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Biochemistry

Enzymes 3

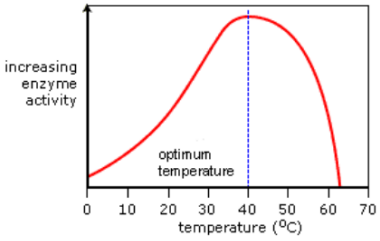
❖ Enzyme regulation

- Mechanisms of regulation:
  - **Non-specific regulation** which affects many enzymes, including:
    - ✓ Temperature, pH
    - ✓ Localization (compartmentalization and complexing of enzymes) which affects diffusion
    - ✓ Expression of isoenzymes
  - **Regulation of enzymatic activity**, including inhibitors and conformational changes (such as modulators, reversible covalent modification, irreversible covalent modification, allostery)

❖ Non-specific regulation

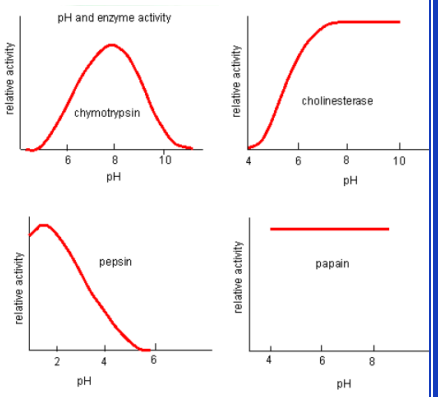
♦ Temperature

- Reaction rates increase with temperature due to increased kinetic energy of the molecules resulting in more collisions between enzymes and substrates, but **very high** temperatures lead to **protein denaturation**
- Each enzyme has an **optimal temperature**
  - For thermophilic bacteria, optimal temperature is about 65°C
  - For human enzymes, optimal temperature is about 37°C



♦ pH

- pH alters the protonation (ionization) state of the substrate and/or the enzyme and their binding
- The effect of pH is enzyme-dependent
  - **Chymotrypsin:** works at best on **pH=8**
  - **Cholinesterase:** works at almost **pH=7 and higher**
  - **Pepsin:** works at best on **pH=2**
  - **Papain:** it is **not affected** by pH at all

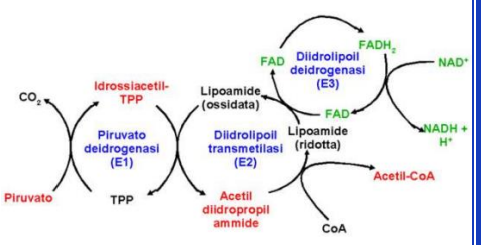


♦ Regulation of enzyme amount

- It includes 3 mechanisms:
  - Enzyme synthesis at the gene level by affecting the transcription and translation of a gene
  - Enzyme degradation by proteases
  - Synthesis of isozymes
- They are comparatively **slow mechanisms** for regulating enzyme concentration (hours-weeks)
  - They affect the **half-life** of the enzyme

♦ Compartmentalization

- Compartmentalization reduces the area of diffusion of both enzyme and substrate increasing the probability that they collide, such as lysosomal enzymes and the enzymes of lipids metabolism (where lipids are synthesized in the cytosol and broken down in the mitochondria)
- **Enzyme complexing:** It is the formation of a complex of multiple enzymes which reduces diffusion
  - Such as pyruvate dehydrogenase in the mitochondria is composed of 3 enzymes (decarboxylation, oxidation and transfer of the acyl group to CoA)



♦ Isoenzymes (isozymes)

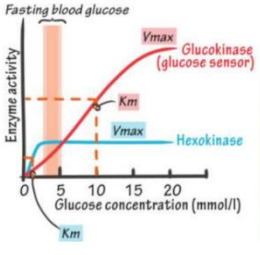
- Isoenzymes are enzymes that can act on the same substrates producing the same products
  - They are produced by different genes that vary only slightly
  - Often, various isozymes are present in different tissues of the body
  - They can be regulated differently, and can have different catalytic activities

