Enzymes, Reactions, Metabolism and Homeostasis

Background

Enzymes, which are comprised of amino acids, are very important macromolecules found in every cell. The sequence of amino acids is genetically determined and is part of the “information” theme discussed in lecture. Enzymes generally act as catalysts that increase the rate at which substances in a cell are converted into other substances. Without enzymes, some reactions would take place too slowly—or might not take place at all. As discussed in class, enzymes play a pivotal role in a cell’s regulatory capacity and are one of the means by which cells “feedback” on metabolism in order to achieve homeostasis.

Each enzyme has a different job and many enzymes work synchronously to keep an organism alive and healthy. In the liver, for example, there are multiple enzymes that act on toxic or poisonous compounds, and detoxification is one of the primary functions of the liver. One of the most common “poisons” is hydrogen peroxide ($H_2O_2$), which is very reactive and harmful to membranes in particular. As a sidebar, it is interesting to note how closely related $H_2O_2$ is to water ($H_2O$) and how the addition of one atom of oxygen can change a molecule of $H_2O$ (which is essential to life) into a molecule that is toxic. As a poison, $H_2O_2$ accumulates in the body due to natural events (e.g., aging; aerobic metabolism; exercise) and other processes (e.g., smoking; air pollution).

Fortunately, there is an enzyme in the liver that breaks down the $H_2O_2$ into water and oxygen. This enzyme is known as catalase. The catalase enzyme reduces the substrate - $H_2O_2$ - to two products, $H_2O$ and oxygen ($O_2$), by the following decomposition reaction:

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2 \text{(gas)}$$

Like all enzymes, catalase helps the reaction but it is not consumed in the reaction; this is a characteristic of a catalysis reaction. Also like other enzymes, catalase must have a proper environment in which to work. For example, catalase works best when the temperature is normal (around 37°C) and when the pH is between 7.3 and 7.4. If the environmental conditions are outside this range, catalase is less effective in decomposing $H_2O_2$ to $H_2O + O_2$. In addition to the efficacy being less due to pH or temperature, catalase can also be interfered with by chemicals that modify the physical structure of the protein, thereby altering the ability of the enzyme to bind to the substrate. These chemicals are called inhibitors.

Since the breakdown of $H_2O_2$ produces $O_2$ gas, this laboratory will be based on a simple technology that measures the build-up of the gas, which in turn results in a pressure increase in a closed system (a test tube).

For You To Do

Use the Pressure Sensor to measure the change in gas pressure inside a flask containing $H_2O_2$ and a source of catalase enzyme. The rate of activity for the mixture will be investigated under multiple conditions: change in pH, change in temperature, and in the presence of an inhibitor (sodium fluoride).

Use DataStudio to record and analyze the data.
Part I: Computer Setup

1. Connect the ScienceWorkshop interface to the computer, turn on the interface, and turn on the computer.
2. Connect the Pressure Sensor DIN plug into Analog Channel A on the interface.
3. Open the file titled as shown: C/Program Files/Data Studio/Library/Biology/BO4Catalase
   - The DataStudio file has a Workbook display. Read the instructions in the Workbook. The file has a Graph of Pressure versus time and a Digits display of Pressure.
   - The ScienceWorkshop document opens with a Graph display of Pressure (kPa) versus Time (s).
   - Data recording is set for 1 measurement per second with a ‘Stop’ condition at 150 s.

Part II: Data Recording

There are six parts the to the data recording.

Part IIA: Catalase + Hydrogen Peroxide

Offer an hypothesis:
What effect do you think adding an inhibitor to the hydrogen peroxide will have on the enzyme’s ability to catalyze the breakdown of the peroxide? State your hypothesis in the Lab Report.

Prepare the Mixture
1. Pour 7.5 mL of 3% hydrogen peroxide in a 100 mL graduated cylinder. Fill the cylinder to the 50-mL mark with 42.5 ML of distilled water.
2. Transfer the diluted peroxide solution to the flask.
3. Turn on the stirrer.
4. Add 1/2 mL of catalase (10 drops) extract to the dilute peroxide solution in the flask.
5. Put the one-hole stopper into the flask.
6. Align the quick-release connector on the end of the plastic tubing with the connector on the pressure port of the Pressure Sensor. Push the connector onto the port, and then turn the connector clockwise until it clicks (about one-eighth turn).

Record the Data
7. Start recording data. (Hint: Click ‘Start’ in DataStudio or click ‘REC’ in ScienceWorkshop.)
   - Data recording will stop automatically at 150 seconds.

Clean up
8. Disconnect the tubing from the Pressure Sensor. Remove the stopper from the flask.
9. Dispose of the peroxide mixture as directed and clean the flask thoroughly.

Part IIB: Catalase + Hydrogen Peroxide + Inhibitor

Offer an hypothesis:
What effect do you think adding an inhibitor to the hydrogen peroxide will have on the enzyme’s ability to catalyze the breakdown of the peroxide? State your hypothesis in the Lab Report.

Prepare the mixture
1. Repeat the data recording procedure. Put 50 mL of dilute (3%) peroxide solution in the flask. Add the spin bar.
2. Add 0.1 g of sodium fluoride to the peroxide solution. Add 1/2 mL of catalase extract to the flask and stopper the flask.
3. Re-connect the tubing to the Pressure Sensor.
Record the data
4. Start recording data.
   • Data recording will stop automatically at 150 seconds.

Clean Up
5. Disconnect the tubing from the Pressure Sensor. Remove the stopper from the flask.
6. Dispose of the peroxide mixture as directed and clean the flask thoroughly.

