Enzymes, ATP and Bioenergetics

Bioenergetics – Bioenergetics can be defined as energy transfer mechanisms occurring within living organisms.

Energy, the ability to do work or bring about change, occurs in a variety of forms including heat, light, chemical and electrical. According to the first law of thermodynamics, **energy can change form, but it cannot be created and cannot be destroyed**. Microorganisms cannot produce energy, but they can form high-energy compounds (ATP) by transferring energy from external sources. Since during such transfers, a certain amount of energy is lost due to **entropy**, an external energy source is essential to life. Energy flows through living systems.

Metabolism – Metabolism can be defined as all the chemical reactions occurring within living organisms, and can be divided into two categories; anabolic reactions or **anabolism** (building reactions), and catabolic reactions or **catabolism** (breakdown reactions).

Metabolism and bioenergetics are linked, because chemical reactions involve energy transfers. Although catabolic reactions require energy to initiate, they ultimately release more energy than they require, so are **exergonic**. Anabolic reactions require or take in energy, so are **endergonic**.

As described earlier, living organisms obtain energy either from chemicals or from light. In either case they use these external energy sources to form ATP, and then use ATP to drive their metabolic processes.

Adenosine triphosphate (ATP) – Adenosine triphosphate is a high-energy compound or **nucleoside triphosphate** formed by adding two additional phosphate groups to a nucleotide containing the base adenine and the pentose monosaccharide ribose. Sometimes referred to as the **energy currency** within cells, ATP is formed and used on a regular basis. According to some sources, a typical eukaryotic cell may use as many as 1 million ATP molecules per second. Energy is stored in the covalent bonds holding the phosphate groups together, particularly in the one between the 2^{nd} and 3^{rd} phosphates. This is called a **pyrophosphate bond**, and releases considerable energy when broken.

Though cells use ATP on a regular basis, they do not store it, so it is constantly being made. The chemical equation representing ATP synthesis looks something like this, ADP + Pi + Energy = ATP. In this equation, Pi represents **inorganic phosphate** and ADP is **adenosine diphosphate**. The energy is provided by chemicals (which hold potential energy in their bonds), or by light. When ATP is used, the pyrophosphate bond is broken and ADP, Pi and energy are released. The energy released is used to drive cellular processes such as active transport, flagellar movement and a wide variety of synthesis reactions.

Chemical reactions that yield ATP are called **phosphorylation reactions** and may be categorized as **substrate level, oxidative** and **photophosphorylation**. Chemoheterotrophs such as fungi, protozoa, animals and many bacteria use either substrate level or oxidative phosphorylation (or both) to make ATP depending on whether their metabolism is fermentative or respiratory (oxidative). Phototrophs such as algae and cyanobacteria use photophosphorylation when light is available, but can also use oxidative phosphorylation during periods of darkness.

Though ATP is the most common form of nucleoside triphosphate, it is not the only one. Nucleotides containing the bases guanine, cytosine, thymine and uracil can also take on extra phosphate groups to form high-energy compounds. Nucleoside triphosphates (NTPs) may contain either ribose or deoxyribose and are sometimes referred to as r-NTPs and d-NTPs respectively. Other high-energy compounds associated with metabolic processes in prokaryotes include acetyl-coenzyme A (acetyl-CoA) and succinyl-coenzyme A (succinyl-CoA).

Enzymes:

Enzymes – Enzymes are proteins (usually) that catalyze biochemical reactions. They are typically globular in form, are often quaternary, and **interact specifically** with only certain types of substrate molecules, i.e., each different type of chemical reaction requires a different type of enzyme. As catalysts, enzymes **increase the rate of chemical reactions**, sometimes hundreds or thousands of times; however, they cannot cause chemical reactions to occur that would not otherwise be possible. Enzymes are not changed by the reactions they catalyze, so **can be used over and over again**.

Though molecular interactions cannot be directly observed, models can be used to explain enzyme activity. In one example, known as the lock and key model enzymes are described as **increasing the interactions between molecules** as explained below.

