Enzymes and Metabolic Pathways

Enzyme characteristics

- Made of protein
- **Catalysts**:
  - reactions occur 1,000,000 times faster with enzymes
  - Not part of reaction
    - Not changed or affected by reaction
    - Used over and over

Why do cells need enzymes?

- Not enough time to leave things to chance
- Most probable reactions are not the required
- Important molecules do not always occur in large quantities

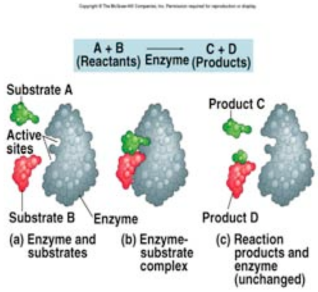
But since life processes are dependent upon enzymes, organisms cannot survive at high temperature
- Heat denatures proteins

Mechanism of Enzyme Action

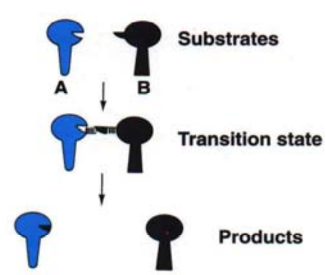
- Ability of enzymes to lower activation energy due to structure.
- Each type of enzyme has has a highly-ordered, characteristic 3-dimensional shape (conformation).
  - Ridges, grooves, and pockets lined with specific amino acids.
  - Pockets active in catalyzing a reaction are called the **active sites** of the enzyme.

Mechanism of Enzyme Action (continued)

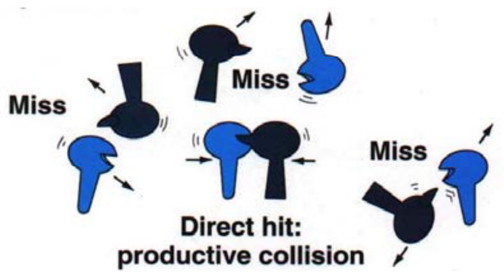
- Substrates have specific shapes to fit into the active sites (lock-and-key model):
  - Substrate fits into active sites in enzyme.
  - Perfect fit may be induced:
    - Enzyme undergoes structural change.
  - Enzyme-substrate complex formed, then dissociates.
  - Products formed and enzyme is unaltered.



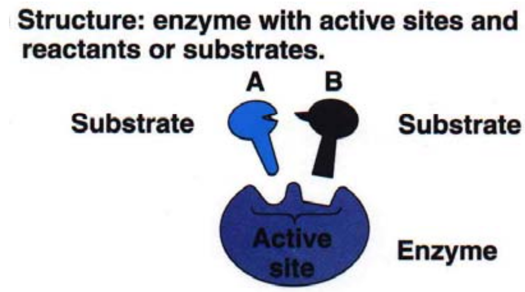
Reactants must collide for reaction to occur



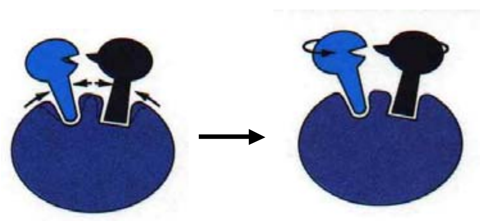
Without enzymes, collisions are random



An enzyme brings reactants together by binding to them



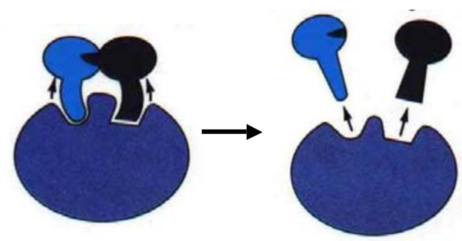
Induced Fit Model



1. Enzyme binds substrate, forms complex

2. Substrates orient for productive collision

Induced Fit Model



3. Productive collision produces transition state

4. Reaction occurs, products are released

Naming of Enzymes

- Enzyme name ends with suffix "*-ase*."
- Name = substrate – action – "*-ase*"
  - E.g., *glucose phosphorylase* is an enzyme that adds a phosphate to glucose.
  - If the "action" is left out of the name, assume the action is hydrolysis. E.g., a *protease* catalyzes the hydrolysis of proteins into oligopeptides or amino acids.
- Different organs may make different enzymes (isoenzymes) that have the same activity.
  - Differences in structure do not affect the active sites.

