SBI4U-C

Lesson 3

Enzymes

Introduction

In the first two lessons of this course, you were introduced to many of the features of biological molecules. In this lesson, you will learn how proteins function as enzymes. Enzymes are protein catalysts that speed up reactions within cells.

For thousands of years, people have used the enzymes produced by naturally-occurring micro-organisms, such as bacteria, yeasts, and moulds, to make foods like bread and cheese, and drinks like beer and wine. Currently, enzymes are used for a range of applications, such as baking, cheese-making, starch processing, and the production of fruit juices and other drinks. Enzymes are used in these products to improve their texture, appearance, and nutritional value, and to give them desirable flavours and aromas. Enzymes are also important in drug manufacture and in the alternative fuel industry.

In this lesson, you will learn more about enzymes, how they operate, and how their activity is "monitored" within cells. You will also conduct an investigation that examines the factors controlling enzyme activity.

Planning Your Study

You may find this time grid helpful in planning when and how you will work through this lesson.

Suggested Timing for This Lesson (Hours)	
Enzymes and Their Importance	1
Enzyme Activation and Inhibition	1
Factors that Affect Enzyme Activity	½
Activity: Investigating Enzyme Activity	1
Key Questions	1

What You Will Learn

After completing this lesson, you will be able to

- describe the chemical structures and mechanisms of various enzymes
- plan and conduct an investigation on the factors that affect enzyme activity using appropriate laboratory equipment and techniques, and report the results in an appropriate format
- describe the structure and function of important biochemical compounds in cells
- analyze technological applications related to enzyme activity in the food and pharmaceutical industries

Enzymes and Their Importance

Millions of reactions take place every day in cells, and most of these reactions are able to occur at an optimal rate, thanks to the work of enzymes. Enzymes are protein catalysts that speed up chemical reactions, without being consumed by the reactions themselves. In most cases, enzymes make reactions happen millions of times faster than they would without a catalyst.

Enzymes are proteins; they are composed of amino acids arranged in tertiary or quaternary structures, with complex conformations (shapes). Enzymes can be used to join together two molecules or break one molecule into two parts. The names of enzymes usually end in "-*ase*." For example, there is a specific enzyme called malt*ase*, which is used to hydrolyze (or break down) maltose, and another called sucr*ase*, which is used to break down sucrose. In these cases, the enzymes involved break the complex sugar molecules down into their monosaccharide monomers.

Enzymes are specific to a particular substrate (reactant). For instance, the enzyme maltase cannot hydrolyze the substrate sucrose, and sucrase cannot hydrolyze maltose.

In order for a reaction to occur, the reactions have to overcome the activation energy (E_a) barrier. The activation energy is the amount of energy that must be available in order for a reaction to occur. Enzymes work by lowering the activation energy. This is shown in Figure 3.1.

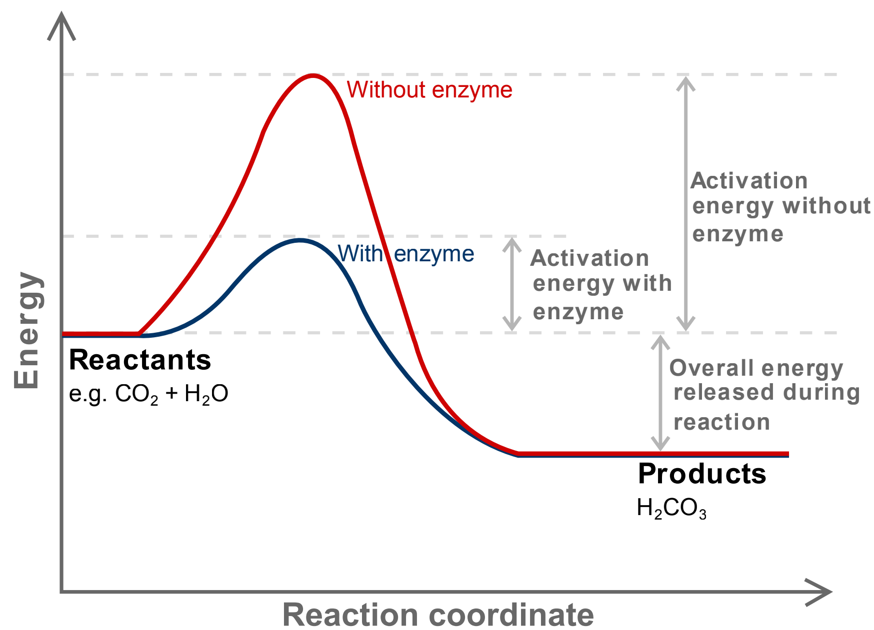

Figure 3.1: Enzymes lower the activation energy required for chemical reactions to occur.

Enzymes lower the activation energy required for the reaction to occur by attaching to the reactants and positioning them so that they are in the optimal orientation to break or make chemical bonds between them. The reactants attach to special sites on the enzyme called "active sites." When reactants begin to interact with enzymes, they are called substrates. Each substrate binds to the active site that is shaped exactly to fit that particular substrate, and no other. This is what makes enzymes very specific. The enzyme and its attached substrate form a structure called an "enzyme-substrate complex."

Once the substrate begins to attach, the active site changes shape slightly to hold on to the substrate and fine-tune its position. It's similar to what happens with your hand when you catch a ball: your fingers automatically wrap around the ball to ensure that you don't drop it. This change in shape (conformation) is called "induced fit." The active site continues to change shape until the substrate is completely bound. Once the substrate is bound and optimally positioned by induced fit, the reaction can proceed to form or break chemical bonds. The process of induced fit for splitting apart a molecule into two parts is shown in Figure 3.2 below. The process works in a similar way to combine two molecules into one.

