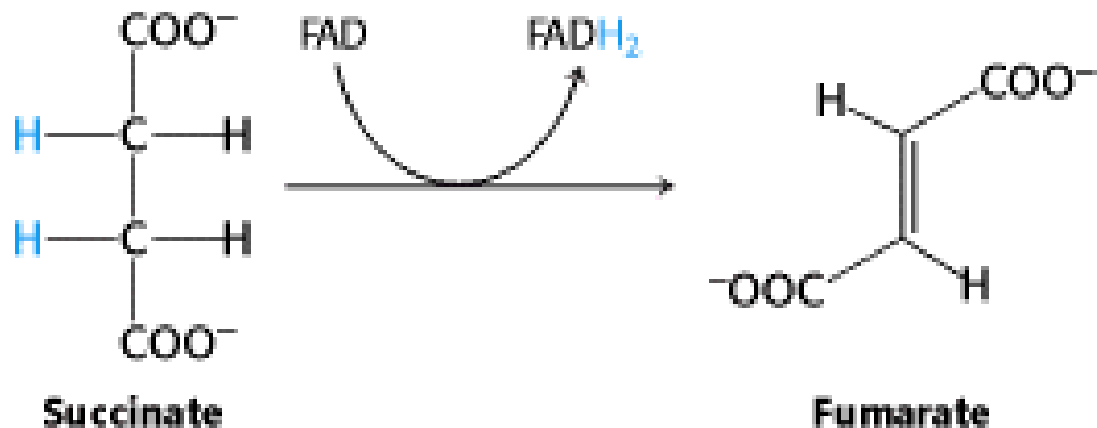
- Functional group (C opposite to N)
- Accepts a hydride ion
- The H<sup>+</sup> from substrate dissociates, & a keto group (CO) is formed
- (ADP) portion of the molecule binds tightly
- The role of enzymes' Histidine



# OXIDATION-REDUCTION COENZYMES

## 2 – FAD & FMN

- Source: Riboflavin (B2)
- FMNH<sub>2</sub> and FADH<sub>2</sub>
- Flavoproteins
- FAD and FMN are prosthetic groups (tightly bound)
- Succinate dehydrogenase
- Pyruvate dehydrogenase complex



# 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 <sub>12</sub>	Coenzyme B <sub>12</sub>	1,2 shift hydrogen atoms
Lipoic Acid	Lipoyllysine	Hydrogen-Atom and Acyl-group transfer
Ascorbic Acid	Ascorbic acid, dehydroascorbic acid	Cofactor in hydroxylation

# Catalytic Metals

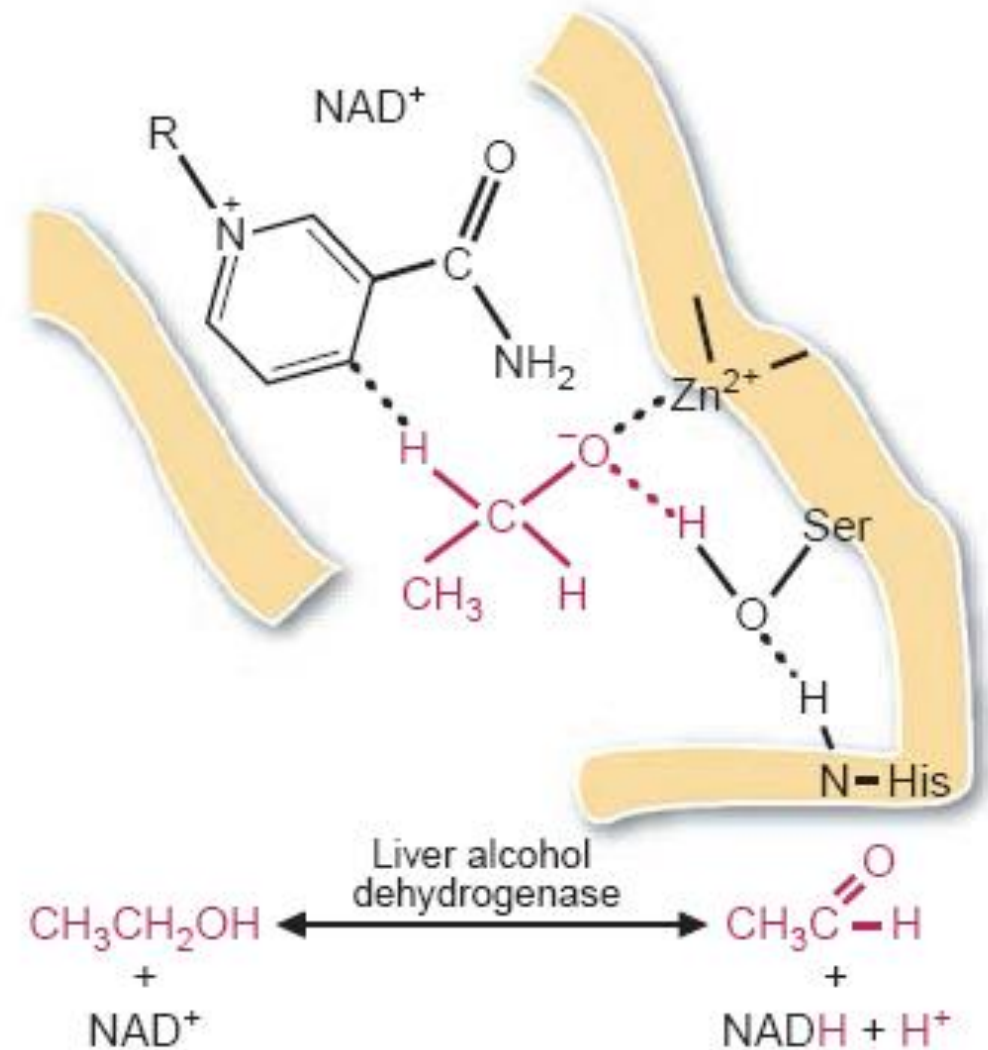
- Metals can be **tightly bound (metalloenzymes)** or **loosely bound (metal-activated enzymes)**
- Acting as electrophiles
- Metal-activated enzymes; the metal either required or enhances activity ( $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ca}^{2+}$ , &  $\text{K}^{+}$ )
- Phosphofructokinase & TPP; ( $\text{Mg}^{2+}$ ) is required to coordinate the phosphate groups on the ATP for a successful reaction (chelation)

