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Section One: Chapter 3: Enzymes in the Body

Introduction to Enzymes

In order to understand the more involved organ systems of the body we must first become familiar with, or re-familiarize ourselves with the tremendously important role of **enzymes** in the human body.

Since there are thousands of reactions occurring in our bodies at any time, and the metabolic processes within cells must occur fast enough to support life, then our bodies require catalysts (in the form of enzymes) in order to conduct those reactions at rates that will sustain us! As we will see, enzymes are catalysts and all enzymes are proteins. They diligently assist in spontaneous chemical reactions that occurs in our body. It is estimated that there are over 1,000 different enzymes found in a typical human cell. This gives some indication of how vital enzymes are to all levels of functioning. This also reveals that there is a bit of information involved in this topic, but it is not too bad. The best way to fully understand anything is to start with the basics and cover the simple elements well and thoroughly. We will see that there are few ways to organize and categorize enzymes in the body that make them easier to understand.

Chemical Reactions in the Body

A good place to start is by knowing the terminology and vocabulary for enzymes.

Catalysts function to speed up the rate of a chemical reaction by lowering the activation energy required for the reaction to take place. The catalyst is not altered or consumed in the process.

Enzymes are proteins that function as *biological catalysts*. The human body contains 1000's of different types of enzymes because all kinds of chemical reactions occur in the body that require them.

A **Substrate** is a molecule that can bind to an enzyme in a specific way and undergo a chemical reaction as a result. They are like *reactants* in a spontaneous chemical reaction (but are called *substrates* when enzymes are involved) and they are transformed into **products**.

All enzymes are proteins with specific three dimensional (3-D) shapes and they contain small regions where substrates bind to the enzyme called the **active site** (see **Fig. 3.1**). Enzymes carry out catalysis by binding to substrate molecules and bringing them physically close together. The close proximity stresses certain substrate chemical bonds allowing them to react with each other more easily. Another way of saying this is that the energy required to reach a certain *transition state* is lowered. The result is a reaction that occurs *much more rapidly* (typically millions of times faster) than it would without enzymes. Thank you enzymes.
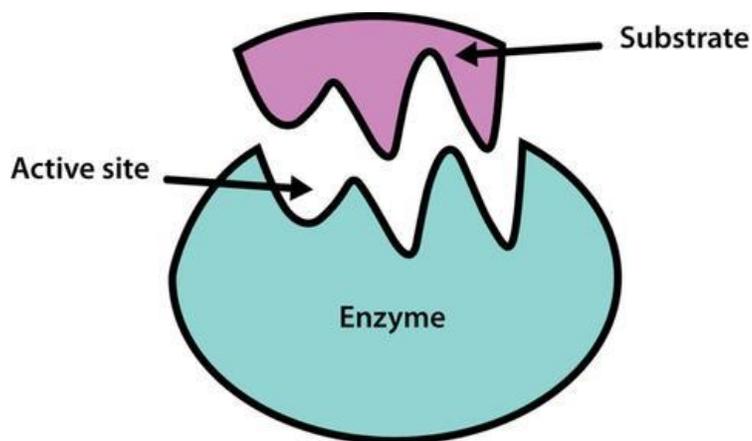
Figure 3.1 Seen here is a diagram of an enzyme, showing its active site where it can bind with a substrate (the substance being acted on by the enzyme) and speed up the chemical reaction.

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The *active site* of an enzyme is often a small region within the larger *binding site*. In some cases, the active site is not part of the binding site but is adjacent to it, or is in a position that allows it to interact with the substrates once they're bound to the binding site.

Enzyme-Substrate Complex - is the combination of an enzyme and it substrates bound to it at the active site. When they dissociate, the products are formed and the enzyme is free to bind new substrates (see **Fig 3.2** below). Substrates can be larger or smaller molecules than their products.

a) Enzyme (unbound)   **b)** Enzyme-Substrate Complex   **c)** Release of Product
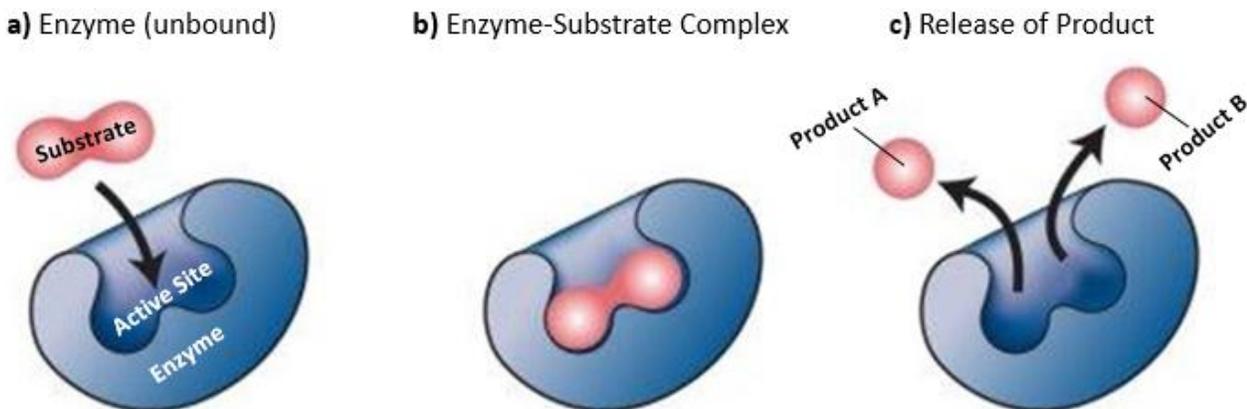
Figure 3.2 In **a)** the substrate is moving into the active site to bind the enzyme. In **b)** the formation of the enzyme-substrate complex occurs, wherein the enzyme can now act on the substrate. Finally, in **c)** the products (A and B), which are chemically different from the original substrate (reactants), are released from the enzyme.

Enzyme Specificity

Specificity is an important concept in physiology across many levels of organization. In terms of enzymology, **specificity is the ability of an enzyme to catalyze only certain reactions**. It also describes how restrictive an enzyme is in its choice of substrate; a completely specific enzyme would have **only one substrate**, while a less specific enzyme may work on **a group** of similar molecules. This exemplifies the two broad types of enzyme specificity: Absolute specificity - when an enzyme only catalyzes one reaction; and Group specificity - when an enzyme will act can on molecules with specific functional groups, such as a phosphate, an amino, or methyl groups. Ultimately, specificity of an enzyme is due to the precise interaction of the substrate with the intricate three-dimensional (3D) structure of the enzyme. An enormously important example of a very specific enzyme is *glucokinase*, it catalyzes this reaction:

$$\text{Glucose + Pi} \longrightarrow \text{Glucose 6-P}$$

As we will see shortly, a 'kinase' is an enzyme that sticks phosphates onto to things, in this case a glucose. Since this is an important enzyme and an extremely common reaction, let's just visualize it a little more thoroughly. Another consistent theme is that often when there is one reaction, there is usually a complementary (or **opposite**) reaction which is also commonly required.

For example, note in **Fig. 3.3** the enzyme **glucokinase** gets a phosphate (H_3PO_4) gets it from adenosine tri-phosphate (**ATP**), which becomes adenosine di-phosphate (**ADP**). The reverse of this action would be how the enzyme **gluco-6-phosphotase** can take off that same phosphate and make ATP and glucose again. Quite often the ATP, which can be used by the cell, is the provider of the high energy phosphate group. When that phosphate needs to be removed, ADP is a happy recipient to become ATP again!