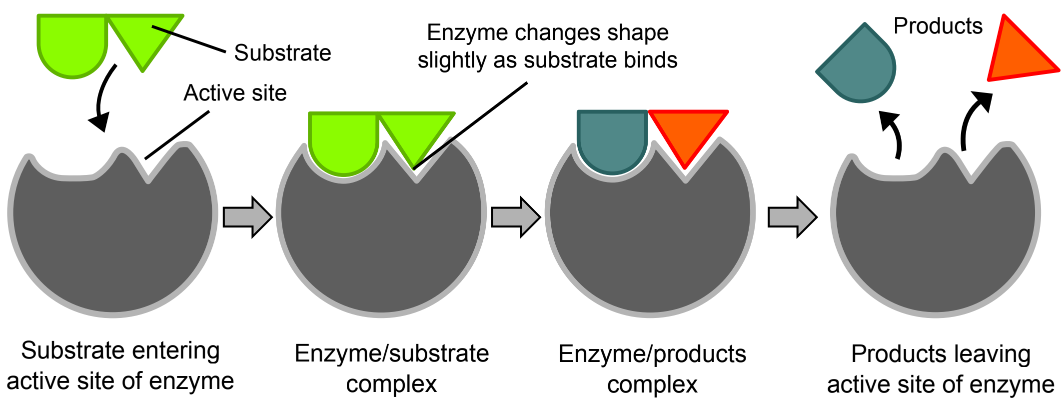

Figure 3.2: The induced fit model for enzyme activity. In this example, two products (glucose and fructose) are created from one substrate (sucrose).

As shown in Figure 3.2, the enzyme and substrate first combine on a specific area of the enzyme called the active site, to form the enzyme substrate complex. In this example, the enzyme is sucrase and the substrate is sucrose. Then, the enzyme substrate complex combines with water (hydrolysis), which changes the shape of the enzyme slightly so that the substrate can break apart into glucose and fructose more easily. Next, the reaction takes place and the sucrose molecule is broken up into the two monomers, fructose and glucose. Finally, the enzyme is released unchanged, ready to react again with a new substrate molecule.

When splitting a molecule apart, enzymes decrease the energy of activation by stretching and bending chemical bonds that need to break during the reaction. This is similar to bending a thin twig so that it won't take much additional energy to snap it. When joining two molecules together, enzymes decrease the energy of activation by forcing the molecules together in just the right way to cause bonds to form more easily. This is similar to what you do when you snap a button together: only when the two parts are perfectly positioned can you easily snap them together.

Enzymes can also change the chemical environment around a molecule to encourage a reaction. For example, it is thought that the acidic active site of the enzyme may provide the low pH environment needed for certain reactions to take place. Enzymes may also need to work with other molecules as helpers. Some enzymes require either non-protein cofactors, such as inorganic substances (Zn^{2+}, Ca^{2+}) or organic coenzymes, before they can work properly. Coenzymes help by moving molecules from one enzyme to another. Many coenzymes are derived from vitamins. For example, the coenzyme NAD^{-} is a derivative of vitamin B_3 (niacin).

Enzymes in Your Body

Enzymes are critical to the proper functioning of your body. Enzymes play major roles in the digestive system, the respiratory system, and the nervous system.

Digestion involves thousands of chemical reactions that serve to break down your food into the basic building blocks of glucose, fats, and amino acids that your body needs. One critical enzyme in this process is called pepsin. This enzyme is secreted into the stomach, where it breaks down proteins into smaller fragments called peptides, so that they can be broken down further in the small intestine. If this enzyme's levels are too low, or its action is blocked (or "inhibited"), then many proteins, like gluten (found in wheat and other grain products) and casein (found in dairy products), are not completely digested, resulting in many health problems.

Respiration is the cellular process in which glucose is reacted with oxygen to produce carbon dioxide and water, while releasing energy in controlled stages. One enzyme critical in this process is called cytochrome c oxidase. This enzyme helps to combine the highly reactive waste oxygen molecules produced during this process with hydrogen, to create water. If this enzyme is absent, or its function is inhibited, then the process of cellular respiration stops within seconds and death is rapid. Carbon monoxide is a potent inhibitor of this enzyme. Because this gas is potentially so lethal, it is now mandatory to have carbon monoxide detectors in houses.

Your brain also relies on many enzymes to maintain optimal conditions for neural processing. For example, the enzyme acetylcholinesterase breaks down acetylcholine, which is a neural transmitter. Acetylcholine is a chemical that helps signals to pass between nerve cells, but the system only works if the amount of acetylcholine is strictly regulated. If this enzyme is absent or inhibited, then excessive acetylcholine will accumulate in the nerve synapses. This will cause paralysis in the muscles, including those that control breathing, and lead to death. The venom of many poisonous snakes includes chemicals that block the activity of acetylcholinesterase.

Enzymes in Industry

Enzymes are used in many industrial processes, from food to drug production. For example, in bread-making, the enzyme amylase is used to break down flour (starch) into simple sugars, which are then converted by yeast into alcohol and carbon dioxide. The carbon dioxide produced makes the bread rise.

Food Industry

Enzyme applications are currently being researched and their function is widely exploited for a variety of applications in the food industry, such as baking, cheese production, starch processing, and the production of fruit juices. Enzymes can improve the food's texture and appearance, enhance its nutritional value, and give it desirable flavours and aromas.

Where do nutritional scientists obtain these enzymes? Most come from bacteria or yeast, but a few are obtained from plant and animal cells. For instance, amylase, which is used in bread-making, can be isolated from germinating barley seeds.

