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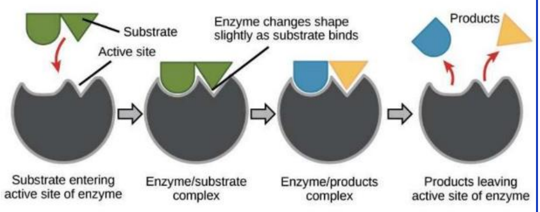
Biochemistry

Enzymes 1

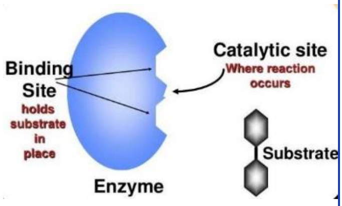
❖ Enzymes

- The function of proteins depends on their ability to bind other molecules (ligands)
  - **Ligand:** a substance that forms a complex with a biomolecule, usually via non-covalent interactions, to serve a biological purpose
- Two properties of a protein characterize its interaction with ligands:
  - **Affinity:** the strength of binding between a protein and other molecules
    - Kd is inversely related to affinity
  - **Specificity:** the ability of a protein to bind one molecule in preference to other molecules
    - Most proteins bind only to specific ligands, also there are non-specific proteins such as **albumin**
    - Usually, the interactions between proteins and molecules are reversible (non-covalent)

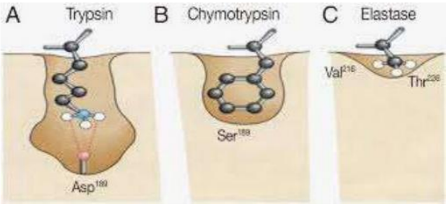
- **Enzymes:** Specialized **proteins** that conduct **(catalyze) chemical reactions** under biological conditions
  - Ribozymes are the only non-protein enzymes, where they are RNA
  - In enzymatic reactions, reactants are known as substrates
- Enzymes are catalysts that exist in small amounts relative to the reactants
  - They increase the rate of a reaction
  - At the end of the reaction, they are **unchanged from the original** conformation
- Most enzymes have very specific functions in converting specific substrates to products
  - Enzymatic reactions are expressed as follows: $E + S \leftrightarrows ES \leftrightarrows EP \leftrightarrows E + P$ or $E + S \leftrightarrows ES \leftrightarrows E + P$
  - E = free enzyme, S = free substrate, ES = enzyme-substrate complex, P = free products, EP = Enzyme-product complex before products are released



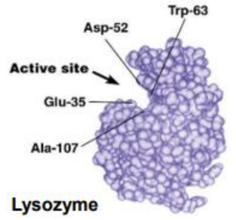
- Enzymes accelerate reactions usually within range of 10^6-10^{14}
  - Catalase (10^8)
  - Carbonic anhydrase (10^6): One enzyme molecule hydrates 10^6 molecules of CO_2 per second (versus 1 per 10 seconds for uncatalyzed reactions)
- The reaction occurs in the **active site**
  - **Active site:** A region with specific 3D shape where the biochemical reaction takes place
  - The active site contains a specialized amino acid sequence that facilitates the reaction, where their R groups interact with the substrate
- Within the active site are two sub-sites:
  - **Binding site:** Bind, orient and stabilize the interactions with the substrate
  - **Catalytic site:** contains amino acid residues that catalyze (carry out) the actual reaction by reducing the activation energy
    - In some enzymes, the binding and catalytic sites are the same



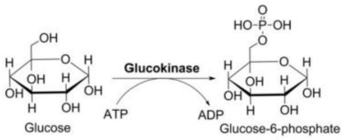
- Binding specificity and selectivity of enzymes are due to their **precise interaction** of active sites to their substrates and the degree of **compatibility** for this interaction which depends on the specific amino acid sequence of the active site
  - The specificity of enzymes varies between different enzymes, where some enzymes can catalyze the reaction with only one substrate (catalase and carbonic anhydrase), but other can catalyze the same reaction for many different substrates (hexokinase)
- Trypsin, chymotrypsin, elastase are pancreatic enzymes secreted into the intestines and digest proteins



