1. Amylase: One of Your Enzymes

Introduction

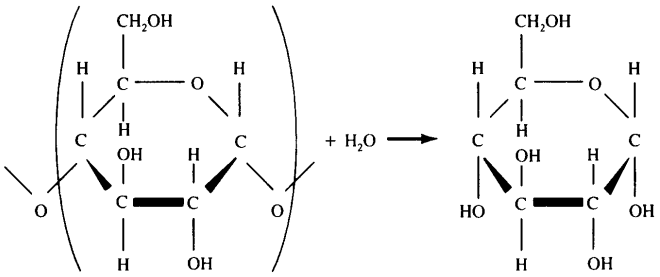
Starch, a biological polymer (Ebbing/Gammon, Chapter 25), is one of the most important sources of carbohydrates in our diet. The digestion of starch begins in the mouth with a process called *hydrolysis* (reaction with water). This reaction, which really consists of a series of steps, is outlined in Figure 1.1. Sugars are the ultimate products of the reaction.

The hydrolysis of starch is very slow in the absence of *amylase*, an *enzyme* found in our saliva. Enzymes are proteins that are efficient and specific catalysts of biochemical reactions. Amylase functions most efficiently at the pH and temperature of the mouth. Extreme deviation from these conditions can cause structural changes that inactivate the enzyme.

Purpose

This experiment will allow you to examine the influence of active and inactive forms of amylase on the hydrolysis of starch. Your own saliva will be the source of the enzyme.

FIGURE 1.1
The hydrolysis of starch.



A special note

The activity of salivary amylase can vary widely among different individuals and for the same individual at different times. A small percentage of individuals (perhaps 3 to 4%) may find that they are unable to do this experiment at all because they have an inactive enzyme. Working with a partner who has active amylase can be an acceptable alternative.

Concept of the experiment

Throughout this experiment, iodine will be used as an indicator for the presence of starch. Iodine and starch interact to form a blue color whose intensity is proportional to the quantity of starch that is present. During hydrolysis, the quantity of starch will decrease as the reaction progresses, and the blue color will fade. If the intensity of the blue color does not diminish, hydrolysis has not occurred (at least to any appreciable extent).

You will use four dilute saliva solutions, called A, B, C, and D, in this experiment. Their uses are outlined in Table 1.1. You will use one or more numbered test tubes with each of these solutions. These numbers are also given in the table. You can avoid confusion about the ultimate purpose of any solution by referring to this table.

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Table 1.1 Purpose of Solutions

Solution	Use	Test Tube No.
A	Qualitative rate study	1–6
B	Effect of acid	7
C, D	Effect of heat	8

Solution A will be used to examine the progress of the enzyme-catalyzed hydrolysis of starch during a period of 10 min. The activity of your salivary amylase will become apparent during this part of the experiment.

Solution B will be used to determine the effect of acidity on the active enzyme, and solutions C and D will be used to determine the effect of heat. If acid or heat makes your amylase totally inactive, catalysis will no longer occur.

Procedure

Getting started

1. Obtain about 2 mL of your saliva in a 5-mL or 10-mL graduated cylinder.
2. Transfer the saliva to a clean Erlenmeyer flask containing 12 mL of distilled water. Agitate the flask thoroughly to homogenize the solution.
3. Mark 2 clean beakers with identifying letters (A and B).
4. Obtain 7 small test tubes.
5. Mark 6 of these test tubes with identifying numbers (1 through 6). Add 1 mL of distilled water to each, and mark the height of the water with a marking pencil. These marks will allow you to add 1 mL of a solution quickly without using a graduated cylinder. Pour the water into a sink.
6. Mark the remaining test tube with an identifying letter (C).
7. Set up a ring stand, an iron ring, a wire gauze, and your laboratory burner. Adjust the height of the ring so that the wire gauze will be in the hottest part of the flame from the burner. Do not light the burner until you have made this adjustment.

   CAUTION: Do not touch the iron ring at any time after the burner has been lit. Avoid burning your fingers.

8. Place a 250-mL beaker containing distilled water on the wire gauze. Heat the water to a gentle boil.
9. Handle the solution of hydrochloric acid carefully.

