Michaelis-Menten Kinetics

\[
E + S \xleftrightarrow{k_1}{k_{-1}} ES \xleftrightarrow{k_2}{k_{-2}} EP \xleftrightarrow{k_3}{k_{-3}} E + P
\]

Turnover is controlled by \( k_2 \).

Satisfied by initial rates or velocities and initial \([S]\) \(>>\) \(E_0\), whence \([P] \to 0\).

\[
\text{Rate} = \text{velocity} = v \equiv k_2 [ES] = \frac{k_2 E_0 [S]}{K_M + [S]} = \frac{V_{\text{max}} [S]}{K_M + [S]}
\]

where \( K_M = \frac{k_{-1} + k_2}{k_1} \) \(\leftarrow\) Michaelis constant

and \( V_{\text{max}} = k_2 E_0 \) \(\Rightarrow\) \(k_2\) is sometimes called \(k_{\text{cat}}\)

\[
\begin{align*}
\text{hyperbolic kinetics} \\
V_{\text{max}} & \quad v \\
1/2 V_{\text{max}} & \quad K_M \\
[S] & \\
\end{align*}
\]

Figure 7-1

Kinetic Plots

\[
v = \frac{V_{\text{max}} [S]}{K_M + [S]} \quad \text{hyperbolic}
\]

where \( v \equiv \) initial rates

\([S] \equiv \) initial substrate concentrations \(>>E_0\)
Linearized Plots

(1) **Lineweaver-Burk**: $1/v$ vs $1/[S]$ — Double Reciprocal

\[
\frac{1}{v} = \frac{1}{V_{\text{max}}} + \frac{K_M}{V_{\text{max}}} \cdot \frac{1}{[S]} 
\]

Figure 7-2

(2) **Eadie-Hofsetr**: $v/[S]$ vs $v$ — Single Reciprocal

\[
\frac{v}{[S]} = \frac{V_{\text{max}} - v}{K_M} 
\]

Figure 7-3
(3) **Dixon:** $\frac{[S]}{v}$ vs $[S]$

$$\frac{[S]}{v} = \frac{[S]}{V_{\text{max}}} + \frac{K_M}{V_{\text{max}}}$$

![Graph](image)

**Inhibition of Enzymatic Reactions**

(1) **Competitive Inhibition**

A competitive inhibitor is a molecule that resembles the substrate and occupies the catalytic site because of its similarity in structure, but is completely unreactive. By occupying the active site, the inhibitor prevents normal substrates from binding and being catalyzed. Operationally, competitive inhibitors bind reversibly to the active site. Hence, inhibition can be reversed by (1) diluting the inhibitor, or (2) swamping the system with excess substrate.

**Reaction Mechanism**

$$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow[k_2]{k_{-2}} E + P$$

Define $K_I = \frac{[E][I]}{[EI]}$
**Expectation**

A competitive inhibitor increases $K_M$, but does not affect $V_{\text{max}}$ (because of sufficiently high $[S]$, $S$ will displace $I$).

\[ v = \frac{V_{\text{max}} [S]}{[S] + K_M \left[ 1 + \frac{[I]}{K_I} \right]} = \frac{V_{\text{max}} [S]}{[S] + K_M' \left[ 1 + \frac{[I]}{K_I} \right]} \]

where $K_M' = K_M \left[ 1 + \frac{[I]}{K_I} \right] \Leftarrow \text{modified } K_M$
\[ \text{Slope} = \frac{V_{\text{max}}}{K_M} - \frac{V_{\text{max0}}}{K_M^2} \]

Figure 7-7

\[ \frac{[S]}{v} = \frac{K_M}{V_{\text{max}}} \]

\[ \frac{[I]}{v} = 0 \]

Parallel

\[ \text{Slope} = \frac{1}{V_{\text{max}}} \]

