Instructor Notes

Lead and Mercury Ion Catalase Inhibition

This laboratory investigation allows participants to observe the effect of temperature and pH on enzyme activity. The investigation also introduces an environmental factor—exposure to heavy metal ions—and the effect that this factor may have on enzyme activity.

Catalase, the enzyme used in this activity, is composed of four tetrahedrally arranged, same-size subunits. Each of these subunits is a combination of a large polypeptide chain and a prosthetic group (the nonprotein portion of the enzyme), ferric protoporphyrin IX or heme. The structure of hemoglobin, an oxygen-transport protein found in the blood, is similar. The structure of heme is shown in the Activity Instructions.

➤ *This activity is written for workshop participants and may need modification for classroom use.*

Suggested Background Reading

- An Introduction to Toxicology

National Science Education Standards for Grades 5–12

Science as Inquiry

- Abilities Necessary to Do Scientific Inquiry
  Conduct scientific investigations. Students test the impact of lead(II) and mercury(II) ions on the catalase-catalyzed decomposition of hydrogen peroxide. Variables are controlled by substituting, one at a time, the lead(II) nitrate solution and the mercury(II) chloride solution for water and then measuring the relative rates of oxygen gas production.

Physical Science

- Properties and Changes of Properties in Matter
  Substances react chemically in characteristic ways with other substances to form new substances with different characteristic properties. Students learn that in the presence of catalase, hydrogen peroxide decomposes rapidly to form oxygen gas and water. Catalase significantly loses its ability to catalyze in the presence of mercury(II) ions.
- Chemical Reactions
  Chemical reactions occur all around us. Students observe the decomposition of hydrogen peroxide in the presence of catalase. Since hydrogen peroxide is a by-product of a variety of chemical reactions within the human body, its decomposition in the presence of catalase occurs constantly throughout our bodies.

Catalysts accelerate chemical reactions. Students learn that chemical reactions in living systems are catalyzed by protein molecules called enzymes.

Life Science

- Regulation and Behavior
  Regulation of an organism's internal environment involves sensing the internal environment and changing physiological activities to keep conditions within the range required to survive. Students learn that hydrogen peroxide is toxic to cells. As the amount of hydrogen peroxide builds up, the catalase activity in the cells speeds the decomposition of the hydrogen peroxide to oxygen and water.

- The Cell
  Inside the cell is a concentrated mixture of thousands of different molecules that form a variety of specialized structures to carry out cell functions such as waste disposal. Students learn that cells in a potato and in our bodies contain catalase, which catalyzes the removal of hydrogen peroxide.

  Most cell functions involve chemical reactions. Students learn that both the breakdown and synthesis of molecules are made possible by a large set of protein catalysts, called enzymes. At the molecular level, all life processes are dependent on enzymes. The breakdown of hydrogen peroxide is catalyzed by an enzyme, catalase. This activity illustrates that enzymes operate most effectively within a defined range of conditions.

Science in Personal and Social Perspectives

- Natural and Human-Induced Hazards
  Human activities such as waste disposal can induce hazards. Students learn that lead(II) and mercury(II) ions dumped into the environment can be taken up by organisms, including humans, and interfere with chemical reactions necessary for proper body function.

Safety

As the instructor, you are expected to provide participants with the necessary safety equipment (including personal protective equipment such as goggles, gloves, aprons, etc.) and appropriate safety instruction to allow them to work safely in the laboratory. Always follow local, state, and school policies. Read and follow all precautions on labels and MSDSs provided by the manufacturer for all chemicals used.

Goggles and gloves should be worn when preparing the lead and mercury solutions and when performing the activity. Lead(II) and mercury(II) ions are poisonous. Mercury(II) chloride may be fatal if as little as 1 g is swallowed. Do not breathe its dust. Purchase lead(II) nitrate and mercury(II) chloride in as small quantities as possible, preferably 1 g or less. Store both tightly closed in a safe area. Follow local, state, and school policies regarding disposal. Read

and follow all precautions on labels and MSDS sheets provided by the manufacturer for all chemicals used.

Materials

Per group

- 2, 24-well plates
- 1 sheet of white paper
- 4, 2-mL pipets
- 40 mL 3% hydrogen peroxide (H_2O_2)
  This concentration is available in drugstores. Be sure that it is fresh. It is best to start with a previously unopened bottle.
- 40 mL freshly prepared potato extract (prepared in Getting Ready)
- water in labeled plastic pipet (prepared in Getting Ready)
- 18 toothpicks
- 18 filter paper disks (prepared in Getting Ready)
- 2 forceps
- stopwatch or timer with a second hand
- 0.05 M lead(II) nitrate ($Pb(NO_3)_2$) solution in labeled plastic pipet (prepared in Getting Ready)
- 0.05 M mercury(II) chloride ($HgCl_2$) solution in labeled plastic pipet (prepared in Getting Ready)

Getting Ready

1. Prepare the 0.05 M mercury(II) chloride solution by dissolving 0.34 g $HgCl_2$ in enough water to make 25 mL solution.
2. Prepare the 0.05 M lead(II) nitrate solution by dissolving 0.41 g $Pb(NO_3)_2$ in enough water to make 25 mL solution.
3. Prepare three pipets for each group as follows: Label the pipets "water," "lead nitrate solution," and "mercury chloride solution." Collect a small amount of the appropriate liquid in each pipet. Store the three pipets upside down in a small beaker. Each group will need only 6 drops of each solution.
4. Prepare the potato extract (source of catalase):
   a. Cut a fresh potato into small cubes.
   b. For each group, place at least 60 g of the potato cubes in a blender with 200 mL distilled water and homogenize for 30 seconds.
   c. Strain through several layers of cheesecloth.
   d. Discard the pulp and keep the filtrate on ice until ready to use. (This process should be done as close to class time as possible.)