- **Lactate dehydrogenases (LDH)**
  - A tetrameric enzyme composed of a combination of protein subunits: H (heart) and M (skeletal muscle and liver), which combine in various ways leading to 5 distinct isozymes leading to 5 distinct isozymes (LDH 1-5) with different combinations of the M and H subunits
  - All the 5 isomers catalyze the conversion between lactate and pyruvate, but they have slightly different primary structure, properties and affinity
    - ✓ H subunit has a net charge of (+1) and a higher affinity towards lactate, resulting in a preferential conversion of lactate to pyruvate (and NAD^+ to NADH)
    - ✓ M subunit has a net charge of (-6) and higher affinity towards pyruvate, thus converting pyruvate to lactate (and NADH to NAD^+)
  - The function and importance of LDH isozymes:
    - ✓ Muscles can function anaerobically, but heart tissues cannot
    - ✓ Whereas the all-M isozyme (M4) functions anaerobically and catalyzes the reduction of pyruvate into lactate, the all-H enzyme (H4) functions aerobically and catalyzes the reverse reaction

Isoenzyme	Structure	Present in	Elevated in
LDH1	(H_4)	Myocardium	myocardial infarction
LDH2	(H_3M_1)	RBC	
LDH3	(H_2M_2)	Lungs	
LDH4	(H_1M_3)	Kidney	
LDH5	(M_4)	Skeletal muscle, Liver	Skeletal muscle and liver diseases

- **Regulation of LDH:**
  - **H4** LDH has a low K_m for lactate, high K_m for pyruvate, and is inhibited by high levels of pyruvate
    - ✓ H4 isoenzyme favors lactate to pyruvate
    - ✓ So, the heart is always aerobic
  - **M4** LDH enzyme has a high K_m for pyruvate and is not inhibited by pyruvate
    - ✓ M4 LDH is always active even at high levels of pyruvate ensuring that pyruvate is always funneled to anaerobic metabolism (favors pyruvate to lactate)

- **Hexokinase and glucokinase**
  - Hexokinase and glucokinase (hexokinase IV) are allosteric isozymes that catalyze Glucose → Glucose-6-Phosphate
  - Glucokinase is a liver (and pancreatic) enzyme, whereas hexokinase in RBC (and skeletal muscle)
    - ✓ The purpose of liver enzyme is to balance glucose level in the blood by forming glycogen
    - ✓ The purpose of RBC enzyme is to produce energy by entering the process of glycolysis
  - The biological significance of glucose-6-phosphate is that once glucose is **phosphorylated**, it cannot cross plasma membrane out of cells
    - ✓ In the **liver,** the enzyme has a **low efficiency** so allowing glucose to cross the membrane before being phosphorylated (to **provide glucose** to other organs)
    - ✓ In **RBC and skeletal muscles,** the enzyme has a **high efficiency** enzyme to **trap glucose** in the cells and break it down to produce energy
  - **Hexokinase** is inhibited by glucose-6-phosphate, but **glucokinase** is not
  - Glucokinase is activated by insulin and inhibited by glucagon
    - ✓ At fasting state, glucose is not stored
    - ✓ At well-fed state, RBCs and skeletal muscles do not consume all glucose in blood and liver can convert excess glucose in glycogen for storage



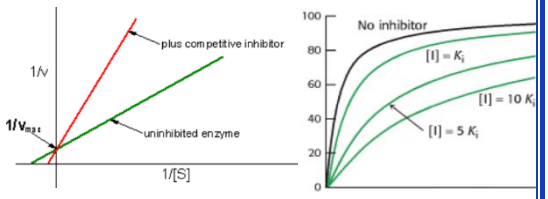
❖ Regulation of enzymatic activity

♦ Inhibitors

- Enzyme inhibition can be either reversible or irreversible

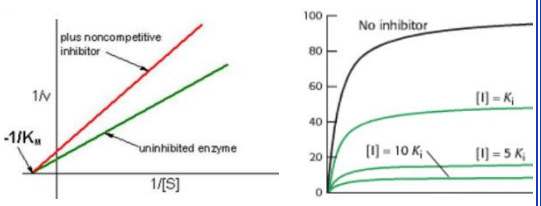
A) Reversible inhibitors rapidly dissociate from enzymes (non-covalent binding), and they include:

1) Competitive inhibitors



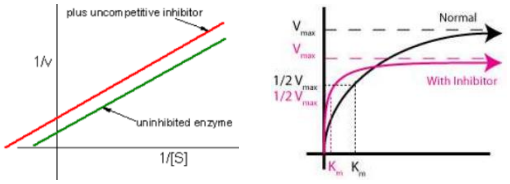
- They compete with the substrate for the active site
- Increasing substrate can overcome inhibition
- Same V_{max}, but higher K_M

2) Noncompetitive inhibition



- They bind E or ES complex at a site other than the catalytic site
- Substrate can bind to the enzyme-inhibitor complex, but ESI cannot form a product
- Lower V_{max}, but same K_M