- Features of the active site:
  - It is **internal** relative to the enzyme (not highly exposed to the surface) and looks like a canal
  - It is a three-dimensional pocket formed by amino acid groups that come from different parts of the primary structure usually forming a domain
  - The amino acid residues can be nonpolar and polar, and **water is usually excluded** unless it is part of the reaction
  - Substrates bind to enzymes by **multiple weak attractions**
    - ✓ Interactions between the binding site and the substrate are non-covalent
  - **Chirality** is important regarding the specificity, so binding of substrates to active sites occurs at least, at three points
  - It is **small** relative to the total structure of an enzyme
    - ✓ The “extra” amino acids create the 3D active site, and may form regulatory sites

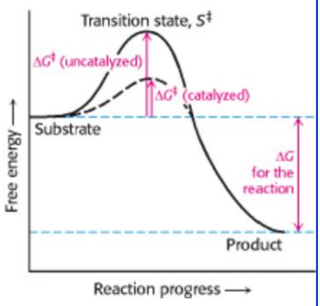


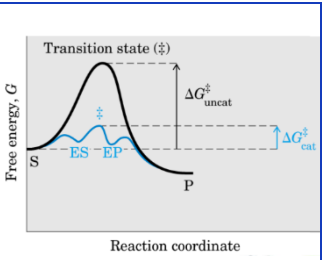
- How does substrates fit into the active site of the enzyme?
  - **Lock and key model:** The substrate fits directly into the active site
  - **Induced fit model:** Enzymes are flexible and active sites can be modified by binding of substrate
- **Glucokinase** follows both models
  - Glucose binds the active site by the lock and key model, inducing changes in the conformation where the ATP binds (which is follows induced fit model)



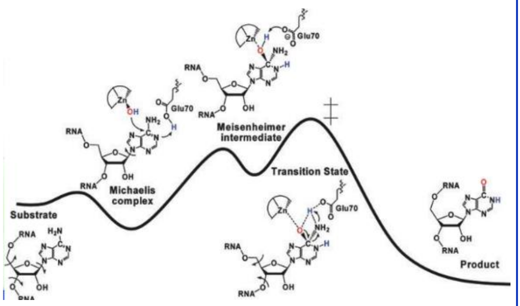
Enzymes and energy

- Types of energy:
  - Kinetic: energy of motion
  - Potential: capacity to do work (stored), which is more important in studying biological systems
- Molecules have their own potential energy stored in the bonds connecting atoms in molecules
  - **Free energy (G):** is the potential energy stored in bonds which is the **energy available** for reactions
- **Free energy change (ΔG)** is the difference between the free energy values between reactants and products ($\Delta G = G_{Products} - G_{Reactants}$)
  - ΔG accounts for the equilibrium of the reaction and enzymes accelerate how quickly this equilibrium is reached
  - If ΔG is **negative**, $G_{products}$ is less than $G_{reactants}$, **energy is not needed** to drive the reaction, but **released**, making the forward reaction **spontaneous** (the reaction is called **exergonic**)
  - If ΔG is **positive**, $G_{products}$ is more than $G_{reactants}$, an input of **energy is needed**, making the reaction **not spontaneous** (the reaction is called **endergonic**)
  - If **ΔG is zero**, both forward and reverse reactions occur at equal rates; the reaction is at **equilibrium**
- Any enzymatic reaction goes through a **transition state (ES)** that has a higher free energy than does either S or P
  - At the highest energy level, the substrate configuration is **most unstable** and is most tightly bound to the enzyme (**bonds are maximally strained**)
- **Activation energy ($\Delta G^{\ddagger}$):** The difference in free energy between the transition state and the substrates
- Enzymes **lower the activation energy** and enzymes facilitate the formation of the transition state at a lower energy