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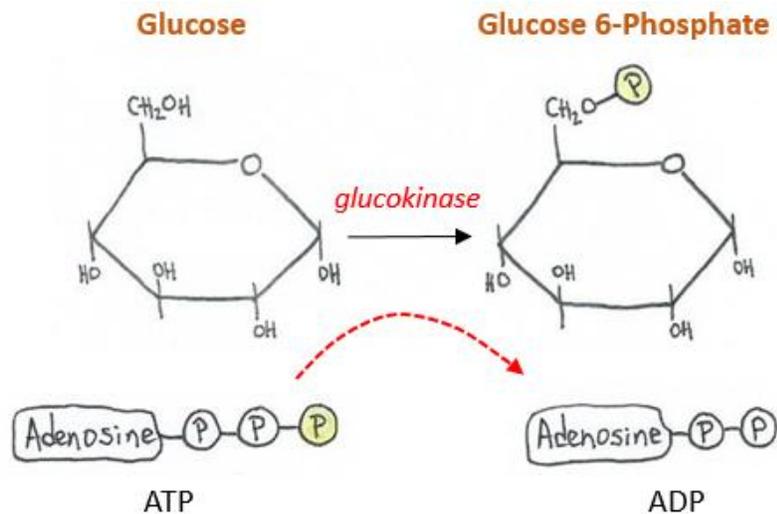
Figure 3.3 Glucose can be phosphorylated by adding the phosphate group (the yellow colored P) to it, this requires the enzyme glucokinase. In the body, this phosphate group comes from adenosine tri-phosphate or ATP. Once the phosphate group is transferred onto the glucose it becomes the molecule glucose 6-phosphate, and the ATP become adenosine di-phosphate or ADP.

Enzymatic Glucose Cycling

This process of **phosphorylating glucose** is very important inside cells. Firstly by phosphorylating glucose it changes it and it is no longer glucose. This may seem obvious, or trivial, however as we will also see soon, most of the glucose moves into cells **down its concertation gradient**, thus by lowering the glucose concentration inside the cell (intracellularly), a cell can now get more glucose from the blood if needed. Also, by sticking a big phosphate group on it and changing it to **glucose-6-phosphate** (**G-6-P**), it cannot leave the cell. These two enzymes have opposite actions: **1) Glucokinase** sticks a phosphate on glucose and makes G-6-P; and **2) Glucose-6-phosphatase** takes that phosphate off and makes glucose again!

Glucokinase

The enzyme glucokinase is primarily found in the **liver** and in **pancreas** (beta cells), but most tissues use it. In the liver, glucokinase is critical for storing glucose as **glycogen**, particularly in the postprandial (after eating) state. The pancreatic beta cells make and release the hormone **insulin**, which regulates the uptake of glucose from the blood into most cells of the body, so the glucokinase acts as the 'glucose sensor' in these beta cells of the pancreas by controlling the rate of glucose metabolism.
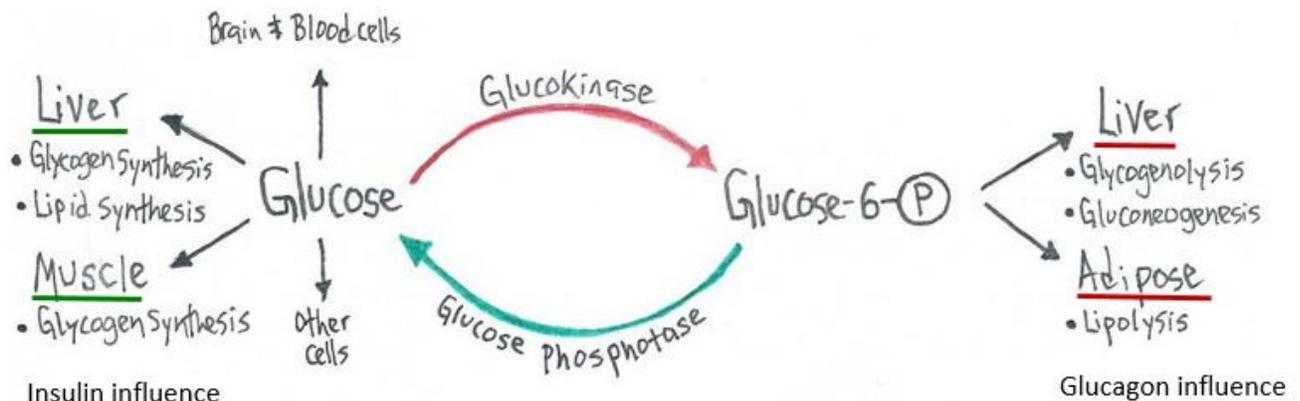
Figure 3.4 Shows the cyclic nature of glucose phosphorylation. Glucokinase promotes glucose storage, whereas the glucose-6- phosphatase promotes glucose liberation and utilization

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Pancreas and Liver Involvement in Glucose Regulation

When blood glucose concentration falls to **hypoglycemic** levels, alpha cells in the **pancreas** release the peptide hormone **glucagon** which blocks the effect of **insulin** on **liver** hepatocytes, which then induces **glycogenolysis** and **gluconeogenesis**, which are both mechanisms of making more free glucose available for the body to use. Glucagon also reduces glucokinase activity in hepatocytes.

Let's take a moment to understand two very important and complicated sounding terms. By looking more closely at the word, and knowing the meaning of the prefix and suffix, they will become much more simple and memorable terms!

> **1) Glycogenolysis**: This term tells us about this process; it is composed of two parts, **glycogen** and **lysis**. The 'o' brings the two parts together. Glycogen is the storage molecule of glucose in animals, and lysis means to cut or breakdown. Therefore, during glycogenolysis the glycogen (which is a polymer of glucose, thus contains *only* glucose), is cleaved or lysed or cut up to release, yes glucose! This process is triggered when the body needs to elevate blood glucose levels.

> Glycogenolysis occurs in the liver (in hepatocytes), where most of our glycogen is stored, and also in muscle cells (myocytes). The process is under the regulation of two key enzymes: phosphorylase kinase and glycogen phosphorylase.

> **2) Gluconeogenesis**: This term tells also us what is occurring during this process; it is composed of three parts, **gluco**, **neo** and **genesis**. Gluco means glucose, neo means new, and genesis means to create or make. Therefore during gluconeogenesis the body is creating new glucose from non-carbohydrate sources, such as proteins and fats.

> Gluconeogenesis predominantly occurs in the liver and kidney. It provides glucose for the body when glucose is scarce or absent. This is why if a person does not consume many carbohydrates, their body will switch to using lipids and proteins as fuel in order to make glucose, thus using up stored lipids and proteins. This process is also used when it needs to elevate blood glucose levels.

Glucose-6-phosphatase

The enzyme **glucose-6-phosphatase** (G-6-P) is found only in the **liver**, **kidneys**, and areas of the **intestine**. This enzymes is pivotal for converting **G-6-P** into glucose (see **Fig.3.4** above), it enables the provision of glucose when needed, for example during starvation. The role of glucose-6-phosphatase in the liver and the kidney is for the **production of glucose** for release into blood. In the liver, glucose-6-phosphatase catalyzes the last step of glycogenolysis and gluconeogenesis. Glucose-6-phosphatase is thought to be regulated only by the levels of glucose-6-phosphate, which are increased by **glucagon** (plus glucocorticoids and thyroxine), that it, it is increased when the body needs more free glucose.