5. Use a single-hole punch to make at least 18 filter paper disks per group.

Procedure Notes and Outcomes

This experiment works nicely with cooperative groups of four participants:

- director
- operator
- timer
- recorder

The effectiveness of the enzyme will be determined by the rate at which oxygen gas (O_2) is released. A filter paper disk sinks in the solutions used in this activity. However, when O_2 is produced from the decomposition of hydrogen peroxide (H_2O_2), some O_2 adheres to the disk. When enough gas bubbles adhere to the disk, the disk-plus-gas system becomes less dense than the solution and the disk floats to the top. Thus the time it takes the disk to float is a measure of the rate of the decomposition of the H_2O_2. The more effective the catalyst, the faster the decomposition, the faster O_2 is produced and adheres to the disk, and the faster the disk floats to the surface.

Enzyme inhibitors decrease the activity of an enzyme. The better the inhibitor is, the greater the decrease in the catalysis. In this activity, participants will test the abilities of two heavy metal ions, lead(II) ion (Pb^{2+}), and mercury(II) ion (Hg^{2+}), to inhibit catalase (obtained from potatoes). If either is a catalase inhibitor, the rate of the decomposition reaction will decrease when it is added to the reaction mixture. This decreased rate of reaction will be observed by the disk taking longer to accumulate enough O_2 bubbles to float to the top.

Sample Data

Sample Data Table (Time in Seconds)							
well	1	2	3	4	5	6	Average
row 1 catalase + water	10	12	11	10	11	12	11
row 2 catalase + Pb^{2+}	15	14	13	15	13	14	14
row 3 catalase + Hg^{2+}	34	31	35	31	33	34	33

Plausible Answers to Questions

1. What was the purpose of the trials in row 1?
   The trials in row 1 were controls. They showed that the water in the solutions did not inhibit the catalase enzyme.

2. What was the purpose of repeating each experiment six times and calculating the averages?
   Repeating the experiments and averaging the results minimizes the impact of an error in an individual experiment.

3. What evidence was there that a chemical reaction was taking place?
   The decomposition reaction of hydrogen peroxide produced oxygen gas and water. The gas production was visible as bubbles.

4. Did the addition of either heavy metal ion solution have any effect on the activity of the enzyme? Support your answer with your experimental results.
   Answers here should reflect the data obtained by the participants. The heavy metal salts are expected to inhibit the activity of the enzyme. The sample data provided in the table shows general trends that participants should observe. Typical results are 10–12 seconds for row 1 and 13–15 seconds for row 2. Results for row 3 using the mercury chloride solution are significantly greater and typically in the range of 30–35 seconds.

5. Did one of the heavy metal ion solutions have a greater effect than the other on the enzyme activity? Support your answer with your experimental results.
   Answers here should reflect the data obtained by the participants. Mercury(II) ions are generally found to be much stronger than lead(II) ions as inhibitors of catalase.

Activity Instructions

Lead and Mercury Ion Catalase Inhibition

An enzyme is a protein that catalyzes a reaction without itself being used up. The substrate is the material that the enzyme acts upon. Catalase is one of several enzymes that catalyze the decomposition of the substrate hydrogen peroxide (H_2O_2) to water and oxygen gas (O_2).

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2(g)$$

H_2O_2 is produced in the body as a by-product of a variety of reactions. Under ideal conditions, a single catalase molecule can catalyze the decomposition of as many as 40 million H_2O_2 molecules per second. Catalase is found in very low concentrations throughout the human body and other biological systems. It prevents the accumulation of H_2O_2, which—as a strong oxidizing agent—tends to disrupt the delicate balance of cell chemistry.

The heme structure shown in Figure 1 is only a small portion of the catalase enzyme but is central to its function. (There are four such structures, each with a single large polypeptide chain. Together these four segments make up one unit of catalase.) The iron(III) ion (Fe^{3+}) in the heme group is critical to the catalysis. If the Fe^{3+} is removed by, for example, replacement by a heavy metal ion such as lead(II) (Pb^{2+}) or mercury(II) (Hg^{2+}) the catalytic activity stops.