Enzymes as Catalysts

Regulation of Enzyme Activity

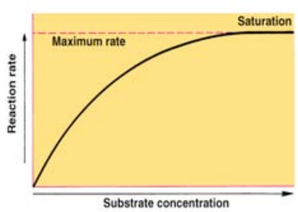
- Rate of enzyme-catalyzed reactions measured by the rate that substrates (reactants) are converted to products.
- Factors regulating enzyme activity:
  1. Mass action: concentration of substrates and products.
  2. Concentration of enzyme.
     - Gene expression.
     - Post-translational modification.
     - Proteolysis.
  3. Alteration of enzyme structure.
     - Activation.
     - Temperature.
     - pH.
  4. Cofactors and Coenzymes.
  5. Inhibitors.

Reversible Reactions

- Some enzymatic reactions are reversible.
  – Both forward and backward reactions are catalyzed by same enzyme.
- $H_20 + C0_2 \rightleftharpoons H_2C0_3$ (carbonic anhydrase)
- Law of mass action:
  – Principal that reversible reactions will be driven from the side of the equation where concentration is higher to side where concentration is lower.
- But most biological reactions have complex organic substrates which require separate enzymes to catalyze the reverse reaction.

Substrate Concentration

- At a specific [enzyme], rate of product formation increases as the [substrate] increases.
- Plateau of maximum velocity occurs when enzyme is **saturated**.
- Additional [substrate] does not not increase reaction rate.

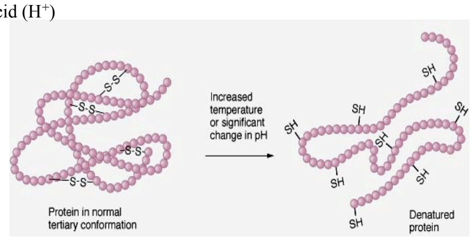


Enzyme Activation

- Enzymes may be produced in an inactive form.
- Final post-translational modification occurs only when enzyme is needed.
  – In pancreas, digestive enzymes are produced as inactive zymogens, which are activated in lumen of intestine.
    - Part of the polypeptide is hydrolyzed off.
    - Protects against self-digestion.
  – In liver cells, enzyme is inactive when produced and is activated by addition of phosphate group.
    - Phosphorylation/dephosphorylation: Activation/inactivation of an enzyme.

Enzyme Shape

- Function of the enzyme is dependent on its shape
- Destruction of shape destroys function
  – Heat
  – Acid (H^+)



Functional shape Denatured

Effect of Temperature

- Rate of reaction increases as temperature increases.
- Reaction rate plateaus, slightly above body temperature (37º C).
- Reaction rate decreases as temperature increases.
- Enzymes denature at high temperatures.

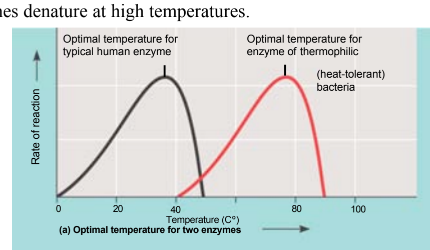
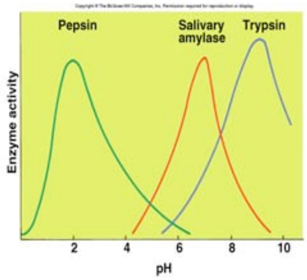


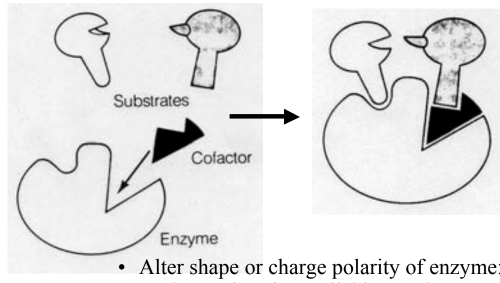
Figure 8.18

Effect of pH

- Each enzyme exhibits peak activity at narrow pH range (pH optimum).
- pH optimum reflects the pH of the body fluid in which the enzyme is found.
- If pH changed, so is no longer within the enzyme range; reaction will decrease.



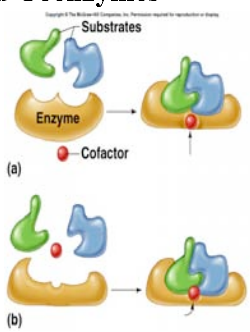
Enzyme helpers: Cofactors

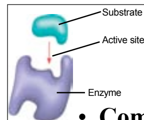


- Alter shape or charge polarity of enzyme: makes active site available to substrate
- Metal ions from dietary minerals.