A deficiency or the absence of the glucose-6-phosphatase can results in an abnormal accumulation of **glycogen** in the liver, so much that the liver swells and enlarges while at the same time producing symptoms of hypoglycemia (low blood sugar) and hyperuricemia (elevated uric acid level in the blood) which is a condition also found in **gout**. This can be related to **gouty arthritis**, which restricts joint movement often affecting the feet. These conditions arise when body pH declines below normal, thus an effective prevention is to remain basic (alkali) and keep hydrated with good water.

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Enzyme and Substrate Fitting

There are two ways that enzyme-substrate specificity can be described: **1)** Lock and Key or **2)** Induced Fit.

1) Lock and Key - describes when the active site of the enzyme is highly specific to the shape of the substrate, with a *complimentary fit*, like a lock and key arrangement. This is a convenient way to think about enzyme-substrate binding, as we are familiar with locks and keys. For example, we understand that very small changes in the 'cut' of a key will mean it won't work anymore, as it is not an exact match for it. Enzyme and substrate binding behavior is much more complex than this, but this is a good place to start.

2) Induced Fit - describes when the initial shape of the active site is not necessarily highly specific to the substrate. However, as the substrate approaches the enzyme, it **induces a change in the shape of the active site**, for a better fit. These are also called ***conformational changes***. This model can accommodate for a wider range of substrates and explains reversible reactions better than the lock and key model.

Enzyme 'Optimal' Conditions and Denaturation

Enzymes work most effectively under certain conditions that are referred to as '**optimal**' conditions, which will depend on the enzyme, the location in the body and the organism it is found in. For example, most enzymes in the human body work best at around **37°C** or **97.6°F**, which makes sense because that is normal body temperature (T_b). At lower temperatures the digestive enzymes in the small intestine will still work but much more slowly than at the optimal temperature. If body temperature should increase due to exercise or a fever, enzymes will work more quickly (due to the increase in kinetic energy) but only to a certain degree, which we will see below!

Similarly, enzymes can only function in a certain pH (acidic/alkaline) range. Their optimal pH range will depend on their function and where they are found in the body. Enzymes in the intestines work best at alkaline pH, e.g., a pH of 8, whereas enzymes in the much more acidic conditions of the stomach work best at highly acidic levels, e.g., a pH of 2.

If the temperature is too high or if the environment is too acidic or alkaline for that particular enzyme, then the enzyme changes shape in response to the conditions; this alters the shape of the active site so that substrates cannot bind to it, in this way the enzyme has become **denatured**. Depending on the conditions, enzymes can be temporarily denatured and recover, or they can be permanently denatured.
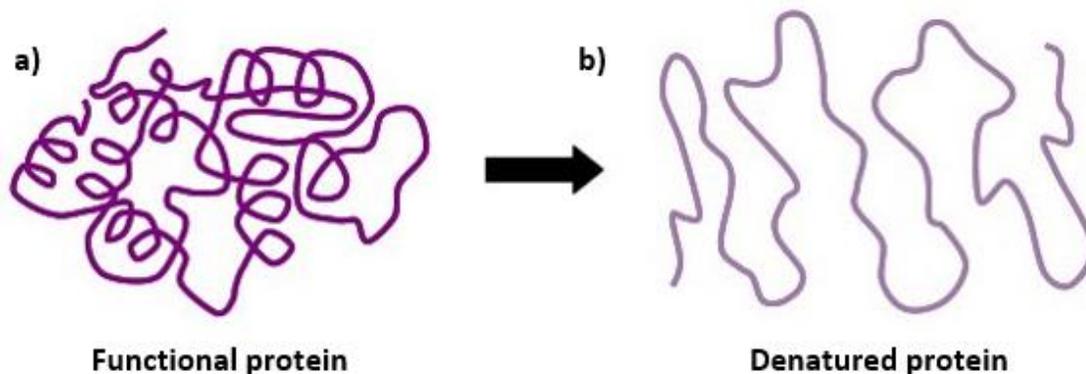
Figure 3.5 Shown above is a representation of a functional protein **a)** that has the necessary folding regions and patterns to function as required. In **b)** it becomes denatured, losing its original shape and thus functional form. Notice the significant change in the spatial structure of the amino acid sequence (shown by the continuous line in both drawings) that have been significantly altered and will then impair the function of the protein.

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A good everyday example the **permanent** and **irreversible** denaturation and destruction of a protein when egg white, the albumin portion of an egg, is heated up to 184º F (see **Fig. 3.6**). It changes from the slippery clear viscus fluid seen in photo **a)** to the right, into a firm white rubbery texture seen in **b)**.

It is useful to note that it is not just heat that can cause denaturation of albumin. For example, if you have ever made a lemon meringue pie you have witnessed how raw egg whites, if whisked harshly enough, will become stiff because they are denatured completely and they cannot regain their original former state. If egg whites are beaten only until they form soft peaks, the proteins are only partially denatured and retain some of their elasticity.
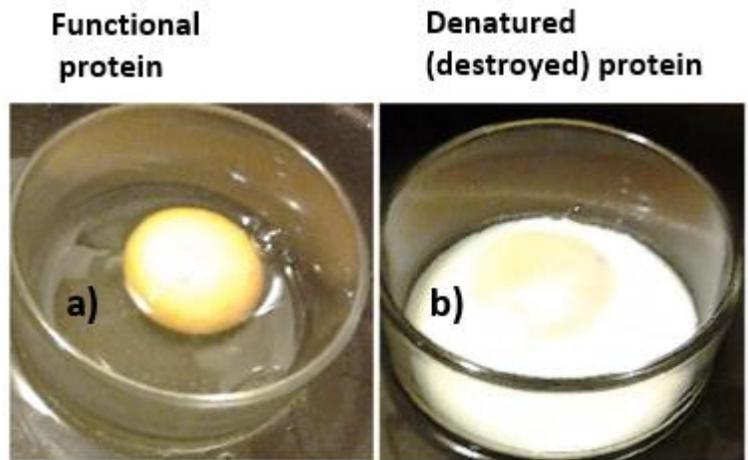
Figure 3.6 In the bowl **a)** on the left, the albumin portion of an egg (the egg white) is raw and has a viscus, slippery texture which is transparent. When this is heated up to 184º F it changes into **b)** a rubbery white texture.

