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:

\[ [E_o] \ll [S_o] \]

\[ [S_t] \approx [S_o], \ t = \text{early times} \]

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

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

Briggs-Haldane Treatment

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

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

1. \( V_o \% E_o \): linear dependence

\[ k_1 \quad \text{constant } S_o \]

\[ V_o \]

\[ E_o \]

1. \( V_o \% [E_o] \)

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

\[ V_{\text{max}} = k_2 [E_o] \]

\[ V_o = \frac{k_2 [E_o][S_o]}{K_m + [S_o]} \]
How does either equation explain the basic observations of enzyme kinetics?

\[
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} P + E \quad \text{K}_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}
\]

2. \(V_o\) vs \(S_o\): Hyperbolic Dependence

Case 1: Low \([S_o]\)

Case 2: High \([S_o]\)

\[
V_o = V_{max}
\]

Case 1: Low \([S_o]\)

Case 2: High \([S_o]\)

\[
V_o = \frac{V_{max}[S_o]}{K_m + [S_o]} \quad \text{Choose} \quad [S_o] \ll K_m
\]

\[
V_o \propto \frac{V_{max}[S_o]}{K_m}
\]
How does either equation explain the basic observations of enzyme kinetics?

\[
E + S \xrightarrow{k_1} ES \xrightarrow{k_2} P + E \quad \text{K}_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}
\]

2. \(V_o\) vs \(S_o\): Hyperbolic Dependence

Case 2: High \([S_o]\)

\(V_o = \text{constant} = V_{\text{max}} = k_2[E_o]\)

Choose \([S_o] \gg K_m\)

\(V_o = \frac{V_{\text{max}}[S_o]}{K_m + [S_o]}\)

What is the value of \(S_o\) when \(V_o = V_{\text{max}}/2\)?

Briggs-Haldane Treatment

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

\[
\frac{V_{\text{max}}}{2} = \frac{V_{\text{max}}[S_o]}{K_m + [S_o]}
\]
What is the value of $S_o$ when $V_o = V_{\text{max}}/2$?

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

2. \[
2[S_o] = K_m + [S_o]
\]

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

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

Method to approximate a value for $K_m$

Recall when we chose $[S_o] \ll K_m$?

\[
E + S \xrightleftharpoons[k_1]{k_-} ES \xrightarrow{k_2} P + E \quad \text{Keq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}
\]

2. $V_o$ vs $S_o$: Hyperbolic Dependence

Case 1: Low $[S_o]$

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

Choose $[S_o] \ll K_m$

\[
V_o = \frac{V_{\text{max}}[S_o]}{K_m}
\]
Recall when we chose $[S_o] >> K_m$?

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

2. $V_o$ vs $S_o$: Hyperbolic Dependence

Case 2: High $[S_o]$

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

Choose $[S_o] >> K_m$

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

Point of Comparison: $S_o$

Constant $E_o$

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

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:**

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

**Lineweaver-Burk Equation:**

\[ \frac{1}{V_o} = \frac{K_m}{V_{max}} \left( \frac{1}{S_o} \right) + \frac{1}{V_{max}} \]

\[ Y = \frac{1}{V_o} \quad y = mx + b \]

\[ X = \frac{1}{S_o} \quad m = \frac{K_m}{V_{max}} \]

\[ x = \frac{1}{S_o} \quad b = \frac{1}{V_{max}} \]

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

\[ V_{max} = \frac{1}{y} \]

\[ K_m = \frac{1}{x} \]

---

**What is the interpretation of \( K_m \)? Case #IV**

\[ E + S \xrightarrow{k_1} ES \xrightarrow{k_2} P + E \]

**Recall**

\[ [S_o] >> [E_o] \]

\[ K_m = \frac{k_1 + k_2}{k_1} \]

**Classical Briggs-Haldane, or Michaelis-Menten Kinetics**

\[ [ES] \text{ does not rapidly reach equilibrium} \]

\[ [ES] \text{ builds up to a small level depending upon the magnitude of } K_m \]

\[ \text{initial rate} = \frac{k_2[E_o]}{(K_m + S_o)} = \frac{V_{max}}{(K_m + S_o)} \]

\[ k_2 \text{ is called } "k_{cat}" \]
**What is the interpretation of $K_m$? Case #I**

$$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} P + E \quad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

