

Chapter 6: Energy, Enzymes, and Metabolism

I Energy and Energy Conversions

To physicists, energy is the capacity to do work.

To biochemists, energy is the capacity for change.

- Cells must acquire energy from their environment.
- Cells cannot make energy; energy is neither created nor destroyed.
- Energy can be transformed.
- In life, energy transformations are primarily molecular movement and changes in chemical bonds.
- Metabolism is the term used for all chemical changes that occur within a cell.

A. Energy changes are related to changes in matter

- Two main types of energy are kinetic and potential energy. (*See Figure 6.1*)
 - Potential energy is energy of state or position - it is stored energy. Potential energy is like money in the bank. It is there until you are spending it, which is when it becomes active or kinetic energy.
 - Kinetic energy is energy in action. It is doing work that alters the state or motion of matter. It is like spending your savings. Energy must be present to be spent.
- The chemical reactions of biology are called metabolism
- Metabolism can be divided into two types of activities: anabolic and catabolic reactions.
 - Anabolic reactions are those that link simple molecules together to make complex ones. These are energy investing reactions.
 - Catabolic reactions are those which break down complex molecules into simpler ones. Some of these reactions provide the energy for anabolic reactions.
- Life must follow all the laws of physics, including the laws of thermodynamics.

B. The first law: Energy is neither created nor destroyed

- Conservation of energy and the characteristics of energy conversions apply universally.
- First Law of Thermodynamics: During any inter-conversion of forms of energy, total initial energy will equal the total final energy. (*See Figure 6.3a*)

C. The second law: Not all energy can be used, and disorder tends to increase

Second Law of Thermodynamics: (*See Figure 6.3b*)

- When energy is being transformed, some is unavailable to do work.

- Theoretically, a process could be 100% efficient, certainly not greater than 100%, since this would mean more energy was available than was originally present.
- However, energy is never utilized perfectly, so the efficiency is less than 100%.
- Total energy = usable energy + unusable energy.
- $H = G + TS$
 - Enthalpy is the total energy of the system, H.
 - Free energy, G, is usable energy.
 - Entropy is the unusable energy, S, multiplied by the absolute temperature.
 - Because it is the usable energy we are interested in: $G = H - TS$.
 - G, H or S cannot be measured precisely.
 - Change at a constant temperature can.
 - The energy changes are calculated in calories or joules.
 - The symbol Δ is delta, and represents the change.
- The equation $\Delta G = \Delta H - T\Delta S$ describes the events in terms of energy that occur during chemical reactions.
 - Notice there is no Δ in front of T since this must be constant in order to calculate other variables.
 - If ΔG is positive (+), then the reaction requires an input of energy. This is the case for anabolic reactions.
 - If ΔG is negative (-), energy is released. This is the case for catabolic reactions.

- The efficiency of energy use is dependent on the system. Cars use the energy in gasoline to move along highways. Two different types of automobiles that weigh the same amount probably consume different amounts of fuel to go the same distance. The one that uses the least amount of fuel is most efficient.
- Some biological systems are amazingly efficient, although never 100%. An example of an efficient use of free energy in living cells is the generation of ATP from glucose, when oxygen is used as the final electron acceptor. The details of this system are covered in Chapter 7. In spite of there being almost two dozen enzyme catalyzed reactions, the net capture of energy is about 68%!

- If more products than reactants are generated entropy has increased.
- When proteins are made from amino acids, energy is used, there are fewer products, and S is negative.
- When amino acids are freed from proteins, energy is released, the ΔG is negative, and although the released energy is usually not captured in a useful form for the cell, there is an increase in entropy and a release of heat.

Open versus closed systems (See Figure 6.4a,b):

- The universe can be simply viewed as a closed system. It has a finite amount of energy.