Cofactors and Coenzymes

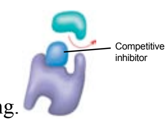
- Needed for the activity of specific enzymes.
- Cofactor:
  - Attachment of cofactor causes a conformational change of active site.
  - Participate in temporary bonds between enzyme and substrate.
- Coenzymes:
  - Organic molecules derived from H_20 soluble vitamins.
  - Transport H^+ and small molecules from one enzyme to another.





Enzyme Inhibitors

- **Competitive Inhibitors**:
  - Shape at least partially fits in the enzymes active site ➩ blocks substrate from binding.



- **Allosteric** ***"different shape"*** **Inhibitors**:
  - Bind to a different location of the enzyme protein — the *allosteric site* ➩ alters the shape of the protein ➩ active site no longer fits substrates

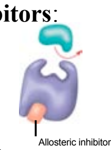


Figure 8.19

Most enzymes have allosteric regulation

- Proteins change shape when regulatory molecules bind to sites.
  - Allosteric activators (cofactors) and/or inhibitors

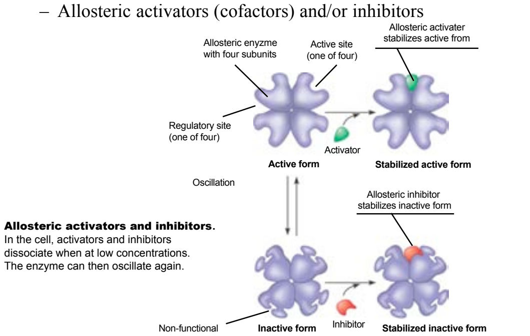


Figure 8.20

Enzymes are Binding Proteins

- I.e., they function by binding to a substrate (ligand)
- Other important biological regulation by means of binding proteins:
  - Receptors for hormones & neurotransmitters
  - Transporters across cell membranes
  - Carriers of low-solubility or fragile substances
  - Immune recognition

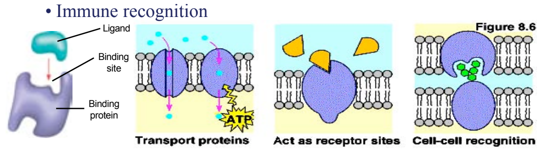


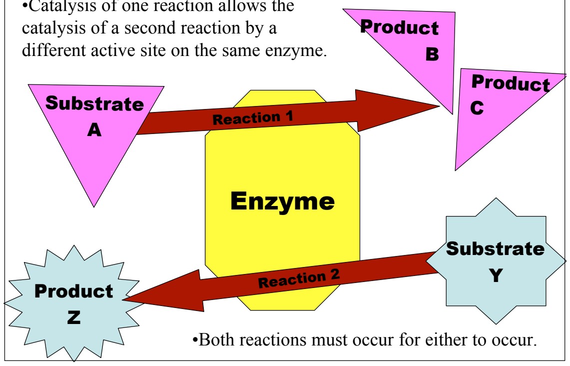
Figure 8.6

All binding proteins have similar characteristics:

1. **Specificity**
2. **Saturation**
3. **Competitive Inhibition**

Coupled Reactions

•Catalysis of one reaction allows the catalysis of a second reaction by a different active site on the same enzyme.



•Both reactions must occur for either to occur.

Coupled Reactions: Bioenergetics

- Energy transfer from one molecule to another couples chemical reactions
- **Exergonic reaction**: reaction releases energy
- **Endergonic reaction**: reaction requires energy
- **Coupled bioenergetic reactions**: the energy released by the exergonic reaction is used to power the endergonic reaction.

Bioenergetics

- Flow of energy in living systems obeys:
- 1st law of thermodynamics:
  - Energy can be transformed, but it cannot be created or destroyed.
- 2nd law of thermodynamics:
  - Energy transformations increase entropy (degree of disorganization of a system).
  - Only free energy (energy in organized state) can be used to do work.
    - Systems tend to go from states of higher free energy to states of lower free energy.

Free Energy & Entropy

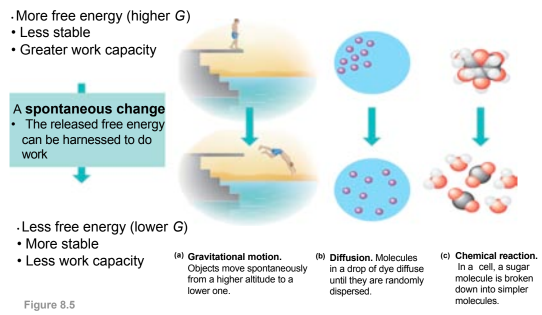


Figure 8.5

Exergonic and Endergonic reactions in metabolism

- An exergonic reaction
  - Proceeds with a net release of free energy and is spontaneous
- An endergonic reaction
  - Is one that absorbs free energy from its surroundings and is nonspontaneous

