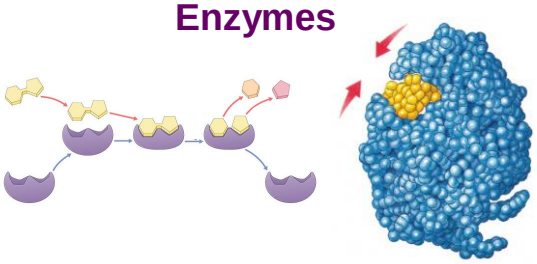
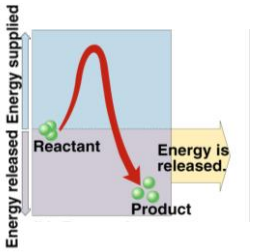
Chapter 8.3 – 8.5

Enzymes



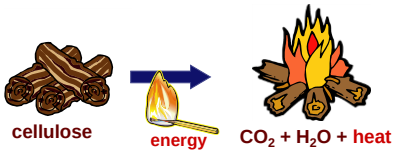
Spontaneous reactions?

- If reactions are "downhill", why don't they just happen spontaneously?
  - because covalent bonds are stable



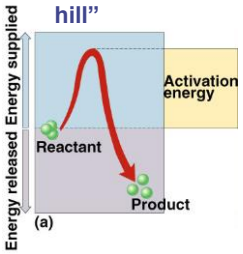
Activation Energy

- Breaking down large molecules requires an initial input of energy
  - activation energy
  - large biomolecules are stable
  - must absorb energy to break bonds



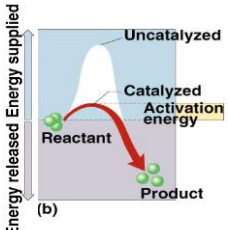
Activation Energy

- the amount of energy needed to destabilize the bonds of a molecule
  - moves the reaction over an "energy hill"



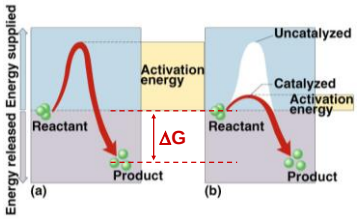
Reducing Activation Energy

- Catalysts
  - reducing the amount of energy to start a reaction

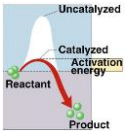


"Organic" Catalysts

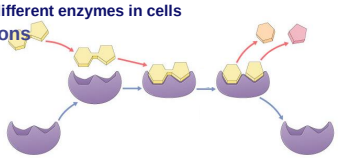
- So what's a cell to do to reduce activation energy?
  - get help! … chemical help… ENZYMES



Enzymes



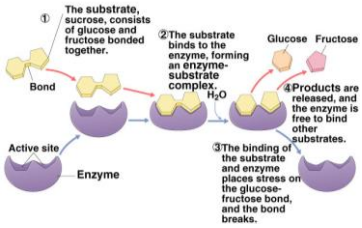
- Biological catalysts
  - proteins (& RNA – ribozymes!)
  - facilitate chemical reactions
    - increase rate of reaction without being consumed
    - reduce activation energy
    - don't change free energy (ΔG) released or required
  - required for most biological reactions
  - highly specific
    - thousands of different enzymes in cells
  - 'control' reactions



Enzymes & Substrates

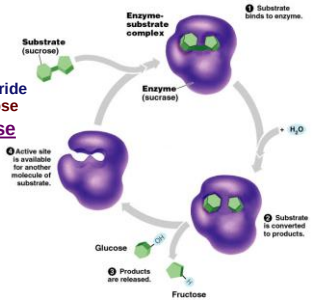
substrate
- reactant which binds to enzyme
- enzyme-substrate complex: temporary association between substrate and enzyme

product
- end result (molecules) of reaction



Enzymes & Substrates

- Enzyme + substrates → products
  - ex. sucrase
    - enzyme breaks down sucrose
    - binds to sucrose and breaks disaccharide into fructose & glucose
  - ex. DNA polymerase
    - enzyme builds DNA
    - adds nucleotides to a growing DNA strand