   CAUTION: A 6 *M* solution of hydrochloric acid can cause chemical burns in addition to ruining your clothes. If you spill any of this solution on you, wash the contaminated area thoroughly and report the incident to your laboratory instructor. You may require further treatment.

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Preparing solution C

1. Add 1 mL of the saliva solution to test tube C. This is solution C.
2. Heat it in the boiling water for 20 min. During this time, go on to the next part of this experiment. However, when 20 min have elapsed, remove the test tube from the bath and allow the saliva solution to cool.

Working with solution A

1. Obtain 6 mL of a 1% starch solution in beaker A. This is solution A.
2. Add 1 drop of the iodine solution and 1 drop of 6 *M* HCl to each of test tubes 1 through 6.
3. Pour 1 mL of solution A into test tube 1, using the mark that you have made. *Save this test tube and its contents.* You will use it as a reference throughout the experiment.
4. Read Steps 5 through 9 before proceeding.
5. Add 1 mL of the saliva solution to solution A, using a 5-mL or 10-mL graduated cylinder and noting the time to the nearest second. Swirl the solution immediately and thoroughly.
6. When 1 min has elapsed, pour 1 mL of the solution from Step 5 into test tube 2. Shake the test tube gently.
7. Repeat Step 6 after 2, 4, 6, and 10 min, using test tubes 3, 4, 5, and 6, respectively.
8. Compare the colors in test tubes 1 through 6 and record the results. If the color has lessened as time progressed, hydrolysis of starch has occurred. Record the degree of hydrolysis (none, some, or complete).
9. *Save test tube 6 with its solution.* You will use it as a reference throughout the remainder of this experiment. This test tube shows the maximum amount of hydrolysis that can occur during 10 min with your amylase.
10. Wash, rinse, and dry test tubes 2 through 5. Renumber two of them (7 and 8).

Working with solution B

1. Obtain 6 mL of the 1% starch solution in beaker B. This is solution B.
2. Add 4 drops of 6 *M* HCl to solution B.
3. Add 1 mL of the saliva solution to solution B. Note the time while swirling to obtain a homogeneous solution.
4. Set the solution from Step 3 aside for exactly 10 min.
5. Add 1 drop of iodine solution to test tube 7.
6. After 10 min have elapsed, pour 1 mL of the solution from Step 4 into test tube 7. Shake the test tube gently.
7. Record the color in test tube 7. Compare the color with those in test tubes 1 and 6. Has any hydrolysis occurred?
8. Wash, rinse, and dry beakers A and B. Identify one of them with a new letter (D).

Working with solutions C and D

1. Obtain 6 mL of the 1% starch solution in beaker D. This is solution D.
2. When solution C has cooled to room temperature, add it to solution D, noting the time. Swirl.

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3. Set solution D aside for exactly 10 min.
4. Add 1 drop of iodine solution to test tube 8.
5. Pour 1 mL of the solution from Step 2 into test tube 8. Shake the test tube gently.
6. Record the color in test tube 8. Compare the color with those in test tubes 1 and 6. Has any hydrolysis occurred?

CAUTION: Before you leave the laboratory, make sure that your gas outlet and those of your neighbors are closed.

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Date: ..... Student name: .....

Course: ..... Team members: .....

Section: .....

Instructor: .....

Prelaboratory assignment

1. Provide definitions for the following terms:

   a. Enzyme

   b. Catalyst

   c. Amylase

   d. Hydrolysis

2. How will you know if the hydrolysis of starch has occurred in this experiment?

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3. Provide a detailed description of the purposes of the contents of the 8 test tubes listed in Table 1.1.

4. What special safety precautions are cited in this experiment?

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Results

1. *Solution A*

Test Tube	Reaction Time (min)	Description of Color	Degree of Hydrolysis
1	0		
2	1		
3	2		
4	4		
5	6		
6	10		

2. *Solution B*

Test Tube	Reaction Time (min)	Description of Color	Degree of Hydrolysis
7	10		

3. *Solutions C and D*

Test Tube	Reaction Time (min)	Description of Color	Degree of Hydrolysis
8	10		

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Questions

1. a. Summarize the effects of acidity and heat on your amylase.

   b. What reason can you give for the effects of acidity and heat on the activity of your amylase?

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