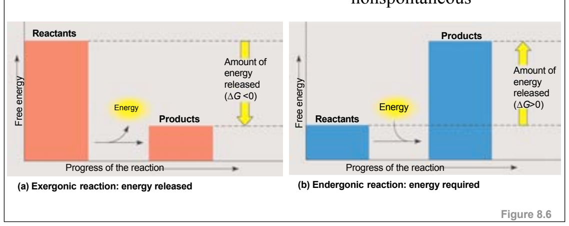
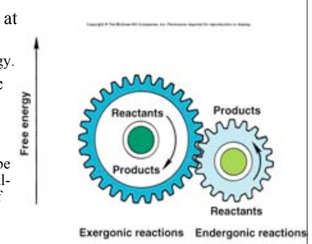


Figure 8.6

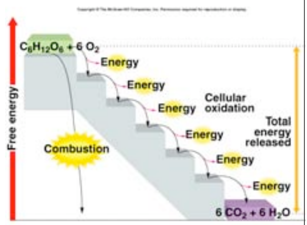
Coupled Reactions: Bioenergetics

- Cells must maintain highly organized, low-entropy state at the expense of free energy.
  - Cells cannot use heat for energy.
- Energy released in exergonic reactions used to drive endergonic reactions.
  - Require energy released in exergonic reactions (ATP) to be directly transferred to chemical-bond energy in the products of endergonic reactions.



Endergonic and Exergonic Reactions

- Endergonic:
  - Chemical reactions that require an input of energy to make reaction "go."
    - Products must contain more free energy than reactants.
- Exergonic:
  - Convert molecules with more free energy to molecules with less.
  - Release energy in the form of heat.
    - Heat is measured in calories.



The Role of ATP:
Energy transfer from one molecule to another couples chemical reactions

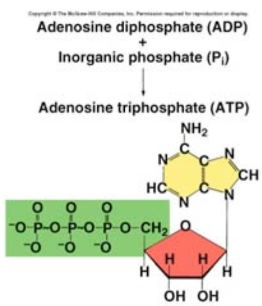
A-P-P~P + glucose -->

glucose-6~phosphate + A-P-P

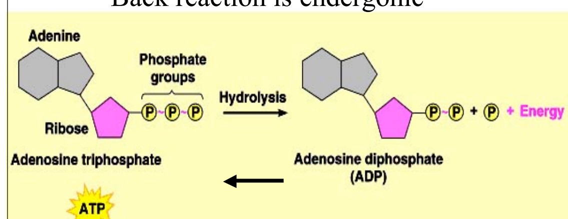
- **Exergonic reaction**: removing phosphate from ATP releases energy
- **Endergonic reaction**: transfer of phosphate to glucose stores energy

Formation of ATP

- Formation of ATP requires the input of a large amount of energy.
  - Energy must be conserved, the bond produced by joining P_i to ADP must contain a part of this energy.
- This energy released when ATP converted to ADP and P_i.
- ATP is the universal energy carrier of the cell.

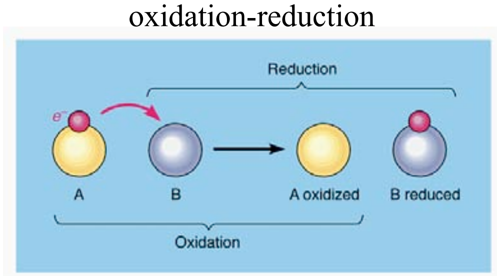


Forward reaction is exergonic
Back reaction is endergonic



- Cells use ATP by breaking phosphate bond and transferring energy to other compounds
- Cells make ATP by transferring energy from other compounds to form phosphate bond

Coupled Reactions: *Redox*
Transfer of electrons is called oxidation-reduction



- AKA, Reduction–oxidation ["Redox"]

Oxidation-Reduction

- May involve the transfer of hydrogen atom [$H^+ + e^-$] rather than free electrons.
- Reduced:
  - Molecule/atom gains electrons or hydrogen.
- Reducing agent:
  - Molecule/atom that donates electrons or hydrogen.
- Oxidized:
  - Molecule/atom loses electrons or hydrogen.
- Oxidizing agent:
  - Molecule/atom that accepts electrons or hydrogen.
- Reduction and oxidation are always coupled reactions.
  - The reducing agent is oxidized.
  - The oxidizing agent is reduced.

