

Abstract:

This laboratory explores the affects pH has on a reaction rate. The reaction studied was the breakdown of hydrogen peroxide catalyzed by the enzyme peroxidase. Three trials were run at pH levels of 6, 7 and 8. It was hypothesized that the reaction would run very quickly at a pH of 7, since that is the normal condition of cells where peroxidase is found. It was further hypothesized that the reaction would slow significantly at pH's of 6 and 8 since they are not within the normal pH range of a cell. All hypotheses were found to be correct. Altering the pH of the solution into which the enzyme was added slowed the reaction rates significantly. The breakdown of peroxide occurred most rapidly in the solution with a pH of 7. The slower reaction rates are due to the addition of hydrogen or hydroxide ions which bond to positively or negatively charged side chains of the amino acids within the protein. Since the side chains are bonded to ions in solution, they are unavailable to bond with each other. This lack of bonding amongst the side chains affects the tertiary structure of the protein, changing its shape. The shape change makes many of the peroxidase molecules unavailable to correctly bind with the hydrogen peroxide substrate, thus slowing the rate of the reaction.

Materials and Methods:

Materials:

9 test tubes	4 small beakers	One larger beaker with ice
LabPro hardware	laptop computer w/	3 graduated cylinders
Disposable pipette	Loggerpro software	30 ml H_2O_2
15 ml each of pH buffer 6, 7,8		enzyme source (ground liver)

Methods:

1. Mark the 3 sets of test tubes with a 6, 7 and 8. You should have 3 test tubes of each number (three 6's, three 7's, and three 8's). These numbers represent the pH of the solution that will be in that tube.
2. Use the graduated cylinder to measure out 3 ml of H_2O_2 . Pour into one of the test tubes. Measure and pour 3 ml of peroxide into each tube.
3. Obtain about 25 ml of enzyme source. Place in a beaker of ice to keep chilled.*We did not do this-a likely source of error discussed below
4. Set up LabPro interface according to the following directions:
 - a. Attach the tubing and stopper to the gas pressure sensor. Plug the sensor into Channel 1 on the LabPro interface, and attach the LabPro to your laptop using the USB cable. Plug in the LabPro to a power source, and start up the LoggerPro software. The software should recognize the sensor and begin taking measurements.
5. Obtain about 15 ml of each pH buffer needed; pH 6, 7 and 8.
6. Using a clean graduated cylinder, measure 3 ml of pH 7 buffer solution. Pour into one of the test tubes marked "7" that contains H_2O_2 .
7. Use a disposable pipette to add 2 drops of enzyme source to the test tube. Place the LabPro stopper into the test tube. Gently swirl the contents of the tube. Immediately hit "Start" in the LoggerPro software to begin plotting data.
8. Watch the pressure readings on your laptop screen. If the pressure exceeds about 130 kPa, the stopper is likely to pop off the tube. Continue data collection until the rate is no longer linear, the pressure reaches 130 kPa, or about 3 minutes have elapsed (whichever comes first).
9. Send your data to the printer, then clear it.

10. Repeat procedure steps 6-9 with another of the test tubes marked "7".
11. As a control, run the final test with the pH 7 buffer but do NOT add any enzyme.
12. Repeat procedure steps 6-11, replacing the 7 pH buffer with the pH 6 buffer, then the pH 8 buffer.
13. Be sure to print all test results.
14. Clean up all materials before leaving the lab.

Results:

pH 7:

	Observations	Rate of O_2 formation (increase in pressure)
Trial 1 w/enzyme	Many bubbles Top popped off Test tube felt slightly warm	.1997 kPa/sec
Trial 2 with enzyme	Very few bubbles No heat generated	.02882 kPa/sec
Trial 3 no enzyme	No bubbles No heat generated	2.538×10^{-5} kPa/sec

pH 6:

	Observations	Rate of O_2 formation (increase in pressure)
Trial 1 w/enzyme	Some bubbles Test tube felt slightly warm	.1485 kPa/sec
Trial 2 with enzyme	Very few bubbles No heat generated	..05601 kPa/sec
Trial 3 no enzyme	No bubbles No heat generated	-.003980 kPa/sec

pH 8:

	Observations	Rate of O_2 formation (increase in pressure)
Trial 1 w/enzyme	Very few bubbles Test tube felt slightly warm	.01221 kPa/sec
Trial 2 with enzyme	Very few bubbles No heat generated	-.0002528 kPa/sec
Trial 3 no enzyme	No bubbles No heat generated	.002336 kPa/sec

Loggerpro generated data tables are attached for review. Rate of oxygen formation was calculated based on the slope of the line as the pressure of oxygen increased within the test tube. The steepest slope, and thus the reaction that had the fastest rate, was the first tube with a pH solution of 7. This tube also had the most visible signs of a reaction taking place such as the formation of many bubbles and the top popping off. The three test trials that were run without enzyme were used as a standard for comparison.

Discussion:

This lab was run to determine whether pH would affect the rate of an enzyme catalyzed reaction. The reaction in question was the breakdown of hydrogen peroxide catalyzed by the enzyme peroxidase into oxygen and water. Peroxidase is an enzyme normally found in cells. Without it, cells would undergo oxidative damage (Jacobs, 2007). Since the pH of human cells is around 7, I hypothesized that both raising and lowering the pH of the solution would slow the reaction rate. By examining the data, this hypothesis was found to be correct. The rate of reaction, as measured by the rate of oxygen production, was fastest at a pH of 7. The slope of the lines for the pH of 6 and 8 was much less steep, signifying a much slower rate of reaction. These results determine

that pH does affect the enzyme catalyzed breakdown of hydrogen peroxide. If the pH is not that which is seen in normal cells, 7, the reaction is very slow.

Changing the pH of the peroxide also changed the pH of the enzyme peroxidase once it was added to the solution. Peroxidase is a protein. Proteins are chains of amino acids with a backbone of an amine group bonded to a carboxylic acid group. Varied side chains branch off of the connected backbone molecules. These side chains are involved in forming the enzyme's tertiary structure by using varied IMF's to create a specific shape.

Tertiary structure is important because for an enzyme to work, it must have a very specific shape to fit, lock and key style, onto the substrate. As the substrate and enzyme bind, the shape of the substrate molecule slightly bends. This strains the bonds of the substrate, allowing them to be broken easier. By straining the bonds, the enzyme is lowering the amount of energy needed for the reaction to proceed, in effect, lowering the activation energy. Once the activation energy is lowered, more molecules can now get over the E_a "hump" and react. This creates a faster rate of reaction. If the tertiary structure of the enzyme is disturbed in some way, the enzyme cannot properly bind to the substrate. If there is no enzyme straining the bonds of the substrate, the activation energy of the reaction is not lowered. With a higher E_a , the reaction rate is very slow. This is what altering the pH of the solutions did. It altered the tertiary structure of the enzyme, slowing the reaction rate.

Altering the pH of the solution altered the tertiary structure of the enzyme by changing how the side chains bonded with each other. Some amino acid side chains have ionic charges and use ionic bonds to help create the tertiary structure described above. Changing the pH of the solution changes the charges of the ionic side chains. A higher

pH means there are fewer H^+ ions and more OH^- ions available in solution. The OH^- ions bond to many of the positively charged side chains. This now makes them unavailable for bonding to negative side chains of amino acids within the protein. Since the side chains are not bonding as they should, the proteins tertiary structure is altered as described above. Lowering the pH of the solution adds more H^+ ions to the solution. These ions bond with the negatively charged amino acid side chains within the protein. They now are no longer available to bond to positively charged amino acid side chains, again changing the tertiary structure of the protein. When the pH of the solution was kept at 7, there was some bonding of H^+ and OH^- ions to charged side chains. However, not enough side chains were involved in these bonds to alter the tertiary structure of the protein, allowing it to have the correct shape necessary to bind to the hydrogen peroxide substrate molecule, thus creating a faster reaction rate.

It is extremely important that the enzyme source be kept cooled. As enzymes heat up, they become denatured, changing shape (not unlike the shape change described above). We did not keep our enzyme source cooled. This explains why the rates of our second trials of each pH with enzyme added were almost as slow as the reaction with no enzyme. To further justify the conclusions drawn by this experiment, it should be repeated with a consistently cooled enzyme source.

Resource:

Jacobs, C. (2007). *Enzyme catalysis lab*. MISEP Chemistry 512