PRELIMINARY ACTIVITY FOR

Evolution of Cellobiase

Open Inquiry Version

The kingdom Fungi contains the Basidiomycota or club fungi, a group that includes what we call mushrooms. Mushrooms are decomposers that have evolved to grow in diverse environments. The button mushroom (*Agaricus bisporus*), also known under various names including: common mushroom, table mushroom, and champignon mushroom, is native to grasslands. The oyster mushroom (*Pleurotus ostreatus*) is typically found on trees and wood. Other mushrooms are mycorhizal, which means that they have evolved symbiotic relationships with the roots of specific trees. The matsutake (*Tricholoma magnivelare)* and chanterelles *(Cantharellus sp.)* are examples of mycorhizal mushrooms. Morels, false morels, and truffles are also decomposers, but they are not true mushrooms, they are classified as cup fungi and belong to the group Ascomycota.

In this investigation, you will be studying the cellobiase activity found in different types of club and/or cup fungi. *Cellobiase* is involved in the last step of the process of breaking down cellulose, a molecule made up of bundled long chains of glucose that are found in plant cell walls, to glucose. This is a natural process that is used by fungi to produce glucose as a food source.

The natural substrate for the enzyme cellobiase is cellobiose (Figure 1). This is a disaccharide composed of two beta glucose molecules. However, when scientists study enzyme function, it is best if there is an easy way to detect either the amount of substrate that is used up or the amount of product that is formed. Solutions of cellobiose (substrate) and glucose (product) are colorless, and there are not many simple methods to detect these molecules quantitatively.

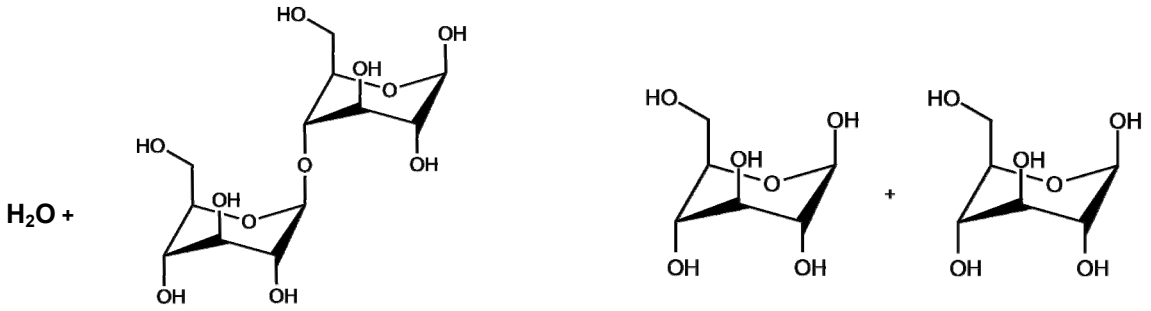
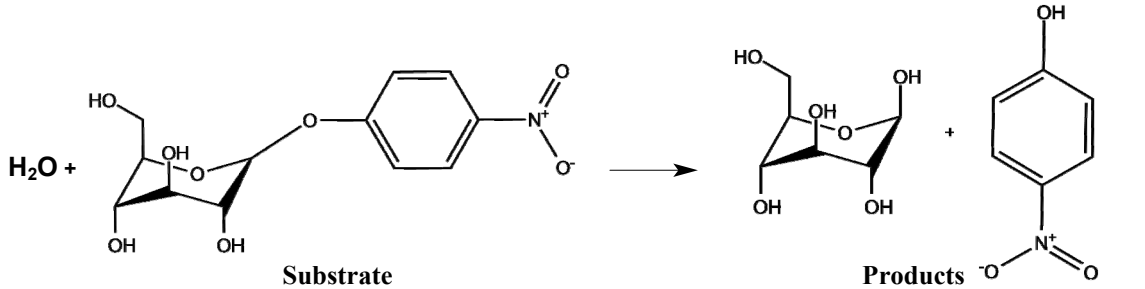


Figure 1

To make this reaction easier to follow, an artificial substrate, *p*-nitrophenyl glucopyranoside, will be used. This artificial substrate can also bind to the enzyme and be broken down in a manner similar to the natural substrate cellobiose. When the artificial substrate, *p*-nitrophenyl glucopyranoside, is broken down by cellobiase, it produces glucose and *p*-nitrophenol (see Figure 2, below). When *p*-nitrophenol is mixed with a basic solution referred to as the *stop solution*, it will stop the reaction and turn the solution yellow. The amount of yellow color is proportional to the amount of *p*-nitrophenol present. For every molecule of *p*-nitrophenol present, one molecule of *p*-nitrophenyl glucopyranoside is broken apart. For the cellobiase

reactions being run, another advantage of using a basic solution to develop the color of the *p*-nitrophenol is that the basic pH will also denature the enzyme and stop the reaction.



Substrate
p-nitrophenyl glucopyranoside
Colorless Solution

Products
Glucose + *p*-nitrophenol
Yellow in Stop Solution

Figure 2

In this Preliminary Activity, you will (1) produce a button mushroom extract, (2) react this cellobiase-containing extract with substrate, (3) collect samples after 1, 2, 3, 4, and 6 minutes of reaction, (4) use a spectrophotometer to measure the absorbances of the colored samples, (5) plot a graph of absorbance *vs*. time, and (6) use the graph to determine the rate of the cellobiase catalyzed reaction.