Enzymes in Biochemical and Metabolic Pathways

Enzymes are crucial in creating and maintaining **metabolic pathways** - these are discrete and ordered sequences of enzymatically mediated chemical reactions, in which the products of one reaction become the substrates for the next in the sequence, and so on, until the end. An example is shown in **Fig. 3.7**.
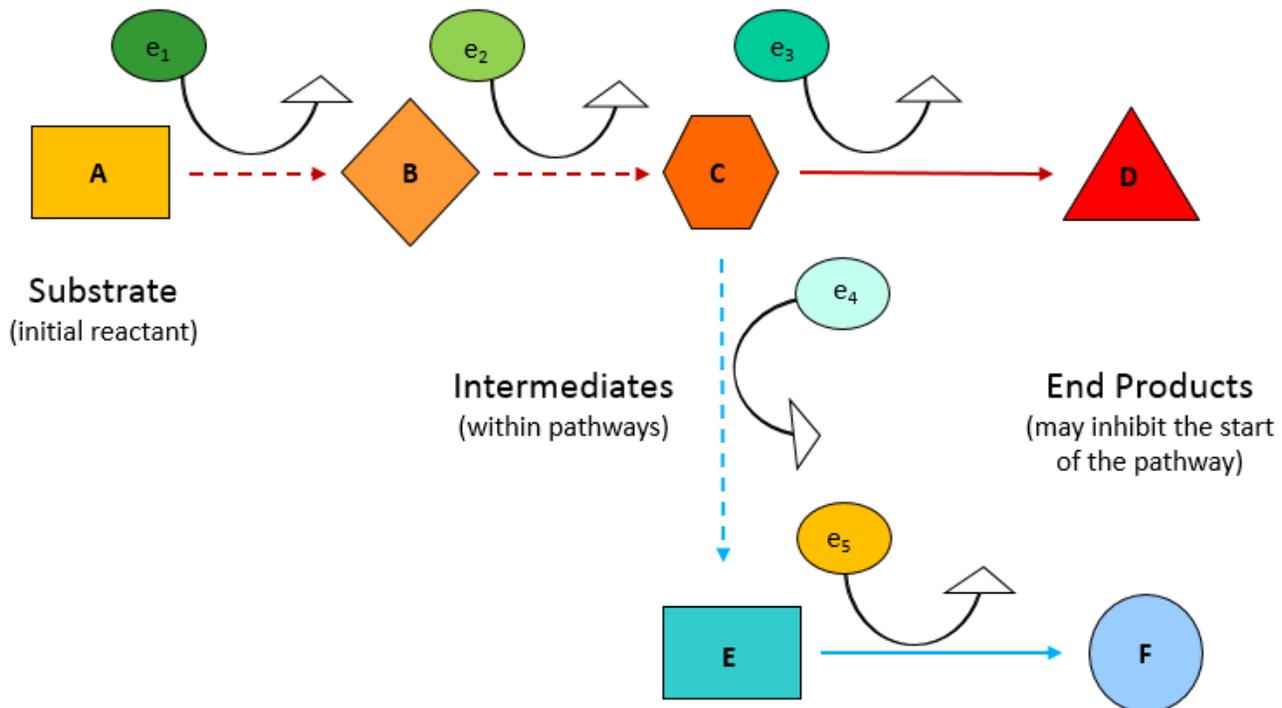
Figure 3.7 This is an example of a general metabolic pathway in which a substrate is the initial reactant that is converted into intermediates and finally into products. In this particular case, there are two pathways here, indicated by the color of the arrows (→ red yields **D** and → blue yields **F** as products), which make different end products using different enzymes (and here have different intermediates).

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Allosteric Modulation and Allosteric Inhibition (End Product Inhibition)

Let's start the practice of making sure we know what new words and terms mean when they come up for the first time. Etymologically, **allosteric** comes from the Greek words allo meaning 'other', and steric meaning 'solid' or 'spatial arrangement'. Hopefully most of the important new words encountered will be in the glossary (Appendix A). Thus allosteric means an arrangement of objects in space. Allosteric enzyme modulation means changing the shape of an enzyme to change the activity of that enzyme.

Allosteric Modulation is a way of changing the shape of something and therefore changing its activity. To modulate is to vary up or down, so allosteric modulators that affect enzymes can increase or decrease an enzyme's activity. The body is naturally very conservative and avoids wasting resources and energy. **Allosteric Inhibition** is a way of changing the shape of enzymes to decrease (*inhibit*) their activity. Therefore, a fundamental aspect of regulating enzymatically controlled pathways is to *inhibit* enzyme activity in order to decrease or turn off an unnecessary pathways. This effectively controls the rate of production of a metabolic pathway.

Allosteric Inhibition or End Product Inhibition

End Product Inhibition
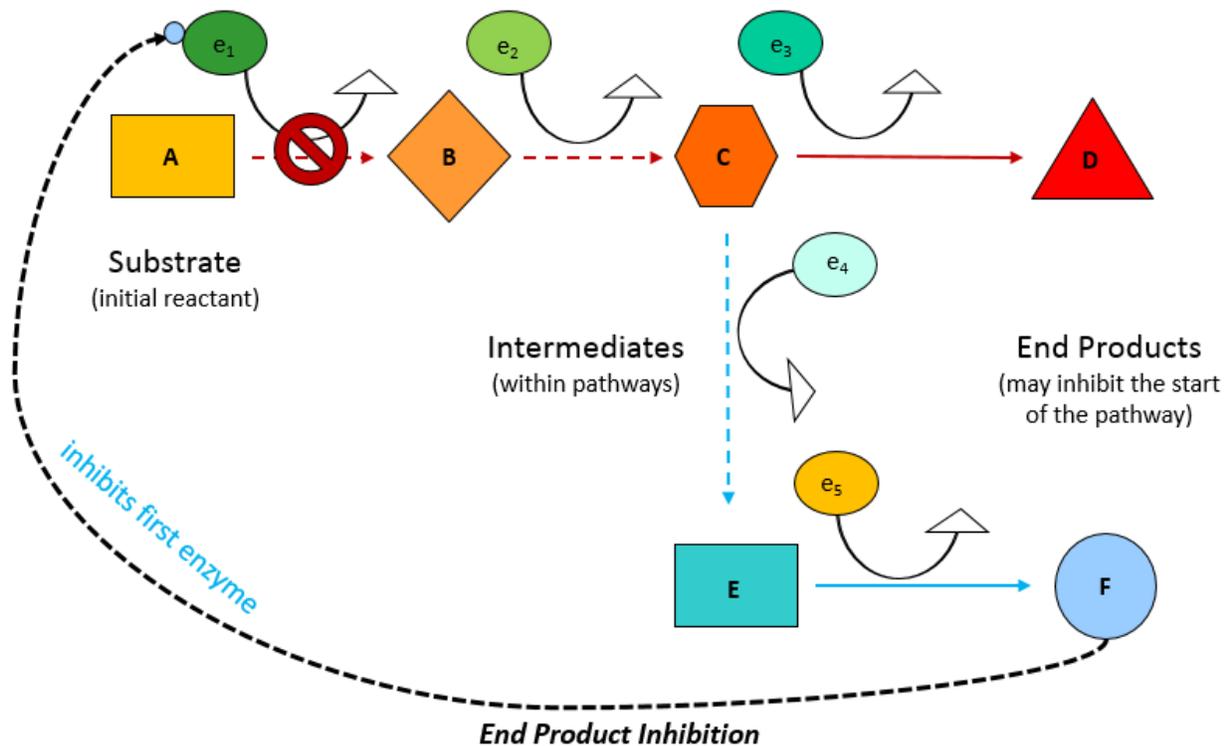
Figure 3.8 An important aspect of all the metabolic pathways in the human body is that they are highly regulated. A typical way to control these types of pathways is by altering the activity of the initial enzyme in the pathway. By inhibiting it or activating it, this will then alter the production of intermediates and end products for all pathways downstream of that enzyme. Shown above is allosteric inhibition (or "**End Product Inhibition**") where the end product of a pathway (when produced in excess) binds to the initial enzyme in that pathway and inhibits that enzyme and hence decreasing that end product pathway.

As seen in **Fig. 3.8** above, if the concentration of the end product in a pathway increases (**F** in diagram), it binds to the enzyme catalyzing the first step in the pathway (e_1), at a site <u>other than the active site</u>, and in doing so it reduces the ability of the enzyme to bind substrates at the active site, slowing down or stopping the metabolic pathway at the beginning. Therefore, the production of the *end product* is reduced

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or no longer made at all. If this end product begins to get used up again, the concentration will decrease enough so as to no longer inhibit **e₁** and the pathway can resume and more end product is made.