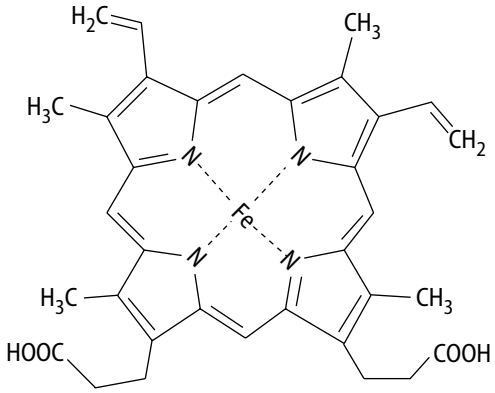

Figure 1: Structure of heme

Safety

In a laboratory setting, you are ultimately responsible for your own safety and for the safety of those around you. It is your responsibility to specifically follow the standard operating procedures (SOPs) which apply to you, including all local, state, and national guidelines on safe handling, storage, and disposal of all chemicals and equipment you may use in the

labs. This includes determining and using the appropriate personal protective equipment (e.g., goggles, gloves, apron). If you are at any time unsure about an SOP or other regulation, check with the course instructor.

Goggles and gloves must be worn during this activity. Lead(II) and mercury(II) ions are poisonous. Mercury(II) chloride may be fatal if swallowed. Dispose of the reaction solutions as directed by your instructor. Do not dump them down the drain. Return leftover lead and mercury solutions to your instructor.

Procedure

1. Place the two 24-well plates on top of a white sheet of paper, oriented as shown in Figure 2. Label one plate "A" and the other plate "B," and the rows as shown.

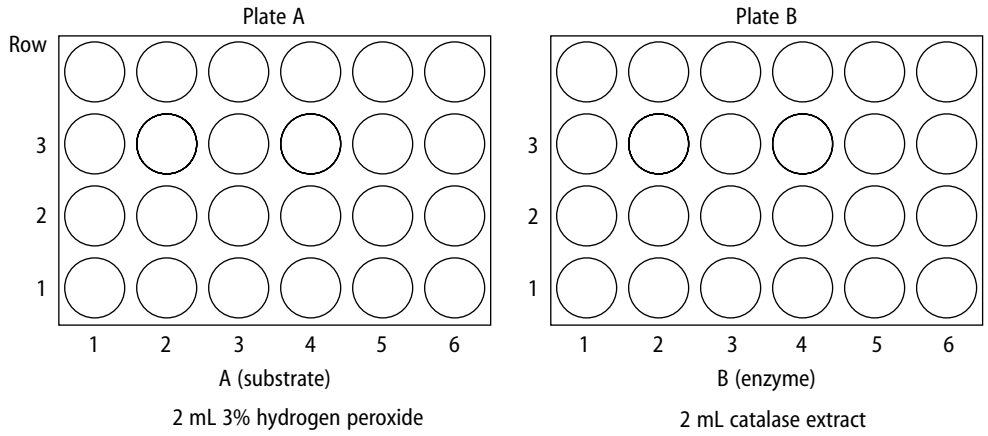

Figure 2: Arrangement of well plates

2. Fill each of the six wells in row 1 of plate A with 2 mL hydrogen peroxide (H_2O_2).
3. Fill each of the six wells in row 1 of plate B with 2 mL catalase extract.
4. Add 1 drop water from the pipet labeled "water" to the catalase extract in well 1:1 (row 1, cell 1) of plate B. Stir quickly with a clean toothpick.
5. After 15 seconds, use the forceps to dip a filter paper disk into well 1:1 of plate B.
6. Drop the disk from step 5 into the diluted H_2O_2 solution in well 1:1 of plate A. Start timing as soon as the disk hits the surface and sinks. (If surface tension keeps the disk afloat, immediately submerge it with the forceps.) Stop timing when the disk floats to the surface of the well. Record the time in the data table.
7. Repeat steps 4–6 using well 1:2 in each plate.
8. Continue this process using the wells in row 1 of each plate until six trials have been completed.

9. Repeat steps 2 and 3 using the six wells in row 2 in each plate.
10. Use the pipet labeled "lead nitrate solution" to add 1 drop 0.05 M lead nitrate solution to well 2:1 in row 2 of plate B. Immediately stir with a clean toothpick.
11. Repeat steps 5 and 6 using the 2:1 well in each plate.
12. Repeat steps 10 and 11 for each of the remaining five sets of row 2 wells.
13. Repeat steps 2 and 3 using the six wells in row 3 in each plate.
14. Use the pipet labeled "mercury chloride solution" to add 1 drop 0.05 M mercury(II) chloride solution to well 3:1 in row 3 of plate B. Immediately stir with a clean toothpick.
15. Repeat steps 5 and 6 using the 3:1 wells in each plate.
16. Repeat steps 14 and 15 for each of the remaining five sets of row 3 wells.
17. Calculate and record the average time for each row on the data table.

Data Table (Time in Seconds)							
well	1	2	3	4	5	6	Average
row 1 catalase + water							
row 2 catalase + Pb^{2+}							
row 3 catalase + Hg^{2+}							

Questions

1. What was the purpose of the trials in row 1?
2. What was the purpose of repeating each experiment six times and calculating the averages?
3. What evidence was there that a chemical reaction was taking place?
4. Did the addition of either heavy metal ion solution have any effect on the activity of the enzyme? Support your answer with your experimental results.
5. Did one of the heavy metal ion solutions have a greater effect than the other on the enzyme activity? Support your answer with your experimental results.