3) Uncompetitive inhibition



- They bind to the enzyme-substrate complex only reducing both V_{max} and K_M

B) Irreversible inhibitors (Mechanism-based inhibitors)

- They decrease the concentration of active enzyme
- They mimic or participate in an intermediate step of the catalytic reaction, and they include:

1) Covalent inhibitors

- They form covalent or extremely tight bonds with active site amino acids, such as:
  - ➢ Diisopropyl fluorophosphate (DFP) which is an organophosphate that inhibits acetylcholinesterase preventing degradation of Ach, and can inhibit serine proteases
  - ➢ Sarin nerve gas and insecticides (malathion and parathion)
  - ➢ Aspirin which acetylates a serine residue in the active site of cyclooxygenase
    - ✓ Aspirin resembles a portion of the prostaglandin precursor

2) Substrate and transition-State analogs

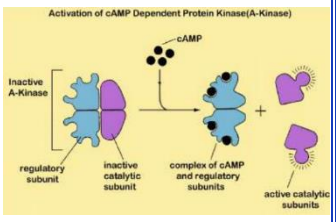
- These inhibitors bind **more tightly** than substrates, so this inhibition is called **suicide inhibition**
- Drugs can't be designed that precisely mimic the transition state because it is a highly unstable structure
- Examples:
  - ➢ Methotrexate which is a synthetic inhibitor used to treat cancer, inflammation (rheumatoid arthritis)
    - ✓ It is a structural analog of folate, a substrate for the enzyme **dihydrofolate reductase**, and a coenzyme for thymidylate kinase, both of which are responsible for the synthesis of nucleotides
    - ✓ It binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate and **inhibits nucleotide base synthesis**
  - ➢ Penicillin which inhibits the glycopeptidyl transpeptidase in the **bacterial cell wall**
    - ✓ The cell wall is the outer covering of the bacteria-containing peptidoglycan layer which is made up of peptides that are cross-linked by glycopeptidyl transpeptidase
    - ✓ The amide bond in the **β-lactam ring** of penicillin looks like the natural transition-state complex

3) Heavy Metals

- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe) tightly bind to a functional group in an enzyme
  - They can undergo Nonspecific inhibition **at high doses**
    - ✓ Hg binds to reactive sulfhydryl groups away from the active site and affect binding of substrates
    - ✓ Unknown enzymes are inhibited in mercury toxicity
  - They can be specific
    - ✓ Lead replaces the normal functional metal in an enzyme such as calcium, iron, or zinc by an irreversible mechanism
    - ✓ Its developmental and neurologic toxicity may be caused by its ability to replace Ca^{+2} in several regulatory proteins that are important in the central nervous system and other tissues

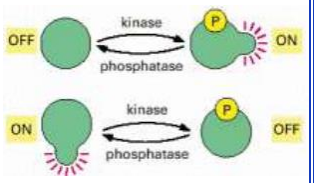
♦ Regulation through conformational changes

- The most Important example is **PKA**
  - PKA (Protein kinase A) is a serine/threonine kinase that consists of four subunits (R2C2) and can phosphorylate many types of proteins (such as glycogen synthase, glycogen phosphorylase)
- Some hormones such as glucagon and epinephrine increase the production of cAMP in the cell
  - cAMP activates PKA by inducing its subunits to **dissociate (2R and 2C)** where the catalytic subunits become **active** after dissociation
  - When PKA is activated, it phosphorylates glycogen synthase (inhibiting it) and glycogen phosphorylase (activating) which induces the **release of glucose** from its stores



♦ Reversible covalent modification

- They are rapid and transient addition of certain groups (mostly **phosphate**) to the enzyme
  - Phosphorylation of an enzyme occurs usually on serine, threonine and tyrosine residues
  - **Phosphorylation** of enzymes is done by protein kinases, where ATP is the phosphoryl donor
  - **Dephosphorylation** of enzyme is done by phosphatases where phosphate is removed
    - ✓ Dephosphorylation is not the reversal of phosphorylation
    - ✓ Addition or removal of a phosphate group to an enzyme may activate or inactivate these enzymes, because the formation or removal of new electrostatic interactions and/or hydrogen bonds altering substrate binding and catalytic activity
    - ✓ It can happen in less than a second or over a span of hours
    - ✓ Phosphorylation often causes **highly amplified** effects



