enzyme kinetics

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0.1 Initial rate

Initial rate V_0 corresponds to a known fixed substrate. As time proceeds $[S]$ will drop.

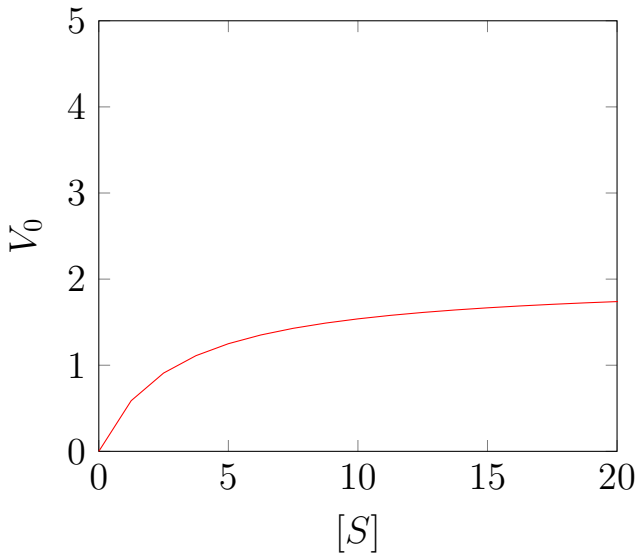

Figure 1: Fig 1: At high substrate concentration the curve flattens out, i.e. all the enzyme molecules are bound to the substrate molecules and the rate becomes zero order in substrate concentration.

It is rectangular hyperbola

$$V_0 = \frac{a[S]}{b + [S]} \tag{1}$$

0.2 Michaelis-Menten Kinetics

It explains the dependence of the initial rate of enzyme-catalyzed reactions on concentration.

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

initial rate of product formation, V_0 :

$$V_0 = \left(\frac{d[P]}{dt}\right)_0 = k_2[ES] \tag{2}$$

We need to calculate $[ES]$. The dissociation constant, k_s:

$$k_s = \frac{k_-1}{k_1} = \frac{[E][S]}{[ES]}$$

Total concentration of enzyme at a time shortly after the start of the reaction is:

$$[E]_0 = [E] + [ES]$$

$$k_s = \frac{([E]_0 - [ES])[S]}{[ES]} \tag{3}$$

$$[ES] = \frac{[E]_0[S]}{k_s + [S]} \tag{4}$$

substituting 4 in 2

$$V_0 = \left(\frac{d[P]}{dt}\right)_0 = k_2 \frac{[E]_0[S]}{k_s + [S]} \tag{5}$$

thus the rate is always proportional to the total concentration of the enzyme.
Equation 5 has the same form as equation 1

$$V_0 = \frac{a[S]}{b + [S]}$$
$$V_0 = k_2 \frac{[E]_0[S]}{k_s + [S]}$$

where a = $k_2[E]_0$ and b = k_s

At low substrate concentrations [S] << k_s, equation 5 becomes :

$$V_0 = K_2 \frac{[E]_0[S]}{k_s}$$

that is, it is a second order reaction, first order in $[E]_0$ and first order in [S]. It corresponds to initial linear portion of the plot in figure 1

At high substrate concentration [S] >> k_s, equation 5 becomes:

$$V_0 = k_2[E]_0$$

at this stage all the enzyme molecules are in the enzyme-substrate complex, that is, the reacting system is saturated with substrate(S). Consequently, the initial rate is zero order in [S]. This rate law corresponds to the horizontal portion of the plot in figure 1. The curved

portion in the plot represents the transition from low to high substrate concentration.

When all enzyme molecules are complexed with substrate as ES, the measure of initial rate must be at it's maximum value (V_{max} = maximum rate), so that:

$$V_{max} = k_2[E]_0 \tag{6}$$

In equation 5, when [S] = k_s

$$V_0 = \left(\frac{d[P]}{dt}\right)_0 = k_2\frac{[E]_0[S]}{k_s + [S]}$$

$$V_0 = k_2\frac{[E]_0[S]}{2[S]} = \frac{k_2[E]_0}{2}$$

$$V_0 = \frac{V_{max}}{2}$$

so, k_s equals the concentration of substrate when the initial rate is half of maximum rate.

