

NZ Enzyme Kinetics

Objective

The objective is to measure the rate of an enzyme catalyzed reaction as the concentration of the substrate is varied, and utilizing the Michaelis-Menten Equation to represent the enzyme kinetics, determine the Michaelis constant K_M , the maximum velocity (maximum rate) V_{max} , and the turnover number. The reaction to be studied will be the conversion of peroxide to water and oxygen using the enzyme catalase. $2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$. Present results and answers for each of the 10 parts in the Data and Analysis section.

Materials

Catalase Enzyme (obtained from Sigma Chemical — extracted from Bovine Liver) Activity listed as 1.0 unit of enzyme will decompose $1.0 \mu\text{mol}$ of H_2O_2 per minute at $\text{pH}=7.0$ at 25°C while H_2O_2 concentration falls from 10.3 to 9.2 mM. Observe the label on Sigma bottle to determine activity of enzyme. It probably lists activity between 2000 to 5000 units. For example if the Sigma label list activity as 2200units/mg of solid and 2860units/mg of protein, then the solid taken from bottle that you would have an activity of 2200 units per mg since it is not pure protein. (less than 0.1g)

3.0% solution of H_2O_2 in water (less than 50 mL)

Water and Ice

Equipment and Supplies

8 125mL Erlenmeyer flasks

2 1000mL beakers

2 600mL beakers

2 1mL pipet

2, 3, 4, 5, 10, and 25mL pipet

Heater/Stir Plate with magnetic bar

1 50mL graduate cylinder

2 50mL buret

2 buret stands and clamps (use one buret to dispense water to flasks and one to measure O_2)

rubber tubing and glass connection from flask to buret

analytical balance

Special Instructions:

Experiment

1) Using pipets and/or burets prepare flasks containing the following amounts of fresh 3% hydrogen peroxide solution and water, respectively: Flask #1 1mL and 48mL, Flask #2 2mL and 47mL, Flask #3 3 mL and 46 mL, Flask #4 4mL and 45mL, Flask #5 5mL and 44mL, Flask #6 10mL and 39mL, Flask #7 15mL and 34mL, Flask #8 20mL and 29mL.

2) Dissolve about 0.02 g of catalase in 50mL of water. Note you need to record exact amount of catalase dissolved but it need not be exactly 0.0200g. Instructor will obtain enzyme from freezer

for you. As soon as you have obtained amount you need, return the bottle to the instructor to return to the freezer. Enzyme must be kept cold. Note the decimal place - you only need a tiny amount of catalyst. As soon as catalase is dissolved in water, place in graduated cylinder and place graduated cylinder in beaker of ice to keep the enzyme cold.

3) See Instructor for directions of how to set up buret to monitor amount of oxygen released from reaction.

4) Carry out series of reactions where exactly 1mL of enzyme is added to a flask, quickly stoppered, and swirled so that the oxygen produced in the reaction is directed to and trapped in an inverted buret. You should continue to swirl flask so enzyme and substrate are well mixed.

Make a reading of initial water level of inverted buret (near top, but on scale) and final value after 30, 45, 60, or 75, seconds and for slowest reactions you may need to take readings at 90, 120, or 180 as appropriate to determine rate expressed in mL of oxygen produced per minute. In other words, if the reaction is very fast, you need to use a short time, and if reaction is slow, you need to use longer time interval. You will read volume at several different time intervals and for each one calculate the velocity (rate) in mL/min. You will then average the ones that are consistent with each other. For slower reactions, you may need to ignore first reading you get if slower than later readings.

To prepare for next reaction you can draw water back up the inverted buret using a pipet bulb so it is not necessary to disassemble apparatus.

5) Record room temperature and atmospheric pressure.

Data and Analysis

Use numbers below 1, 2, 3 etc. to identify parts of your data and analysis presentation.

1) Prepare table (Table 1) with columns of the following data: Vol. H_2O_2 (mL), Vol enzyme soln (mL), Vol. H_2O (mL), Vol. of O_2 gas (mL), time (s), Rate of O_2 produced (mL/min). Discard values of the rate that are too large or small and average the remaining values for use in Table 2.

