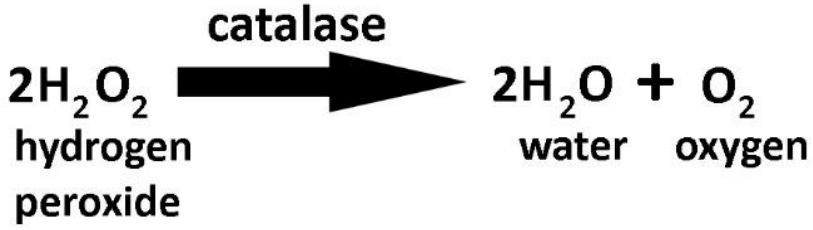
Effect of Temperature on Enzyme Activity: Catalase

As you recall from reading the section on naming and preparation of enzymes, catalase is an enzyme found in nearly every organism. It breaks down hydrogen peroxide to produce water and oxygen. If you haven't read the section on naming and preparing enzymes, please read it now before proceeding with the lab.



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, enzyme 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 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 the effect of temperature on enzyme activity. You will be measuring the activity of catalase from either potato or liver extract at various temperatures (0, 25, 37, and 60 degrees Celsius) in this activity. Write a hypothesis for your experiment here:

_____

_____

Materials

Test tubes – 5
Distilled water
Pipettes
Catalase extract
Hydrogen peroxide
Warm water bath (60^0C)

Warm water bath (37^0C)
Ice bath
Beaker for storing test tube in freezer
Test tube clamps
Sharpie
Test tube rack
1 Small beaker - 15 mL

Ruler
Hot plate
Beaker for 60^0 C bath
Thermometer
Graduated cylinder
Stopwatch

IT IS IMPORTANT TO KNOW THE TEMPERATURE OF THE WATER BATH YOU ARE USING! The 0^0 incubator will be either the laboratory freezer or ice bath. The 25^0 (room temperature) incubator is the lab bench top. The 37^0 incubator will be either a water bath or incubator. The 60^0 incubator will be a water bath, hot plate or incubator.

Procedure

1. A hot plate and beaker can be used as a 60^0 C incubator if the laboratory does not have one. Plug in the hot plate. Fill the beaker half full of water, place the beaker on the hot plate. Turn the hot plate on about 1/4 of the way on. After 10 minutes insert the thermometer into the beaker. If the temperature is too low, turn the knob to increase it slightly. If it is too high, turn the knob to decrease the temperature; then add some DI water to bring the temperature down to 60^0C. Recheck and adjust the temperature frequently. Locate the other water baths or temperature incubators.

2. Pick up 5 test tubes and a test tube rack.

3. Label the test tubes as follows: water RT, 0^0, RT, 37^0, and 60^0.

4. Pour ~ 6 mL of catalase extract into a small beaker and take it back to your bench.

5. Use a pipette to add 20 drops of water to the test tube labeled 'water RT'. Use the same pipette to add 20 drops of catalase extract to the remaining test tubes.

6. Place each test tube into the appropriate water bath, i.e., the test tube labeled 0^0 should be place in the ice bath or freezer. The RT (room temperature) tubes remain in the test tube rack on the bench.

7. After 5 minutes, use the graduated cylinder to acquire 3 mL of hydrogen peroxide from the supply bench.

8. Pour the hydrogen peroxide into the test tube labeled 'water RT' and start the stop watch. Measure the height of the bubbles (if any) after 45 seconds. Record the height of the bubbles (above the solution) in mms. _____

9. Use the graduated cylinder to get another 3 mL of hydrogen peroxide from the supply bench.

10. Pour the hydrogen peroxide into the test tube labeled 'RT' and start the stop watch. Measure the height of the bubbles (if any) after 45 seconds. Record the height of the bubbles (above the solution) in mms. _____

11. Repeat this procedure for the remaining test tubes. Remove the test tubes from the water baths immediately before adding the hydrogen peroxide. Record your results below.

	mms of bubbles	**Was catalase active?**
Water RT		
Catalase RT		
Catalase 0^0		
Catalase 37^0		
Catalase 60^0		
Catalase 100^0		

How do you explain your results?

Was your hypothesis supported?

What was your dependent variable?

What was/were your independent variables?

Why do you think 100^0 C not one of the conditions used in this activity?

Graph your results.

What is the temperature optimum for your enzyme?

A patient checked into the emergency room with a temperature of 104.8 ^{o}. Before the attending physician ordered any tests or bloodwork, they ordered that the patient be submerged in a cool bath. Why?

Name the enzyme used in this activity.

What is the enzyme's substrate?

What is the enzyme's product?