Experiment 8. Enzyme Kinetics: Catalase–Hydrogen Peroxide Decomposition Kinetics

Background

Enzymes are large, three-dimensional proteins that catalyze a wide range of biochemical reactions. Enzymes normally show a remarkable specificity for catalyzing the reaction of only certain substrates. This specificity exists because the mechanism for catalysis involves the binding of the enzyme and a particular substrate in a “lock and key” fashion (i.e. evolution has favored the development of certain enzymes that have a three-dimensional surface that contours to the shape of a specific substrate). The rate of the reaction increases because intermolecular interactions that accompany binding lower the activation energy for the substrate reaction.

A simple model for enzyme action is represented by

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P + E,$$

where *E* is the enzyme, *S* is the substrate, *ES* is called the enzyme-substrate complex, and *P* is the product. The rate equation associated with this mechanism is worked out in many physical chemistry and biochemistry textbooks (and is not shown here), and is given by

$$R = \frac{k_2[E]_t[S]}{\frac{k_{-1}+k_2}{k_1}+[S]}, \qquad (1)$$

where R is the rate of reaction and $[E]_t$ represents the concentration of both bound and unbound enzyme in solution (i.e. $[E]_t = [E] + [ES]$). The ratio of rate constants that appears in the denominator of equation 1 is simply equal to another constant. Consequently, equation 1 can be written in a more compact form as

$$R = \frac{k_2[E]_t[S]}{K_m+[S]}, \qquad (2)$$

where K_m is called the Michaelis constant, and equation 2 is called the Michaelis-Menten equation.

In real biochemical systems, the enzyme is normally present at extraordinarily small concentrations in comparison to the substrate (i.e. $[E] << [S]$). As the substrate concentration is increased, very little unbound enzyme will remain. In other words, the enzyme in the system will eventually become saturated with substrate (i.e. $[E]_t = [ES]$) and the maximum possible rate for the reaction will be observed in this limit. Referring to equation 2, the maximum rate is achieved when $[S] >> K_m$, meaning K_m can be neglected in the denominator and equation 2 becomes

$$R_{\max} \approx \frac{k_2[E]_t[S]}{[S]} = k_2[E]_t. \qquad (3)$$

(Please note that R_{max} is often referred to as V_{max} in many literature sources, particularly within the biochemical community). With the definition of R_{max}, we can rewrite the Michaelis-Menten rate equation in the form

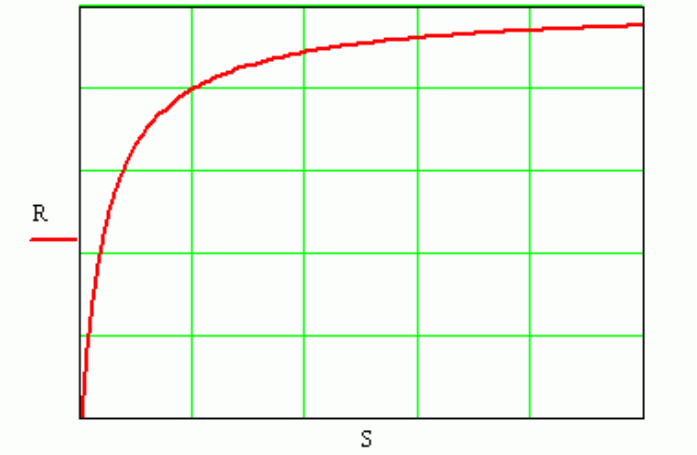
$$R = \frac{R_{\max}[S]}{K_m + [S]}. \qquad (4)$$

The constants K_m and R_{max} each quantify a characteristic of our enzyme-substrate system. K_m can be interpreted as the concentration of substrate that is required to achieve one-half the maximum rate. This can be verified by substituting $K_m = [S]$ in equation 4 which yields

$$R = \frac{R_{\max}[S]}{[S]+[S]} = \frac{1}{2}R_{\max}. \qquad (5)$$

If an enzyme-substrate system has a relative small value of K_m, then that enzyme has a high affinity for binding that substrate. Alternatively, the constant R_{max} is simply a measure of the inherent ability of the enzyme to act as a catalyst (an enzyme-substrate system with a large R_{max} value is going to occur at a relatively fast rate). Equation 4 is plotted in figure 1 for arbitrary values of K_m and R_{max}; initially, the rate increases at a rapid rate with increasing [S], but levels out as the enzyme becomes saturated (i.e. R converges on R_{max} as [S] becomes large).

Figure 1



How does one evaluate the constants K_m and R_{max} for a particular enzyme-substrate system? The most common method involves measuring the rate of reaction at several different concentrations of substrate (holding the concentration of enzyme constant from trial to trial) and subsequently fitting the data to equation 4. This process is made easier by taking the reciprocal of equation 4, which yields

$$\frac{1}{R} = \left(\frac{K_m}{R_{\max}} \right) \frac{1}{[S]} + \frac{1}{R_{\max}}. \quad (6)$$

In other words, a plot of $1/R$ versus $1/[S]$ should be linear (if the enzyme-substrate system obeys the Michaelis-Menten mechanism), and the slope and intercept can be used to evaluate K_m and R_{max}. Such a plot is called a Lineweaver-Burk plot.