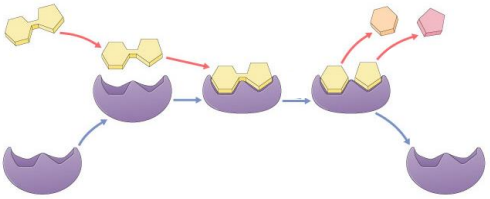


Specificity of Enzymes

- Reaction specific
  - each enzyme is substrate-specific
    - due to fit between active site & substrate
      - substrates held in active site by weak interactions
        - H bonds
        - ionic bonds
  - enzymes named for reaction they catalyze
    - sucrase breaks down sucrose
    - proteases break down proteins
    - lipases break down lipids
    - DNA polymerase builds DNA
    - pepsin breaks down proteins (polypeptides)

Reusable

- Not consumed in reaction!
  - single enzyme molecule can catalyze thousands or more reactions per second
  - enzymes unaffected by the reaction

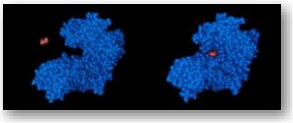


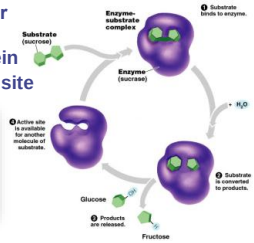
How does it work?

- Variety of mechanisms to lower activation energy & speed up reaction
  - active site orients substrates in correct position for reaction
    - enzyme brings substrate closer together
  - active site binds substrate & puts stress on bonds that must be broken, making it easier to separate molecules
  - groups near the active site can add a chemical charge for re-dox reactions

Lock and Key Model

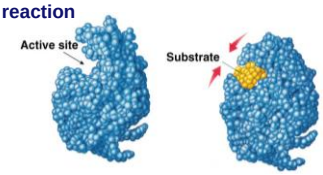
- Simplistic model of enzyme action
  - 3-D structure of enzyme fits substrate
- Active site
  - enzyme's catalytic center
  - pocket or groove on surface of globular protein
  - substrate fits into active site





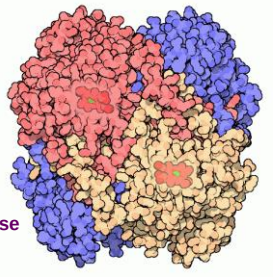
Induced Fit Model

- More accurate model of enzyme action
  - 3-D structure of enzyme fits substrate
  - as substrate binds, enzyme changes shape leading to a tighter fit
    - "conformational change"
    - bring chemical groups in position to catalyze reaction



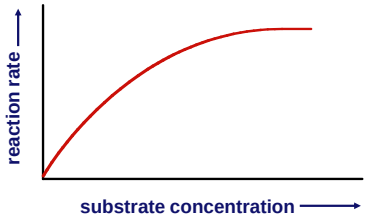
Factors Affecting Enzymes

- Substrate concentration
- Enzyme concentration
- Temperature
- pH
- Salinity
- Activators
- Inhibitors



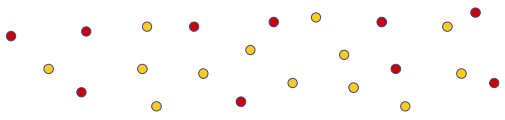
catalase

[Substrate]

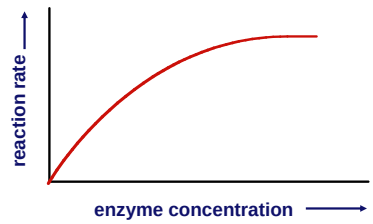


[Substrate]

- Effect on rates of enzyme activity
  - as ↑ substrate = ↑ reaction rate
    - more substrate = more frequently collide with enzymes
  - reaction rate levels off
    - all enzymes have active site engaged
    - enzyme is saturated; is the limiting factor
    - maximum rate of reaction

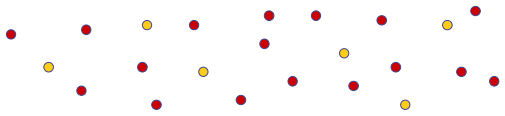


[Enzyme]

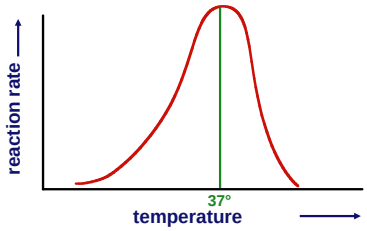


[Enzyme]

- Effect on rates of enzyme activity
  - as ↑ enzyme = ↑ reaction rate
    - more enzymes = more frequently collide with substrate
  - reaction rate levels off
    - substrate becomes limiting factor
    - not all enzyme molecules can 'find' substrate



