



The Enzymatic Activity of Lactase

OBJECTIVES

The purpose of this lab is to explore the properties of the enzyme lactase.

By the end of this lab, students will understand the biological function of enzymes and why they are important in the body.

Students should also examine the specificity of an enzyme to a specific substrate.

ESSENTIAL QUESTIONS

- What is lactose?
- How do our bodies metabolize lactose?
- How have humans evolved to digest lactose beyond our childhood years?

MATERIALS NEEDED

- Lactase Tablet
- 15 mL of milk
- water
- sucrose
- 100 mL graduated cylinder
- 10 mL graduated cylinder
- 3 400-mL beakers
- 4 medium test tubes
- test tube rack
- marking pencil
- clock
- glucose test strips
- stirring rod
- electronic balance

MA STATE FRAMEWORK(S)

HS-LS1-6. Construct an explanation based on evidence that organic molecules are primarily composed of six elements, where carbon, hydrogen, and oxygen atoms may combine with nitrogen, sulfur, and phosphorus to form monomers that can further combine to form large carbon-based macromolecules.

HS-LS1-7. Use a model to illustrate that aerobic cellular respiration is a chemical process whereby the bonds of food molecules and oxygen molecules are broken and new bonds form, resulting in new compounds and a net transfer of energy

PROCEDURE

Introduction for students:

Enzymes are a class of protein molecules that act as biological catalysts. You may already know some properties of chemical catalysts. They increase the rate of a reaction, but are not used up in the reaction. Enzymes share these features with chemical catalysts, but also have some important differences. One of the most important differences is that enzymes are usually much more specific than chemical catalysts. Enzymes recognize particular reactant molecules, called substrates, and convert them into products. In contrast, chemical catalysts usually act on a broad range of reactants. In an enzymatic reaction, the enzyme "recognizes" the substrate by its shape and by the position of its hydrogen bonding sites and binds the substrate to itself at the active site. Once at the active site, the substrate rapidly undergoes the enzyme catalyzed reaction.

The enzyme you will be studying in this experiment is lactase, and the reaction it catalyzes is the hydrolysis of the disaccharide lactose into the monosaccharides galactose and glucose. Humans require this enzyme

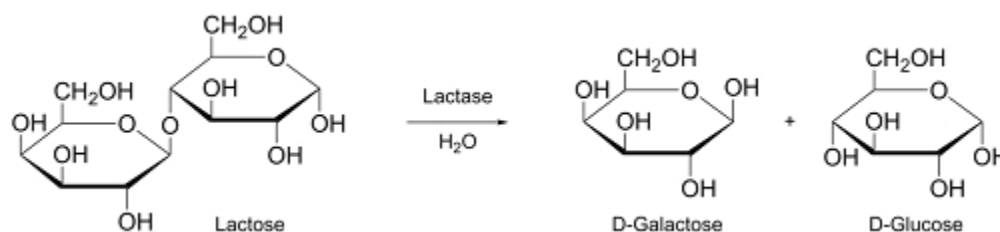
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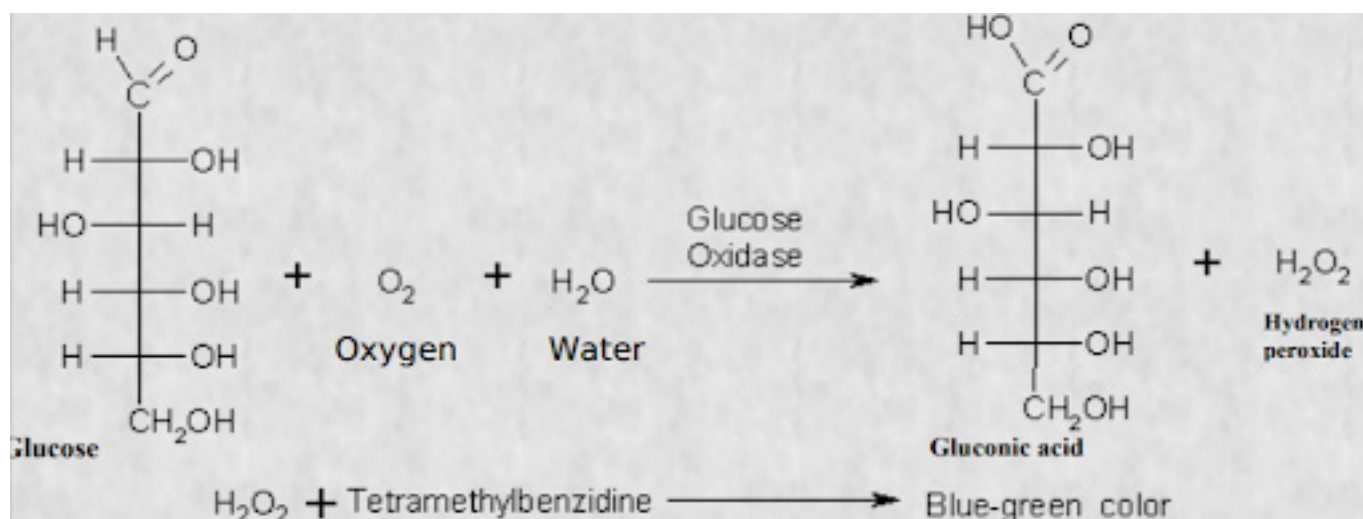
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for digestion of lactose found in milk and other dairy products. The medical condition known as lactose intolerance results when the body produces insufficient amounts of lactase to digest the lactose found in these foods. Lactose intolerance can cause intestinal discomfort and symptoms such as cramps, bloating and diarrhea. In the dairy case at the grocery store, you may have noticed "lactose-free" milk. This type of milk has lactase enzyme added to it to hydrolyze the lactose before you drink it. Individuals with lactose intolerance can then enjoy the benefits of milk without the unpleasant side effects.



