

Enzyme kinetics: the alcohol dehydrogenase catalyzed oxidation of ethanol

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Goal: Kinetic data is presented for the alcohol dehydrogenase catalyzed oxidation of ethanol to acetaldehyde. The results are analyzed within the context of the Michaelis-Menten mechanism for enzyme action.

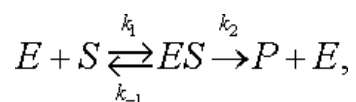
Prerequisites: An introductory knowledge of kinetics, including a few examples of mechanisms and how they give rise to a specific rate equation. While not necessary, background preparation for this exercise might include the derivation of the Michaelis-Menten equation.

Resources you will need: This exercise should be carried out within a software environment that is capable of data manipulation, which can generate a best-fit line for an x-y data set, and can also determine the uncertainties in the slope and intercept of the best-fit line. You will also be graphing the data along with the fitted function.

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.

A simple model for enzyme action is represented by



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 most physical chemistry textbooks, and is given by

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

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 more compactly as

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

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. $[E] \ll [S]$). 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 $[S] \gg K_m$, meaning K_m can be neglected in the denominator and equation (2) becomes

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

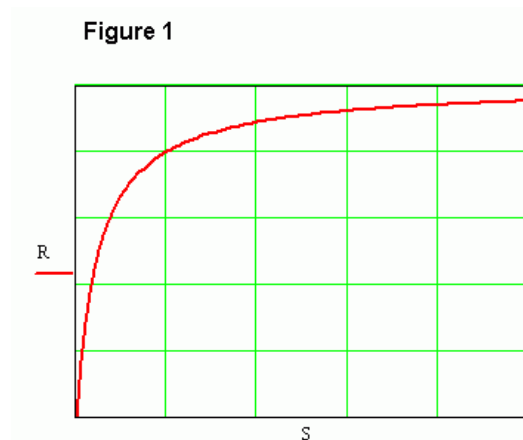
With the definition of R_{\max} , we can rewrite the Michaelis-Menten rate equation in the form

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

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

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

If an enzyme-substrate system has a relatively small value of K_m , then we know that that enzyme has a very 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).



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 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 the parameters K_m

and R_{max} . Such a plot is called a Lineweaver-Burk plot.

In the exercise below, you will be presented with some kinetic data for the oxidation of ethanol to acetaldehyde. This reaction is catalyzed in the human body by an enzyme called alcohol dehydrogenase. The data will be analyzed within the context of the Michaelis-Menten mechanism to determine the constants K_m and R_{max} .

Experimental Data:

The following data were obtained from K. Bendinskas, C. DiJiacomo, A. Krill, and E. Vitz, *Journal of Chemical Education*, **82(7)**, 1068 (2005). In this study, the enzyme alcohol dehydrogenase (ADH) is used to catalyze the conversion of ethanol (the substrate) to acetaldehyde (the product). Eight kinetic trials were carried out in a pH 9.0 buffer; only the concentration of ethanol was varied from one trial to the next. The reaction was followed spectrophotometrically, although in an indirect fashion. Specifically, the direct conversion of ethanol to acetaldehyde only gives rise to absorbance changes in the UV portion of the spectrum, which is difficult to follow with standard lab spectrometers and cuvettes. The authors were able to couple the ethanol reaction to another reaction that gives rise to a color change in the visible region (through a coupling scheme that can be read about in the above reference, dichlorophenolindophenol undergoes a synchronous redox reaction which causes the solution to go from blue to colorless; the authors monitored this change at 635 nm).

The initial rate of reaction was defined as the slope of absorbance at 635 nm versus time plot that was recorded for each trial (these dA/dt values are given in the table below, and represent the rate of reaction, R).

<u>Trial</u>	<u>[Ethanol] - (mol/L)</u>	<u>Rate - Absorbance change - (dA/dt) - (1/min)</u>
1	0.007	0.06
2	0.015	0.11
3	0.031	0.16
4	0.068	0.21
5	0.100	0.23
6	0.200	0.28
7	0.300	0.29
8	0.400	0.28

Exercise:

1. Enter the raw data into an appropriate quantitative analysis software environment and plot R versus $[S]$. Estimate values for K_m and R_{max} for this system by simply inspecting the graph.
2. Manipulate the data into a form that will allow you to generate a Lineweaver-Burk plot. Generate this plot, include a best-fit line, and use the slope and intercept to calculate values of K_m and R_{max} . Do these numbers agree with the estimates that you obtained in step (1)?
3. Lineweaver-Burk plots, which are double-reciprocal plots, are sometimes problematic because they tend to cluster all the large substrate concentration data to one side of the plot. Also, the few points corresponding to smaller substrate concentrations tend to be noisy because they are obtained by taking the reciprocal of a small number that contains a relatively high degree of random error. These difficulties can sometimes be avoided by plotting the data in an alternate fashion. One such method is known as a Hanes-Woolf plot, and is obtained by multiplying both sides of equation (6) by $[S]$, which yields

$$\frac{[S]}{R} = \left(\frac{K_m}{R_{max}} \right) + \frac{[S]}{R_{max}}. \quad (7)$$

Generate a Hanes-Woolf plot, include a best-fit line, and calculate values of K_m and R_{max} . Comment about the similarities (dissimilarities) of the plots and the values of K_m and R_{max} obtained in steps (2) and (3).

4. MORE CHALLENGING: Using a software environment that is capable of determining the standard deviation in the slope and intercept of a best-fit line, and by propagating that error into K_m and R_{max} , determine whether the Hanes-Woolf method yields K_m and R_{max} values with more or less uncertainty in comparison to the Lineweaver-Burk method.

Suggestions for improving this web site are welcome. You are also encouraged to submit your own data-driven exercise to this web archive. All inquiries should be directed to the curator: Tandy Grubbs, Department of Chemistry, Unit 8271, Stetson University, DeLand, FL 32720.

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