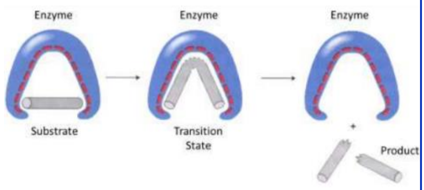


- Substrates often undergo several transformations and intermediates when associated with the enzyme and each form has its own free energy value
  - ➢ The activation energy corresponds to the complex with the highest energy
  - ➢ Activation energy does not enter into the final ΔG calculation for a reaction
- Adenosine deaminase is an enzyme that converts adenosine into inosine, in a reaction that involves the formation of 3 intermediates
  - ➢ **ΔG remains is not affected by the enzymatic reaction**



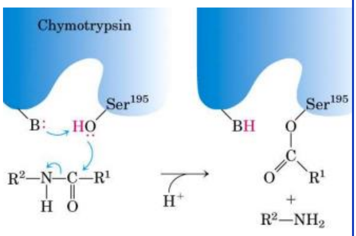
- Enzymes can reduce the activation energy in many ways including bring substrates together in a near proximity by orienting the active site to fit the substrate in the best fit possible or changing the energy within bonds allowing the breakup and formation of bonds
- Examples of possible mechanisms to reduce $\Delta G^\ddagger$:

1) Catalysis by bond strain



- The induced structural rearrangements produce **strained substrate bonds**, which **more easily** attain the transition state
- The new conformation makes the substrate bonds **vulnerable (weaker)**
- Such as **lysozymes** which break down sugars by distorting them from the typical chair hexose ring into the sofa conformation, which is similar in shape to the transition state

2) Catalysis involves acid/base reactions



- The R groups of amino acids act as donors (acids) or acceptors (bases) of H^+
- **Histidine** is the main amino acid used in this mechanism at physiological pH
- **Serine** is used in catalysis in the **serine proteases (such as chymotrypsin)**
- Also, aspartate, glutamate and threonine are utilized in this mechanism

3) Covalent catalysis

- A **covalent intermediate is formed** between the enzyme or coenzyme and the substrate
- Examples of this mechanism is proteolysis by **serine proteases**, which include digestive enzymes (trypsin, chymotrypsin, and elastase)

❖ Enzymes classification

- Enzymes can be:
  - ➢ **Simple (Apoenzyme):** only the protein part of the enzyme
  - ➢ **Complex (Holoenzyme, conjugated):** associated with non-protein molecule (such as coenzyme)
- In general, enzymes end with the suffix (-ase)
- Most other enzymes are named for their substrates and for the type of reactions they catalyze, such as:
  - ➢ ATPase which breaks down ATP
  - ➢ ATP synthase synthesizes ATP
- Some enzymes have common names
  - ➢ Such as: The proteolytic enzyme trypsin

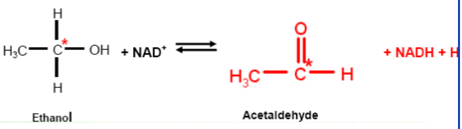
- Enzymes are classified according to their function into:

♦ Oxidoreductases

- They catalyze oxidation/reduction reactions involving the transfer of hydrogen atoms or electrons
- They include:

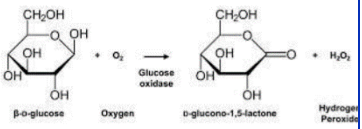
1) Dehydrogenases

- They transfer electrons in the form of hydride ions (H) or hydrogen atoms using an electron-transferring coenzyme, such as NAD^+, NADH, FMN and $FADH_2$
  - ➢ **Lactate dehydrogenase** (converts lactate into pyruvate using NAD^+ and NADH)
  - ➢ **Alcohol dehydrogenase** (convert Ethanol into acetaldehyde)



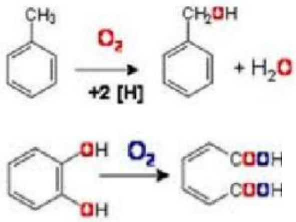
2) Oxidase

- They catalyze hydrogen transfer from the substrate to molecular oxygen (O_2) producing hydrogen peroxide (H_2O_2) as a by-product
  - ➢ **Glucose oxidase**