In food production, enzymes provide several benefits, such as:

- Replacing synthetic chemicals, which lowers the amount of waste and energy consumed to produce the food product
- Allowing some reactions to occur that otherwise may not have occurred naturally. A good example of this is the enzyme pectinase, which is used to produce clear (not cloudy) apple juice.
- Providing specific action that allows for very specialized food products to be produced efficiently

Alternative Fuels

Enzymes are at the forefront in the search for alternative fuels like ethanol. A promising source of ethanol comes from agricultural waste, such as the corn stalks left behind after harvesting, or from a native grass called switchgrass (*Panicum virgatum*) (see Figure 3.3). This ethanol is called cellulosic ethanol, because it comes from cellulose. Using agricultural waste or switchgrass to produce ethanol would not compete with food production. This means that the critical food supplies needed to feed people would not be used for producing fuel instead. Currently, however, there is a problem with using agricultural waste and switchgrass for ethanol production because it does not ferment as easily as other agricultural products, like corn. Because of this, it does not yet produce ethanol abundantly or cheaply.

The secret to improving cellulosic ethanol production is to find enzymes that optimize the biochemical pathways that produce ethanol. Many Canadian companies are in the race to find or design new enzymes to make this type of alternative fuel production commercially practical.

Figure 3.3: Switchgrass (*Panicum virgatum*) could be an important new source of ethanol fuel. It grows on land that is unsuitable for agriculture, so its cultivation would not harm food production.

Support Question

Be sure to try the Support Questions on your own before looking at the suggested answers provided.

19. Fill in the blanks in the following paragraph. You can print this out or answer elsewhere.

Enzymes are ___________________________ catalysts, and as such they ___________________________ a chemical reaction, without being ___________________________ in the process. Enzymes work by reducing the ___________________________. The ___________________________ is the reactant on which an enzyme acts. This reactant binds to a particular spot on the enzyme known as the ___________________________. Once bound, the reactant and enzyme form the ___________________________ complex, which allows the reaction to proceed at a lower energy. Enzymes are very ___________________________ to the reactant to which they bind. The names of enzymes usually end in ___________________________.

Suggested answer

Enzyme Activation and Inhibition

Although enzymes are important molecules involved in chemical reactions, they need to be monitored, in terms of when to be active (which is similar to having an "on" and "off" light switch in a room). For example, the enzyme lipase catalyzes the breakdown of triglycerides into glycerol and fatty acids, but this enzyme does not always need to be active. When your body requires energy from triglycerides, it will "activate" the lipase enzyme to break down fats. When it does not require the lipid energy, it will inhibit the lipase or "turn it off." How does this happen?

Enzyme Inhibition

Enzymes can be inhibited or prevented from carrying out their function, in a variety of ways. Some of the inhibition occurs at the enzyme's active site, which is where the substrate binds when the enzyme is actively catalyzing cell reactions. Large enzymes with more than one subunit contain more than one active site. Most enzymes also contain an additional site, called the allosteric site. The allosteric site is involved in turning the enzyme "on" and "off."

If the enzyme inhibition happens on an active site, it is called competitive inhibition. If it happens as a result of changes on the allosteric site, it is called non-competitive inhibition.

Competitive Inhibitors

Competitive inhibitors are molecules that are so similar in shape to an enzyme's substrate that they can bind to the active site and block the normal substrate from binding. This process is demonstrated in Figure 3.4 below.

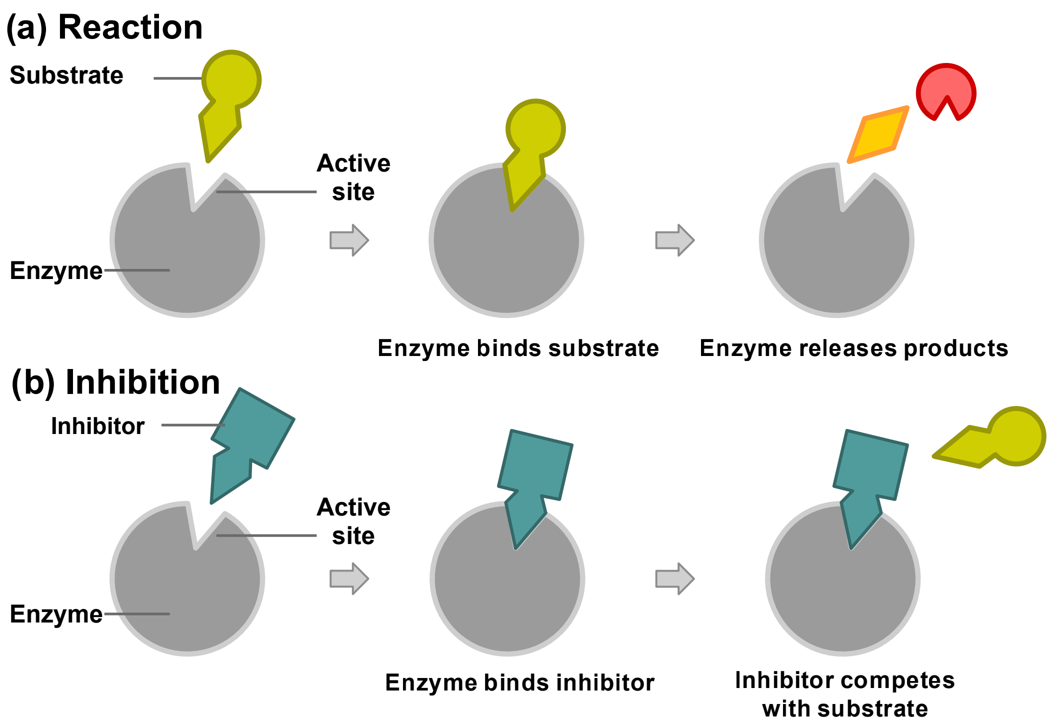

Figure 3.4: Competitive inhibition. The competitive inhibitor molecule has a similar shape to that of the substrate. It binds to the active site on the enzyme, preventing the normal substrate from binding to the site.