Examples in the Body

A specific example of this process is seen in the production of the important neurotransmitter called **norepinephrine** (**NE**), it used to be called noradrenaline, which is derived from the amino acid tyrosine. By the way, norepinephrine (epi = 'above', and nephro = 'kidney') is made by the paired adrenal glands (the adrenal medulla to be precise) which are endocrine glands that sit atop each of the two kidneys! Norepinephrine (NE) is released when there is a perception of stress or excitement by the body,

In the NE metabolic pathway, a series of enzymatically are involved, just like as shown in **Figure 3.7**. The NE is the **end product** and therefore the amount being made is an important signal to that pathway making it. Since the body does not want to make more of something than it needs, if NE is not being used at the rate it's being synthesized, then the excess NE will inhibit the first enzyme in the pathway and shut production of itself off, seen in **Figure 3.8**. If the NE levels become too low, the lack of excess NE will allow the restoration of the enzyme activity and re-instigate the production of that pathway.

Questions to Consider:

Here are some questions to consider regarding the specific example above involving norepinephrine, and about Allosteric Inhibition or End Product Inhibition in general.

1. What type of homeostatic mechanism is End Product Inhibitions? A ___________ Feedback Loop.

2. What would happen to the production of NE if the body lacked tyrosine?

3. Name a gland in the body that makes NE. Where is it located?

4. What kind of stimulation would cause an increase in the release of NE?

5. Looking at Figure 3.8, would inhibition of e₄ (enzyme 4) cause changes in product D?

As a side note, **metabolic pathways** in physiology are often extremely complex because they are integrated into many different aspects of a cell's function.

See **Figure 3.9** to the right, one of many examples of research done to map out pathways within our cells. Although reactions and pathways are very important, often they are examined in isolated cells and may be completely disconnected from the entire body and any real meaning. Fortunately for us, we do not have to view the cell or pathways this way, but we can look for basic patterns in order to see the big picture and the whole organism.
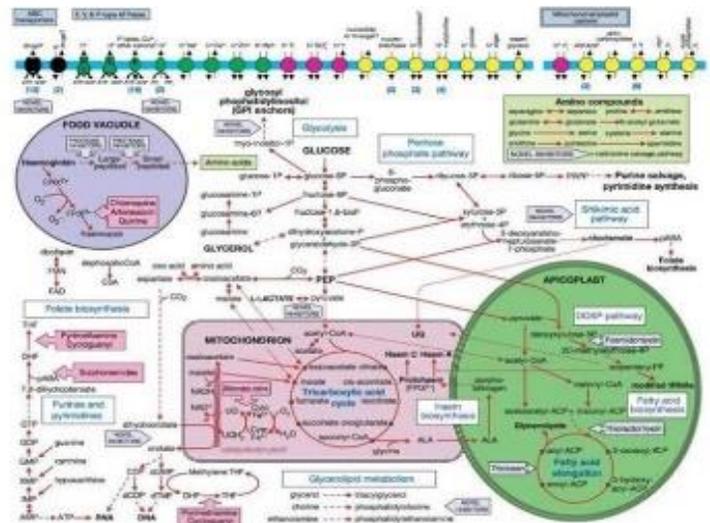
Figure 3.9 An example of an extremely elaborate and complex description of a metabolic pathway.

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Naming Enzymes

What's in a Name? Answer: Everything!

By convention, most enzymes names end with **_ase_** and typically their name indicates their function or the substrates they bind, or the product they make. This is a very useful way of naming things because very often the name can give us a clue about the action, substrate or product involved.

Before we begin to explore the -ase name endings, it is important to note that there are exceptions to this naming convention. We will encounter another type of naming system shortly, and that is the usage of the "**-ogen**" ending to denote the changes in the length of the enzyme (as in the linear sequence of amino acids) and how this is related to the cutting and activation of inert enzymes.

The -ase Endings for Enzymes

With regard to the **-ase** name ending for most enzymes, here are some common enzymes categories and functions that students will need to know.

- ☐ **_Phosphatase_** - removes phosphates from molecules.
- ☐ **_Kinase_** - adds *phosphates* to molecules.
- ☐ **_Dehydrogenase_** - removes hydrogens from molecules.
- ☐ **_Hydrolase_** - adds H_2O to molecules, to breaks down into smaller molecules.
- ☐ **_Polymerase_** - assembles polymers, composed of many repeating subunits.
- ☐ **_Isomerase_** - rearranges atoms in a molecule to make isomers.
- ☐ **_Synthase_** - removes H_2O from molecules, to make larger molecules.
- ☐ **_Carbonic anhydrase_** - removes H_2O from carbonic acid; found in erythrocytes.
- ☐ **_Amylase_** - digests the starch amylose into maltose and glucose
- ☐ **_Maltase_** - breaks down the disaccharide maltose into glucose.
- ☐ **_Lipases_** - group of enzymes that help digest lipids (fats).
- ☐ **_Proteases_** - group of enzymes that hydrolyze peptide bonds in proteins.
- ☐ **_Peptidases_** - hydrolyze (cleave) peptide bonds at the terminal amino acid.

Our study of the enzyme names and categories will be fairly limited, but enzymes in our body can be classified into seven (7) categories, based on the type of reactions that the enzymes catalyze. Although we will **not** go into details about these categories, they are as follows:

1) oxidoreductases, **2)** transferases, **3)** hydrolases, **4)** lyases, **5)** isomerases, **6)** ligases, and **7)** translocases.

The first three are the most abundant forms of enzymes.

As an exercise, use the information above to try to figure out what the names of these enzymes might be telling us:

- **a)** Glucokinase:
- **b)** Gluco-6-phosphotase:
- **c)** Reverse transcriptase:
- **d)** Alcohol dehydrogenase:

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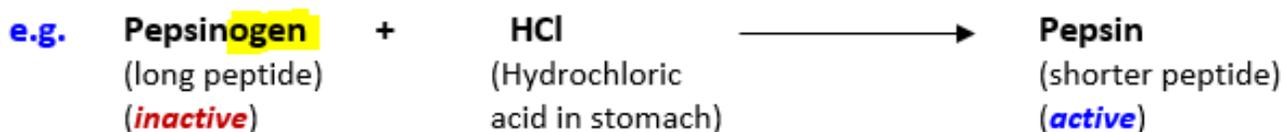
Enzyme Activation

Not all enzymes are synthesized in a form that is ready to catalyze a reaction. There are 3 main ways in which an enzyme can be '**activated**' if it is initially synthesized in its <u>inactive</u> form.

1. Proteolytic Activation - Some enzymes require proteolytic ('breaking of protein') activation. If an enzyme is made in its inactive form it will remain that way until it is cleaved (cut) by other enzymes or conditions in order to become activated. This typically involves a shortening the polypeptide chain.

e.g.   **Pepsinogen**   +   **HCl**   ⟶   **Pepsin**
(long peptide)   (Hydrochloric   (shorter peptide)
(*inactive*)   acid in stomach)   (*active*)

Often the enzymes requiring proteolytic activation do not have the conventional -*ase* ending to their names. The inactive forms of the protein often have suffix (ending) of -*ogen* attached at the very end of the name, e.g., pepsin*ogen* (inactive) and pepsin (active).

As we will see later, there are several other examples of proteolytic activation that we will encounter in future systems. And all of the examples we cover will follow the same pattern. Imagine that by cleaving the -ogen off you are not only shortening the name but also shortening the length of the protein.