- **Adenylylation** (addition of adenylyl group) where AMP is transferred to Tyr residues through phosphodiester linkage, which inhibits cytosolic enzymes
- **Uridylylation** (addition of uridylyl group)
- **ADP-ribosylation** (addition of adenosine diphosphate ribosyl group) inactivates enzymes
- **Methylation** of carboxylate side chains masking negative charges
- **Acetylation** (from acetyl Co) to lysine residues masking positive charges

- **Glycogen phosphorylase (GP)** catalyzes the removal of glucose molecules from glycogen where the phosphorylated Ser residue is remote from the active site
  - It can be either glycogen phosphorylase a (phosphorylated) or b (dephosphorylated)
  - It can be active (R) or inactive (T)

- Phosphorylase **a** is usually **active** because the equilibrium favors the R state
- Phosphorylase **b** is usually **inactive** because the equilibrium favors the T state
  - The transition of phosphorylase b between the T and the R state is controlled by the energy charge (ATP and AMP) of the muscle cell and the availability of glucose-6- phosphate
  - Muscle phosphorylase b is active only in the presence of **high concentrations of AMP**, which binds to a nucleotide-binding site and stabilizes the conformation of phosphorylase b in the R state
  - **ATP** acts as a **negative allosteric effector** by competing with AMP and so favors the T state
  - **Glucose 6-phosphate** favors the T state of phosphorylase b, an example of **feedback inhibition**

- Large and small regulatory modulators

1) **Small monomeric G proteins**
2) **Large trimeric G proteins:** a family of membrane-bound proteins causing changes inside the cell
   - They communicate signals from hormones, neurotransmitters, and other signaling factors through G protein-coupled receptors (GPCRs)
   - When they bind **GTP**, they are **'on'**, and, when they bind **GDP**, they are **'off '**
     - ✓ When active it binds target proteins, which are then activated or inhibited
     - ✓ The G protein hydrolyzes a phosphate from GTP to form GDP, which changes the G protein conformation and causes it to dissociate from the target protein
   - The **α subunit** binds to effectors stimulating or inhibiting them

- The activity of many monomeric G proteins is regulated by:
  - **GAPs** [GTPase-activating proteins]
  - **GEFs** [guanine nucleotide exchange factors]
  - **GDIs** [GDP dissociation inhibitors]

♦ Irreversible covalent modification (proteolytic activation)

- **Zymogens or proenzymes** are **inactive** precursors of enzymes
  - Activation is done by irreversibly removing part of the enzyme (usually known as the **pro region** present at the **N-terminus**)
  - Examples: digestive enzymes such as chymotrypsin, trypsin, and pepsin that get activated when food is ingested. Trypsinogen is activated via removal of the first six amino acids at the N-terminus

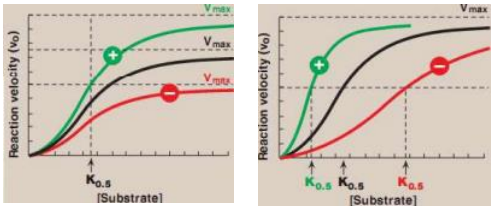
♦ Regulation including conformational changes

- These regulatory mechanisms include: Allostery, Covalent modulation, Protein-protein interactions between regulatory & catalytic subunits or between two proteins and Proteolytic cleavage
  - Rapidly change from inactive to fully active enzyme

- **Allosteric enzymes:** they are multi-subunit proteins where one subunit contains the active site (catalytic subunit) and another containing the regulatory site (regulatory subunit).
  - Multiple active sites can exist on multiple subunits
  - The binding of regulatory molecules triggers conformational changes in the active site via modifying non-covalent interactions
  - Allosteric enzymes bind modifiers at the allosteric site (which is a site that is physically separate from the catalytic site)
    - ✓ A **negative allosteric modifier** (**inhibitor**) causes the enzyme to have less activity
    - ✓ A **positive allosteric modifier** (**activator**) causes the enzyme to be more active

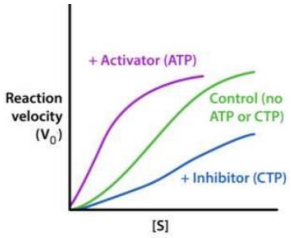
- When the modifier is a molecule other than the substrate, then it is known as **heterotropic**
- If the modifier is same as the substrate, then it called **homotropic**
- If the binding of the substrate causes the enzyme to become more active and binds to a second substrate at a different active site with more ease, it is called **positive cooperativity**

