Activity of Lactase Enzyme Lab

Carbohydrates are usually classified into groups based on the number of repeating units that make up the compound. Monosaccharides (simple sugars) are composed of only a single sugar unit. Disaccharides are composed of two bonded simple sugar units. Polysaccharides (complex carbohydrates) are composed of more than two sugar units (often hundreds of units in length). In this lab, you will observe lactose, the sugar found in milk. It is a disaccharide composed of a glucose and a galactose (both are six-sided simple sugars). In addition, you will observe sucrose – more commonly known as table sugar. Sucrose is also a disaccharide, but it is composed of a fructose and a glucose (fructose is a five-sided simple sugar).

Lactase is an enzyme, as the –ase should imply. Lactase breaks lactose sugar down into galactose and glucose, as shown in the equation below:

\[ C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6 + \text{heat} \]

The structure of the lactase enzyme has been highly conserved (unmodified) across most mammalian species, as well as through other kingdoms (fungi). Due to this, we can inexpensively synthesize and isolate lactase from yeast and other fungi for use in humans. The isolated enzyme is readily available and can be purchased in pill form by people who are lactose intolerant (lack a functional form of the enzyme and are unable to break down the sugar into its components).

In this lab, you will test for breakdown of sugars using lactase. In addition, you will test how a change to environmental conditions may change the activity of an enzyme.

Purpose
Examine enzyme specificity to a specific substrate
Observe how environmental conditions may affect enzyme activity

Materials
- lactase tablet
- 15 mL of skim milk
- water
- sucrose
- glucose
- 100 mL graduated cylinder
- 10 mL graduated cylinder
- four 400 mL beakers
- six test tubes & rack
- clock
- hot plate
- glucose test strips
- glass stir rod

Safety
- Wear your safety goggles (general – do not write)
- Do not consume any of the materials (milk, sucrose, lactase, etc)
- Do not touch the hot plate
- Return or dispose of all materials properly (general - do not write)
- Wipe down table once materials are returned (general – do not write)
- Wash hands thoroughly after completing the experiment (general – do not write)
Hypothesis
Record a hypothesis that will test the purpose above
Use the criteria for a good hypothesis to direct your writing

Prelab
Write out the questions entirely & use complete sentences for your answers
1. Describe the composition of lactose.
2. How does the composition of lactose and sucrose (the two sugars in this lab) differ?
3. From an evolutionary perspective, what does a highly conserved structure for the lactase enzyme indicate about its importance?

Procedure
Part I – Solution Preparation
May be prepared prior to class to save time
1. Enzyme Solution
   A. Add 1 lactase tablet to 200 mL of water
   B. Stir until the tablet has dissolved
2. Skim Milk
3. Sucrose Solution
   A. Add 5 grams of sugar to 100 mL of water
   B. Stir until the sugar has dissolved
4. Glucose Solution
   A. Add 5 grams of sugar to 100 mL of water
5. Denatured Enzyme Solution
   A. Place 20 mL of Enzyme Solution into a test tube
   B. Add 400-500 mL of water to a 600 mL beaker
   C. Place the test tube in the beaker
      Gently lay the test tube so it rests on the side of the beaker
   D. Place the beaker and test tube on the hot plate
   E. Boil the water in the beaker for 30 minutes
   F. Let the solution cool to room temperature
**Procedure, cont.**

**Part II – Lab**

1. Label the test tubes as follows:
   A. Skim Milk & Enzyme Solution
   B. Skim Milk & Water
   C. Skim Milk & Denatured Enzyme Solution
   D. Sucrose & Enzyme Solution
   E. Sucrose & Water
   F. Glucose & Water

2. In test tube A, add 2 mL of skim milk and 1 mL of enzyme solution

3. Allow the possible reaction to run for 2 minutes

4. Test for glucose with the glucose test strip

5. Record results in your data table
   *If there was glucose present mark '+'; if glucose was absent, mark '-'*

6. Repeat steps 3-5 for each of the remaining set ups:
   - Test tube B – add 2 mL of skim milk and 1 mL of water
   - Test tube C – add 2 mL of skim milk and 1 mL of denatured enzyme solution
   - Test tube D – add 2 mL of the sucrose solution and 1 mL of enzyme solution
   - Test tube E – add 2 mL of the sucrose solution and 1 mL of water
   - Test tube F – add 2 mL of the glucose solution and 1 mL of water

**Observations & Data**

*Design & complete a data table that will incorporate all the necessary information from the lab*

*Be sure to include a column for any possible observations*

**Conclusion Questions**

*DO NOT WRITE IN NOTEBOOK UNTIL COMPLETED WITH THE LAB*

*Must be answered in your notebook using complete sentences – Answers Only, don’t write questions*

1. Diagram (a simple sketch is fine) and describe the lactose/lactase reaction.

2. If there was a difference in reactivity between the two sugars, explain your results.

3. If there was a difference in reactivity between the unboiled and boiled enzymes, explain your results.

4. Describe what happened to the enzyme when it was boiled.

5. Temperature often affects enzyme activity. Another way to affect the enzyme is by altering the pH level. Depending on the enzyme, the pH of the solution may be lowered or raised outside its functional range. In the case of lactase, it normally functions within the small intestine (pH around 6). Since lactase pills must pass through the stomach, the enzyme must be able to survive such an environment (pH typically around 3). Describe how lowering the pH of the enzyme solution would affect the enzyme.

6. What type of reaction is this? Dehydration or Hydrolysis?