Part IIC: Catalase + Hydrogen Peroxide + Base (pH Change)

Offer an hypothesis:
What effect do you think adding a base to the hydrogen peroxide will have on the enzyme’s ability to catalyze the breakdown of the peroxide? State your hypothesis in the Lab Report.

Prepare the mixture
1. Repeat the data recording procedure. Put 50 mL of dilute peroxide solution in the flask. Add the spin bar.
2. Add 8 mL of 1 Molar sodium hydroxide (NaOH) to the peroxide to raise the pH before you add the catalase. Add 1/2 mL of catalase extract to the flask and stopper the flask.
3. Re-Connect the tubing to the Pressure Sensor.

Record the data.
4. Start recording data.
   • Data recording will stop automatically at 150 seconds.

Clean Up
5. Disconnect the tubing from the Pressure Sensor. Remove the stopper from the flask.
6. Dispose of the peroxide mixture as directed and clean the flask thoroughly.

Part IID: Catalase + Hydrogen Peroxide + Acid (pH Change)

Offer an hypothesis:
What effect do you think adding acid to the hydrogen peroxide will have on the enzyme’s ability to catalyze the breakdown of the peroxide? State your hypothesis in the Lab Report.

Prepare the mixture
1. Repeat the data recording procedure. Put 50 mL of dilute peroxide solution in the flask. Add the spin bar.
2. Add 5 mL of 1 Molar hydrochloric acid (HCL) to the peroxide to lower the pH before you add the catalase. Add 1/2 mL of catalase extract to the flask and stopper the flask.
3. Re-connect the tubing to the Pressure Sensor.

Record the data.
4. Start recording data.
   o Data recording will stop automatically at 150 seconds.

Clean Up
5. Disconnect the tubing from the Pressure Sensor. Remove the stopper from the flask.
6. Dispose of the peroxide mixture as directed and clean the flask thoroughly.

Part IIE: Chilled Catalase + Hydrogen Peroxide (Temperature Effect)

Offer an hypothesis:
What effect do you think decreasing the temperature of the catalase will have on the enzyme’s ability to catalyze the breakdown of the peroxide? State your hypothesis in the Lab Report.
Prepare the mixture
1. Repeat the data recording procedure. Put 50 mL of dilute peroxide solution in the flask. Add the spin bar.
2. Put 1/2 mL of catalase extract into a test tube. Put the test tube into a beaker and pack crushed ice around the test tube. **Cool the test tube in the ice for 5 minutes.**
3. Add the chilled catalase extract to the flask and stopper the flask.
4. Re-connect the tubing to the Pressure Sensor.

Record the data
5. Start recording data.
   - Data recording will stop automatically at 150 seconds.

Clean Up
6. Disconnect the tubing from the Pressure Sensor. Remove the stopper from the flask.
7. Dispose of the peroxide mixture as directed and clean the flask thoroughly.

**Part II: Heated Catalase + Hydrogen Peroxide (Temperature Effect)** – We do not have the equipment for this, so I am deleting the section.
Lab Report – Catalase Enzyme Activity

*Hypotheses*

**Part IIA: Catalase + Hydrogen Peroxide**
What do you think will happen to the hydrogen peroxide and the oxygen gas when the catalase is added? (Remember how hypotheses are formulated)

Hypothesis:

**Part IIB: Catalase + Hydrogen Peroxide + Inhibitor**
What do you think adding an inhibitor to the hydrogen peroxide will have on the enzyme’s ability to catalyze the breakdown of the peroxide?

Hypothesis:

**Part IIC: Catalase + Hydrogen Peroxide + Base**
What do you think adding a base to the hydrogen peroxide will have on the enzyme’s ability to catalyze the breakdown of the peroxide?

Hypothesis:

**Part IID: Catalase + Hydrogen Peroxide + Acid**
What do you think adding acid to the hydrogen peroxide will have on the enzyme’s ability to catalyze the breakdown of the peroxide?

Hypothesis:
Part IIE: Chilled Catalase + Hydrogen Peroxide
What do you think decreasing the temperature of the catalase will have on the enzyme’s ability to catalyze the breakdown of the peroxide?

Hypothesis:

Part IIF: Heated Catalase + Hydrogen Peroxide
What do you think boiling the catalase will have on the enzyme’s ability to catalyze the breakdown of the peroxide?

Hypothesis:

Questions

1. What does the graph of the reaction between hydrogen peroxide and catalase tell you about enzyme activity?

2. Describe the effect of adding the inhibitor (sodium fluoride) to the peroxide before you add the catalase to the solution of peroxide? What explanation can you give for the results?
3. Describe the effect of adding the base (sodium hydroxide) to the solution of peroxide? What did the sodium hydroxide do to the pH of the solution in the flask? What does this tell you about the range of conditions in which catalase may be effective?

4. Describe the effect of adding the acid (hydrochloric acid) to the solution of peroxide? What did the hydrochloric acid do to the pH of the solution in the flask? What does this tell you about the range of conditions in which catalase may be effective?

5. Describe the effect of cooling the catalase before adding it to the solution of peroxide?

1. Describe the effect of heating the catalase to boiling before adding it to the solution of peroxide?

2. How did the effect of cooling compare to the effect of boiling the catalase? How can you explain the difference between these two trials?
3. Provide an explanation for how catalase (or any enzyme) affects the rate of reactions using the information from the lecture and text. Use a diagram to help with your explanation.

4. Discuss how enzymes participate in the regulatory capacity of a cell and the principle of homeostasis.