- The universe seems to have had a beginning, which is evident when looking at the night sky, and seeing distinct stars.
- If stars had existed forever, light from them would have had time to reach the sky of earth and there would be a confluent glow of starlight.
- There is directionality to the universe in terms of energy. The universe is moving in the direction of greater entropy.
 - This because every time an energy transformation occurs, some of the energy is unusable and adds to entropy.
 - It is as if a great watch was wound up at the beginning of time and has been unwinding ever since.
- Hydroelectric dams and living systems behave like open systems. Energy flows into and out of them. This is unlike a thermos bottle (*see Figure 6.4*) or the universe as a whole. However, both the dam and the cell are part of the universe and contribute to the increase in universal entropy. Not all the energy of either is directed perfectly to do work. With each energy transformation, there is an increase in entropy.
- The earth acts as an open system, which receives its energy from the environment, mostly the sun.

D. Chemical Reactions release or take up energy

Life is a series of startling chemical reactions.

- Each reaction either consumes or releases energy.
 - Anabolic reactions make single products from many smaller units and consume energy.
 - Catabolic reactions reduce an organized substance such as a glucose molecule into smaller more randomly distributed substances, such as carbon dioxide and water, and release energy.
- Energy released is $-\Delta G$; amount taken up is $+\Delta G$.
- Some reactions are classified as spontaneous. (*See Figure 6.4a*)
 - A spontaneous reaction goes more than halfway to completion *without* input of energy.
 - Nonspontaneous reactions proceed that far only *with* an input of energy.
 - Spontaneous reactions are called exergonic and have negative ΔG values.
 - Nonspontaneous reactions are called endergonic and have positive ΔG values. (*See Figure 6.4b*)
 - If under certain conditions $A \rightarrow B$ is spontaneous (and exergonic) then $B \rightarrow A$ is nonspontaneous (and endergonic).
 - Making protein is endergonic; hydrolyzing protein is exergonic.
- All reactions can be conceptualized as reversible.
 - The two, forward and reverse, reactions both occur $A \rightleftharpoons B$.
 - Adding more A speeds up the forward reaction, $A \rightarrow B$; adding more B speeds up the reverse reaction, $A \leftarrow B$.

- At some relative concentration of A and B, forward and reverse reactions take place at the same rate.
- At this point, no further net change occurs. However, reactions of individual molecules continue.
- This point is called chemical equilibrium. (*See Figure 6.6*)

E. Chemical Equilibrium and Free Energy

- An example of equilibrium is glucose 1-phosphate \rightleftharpoons glucose 6-phosphate.
 - At pH 7.0 and 25°C, equilibrium of product-to-reactant is 19:1.
 - The forward reaction has gone 95% to completion.
- The further toward completion a reaction goes in order to reach equilibrium, the greater the amount of free energy released.
- The reverse is true for the reverse reaction.

II ATP: Transferring Energy in Cells

- All living cells use ATP for capture, transfer and short-term storage of energy.
- ATP is adenosine triphosphate. *Figure 6.8* has the chemical diagram of ATP.
- ATP consists of the nitrogenous base adenine bonded to ribose. The 5th carbon of the ribose has three phosphate groups attached.
- ATP can hydrolyze to yield ADP and an inorganic phosphate ion (P_i , short for HPO_4^{+2}).

A. ATP hydrolysis releases energy

- $ATP + H_2O \rightarrow ADP + P_i + \text{free energy}$. Change in free energy (ΔG) is -12kCal/mole at a typical temperature and pH.
- The equilibrium is far to the right, 10×10^6 ADP molecules to each remaining ATP.
- Making ADP from AMP involves overcoming repulsive negative charges. This energy is stored in the molecule. (*See Figure 6.9*)
- The same is true for ADP to ATP.

B. ATP (ADP) couples exergonic and endergonic reactions

Where does the energy come from to make ATP?

- $ADP + P_i + \text{free energy} \rightarrow ATP + H_2O$
- Exergonic reactions are coupled to the endergonic reaction of making ATP. The energy is captured in ATP.
- The energy to make ATP comes from the energy released from fuel molecules such as glucose.
- ATP shuttles energy from exergonic reactions to endergonic reactions.
 - *Figure 6.10* provides a sketch of a coupled reaction that uses ATP to provide energy for the production of glutamine from an ammonium ion and glutamate.

