An investigation with catalase

Relation to topics / curriculum link:
- cellular energetics

Prior knowledge and skills needed:
- properties, actions and roles of enzyme
- test for oxygen

Concept:

Enzymes are biological catalysts that speed up chemical reactions in organisms.

Factors such as temperature and pH, affect the actions of enzymes.

Introduction

Catalase is a common enzyme found in animal and plant tissues. It catalyses the breakdown of hydrogen peroxide into water and oxygen. Since hydrogen peroxide is a toxic by-product of metabolism in certain plant cells and animal cells, catalase helps to protect the cell from toxic effects of hydrogen peroxide.

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\text{hydrogen peroxide} \xrightarrow{\text{catalase}} \text{water} + \text{oxygen}
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Catalase is often used in investigations to study the action of enzymes. Sometimes it is difficult to measure the catalase activity of living tissues in an objective way by just observing bubble formation. This experiment uses a rather simple and objective method to estimate the rate of catalase reaction. The rate of catalase activity is estimated by measuring the time taken for a paper disc soaked with enzyme extract to rise from the bottom of hydrogen peroxide solution to the surface. The rising of the paper disc is due to the formation of oxygen bubbles from the catalase reaction.
Materials

- fresh tissue (e.g. apple, potato)
- buffer solution (pH 7)
- hydrogen peroxide solution (1%)
- beaker (50 ml)
- filter paper
- forceps
- hole puncher
- knife
- plastic vial
- stop-watch
- mortar and pestle (optional)
- centrifuge and centrifuge tube (optional)

Activity: Comparing the rate of catalase activity in living tissues

1. Grind the fresh tissue in a mortar with a minimum amount of buffer solution.*
2. Scrape the paste into a centrifuge tube and centrifuge until a pellet is formed.*
3. Transfer the supernatant to a clean and dry centrifuge tube.*

*(Steps 1-3 are optional, depending on the type of tissue used.)*

4. Get a piece of dry filter paper and use a hole puncher to punch out several discs. Put a disc into the extract. If steps 1-3 are skipped, just put a disc in contact with the freshly cut surface of a fruit or vegetable. Blot the soaked disc slightly on a sheet of filter paper.
5. Use forceps to transfer the soaked disc into a plastic vial containing 1% H₂O₂ solution of at least 1 cm in depth. Make sure it settles at the bottom, and start timing.
6. Note the time taken for the disc to rise to the surface of the solution and then take away the disc.
7. Repeat steps 1 to 6 for another tissue sample.
Questions for discussion:

1. What gas is evolved when fresh tissues are added to hydrogen peroxide solution? How do you test for it?
2. Describe what happened biochemically to catalase and hydrogen peroxide during this experiment.

Further investigation

1. Investigating the effect of pH on the rate of catalase activity
   pH affects the rate of enzyme activity. Design a simple experiment to investigate how catalase activity in potato is affected by pH. Discuss the experimental design with your classmates before carrying out the investigation. Write a report to explain your conclusion.

2. Investigating the effect of temperature on the rate of catalase activity
   Temperature also affects the rate of enzyme activity. Design a simple experiment to investigate how catalase activity in apple is affected by temperature. Discuss the experimental design with your classmates before carrying out the investigation. Write a report to explain your conclusion.

Reference