Figure 7-8
**Classical Example of Competitive Inhibition**

Enzyme: succinic dehydrogenase (SDH), which catalyzes the oxidation of succinic acid to fumaric acid.

```
CH3\text{COOH} \underset{SDH}{\xrightarrow{k_1}} \text{CH2HC} \text{COOH}
```

Here substrate $\equiv$ succinic acid

```
\text{COOH} \quad \text{fumaric acid}
```

inhibitor $\equiv$ malonic acid

\[ K_I = 1 \times 10^{-5} \text{ M}, \quad \text{which means that at } [\text{malmate}] = 10^{-5} \text{ M, the apparent affinity of the enzyme for succinate decreases by a factor of 2!} \]

(2) **Noncompetitive Inhibition**

A noncompetitive inhibitor is one that binds reversibly to the enzyme, but not at the active site itself, so that the substrate can still bind at the active site, but there’s no catalyzed transformation.

This type of inhibition cannot be overcome by a large amount of substrate, thus noncompetitive inhibition.

**Reaction Mechanism**

```
E + S \xrightleftharpoons[k^{-1}_1]{k_1} ES \xrightleftharpoons[k^{-2}_2]{k_2} E + P
```

```
I \quad I
```

Define $K_I = \left( \frac{[E][I]}{[EI]} \right)_{eq}$, $K_{SI} = \left( \frac{[ES][I]}{[ES \cdot I]} \right)_{eq}$
**Expectation**

The rate or velocity decreases to the extent that $E$ is complexed by inhibitor, irrespective of $EI$ or $ES \cdot I$. Thus, a noncompetitive inhibitor decreases $V_{\text{max}}$ without affecting the apparent $K_M$. The situation is not overcome by swamping the system with substrate.

![Hyperbolic Plots](image)

**Typical Case**

$K_I = K_{SI}$, i.e., the affinity of the inhibitor site for the inhibitor does not depend on whether $E$ is bound with $S$. In this instance

$$v = \frac{V_{\text{max}} [S]}{1 + \frac{[I]}{K_I} ([S] + K_M)} = \frac{V'_{\text{max}} [S]}{K_M + [S]}$$

where $V'_{\text{max}} = \frac{1}{1 + \frac{[I]}{K_I}} \cdot V_{\text{max}}$
Lineweaver-Burk Plots

\[ \frac{1}{v} \]

\[ \frac{1}{v_\text{max}} - \frac{1}{v} \]

\[ \frac{1}{K_M} - \frac{1}{[S]} \]

inhibitor

no inhibitor

Figure 7-10

Eadie-Hofster Plot

\[ \frac{v}{[S]} \]

\[ \frac{1}{v} \]

\[ [I] \]

0

\[ v \]

lines parallel

no inhibitor

Figure 7-11
More General Case

$K_I \neq K_{SI}$ allosteric interaction between catalytic and inhibitor sites.

\[
v = \frac{k_2 E_0 [S]}{1 + \frac{[I]}{K_{SI}}} \cdot \frac{1}{\left( \frac{\alpha}{\beta} \right) K_M + [S]} = \frac{V'_\text{max} [S]}{1 + \frac{[I]}{K_M}} \cdot \frac{1}{\left( \frac{\alpha}{\beta} \right) K_M + [S]}
\]

where \( \left( \frac{\alpha}{\beta} \right) = \frac{1 + \frac{[I]}{K_I}}{1 + \frac{[I]}{K_{SI}}} \) and $V'_\text{max} = \frac{V_\text{max}}{\beta}$

Both $V'_\text{max}$ and $K_M$ can be affected!
Case of $K_{SI} \gg K_I$

This is an interesting situation where the allosteric interaction between catalytic and inhibitor sites is so strong that binding is mutually exclusive.

$$v = \frac{1}{\beta} \left( \frac{V_{\text{max}} [S]}{\left( \frac{\alpha}{\beta} \right) K_M + [S]} \right)$$

If $K_{SI} \gg [I]$, $\beta \to 1$

then

$$v = \frac{V_{\text{max}} [S]}{\left( 1 + \frac{[I]}{K_I} \right) K_M + [S]} = \frac{V_{\text{max}} [S]}{K_M' + [S]}$$

which is the result for competitive inhibition!