Temperature

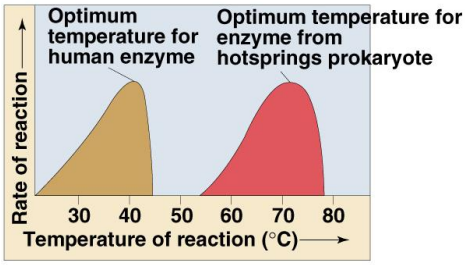


Temperature

- Effect on rates of enzyme activity
  - Optimum T°
    - greatest number of molecular collisions
    - human enzymes = 35°- 40°C (body temp = 37°C)
  - Increase beyond optimum T°
    - increased agitation of molecules disrupts bonds
      - H, ionic, hydrophobic = weak attractions
    - denaturation = lose 3D shape (3° structure)
  - Decrease T° below optimum T°
    - molecules move slower
    - decrease collisions

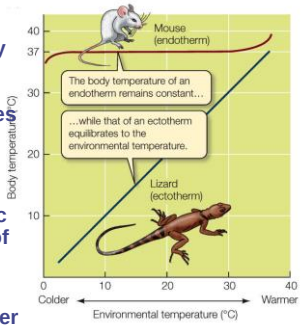
Temperature

- Different enzymes functional in different organisms

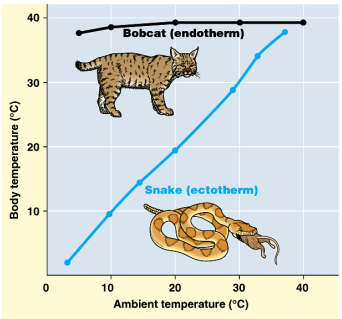


What -therm are you?

- ectotherm
  - animals whose body temperature are determined by external heat sources
- endotherm
  - regulate body temperature by producing metabolic heat or prevention of heat loss
- heterotherm
  - can behave like either
    - hibernating mammals

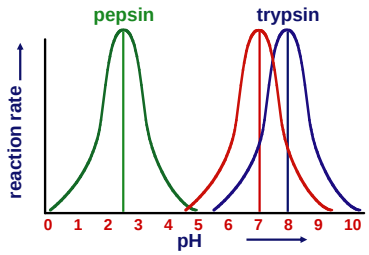


How do ectotherms do it?



ISOZYMES!
different enzymes with different chemical compositions and physical properties, but control the same reaction.

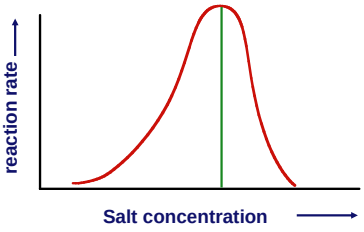
pH



pH

- Effect on rates of enzyme activity
  - pH changes
    - changes charges (add or remove H^+)
    - disrupt bonds, disrupt 3D shape
      - affect 3° structure
  - most human enzymes = pH 6-8
    - depends on localized conditions
    - pepsin (stomach) = pH 3
    - trypsin (small intestines) = pH 8

Salinity (salt concentration)

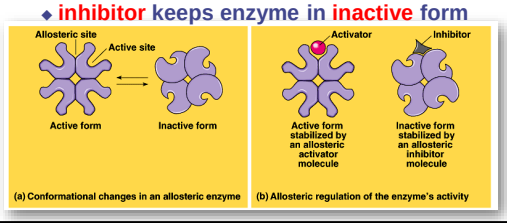


Salinity (salt concentration)

- Effect on rates of enzyme activity
  - protein shape (conformation)
    - depends on attraction of charged amino acids
  - salinity changes
    - change [inorganic ions]
    - changes charges (add + or –)
    - disrupt bonds, disrupt 3D shape
      - affect 3° structure
  - enzymes intolerant of extreme salinity
    - Dead Sea is called dead for a reason!

Action of Allosteric Control

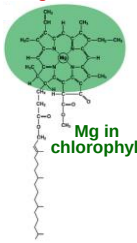
- Activators & Inhibitors
  - regulatory molecules attach to allosteric site (not active site) causing conformational (shape) change
  - activator keeps enzyme in active form
  - inhibitor keeps enzyme in inactive form



Activators

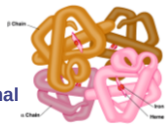
- Compounds which help enzymes
- Prosthetic groups
  - non-amino acid groups bound to enzymes
    - heme group in hemoglobin
- Cofactors
  - non-protein, small inorganic compounds & ions
    - Mg++, K+, Ca++, Zn, Fe, Cu
    - bound in enzyme molecule
- Coenzymes
  - non-protein, organic molecules
    - bind temporarily or permanently to enzyme near active site
  - many vitamins
    - NAD (niacin; B3)
    - FAD (riboflavin; B2)
    - Coenzyme A

