ENZYMES

Increase rate of metabolic reactions by lowering the activation energy required to start the reaction

Enzymes are critical to metabolic function. Enzymes do all of the “work” that gets done in cells. Enzymes are protein molecules that your cells make and then use as “tools” to get their metabolic work done. Enzymes must physically fit together with the molecule they work on. These are called substrate molecules. The analogy of a lock & key is a good one for describing the relationship between enzymes and their substrates.

Enzymes, like keys, are specific to their substrate. The concept is, in fact, called “substrate specificity”. The reason for substrate specificity is that the enzyme and substrate must fit together physically very well for the chemical reactions to take place.

• Enzyme is key
• Substrate is lock

• Enzyme acts (does work) on substrate to change it in some way- either breaks molecules apart (catabolism) or puts molecules together (anabolism)
• Enzymes must have ATP and must maintain their correct structure to work
Enzymes are protein catalysts

- level of specificity varies depending on enzyme involved- some have greater substrate specificity than others
- Usually will only bind to one or a very few similar substrates

Enzymes increase the number of reactions by

- bringing the reactant (substrate) molecules together physically
- Changing their shape while bound to substrate, forcing the substrate molecule apart

- Enzymes are not used up or altered in reactions
- speed up reactions up to $10^{10}$ times!

- Enzymes do not make reactions happen that COULDN’T happen on their own, BUT if enzyme is not present, reaction WILL NOT occur at a rate that is useful to cell
- usually metabolism occurs in “biochemical cascades”---many enzymes organized in sequence
An enzyme can be:

- a pure protein made up of one or more chains
- a holoenzyme, which can consist of an apoenzyme (protein component) plus one or more of the following

- a nonprotein component (cofactor)
  - **Prosthetic group** = a cofactor that is **covalently** attached to the apoenzyme (e.g. folic acid, riboflavin, FAD, heme groups, Zinc, etc.)
  - **Coenzyme** = a cofactor that is **NOT covalently** bound to the apoenzyme; it may dissociate from the apoenzyme and carry one or more of the products of the reaction to another enzyme (e.g. NAD, coenzyme A)
Factors Affecting Enzyme Function

- Substrate or Enzyme Concentration
- Temperature
- pH
- Allosteric Regulators
- Product Inhibition
- Phosphorylation state

ALL enzymes have an optimal temp and pH where they function best.
- Below and above optimal pH, enzyme function declines logarithmically
- Below optimal temp enzyme function also declines logarithmically as with pH
- Above optimal temp function of enzymes MAY decline gradually for a time, but then drops off dramatically when temp get high enough to denature enzyme/protein. Denatured proteins lose all shape and thus, function!!!

- Increasing concentration of enzyme to equal that of substrate will increase # of rxns that can occur at one time. Increasing concentration of enzyme beyond that of substrate will have no additional benefit.

- Allosteric regulators can be co-factors or co-enzymes required for enzyme function- in other words, they physically must be attached to the enzyme for it to work.

- Or allosteric regulators can be inhibitors- if inhibitor is present and attached to enzyme, it will prevent enzyme function.

- Product inhibition is a type of allosteric regulation where the enzyme’s product actually stays around blocking the enzyme from attaching to any new substrate molecules. This is a common way for cells to regulate concentration of the product being made.
• Enzymes must be “phosphorylated” in order to function. This is what is meant when we say that enzymes require ATP to function- the ATP molecule actually attaches a phosphate group to the enzymes, thereby activating them. When an enzyme “does work” on a substrate molecule, it loses its phosphate and becomes inactivated (it actually uses the energy stored in the phosphate bond to do the work). An inactivated enzyme, needs to be re-phosphorylated before it can be activated again.

• Addition of phosphate by substrate-level-phosphorylation actually alters the active site of the enzyme, thus making it functional. During enzyme activity, the enzyme is dephosphorylated which inactivates it. The enzyme must then be phosphorylated again to be reactivated. Other enzymes are required to phosphorylate (phosphatase) and dephosphorylate (kinase) molecules.
Role of ATP in Metabolism

Energy currency is adenosine-triphosphate-ATP

• phosphate bonds HIGH ENERGY!

