Experiment 25

SEARCHING FOR THE CHAMPION OF SALIVA

Objective

The purpose of this experiment is to determine the efficiency of the hydrolysis of starch catalyzed by salivary amylase using simple iodine test. The factors affecting the activities of the enzyme will also be investigated.

Lab techniques

- Operating dispenser, graduated pipet, and spot-test.

Introduction

Everyone must have an experience of continuous secretion of saliva under certain circumstances such as during dental treatment. It is true that one may secrete saliva to different extents according to different conditions in a single day. Approximately 1~1.5 L of saliva may be produced per day. The saliva secreted from the salivary glands is then transported to the mouth through secreting ducts. Saliva has many important functions. It helps us keep our mouth moist and clean, moisten the food for easier swallowing, and help in digestion at the same time. Saliva can help in digestion because it contains a digestive enzyme, amylase, which can catalyze the hydrolysis of starch to form small molecules of oligosaccharide or maltose. Enzymes are very important to all living organisms. It is involved in the control of periodic growth of cells, metabolism, signal transduction, replication of DNA, transportation of nutrients, and maintenance of the growth of cells etc. The enzymatic catalysis is highly specific and effective. However, as enzymes are protein in nature, environmental conditions such as pH value and temperature affect their structures. Therefore, enzymatic catalysis is highly affected by environmental factors. For example, gastric enzymes in human stomach operate in an acidic environment, and some bacteria found in Yellowstone National Park can even survive at 90^{o}C. In this experiment, the most easily obtained salivary amylase will be used to investigate factors affecting the catalytic efficiency of enzymes.

Due to the formation of a blue-black complex of starch and triiodide ion (I_3^-), iodine solution can be used to mix with the reaction solution, and the color change can then be observed to identify the disappearance of starch (Fig. 25-1). Thus, the extent of hydrolysis of starch by salivary amylase can be determined. After mixing

the reactant solution and the iodine indicator, record the time required to complete hydrolysis of a fixed amount of starch. The end point is the time that the blue-black color just disappears. The result represents the rate of enzymatic catalysis. The shorter the time required to complete hydrolysis means the higher efficiency of the enzyme's catalytic activity and vice versa.

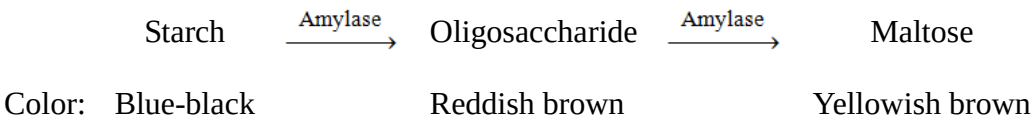

Figure 25-1 The color changes of starch/amylase with iodine solution

Apparatus

Test tubes (10), test tube rack, beaker (100 mL), glass rod, graduated cylinder (10 mL), funnel, graduated pipet (2 mL), pipet filler, plastic dropper, glass dropper (2), Styrofoam cup (2), thermometer, timer, transparency, and supporting sheet.

Chemicals

0.5% Sodium chloride, NaCl
Iodine test solution, 1% I_2/2% KI
2% Starch
Buffer solutions of pH 5.0, 7.0, and 9.0

Procedure

I. Pre-determination of the activity of salivary amylase

1. Wash and dry 10 test tubes, and allow them to cool down before use. Then, take a sheet of amylase activity determining transparency attached to a sheet of white paper for iodine spot-test.
2. Prepare a solution of salivary amylase: Collect 1.0 mL of your saliva in a graduated cylinder and transfer it to a 100 mL beaker. Add 25 mL of a 0.5% sodium chloride solution to it and mix the solution thoroughly with a glass rod. Measure the temperature of the solution as the reaction temperature.
3. Iodine test solution: Withdraw some iodine test solution in a clean plastic dropper. Invert the dropper and let it stand in a test tube rack before using it (Fig. 25-2). Add a drop of iodine test solution onto each cell on the amylase activity test transparency sheet for 5-10 cells (Fig. 25-3).
4. Prepare the reaction solution: Transfer 1 mL of a pH 7.0 buffer solution to a clean test tube and add 1 mL of a 2% starch solution to it. The resulting mixture is the reaction solution.

5. Transfer 2 mL of the salivary amylase solution to the reaction solution using a 2 mL graduated pipet. Then, mix the solution thoroughly by shaking the test tube and start recording the reaction time immediately.
   Note: Refer to experimental skills videos to learn how to use a graduated pipet.
6. After mixing the solution, immediately transfer one drop of the reaction mixture to the transparency to mix with an iodine test solution droplet. Then, repeat the sampling and iodine-test process every 30 s. Record the color change observed as time goes by until the blue-black color disappears and only the yellowish brown iodine solution can be observed.
   Note 1: The reaction mixture should be dropped without direct contact with the iodine-test solution droplet to avoid contamination. For each test, a fresh portion of the reaction mixture should be taken.
   Note 2: If the reaction rate is too slow, the test frequency can be extended to once a minute.
7. Record the time required for the iodine-test to change from blue-black to reddish brown and yellowish brown on your notebook.

II. The effect of pH value on the activity of the salivary amylase

8. Transfer 1 mL of a buffer solution with pH 5.0 and 9.0 to two test tubes, separately, and add 1 mL of a 2% starch solution to each of them.
9. Repeat step 5~7 to determine the catalytic efficiency of the salivary amylase under various pH values.
   Note: The glass dropper should be washed and rinsed thoroughly once the reaction condition is changed.

III. The effect of temperature on the activity of the salivary amylase

10. Take a 100 mL beaker, add 70 mL of hot water. Use the hot plate to heat it and keep the temperature at 80^{o}C.
11. Transfer 2 mL of the salivary amylase solution and 2 mL of the reaction solution (1 mL of buffer solution with pH 7.0 and 1 mL of a 2% starch solution) to 2 test tubes, respectively. Then, place them in the 80^{o}C hot water bath for 5 min. Measure the temperature of the reaction solution until it reaches an equilibrium.
12. Transfer the salivary amylase solution to the reaction solution. Mix them quickly and place it back to the hot water bath, start the timer at the same time. Then, follow step 6 and 7 to determine the catalytic efficiency of the amylase. Record the change in temperature of the solution during the determination.
13. After proceeding for 10 min, remove the test tube from the hot water bath, let the reaction tube to cool to room temperature, and continue testing for another 5

min.

14. Start another trial by repeating steps 10~12 in an ice-water bath, and then follow step 13.
15. Start another trial by repeating steps 10~12 in a 50^{o}C warm water bath.

IV. The effect of alcohol on the activity of the salivary amylase

16. Transfer 1 mL of 95% alcohol solution to a clean tube, and then add 1 mL of a 2% starch solution to it as reaction solution.
17. Repeat step 5~7 to determine the catalytic efficiency of the salivary amylase in alcohol solution. Record the change in temperature of the solution during the determination.
18. After finishing the experiment, wash the transparency with water and wipe it dry with tissue paper. Return it together with the support sheet to the assigned box at the instructor bench. The plastic dropper for the iodine test solution should also be returned for the other classes to use.

References

1. http://faculty.mansfield.edu/bganong/biochemistry/spitlab.htm
2. http://www.science.smith.edu/departments/Biochem/Biochem_353/amylase.htm

Figure 25-2 Iodine test solution in a clean plastic dropper

Figure 25-3 Add drops of iodine test solution on activity test transparency sheet