Enzymatic Digestion of Starch by Amylase

- TEACHER GUIDE -
Enzymatic Digestion of Starch by Amylase

OVERVIEW

- This lab aims to improve students' understanding of enzymes, their properties and functions, as well as their diverse applications.
- Students will design their own experiments in order to investigate factors that affect enzyme-catalyzed reactions. Specifically, students will alter reaction conditions (such as temperature, pH, and source of amylase) and determine the effects on the rate of breakdown of starch into simple sugars by the enzyme amylase.

OBJECTIVES

Students will

- Understand that enzymes are proteins that catalyze biochemical reactions and that the activities of enzymes depend on various conditions, such as temperature and pH.
- Explain the structure and function of enzymes and their applications in human and industrial processes.
- Evaluate the effects of various parameters (e.g., temperature, pH, and source of amylase) on the rate of enzymatic digestion of starch by amylase.

CONTENT STANDARDS

This lesson is appropriate for high school biology and chemistry students and addresses the following Next Generation Science Standards:

Disciplinary Core Ideas:

- HS-LS1 (From Molecules to Organisms: Structure and Processes)
- HS-PS1 (Matter and its Interactions)
    - HS-PS1-5. Apply scientific principles and evidence to provide an explanation about the effects of changing the temperature or concentration of the reacting particles on the rate at which a reaction occurs.

Science and Engineering Practices:

- Asking Questions and Defining Problems
- Planning and Carrying Out Investigations
- Analyzing and Interpreting Data
- Using Mathematics and Computational Thinking
- Constructing Explanations and Designing Solutions
- Engaging in Argument from Evidence
- Obtaining, Evaluating, and Communicating Information

Crosscutting Concepts:

- Structure and function

MATERIALS

- Alpha-amylase
- Starch
- Tap water
- Beakers to prepare starch and amylase stock solutions
- Conical vials (or small beakers) for each group to have their own starch and amylase solutions
- Iodine
- Small vials for each group to have their own iodine
- Spotting plates with wells
- Buffers at various pHs (or other acid/base solutions to test the parameter of pH)
- Hot plate
- Stir rod or stir bar
- Ice
- Thermometer
- Timer
- Beakers for water baths
- Test tubes
- Test tube rack
- Graduated droppers

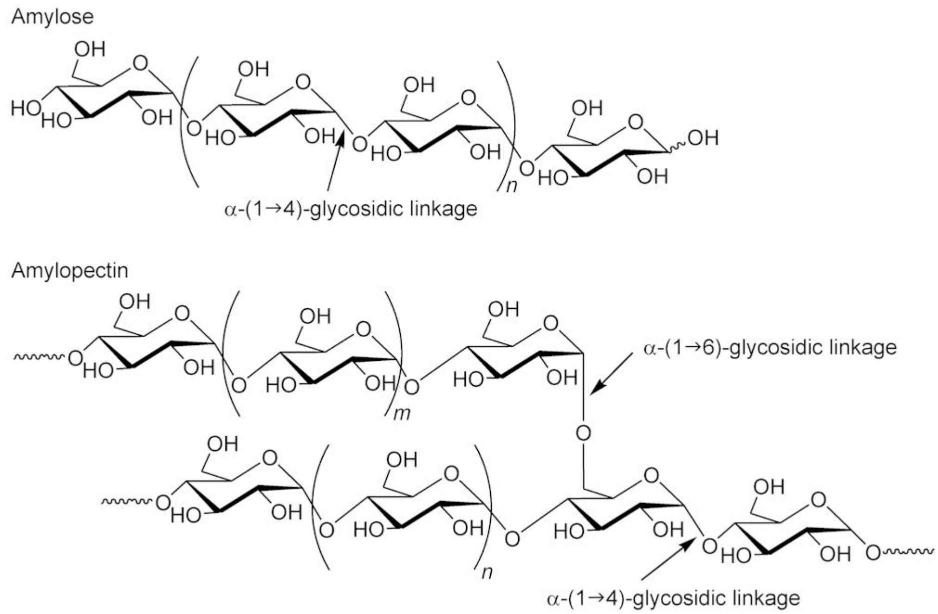
SAFETY

- Students and instructors should wear personal protective equipment such as safety glasses or goggles, gloves, closed-toe shoes, long pants, and lab coats at all times during this lab activity. Long hair and loose clothing should be pulled/tied back.
- Hot water, glassware, and acidic/basic solutions are all potential safety hazards in this lab activity. Students and instructors must handle these materials carefully using appropriate lab safety practices. Broken glassware and reagents should be disposed of properly.
- Always ensure that students understand and adhere to safe laboratory practices when performing any activity in the classroom or lab. Model proper laboratory safety practices for your students, demonstrate the protocol for correctly using the instruments and materials necessary to complete the activities, and emphasize the importance of proper usage.

