ENZYME KINETICS

Go to lecture notes and/or supplementary handouts for the following:

1. **Basic observations in enzyme kinetics**
2. **Michaelis-Menten treatment of enzyme kinetics**
3. **Briggs-Haldane treatment of enzyme kinetics**

Always remember the following:

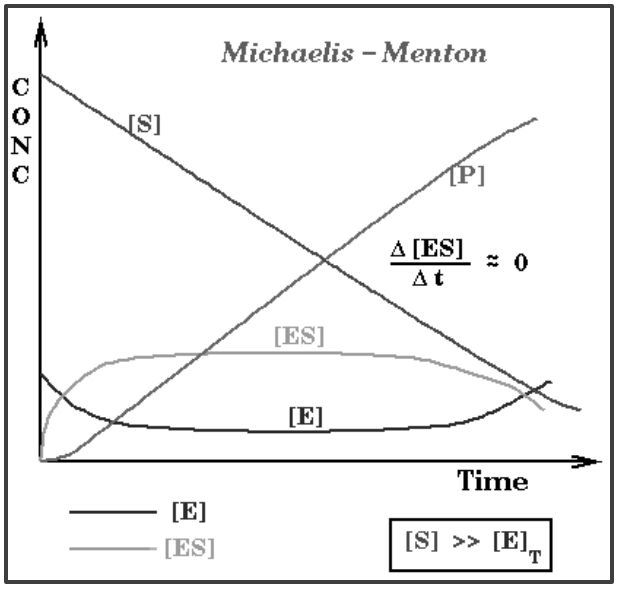
$[E_o] <<<< [S_o]$

$[S_t] \sim [S_o]$, t = early times

rate = k[ES]

$[E_o] = [E] + [ES]$

What happens to S, P, E, ES?



http://dept.physics.upenn.edu/courses/gladney/mathphys/subsection4_1_6.html

Comparison of MM and BH treatments

Michaelis-Menten Treatment

$$V_o = \frac{V_{max}[S_o]}{K_{eq} + [S_o]}$$

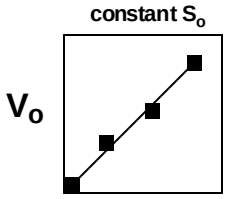
Briggs-Haldane Treatment

$$V_o = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

How does either equation explain the basic observations of enzyme kinetics?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

1. V_o ∝ E_o: linear dependence



$$V_o \propto [E_o]$$

$$V_o = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

$$V_{max} = k_2 [E_o]$$

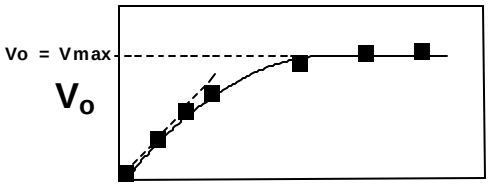
$$V_o = \frac{k_2 [E_o][S_o]}{K_m + [S_o]}$$

How does either equation explain the basic observations of enzyme kinetics?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

2. Vo vs So: Hyperbolic Dependence

constant E_o



S_o

Case 1: Low $[S_o]$

Case 2: High $[S_o]$

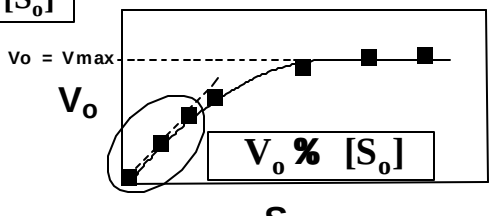
How does either equation explain the basic observations of enzyme kinetics?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

2. Vo vs So: Hyperbolic Dependence

Case 1: Low $[S_o]$

constant E_o



S_o

$$V_o = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

Choose $[S_o] << K_m$

$$V_o \approx \frac{V_{max}[S_o]}{K_m}$$

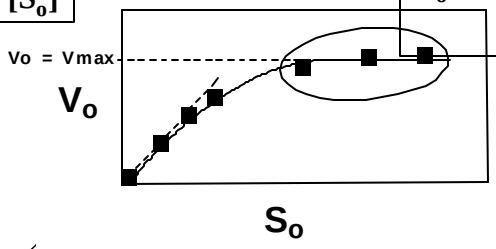
How does either equation explain the basic observations of enzyme kinetics?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

2. Vo vs So: Hyperbolic Dependence

Case 2: High $[S_o]$

constant E_o V_o = constant = V_{max} = $k_2[E_o]$



$$V_o = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

Choose $[S_o] >> K_m$

$$V_o = V_{max}$$

What is the value of S_o when $V_o = V_{max}/2$?