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



# Catalytic Metals

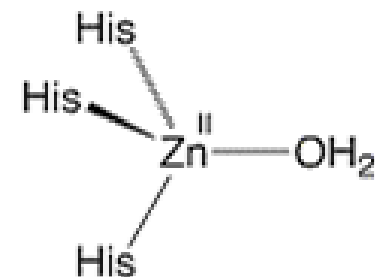
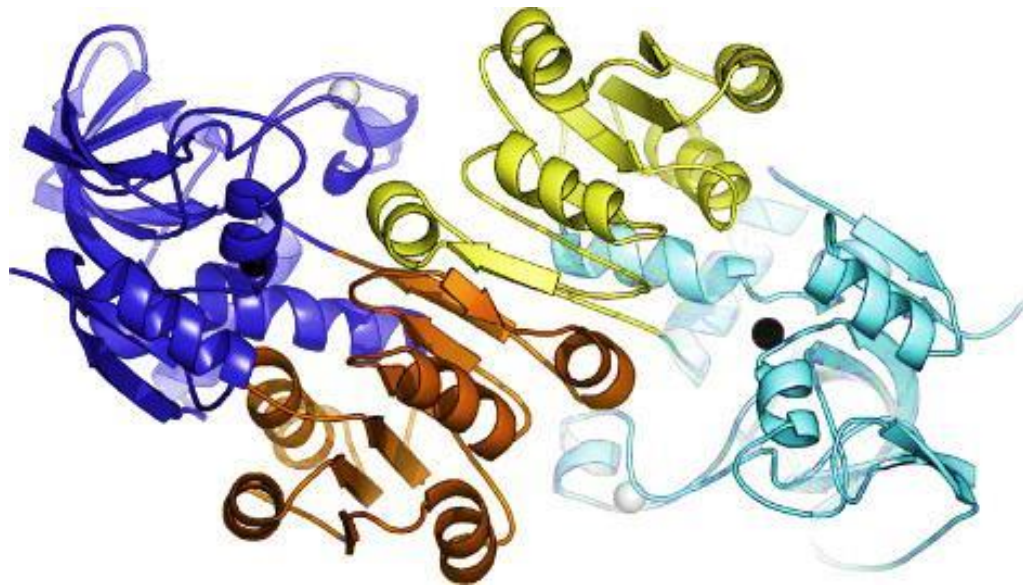
- Alcohol dehydrogenase (ADH)
- Activated serine (pulls a proton off  $\text{-OH}$ )
- Oxyanion is stabilized by zinc
- Transfer of a hydride ion to  $\text{NAD}^+$
- Zinc in ADH as His in lactate dehydrogenase





# Metalloenzymes

- Metal ions are usually incorporated during synthesis & removal of the metal causes denaturation
- These metal ions may contribute either to the structure or the catalytic mechanism
- Liver alcohol dehydrogenase (dimer); 2  $\text{Zn}^{+2}$  in each monomer; one for structural maintenance (joins the two subunits), the other is catalytic
- Carbonic anhydrase; A zinc atom is essentially always bound to four or more groups



**Carbonic Anhydrase**

