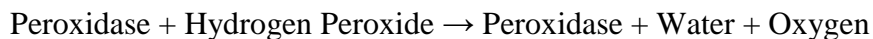


Effect of Temperature on Peroxidase

Peroxide (such as hydrogen peroxide) is a toxic byproduct of aerobic metabolism. Peroxidase is an enzyme that breaks down these peroxides. It is produced by most cells in their peroxisomes. The basic reaction is as follows:



To determine the rate of an enzymatic reaction, you must measure a change in the amount of at least one specific substrate or product over time. In a decomposition reaction of peroxide by peroxidase (as described in the formula above), the easiest molecule to measure would probably be oxygen, a final product. This could be done by measuring the *actual* volume of oxygen gas released or by using an indicator. In this experiment, an indicator for oxygen will be used. The compound guaiacol has a high affinity for oxygen, and in solution, it binds instantly with oxygen, turning yellowish to brown in color. The greater the amount of oxygen gas produced, the darker brown the solution will become. The color change can be recorded as a change in the amount of light absorbed by the solution as measured using a spectrophotometer.

A series of 8 test tubes was prepared as follows:

Table 1 – Preparation of tubes

| Tube | Distilled water (mL) | 0.1% peroxide (mL) | Guaiacol (mL) | Temperature (°C) |
|------|----------------------|--------------------|---------------|------------------|
| 1 | 6 | 0.3 | 0.2 | 5 |
| 2 | 6 | 0.3 | 0.2 | 15 |
| 3 | 6 | 0.3 | 0.2 | 25 |
| 4 | 6 | 0.3 | 0.2 | 40 |
| 5 | 6 | 0.3 | 0.2 | 55 |
| 6 | 6 | 0.3 | 0.2 | 70 |
| 7 | 6 | 0.3 | 0.2 | 100 |
| 8 | 6.5 | - | - | ambient |

To each tube, 1.5 mL of peroxidase was added and timing was begun immediately. After 5 minutes, the absorbance of each tube is measured using the spectrophotometer.

Questions

1. Identify the enzyme, substrate and product in this reaction.
2. Why are we able to use guaiacol as a measure of enzyme activity?
3. What is the purpose of tube 8?
4. Graph the sample data from Table 2. Based on the data, what can you say about the effect of temperature on enzyme reaction rate?

Table 2 – Absorbance reading for each tube

| | | | | | | | | |
|------------|-------|-------|-------|-------|-------|-------|-----|---------|
| Tube | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Temp (°C) | 5 | 15 | 25 | 40 | 55 | 70 | 100 | Ambient |
| Absorbance | 0.106 | 0.177 | 0.251 | 0.312 | 0.289 | 0.164 | 0 | 0 |

5. Explain the lack of absorbance in Tube 7.
6. a) Name three or four factors (including both biotic and abiotic) that vary in the environment in which organisms live.
b) Which of those factors do you think could affect enzyme activity?
c) How would you modify your basic assay to test your hypothesis?
7. Choose one factor you identified in Q. 6 and write a hypothesis about the effect of that factor on enzyme activity. Justify your hypothesis from what you know about enzymes.
8. Design an experiment that uses this assay to determine the effect of the factor you chose on enzyme activity. Describe your protocol and identify the independent variable, dependent variable, controlled variables and control group.