Once the inhibitor has attached to the active site, the enzyme is unable to catalyze any reactions at that site. This process happens very quickly. Many poisons act as competitive inhibitors on critical enzymes. For example, the poison cyanide blocks the active site on cytochrome c oxidase and so stops cellular respiration almost instantly.

Competitive inhibition does not necessarily result in the enzyme being fully "turned off." If an enzyme has more than one active site, then competitive inhibition can act to slow down the rate of reaction of the enzyme by blocking some active sites, but leaving other active sites open to hold substrates. The more active sites there are that are blocked by inhibitors, the slower the overall rate of reaction will be. In this way, the overall reaction rate can be controlled gradually. The enzyme becomes completely inactive only if all of the active sites are blocked by inhibitors.

Non-competitive Inhibitors

Non-competitive inhibitors are molecules that bind to the enzyme at another site on the enzyme, called the allosteric site (not the active site). This causes conformation (shape) change in the enzyme, preventing the normal substrate from binding at any active site. This method of enzyme regulation results in the enzyme being turned off. This process is demonstrated in Figure 3.5 below.

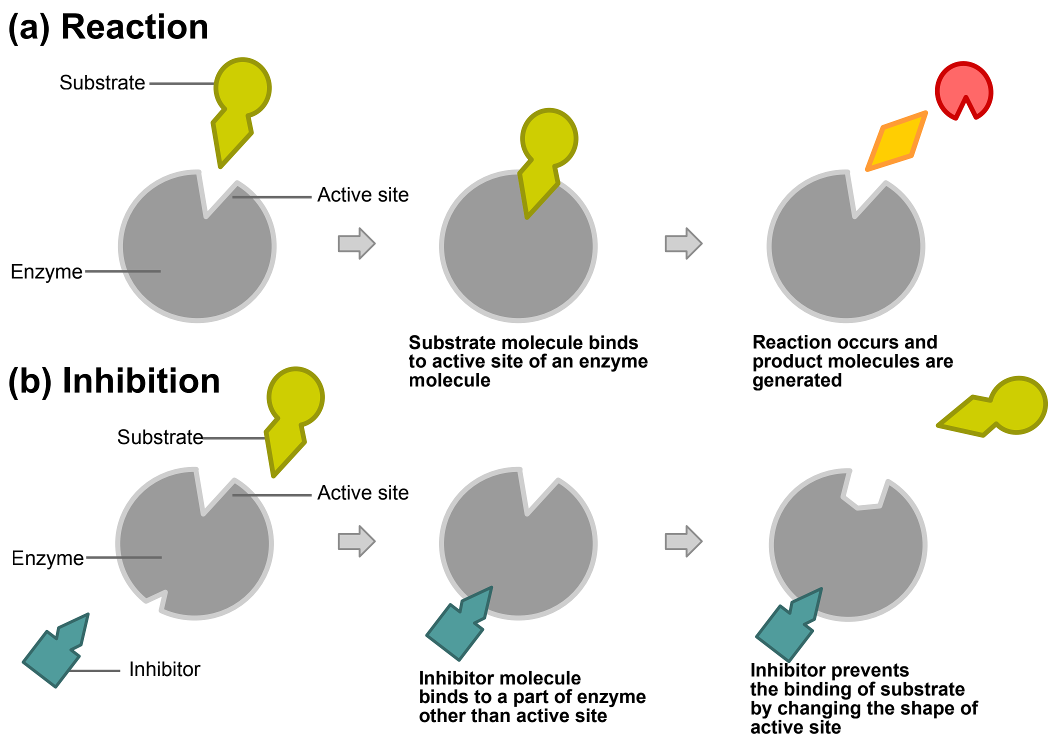

Figure 3.5: In non-competitive inhibition, the inhibitor molecule binds to the allosteric site on the enzyme. This changes the shape of the active site, thereby preventing the normal substrate from successfully binding. This makes the enzyme inactive.

This process can also be used to activate an enzyme. Non-competitive inhibition or non-competitive activation via the allosteric site can provide a very sophisticated way to fine-tune the behaviour of an enzyme. This method of enzyme control is called allosteric regulation and is described in the next section.

Allosteric Regulation

Enzymes can oscillate in shape between the "on" and "off" conformation. They can be temporarily locked into the "on" or "off" conformation by interacting with other molecules called allosteric activators and inhibitors. When these regulatory molecules bind to the allosteric or regulatory site on the enzyme, they force a change in the shape of the enzyme.

Allosteric activators: These change the enzyme's shape and stabilize it, which keeps all of the active sites available for substrates to bind to (the enzyme is "on"). Figure 3.6a shows how this activation process works.

