

Problem Set 25: Enzyme Kinetics - Intermediate Concepts

Due Dec 4, 2008

1. Once an enzyme kinetics experiment is finished, the first step in analyzing the data is to graph the results. Since straight lines are easier to interpret than hyperbolic equations, the Michaelis-Menton equation is rewritten in the form of a straight line. Start with the following version of the Michaelis-Menton equation and rewrite it in terms of a straight line where the y-axis will be $1/v$ and the x-axis will be $1/[S]$:

$$v = \frac{V_{\max}[S]}{K_M + [S]}$$

A graph of $1/v$ versus $1/[S]$ is called a Lineweaver-Burk plot.

What are the slope and intercept of this line?

2. Suppose that you were studying an enzyme which had a single substrate and was known to follow the Michaelis-Menton mechanism. You took the wild-type enzyme and made a site-directed mutation to it. Your mutation significantly INCREASED k_1 for the reaction but did not alter either k_1 or k_2 .
- Would K_M for your mutant be different than for the wild-type enzyme?
 - Would V_{\max} for your mutant be different than for the wild-type enzyme?
 - Draw a Lineweaver-Burk plot showing both the data you would expect for both the wild-type enzyme and the mutant.
3. In a separate experiment, the following rates for Michaelis-Menton kinetics were measured for a wild-type enzyme and a mutant:

| <u>Rate constant</u> | <u>Wild-Type</u> | <u>Mutant</u> |
|----------------------|------------------|---------------|
| k_1 | 18 | 3 |
| k_{-1} | 916 | 1 |
| k_2 | 26 | 156 |

- Do K_M and V_{\max} change for these two enzymes?
 - Considering just the first step of the Michaelis-Menton mechanism, does S bind more strongly to the mutant or to the wild type enzyme?
 - Is K_M a useful measure of substrate binding?
4. Two different enzyme kineticists were studying the same enzyme. Previous experiments had suggested that K_M for the enzyme was around 15 mM and that k_{cat} was approximately 400 s^{-1} . Both kineticists were using an initial enzyme concentration of $1 \times 10^{-6} \text{ M}$.

- What is V_{\max} for this experiment?
- In order to get a more reliable estimate of the steady state kinetic parameters, both kineticists constructed a Lineweaver Burk plot. Scientist #1 used the data on the left, while scientist #2 used the data on the right:

| <u>[Substrate]</u> | <u>velocity</u> | <u>[Substrate]</u> | <u>velocity</u> |
|--------------------|------------------------|--------------------|------------------------|
| 1.5 mM | 3.636×10^{-5} | 1.5 mM | 3.636×10^{-5} |
| 39 mM | 2.889×10^{-4} | 2 mM | 4.706×10^{-5} |
| 75 mM | 3.333×10^{-4} | 3 mM | 6.667×10^{-5} |
| 113 mM | 3.531×10^{-4} | 5.8 mM | 1.115×10^{-4} |
| 150 mM | 3.636×10^{-4} | 150 mM | 3.636×10^{-4} |

Who did the better experiment? Why?

5. Explain the difference between a Lineweaver-Burk plot and an Eadie-Hofstee plot, and explain why an Eadie-Hofstee plot is preferable.