Properties of Egg White and Pure Lysozyme Enzyme

Introduction:

This laboratory demonstrates enzyme specific activity, and the effect of substrate concentration on enzyme activity. The Michaelis constant is determined for the enzyme reaction based on the Lineweaver–Burk plot of the Michaelis-Menten equation.

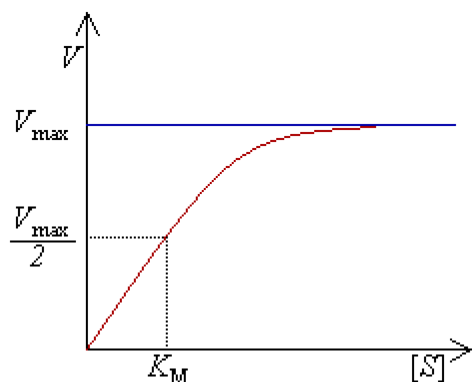
Pre-lab questions: (Questions to be answered before doing the experiment. The answers are due at the beginning of each experiment. The questions are for credit and some may appear on your final exam).

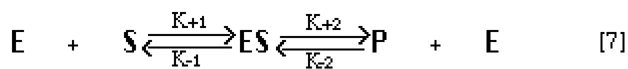
1. In studying enzyme kinetics in a lab setting, which factors do you think would have to be controlled?
2. Which factors increase or decrease the rate of an enzyme process?
3. How do enzymes affect chemical reactions?
4. Are enzymes changed during a reaction? Explain.

Substrate concentration:

Normally in a reaction mixture if the amount of enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity (ΔA/Δt).

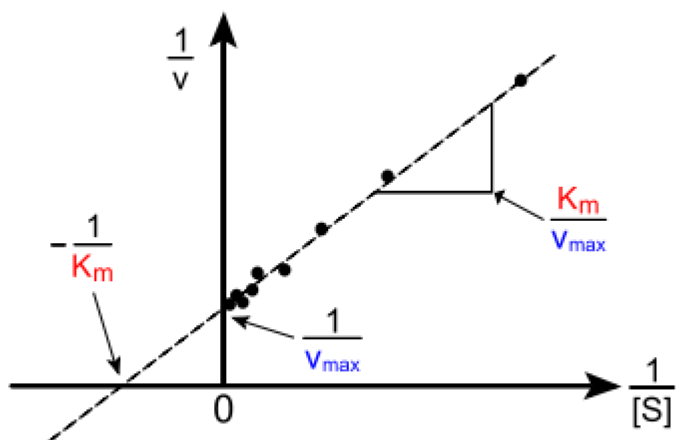
It is theorized that when this maximum velocity had been reached, all available enzyme has been converted to ES, the enzyme substrate complex. This is the maximum velocity and is designated V_{max}. Using the maximum velocity and the equation below, Michaelis developed a set of mathematical expressions to calculate enzyme activity in terms of reaction speed from measurable laboratory data.





The Michaelis constant Km is defined as the substrate concentration at 1/2 the maximum velocity. Using this constant and the fact that Km can also be defined as:

$\mathbf{K_m = K_{-1} + K_2 / K_1}$



Lineweaver–Burk plot

The plot provides a useful graphical method for analysis of the Michaelis–Menten equation:

$$V = V_{\max}\frac{[S]}{K_m + [S]}.$$

Taking the reciprocal gives

$$\frac{1}{V} = \frac{K_m + [S]}{V_{\max}[S]} = \frac{K_m}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}}$$

where V is the reaction velocity (the reaction rate), K_m is the Michaelis–Menten constant, $V_{\max}$ is the maximum reaction velocity, and $[S]$ is the substrate concentration.

Michaelis constants have been determined for many of the commonly used enzymes. The size of Km tells us several things about a particular enzyme.

- A small Km indicates that the enzyme requires only a small amount of substrate to become saturated. Hence, the maximum velocity is reached at relatively low substrate concentrations.

- A large Km indicates the need for high substrate concentrations to achieve maximum reaction velocity.
- The substrate with the lowest Km upon which the enzyme acts as a catalyst is frequently assumed to be enzyme's natural substrate, though this is not true for all enzymes.

Method:

Exp: 1

Break 1 egg and separate the egg white using the egg shell. Keep the runny part of the egg white. To 1ml. of egg white add 1 ml of 0.2 M potassium phosphate buffer (ph 6.2). Make a 0.015 g in 25 ml. suspension of *micrococcus lsodeikticus* (Sigma Chem. Co.) cells in 0.1 M potassium phosphate buffer (ph 6.2). The absorption of the bacteria suspension at wavelength 480 should be approximately 0.8.

Lysozyme assay:

The change in absorbance per unit time is the measure of the lysozyme activity. Measure the increase in absorbance over 5 minutes using kinetics option on Cary 50 or Cary 60. Add increasing levels of substrate with constant enzyme. To a **glass** cuvette add subtrate and buffer. Start measuring absorbance to get a starting value. Add enzyme quickly and stir with plastic pipette tip. Follow reaction rate. Activity can be measured as the change in absorbance over time (5 min). **The table values (below) can be changed depending on initial reaction rate.**

A Michaelis constant (substrate concentration at ½ maximum enzyme activity) for lysozyme under these conditions may be determined by increasing the substrate concentration at constant enzyme concentration if a maximum rate can be determined. Students may have to change concentrations to try and determine a Km.

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Enzyme (diluted egg white)	Buffer (0.1M phosphate ph 6.2)	Substrate (Bacteria)	Activity ($\Delta A/\Delta t$)
50 ul.	2.5 ml.	0.5 ml.	
50	2.0	1.0	
50	1.0	2.0	
50	0.0	3.0	

Exp: 2

Pure enzyme activity:

Weigh 0.01 g of pure lysozyme (Sigma Chem. Co.) and dissolve in 25 ml. of 0.1 M phosphate buffer (ph 6.2). Make a 0.015 g in 25 ml. suspension of *micrococcus lsodeikticus* (Sigma Chem. Co.) cells in 0.1 M potassium phosphate buffer (ph 6.2).

To a **glass** cuvette add sustrate and buffer. Add enzyme quickly and stir with plastic pipette tip. Follow reaction rate using kinetics option on Cary 50. Activity can ne measured as the change in absorbance over time (5 min or where best change in slope over time)). These table values can be changed depending on initial reaction rate.

Enzyme	Buffer	Substrate	Activity
20 ul.	2.5 ml.	0.5 ml.	
20	2.0	1.0	
20	1.0	2.0	
20	0.0	3.0	

A Michaelis constant (substrate concentration at ½ maximum enzyme activity) for lysozyme under these conditions may be determined by increasing the substrate concentration at constant enzyme concentration if a maximum rate can be determined. Students may have to change concentrations to try and determine a Km.

Plot reaction rates with adding substrate. Determine Km and enzyme constant. Use Lineweaver-Burke plot for determinations. Compare reaction of pure enzyme verses egg. Determine Km values and in both pure and egg solution.

Questions:

1) What are enzyme co-factors and how might they affect enzyme activity.
2) How might you follow or measure other enzyme reaction rates?
3) In kinetic studies why do reaction rates change?
4) What controls enzyme reaction rates in the body of a person or animal?