- The reaction actually involves several unstated intermediate reactions, one, which is when glutamate gets phosphorylated and becomes a compound called glutamyl phosphate.
- The phosphate is transferred from the ATP in an exergonic reaction to the glutamate.
- This is very typical of how ATP is used in synthesis reactions.
- Each cell requires millions of molecules of ATP per second to drive its biochemical machinery.
- Each ATP molecule undergoes about 10,000 cycles of synthesis and hydrolysis every day.

III Enzymes: Biological Catalyst

- A catalyst is any substance that speeds up a chemical reaction without itself being used up.
- An enzyme is a biological catalyst. Almost all are proteins. A rare few are made of RNA and are called ribozymes.

A. For a reaction to proceed, an energy barrier must be overcome

- It is easily possible to predict the direction a spontaneous reaction will go but not the likelihood or rate.

- The direction of the spontaneous reaction of wood with oxygen and the levels of end-products in our environment predict that forests will burn. However, it might be a few years or a few hundred years before it occurs.
- An initial investment of energy must be made, like the energy from a lightning strike.
- When the energy from the combustion of wood is released, some of the energy is invested in the unburned molecules of wood to perpetuate the fire.

- The energy that must be invested to initiate a reaction is called its activation energy.
- All reactions have activation energy requirements, even extremely exergonic ones.
 - Different reactions have different activation energy requirements.
 - Activation energy is the energy needed to put molecules into a transition state. The molecules must become unstable transition-state species.
 - Transition-state species have higher free energy than either reactants or products. (*See Figure 6.11*)
- Adding enough heat to increase the average kinetic energy of the molecules is often how exergonic reactions are initiated, like the lightning strike.
- This is not an appropriate way for biological systems to drive reactions. Enzymes, biological catalysts, solve this problem.

- Catalysts do not cause a reaction to take place that could not take place eventually without it.
- Catalysts lower, substantially, the required energy of activation.
- Catalysts make it more likely that unstable intermediates form.

B. Enzymes bind specific reactant molecules

- Almost all enzymes are proteins, made of simple amino acids. (The exceptions are the few interesting ribozymes made of RNA.)
- Although made of amino acids, some enzymes have important contributing molecules that participate in the catalysis such as some types of vitamins and metal ions.
- Enzymes allow reactions to occur under physiological conditions.
 - Enzymes not only catalyze reactions, they are designed to function under the conditions of each particular organism.
 - An enzyme that catalyzes a certain reaction in one species might differ in amino acid composition in another species, especially if they live at different temperatures and/or ionic environments.
- Enzymes bind specific reactant molecules.
- The reactants are called substrates.
- Substrates bind to a particular site on the enzyme surface called the active site.
- Enzymes are specific. They bind specific substrates and catalyze particular reactions under certain conditions.
- The specificity for substrates comes from the 3-D shape of the enzyme.
 - The amino acid sequence, temperature and other solution or environmental conditions determine the 3-D shape.
 - Sometimes, the shape is also generated in part by the process the enzyme goes through during synthesis.
- The name of the enzyme relates to its function.
 - RNA polymerase catalyzes formation of RNA and not DNA.
 - RNA nuclease hydrolyzes RNA polymers.
 - Hexokinase accelerates phosphorylation of hexose. (All kinases add phosphates. All phosphatases remove phosphates.)
- Binding substrate to the active site produces an enzyme-substrate complex.
 - Hydrogen bonding, ionic attraction or even covalent bonding can stabilize these complexes.
 - The enzyme-substrate complex generates the product and free enzyme: $E+S \rightarrow ES \rightarrow E+P$. (See Figure 6.13)

C. Enzymes lower the activation energy barrier but do not affect equilibrium

- Enzymes, like all catalysts, lower activation energy requirements but do not affect equilibrium. (See Figure 6.14)

- The equilibrium is determined by the nature of the reaction, and the conditions; and, is the relative concentration of reactants and products, when no further net change is observed.
- Enzymes accelerate both the forward and reverse reactions and reduce the time it takes to reach equilibrium.
- Enzymes do not, singularly, alter the final relative concentration of reactants to products.
 - An example is lactate dehydrogenase. This enzyme catalyzes pyruvate to lactate, and lactate to pyruvate conversions.
 - Which is the substrate? Both can be the substrate or product. Which is mostly the case depends on the relative concentration of each. If lactate concentrations are high then it is the substrate.
- Enzymes can have a profound effect on rates toward equilibrium. Reactions that might take years to happen can occur in a fraction of a second.