Examples in the Body
From the names of these inactive enzymes below, suggest the new name for what each will become.

- o   Fibrinogen becomes - >
- o   Thrombinogen becomes - >
- o   Angiotensinogen becomes - >

Some enzymes require **cofactors** or **coenzymes** in order for the enzyme to function properly.

2. Cofactors - are **inorganic**, non-protein components required for substrate binding at the active site. Typically it is a **mineral** or metal ion that binds to the enzyme and activates its catalytic function. These include: Fe^{3+}, Fe^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+} and Mn^{2+}. All induce conformational changes. For example, *carbonic anhydrase,* an enzyme that helps maintain the pH of the body, cannot function unless it is attached to a **zinc ion** (see **Table 3.1**).

Table 3.1. Enzymes that require mineral cofactors.

Cofactor	Example Enzymes
Zn^{2+}	carbonic anhydrase
Zn^{2+}	alcohol dehydrogenase
Cu^{2+}	cytochrome oxidase
K^+ and Mg^{2+}	pyruvate phosphokinase

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3. Coenzymes – are usually small **organic** molecules typically derived from a **vitamin**, which is needed to make an enzyme catalytically active. They act by accepting electrons from an enzymatic reaction and transferring them to a different reaction chain. For example, **FAD** (flavin adenine dinucleotide) is a very common coenzyme. It carries electrons and participates in many important chemical reactions that flavoproteins carry out. Similarly, NAD^+ (nicotinamide adenine dinucleotide) shuttles electrons from glycolysis to the electron transport chain for aerobic respiration. Another important example is NADP (nicotinamide adenine dinucleotide phosphate).

Examples of how a variety of **whole foods** provide you with the required **co-enzymes**:

- **FAD** containing riboflavin, also known as vitamin B_2.
  Found in **eggs**, **meats**, **milk** and **broccoli**.

- **NAD**, **NADP**, both contain **niacin** (nicotinic acid) also known as vitamin B_1.
  Found in **meats**, **whole grains** and **leafy greens**.

- **Coenzyme A** is a pantothenic acid (B_5) precursor.
  Found in **meat**, **whole grains**, **milk**, **eggs** and a variety of **vegetables**.

Apoenzymes and Holoenzymes

Cofactors and coenzymes have an important similarity in that if either the cofactor or coenzyme is not present, the **enzymes that require them cannot function**. As such, these enzymes have terms that denote either the absence or presence of the cofactor or coenzyme (see **Fig. 3.10**)

Apoenzymes are **inactive** enzymes which are not bound to their cofactor/coenzyme. The term 'apo' means away from, separate, apart from.

Holoenzymes are catalytically **active** enzymes because they are bound to their cofactors/coenzymes and are therefore 'whole'. The term 'holo' means entire, complete or whole.
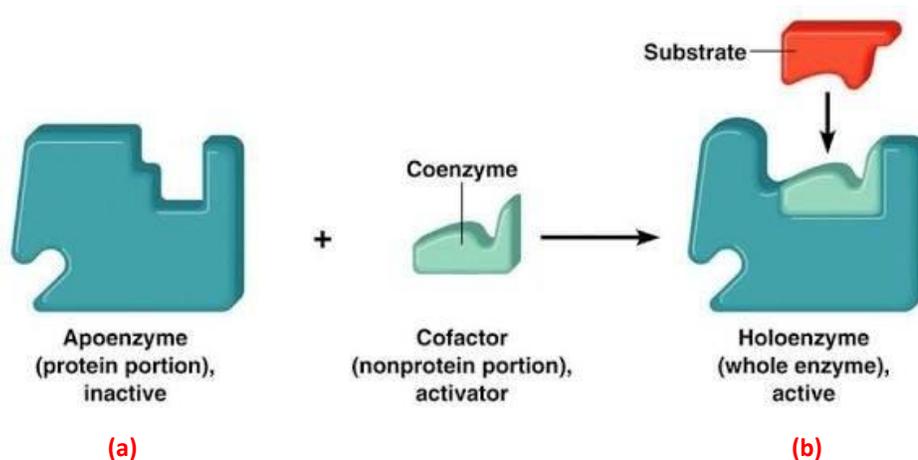
Figure 3.10 Shown above are representations of **(a)** an **Apoenzyme** that requires a cofactor/coenzyme but it is not bound, and therefore the enzyme is inactive in this form. When the required cofactor/coenzyme is present and bound, then it is **(b)** a **Holoenzyme** and is catalytically active.

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Factors that Effect Enzyme Activity

Once an enzyme is active, there are several things that can modulate (change) the level of activity of that enzyme. These modulators can include **pH**, **temperature**, other **molecules** (inhibitors) or the addition or removal of **covalent bonds**. These factors can inhibit or stimulate enzyme activity.

1. The pH of surroundings (Acidity/Alkali)

Enzymes function within certain pH ranges. Changes in pH alter the tertiary structure of an enzyme. Therefore, even small changes in pH can **increase or decrease enzyme activity**. Essentially most enzymes have an **optimal pH** which is really a *pH range* that they function most effectively within. Beyond a critical level, above or below its optimal pH range, the enzyme will become **denatured** (like changes to the egg white!). Consider the different pH environments of the body - mouth, stomach and small intestine, etc.

For example, **salivary amylase** is an enzyme made by the salivary glands of the mouth and is released into the oral cavity (mouth) to chemically break down ingested **starch** into the disaccharide **maltose**. In humans the optimum pH range for salivary amylase is a pH between **6.7** and **7.0**. Note that there is another enzyme called **pancreatic amylase**, made by, yes, the pancreas! Similarly, although this enzyme is stable over a pH range of **5.0** to **10.5**, its optimal pH is *7.0*.

2. Temperature

Enzymes also function within certain temperature ranges, typically most enzymes in the human body have an optimal level of activity around **normal body temperature** (37.6°C or 96.7°F). Again, changes in body temperature alter the tertiary structure of the enzyme and small changes in temperature can increase or decrease enzyme activity. For instance, the effect of a fever on enzymes of the body is to increase enzyme activity until temperatures exceed optimal ranges. Beyond a critical level, the enzyme is **denatured**.

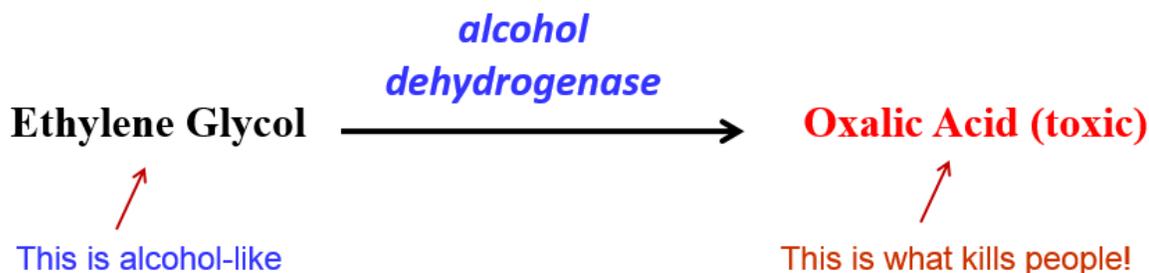
3. Inhibitors

a) Competitive Inhibitors are molecules that bind to the active site without being acted on by the enzyme. Many are similar enough in structure to the *true substrate* and bind to the active site, blocking the true substrate from occupying that site, thereby inhibiting the chemical reaction. In other cases, the competing molecule is a substrate that can be acted on by the enzyme and a reaction will take place, but again the reaction for the true substrate will be inhibited.