0.3 Steady-State kinetics

rate of formation of ES = rate of disappearance of ES

$$k_1[E][S] = k\_1[ES] + k_2[ES]$$

$$\frac{d[E][S]}{dt} = 0$$

$$\frac{d[E][S]}{dt} = 0 = k_1[E][S] - k\_1[ES] - k_2[ES]$$

$$[E]_0 = [E] + [ES]$$

$$\frac{d[E][S]}{dt} = k_1([E]_0 - [ES])[S] - (k\_1 + k_2)[ES] = 0$$

Solving for ES, we get

$$[ES] = \frac{k_1[E]_0[S]}{k_1[S] + k\_1 + k_2} \tag{7}$$

Substituting equation 7 in equation 2

$$V_0 = \left(\frac{d[P]}{dt}\right)_0 = k_2[ES] = k2\frac{k_1[E]_0[S]}{k_1[S] + k\_1 + k_2}$$

dividing numerator and denominator by k_1

$$\frac{k_2[E]_0[S]}{\frac{k\_1+k_2}{k_1}+[S]}$$

$$V_0 = \frac{k_2[E]_0[S]}{k_M + [S]} \tag{8}$$

k_M is the Michaelis-Menten constant, defined as

$$k_m = \frac{k\_1 + k_2}{k_1} \tag{9}$$

Equation 8 and 5 have similar dependency on substrate concentration; however $k_m \neq k_s$ in general, unless $k\_1 >> k_2$.

Since $k_2[E]_0 = V_{max}$:

$$V_0 = \frac{V_{max}[S]}{k_M + [S]} \tag{10}$$

When $V_0 = \frac{V_{max}}{2}$

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{k_M + [S]}$$
$$k_m = [S]$$

0.4 Graphical determination of V_{max} and k_m

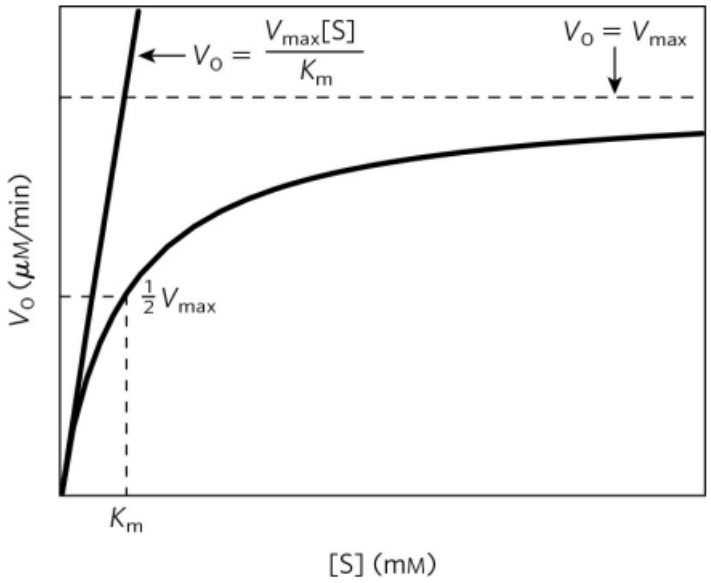

Figure 2: V_0 - [S] curve. Locating the asymptote value V_{max} at very high [S] is difficult.

This graph shows the kinetic parameters that define the limits of the curve at high and low [S]. At low [S], $K_M >>$ [S], and the [S] term in the denominator of the Michaelis-Menten equation (10) becomes insignificant. The equation simplifies to $V_0 = \frac{V_{max}[S]}{k_M}$, and V_0 exhibits a linear dependence on [S], as observed here. At high [S], where [S] $>> K_m$, the K_m term in the denominator of the Michaelis-Menten equation (10) becomes insignificant and the equation simplifies to $V_0 = V_{max}$; this is consistent with the plateau observed at high [S]. The Michaelis-Menten equation is therefore consistent with the observed dependence of V_0 on [S], and the shape of the curve is defined by the terms $\frac{V_{max}}{k_M}$ at low [S] and V_{max} at high [S].

$$\frac{1}{V_0} = \frac{k_m + [S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \frac{k_m}{V_{max}[S]} + \frac{1}{V_{max}} \tag{11}$$

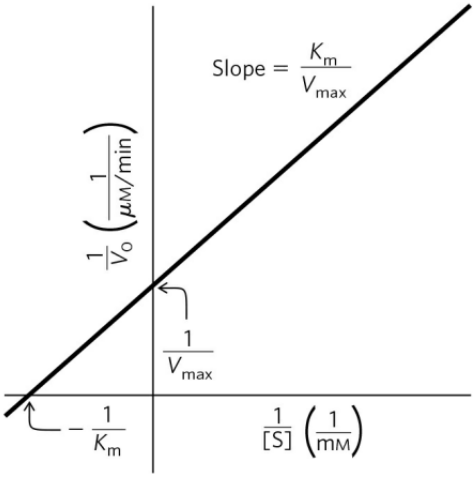

Figure 3: Lineweaver-Burk plot

Multiplying equation 11 by $V_0 V_{max}$

$$\frac{V_0 V_{max}}{V_0} = V_0 V_{max}\left[\frac{k_M}{V_{max}[S]} + \frac{1}{V_{max}}\right]$$

