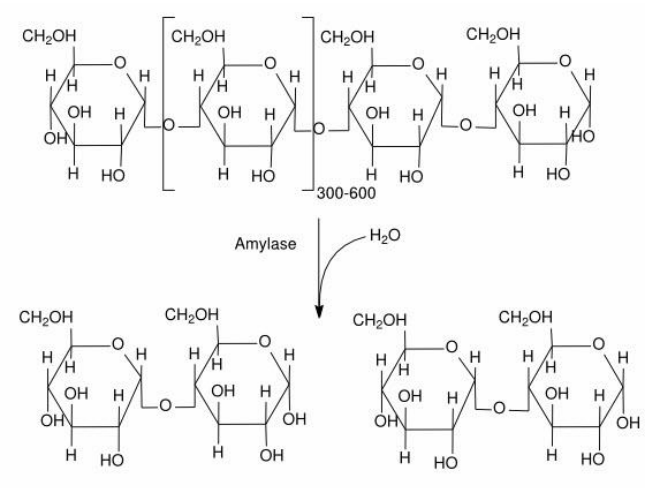
Effects of pH on Enzyme Activity: Amylase

As you recall from reading the section on naming and preparation of enzymes, amylase is an enzyme that breaks down starch to produce smaller molecules called dextrins and an even smaller disaccharide, maltose.



If you haven't read the section on naming and preparing enzymes, please read it now before proceeding with the lab.

In today's activity you will be examining the effect of pH on enzyme activity using pH buffers, the enzyme amylase and starch. Acid and base functional groups compete with hydrogen bonds and alter the ionization of R-groups that are critical to protein structure. By competing with established bonds within the molecules and altering existing bonds, pH changes can potentially change the shape of the active site and therefore potentially change the activity of the enzyme. Protein structure involves at least three levels of structure and in some cases a fourth level of structure. Secondary structure (alpha helix, beta pleated sheet) is created by the interaction of polar entities within the individual amino acids and the formation of hydrogen bonds. Tertiary structure results from the interaction of the R-groups.

When doing enzyme studies researchers can either look for the presence of the enzyme's product or the absence or disappearance of the enzyme's substrate. In this activity we are going to going to identify the appearance of the product, maltose, a reducing sugar. If you recall from the earlier module on carbohydrates Benedict's reagent can be used to identify the presence of reducing sugars. Benedict's reagent identifies monosaccharides and some disaccharides. The light blue colored reagent is added to the test solution and boiled. If the solution changes color, from light blue to yellow or orange or dark red then reducing sugars are present. The redder the color, the greater the concentration of reducing sugars.

In this activity you will be looking at the effect of pH on enzyme activity by observing the production of one of the enzyme's products.

Materials:

Saltines - crushed
Test tubes – 13
Distilled water
Test tube rack

Benedict's reagent
Hot plate
Beaker with boiling beads and water
Amylase
pH buffers
Weigh boat
Balance

1. Pick up 7 test tubes and test tube rack from the supply table.
2. Plug in and turn on the hot plate. Half fill the beaker with water. Add boiling beads and place the beaker on the hot plate.
3. Label test tubes, CA, CS, 2, 4, 7, 10 and 12. Label each test tube with the group members' names.
4. Add 20 drops amylase to the test tubes labeled CA, 2, 4, 7, 10, and 12.
5. Add 20 drops of distilled water to the tubes labeled CA. Add 40 drops of DI water to the test tube labeled CS.
6. Add 20 drops of the appropriate buffer to each tube, i.e., add 20 drops of buffer pH 2 to test tube 2, add 20 drops of buffer pH 4 to test tube 4, etc. Swirl each tube.
7. Allow the tubes to incubate on the benchtop for at least 10 minutes, swirling occasionally.
8. While the tubes are incubating on your desk, pick up 6 more test tubes from the supply desk
9. Use the balance and weigh boat to weigh .5 gm of crushed saltines. Add the crushed saltines to a test tube. Repeat this procedure until you have added .5 gm of crushed saltines to each of the 6 test tubes.
10. After the 10 minute incubation period, add a test tube of crushed saltines to the test tube labeled CS. Add a test tube of crushed saltines to the test tube labeled 2. Add a test tube of crushed saltines to the test tube labeled 4. Add a test tube of crushed saltines to the test tube labeled 7. Add a test tube of crushed saltines to the test tube labeled 11. Add a test tube of crushed saltines to the test tube labeled 12. Swirl to mix the crushed saltines and enzyme mixture.
12. Allow these tubes to incubate on the benchtop for 15 minutes.
13. Add 3 mL of Benedict's solution to each test tube and place each tube in the boiling water bath. Boil the tubes for 5 minutes.
14. Record the color of each tube below.

Record the results below.

	Amylase present	Starch present	Color with Benedict's Reagent	Reducing Sugar Present?
CA				
CS				
2				
4				
7				
10				
12				

1. What was your independent variable/s?

2. What is your dependent variable?

3. What are CA and CS?

4. The positive reaction with Benedicts indicates what has been produced?

5. Name the enzyme whose action is being studied in this activity?

6. What is the product of the enzyme?

7. Based on color, what is the apparent pH optimum for this enzyme?