An interesting example of competitive inhibition can be seen with **ethylene glycol**, better known as **antifreeze**. It is a poison that kills about fifty people each year.

alcohol dehydrogenase

Ethylene Glycol ⟶ **Oxalic Acid (toxic)**

This is alcohol-like   This is what kills people!

In the body, the enzyme *alcohol dehydrogenase* acts on ethylene glycol to convert it to a toxic compound called **oxalic acid**. It is the oxalic acid that kills people, not the ethylene glycol per se. The treatment for ethylene glycol poisoning is administration of ethanol. That's right, the patient's treatment is to get intoxicated. Ethanol is the natural substrate for alcohol dehydrogenase so it competes with ethylene glycol for the alcohol dehydrogenase binding site. When ethanol is administered after ingestion of ethylene

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glycol, it binds to alcohol dehydrogenase preferentially in place of ethylene glycol. This blocks the production of the deadly oxalic acid and decreases the toxic effects of ethylene glycol ingestion.

b) Non-Competitive Inhibitors bind to the enzyme *at some site other than the active site*. They do not affect enzyme-substrate binding but in some other manner inhibit the enzyme from catalyzing the reaction. Some noncompetitive inhibitors act by binding to the inorganic ion cofactors of enzymes.

*Irreversible inhibitors - bind to an enzyme and permanently inactivate it. Gee that is harsh!
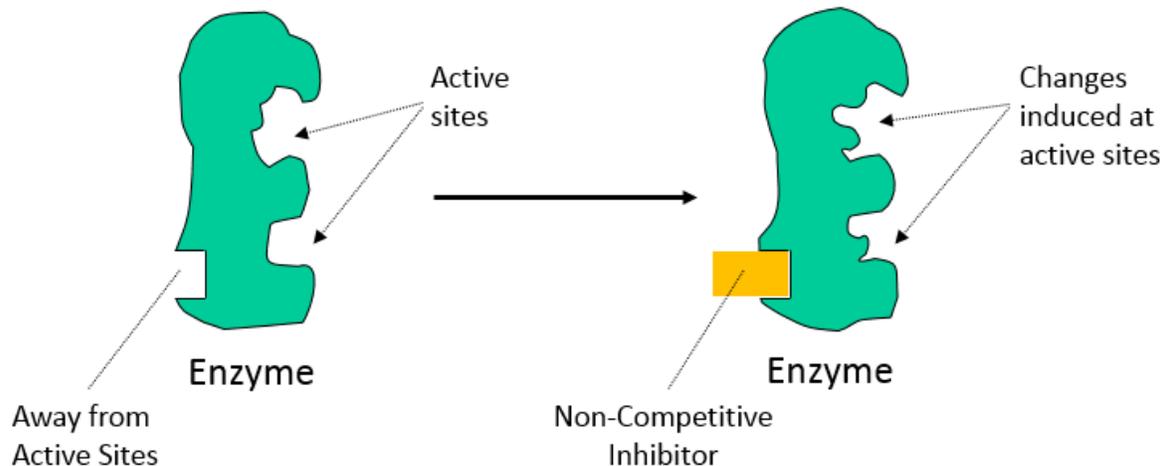
Figure 3.11 On the left is the enzyme that has nothing bound to it, although there is a region away from the active site for a **non-competitive inhibitor** to bind. On the right, the enzyme is now bound by a non-competitive inhibitor away from the active sites; however, this binding away from the active sites changes the configuration of the active sites and will therefore prevent the true substrate from binding, thus the enzyme has been inhibited.

4. Modulators - these bind away from active site but alter its configuration and can *increase* or *decrease* the enzyme's affinity for substrates. **Covalent modulators** bind *covalently* to an enzyme. They do not make covalent bonds at the active site, but when the covalent bond is formed the shape of the enzyme changes. This change in shape alters the 3-D shape of the binding site, thus altering the affinity that the enzyme will have for the substrate(s). Two very important covalent modulators are: **1)** adding or removing phosphate groups and **2)** changes in the concentration of intracellular calcium ions ($[Ca^{2+}]_i$)
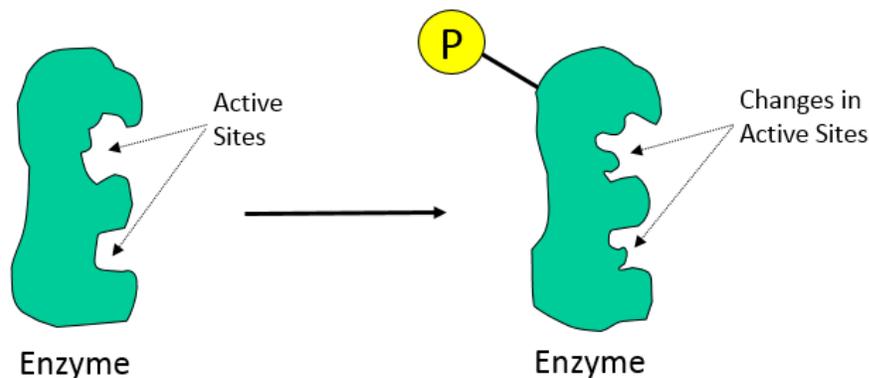
Figure 3.12 On the left is the enzyme that has nothing bound to it, and on the right, the enzyme is now bound by a **covalent modulator**, away from the active sites. Here we see the addition of a phosphate group to the enzyme, thus the enzyme has been phosphorylated. The covalent bond changes the configuration of the active sites, but this can increase or decrease the enzymatic activity, and therefore is called a covalent modulator.

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Enzyme and Substrate Concentration Affect the Reaction Rate

The rate of enzymatically catalyzed reactions is assessed by measuring the amount of product being synthesized (the rate of product). It can also be measured by the substrate consumption.

This last section is a brief examination of how the rate of a chemical reaction can be varied and how it can be dependent on the concentration of the enzyme and the substrate. We will examine how changes in the amount of **Enzyme**, and the amount of **Substrate** effect the rate of reactions.

1. **The Reaction Rate is Directly Related to the Amount of Enzyme Present.**
If the substrate concentration ([substrate]) is kept constant, then the more enzyme that is present, the greater the rate of the reaction, i.e., the more product is produced. Ultimately there is a limit to how much the reaction rate can continue to increase with increases in enzyme concentration, but the graph on the left in **Figure 3.13** shows how the rate of reaction proceeds in general.