Each enzyme has an **active** or **reactive site** on its surface that fits with a specific set of **substrate molecules** or **reactants** like a lock fits with a specific key. Though the substrates rarely interact on their own, when held within the enzyme's reactive site, they bind together forming a product. When the product is released, the enzyme is free to bind more substrate and the reaction is repeated.

Enzymes also influence chemical reactions by **decreasing the increments of activation energy** necessary to initiate them. For example, glucose can interact with oxygen to form carbon dioxide and water, but the reaction is unlikely to occur if a spoonful of glucose is simply exposed to the oxygen present in air. The energy required to initiate the interaction is called **activation energy** or the **energy of activation**. Raising the energy level by heating the glucose, e.g., by holding the spoon over a Bunsen burner, will cause the reaction to occur, with energy released in the form of heat and light. Most of the glucose will be converted into carbon dioxide and some oxygen will be bound in water (though some carbon is likely to remain in the spoon in the form of ash).

Since heating protoplasm over a Bunsen burner is not conducive to maintaining life processes, an energy form other than heat is required for metabolism. Enzymes allow chemical reactions to proceed with activation energy provided by the catabolism of ATP. When cells convert glucose and oxygen into carbon dioxide and water, they use 2 molecules of ATP as activation energy and gain 36 to 38 molecules of ATP in return. Without enzymes, this would not be possible.

Enzyme categories:

Enzymes can be categorized in a variety of ways depending on their composition, their regulation, the types of reactions they catalyze and where they are. Some examples of enzyme categories are listed below.

1. **Endoenzymes Vs exoenzymes** – **Endoenzymes** are those active within living cells, while **exoenzymes** are active outside. Metabolic processes involve endoenzymes, but exoenzymes are often required to break down food materials needed as a source of energy. Bacteria, fungi and many types of multicellular organisms, including humans, release digestive enzymes into their environments so that nutrient materials (reduced to small molecules) can be taken through their cell membranes more readily.

2. **Simple enzymes Vs conjugated enzymes** – **Simple enzymes** are those active as protein along, i.e., those that do not require non-protein "helpers". **Conjugated enzymes** are those requiring some form of non-protein helper in order to be active. The protein portion of a conjugated enzyme is called an **apoenzyme** and is inactive; the active form of a conjugated enzyme is called a **holoenzyme** and includes both protein and non-protein constituents. Helper groups (sometimes called cofactors) occur in three basic forms including:
   a) **Coenzymes** – Coenzymes are non-protein organic groups that can bind with apoenzymes and convert them into holoenzymes. Some examples of coenzymes include NAD, FAD, NADP, coenzyme A and coenzyme Q (ubiquinone). NAD and FAD are derived from B-complex vitamins, niacin and riboflavin respectively.
   b) **Cofactors** – Cofactors are inorganic groups that can bind with apoenzymes and convert them into holoenzymes. Minerals such as calcium, magnesium and manganese often serve as cofactors.
   c) **Prosthetic groups** – Prosthetic groups are inorganic and permanently bound to their enzyme conjugates. Iron and copper often serve as prosthetic groups. An important group of conjugated enzymes called **cytochromes** (cytochrome = cell color), are pigmented enzymes with iron prosthetic groups. These play an essential role in the electron transport chains associated with oxidative and photophosphorylation.

   Although these enzyme helpers can serve a variety of functions, they often act as electron acceptors and so play an essential role in oxidation and reduction reactions. Unlike enzymes, coenzymes and cofactors are not specific, and the same ones can be found interacting with a variety of different enzymes. Like enzymes they can be used over and over again, but they are changed during many reactions because they are alternately oxidized and reduced. NAD is commonly associated with enzymes involved in catabolic processes, while NADP is associated with enzymes involved in anabolism.

   Oxidation reactions – Oxidation reactions are those involving the addition of oxygen to or the removal of electrons and hydrogen protons from atoms or molecules.
   Reduction reactions – Reduction reactions are those involving the removal of oxygen from or the addition of electrons and hydrogen protons to atoms or molecules.