In the following experiment, the catalytic decomposition of hydrogen peroxide by the enzyme catalase is investigated;

$$2\ H_2O_2(aq) \rightarrow O_2(g) + 2\ H_2O(l). \quad (7)$$

Catalase is present in nearly all plant and animal organisms as part of a biochemical defense mechanism that removes trace amounts of hydrogen peroxide generated during metabolism (hydrogen peroxide that would otherwise build up and oxidatively damage tissues). Equation 7 reveals that the decomposition reaction yields one equivalent of $O_2(g)$ for every two equivalents of H_2O_2 that decomposes. The reaction is studied here for a series of trials containing different initial concentrations of H_2O_2 (and a constant concentration of catalase enzyme). The rate of reaction is measured by using a computer interfaced gas pressure sensor to follow the pressure increase that occurs inside a closed reaction vessel.

Hazards

Hydrogen peroxide solution will be utilized in this experiment. It is a strong oxidant and an irritant to the skin and eyes. Contact with any hydrogen peroxide solution should be avoided by wearing gloves and goggles. Particular care should be taken if you will be preparing the 4% H_2O_2 stock solution that is in turn made by diluting an appropriate volume of 30% H_2O_2 (a strongly oxidizing solution that can quickly burn the skin). If skin or eye contact occurs, flush the area with plenty of water. Waste H_2O_2 and catalase solutions can normally be flushed down the drain with plenty of water or otherwise disposed of according to local ordinances.

Experimental

Unless instructed otherwise, assemble the apparatus illustrated in Figure 2. The manometric reaction vessel consists of a heavy-walled glass pressure tube that can be rapidly sealed by spinning in place a threaded Teflon ® plug that is fitted with a rubber

O-ring. The Teflon plug in turn has a threaded outlet through which a connection is made to the computer interfaced pressure sensor. About 80% of the pressure tube is submerged in a large beaker of water during data collection to promote isothermal conditions from trial to trial. A stir-bar/stir-plate is utilized to vigorously mix the solution as it reacts and data is collected.

Referring to the amounts in Table 1 and the stock solutions of 4% H_2O_2, pH = 6.8 phosphate buffer, and catalase enzyme supplied by your instructor, carry out each of the kinetic trials as follows:

1. The stir bar and the indicated aliquots of 4% H_2O_2 and working phosphate buffer solutions (collectively referred to as '*Solution A*') are placed into the pressure tube. Pressure-time data acquisition is then initiated on the computer.
2. Then, in rapid sequence, 1.0 mL of enzyme stock solution ('*Solution B*') is injected into the pressure tube, the tube is spun onto the threaded Teflon ® plug and tightened, and the stir rate on the stir plate is increased to a value with a goal of producing a vigorous, uniform, and reproducible vortex from trial to trial. A slight upward inflection will be observed in the pressure-time data just as the Teflon ® plug is tightened; this inflection provides a convenient reference point in the data set for identifying the beginning of the pressure-time kinetic trial.
3. Data acquisition is performed for approximately 6 minutes per trial, or until no further pressure increase is evident.
4. At the conclusion of the data collection for a given trial, save the data set to an appropriate folder on the computer (so that it can be recalled later if further analysis is called for) and then use the data analysis options within the software to determine the slope of the tangent line (*m*) corresponding to the point of steepest accent on the pressure-time curve. Record this slope in Table 1 below

for each trial. NOTE: If the data acquisition software that you are using does not have a feature for determining the slope for a segment of data, they you will need to export each data set to an appropriate software environment (such as Microsoft Excel) to accomplish this task.

5. Be certain to rinse the stir-bar/reaction tube with distilled water between trials.
6. Record the temperature of the water bath before concluding the experiment.

Table 1:

Solution A		Solution B		
Stock 4% H_2O_2 (mL) (use adjustable micropipet)	Working Buffer (mL) (use 25 mL Mohr pipet)	Stock Enzyme (mL) (use 1.0 mL micropipet)	**Total Volume (mL)**	**Slope of Tangent Line - *m***
0.1	18.9	1.0	**20.0**	
0.2	18.8	1.0	**20.0**	
0.35	18.65	1.0	**20.0**	
0.5	18.5	1.0	**20.0**	
0.75	18.25	1.0	**20.0**	
1.0	18.0	1.0	**20.0**	

Figure 2: Apparatus for Measuring Catalase-H_2O_2 Decomposition Kinetics

Calculations and Questions to Address

1. Using the amounts indicated in Table 1, calculate the molar concentration of H_2O_2 in each trial (to accomplish this, you will first need to determine the molar concentration of the 4% H_2O_2 stock solution).
2. Assuming that the airspace above the reacting solution corresponds to a total volume of 52 mL (at the temperature of your water bath) and that the produced $O_2(g)$ is behaving according to the ideal gas law, calculate the maximum pressure increase (ΔP_{max}) that one would expect to observe if all the H_2O_2 decomposes according to equation (7). Does this calculated pressure increase match the measured pressure increase? Comment on any differences observed.
3. Calculate the initial rate of reaction for each trial by dividing the observed slope of the tangent line (m) by the corresponding calculated value of ΔP_{max} found above;

$$Rate = \frac{m}{\Delta P_{max}}. \qquad (8)$$

4. Perform a Lineweaver-Burk analysis of the data by generating a plot of 1/*Rate* versus 1/[H_2O_2], fitting a best-fit line to the data, and using the slope and intercept of that best-fit line to determine the Michaelis constant (K_m) and the maximum rate (R_{max}).
5. Comment about whether your results support the notion that the catalase-H_2O_2 system obeys the Michaelis-Menton mechanism. Compare your K_m value to an appropriate literature value.
6. If the experiment is performed by several groups, pool the class data and calculate the average and standard deviation of K_m. Comment about the degree

of any random and/or systematic error that may be present in K_m, and the possible sources of these errors.