• used to store energy SHORT TERM

• (Save energy) Cells must efficiently transfer energy from their energy-trapping systems to systems that actually carry out work

• (Spend energy) Cells must also use various metabolic processes to replace energy used in doing work

• ATP is the only* molecule that can directly activate enzymes, since they need to be phosphorylated

• The macromolecules in food, carbs, fats, etc are fuel used to make ATP.

• Breakdown

  • one phosphate cleaved from ATP
    • Ps negatively charged - repel each other so pretty easy to remove one
    • energy released: 7300 kcal/mol

• (Exergonic) breakdown of ATP can be coupled with various endergonic reactions to facilitate their completion

• *Note: there are also UTP, GTP, and CTP in cell; pools of nucleotide phosphate groups can be interchanged by appropriate enzymes (used rarely)

• Metabolic-energy-trapping processes- glycolysis, oxidative-phophorylation, etc.
  • used to catalyze formation of ATP from ADP & P_i, & thus to restore energy balance of cell
Metabolism

- **Anabolism**: synthesis, building up of molecules, cells, structures, tissues
  - requires energy
  - Makes larger molecules out of smaller ones

- **Catabolism**: degradation, destruction, breakdown of structures
  - releases energy
  - Makes smaller molecules out of larger ones

ENERGY IS THE ABILITY TO DO WORK
- usually expressed in units of calories, or joules
• All energy forms are, in principle, interconvertible.
• In all these processes the total amount of energy is conserved;
• from the height and weight of the brick in the first example, we can predict exactly how much heat will be released when it hits the floor.

• Bricks are a good analogy to macromolecules!!
• think of anabolism like the building of a brick building with the smaller components (bricks)– it takes a lot of energy input-

• THOSE GUYS ARE WORKING HARD!!!
- Once built the building is storing a lot of potential energy in its structure - the bricks have a lot of potential energy just based on height.
• As it collapses the building releases a tremendous amount of energy in the form of heat and sound waves.
• The bonds between the carbohydrate molecules that make up this bread are just like the bricks in this building (although, they taste better!!)

• As bread, they are storing potential energy, but once the molecules start to get digested and broken down, they release energy. Chemical bonds store potential energy.

• Just like with bricks falling and hitting the ground, the breaking of chemical bonds in digestion releases a lot of the energy as heat.

• Bond formation requires energy, bond breaking releases energy
In order to avoid losing most of the energy from our food molecules as heat, our metabolism involves the use of carrier molecules. These are molecules which allow for the stepwise destruction of food molecules and the short term storage of the energy that has been liberated.

Metabolism uses the coupling of oxidation and reduction reactions to accomplish this stepwise process.

Oxidation releases energy, while reduction “stores” it.

Compare oxidation vs. burning of sugar

- oxidation & burning are both mechanisms for energy release
- note that same amount of energy released from both mechanisms
- in burning, all energy lost as HEAT
- in oxidation, energy released in small steps as individual bonds are broken
  - energy may be used to make ATP right there on the spot OR
  - released in form of high energy protons and electrons
    - transferred to/trapped by carrier molecules (usually NADH or FADH2)
Pages 112-113 has a nice explanation of redox reactions

Remember- Bond formation requires energy, bond breaking releases energy

Oxidation and reduction reactions are reactions in which bonds are broken and formed.
These types of reactions are coupled so that (potential) bond energy can be transferred from one type of molecule to another

Oxidation & Reduction
• all catabolic reactions involve electron transfer
• Important to know here:
• In oxidation, electrons are lost and energy is released
• In reduction electrons are gained and this requires energy

WHY??
• Carrier molecules are used to carry energy in the form of electrons
  • 2 carriers used in cells are NADH and FADH2 (these are the B vitamins, niacin and riboflavin)
  • they carry electrons in the form of hydrogen atoms
  • When carrying electrons, they are “reduced” and called NADH and FADH2 respectively
  • When they have released their electrons, they are “oxidized” and are called NAD+ and FAD+ respectively

• e.g. coenzyme FAD+ (aka riboflavin)
  • receives two H atoms to become FADH₂ (reduced)

• e.g. NAD+ (aka niacin)
  • carries one hydrogen atom and one electron (from another H-atom) [proton remains in cellular fluids]
Three steps of cellular respiration (catabolism of glucose)