   In biological systems, the transfer of electrons and hydrogen protons often occurs in association with energy transfers, so oxidation-reduction reactions (redox reactions) occur frequently. Though different in outcome, the two processes are linked because when one type of molecule is reduced, another is oxidized. Students may find it helpful to remember the saying "LEO the lion goes GER" when studying oxidation and reduction reactions. When atoms or molecules lose electrons they are oxidized (LEO), and when they gain electrons they are reduced (GER).

Coenzymes in their reduced form ($NADH + H^+$ and $FADH_2$) have a higher energy potential than they do when in their oxidized form (NAD and FAD).

3. **Constitutive Vs repressible Vs inducible enzymes -** Enzymes can be categorized as constitutive, repressible or inducible on the basis of regulatory mechanisms associated with their production.

    a) **Constitutive enzymes** – Constitutive enzymes are those essential to cell function and so always being made. The synthesis of constitutive enzymes is not repressible, i.e., it cannot be blocked at the gene level.
    b) **Repressible enzymes** – Repressible enzymes are those coded for by repressible genes, i.e., those for which transcription can be repressed.
    c) **Inducible enzymes** – Inducible enzymes are those coded for by inducible genes, i.e., those for which transcription can be induced.

    Note – The information provided above relative to repressible and inducible enzymes will make more sense after genetic regulation has been described. It is not significant at this point in time.

Factors influencing enzyme activity:

A variety of factors influence enzyme activity and the rate at which metabolic processes can occur. Some of these are listed below.

1. **Temperature** – Temperature influences enzyme activity in part by increasing the number of collisions occurring between enzymes and substrate molecules; however, multiple other factors can be involved. Different types of enzymes have different **temperature optimums**, i.e., temperature ranges within which they work best. This is why different organisms grow best at different temperatures (e.g., psychrophiles, mesophiles, thermophiles, etc.). Excess heat will cause enzyme activity to be lost because the proteins are denatured.
2. **pH** – The pH of the environment can significantly influence enzyme activity by changing the charge on various groups along the enzyme surface. Increases in the production of acidic or alkaline substances associated with metabolic processes can vary how readily enzymes bind with their substrates. Different enzymes have different pH optimums, i.e., work best at different pH levels.
3. **Concentration** – The concentration of either enzyme or substrate present in an environment can influence enzyme activity. If substrate is plentiful, increasing enzyme concentration will increase reaction rate, but if the substrate is limited, many enzymes have nothing to interact with and they can catalyze no reactions.
4. **Light** – Some enzymes are activated or inactivated by exposure to light. Enzymes involved in light repair (a process used to repair damaged DNA molecules), are activated by visible light. Some enzymes involved in pigment production are also activated by light.

5. **Inhibitors and enhancers** (activators) – Inhibitors are substances that decrease or block enzyme activity, while enhancers or activators are those that increase enzyme activity. Inhibitors can exert their influence in different ways.
   a) **Competitive inhibition** – Competitive inhibition involves an inhibitor binding to the active site of an enzyme in place of the normal substrate. When the inhibitor is bound, the substrate cannot be acted upon and enzyme activity is blocked. A variety of substances recognized as toxic, including arsenic, cyanide and sarin (nerve gas) act as **competitive inhibitors** of important enzymes.
   b) **Allosteric inhibition** – Allosteric inhibition involves an inhibitor binding to a site on an enzyme other than the active site, i.e., the **allosteric site**. When the inhibitor binds, it changes the molecular configuration of the enzyme so that the active site is no longer active, i.e., can no longer bind the normal substrate.

   Enhancers or activators typically bind to allosteric sites on enzymes and thereby increase the enzymes ability to interact with substrate molecules.

Enzyme names:

Enzyme names typically (though not always) end in "ase" and often provide information about the enzyme, e.g., what it does or what it acts on. Recall that **luciferase** enzymes are associated with bioluminescence or light production. In this case the enzymes are named after Lucifer, the angle of light. **Polymerase enzymes** catalyze reactions associated with the synthesis of polymers (DNA and RNA). **Isomerase** enzymes convert molecules into their chemical isomers, and aminoacyl-t-RNA-synthase enzymes catalyze reactions resulting in the formation of aminoacyl-t-RNA. Unfortunately, some enzymes have more than one name, and this can cause confusion.