Teaching Enzyme Kinetics with Turnip Peroxidase

Variation of Bio Lab Investigation 13 Enzyme Activity

Karin Knisely

MATERIALS AND EQUIPMENT

Turnips

1. Use a freshly bought turnip. After 2 months in the refrigerator, turnip lost a lot of its activity.
2. 1 medium-sized turnip weighs approx. 350 g.
3. Blend approx. 12 g peeled-and-diced turnip in 310 mL deionized water (approx. 0.04 g/mL) for 30 sec. Each assay requires 1.5 mL, so this amount is enough for approx. 200 assays.
4. Filter through cheesecloth. Filtrate contains peroxidase. Keep on ice.

Hydrogen peroxide

1. 3% standard solution is available at drug stores and supermarkets
2. Make a range of dilutions: 0.5%, 0.25%, 0.1%, 0.05%, 0.025%, 0.01%
3. Use V1C1=V2C2, where
   a. **V1 is the unknown** (the volume of stock solution to use)
   b. **C1 is the concentration of stock solution** (Notice that in the calculations below, I make the most concentrated solution (0.5%) first, and then I use that solution to make the second most concentrated solution (0.25%), and so on. This serial dilution method ensures that the volumes you have to pipet will always be within the range and precision of the measuring pipets.)
   c. **V2 is the volume of diluted solution that you need.** Each assay requires 0.3 mL H_2O_2 so if you make 10 mL of each H_2O_2 solution, you will have enough to make the next solution in the series and still be able to do the assay for that particular H_2O_2 solution 16 times.
   d. **C2 is the concentration of the diluted solution that you need**.
   e. **The difference between V1 and V2 is the volume of water you need for the dilution**.

X (3%) = (10.2 mL) (0.5%) X = 1.7 mL of 3% H_2O_2 + 8.5 mL deionized water	X (0.25%) = (10 mL) (0.1%) X = 4 mL of 0.25% H_2O_2 + 6 mL deionized water	X (0.05%) = (10 mL) (0.025%) X = 5 mL of 0.05% H_2O_2 + 5 mL deionized water
X (0.5%) = (10 mL) (0.25%) X = 5 mL of 0.5% H_2O_2 + 5 mL deionized water	X (0.1%) = (10 mL) (0.05%) X = 5 mL of 0.1% H_2O_2 + 5 mL deionized water	X (0.025%) = (10 mL) (0.01%) X = 4 mL of 0.025% H_2O_2 + 6 mL deionized water

Guaiacol (Sigma-Aldrich G5502-100G, 0.3%)

1. Each assay requires 0.2 mL guaiacol. 10 mL will allow you to do about 50 assays. Combine 0.03 mL stock with 9.97 mL dH_2O. Keep in a brown bottle (guaiacol is light sensitive).

Spectrophotometer (470 nm)

ASSAY FOR MEASURING EFFECT OF H_2O_2 (SUBSTRATE) CONCENTRATION ON REACTION RATE

1. Label seven 1.5x16 cm test tubes as follows: B for the blank and the rest for the H_2O_2 solutions to test: 0.5%, 0.25%, 0.1%, 0.05%, 0.025%, 0.01%
2. Into the test tube labeled B, add 13 mL d H_2O, 1.5 mL turnip extract, and 0.2 mL guaiacol. Mix by pouring this solution into another test tube and back.
3. Pour about 3 mL of (2) into the cuvette. Wipe the cuvette with a tissue and put the cuvette into the spectrophotometer.
4. Zero the spectrophotometer at 470 nm with the blank. Empty the cuvette so that you can use it for Step 6. It does not have to be rinsed because it does not contain any brown product (tetraguaiacol) because the reaction cannot take place without H_2O_2.
5. Into the test tube labeled 0.5% H_2O_2, add 13 mL d H_2O, 1.5 mL turnip extract, and 0.2 mL guaiacol.
6. The next series of steps must be done within 30 sec.
   a. Add 0.3 mL of the 0.5% H_2O_2 solution to (5). Start the stop watch immediately.
   b. Mix by pouring this solution into another test tube and back.
   c. Pour about 3 mL of the reaction mixture into the cuvette.
   d. Wipe the cuvette with a tissue and put the cuvette into the spectrophotometer.
   e. Record the absorbance at 30 sec.
7. Leave the cuvette in the spectrophotometer and record the absorbance every 30 sec for 3 min.

CALCULATE REACTION RATE (ALSO CALLED INITIAL VELOCITY)

1. For each H_2O_2 concentration tested, calculate the initial velocity as follows:

Initial velocity = $\frac{Absorbance\ at\ 3\ \mathrm{min} - Absorbance\ at\ 0.5\ min}{3-0.5}$ = units of product formed/min

2. Plot initial velocity on the y-axis and H_2O_2 concentration on the x-axis.
3. Estimate the initial velocity where the curve leveled off and include the appropriate units. This is the V_{max}, the maximum reaction rate assuming "unlimited" substrate.
4. Estimate the H_2O_2 concentration at ½ V_{max}. This concentration is called K_m, the Michaelis constant. It represents the concentration of substrate at which the enzyme works most efficiently.

RESULTS AND DISCUSSION

1. Describe in words the shape of the curve. As hydrogen peroxide concentration increased, what happened to the initial velocity?
2. Explain in terms of enzyme-substrate interactions why initial velocity initially increased linearly with substrate concentration.
3. Explain in terms of enzyme-substrate interactions why initial velocity leveled off beyond a certain substrate concentration.
4. What factors could be varied to increase the V_{max} beyond the rate determined in this experiment? Explain your reasoning.