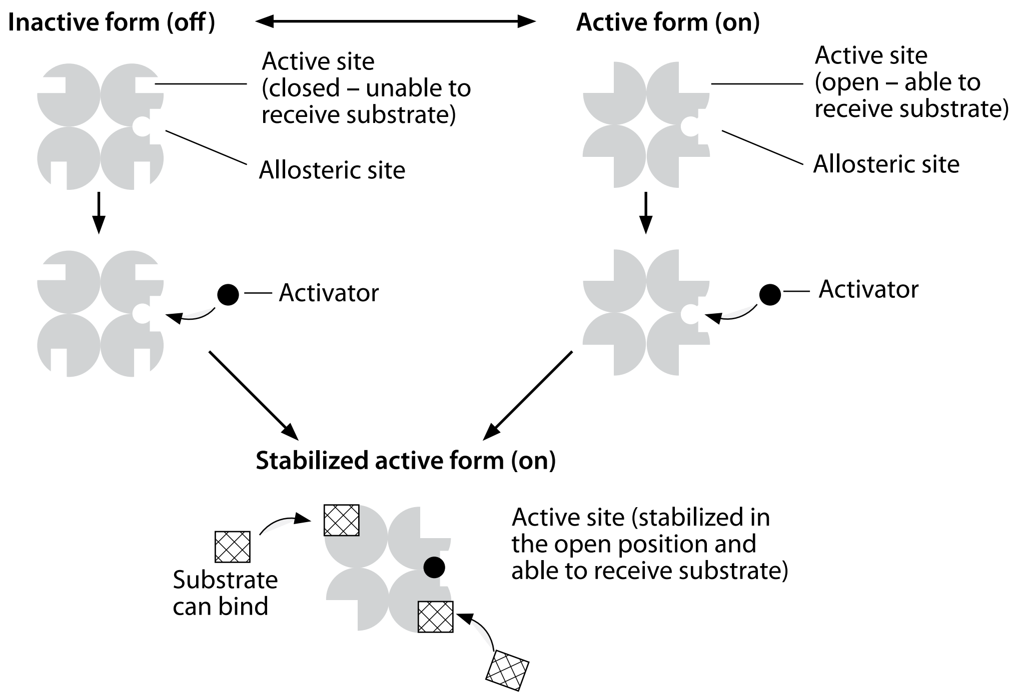

Figure 3.6a: Some enzymes can switch between their active and inactive forms, but can be locked into the active position using allosteric activators. The activator molecule attaches to the allosteric site to stabilize the enzyme in the "on" position, regardless of the enzyme's original conformation. In this example, the enzyme has four active sites and all are turned on by the one activator.

Allosteric inhibitors: These change the enzyme's shape and stabilize it to make all of the active sites unavailable to substrates (the enzyme is "off"), as shown in Figure 3.6b.

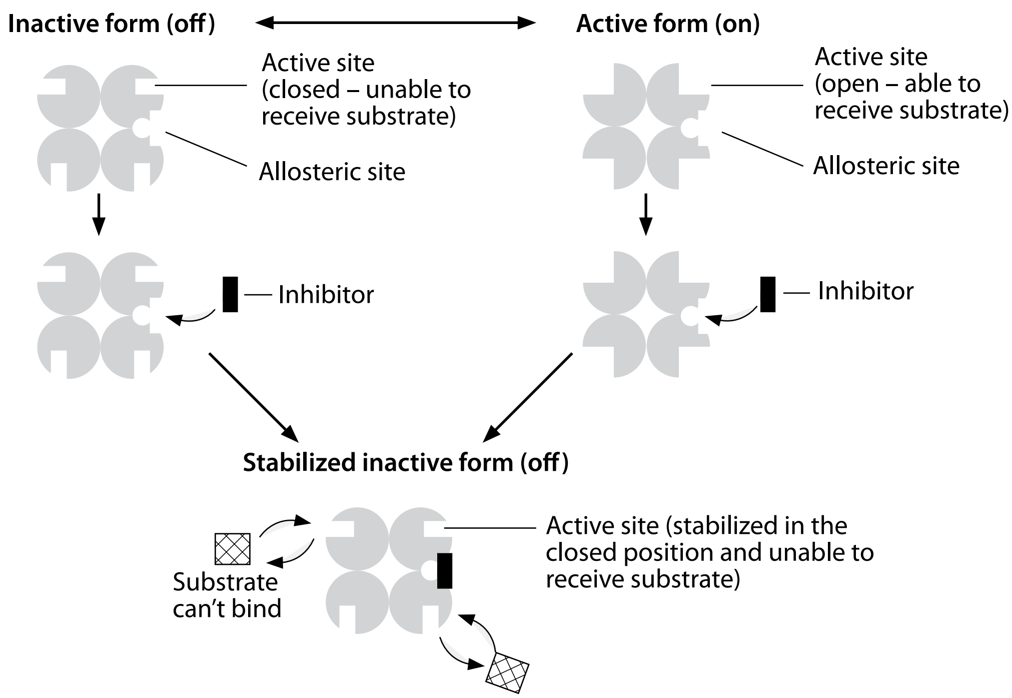

Figure 3.6b: The allosteric inhibitor molecule attaches to the allosteric site to stabilize the enzyme in the "off" position, regardless of the enzyme's original conformation. In this example, the enzyme has four active sites and all are turned off by the one inhibitor.

The enzyme stays in the "on" or "off" position until the allosteric regulator is removed. Because only one regulatory molecule is needed to turn all of the active sites on the enzyme on or off, this is an efficient way to control enzyme function.

Feedback Inhibition

Feedback inhibition is a method that cells use to control metabolic pathways, involving a series of sequential reactions in which each step is catalyzed by a specific enzyme. In this method of metabolic control, a product that is formed later in a sequence of reactions allosterically inhibits an enzyme that catalyzes a reaction occurring earlier in the process. It is a negative feedback process, because as the amount of product produced increases, the rate of the reaction decreases. This feedback inhibition process is shown in Figure 3.7 on the next page.

