ENZYME KINETICS

Many enzymes show a typical hyperbolic relationship between reaction velocity and substrate concentration, with a gradual approach to saturation of the enzyme with high substrate levels (Fig 1). The Michaelis-Menten equation is an algebraic expression of the hyperbolic relationship in which the important terms are substrate concentration (S), initial velocity (v), maximum velocity (Vmax) and Michaelis constant (K_M).

Two basic reactions are involved in the formation and breakdown of the enzyme-substrate complex:

1) \[ E + S \rightleftharpoons ES \]
\[ k_1 \]
\[ k_{-1} \]

2) \[ ES \rightleftharpoons E + P \]
\[ k_2 \]
\[ k_{-2} \]

If [E_t] represents the total enzyme concentration (the sum of free and bound enzyme) and [ES] is the concentration of enzyme-substrate complex, then [E_t] - [ES] represents the concentration of free or unbound enzyme. The substrate concentration, [S], is usually greater than [E_t] and therefore is not limiting ([S] >> [ES]).

I. The rate of formation of ES is

3) \[ \text{RATE} = k_1 ([E_t] - [ES]) [S] \]

where \( k_1 \) is the rate constant of reaction 1.

II. The rate of breakdown of ES is

4) \[ \text{RATE} = k_{-1} [ES] - k_2 [ES] \]

where \( k_{-1} \) and \( k_2 \) are the rate constants for the reverse of reaction 1 and the forward direction of reaction 2 respectively.

III. When the rate of formation of ES is equal to the rate of its breakdown, [ES] will be constant and the reaction is in a steady state:

5) \[ k_1 ([E_t] - [ES]) [S] = k_{-1} [ES] + k_2 [ES] \]

6) \[ k_1 [E_t] [S] - k_{-1} [ES] [S] = (k_1 + k_2) [ES] \]
7) \[ k_1[E_t][S] = k_1[ES][S] + (k_1 + k_2)[ES] \]

8) \[ k_1[E_t][S] = (k_1[S] + k_1 + k_2)[ES] \]

9) \[ [ES] = \frac{[E_t][S]}{[S] + (k_2 + k_1)[S]/k_1} \]

10) \[ [ES] = \frac{[E_t][S]}{[S] + (k_2 + k_1)/k_1} \]

IV. The initial velocity is determined by the breakdown of [ES] in reaction (2), whose rate is \( k_2 \). Therefore,

11) \[ v = k_2[ES] \]

12) \[ v = \frac{k_2[E_t][S]}{[S] + (k_2 + k_1)[S]/k_1} \]

If we define \( K_M \) as \( (k_2 + k_1)/k_1 \) and \( V_{max} \) as \( k_2[E_t] \) (i.e., the rate when all available E is present as ES) we get

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

This is the Michaelis-Menten rate for a one-substrate enzyme-catalyzed reaction. It is a statement of the quantitative relationship between initial velocity, \( v \), the maximum velocity, \( V_{max} \), and the initial substrate concentration (S), all related through the Michaelis constant, \( K_M \). \( K_M \) can be most simply defined as the concentration of the specific substrate at which an enzyme yields 1/2 its maximum velocity.

In the case where \( v = V_{max}/2 \)

14) \[ \frac{V_{max}}{2} = \frac{V_{max}[S]}{K_M + [S]} \]

15) \[ K_M + [S] = 2[S] \]

16) \[ K_M = [S] \]

We may now consider methods for determining \( V_{max} \) and \( K_M \) from measurements of \( v \) at different values of [S]. \( K_M \) could be estimated from the hyperbolic curve in Fig.1 but \( V_{max} \) cannot be obtained accurately since it is often difficult to saturate an enzyme experimentally.
However, by taking the reciprocal of equation 13 we get

\[
17) \quad \frac{1}{v} = \frac{K_M + [S]}{V_{max}[S]}
\]

\[
18) \quad \frac{1}{v} = \frac{K_M}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}
\]

\[
19) \quad \frac{1}{v} = \frac{K_M}{V_{max}[S]} + \frac{1}{V_{max}}
\]

Equation 19 is called the Lineweaver-Burk equation. A plot of \(1/v\) vs \(1/[S]\) (double reciprocal plot) yields a straight line with slope \(K_M/V_{max}\) and y-intercept \(1/V_{max}\) (Fig. 2). Since the slope and intercept can be readily measured from the graph, \(V_{max}\) and \(K_M\) can be accurately determined.

In analyzing the Lineweaver-Burk plot it can be seen that the lowest substrate concentrations have the greatest affect on slope and intercept. These concentrations are also the greatest source of error in rate measurements. Therefore, extreme care must be taken in experimentally gathering data for kinetic analysis. Substrate concentrations must be chosen to yield equally spaced points in the double reciprocal plot.

Another way to linearize the Michaelis-Menten equation is generally used to analyze kinetic data. Rearrangement of Eq 19 gives

\[
20) \quad v(K_M + [S]) = V_{max}[S]
\]

\[
21) \quad vK_M + v[S] = V_{max}[S]
\]

\[
22) \quad (v/[S]) K_M + v = V_{max}
\]

\[
23) \quad v = -K_M (v/[S]) + V_{max}
\]

Equation 23 is the Woolf-Hofstee equation. A plot of \(v\) vs \(v/[S]\) gives a straight line with slope = \(-K_M\) and y-intercept = \(V_{max}\) (Fig. 3). Both slope and intercept can be easily determined from experimental data using substrate concentrations equally spaced above and below the \(K_M\).
EXERCISE:

A. Given a hypothetical enzyme with a $K_M = 20 \, \mu\text{M}$ and $V_{\text{max}} = 10 \, \text{nmol/mg protein/min}$, calculate substrate concentrations bracketing the $K_M$ and giving $v = 15\% - 85\%$ of $V_{\text{max}}$ in 10\% intervals).

B. Plot $v$ vs $[S]$ 

C. Plot $v$ vs $v/[S]$ 

D. Using the low and high rates calculated above, calculate the substrate concentrations that will give you 8 equally spaced points on a Lineweaver-Burk plot.

E. Plot $1/v$ vs $1/[S]$