Briggs-Haldane Treatment

$$V_o = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

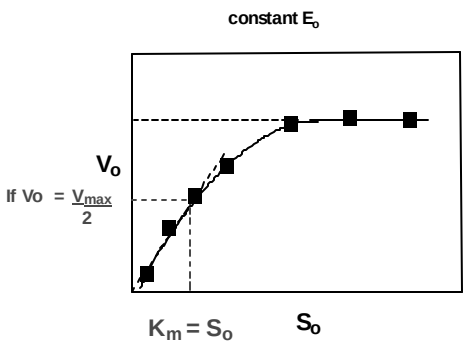
$$\frac{V_{max}}{2} = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

What is the value of S_o when $V_o = V_{max}/2$?

$$\frac{1}{2} = \frac{[S_o]}{K_m + [S_o]}$$

$$2[S_o] = K_m + [S_o]$$

$$K_m = [S_o]$$



This is a numerical relationship only at a specific value of $V_o = V_{max}/2$.

This "$K_m = S_o$" when $V_o = V_{max}/2$ is not a conceptual idea!

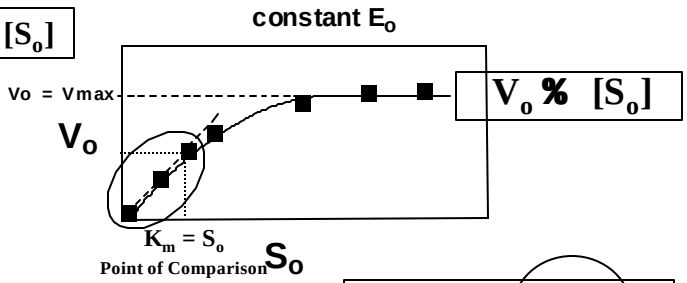
Method to approximates a value for K_m

Recall when we chose [So] << Km?

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

2. Vo vs So: Hyperbolic Dependence

Case 1: Low $[S_o]$



V_o ∝ $[S_o]$

$$V_o = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

Choose $[S_o] << K_m$

$$V_o \cdot \frac{V_{max}[S_o]}{K_m}$$

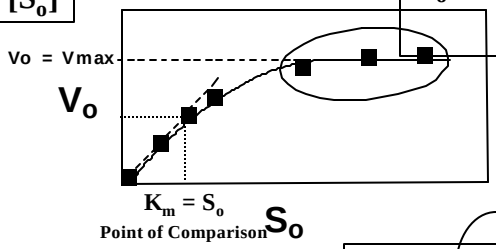
Recall when we chose [So] >> Km??

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

2. Vo vs So: Hyperbolic Dependence

Case 2: High $[S_o]$

constant E_o V_o = constant = V_{max} = $k_2[E_o]$



$$V_o = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

Choose $[S_o] >> K_m$

$$V_o = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

How can we determine if experimental data fits these equations?

Michaelis-Menten Treatment

$$V_o = \frac{V_{max}[S_o]}{K_{eq} + [S_o]}$$

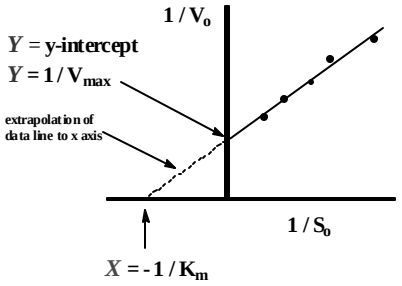
Briggs-Haldane Treatment

$$V_o = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

Transform the equations to a linear form: Lineweaver-Burk

MICHAELIS-MENTEN EQUATION: $V_o = \dfrac{V_{max}[S_o]}{K_m + [S_o]}$

LINEWEAVER-BURK EQUATION: $1/V_o = K_m/V_{max}(1/S_o) + 1/V_{max}$



X = x-intercept (*X* is a negative number)

$y = mx + b$

$y = 1/V_o \quad m = K_m/V_{max}$

$x = 1/S_o \quad b = 1/V_{max}$

NOTE: If data points fit a straight line, the data is said to "fit" the theoretical equation

$V_{max} = 1/Y$

$K_m = 1/X$

What is the interpretation of K_m? Case #IV

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

$$V_o = \frac{k_2[E_o][S_o]}{\left(\frac{k_2 + k_{-1}}{k_1}\right) + [S_o]} = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

Recall

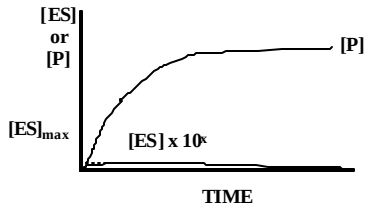
$[S_o] >> [E_o]$ at all $[S_o]$

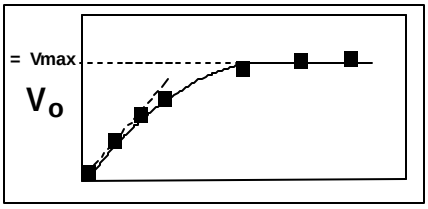
CASE IV: $k_{-1} \sim k_2$; $K_m = (k_1 + k_2)/k_{-1}$