2) Calculate the concentration (M) of a 3.0% by weight solution of H₂O₂. Assume density of solution is 1.0g/mL. Calculate the concentration of your initial enzyme solution (M). The molecular weight of the catalase is given as 240,000 Daltons or (g/mol) for enzyme extracted from bovine liver (*Merck Index 12th edition*).

3) Use the ideal gas law to calculate the moles of O₂ in exactly 1 mL of O₂ gas. When you do this calculation you need to adjust the vapor pressure for the vapor pressure of water ($P_{O_2} = P_{atm} - P_{H_2O}$).

4) Use the balanced chemical equation to calculate the moles of H₂O₂ that disappear for every 1mL of O₂ gas that is formed.

5) In calculations, Vol. of H₂O₂(mL) refers to amount of hydrogen peroxide solution added to water and enzyme. However, concentration of H₂O₂ refers to final 50mL of reacting liquid in the flask containing a mixture of 3%H₂O₂ solution, water, and enzyme solution. Prepare a table (Table 2) with columns of the following data: Vol. of H₂O₂ (mL), dilution factor, [H₂O₂] (M), average rate (mL O₂/min), v(mol H₂O₂/min), 1/[H₂O₂] (L/mol), and 1/v (min/mol). Note that the velocity (or rate) is based on the loss of H₂O₂ per min using value calculated in item 4 previously.

6) Plot (Figure 1) v versus [H₂O₂].

7) Beginning with the Michaelis-Menten Equation

$$v = (V_{max} [S]) / (K_M + [S])$$

where [S] is the substrate concentration, in our case this is the peroxide [H₂O₂], show a derivation to obtain equation

$$1/v = (K_M/V_{max}) (1/[S]) + 1/V_{max}$$

A plot of 1/v versus 1/[S] is referred to as a Lineweaver-Burk plot. Show how K_M and V_{max} can be calculated from the slope and intercept of a Lineweaver-Burk plot. In other words, write K_M and V_{max} in terms of slope and intercept.

8) Plot (Figure 2) 1/v versus 1/[H₂O₂] and use the information from the previous question to determine K_M and V_{max}.

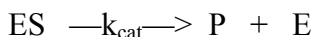
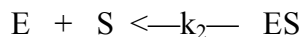
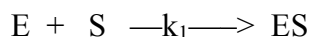
9) Use the results above to calculate the turnover number from the equation

$$\text{Turnover Rate} = V_{max} / [E]_T$$

where [E]_T is the enzyme amount in moles. Define the K_M, V_{max}, and Turnover rate. Under optimal conditions the Turnover Rate (the molecules of H₂O₂ reacted per molecule of catalase) for

the Catalase enzyme has been reported as 5.6×10^6 per min. Explain why your value might be lower than the accepted value.

10) Beginning with the enzyme-substrate kinetics mechanism and symbols used below



and using the information below as a guide, express in your own words a derivation that leads from above equation to the the Michaelis-Menten Equation

$$v = (V_{max} [S]) / (K_M + [S])$$

You will use the steady state approximation $d[ES]/dt = 0$ proposed by Briggs and Haldane in 1925. Consider the substrate to be in excess of enzyme $[S] \gg [E]$, use the velocity v for the rate of product $v = d[P]/dt = k_2 [ES]$. Let the total enzyme be the sum of the free and bound enzyme

$$[E]_T = [E] + [ES]$$

and let the Michaelis constant be

$$K_M = (k_2 + k_{cat}) / k_1$$

and the maximum velocity be

$$V_{max} = k_2 [E]_T.$$

References

D. R. Kimbrough, M. A. Magoun and M. Langfur, *Journal of Chemical Education*. 74, 210-212 (1997).

S. O. Farrell and R. T. Ranallo, *Experiments in Biochemistry*. (Harcourt Brace & Company, Orlando, Florida 2000) pages 83-94 and 179-186.

D. Voet, J. G. Voet, and C. W. Pratt, *Fundamentals of Biochemistry*. (John Wiley & Sons, New York, 1999) pages 326-331.

Enzyme handout pages 630-632