- Types of allosteric enzymes:
  - The **Michaelis-Menten model cannot explain the kinetic properties of allosteric enzymes**
  - **$K_{0.5}$** is used instead of K_M
  - Allosteric enzymes have a **sigmoidal curve**
    - ✓ Activators lead to a near-hyperbolic plot
- regulation of allosteric enzymes has 2 systems:
  - V system where V_{max} is changed and $K_{0.5}$ is constant
  - K system where $K_{0.5}$ is changed and V_{max} is the same



- Allosteric inhibitors usually have a **much stronger** effect on enzyme velocity than competitive and noncompetitive inhibitors
- Allosteric enzymes are not limited to regulation through **inhibition** whereby allosteric effectors may function as **activators**
- The allosteric effector needs **not** bear any **resemblance to substrate** or product of the enzyme
- The effect of an allosteric effector is **rapid** occurring as soon as its concentration changes in the cell
- **Feedback regulation** of metabolic pathways by end products or by signal molecules

- **Aspartate transcarbamoylase (ATCase):** consists of 12 polypeptide chains (six catalytic subunits as two trimers and six regulatory subunits as three dimers) and it catalyzes the first step in the synthesis of pyrimidine nucleotides
  - It exists in two forms: T state (less active) and R state (more active)
  - It is **inhibited by CTP** (the end-product) by inducing a major rearrangement of subunits position stabilizing the T state of the enzyme, decreasing binding affinity for Asp (substrate) at active sites on catalytic subunits increasing $K_{0.5}$ (K system)
  - A non-competitive inhibitor changes $K_{0.5}$
  - ATP, purine activate the enzyme in order to balance the rate of synthesis of purines and pyrimidines



❖ Modes of metabolic regulation

♦ Feedback inhibition

- Feedback inhibition or negative feedback regulation where an enzyme present early in a biochemical pathway is inhibited by a late product of pathway
  - Such as the inhibition of hexokinase by its product (glucose-6-phosphate)

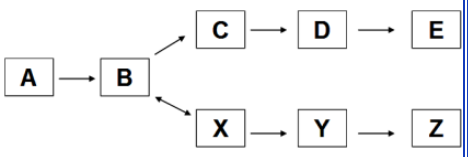
♦ Feedback activation

- Positive feedback regulation where a product stimulates the activity of an enzyme
  - Such as blood clotting (thrombin)

♦ Feed-forward activation

- A substrate produced early in a pathway activates an enzyme downstream of the same pathway
  - Such as glycolysis and the enzymes responsible for the elimination of poisons

- **Committed step:** It is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return
  - Committed steps are exergonic reaction
  - For **glycolysis**, the committed step is the reaction that converts fructose-6-phosphate into fructose-1,6-bisphosphate using the enzyme phosphofructokinase (**PFK**)



- **Rate-limiting reactions** is the step that slows down the rate of the reaction because:
  - Requirement for high amount of energy
  - Strict regulation of enzymes
  - high K_m values of enzyme towards its substrate
  - These reactions are also usually, but not necessarily, committed steps

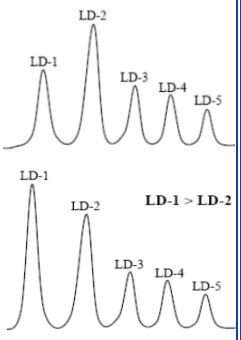
❖ Enzymes in Disease Diagnoses

- Enzymes are considered as **biomarkers**, for example the presence of enzymes in serum indicates that tissue or cellular damage and the amount of an enzyme in serum is of diagnostic significance

♦ AST (aspartate transaminase) and ALT (alanine aminotransferase)

- They are the typical **liver enzymes** to be measured
- **ALT** is predominantly in hepatocytes
- The ratio of ALT/AST is diagnostic
  - Liver disease/damage (not of viral origin) < 1
  - Viral hepatitis > 1

♦ LDH



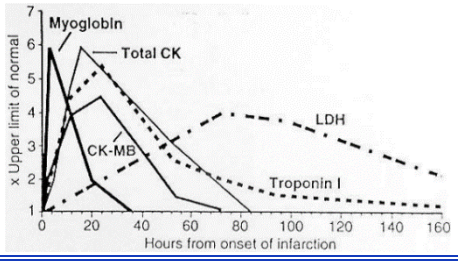
- A comparison of serum levels of LDH-1/LDH-2 ratio is diagnostic for myocardial infarction (heart attacks), where **normally** this ratio should be less than 1
  - Following an **acute myocardial infarct**, the LDH ratio will be more than 1