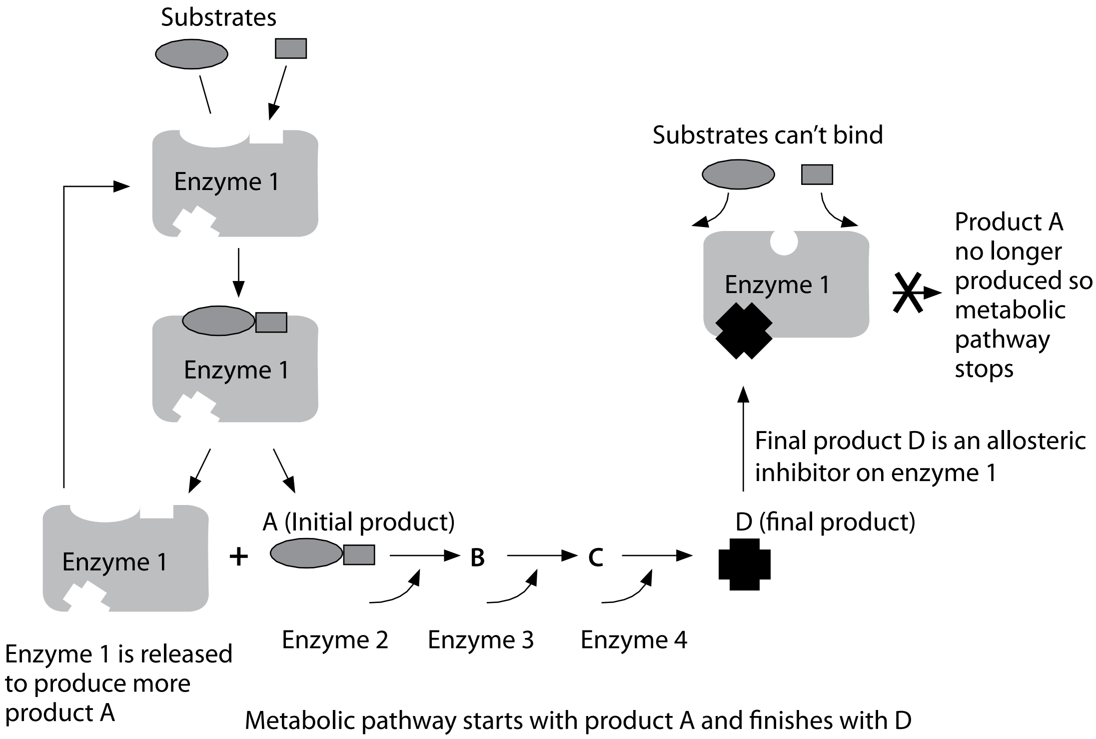

Figure 3.7: In feedback inhibition, the final product is an allosteric inhibitor on the first enzyme in the pathway. This stops the process.

In Figure 3.7, enzyme 1 catalyzes the production of product A from two substrate molecules. A metabolic pathway converts product A into product D, using three other enzymes. Product D can act as an allosteric inhibitor on enzyme 1. When the concentration of product D is high enough, it will inhibit all of the available enzyme 1 molecules, and so stop the production of product A. This effectively stops the metabolic process.

Support Question

20. Compare competitive to non-competitive inhibition.

Suggested answer

Factors that Affect Enzyme Activity

Apart from activators and inhibitors, there are three other important factors that affect how optimally an enzyme will function within the cell. They are:

1. pH
2. Temperature
3. The concentrations of the enzyme and substrate

pH

Enzymes have optimal pH, depending on the environment of the substrate. Some enzymes work best at a neutral pH (7), while others are active at acidic pH ranges (3–4). This is shown in Figure 3.8 below.

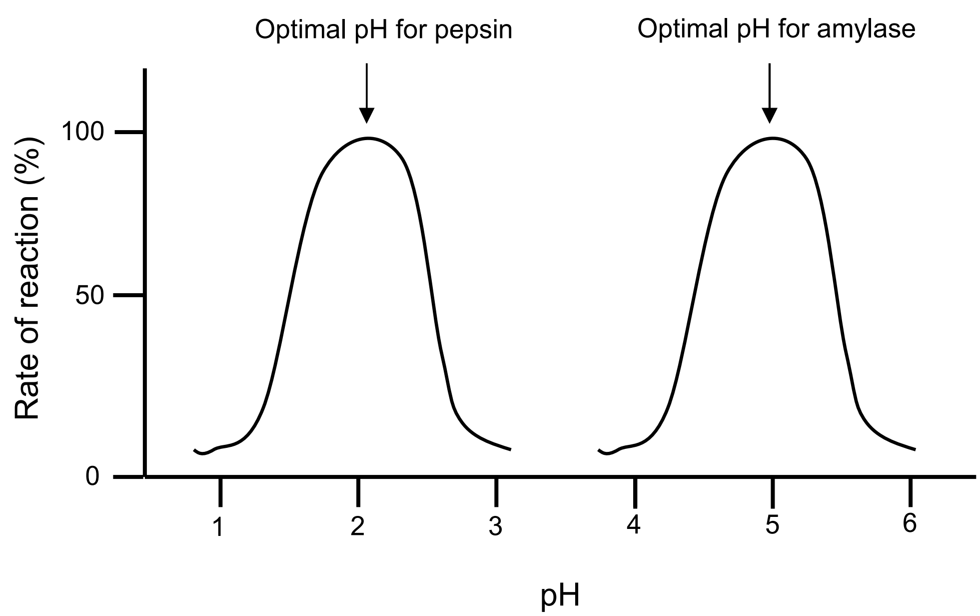

Figure 3.8: pH is a factor that affects how well an enzyme will operate. Pepsin is an enzyme found in the stomach and it works best at around pH 2. Amylase is an enzyme found in the mouth and it works best at around pH 5, which is the normal pH of the mouth.