BACKGROUND INFORMATION FOR TEACHERS

When we think of starch, we commonly think of starchy foods - potatoes, rice, pasta, corn - and their role in the human diet. However, starch from plants has other important and familiar applications. For instance, starch is broken down into its sugar components, which are used in the production of biofuels and bioplastics.

Starch is a polysaccharide made up of two components, amylose and amylopectin. These two polymers consist of glucose monomers that are joined by glycosidic bonds (see figure below).



The enzyme amylase catalyzes the breakdown of starch into glucose molecules. Amylase is found in our saliva, for instance, and initiates the digestive process by starting to break down the starch that we eat. Enzymes are proteins that can (a) carry out a specific reaction and (b) do so multiple times without getting used up. For example, although amylase can break down the alpha-glycosidic bonds that join glucose subunits in starch, it cannot break apart the (β-1→4)-glycosidic bonds that join glucose subunits in cellulose (cellulose is an important structural component in plant cell walls), despite the fact that both starch and cellulose are made up of bonded glucose subunits. Amylase enzymes selectively cleave the type of glucose-to-glucose bond found in starch, not the kind of glucose-to-glucose bond found in cellulose. Another example is that amylase cannot break down proteins, which are chains of amino acids, while proteases, which break down proteins, cannot break down starch. Enzymes are sensitive to their environment. Examples of what we mean by environment are things such as the temperature an enzyme is in or the acidity of the liquid they are in. If an enzyme is heated too much it will misfold and change its shape. Starch is generally insoluble in water at room temperature. However, at higher temperatures (optimal temperature is 37°C), hydrogen bonds in starch are weakened and broken, exposing amylose and amylopectin and allowing amylase to hydrolyze glycosidic bonds.

An easy way to track the rate of reactions catalyzed by enzymes is by means of indicators. For the reaction under study, iodine solution is used as a starch indicator (tests for the presence of starch) and can be used to track the digestion of starch by amylase. Iodine is typically orange-yellow in color, and in the presence of starch it becomes blue-black. When the iodine atoms in the indicator are diluted in a water solution they are arranged in random order in the liquid. When they are randomly distributed they have a light yellow color. However, when starch is added to the water solution it binds to iodine and organizes it in a non-random arrangement. Iodine in this non-random arrangement imposed by the starch chain is blue. When starch gets broken down into glucose it can no longer arrange iodine non-randomly, so the solution turns yellowish clear again.

TIME REQUIREMENTS

- Prep time: 30 minutes
- Class-time: 1 to 2 class periods, depending on class duration (50 or 90 minutes) and level of student inquiry-based investigations

PREPARATION

1. Prepare water baths
    a. To test the effect of temperature on amylase activity, water baths should be prepared at various temperatures. Teachers and students can decide which temperatures to test, but we recommend the following conditions (label water baths accordingly):
        i. Cold: Fill beaker about 1/3 with water and add ice. Approx. 5°C
        ii. Room temperature: Fill beaker about 1/3 with water and allow to equilibrate for a few minutes until room temperature. Approx. 20-25°C
        iii. Optimal temperature: Fill beaker about 1/3 with water and place on hot plate, monitoring temperature. Approx. 37°C
        iv. Hot: Fill beaker about 1/3 with water and place on hot plate, monitoring temperature. Approx. 65°C or above
2. Prepare 1% starch solution (1g of starch per 100mL; scale to the volume that fits the needs of your class)
    a. Weigh out appropriate amount of starch.
    b. Mix starch with room temperature water. Place on a hot plate and heat to dissolve starch (approx. 20 minutes). Mixture should thicken and be slightly cloudy.
    c. Each group of students should have their own small beaker of starch
3. Prepare 1.0% amylase solution (1g per 100mL; scale to the volume that fits the needs of your class)
    a. Weigh out appropriate amount of amylase.
    b. Mix amylase with room temperature water.
    c. Each group of students should have their own small beaker of amylase.
4. Prepare pH solutions
    a. Acid/base solutions (e.g., HCl and NaOH) can be used, or buffers at various pH’s, depending on what is available to you.
5. Make sure each group of students has their own supply of amylase, starch and iodine. Each group should also have their own spotting plate(s) (or small medicine cups, or similar item to test the reaction), several test tubes, graduated droppers, and a timer.

