Chapter 4: Enzymes

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Laboratory Objectives

After completing these exercises, you should be able to:

1. Understand the key concepts of enzyme and substrate interactions.
2. Describe the function of digestive enzymes and how they work.
3. Explain how this procedure can determine whether lactose digestion has occurred.
4. Understand the concepts of denatured enzymes and competitive and noncompetitive inhibition.

Introduction

Cells rely on biological catalyst to help coordinate and regulate biochemical reactions. These biological catalysts found in cells are known as **enzymes**. Genes control which enzymes will be expressed and what their function will be. Therefore, the cell is able to control and direct its own biochemical activity. An enzyme works by recognizing and binding to a specific substrate, and then catalyzes a reaction by lowering the activation energy. The substrate will interact and bind to a specific region on the enzyme called the **active site**. The active site plays a crucial role in enzyme and substrate interactions. Substrates will bind the active site on a specific enzyme and undergo a chemical reaction. Additionally, there are other regions in which a molecule can bind on the enzyme, these are known as allosteric sites. Allosteric sites on the enzyme differ from the active site, these are places where molecules that inhibit enzyme activity can bind. If there is a substrate with a much higher affinity for the active site, then this substrate can outcompete all other substrates for the active site. Similarly, when there is an inhibitor with a high affinity for the active site on the enzyme, it can prevent binding of the substrate, this is known as **competitive inhibition**. Most competitive inhibitors function by binding to the active site in a reversible manner, meaning if there is a substrate with a higher affinity for the active site, then the competitive inhibitor will not bind. Alternatively, there are also **non-competitive inhibitors** that can affect the function of the enzyme by binding to a site other than the active site.

As an example of substrate and enzyme interactions, Figure 1 shows the interaction between the enzyme **sucrase** and its substrate sucrose. The enzyme sucrase binds directly to sucrose and positions it so the covalent bond between the monosaccharides glucose and fructose is completely broken, releasing the two sugar products. Release of the products restores the enzyme to its original form. The enzyme can repeat this reaction over and over, as long as substrate molecules are present.

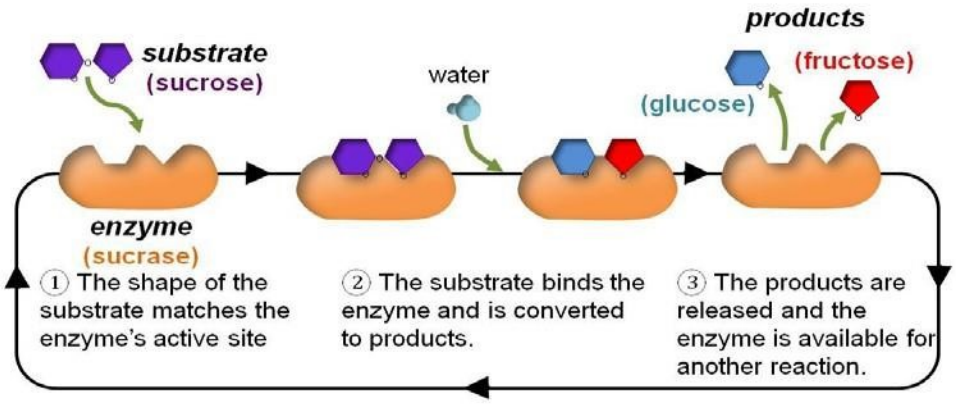

Figure 1. Enzyme and substrate interaction.

There are several factors that can affect protein shape and ultimately protein function. The function of a protein is dependent upon its three-dimensional shape. Anything that alters or affects the shape of the enzyme is said to have **denatured** the enzyme. Factors that can affect the shape of the enzyme and cause it to become non-functional include changes in temperature, pH, salt concentration, inhibitors, activators, *etc.*

Digestive Enzymatic Reactions

The human body has various digestive enzymes which function as biological catalyst and break down specific substrates into smaller molecules. The table below gives examples of several important digestive enzymes, their substrates and the end product produced by the enzymatic reaction. These enzymes are produced in the digestive system, specifically either in the pancreas or small intestine. Lipase, trypsin and amylase are present together within the pancreatic juice, which is a digestive liquid. These enzymes located in the pancreatic juice are used for the complete chemical digestion of food that has been ingested. The complete digestion of food is crucial so that individual monomers (*e.g.* amino acids, monosaccharides, fatty acids) can be absorbed and utilized by biological reactions. **Lactase** is found in the small intestine where it breaks down lactose into glucose and galactose sugars.

Table 1. Examples of digestive enzymes, specific substrates and final enzymatic product.

Enzyme	Substrate	Product
Lipase	Triglycerides	Fatty acids, glycerol
Trypsin	Proteins	Smaller polypeptides
Amylase	Starch	Glucose
Lactase	Lactose	Glucose, Galactose

Exercise 1: Lactose Digestion

Lactose is the main disaccharide found in milk, it is composed of galactose and glucose. Lactase is an enzyme found in the small intestine of mammals that catalyzes the breakdown of lactose by hydrolyzing the β-glycosidic bond in *D*-lactose to form *D*galactose and *D*-glucose. People who are unable to digest the lactose sugar are known to be lactose intolerant, or lack the lactase enzyme and cannot break down the sugar molecule. Symptoms of lactose intolerance include bloating, abdominal pain, gas, cramping and diarrhea. Most people are aware of their condition and avoid dairy products or consume over the counter medications like LACTAID®. LACTAID is a supplement that contains the enzyme lactase for lactose digestion. Sucrose (best known as table sugar) is another disaccharide composed of fructose and glucose and is very similar to lactose. Although lactose and sucrose are similar, lactase will only recognize and bind to lactose because of the structure of the lactose substrate. Recall that enzymes will only bind to specific substrates.