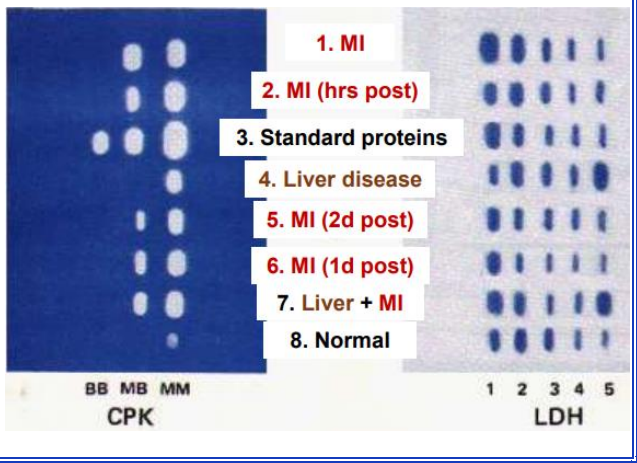
♦ CPK

- CPK is found primarily in heart and skeletal muscle as well as the brain
- Three tissue-specific isozymes of CPK:
  - CPK3 (CPK-MM)
  - CPK2 (CPK-MB)
  - CPK1 (CPK-BB)

Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB	0 trace BB	0% BB	97% BB
<6% MB	1% MB	**20% MB**	3% MB
>94% MM	99% MM	**80% MM**	0%MM

- A significant amount of CPK-MB is released after MI leading to increased CPK-MB/total CPK ratio (diagnostic of an acute infarction), but the increase of total CPK in itself may not indicate infraction
- CPK-MB disappears in 1-3 days, so another elevation is indicative of another event (reinfarction)





- **Troponin** levels rise within four to six hours after the beginning of chest pain or heart damage and stay elevated for at least one week
  - This long elevation allows detection of a myocardial infarction that occurred days earlier but prevents detection of a second infarction if it occurred only days after the first
  - So, to detect a second infraction we use CPK

❖ Cofactors

- Enzymes carry out reactions utilizing different catalytic strategies, where:
  - Some enzymes (such as chymotrypsin) rely on specific amino acid residues within the active site
    - ✓ Almost all polar amino acids participate in **nucleophilic catalysis**
    - ✓ Ser, Cys, Lys, & His can participate in **covalent catalysis**
    - ✓ Histidine usually participates in the **acid-base catalysis**
  - Other enzymes need cofactors (nonprotein compounds that participate in the catalytic process) so they are called conjugated enzymes (Holoenzyme)

Name	Coenzyme or Active Form
Thiamin	Thiamine pyrophosphate (TPP)
Riboflavin	Flavin mononucleotide (FMN) Flavin adenine dinucleotide (FAD)
Nicotinic Acid	Nicotinamide adenine dinucleotide (NAD) Nicotinamide adenine dinucleotide phosphate (NADP)
Pantothenic Acid	Coenzyme A (CoA)
Pyridoxine	Pyridoxal Phosphate
Biotin	Biocytin
Folate	Tetrahydrofolate
Vitamin B_{12}	Coenzyme B_{12}
Lipoic Acid	Lipoyllysine
Ascorbic Acid	Ascorbic acid, dehydroascorbic acid

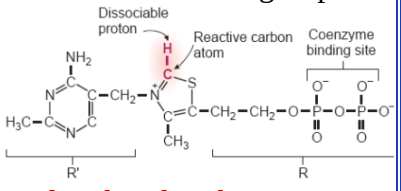
- Cofactors can be organic (such as vitamins) or inorganic (such as metals)
  - **Co-enzymes:** they are **organic** cofactors which can be:
    - ✓ Prosthetic group: tightly (covalently) bound
    - ✓ Co-substrate: loosely bound
    - ✓ They include activation-transfer coenzymes and oxidation-reduction coenzymes
  - Metals can be tightly bound forming metallo-proteins, or loosely bound forming metallo-associated proteins

♦ Activation-transfer coenzymes

- The functional group of the coenzyme directly participates in catalysis
- They contain 2 groups: A functional (reactive) group that **forms a covalent bond with substrate** and a binding group that **binds tightly to the enzyme**
  - Dependence on the enzyme for additional specificity of substrate & additional catalytic power
- Examples:

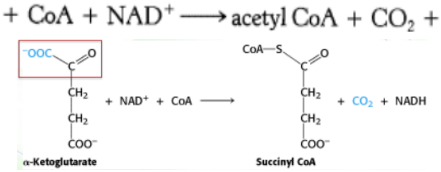
1) Thiamin pyrophosphate, TPP

- **Thiamin (vitamin B1)** is converted to **thiamin pyrophosphate (TPP)**, in the brain & liver
- It is involved in **decarboxylation** reactions, where the reactive thiamin carbon binds to the keto group of substrates releasing CO_2
- The pyrophosphate is the **binding group** which provides negatively charged oxygen atoms and **chelates Mg^{2+}** which is tightly bound to the enzyme

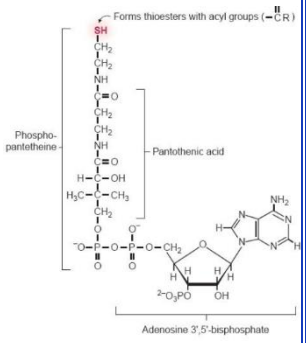


- The **functional group** is the reactive carbon (carbanion) atom that forms a covalent bond with a substrate's keto group while cleaving the adjacent carbon–carbon bond
- It is used by:
  - **Pyruvate dehydrogenase complex:** which undergo the decarboxylation of pyruvate into acetyl CoA
  - **α-ketoglutarate dehydrogenase:** Decarboxylation of α-ketoglutarate into succinyl CoA

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \longrightarrow \text{acetyl CoA} + CO_2 + \text{NADH}$$

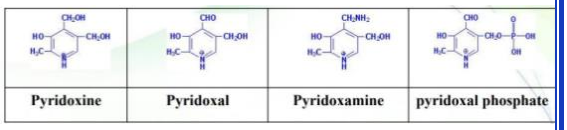


2) Coenzyme A (CoA)



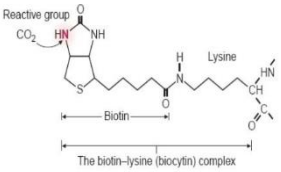
- Source: **pantothenate (B5)** which is made of al β-alanine and pantoic acid
- Participate in the **metabolism of carbohydrate, fats, and proteins**
  - **Functional group:** sulfhydryl group (nucleophile) where it attacks carbonyl groups and forms acyl thioesters, and when a molecule is conjugated with CoA it becomes an energy-rich molecule
  - **Binding group:** adenosine 3' ,5'- bisphosphate (tight & reversible)
- It is used by:
  - **Pyruvate dehydrogenase** complex converts pyruvate + CoA into acetyl CoA and CO_2, where it can be used in the next step of the reaction which is catalyzed by dihydrolipoamide acetyltransferase
  - Condensation of acetyl CoA and oxaloacetate into citrate by **citrate synthase** (a transferase) in the mitochondria

3) Pyridoxal phosphate



- Sources: **pyridoxal (B6)**, pyridoxamine and pyridoxine
- Participate in the **metabolism of amino acids** via reversible transamination reactions
  - Such as the conversion between glutamate and α-ketoglutarate, alanine and pyruvate or aspartate and oxaloacetate
  - The **functional** and **binding group** are the same (the reactive adehyde group)
  - The reactive aldehyde forms a covalent bond with the amino groups, then the ring nitrogen withdraws electrons from bound amino acid (cleavage of bond)

4) Biocytin



- Source: **Biotin (vitamin B7)** from food & intestinal bacteria
  - It is required for **carboxylation** reactions
  - The **functional group** is the N in the biotin ring
  - The **binding group** is the lysine residue
  - Deficiencies are seen after long antibiotic therapies or excessive consumption of raw eggs (egg white protein (avidin) has a high affinity for biotin)
- It is used by:
  - **Pyruvate carboxylase** (converts pyruvate into oxaloacetate)
  - **Acetyl CoA carboxylase** (fatty acid synthesis)

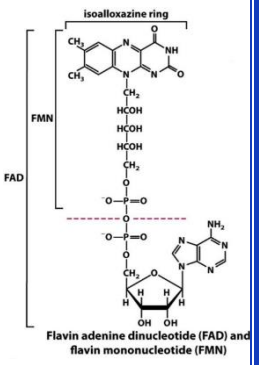
♦ Oxidation–reduction coenzymes

- A number of coenzymes work within oxidoreductases
- Each coenzyme has a unique functional group that accepts and donates electrons and is specific for the form of electrons it transfers (hydride ions, hydrogen atoms, oxygen)
- These do not form covalent bonds with the substrate, a portion of the coenzyme binds the enzyme
- The most common: **NAD^+** & **FAD**
  - Others work with metals to transfer single electrons to O_2 (Vitamins E & C)
  - Coenzymes are dependent on the enzyme for additional specificity of substrate & catalytic power