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

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

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**

$$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} P + E \quad K_{eq} = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

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

Recall

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

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

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

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

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

$V_o = \frac{k_2[E_o][S_o]}{k_2 + k_{-1}} + [S_o]$

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

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 $K_d >> S_o$ (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>
<thead>
<tr>
<th>$K_m$ for Some Enzymes and Substrates</th>
<th># x 10^{-3}$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>Substrate</td>
</tr>
<tr>
<td>Catalase</td>
<td>$H_2O_2$</td>
</tr>
<tr>
<td>Hexokinase (brain)</td>
<td>$ATP$</td>
</tr>
<tr>
<td></td>
<td>$\alpha$-Glucose</td>
</tr>
<tr>
<td></td>
<td>$\alpha$-Fructose</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>$HCO_3$</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Glycyltyrosinglycine</td>
</tr>
<tr>
<td></td>
<td>$N$-Benzoyltyrosinamide</td>
</tr>
<tr>
<td>$\beta$-Galactosidase</td>
<td>$\alpha$-Lactose</td>
</tr>
<tr>
<td>Threonine dehydratase</td>
<td>L-Threonine</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>H$_2$O$_2$</td>
<td>40,000,000</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>HCO$_3^-$</td>
<td>400,000</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>Acetylcholine</td>
<td>14,000</td>
</tr>
<tr>
<td>$\beta$-Lactamase</td>
<td>Benzylpenicillin</td>
<td>2,000</td>
</tr>
<tr>
<td>Fumarase</td>
<td>Fumarate</td>
<td>800</td>
</tr>
<tr>
<td>RecA protein (an ATPase)</td>
<td>ATP</td>
<td>0.4</td>
</tr>
</tbody>
</table>

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 2$^{nd}$ 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:

$$\frac{k_{cat}}{K_m} = s^{-1} = M^{-1}s^{-1}$$

$$-\frac{dP}{dt} = k[E_o][S_o] = M^{-1}s^{-1}$$

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$k_{cat}$</th>
<th>$K_m$</th>
<th>$k_{cat}/K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td>Acetylcholine</td>
<td>$1.4 \times 10^5$</td>
<td>$5 \times 10^{-1}$</td>
<td>$1.6 \times 10^4$</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>CO$_2$</td>
<td>$1 \times 10^8$</td>
<td>$1.2 \times 10^{-2}$</td>
<td>$8.3 \times 10^7$</td>
</tr>
<tr>
<td>Catalase</td>
<td>HCO$_3^-$</td>
<td>$4 \times 10^5$</td>
<td>$2.6 \times 10^{-3}$</td>
<td>$1.5 \times 10^5$</td>
</tr>
<tr>
<td>Cysteine oxidase</td>
<td>Cysteine oxidase</td>
<td>$4 \times 10^5$</td>
<td>$1.1$</td>
<td>$4 \times 10^5$</td>
</tr>
<tr>
<td>Fumarase</td>
<td>Fumarate</td>
<td>$5.7 \times 10^4$</td>
<td>$2 \times 10^{-1}$</td>
<td>$2.8 \times 10^4$</td>
</tr>
<tr>
<td>Malate</td>
<td>Malate</td>
<td>$8 \times 10^5$</td>
<td>$5 \times 10^{-3}$</td>
<td>$1.6 \times 10^5$</td>
</tr>
<tr>
<td>β-Lactamase</td>
<td>Benzylpenicilline</td>
<td>$9 \times 10^5$</td>
<td>$2.5 \times 10^{-3}$</td>
<td>$3.6 \times 10^5$</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>Glyceraldehyde 3-phosphate</td>
<td>$2.0 \times 10^5$</td>
<td>$2 \times 10^{-3}$</td>
<td>$1 \times 10^5$</td>
</tr>
</tbody>
</table>

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