Fe in hemoglobin's heme



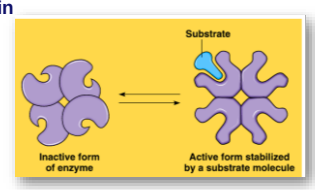
Mg in chlorophyll

Substrate Cooperativity



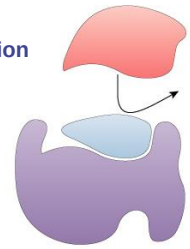
- Substrate acts as an activator
  - substrate causes conformational change in enzyme
    - induced fit
  - favors binding of substrate at 2nd site
  - makes enzyme more active & effective
    - ex: hemoglobin

4 polypeptide chains:
- bind 4 O_2;
- 1st O_2 binds
- makes it easier for other 3 O_2 to bind



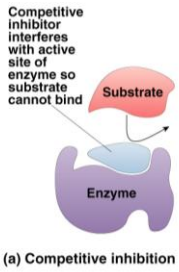
Inhibitors

- Regulation of enzyme activity
  - other molecules that affect enzyme activity
- Selective inhibition & activation
  - competitive inhibition
  - noncompetitive inhibition
  - irreversible inhibition
  - feedback inhibition



Competitive Inhibitor

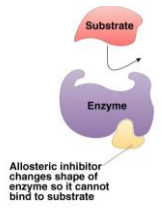
- Effect
  - inhibitor & substrate "compete" for active site
    - ex: disulfiram (Antabuse) to overcome alcoholism
  - overcome by increasing substrate concentration
    - saturate solution with 'alternate substrate' so it out-competes for active site on enzyme
    - ex: methanol poisoning



(a) Competitive inhibition

Non-Competitive Inhibitor

- Effect
  - inhibitor binds to site other than active site
    - allosteric site
    - called allosteric inhibitor
      - ex: some anti-cancer drugs (methotrexate & FdUMP) inhibit enzymes involved in synthesis of nucleotides & therefore in building of DNA = stop DNA production, stopping abnormal division
      - ex: heavy metal poisoning
      - ex: cyanide poisoning
    - causes enzyme to have a conformational shape change
    - renders active site unreceptive



(b) Noncompetitive inhibition

Irreversible Inhibition

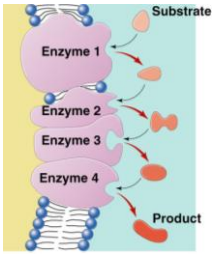
- Inhibitor permanently binds to enzyme
  - competitor
    - permanently binds to active site
  - allosteric
    - permanently changes shape of enzyme
    - ex: nerve gas DIPF, sarin, many insecticides (malathion, parathion…)
      - DIPF (diisopropylphosphorofluoridate) is an…
      - acetylecholinesterase inhibitor—doesn't breakdown the neurotransmitter, acetylcholine, which is vital for muscle contraction

Metabolic Pathways

A → B → C → D → E → F → G

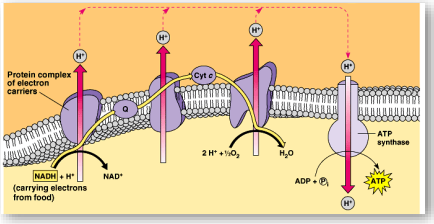
enzyme 1, enzyme 2, enzyme 3, enzyme 4, enzyme 5, enzyme 6

- Chemical reactions of life are organized in pathways
  - divide chemical reaction into many small steps
    - efficiency
    - control = regulation



Efficiency

- Groups of enzymes organized
  - if enzymes are embedded in membrane they are arranged sequentially
- Link endergonic & exergonic reactions



Feedback Inhibition

- Regulation & coordination of production
  - product is used by next step in pathway
  - final product is inhibitor of earlier step
    - allosteric inhibitor of earlier enzyme
    - feedback inhibition
  - no unnecessary accumulation of product

A → B → C → D → E → F → G

enzyme 1, enzyme 2, enzyme 3, enzyme 4, enzyme 5, enzyme 6

G is an allosteric inhibitor of enzyme 1

Feedback Inhibition

- Example
  - synthesis of amino acid, isoleucine from amino acid, threonine