D. What are the chemical events at the active sites of enzymes?

(See Figure 6.15 to help clarify the following discussion,)

- Enzymes orient substrates.
 - While free in solution, substrates tumble and collide.
 - The probability for the collision at the angle necessary to change chemical interactions is low.
 - When held by enzymes, two reactants can be oriented such that a reaction is more likely.
- Enzymes add charges to substrates.
 - The R groups of enzymes' amino acids may directly participate in making substrates more reactive.
 - Some enzymes work by what is called acid-base catalysis.
 - Acidic or basic side chains of amino acids form the active site and transfer H^+ to or from the substrate, destabilizing a covalent bond in a substrate.
- Some enzymes use covalent catalysis. A functional group side chain forms a temporary covalent bond with the substrate.
- Some have metal cofactors. These involve themselves in the gain or loss of electrons, called redox reactions.
- Some enzymes induce strain in the substrate.
 - For example, the carbohydrate substrate for the enzyme lysozyme enters the active site in a flat-ringed "chain" shape.
 - Then, the active site causes it to flatten out into a "sofa" shape.
 - The stretching of the bonds decreases their stability, making them reactive to water.

E. Substrate concentration affects reaction rate.

- The rate of an uncatalyzed reaction is directly proportional to the concentration of reactants.

- This is true to a point with catalyzed reactions. At some point the enzyme will have all active sites occupied.
- Saturating an enzyme makes it possible to determine how many molecules are converted per unit time. (*See Figure 6.16*)
- The turnover number ranges from 1 molecule per 2 seconds, to 40 million per second for the liver enzyme, catalase.

IV Enzyme structure determines its function.

- The active site is specific to the substrate.
- Most enzymes are much larger than their substrate.
- The active site of most enzymes is only a small region of the whole protein.

A. The active site is specific to the substrate

- Emil Fisher in 1894 compared the fit with a lock and key.
- David Phillips in 1965 observed a pocket in the enzyme lysozyme using X-ray crystallography. (*See Figure 6.17*)

B. An enzyme changes shape when it binds a substrate

- Studies on enzyme inhibitors showed that purposely-modified substrates could also bind the enzyme but failed to be affected by the enzymes.
- Some enzyme inhibitors were larger than the normal substrate, yet could occupy the binding site.
 - The conclusion is that some enzymes are flexible.
 - The active site can change shape.
 - This is called induced fit.
- Enzymes are much larger than their substrates or reactants.
 - Part of the larger size might be what allows induced fit. (*See Figure 6.18*)
 - Some regions of an enzyme tolerate little change without the enzyme losing its activity. This is the case within the active site.
 - Some enzymes can tolerate changes in amino acid composition outside the active site.

C. To operate, some enzymes require added molecules

Table 6.1 is a list of examples of cofactors, coenzymes and prosthetic groups.

- Cofactors: Inorganic ions such as copper, zinc, iron and magnesium that bind to certain enzymes and are essential to their function
- Coenzymes: Carbon containing molecules that are required for the action of one or more enzymes (*See Figure 6.19*)
- Prosthetic groups: These are permanently bound to enzymes. They include heme groups (iron containing organic molecules) that are attached to hemoglobin and myoglobin.
- Coenzymes must react, separate and then participate in other reactions.

- ATP and ADP are coenzymes. They are also substrates of the reactions.
- Another is NAD, which will be covered in the next chapter.

V Metabolism and Regulation of Enzyme Activity

- In general, enzyme activity must be regulated. There is a time for such things as RNA synthesis and glucose breakdown.
- Organisms must do just the things that need to be done and when they need to be done.