$$V_{max} = \frac{V_0 k_M}{[S]} + V_0$$

$$V_0 = V_{max} - \frac{V_0 k_M}{[S]} \tag{12}$$

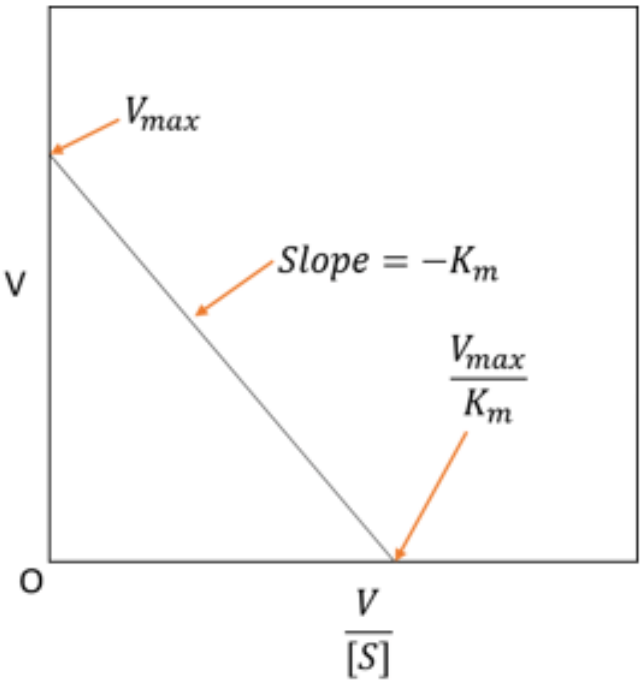

Figure 4: Eadie-Hofstee plot

0.5 Significance of k_M and V_{max}

k_M varies from one enzyme to another, and also with different substrates for the same enzyme. k_M is equal to the substrate concentration at half the maximum rate or k_M is equal to the substrate concentration at which half the enzyme active sites are filled with substrate molecules.
The value of k_M is sometimes equated with the dissociation constant of the enzyme-substrate complex ES. The larger the k_M, the weaker the binding. This is true only when $k_2 << k\_1$, so that equation 9 becomes

$$k_M = \frac{k\_1}{k_1}$$

k_M depends on temperature, nature of substrate, pH, ionic strength etc. Any variation in k_M (for same enzyme and substrate) is often an indication of the presence of an inhibitor or activator.

V_{max} is maximum rate attainable, it is the rate at which the total enzyme concentration is present as enzyme-substrate complex.

$$V_{max} = k_2[E]_0$$

If $[E]_0$ is known, the value of k_2 can be determined from value of V_{max} measured from one of the plots.
k_2 is a fixed first order rate constant has the unit of per unit time (s^{-1} or min^{-1}). It is called the *turnover number* or k_{cat} or *catalytic constant*. The turnover number of an enzyme is the number of substrate molecules that are converted to product per unit time, when the enzyme is fully saturated with the substrate.

Carbonic anhydrase catalyses

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

hydration of CO_2 and dehydration of H_2CO_3. It's $k_2 = k_{cat} = 1 * 10^6\ s^{-1}$ at 25 C. Suppose, 1 X 10^6 M solution of enzyme can catalyze the formation of 1M H_2CO_3 per second from H_2O and CO_2, then

$$\begin{aligned} V_{max} &= [E]_0 k_2 = [E]_0 k_{cat} \\ &= 1 * 10^{-6} M * 1 * 10^6 s^{-1} \\ &= 1 M s^{-1} \end{aligned}$$

Equation 8

$$V_0 = \frac{k_2[E]_0[S]}{k_M + [S]}$$

under physiological condition [S] << k_M

$$V_0 = \frac{k_2}{K_M}[E]_0[S]$$

$$V_0 = \frac{k_{cat}}{K_M}[E]_0[S] \tag{13}$$

this expresses rate law of a second order reaction.
$\frac{k_{cat}}{k_M}$ $(M^{-1}s^{-1})$ is a measure of the catalytic efficiency of an enzyme. A large ratio favors the formation of the product.

$$\frac{k_{cat}}{K_M} = \frac{k_2}{K_M} = \frac{k_2 k_1}{K_2 + k_{-1}}$$

Upper limit of catalytic efficiency is the maximum of the ratio, which is maximum when $k_2 >> k_{-1}$, that is k_1 is rate determining and the enzyme turns over a product as soon as an ES complex is formed.

0.6 Important takeaways

For a low substrate concentration, the rate of product formation is first order with respect to enzyme and also first order with respect to the substrate.

For a very high substrate concentration, initial rate of product formation is zero order with respect to the substrate.

The rate of product formation is independent of the concentration of enzyme- substrate complex.

Maximum possible rate of product formation is dependent on k_2 and initial concentration of enzyme.

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