Analyzing Figure 3.8, you can see that some digestive enzymes, such as pepsin (found in your stomach), are active at acidic pH ranges. This is useful, since most proteins are digested in your stomach, which is an acidic environment. Amylase, on the other hand, digests optimally at a slightly acid pH similar to that found in your mouth. This allows it to catalyze the breakdown of starch into simple sugars in your mouth.

Temperature

Every enzyme has an optimal temperature at which it works best. Most enzymes work best around 37°C, the normal body temperature for mammals, as shown in Figure 3.9 below. However, some organisms contain enzymes that function best at extremes of hot or cold. In a later unit, you will learn about one of these enzymes, *Taq* polymerase, and why it lies behind the biotechnology revolution.

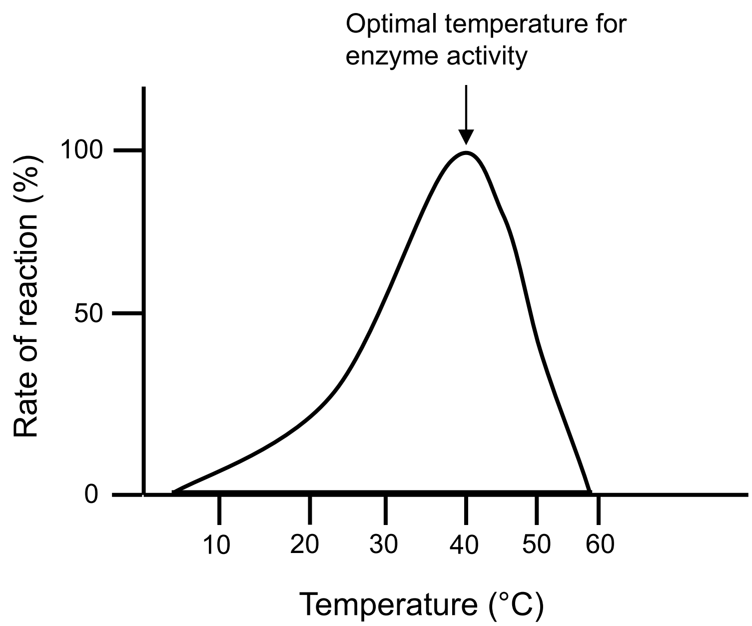

Figure 3.9: Most enzymes work optimally at temperatures similar to your body temperature of 37°C.

Enzyme and Substrate Concentration

Increasing the amount or concentration of a substrate or enzyme will increase the number of successful enzyme-substrate collisions, thereby increasing the enzyme activity. There is a threshold point, however, beyond which there is no further increase in the enzyme's activity. This is the point of saturation, shown in Figure 3.10. Saturation means that all of the enzyme molecules are occupied with substrate. Adding more substrate will not increase the rate of reaction because the enzymes are already working at full capacity.

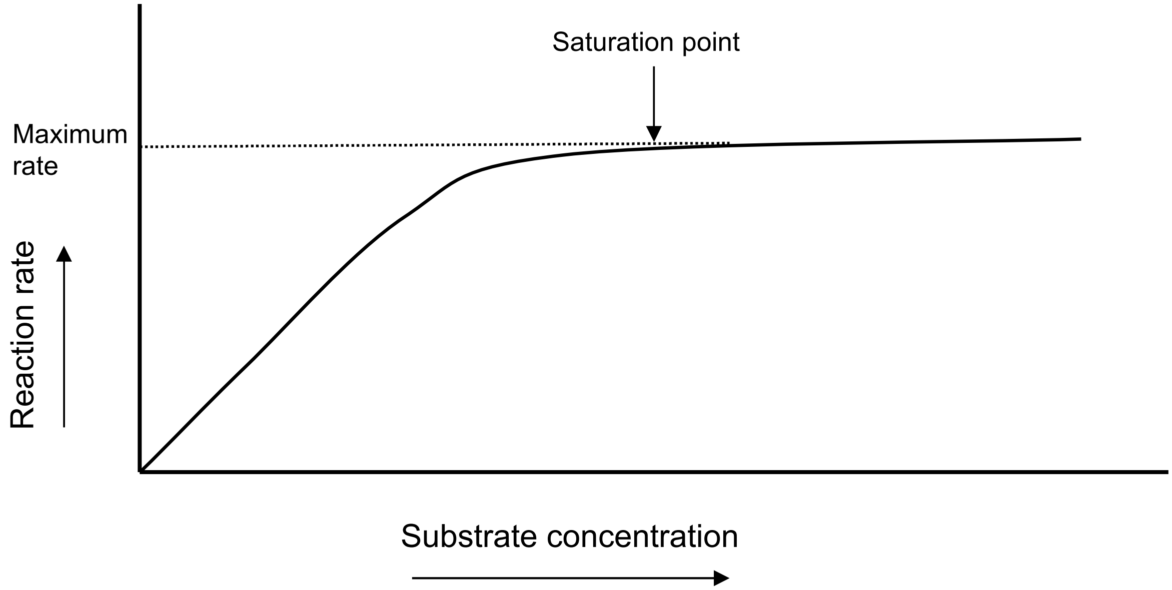

Figure 3.10: The increasing concentration of an enzyme and/or substrate will speed up the reaction until the point of saturation is reached.

Support Question

21. Explain how temperature affects an enzyme's rate of reaction.

Suggested answer

Enzymes: The Body's Catalysts is an animated tutorial on enzymes. This **optional** tutorial reviews enzyme structure, function, and control. You may find this helpful for reinforcing your understanding of enzymes.

Activity: Investigating Enzyme Activity

Background

Hydrogen peroxide (H_2O_2) is a product of some cell processes and since it is toxic to cells, it must be inactivated immediately. This breakdown is illustrated in the following balanced chemical reaction:

$2H_2O_2 \rightarrow 2H_2O + O_2$

However, the natural rate of this reaction is slow enough to still cause some damage to cells before the hydrogen peroxide is completely broken down. An enzyme in cells, called catalase, speeds up the rate of this reaction to reduce the risk of exposure to hydrogen peroxide.