A. Metabolism is organized into pathways

- Metabolism is organized into biochemically related enzyme catalyzed reactions called biochemical pathways.
 - These pathways are series of enzyme-catalyzed reactions that together generate the necessary, but otherwise incredibly unlikely to occur, molecules of life.
 - A simple diagram of a biochemical pathway is: $A \rightarrow B \rightarrow C \rightarrow D$.
 - Each step A to B to C to D occurs appropriately because of enzymes. For example, Enzyme 1 converts A to B; Enzyme 2 converts B to C.
 - Some metabolic pathways are anabolic and synthesize macromolecules.
 - Some are catabolic and breakdown macromolecules and fuel molecules.
- In life, all reactions, taken together, are net exergonic, or energy releasing.
 - All anabolic reactions are coupled to catabolic ones.
 - The life we are most familiar with uses one important reaction to get the energy for the rest - the capture of a photon of light into a chemical bond.
 - Some other less familiar life forms use the energy of methane or reduced inorganic substances as the starting energy source for all other reactions.

B. Enzyme activity is subject to regulation

- Enzymes can have their activity inhibited.
 - Reversible inhibition: These molecules inhibit the enzyme temporarily. (*See Figure 6.21*)
 - If they bind to the active site, they compete for the binding site and are called competitive inhibitors.
 - If they bind at a location other than the active site, they are called non-competitive inhibitors.
- Irreversible inhibition is when the inhibitor in essence destroys the enzyme, rendering it unable to interact with its normal substrate(s).
 - Diisopropylphosphorofluoridate, for example, reacts with the OH group of the serine residues found in active sites. This eliminates the activity of the enzyme.
 - Sarin, a related compound, is the nerve gas that was released in the Tokyo subway, causing death and illness to many people.

- Irreversible inhibitors are usually man-made compounds. Nature uses reversible inhibition.

C. Allosteric enzymes have interacting subunits

- Allosteric enzymes have interacting subunits that modulate their catalytic activity. Allo means different and steric means shapes.
 - Allosteric enzymes are controlled by effector molecules.
 - Effector molecules bind to an allosteric site, which is separate from the active site.
 - This changes the structure and function of the enzyme.
 - Depending on the particular enzyme, the binding may enhance or diminish reactions at the active site.
- Some allosteric enzymes have multiple active sites.
 - When one binding site is occupied, it changes the other(s) so they bind additional substrate molecules more readily.
 - This changes the shape of the reaction rate versus concentration curve compared to non-allosteric enzymes. (*See Figure 6.22*)
 - The advantage to the system is that the enzyme's catalytic rate becomes concentration sensitive and responsive.

Some allosteric enzymes have more than one type of subunit.

- A catalytic subunit(s) has an active site that binds the enzyme's substrate.
- A regulatory subunit(s) has one or more allosteric sites that bind specific effector molecules.
- The enzyme exists in an active or inactive form, so it is like an enzyme with an on/off switch.
- In the active state, the active site can bind substrate

See *Figure 6.23* for more information on the kind of subunits and nature of regulatory effects.

Allosteric effects regulate metabolism.

- Pathways typically involve a starting material, intermediates, and an end-product.
- The first step in the pathway is called the commitment step.
- Once this step occurs, other enzyme-catalyzed reactions follow until the product of the series builds up.
- One way to control the whole pathway is to have the end-product inhibit the first step in the pathway, the commitment step.
- This is called end-product inhibition.

See *Figure 6.24* for an example of a pathway regulated by end-product inhibition.

D. Enzymes and their environments

- The pH and temperature can affect enzyme activity.

- Some enzymes are tolerant to a wide range of pH and temperatures, while other enzymes are very sensitive.
- Each enzyme is most active at a certain pH and temperature. (*See Figure 6.26*)
- The pH can influence carboxyl and amino groups' charges.
- Temperature can influence shape by breaking hydrogen bonds, perturbing ionic interactions and hydrophobic interactions.
- If heat destroys the enzyme, it is called denatured.
- Some organisms that live at different temperatures generate different forms of an enzyme, called isozymes of the same enzyme.
- Even different organs in a human produce different isozymes.