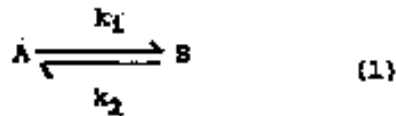


Derivation of the Michaeli-Menten equation describing the kinetics of enzyme-catalyzed reactions.

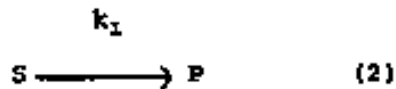
Kinetics Hand Out

1. Conventions

Suppose you have a reaction:



In discussions of enzyme kinetics it is usually assumed that the reaction is irreversible because B is removed and so the reaction is usually written:



S= starting material (or substrate)

P= product

k_1 = rate constant for forward reaction

2. Rate of Uncatalyzed Reaction

For an uncatalyzed reaction

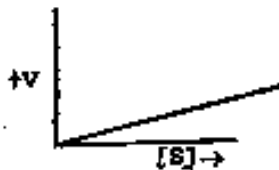
Rate of reaction = V (for velocity)

= rate of S disappearance

= rate of P formation (the way the rate is usually measured experimentally)

= $k_1 [S]$ (by Law of Mass Action) (3)

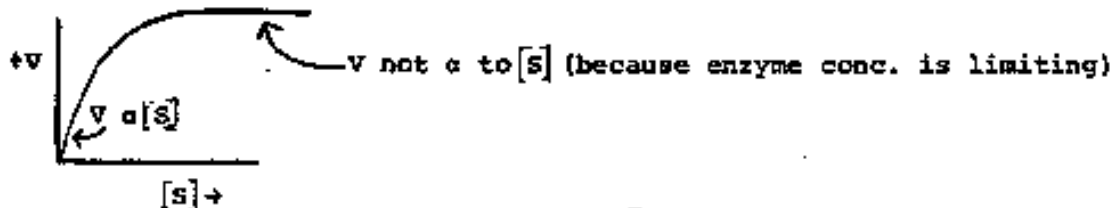
Or V is proportional to $[S]$ for all value of $[S]$:



3. Rate of an Enzyme-Catalyzed Reaction

For an enzyme catalyzed rx

V is not proportional to $[S]$ at all values of $[S]$; for a fixed enzyme concentration (E_0) the relationship of V to S looks like this:



To derive the formula that relates V to $[S]$ and E_0 (enzyme concentration) it is necessary to make four assumptions.

Assumptions:

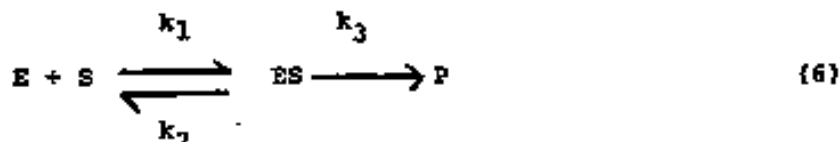
1. The enzyme (E) forms a complex (ES) with the substrate (S):



2. The complex decomposes irreversibly to form the product:



Combining assumptions 1 and 2 the overall reaction becomes:



3. The formation of the complex ($E + S \rightleftharpoons ES$) is much faster than the decomposition of the complex ($ES \rightarrow P$) so rx (5) is rate limiting. This assumption is equivalent to the statement that the rate of the overall reaction is proportional to [ES] and not to [E] and/or [S].
4. The reaction ($E + S \rightleftharpoons ES \rightarrow P$) runs in a steady state so the concentration of ES is constant.

Putting all these assumptions together* produces the following equation:

$$v = \frac{k_3 E_0 [S]}{\frac{k_2 + k_3}{k_1} + [S]} \quad E_0 = \text{initial enzyme concentration} \quad (7)$$

To simplify this equation (and for various other reasons) the 2 quantities V_{max} and K_M are defined:

$$V_{\text{max}} = k_3 (E_0)$$

$$K_M = \frac{k_2 + k_3}{k_1}$$

when these are substituted in equation 7, it becomes:

$$v = \frac{V_{\text{max}} [S]}{K_M + [S]} \quad (8)$$

or

$$v = \frac{V_{\text{max}}}{\frac{K_M}{[S]} + 1} \quad (\text{SA or Michaelis-Menten equation})$$

* see last page for derivation if you want to know the details

Important points to notice about the M.M. equation (8A)

1. V is proportional to $[S]$ at $[S] \ll K_m$
2. V is independent of $[S]$ at $[S] \gg K_m$
3. $V = \frac{V_{max}}{2}$ when $[S] = K_m$

(Convince yourself of these points by substituting the appropriate values in equation (8A) and by consulting the graph of V vs S on the bottom of p.1)

4. Significance of K_m , V_{max} and k_3

V_{max} = speed of reaction at $[S] = \infty$

$V_{max}/2$ = speed of reaction when $[S] = K_m$

k_3 = turnover # of the enzyme
 = the maximum # of molecules of substrate converted per molecule of enzyme per second

when k_2, k_1 are much greater than k_3 (see assumption 3)

$$K_m = \frac{k_2}{k_1}$$

= K_{eq} for $ES \rightleftharpoons E + S$

= dissociation constant for ES

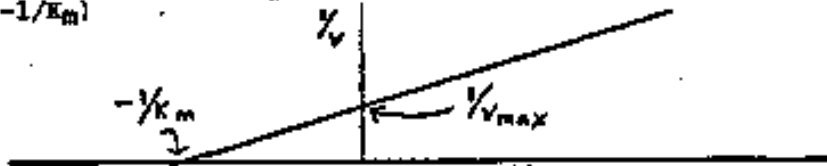
so the lower the K_m , the higher the ability of the enzyme to bind its substr:

5. Determination of V_{max} and K_m

The Michaelis-Menten equation can be rearranged to give:

$$\frac{1}{v} = \left(\frac{K_m}{V_{max}} \right) \left(\frac{1}{s} \right) + \frac{1}{V_{max}} \quad \text{Lineweaver-Burk equation}$$

This equation represents a plot of $1/v$ vs $1/s$ which is a straight line with slope = K_m/V_{max} and y intercept = $1/V_{max}$ (and also x intercept = $-1/K_m$)



∴ The usual way to find a V_{max} and/or K_m is to measure V at different values of S , plot $1/v$ vs $1/s$ (= a Lineweaver-Burk plot) and read the values of $1/V_{max}$ (y intercept) & $-1/K_m$ (x intercept) off the graph.

*Derivation of Michaelis-Menten equation

$$\frac{d(ES)}{dt} = k_1(S)(E) - k_2(ES) - k_3(ES) \quad \text{by law of mass action}$$

$$\frac{d(ES)}{dt} = 0 \quad \text{by assumption 4}$$

$$\text{so } 0 = k_1(S)(E) - k_2(ES) - k_3(ES)$$

$$(E_t) = (E) + (ES) = \text{total enzyme concentration}$$

$$\text{so } (E) = (E_t) - (ES)$$

substituting for (E) and rearranging

$$(ES) = \frac{k_1(E_t)(S)}{k_1(S) + k_2 + k_3}$$

$$v = \frac{dP}{dt} = k_3(ES) \quad \text{by assumption 3}$$

substituting the value for (ES)

$$v = \frac{k_3 \cdot k_1(E_t)(S)}{k_1(S) + k_2 + k_3}$$

which gives equation 7 when rearranged