2. **The Reaction Rate is Related to the Amount of Substrate Present and can Reach a Maximum.**
If the enzyme concentration ([enzyme]) is held constant, the reaction rate will increase as [substrate] increases but there is a limit to how fast a reaction can go, if [enzyme] is the limiting factor. The enzyme becomes saturated with substrate and reaches a maximum saturation point. At enzyme saturation, the enzyme is catalyzing reactions as fast as it can and cannot function any faster. The difference between the two graphs in **Figure 3.13** is that the one on the right is limited by a maximum saturation point.
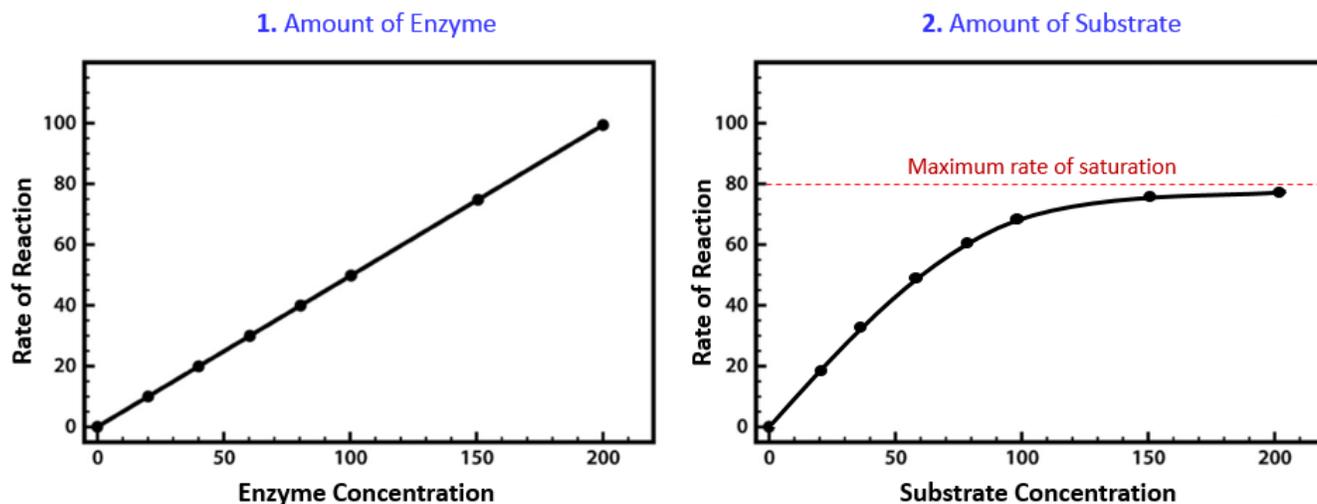
Figure 3.13 The rate of a chemical reaction for two situations: **1.** The enzyme concertation is increased while the substrate concentration is kept constant (left), and **2.** The substrate concertation is increased while the enzyme concentration is kept constant (right). Notice that when the enzyme concentration is increased, the rate of reaction continues to increase, and when the substrate concentration is increased, the rate of reaction continues to increase but only to a point (maximum saturation), and then the reaction rate remains constant.

Reversible Reactions, Reaction Rate, and the Law of Mass Action

Reversible reactions proceed to a state of equilibrium (or balance), where the forward reaction rate = the reverse reaction rate. For example, adding more substrate increases the forward reaction rate and more product forms in order to establish equilibrium.

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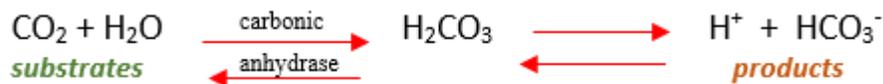
The Law of Mass Action is part of **LeChatelier's principle**, which states that if a system at equilibrium is disturbed by a change in concentration of one of the components or in temperature or pressure, the system will shift until a new equilibrium is reached. Law of Mass Action can also be described this way:

The direction of a reversible equation (forward or reverse) will be driven by the amount of reactant (substrates) or products present; the direction of the reaction will be to decrease whatever is in abundance, until equilibrium is reached. At reaction equilibrium, the ratio of substrates to products is always equivalent; the system adjusts the ratio until equilibrium is restored.

The following enzymatic reaction is an important ***reversible reaction*** which takes place in the body:

$$CO_2 + H_2O \underset{\text{anhydrase}}{\overset{\text{carbonic}}{\rightleftharpoons}} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

substrates   products

Assume that at first this reaction above is at equilibrium, then the concentration of H^+ is increased by some outside force. What will have to happen to the direction of the equation in order to restore equilibrium? ____.

You do not need to know the relative concentrations of the substrates and the products or the equilibrium constant in order to answer this question. Just think of the equation trying to re-balance itself!

Enzymes and Reversible Reactions

The body regulates reversible reactions in order to control metabolism. If a single enzyme controls both the forward and reverse reactions then the product concentration will be governed by ***Law of Mass Action*** only and the reaction can't be closely regulated. If there are separate enzymes, one for the forward and another for the reverse reaction, then these pathways can be regulated more closely.

The synthesis of glucose 6-phosphate, by adding a phosphate to glucose, is an important example of a one-way path in cellular metabolism. All tissues have the pathway needed to add the phosphate to glucose, but only the **liver** and **kidneys** have the enzyme ***glucose phosphatase*** needed to remove the phosphate from glucose to go in the other direction! When only two organs in the entire body can take that phosphate off in order to utilize that glucose - that is finely preserved control.

The presence of irreversible steps in a metabolic pathway provides the cell with an important means of controlling its metabolism. It also requires that cells and tissues and organs **cooperate** with each other.

For example, since most cells can't just make glucose, they must either find alternate sources of energy or send their chemical intermediates to the liver so that the liver can turn them into glucose. The glucose produced by the liver then returns it to the various cell via the blood stream enabling them to function. This is a perfect example of how *cooperation* between the body's individual cells and tissue is the fundamental concept which enables the success of the whole organism.

It is also worth noting that although skeletal muscle cells cannot make glucose, fast twitch muscle cells do have large stores of **glycogen** granules. Glycogen is the storage molecule for glucose (a polymer of glucose). So when glycogen is broken down it releases… glucose! The process by which this occurs is called… **glycogen**lysis, right?!?

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Review Questions for Chapter 3: Enzymes

1. Name an enzyme that digests fat?
   a) sucrase
   b) maltase
   c) lipase
   d) fructose
   e) protease

2. All enzymes are
   a) proteins
   b) biological catalysts
   c) organic molecules
   d) a and b
   e) a b and c

3. The enzyme **glucokinase** has what action?
   a) It removes the phosphate group from glucose-6-phosphate.
   b) It converts glucose to lipids.
   c) It add a phosphate group to glucose.
   d) It adds a phosphate group to glucose-6-phosphotase.
   e) It hydrolyzes glucose for metabolic energy.

4. Inactive enzymes which are not bound to their cofactors are called
   a) apoenzymes
   b) coenzymes
   c) enzyme inhibitors
   d) holoenzymes
   e) denatured

5. If an enzyme has a **non-competitive inhibitor** present, then:
   a) the active site is blocked but the reaction will still occur
   b) there is a binding away from the active site that increases the activity of the enzyme
   c) the active site is blocked and the activity of the enzyme is inhibited
   d) there is a binding away from the active site that inhibits the activity of the enzyme

6. What will happen to the rate of an enzymatic reaction if more **enzyme** is added?
   a) The reaction will happen slower (at a lower rate)
   b) The reaction will happen faster (at a higher rate)
   c) The reaction rate will not change

7. A **Co-factor** for an enzyme is
   a) always organic and consumed in the reaction
   b) slows the rate of reaction down
   c) an inorganic non-protein component, usually a mineral
   d) requires a co-enzyme
   e) is called an apoenzyme

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8. In humans, the enzyme **salivary amylase** breaks down starch in the mouth. What is the **optimum pH** range for this enzyme?

   a) 6 to 7
   b) 6.2 to 6.4
   c) 7.35 to 7.45
   d) 6.7 to 7.0
   e) 7.0 to 7.35

9. Regarding enzymes and the pH of their surroundings:
   a) all enzymes operate optimally within the pH range of 7.35 to 7.45
   b) no enzymes in the body can function if too acidic
   c) the body' enzymes have a variety of optimal pH ranges

10. If the **temperature increases**, enzyme activity
   a) will denature immediately
   b) will slow down
   c) the activity of the enzyme would not be changed
   d) will speed up to a point

Answers in Appendix B