CLASSICAL BRIGGS-HALDANE, OR MICHAELIS-MENTEN KINETICS

[ES] does not rapidly reach equilibrium
[ES] builds up to a small level depending upon the magnitude of K_m
initial rate = $k_2[E_o]/(K_m + S_o) = V_{max}/(K_m + S_o)$

k_2 is called "k_{cat}"





What is the interpretation of K_m? Case #I

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

$$V_o = \frac{k_2[E_o][S_o]}{\left(\frac{k_2 + k_{-1}}{k_1}\right) + [S_o]} = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

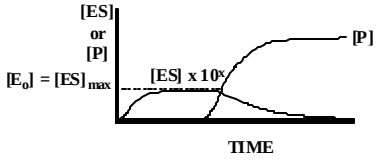
Recall

$[S_o] >> [E_o]$ at all $[S_o]$

CASE I: $k_2 >> k_{-1}$ and $k_2 << k_1$ so k_2 is rate determining step

[ES] builds up to maximum before any P forms
rate = ~~complex function of $[E_o]$ and $[S_o]$~~

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



Under these conditions, the reaction appears to be independent of S_o and only dependent upon E_o

What is the interpretation of K_m? Case #II

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

$$V_o = \frac{k_2[E_o][S_o]}{\left(\frac{k_2 + k_{-1}}{k_1}\right) + [S_o]} = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

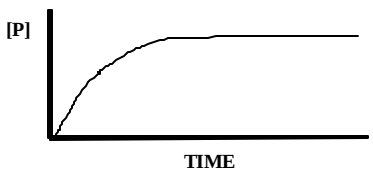
Recall

$[S_o] >> [E_o]$ at all $[S_o]$

CASE II: $k_2 >> k_{-1}$ and $k_2 >> k_1$ so k_1 is rate determining step

[ES] does not build up to any extent
rate = $k_1[E_o][S_o]$

$$E + S \xrightarrow{k_1} ES \xrightarrow{k_2} P + E$$



Under these conditions, the reaction appears to be first order in E_o and S_o and the V_o vs S_o plot would be linear (not hyperbolic)

What is the interpretation of K_m? Case #III

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

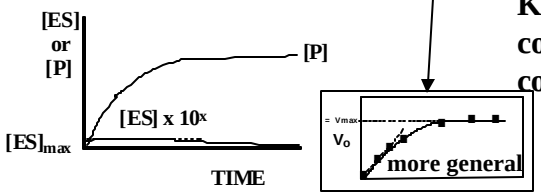
$$V_o = \frac{k_2[E_o][S_o]}{\left(\frac{k_2 + k_{-1}}{k_1}\right) + [S_o]} = \frac{V_{max}[S_o]}{K_m + [S_o]}$$

Recall

$[S_o] >> [E_o]$ at all $[S_o]$

CASE III: $k_{-1} >> k_2$; $K_m = K_d = k_1/k_{-1}$; k_2 is rate determining step

[ES] rapidly reaches equilibrium concentration before any product builds up
[ES] depends upon the magnitude of K_d;
rate = $k_2[ES] = k_2[E_o][S_o]/K_d$ if $K_d >> So$ (Note $K_d << 1$)



Under these conditions, K_m is the dissociation constant for the ES complex:

$$K_m = \frac{k_{-1}}{k_1}$$

Interpretation of K_m

Conceptually, think of K_m as the dissociation constant!

table 8–6

K_m for Some Enzymes and Substrates **# x 10^{-3} M**

Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Higher K_m values = lower affinity of E for S, weaker binding

Lower K_m values = higher affinity of E for S, tighter binding

The Catalytic Constant, k_{cat} or k_2

Conceptually, think of k_{cat} as the ability of E to convert S to P

k_{cat} is called the turnover number

table 8–7

Turnover Numbers (k_{cat}) of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Higher k_{cat} values = higher ability of E to convert S to P

Lower k_{cat} values = lower ability of E to convert S to P

The Meaning of the "Specificity Constant" k_{cat}/K_m

Conceptually, think of k_{cat}/K_m as the 2nd order rate constant if E reacted with S without forming ES

$$E + S \xrightarrow{k} E + P$$

Values for the "Specificity Constant" k_{cat}/K_m

Conceptually, think of k_{cat}/K_m as the 2nd order rate constant if E reacted with S without forming ES ($[S_o] << K_m$)

Units of k_{cat}/K_m are the same as a second order rate constant:

$$(k_{cat} = s^{-1})/(K_m = M) = M^{-1}s^{-1}$$

$$-dP/dt = k[E_o][S_o] \quad Ms^{-1} = (M^{-1}s^{-1})(M)(M)$$

table 8–8

Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8
Triose phosphate isomerase	Glyceraldehyde 3-phosphate	4.3×10^3	4.7×10^{-4}	2.4×10^8

Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science*, p. 166, W.H. Freeman and Company, New York.

Upper limit for 2nd order rxn = diffusion controlled = 10^8 to 10^9 $M^{-1}s^{-1}$