This exercise will examine the ability of the enzyme lactase to interact specifically with the lactose substrate and cleave it into galactose and glucose. Figure 2 shows the enzymatic reaction of lactose being digested into galactose and glucose in the presence of the enzyme lactase and water. Recall that this is a type of **hydrolysis** reaction. To determine whether enzymatic activity occurred, glucose strips will be used to measure the presence or absence of a specific product (glucose).

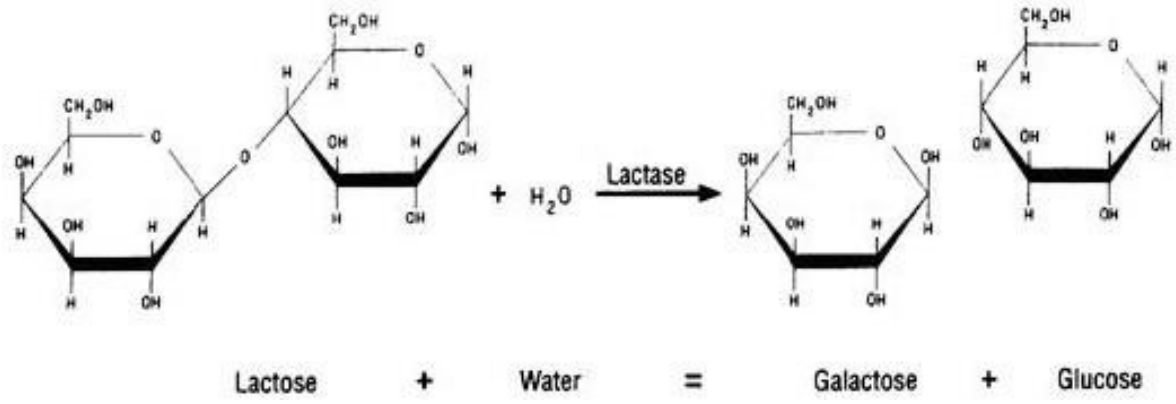

Figure 2. Lactase digestion of lactose into galactose and glucose.

Formulating a hypothesis

You are asked to develop an experiment to answer the following question: **Does lactose content vary by milk type?**

1. What would your answer to the above questions be? What is your hypothesis?

2. What is your independent variable for testing this hypothesis?

3. What could your dependent variable(s) be? What will you measure?

4. What are some of the controlled variables?

Experimental Procedure

Materials

Dairy types of milk – whole, 2%, 1%, non-fat and lactose-free
Non-dairy or plant-based types of milk – soy, almond, coconut
5% sucrose solution
5% lactose solution
Lactase solution (Lactaid tablets; 300mg/200 mL)
Glucose strips
P1000 micropipettes and tips
Tubes in a rack
Small beakers
Forceps
Timer

1. Each group will choose 2 milk samples to test (one dairy and one non-dairy, or lactose-free).

2. A set of 3 replicates will be prepared for each experimental condition. Clearly label all tubes and replicates, especially the ones for different milk samples.

3. Add reagents to each tube as described in **Table 2**. Set up one set of tubes at a time, without adding the enzyme. When all your tubes are set up, then add the enzyme and start the time. After 10 min of digestion measure the presence/absence of glucose by using a glucose strip.

Table 2. Experimental set-up

Tube	Milk	Enzyme solution	Lactose solution	Sucrose Solution	Water
1	1 ml	1 ml	-	-	-
2	1 ml	-	-	-	1 ml
3	-	1 ml	1 ml	-	-
4	-	1 ml	-	1 ml	-

4. Insert the glucose strip into the sample and leave it in for 2-3 sec. Gently shake the glucose strip to remove any excess milk.

5. Place the glucose strip on a piece of paper towel to dry and after 3 min read the results. Make sure to label everything so you don't get your milk samples and replicates mixed up.

6. Observe the color change of the glucose strip and compare it to the glucose concentration chart on the bottle. Write down the glucose concentration for each condition in **Table 3**.

Table 3. Glucose concentration for each condition using various types of milk.

Type of Solution	**Glucose Test (mg/dl)**							
	$MILK_1$				**$MILK_2$**			
	R_1	**R_2**	**R_3**	**AVE**	**R_1**	**R_2**	**R_3**	**AVE**
Test 1: milk + enzyme								
Test 2: milk + water								
Test 3: lactose + enzyme								
Test 4: sucrose + enzyme								

Review Questions

1. Why did the lactase enzyme react with lactose but not sucrose?

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2. What would have happened to lactase if it was boiled before we used it in our experiment?

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3. The lactase enzyme functions in the small intestine where the pH is slightly acidic. Would further lowering the pH of the enzyme solution effect the function of this enzyme? Why or why not?

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4. Name a few other factors that could affect the way an enzyme functions and why.

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5. If you had any problems with the procedure, or you got questionable results, explain how that might have influenced your conclusion.

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Cleanup and Waste Disposal Instructions

- Pour all non-hazardous liquid waste (milk and enzyme solutions) down the sink.

- Remove all sharpie marks from test tubes and beakers with ethanol and paper towels.
- Rinse, dry and leave them upside-down on you bench for the next lab section.

- Discard used glucose strips and the blue pipette tips in the **biohazard bin**.

- Wipe down the bench with Lysol and paper towels.