Glycolysis
- occurs in cytosol of cells
- breaks glucose into two molecules of pyruvic acid
- does not require oxygen (anaerobic)
- produces small quantities of ATP by substrate level phosphorylation and some reduced carrier molecules

Kreb’s Cycle
- occurs in mitochondria of cells
- produces large quantities of reduced carrier molecules and some ATP by substrate level phosphorylation
- does not require oxygen (anaerobic)
- Produces CO2 as a by-product

Electron Transport Chain
- occurs in the mitochondrial membranes
- produces large quantities of ATP by oxidizing the molecules produced by previous steps
•When a phosphate group moves from one molecule directly to another w/o oxidative phosphorylation (more on this in a bit)
Net result of glycolysis:

\[
glucose + 2 \text{ ADP} + 2 \text{ P}_i + 2 \text{ NAD} \rightarrow 2 \text{ pyruvate} + 2 \text{ ATP} + 2 (\text{NADH} + \text{H})
\]

here are some trapped e⁻

Glycolysis- basics to know about glycolysis:

- breakdown of glucose from 6 carbons to 2 3-carbon pyruvates
- Really, it’s the partial oxidation of glucose coupled with the reduction of FAD+ and NAD+
- A small amount of ATP is needed to start the process, but there is a net gain (a small amount of ATP is produced)
- It is anaerobic
- It occurs in the cytoplasm of cells

EXTRA INFO-for “fun”

- total energy yield of 2 ATP = 2(7.3 kcal) = 14 kcal/mol
- total potential energy of glucose: 688 kcal/mol
In between Glycolysis and the Citric Acid Cycle- see page 117

• Pyruvate enters the mitochondria of cells
• Pyruvate undergoes a reaction called decarboxylation (we’ll see more of these later) which is also an oxidation rxn, so more NAD+ gets reduced.
• Decarboxylation means the removal of a carbon. In this case you can see the CO2 being removed in the reaction above.

• In addition, the molecule Coenzyme A (contains vitamin pantothenic acid) is bound to the product and the result is the formation of a 2-carbon molecule called acetyl-CoA

• **pyruvate + NAD+ ---------> acetyl-CoA + CO₂ + NADH**
• this final product is capable of entering the Citric Acid cycle (Kreb’s cycle)
Kreb’s (Citric Acid) Cycle is a series of reactions – see pages 117-120

Basics to know about kreb’s cycle:

DO NOT MEMORIZE WHOLE CYCLE!!! Unless you really want to…

• happens in **mitochondria** of cells

  • Acetyl-CoA (2C) combines with oxaloacetate (4C) to make a 6C molecule - citric acid (thus the name!!)

• Cycle is a series of 8 reactions catalyzed by 8 enzymes.

• There are a few types of reaction that occur:
  • 1 Synthesis (acetyl-coA + oxaloacetate= citric acid)
  • 2 Shape changes (called “isomerization”)
  • 2 Decarboxylations- removal of CO2
  • 4 Redox rxns (2 are also decarbs)
Throughout the processes of glycolysis and Kreb’s cycle, NAD+ and FAD+ were reduced by the simultaneous oxidation of glucose to NADH and FADH2. In other words, they are now carrying lots of energy in the form of electrons (carried as hydrogen atoms). The energy was transferred from the bonds in the glucose to the bonds in the NADH and FADH.

But we know that the only type of “fuel” that cells (read: enzymes) can use is ATP. Remember that enzymes must be phosphorylated in order to be activated.

Okay, so how do we get the energy carried in the NADH and FADH2 transferred into ATP??
See page 120-121

Two ways: fermentation or oxidative phosphorylation

oxidative phosphorylation occurs in the mitochondria of cells at the electron transport chain (ETC) and is an aerobic process (requires oxygen)

ETC is a series of membrane-bound proteins called cytochromes that are repeatedly oxidized and reduced (in turn) and ultimately pass the electrons from NADH and FADH “down the line”.