Coenzymes: Electron Carriers

- **Electron carriers**: shuttles electrons (and hydrogen) between compounds
- Used to transfer electrons to the electron transport chain
- Carbon compounds are oxidized, carriers are reduced

Coenzymes: Electron Carriers

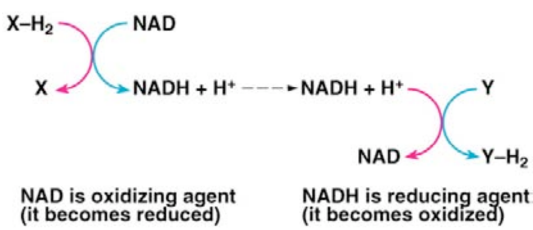
- NAD^+ (nicotinamide adenine dinucleotide)
  - {Derived from vitamin B_3: **n**iacin}

    $NAD^+ + H^+ + 2e^- \rightarrow NADH$
- $FADH^+$ (flavin adenine dinucleotide)
  - {Derived from vitamin B_2: ribo**f**lavin}

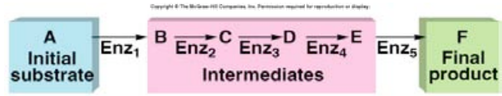
    $FADH^+ + H^+ + 2e^- \rightarrow FADH_2$

- Reminder: Hydrogen = $H^+ + e^-$

Oxidation-Reduction (continued)

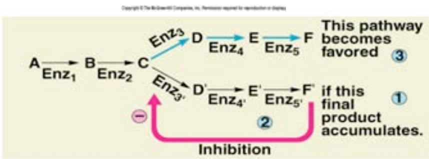


Metabolic Pathways



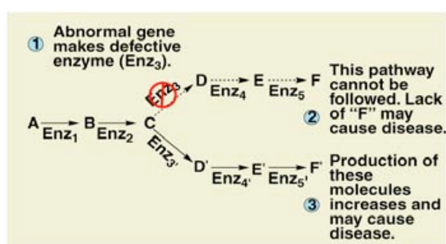
Sequence of enzymatic reactions that begins with initial substrate, progresses through intermediates and ends with a final product.

Branched Pathways



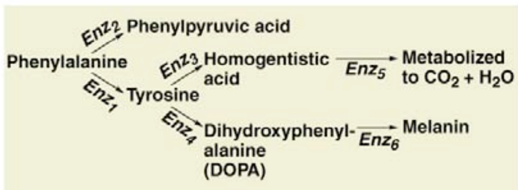
- **End-Product Inhibition**.
- One of the final products in a divergent pathway inhibits the activity of the branch-point enzyme.
  - Prevents final product accumulation.
  - Results in shift to product in alternate pathway.

Inborn Errors of Metabolism



- Inherited defect in a gene for enzyme synthesis.
- Quantity of intermediates formed prior to the defect increases.
- Final product formed after the defect decreases, producing a deficiency.

Inborn Errors of Metabolism
Example: phenylalanine metabolism

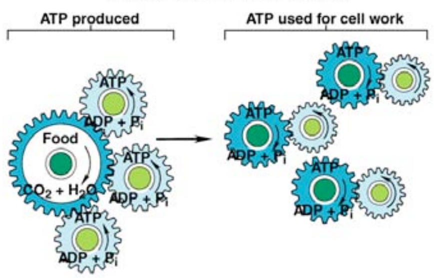


- Defective enzyme$_1$ ➪ phenylketonuria [PKU]
- Defective enzyme$_5$ ➪ alcaptonuria
- Defective enzyme$_6$ ➪ albino

Coupled Pathways: Bioenergetics

- Energy transfer from one metabolic pathway to another by means of ATP.
- **Catabolic pathway (catabolism)**: breaking down of macromolecules. Releases energy which may be used to produce ATP.
- **Anabolic pathway (anabolism)**: building up of macromolecules. Requires energy from ATP.
- **Metabolism**: the balance of catabolism and anabolism in the body.

Coupled Metabolic Pathways: via ATP



ATP drives endergonic reactions

- The three types of cellular work are powered by the hydrolysis of ATP

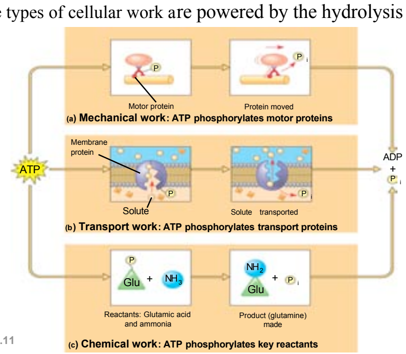


Figure 8.11

Heterotrophic Organisms