1) FAD and FMN

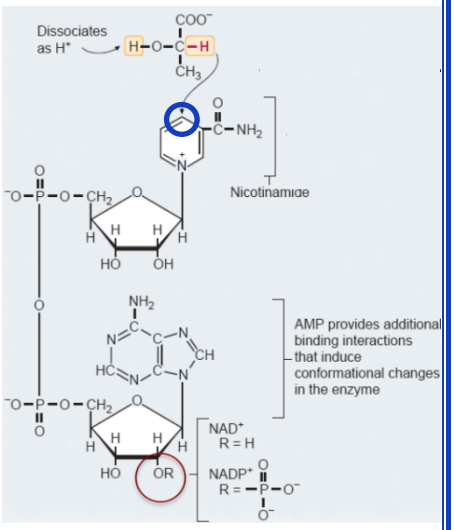
- The precursor is **riboflavin (vitamin B2)**
- Both are prosthetic groups (covalently bound) of flavoproteins
  - They are covalently bound to the enzyme because they are highly reactive

- It accepts electrons in the form of **hydrogen atoms** donated separately & sequentially
  - The phosphate and the adenosine nucleotide are the **binding groups**
  - The **functional group** is the 2 N in the riboflavin rings
  - Their reactions result in the formation of double bonds or disulfide bonds
- FAD is used by **Succinate dehydrogenase** which oxidizes succinate into fumarate



2) NAD^+ and $NADP^+$

- Precursor of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) is **niacin (vitamin B3)**
  - They are **co-substrates (non-covalently bound)** for numerous dehydrogenases
  - The **functional group** is the C opposite to the N in the nicotinamide
    - ✓ It accepts a **hydride ion** from the substrate, and the substrate dissociates, & a keto group (CO) is formed
    - ✓ They are generally involved in the oxidation of alcohols and aldehydes
  - The **binding group** is the nucleotide part



- It is used by **Lactate dehydrogenase**, where the histidine of the enzyme binds the proton of (-OH) on lactate making it easier for NAD+ to pull off the other hydrogen with both electrons (a hydride)

3) Ascorbic acid

- It is Vitamin C
- Used by **Prolyl hydroxylase** which synthesizes 4-hydroxyproline
- It also functions as an **anti-oxidant**, by removing ROS
  - Reactive oxygen species oxidize (take electrons from) ascorbate into a radical ascorbate, which is then oxidized into dehydroascorbate
  - The oxidized forms of ascorbate are relatively stable, unreactive, and do not cause cellular damage
  - The structure of vitamin C (and other anti-oxidants) is preferable due to formation of resonance

♦ Metals

- They act as **electrophiles**
  - They assist in binding of the substrate, or they stabilize developing anions in the reaction
  - They can also accept and donate electrons

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

- Metals carry **positive charges** and can form relatively strong yet kinetically labile bonds, enabling them to participate in binding substrates or coenzymes to enzymes
  - **Mg^{2+}** connects the negatively charged phosphate groups of TPP to basic amino acids in the enzyme
  - The phosphate groups of ATP are usually bound to enzymes through Mg^{2+} chelation
  - They are stable in more than one oxidation state

- **Carbonic anhydrases**, uses **Zinc** as a cofactor
  - Mutations in carbonic anhydrases have been found to cause osteopetrosis (excessive formation of dense bones accompanied by anemia) and mental retardation
  - Zinc is found **only in the +2** state in biological systems

- In carbonic anhydrase, a zinc atom is bound to three imidazole rings (2 His) of three histidine residues and an additional site is occupied by a water molecule

- **Zinc** facilitates the release of a proton from H_2O **generating a hydroxide ion (OH)**
  - The CO_2 substrate binds to the enzyme's active site and is positioned to react with the hydroxide ion where the hydroxide ion attacks CO_2 converting it into a bicarbonate ion then the catalytic site is regenerated with the release of the bicarbonate ion and the binding of another H_2O

- Some metals do not participate in enzyme catalysis directly but facilitate a reaction
  - 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** and a hydride is then transferred to NAD^+