PROCEDURE

1. General procedure
    a. Pipette 5 mL of starch solution into a test tube and label it with an “S” and your group number.
        i. *Water bath at 37°C may be used for enhanced, optimal activity*
    b. Pipette 5 mL of amylase solution into a different test tube and label it with an “A” and your group number.
        i. *Water bath at 37°C may be used for enhanced, optimal activity*
    c. Prepare the spotting plate: On a spotting plate, place one drop of iodine in each well.
    d. Pour the contents of the starch test tube into the amylase test tube. Swirl to mix. Start timer (this is “time zero”). Move to next step immediately.
    e. Immediately at “time zero”, place 3 drops of amylase/starch solution in the first iodine-containing well. Record your observations.
    f. Repeat every 1 minute by placing 3 drops of starch/amylase mixture in a new iodine well and record your results. Continue until there is no color change (color stabilizes).
2. Test the effect of temperature
    a. Temperatures for testing: 5, 20, 65°C
    b. Follow the “General procedure” (see above) at each desired temperature, using water baths.
    c. Be sure to label all test tubes appropriately, with the type of solution (amylase or starch) and the temperature.
    d. Be sure to place starch and amylase test tubes in the appropriate water bath for 5 minutes before combining amylase/starch.
3. Test the effect of pH
    a. pH values for testing: 4, 7, 10
    b. Follow the “General procedure” (see above) at each desired pH.
    c. Note: The original starch solution will be used for all pH testing. The amylase solutions will vary depending on the pH desired.
    d. Perform all testing at room temperature.
4. Test the effect of concentration of amylase
    a. Concentrations for testing: 0.5, 1.0, 5.0% amylase
    b. Follow the “General procedure” (see above) with each concentration.
    c. Perform all testing at room temperature.
    d. If time permits, students can calculate and perform different dilutions of the stock amylase solution.
5. Test the effect of amylase source (salivary amylase)
    a. Each group member: Dispense 3 - 5mL of saliva into a clean 25mL conical tube.
    b. Add DI water to bring volume up to 20mL. Swirl to mix.
    c. The solution with saliva is now your source of amylase.
    d. Follow the “General procedure” (see above).

EXAMPLE 5E OUTLINE

Engage (10 minutes)

1. Intro video: https://youtu.be/oPFLrGKTrw4
2. Enzyme applications in: 3D printing, ethanol fan, leather, sour candy

Explore (15 minutes)

1. Students follow “general procedure” to test amylase activity at body temperature.
2. Teacher floats between groups to ensure student participation and understanding.

Explain (10 minutes)

1. Students interpret their initial results and explain the process and reaction they have observed.
2. Students are able to define and explain the following terms: enzyme, catalyst, substrate, product, starch, glucose, amylase, indicator
3. Transition: what factors influence this reaction and how?

Elaborate (20 minutes)

1. Groups discuss how to design their experiment to test desired variable
2. In groups, test parameter(s).
3. Teacher floats between groups to ensure student participation and understanding.

Evaluate (5 minutes)

1. Discuss their results, how they differed, and why.

EXTENSION IDEAS

- Ideas for possible parameters to test:
    - Temperature, pH, amylase concentration, starch concentration, amylase source
- Determine the concentration of amylase in saliva by having students construct a standard curve of known concentrations of commercial amylase
- Don't tell students what the solutions are and have them design a way to determine what each solution is.
- Quantify sugar content using a Brix meter (can be purchased on Amazon for $25: https://www.amazon.com/Compensation-Refractometer-Ade-Advanced-Optics/dp/B008562GD0/ref=sr_1_3?ie=UTF8&qid=1531708384&sr=8-3&keywords=brix+meter)
    - Brix meters are fun to use since you look through an eyepiece like a telescope. Likely need to increase the initial concentration of starch and enzyme. 1 brix = 1g sugar/100g solution, or 1 brix = 1% sugar. Since we start with a 1% starch stock solution, the sugar concentration will be low after enzyme hydrolysis. Need to increase to 5 or 10% starch solution to get a good reading from the meter. Solutions might have to be filtered before dropping onto the meter.

IDEAS FOR STUDENT QUESTIONS
(Some questions are from https://eng.umd.edu/~nsw/ench485/lab5.htm)

1. Plot the enzyme activity versus pH. From this curve, what is the optimal pH? Explain why enzyme activities depend on pH.
2. Plot the enzyme activity versus temperature. From this curve, what is the optimal temperature? Explain why enzyme activities depend on temperature.
3. To what extent did the heat treatment affect the enzyme activities? What happens to an enzyme when it is subjected to heat?
4. How does the optimal pH for the salivary amylase compare with the pH of the stomach? If the pH of the stomach is not at all favorable for amylase, has the nature made a mistake?
5. Is the enzyme activity directly proportional to the enzyme concentration?
6. What purpose does iodine serve and how does it function?
7. Comment on ways to improve the experiment and explain your rationale.

SUPPLEMENTAL RESOURCES

Animated explanation of the procedure (control)	https://www.youtube.com/watch?v=zsOs3v8Z-P4
Demonstration of the procedure (effect of temperature)	https://www.youtube.com/watch?v=od30lc5oPiM
Demonstration of the procedure (effect of pH)	https://www.youtube.com/watch?v=_wkuUwA4bdA
Similar amylase activity	http://www.seplessons.org/node/3636
Provides in-depth background information and an alternative investigation	http://www.seplessons.org/node/2443
Protocol for alternative variables (source of amylase, temperature, pH, concentration)	https://eng.umd.edu/~nsw/ench485/lab5.htm