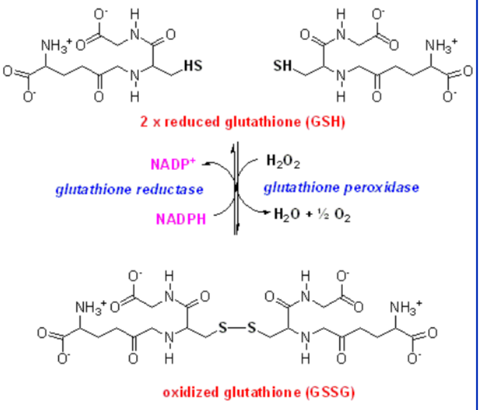
3) Oxygenase

- They catalyze substrate oxidation by molecular oxygen through introducing oxygen into the substrate
- They can be either:
  - ➢ **Monooxygenases:** Introduce only one O atom, and produce H_2O as a by-product (reduced product), such as lactate monooxygenase
  - ➢ **Dioxygenases:** Introduce 2 O atoms, without producing water, such as tryptophan 2,3 dioxygenase



4) Peroxidases

- Peroxidases catalyze oxidation of a substrate by hydrogen peroxide
  - ➢ Such as **Glutathione peroxidase** which oxidizes two molecules of glutathione (GSH) in the presence of hydrogen peroxide
    - ✓ Glutathione peroxidase protects us from reactive oxygen species and its deficiency causes RBC hemolysis
    - ✓ Glutathione reductase (dehydrogenase), reform the 2 glutathione molecules in the reduced form, using NADPH

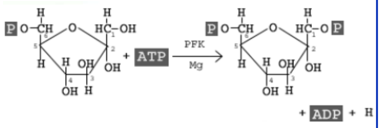


♦ Transferases

- These enzymes transfer a functional group (C, N, P, or S) from one substrate to an acceptor molecule
- They include:

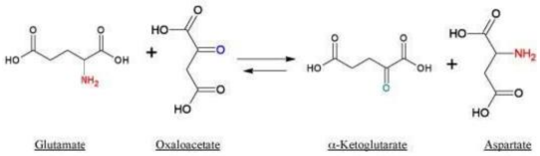
1) Kinases: transfer a phosphate group from (ATP, GTP, CTP…) to an acceptor

  - ➢ Such as **phosphofructokinase** catalyzes the transfer of phosphate from ATP to fructose-6-phosphate forming fructose 1,6 bisphosphate



2) Transaminases: transfer an amino functional group

  - ➢ They convert amino acids into keto acids and vice versa
  - ➢ Such as **aspartate transaminase** which converts between oxaloacetate and aspartate and this reaction is in equilibrium (ΔG is zero)



3) **Synthases:** transfer monomers into the growing end of polymers, so synthesize large polymers

- Such as **glycogen synthase** (UDP glucose-glycogen glycosyltransferase), transfers a glucosyl residue from uridine diphosphate (UDP)-glucose to the end of a glycogen molecule elongating glycogen

Hydrolases

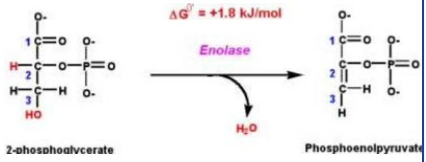
- They catalyze cleavage reactions using water across the bond in the form of OH^- and H^+ to being broken or the fragment condensations
  - Such as **proteases** which catalyze proteolysis (hydrolysis of a peptide bond within proteins)
  - **Trypsin** breaks up peptide bonds on the carboxyl side of only Lys and Arg
  - **Chymotrypsin** hydrolyzes peptide bonds involving bulky aromatic amino acids
  - **Elastase** hydrolyzes peptide bonds involving small, uncharged groups such as Ala, Val, or Gly

