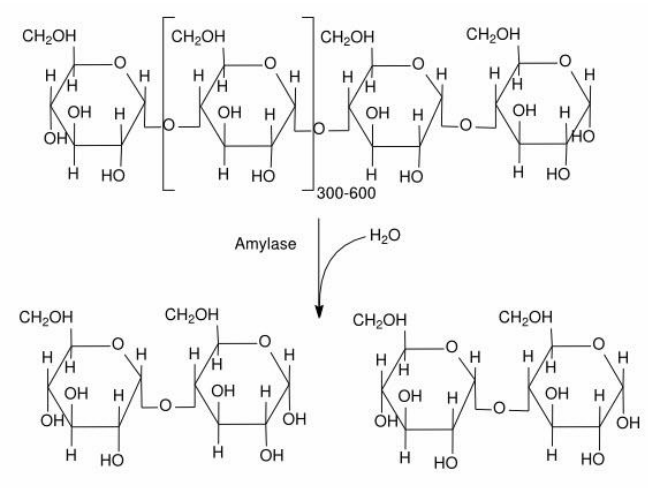
Effects of Temperature on Enzyme Activity: Amylase

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.

When doing enzyme studies researchers can either look for the presence of the enzyme's product or the absence of the enzymes substrate. In this activity we are going to going to observe the disappearance of the substrate by the active enzyme.

Enzymes have evolved to function optimally under specific conditions of pH, temperature and concentration. When enzymes are exposed to conditions well outside their optimum, their activity is impacted. The conditions can be such that the enzyme is permanently damaged or denatured and ceases to function. Conversely, the enzymes activity may be decreased only during the time in which the conditions have been altered. For example, we refrigerate food to slow down spoilage. Bacteria cause spoilage. In the refrigerator the cold conditions inhibit microbial enzymes and slow down bacterial replication and metabolism. If you take something out of the refrigerator and leave it on the counter all day, bacteria and fungi in the food product warm-up and start to actively grow and metabolize. Bacteria replicate very quickly and can spoil food quickly. That is why food scientists recommend that food be thawed in the refrigerator, not on the countertop.

Cold temperatures can disrupt internal bonding in proteins and affect the flexibility of the molecule needed for catalysis. However, the decline in enzyme activity is more likely due to kinetic changes caused by temperature. There simply are fewer interactions occurring between the active site and the substrate. High temperatures up to a point can increase the interactions between the active site and the substrate and can actually increase enzyme activity. At a certain point however, temperature increases damage enzymes/proteins by breaking internal bonds or causing different bonding arrangements within the peptide that lead to denaturation.

The activities in today's lab are designed to exemplify these characteristics. You will be observe enzyme activity at various temperatures (0^0, RT^0, 37^0, 60^0, 100^0) and then test its activity. Write a hypothesis for the digestion of starch by amylase at various temperatures: _____

_____

Materials:

Saltine crackers – finely ground
Test tubes – 5
Distilled water
Test tube rack
Ice bath
Hot plate
Beaker with boiling beads and water
Potassium iodide
Various temperature baths/incubators
Amylase

1. Pick up 5 test tubes and a 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 hot plate.
3. Label test tubes, 0^0, RT^0, 37^0, 60^0, and 100^0. RT or room temperature is ~25^0C. Label each test tube with the group members' names.
4. Add a small amount of finely crushed saltine crackers to each test tube. The cracker level should reach where the curve of the test tube meets the straight upright side of the test tube.
5. Add 2 to 3 mm of distilled water.
6. Add 2 drops of potassium iodide. What happened? What do you observe? _____
7. Place each tube in the appropriate temperature bath. Make sure they are labeled with your group name. The 0 degree bath is either an ice bath or a freezer. RT is room temperature (~25^0 C). 37 degrees is body temperature and is probably a water bath or incubator and the 60 degree incubator could be a water bath or incubator. The hot plate and boiling beaker is the 100 degree bath. If the water is boiling vigorously, you can turn the hot plate off once you have placed the test tube in the beaker.
8. Allow the tubes to equilibrate for 5 minutes and then add 5 drops of amylase. Note the time you added the enzyme. Swirl the tube. Return the tube to its correct temperature incubator.
9. Check back every 2 minutes and note the color of the tube. Do this for 20 minutes. If the tube loses all of its color you can remove the tube and stop recording its color. Record your results below.

Temperature	Time										
	0	2	4	6	8	10	12	14	16	18	20
0											
25											
37											
60											
100											

1. Why are the saltines initially blue?

2. Why did the color disappear?

3. Under which of the conditions does the color disappear faster?

4. Why did the color not disappear at all from some of the tubes?

5. Was your hypothesis supported?

6. What was your dependent variable?

7. What was your independent variable/s?

8. Name the enzyme used in this activity.

9. Name the substrate of the enzyme.

10. Name the products of the enzyme.