Purpose

To investigate whether a potato contains catalase

Note: If you are unable to conduct this experiment, you can use the sample observations provided at the end of this activity to answer the analysis questions.

Materials

- 10 mL (about 2 teaspoons) of 3% hydrogen peroxide (available in drug stores and some grocery stores)
- One small raw peeled potato
- One small boiled peeled potato
- Six small drinking glasses
- A small paring knife
- 10 mL graduated cylinder or a teaspoon

Procedure

1. Set up six labelled glasses containing the following contents:
    - Glass A: Piece of raw potato about 1 cm^3 (about the size of a sugar cube)
    - Glass B: Piece of raw potato about 1 cm^3

- Glass C: Piece of raw potato about 1 cm^3, chopped up into small pieces
- Glass D: Piece of raw potato about 1 cm^3, chopped up into small pieces
- Glass E: Piece of boiled potato about 1 cm^3
- Glass F: Piece of boiled potato about 1 cm^3

2. Add the following to the designated glasses:

   Glasses A, C, and E each receive 2mL or ½ teaspoon of water

   Glasses B, D, and F each receive 2 mL or ½ teaspoon of hydrogen peroxide

3. Observe each glass for evidence of a reaction. You are looking for vigorous bubbling, indicating that oxygen gas is being released.

Results

Use a table similar to the following one to record your observations on the rate of reaction in each glass.

Glass	Contents	Solution added	Observations
A	Raw whole potato	2 mL water	
B	Raw whole potato	2 mL hydrogen peroxide	
C	Raw chopped up potato	2 mL water	
D	Raw chopped up potato	2 mL hydrogen peroxide	
E	Boiled whole potato	2 mL water	
F	Boiled whole potato	2 mL hydrogen peroxide	

Analysis

Answer Support Questions 22–26. Use the sample observation data provided here (click the “Sample data” button), if you were unable to do this experiment.

Sample data

Support Questions

Note: Support Questions 22–26 are based on the activity you have just done.

22. What indication showed that a reaction occurred? Suggested answer

23. What was the purpose of adding only water to half of the glasses? Suggested answer

24. What effect should the catalyst have on the reaction? Suggested answer

25. What effect did chopping up the potato have on the rate of reaction? Suggested answer

26. What effect did boiling the potato have on the rate of reaction? Suggested answer

Note: Support Question 27 is *not* based on the abovementioned activity.

27. Below is a table of results from the reaction of the enzyme called catalase (taken from human cells) with hydrogen peroxide at different temperatures. The results show the amount of oxygen produced by the reaction in a two-minute period. Print out the table and then fill in the missing results by finding the averages for the amount of oxygen produced.

Temperature (°C)	Amount of oxygen produced (mL) Experiment 1	Amount of oxygen produced (mL) Experiment 2	Average amount of oxygen produced
0	0	0	
10	5	4.2	
20	8.3	8.1	
30	12.5	11.7	
40	15.1	14.9	
50	11.0	10.2	
60	3.2	1.5	
70	0	0	

a) Use the data in the table to make a line graph of these results.

b) At which temperature is the most oxygen produced by the reaction?

c) Explain why this temperature is best for the reaction.

d) Using your graph, explain how the rate of the reaction changes as the temperature increases.

e) Why is the experiment done twice?

f) What would you expect to see if you repeated the experiment, using a stronger concentration of hydrogen peroxide?

Suggested answer

Key Questions

Now work on your Key Questions in the online submission tool. You may continue to work at this task over several sessions, but be sure to save your work each time. When you have answered all the unit's Key Questions, submit your work to the ILC.

Total: 17 marks

8. Explain how an enzyme catalyzes the synthesis of a large molecule from two smaller molecules. **(4 marks)**

9. Explain how the following environmental factors affect enzyme activity:

a) pH **(2 marks)**

b) Enzyme and substrate concentration **(2 marks)**

10. Examine the following data for three enzymes (A, B, and C). Each has been tested at a number of temperatures and pH levels to measure their activities. Based on these results, for each enzyme (A, B, and C) determine

a) the optimal temperature.

b) the optimal pH.

(6 marks: 2 marks for each enzyme)

Enzyme activity (mmol/µg of enzyme protein/min) for enzyme A

	T = 4°C	T = 20°C	T = 37°C	T = 45°C	T = 80°C
pH = 1	0	0	0	0	0
pH = 4	0	2	50	4	0
pH = 7	0	5	20	9	0
pH = 10	0	2	5	4	0
pH = 13	0	0	0	0	0

Enzyme activity (mmol/µg of enzyme protein/min) for enzyme B

	T = 4°C	T = 20°C	T = 37°C	T = 45°C	T = 80°C
pH = 1	0	0	0	0	0
pH = 4	2	2	2	2	2
pH = 7	10	10	10	10	10
pH = 10	2	2	2	2	2
pH = 13	0	0	0	0	0

Enzyme activity (mmol/μg of enzyme protein/min) for enzyme C

	T = 4°C	T = 20°C	T = 37°C	T = 45°C	T = 80°C
pH = 1	0	20	10	4	0
pH = 4	0	20	10	4	0
pH = 7	0	20	10	4	0
pH = 10	0	20	10	4	0
pH = 13	0	20	10	4	0

11. Describe three benefits of using enzymes in food technology. **(3 marks)**

Now go on to Lesson 4. Send your answers to the Key Questions to the ILC when you have completed Unit 1 (Lessons 1 to 4).