



T27 Searching for the Champion of Saliva

Collect:

- 2 mL graduated pipet, pipet filler
- Droppers (2), plastic droppers (1)
- Styrofoam cups (2)
- Thermometer and timer (given out by TA)
- Transparency and A4 paper

Prepare:

- 100 mL beaker, glass rod
- Test tubes (10), test tube rack
- 10 mL graduated cylinder
- Funnel



Objective

- Learn the iodine test
- Determine the catalytic efficiency of salivary amylase for the hydrolysis of starch
- Investigate the factors that affect the activity of the enzyme



Amylase in Saliva

Saliva:

- To keep our mouth moist and clean
- Moisten the food for easier swallow
- Helps digestion

Amylase:

Catalyze hydrolysis of starch



Computer modeling of amylase



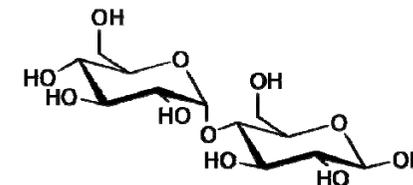
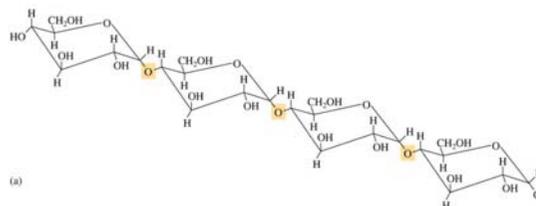
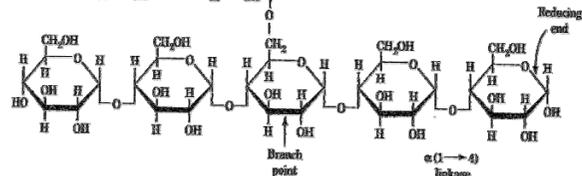
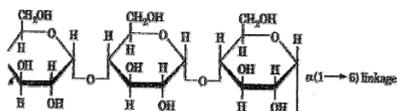
Characteristics of Enzymes

Characteristics of enzymes

- Enzymes are usually **proteins** in nature
- The enzymatic catalysis is **highly specific** (usually work on a specific substrate)
- The enzymatic catalysis is highly **efficient**
- The enzymatic catalysis is highly affected by environmental conditions, such as pH, temperature...etc.



Iodine Test of the Catalytic Activity of Amylase



The higher the efficiency of the catalyst, the shorter time it takes to complete the hydrolysis reaction.



Procedure I. Catalytic Activity of Amylase

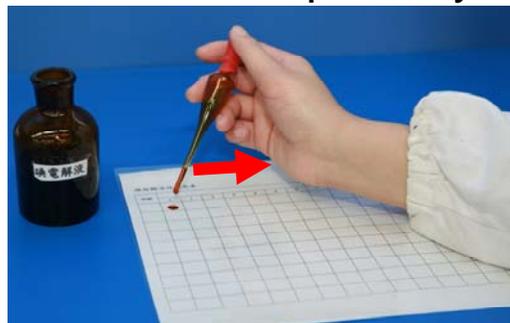
- Clean and dry 10 test tubes
- Prepare salivary amylase soln.
 - **1 mL saliva** (self-produced)
 - Add 25 mL of 0.5% NaCl
 - Mix with glass rod
 - Record soln. temperature



- Take one transparency



- Use plastic dropper to drop iodine soln. on cells of transparency



- Prepare 5~10 drops of iodine testing soln.

- **Prepare reacting soln.**

- 1 mL of 2% **starch soln.**
- Add 1 mL of **pH 7** buffer





Procedure I.

Catalytic Activity of Amylase



1. Obtain 2 mL salivary amylase soln. with 2 mL graduated pipet

↓ Add to soln.
and start timing



**Starch in pH 7
buffer soln.**

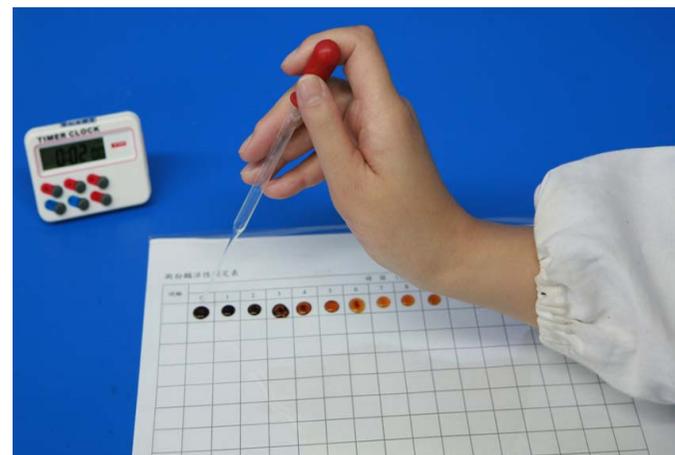
2. Use dropper to take and drop the reaction mixture on the iodine testing soln. on transparency

↓ Test the
activity every
30 s. or 1 min.

3. Observe and record the time until blue-black color disappears

Note:

- Avoid the tip of dropper touching the droplet of reacting soln. for contamination
- Obtain fresh reaction mixture in test tube for each testing





Procedure II. Effect of pH on Catalytic Activity

1 mL of pH 5 or 9 buffer soln
1 mL of 2% starch soln.



pH 5



pH 9 buffer



Add 2 mL salivary amylase soln.



Iodine test

Observe and record the reaction time

Note:

- Wash and rinse the dropper for each trial
- Compare catalytic activity of amylase in pH 5, 7, and 9 buffer solutions



Procedure III. Effect of Temperature on Catalytic Activity

1. Obtain 2 Styrofoam cups and prepare **80 °C** hot-water bath

- Prepare two test tubes
(1) 2 mL salivary amylase
(2) 1 mL starch soln. and 1 mL pH 7 buffer
- Place in hot-water bath for 5 min.



- Mix the soln. in two test tubes to react
- Place the tube back in hot-water bath
- Start timing

**Iodine
test for
10 min.**



- Cool the test tube and bring the temp. of reaction mixture back to room temp.
- Test the activity for another 5 min.

2. Repeat the above steps in 0 and 50 °C water bath



Compare Catalytic Activity of Salivary Amylase with Classmates



Who is the champion of saliva?



Notice

- Salivary amylase solution and reacting solution should be mixed quickly and start timing at the same time
- Hot water is heated in fume hood
- Hot-water bath should be close to or equal to 80 °C (avoid getting burnt)
- Transparency should be rinsed with water, wiped dry, and handed back along with A4 paper
- Hand in plastic droppers used for iodine test to recycle