SO, NADH and FADH2 arrive at ETC in their reduced forms (NADH/FADH2) and get oxidized (think of it as being “unloaded”) by passing the electrons to the first cytochrome in the chain. The NAD+ and FAD+ (oxidized forms) now can go back to the sites of glycolysis and Kreb’s cycles and get reduced (“loaded” up) with electrons (hydrogens) again.
• Electrons passed to cytochromes in ETC, but what about the rest of the hydrogen atom??
• What are hydrogen atoms made of anyway? 1 proton and 1 electron…

• The electrons go from cytochrome to cytochrome along the ETC, meanwhile the protons (aka. H+) are released to the outside of the membrane.
• This causes the formation of a electrochemical gradient.
• A gradient means that the concentration of H+ is higher on one side of the membrane than on the other.
• The laws of thermodynamics demand that the ions “want” to be in equal concentrations on both sides of the membrane and will spontaneously cross wherever there are channels that allow them to pass through.
basic principles:

- molecules/atoms **diffuse (spontaneously move)** from **higher to lower** concentration
- also, electrically-charged molecules (ions) move toward oppositely charged ions
- total difference in concentration and charge = **electrochemical gradient**
- molecules **diffuse DOWN** their electrochemical gradient
  - blocking the diffusion creates potential energy (like water behind a dam)

**Chemiosmosis is the term used to describe the process by which ATP is made using this gradient**
Oxidative Phosphorylation (Chemiosmosis)

• name for process of using energy of an electrochemical gradient(s) to make ATP
• energy generated as electrons passed through ETC used to make ATP (chemical bond energy)
• as cytochromes transport electrons they RELEASE energy
• energy from transfer of electrons used to push protons outside membrane - creates “proton gradient”
  • creates lots of potential energy! - “proton motive force”
  • only place protons can move across membrane and down their gradient is by passing through ATP synthase channels
    • movement of H+ through ATP synthase supplies energy needed to phosphorylate ADP into ATP
      • “oxidative phosphorylation” because components of ETC oxidized as they transfer electrons
      • This distinguishes it from substrate-level phosphorylation

• 3 ATPs made per NADH
• 2 ATPs made per FADH
• NOTE: MUST have an intact membrane!

So, what happens when all the protons all pass through the ATP Synthase channels and the H+ concentration is equal on both sides???

It never gets equal because the protons (H+) are used up (removed) as soon as they get to the other side of the membrane…
• fermentation is anaerobic and will occur when oxygen is not available. It is less efficient than ox-phos
• Described on page 115-117
• Fermentations = regenerate NAD from NADH using some organic molecule as terminal e- acceptor
• pyruvate is available
  • creates waste products for excretion from cell
  • NOTE: waste products still very high in energy content if they could be oxidized further
• some bacteria (e.g. lactic acid bacteria, including strep and lactobacilli) get ALL their energy from fermentation
Catabolism of stuff besides glucose (carbs, proteins, fats, etc)
• **goal**: to produce molecules that can enter common catabolic pathways (glycolysis or TCA cycle)
• Described on pages 122-128
• we’ll cover only the basics for now…
Carbohydrate catabolism

- CHOs are most abundant C-sources in most environments
  - most exist in various polysaccharide forms
- break polysaccharides into mono- or disaccharides that can be transported into cells
- starch, glycogen easily hydrolyzed by amylases
- cellulose
  - difficult to digest, very insoluble, tightly folded
  - many fungi, some bacteria make cellulases, not humans
- once transported into cell
  - converted into some typical glycolytic intermediate (e.g. G-6-P)
  - catabolized by glycolytic enzymes as described in previous slides
Lipid catabolism (page 123-124)

- Biological lipids common as **tri-** or **diglycerides**
- Pancreas secretes lipases to hydrolyze lipids for transport into cell as **free fatty acids & glycerol**
- Fatty acids catabolized by **β-oxidation pathway**
- β-oxidation pathway produces **acetyl-CoA**
  - Since fatty acids have so many more C-H bonds than carbs or proteins, they carry much more energy than carbs or proteins
  - Excessive breakdown of lipids during fasting or starvation can result in ketosis
  - Lipid catabolism controlled by hormones such as glucagon, adrenaline (aka epinephrine) and insulin.

Acetyl coA from beta-oxidation enters kreb’s cycle…
Protein catabolism- pages 125-129

• stomach and pancreas secrete proteases to hydrolyze proteins to amino acids for transport into cell
• [amino group removed by deamination or transamination]
• resulting organic acids converted to pyruvate, acetyl-CoA, or a TCA-cycle intermediate
• Some amino acids can ultimately become part of gluconeogenesis pathways in liver and kidney cells