LAB: Hydrogen Peroxide Metabolism

AP Biology

Activation energy, in the form of ATP, is necessary to initiate all chemical reactions (even those called ‘spontaneous’). Cells employ **enzymes** in order to lower the **activation energy** barrier. The reactant (substrate) binds to the active site of the enzyme, which acts to arrange the substrate in the optimal position for forming or breaking bonds. Each enzyme is specific for a certain reaction and reactant (**substrate**); its **active site** has a specific shape and only specific compounds present in the cell can interact with it. Thus, enzymes are not part of the chemical reaction but rather facilitators of the reaction and so are not ‘used up’ in the reaction. One enzyme can facilitate many reactions per second. Enzyme activity is regulated through feedback mechanisms that involve binding with other small molecules, as well as by environmental factors. Enzyme regulation allows an organism to control metabolism as it responds to changes in the internal or external environment.

Organisms naturally produce hydrogen peroxide (H2O2) as a by-product of respiration. However, H2O2 is damaging to cells and requires immediate metabolism into non-toxic products. Organisms accomplish this in multiple ways. In one pathway, facilitated by the enzyme [**catalase**](http://en.wikipedia.org/wiki/Catalase), one molecule of H2O2 is oxidized and a second reduced via oxygen transfer. The reduced hydrogen peroxide decomposes into water and oxygen gas. A different pathway employs the enzyme **peroxidase** to facilitate a reaction where H2O2 is reduced by the organic molecule guaiacol. This reaction involves the transfer of hydrogen atoms from guaiacol to H2O2; as in the catalase-facilitated reaction, the reduced H2O2 decomposes into water. Both reactions occur promptly due to the presence of their respective enzyme.

***Wear an apron; H2O2 can bleach fabric; wash skin in response to any H2O2 contact.***

**A. H2O2 oxidation & reduction in the presence of catalase**

1. Pour 3-4 mL H2O2 into a test tube and determine the temperature. Add a chunk of raw liver. Monitor the changes in temperature. Verify that the gas that is produced is O2 by popping a bubble with a glowing splint; if the gas is O2, the embers will flame.

2. Repeat the trial with other tissues, omitting the burning splint test.

3. Write out the chemical reaction that occurs, indicating the molecule being oxidized and the molecule being reduced. Balance the equation (it’s the law!). Is the reaction anabolic or catabolic? Endothermic or exothermic? Spontaneous or not? Justify your choices.

**B. Legoase – An enzyme model**

1. Place a bunch of Legos into a shallow bowl. Choose one student to break toothpicks, while others time, count, and record the data.
2. Methods: 10 second intervals; eyes closed, break toothpicks one at a time; leave broken picks in the bowl; no stopping between intervals. Count the number of reactions that occur over time; stop once multiple intervals have passed with no product produced.
3. Graph the rate of the reaction: the amount of product produced over time. Calculate the slope of the line for the linear part of the graph (do not include the very beginning, or the end).
4. Change a condition of the test that you think will impact the reaction rate. Add this data to the graph.

**C. H2O2 oxidation via peroxidase**

A spectrophotometer uses a beam of light to quantify how transparent a solution is. The solutions in part C will all initially be transparent. When the substrate solution is combined with the enzyme solution, the guaiacol molecules are oxidized, each transferring two hydrogen atoms to H2O2. This oxidation turns the solution orange over time, thus transmitting less light. The change in the light transmitted over time can be used to infer and quantify reaction rate.

***Read the entire procedure before beginning. It is imperative that the solutions be correct, that full mixing of the two solutions occurs, and that timing is accurate.***

8. Turn on the spectrophotometer, adjust the wavelength setting to 500nm, set to transmittance mode, and allow the instrument to warm up for 15 minutes.

9. Prepare three small test tubes (cuvettes) as directed in the table below.

|  |  |  |  |
| --- | --- | --- | --- |
|  | *Blank* | *Substrate (S)* | *Enzyme (E)* |
| *pH 5 buffer* | 4mL | 1mL | 2mL |
| *0.02% H2O2* | 2mL | 2mL |  |
| *0.2% Guaiacol* | 1mL | 1mL |  |
| *Enzyme extract* |  |  | 0.5mL |
| *Phosphate buffer* | 2mL |  | 1.5mL |

1. Zero the spectrophotometer (zero absorbance, 100% transmittance) at 500nm using the blank solution. Before every trial, use the blank to ensure the spectrophotometer stays calibrated.
2. When the substrate solution, enzyme solution, and spectrophotometer are ready, carefully pour the S cuvette contents into the E cuvette. Immediately begin timing. Quickly pour the combined contents back into the S tube, wipe the glass with a Kimwipe, and place in the spectrophotometer. Take a reading every 20 seconds, until the transmittance remains nearly constant, indicating the reaction is complete.
3. Graph the rate of the reaction as % transmittance over time. Calculate the rate from the linear slope of the graph early in the trial.
4. Brainstorm with your group variables that might affect the rate of this reaction. Choose one, write a testable question, and discuss a procedure. Note that it is important to keep the total volume of the S and E tubes the same in each trial, and that neither of the buffer solutions be eliminated entirely. Secure the teachers approval before beginning.
5. **Questions for Discussion:**
6. What type of organic molecule is an enzyme? What is the monomer of these polymers? List and describe the stages of formation.
7. Identify the reactant(s) and product(s) in each part of the lab. Identify the substrate and the enzyme.
8. In every case, what happens to enzyme rate over time? Why?
9. Why do turnip, liver, and other tissues produce catalase & perioxidase?
10. In each case, was the reaction an endergonic or an exergonic one? Anabolic or catabolic? Justify your choices.
11. What pattern do you notice about the names of polymers and the enzymes

that act upon them? How can you use the names to determine which is which?

8. Most, but not all, proteins are denatured in environments with extremes of temperature and pH. List examples of extreme environments where proteins (and therefore life) successfully function.

Teacher notes

Flinn kit : instead, order guaiacol (0.2%; two 30mL bottles), mono and dibasic sodium phosphate sol’ns, pH packets

Need: graduated pipettes, 1 and 3 mL; plastic screw cap conical vials - aliquot solns per group to keep down waste, H2O2

Label pipettes! Toss guaiacol/enzyme ones.

Variables: pH via packets (in lieu of pH 5 buffer)

Concentration of H2O2

Enzyme (adjust both enzyme and phosphate buffer amts to equal 2mL.

Recipes:

Phosphate extraction buffer (pH 7) –

1:1:2

0.2M sodium phosphate monobasic : 0.2M sodium phosphate dibasic : DI water

Reaction buffer –

Dissolve packet of pH buffer powder (pH3-8) in 500mL DI water

Dilute H2O2 –

3mL 3% peroxide to a final volume of 500mL with DI water

Enzyme extraction –

Peeled turnip, approx 2g blended into 500 mL pH 7 buffer, from above. Blend in two minute intervals, with 2 minute rests in between. Filter; store in the ’fridge. Pretest to insure the reaction rate is rapid enough to complete a trial in 5 minutes or so.