In this experiment, you will monitor the enzyme activity by determining how much glucose has formed. Because glucose is a product of lactose hydrolysis, measuring the amount of glucose gives a direct measurement of how much lactose has reacted. You will monitor the enzyme activity using test strips that measure the level of glucose in the reaction mixture. Because glucose is a product of lactose hydrolysis, measuring the amount of glucose gives a direct measurement of the extent of the reaction. These are the same strips that diabetics use to monitor glucose levels with a home test kit. The presence of glucose in solution will change the test strip from yellow to a blue-green color. At this point you might be wondering, "How do the test strips work? Glucose isn't green. Is it magic?" The answer is "No." Magic is not involved here, just chemistry. In fact, all test strips and test reagents that you might use for patient diagnosis in the hospital are based on chemical reactions. Here's how the glucose test strips work. First, the glucose is oxidized to form gluconic acid and hydrogen peroxide in a reaction catalyzed by a second enzyme called glucose oxidase. Then the hydrogen peroxide reacts with a dye, tetramethylbenzidine, and changes the dye from yellow to green. All of the detection reagents are impregnated into a pad on the surface of the strip, and the reactions take place inside the pad. By comparing the color of the strip to a standard color chart, the amount of glucose can be determined. The diagram below shows the reactions involved in glucose detection.



Students: Pre-Lab Questions

1. How are enzymes and chemical catalysts in common?
2. How are enzymes and chemical catalysts different?
3. Lactose is composed of what two monosaccharides?
4. How does the industry make "lactose-free" milk?
5. In this experiment, how will you know how much lactose reacted?
6. If glucose is present in a solution, what color change will you notice on a glucose test strip?

Teacher: Prepare the Solutions

1. Enzyme Solution: Add 1 lactase tablet to 200-mL of water. Using a stirring rod, stir until the tablet has dissolved.
2. Milk: this solution contains the lactose.
3. Sucrose Solution: Add 5 grams of sugar to 100-mL of distilled water. Using a stirring rod, stir until the sugar has dissolved.

Students: Lab Procedure

1. Label the test tubes with the following labels:
 - A) skim milk and enzyme solution.
 - B) skim milk and distilled water.
 - C) sucrose solution and enzyme solution.
 - D) sucrose solution and water.
2. In test tube A, add 2 mL of skim milk and 1 mL of enzyme solution. Time for 2 minutes and test for glucose with the glucose test strip. Record results in the Data Table.
3. In test tube B, add 2 mL of skim milk and 1 mL of distilled water. Time for 2 minutes and test for glucose with the glucose test strip. Record results.
4. In test tube C, add 2 mL of sucrose solution and 1 mL of enzyme solution. Time for 2 minutes and test for glucose with the glucose test strip. Record results.
5. In test tube D, add 2 mL of sucrose solution and 1 mL of distilled water. Time for 2 minutes and test for glucose with the glucose test strip. Record results.
6. Clean up your lab station as instructed by your teacher. All solutions can be poured down the sink in this lab. The used glucose test strips should be placed in the trash. Wash out all glassware at the sink using soap and water.

Data Table 1

1. Pour a small amount of the red cabbage indicator liquid into each of the seven cups of different liquids. Add just enough of the indicator until you see a color change. Record your observations, and rank them based on their pH, using this scale: 1= lowest pH, 7 = highest pH

Test Tube	Type of Solution	Color Change	Glucose Result
A	milk & enzyme		
B	milk & distilled water		
C	sucrose & enzyme		
D	sucrose & distilled water		

Concluding Questions

1. Diagram the lactose and lactase reaction. Label the substrate, the active site, the enzyme, the enzyme-substrate complex and the products
2. Sucrose, ordinary table sugar, is a disaccharide like lactose. Sucrose is composed of glucose and fructose. Sucrose and lactose have the same chemical formula, $C_{12}H_{24}O_{11}$. Why then did the enzyme react to lactose but not to sucrose?
3. Lactase is the speeding up of what chemical reaction- dehydration synthesis or hydrolysis?
4. Some humans have the ability to digest lactose, while others do not. Why may this be? How has evolution occurred to change this ability in humans?*

**Please watch the Howard Hughes Medical Institute (HHMI) "The Evolution of Lactose Tolerance" video (<https://youtu.be/MAGbol1qTuk>) for more information on the evolution of humans on selection.*

EXTENSIONS & VARIATIONS

HHMI Lactose Evolution

<https://www.biointeractive.org/classroom-resources/making-fittest-got-lactase-co-evolution-genes-and-culture>

Adapted by N. Berg, NNHS ©2011

(<http://capital2.capital.edu/faculty/wbecktel/Enzyme.htm> and <http://www.learnnc.org/>)