After completing the Preliminary Activity, you will first use reference sources to find out more about mushrooms, cellobiase activity, and the evolution and ecology of mushrooms before you choose and investigate a researchable question dealing with cellobiase and the evolution of mushrooms. Some topics to consider in your reference search are:

- cellulose
- cellobiose
- cellobiase
- basidomycota
- ascomycota
- mycorhizal
- ecology
- evolution

PROCEDURE

Preparation of Cellobiase Containing Samples

1. Obtain and wear goggles.

2. Obtain a stopwatch, which you will use to time the reaction in a later step.

3. Weigh out 1 g of button mushroom and place it in a mortar.

4. Add 2 mL of extraction buffer. Using a pestle, grind your mushroom to produce a slurry.

5. Transfer the slurry into a 1.5 mL centrifuge tube and spin at the highest speed for 3 minutes. **Note:** You will need at least 200 µL of supernatant. The *supernatant* is the liquid remaining above the solid, known as a *pellet*, following centrifugation.

6. Label six 1.5 mL cuvettes 1, 2, 3, 4, 6, and B. **Note:** The label should be restricted to the lid or the upper 1/4 of the cuvette.

7. Pipet 500 µL of stop solution into each cuvette.

8. Place 3 mL of 1.5 mM substrate into a 15 mL conical tube.

9. Pipet 200 µL of your mushroom supernatant into the 15 mL conical tube containing the 3 mL of substrate. **START YOUR TIMER.**

10. When 1 minute has elapsed, remove 500 µL of the mushroom extract/substrate mixture from the 15 ml conical tube, and add it to Cuvette 1.

    To correctly use cuvettes, remember:

    - Wipe the outside of each cuvette with a lint-free tissue.
    - Handle cuvettes only by the top edge of the ribbed sides.
    - Dislodge any bubbles by gently tapping the cuvette on a hard surface.
    - Always position the cuvette so the light passes through the clear sides.

11. When 2 minutes have elapsed, remove 500 µL of the mushroom extract/substrate mixture from the 15 ml conical tube, and add it to cuvette 2.

12. Repeat the Step 11 procedure after 3, 4, and 6 minutes have elapsed, adding the mixture to the cuvettes 3, 4, and 6, respectively.

13. Add 500 µL of 1.5 mM substrate to the Cuvette B. Add one drop of mushroom extract. This will serve as the "blank" for this investigation.

Data Collection

14. Use a USB cable to connect the SpectroVis Plus spectrophotometer to your computer or LabQuest. Start the data-collection program, and then choose New from the File menu.

15. Calibrate the spectrophotometer.

    a. Place the blank, cuvette B, into the cuvette slot of the spectrophotometer.
    b. Choose Calibrate from the Sensors menu of LabQuest or the Experiment menu of Logger *Pro*.
    c. When the warmup period is done, select Finish Calibration. When the calibration procedure is complete, select OK.

16. Determine the optimum wavelength for examining the absorbance of *p*-nitrophenol (the colored product).

    a. Remove the blank cuvette. Place cuvette 6 into the spectrophotometer.
    b. Start data collection. A full spectrum graph of the solution will be displayed. Note that one area of the graph contains a peak absorbance. Stop data collection.

17. Set up the data-collection mode.

    Logger *Pro*

    a. Click Configure Spectrometer Data Collection, . Select Abs *vs.* Concentration as the Collection Mode. The wavelength of maximum absorbance (λ max) will be selected. Verify that the maximum absorbance is close to 405 nm.
    b. Enter **Duration** as the Column Name, **Dur**. as the Short Name, and **min** as the Units.
    c. Select OK. Remove the cuvette from the spectrophotometer and proceed to Step 18.

    LabQuest App

    a. The peak absorbance is automatically selected. On the Meter screen, tap Mode and change the data-collection mode to Events with Entry.

b. Enter **Duration** as the Column Name, and **min** as the Units.
c. Select OK. Remove the cuvette from the spectrophotometer and proceed to Step 18.

18. You are now ready to collect absorbance data for the five samples. Start data collection. Obtain Cuvette 1. Wipe the outside with a tissue and place it in the spectrophotometer. Wait for the absorbance value displayed on the screen to stabilize, and then select Keep to store the data point. Enter **1** as the time and select OK. The data pair you just collected will now be plotted on the graph. Remove the cuvette from the spectrophotometer.

19. Obtain Cuvette 2. Wipe the outside and place it in the spectrophotometer. When the absorbance value stabilizes, keep the data point, enter **2**, and then select OK.

20. Repeat the Step 19 procedure for the cuvettes 3, 4, and 6, entering **3**, **4**, and **6**, respectively.

21. Stop data collection and record the absorbance values in your data table.

Determining the Rate of the Cellobiase Catalyzed Reaction

22. Perform a linear fit on your graph. Record the slope, m, as the rate of the cellobiase catalyzed reaction (in Δ abs/min).

23. Print the graph as directed by your instructor.

QUESTIONS

1. What was the rate of your cellobiase catalyzed reaction?

2. List three varieties of mushrooms available in your area.

3. List two factors that could have affected cellobiase activity in different mushrooms as they evolved.

4. List at least one researchable question concerning cellobiase and the evolution of mushrooms.