Lyases

- They cleave C-C, C-O, C-N, and other bonds by means other than hydrolysis or oxidation, leaving double bonds or rings, or conversely adding groups to double bonds without hydrolysis
- They include:

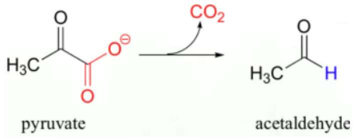
1) Dehydrases

- Removal of H_2O from the substrate to give a double bond
- Such as **enolase**



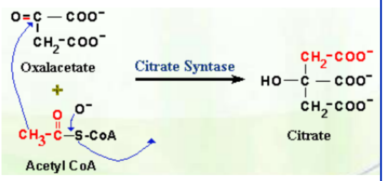
2) Decarboxylases

- Replacement of a carboxyl group by a hydrogen
- Such as **Pyruvate decarboxylase**



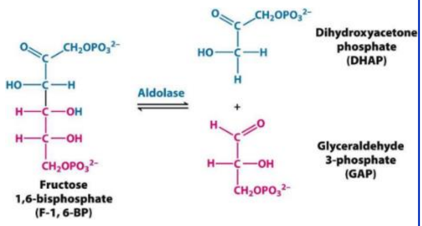
3) Synthases

- Addition of a small molecule to a double bond or when the direction of the reaction favors the formation of a carbon-carbon bond
- Such as **citrate synthase**



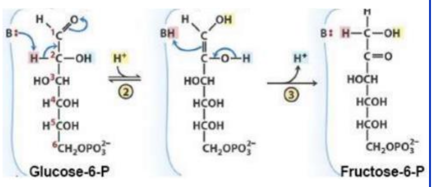
4) Aldolase

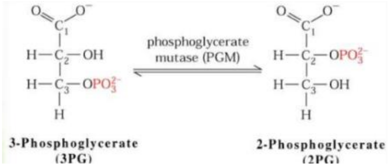
- It breaks down fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehydes-3-phosphate (which are isomers)



Isomerases

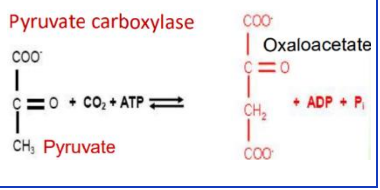
- These enzymes catalyze intramolecular rearrangements
- Enzymes that rearrange the bond structure of a compound are called **isomerases**, whereas enzymes that catalyze the movement of phosphate from one atom to another are called **mutases**
  - **Phosphoglucoisomerase** isomerizes glucose-6-phosphate to fructose-6-phosphate
  - **Phosphoglycerate mutase** transfers a phosphate group from carbon number 3 to carbon number 2 of phosphorylated glycerate





Ligases

- Ligases join C-C, C-O, C-N, C-S, and C-halogen bonds
- The reaction is usually accompanied by the consumption of a high-energy compound such as ATP



- They include **Synthetases** derive the energy from the cleavage of high-energy phosphate bonds
  - Such as **carboxylases**
- Synthases join molecules without the consumption of ATP

❖ Abzymes

- Abzymes are antibodies acting as enzymes
  - They are produced **against transition-state analogs**
  - A host animal is injected with a transition-state analogue. The animal makes antibodies against it (binding with high affinity at specific binding points mimicking an enzyme's active site surrounding a transition state)
- Abzymes with activity similar to cocaine esterase, which degrades cocaine, have been developed against analogs of its transition-state complex. Monthly injections are used to treat addicted individuals by destroying cocaine in the blood and, thereby, decreasing their dependence on cocaine

❖ Ribozymes

- Most enzymes are proteins, but RNA molecules can act as enzymes
- Ribozymes are enzymes made of both protein and RNA
- For some, catalysis is performed by RNA
  - Examples include those involved in **RNA splicing** and **protein synthesis** in ribosomes
  - The catalytic efficiency of RNAs is less than that of protein enzymes, but can be enhanced and stabilized by the presence of protein subunits

- Some scientists think that RNA are the precursors pf life