**Irreversible Modification**

Permanent or irreversible modification of the active site often yields a situation that behaves like the case of simple noncompetitive inhibition!

For example, irreversible modification by the chemical alkylating agent iodoacetamide, which reacts with exposed sulfhydryl groups such as a cysteine to form a covalently modified

$$\text{Enzyme} - \text{S} - \text{CH}_2 - \text{C} - \text{NH}_2$$

or irreversible modification by carbodiimide of carboxyl groups from glutamate and aspartate.
Oftentimes, chemically modified enzyme is inactive catalytically. So, $v \propto [\text{Enzyme}]_{\text{active}}$, i.e., enzyme not chemically modified and unreacted enzyme behave normally with $K_M$ identical to the situation prior to the addition of “inhibitor.”

In fact

$$v = \frac{f_{\text{unmodified}} V_{\text{max}} [S]}{K_M + [S]}$$

like in simple noncompetitive inhibition.

**Well-Studied Enzyme System That Behaves According to Michaelis-Menten Kinetics**

Ferriprotoporphyrin and (Mn)$_2$ protein catalase; catalyze the decomposition of $\text{H}_2\text{O}_2$.

$$2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$$

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Velocity$^a$, $\frac{d[\text{H}_2\text{O}_2]}{dt}$, M S$^{-1}$</th>
<th>$E_a$ (kJ/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>$10^{-8}$</td>
<td>71</td>
</tr>
<tr>
<td>HBr</td>
<td>$10^{-4}$</td>
<td>50</td>
</tr>
<tr>
<td>Fe$^{2+}$/Fe$^{3+}$</td>
<td>$10^{-3}$</td>
<td>42</td>
</tr>
<tr>
<td>Hematin or Hb</td>
<td>$10^{-1}$</td>
<td>—</td>
</tr>
<tr>
<td>Fe(OH)$_2$ TETA$^+$</td>
<td>$10^3$</td>
<td>29</td>
</tr>
<tr>
<td>Catalase</td>
<td>$10^7$</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 7-1. Velocities and Energies for Protein Catalases
for $[\text{H}_2\text{O}_2] = 1 \text{ M}$, $[\text{catalyst}]_{\text{active sites}} = 1 \text{ M}$

triethylenetetramine

$\Delta G_{298}^0 = -103.10 \text{ kJ mol}^{-1}$ for $\text{H}_2\text{O}_2(\text{aq}) \rightarrow \text{H}_2\text{O} (\text{l}) + \frac{1}{2} \text{O}_2(\text{g})$

$\Delta H_{298}^0 = -94.64 \text{ kJ mol}^{-1}$

Turnover number ($S^{-1}$) $\equiv$ maximum velocity divided by the concentration of enzyme active sites
## Table 7-2. Turnover Numbers for Various Enzymes and Substrates

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Turnover No. ((S^{-1})^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>(\text{H}_2\text{O}_2)</td>
<td>(9 \times 10^6)</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>(\text{Acetylcholine})</td>
<td>(1.2 \times 10^4)</td>
</tr>
<tr>
<td>Lactate dehydrogenase (chicken)</td>
<td>(\text{Pyruvate})</td>
<td>(6 \times 10^3)</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>(\text{Acetyl-L-tyrosine ethyl ester})</td>
<td>(4.3 \times 10^2)</td>
</tr>
<tr>
<td>Myosin</td>
<td>ATP</td>
<td>(3)</td>
</tr>
<tr>
<td>Fumarase</td>
<td>(\text{L-Malate})</td>
<td>(1.1 \times 10^3)</td>
</tr>
<tr>
<td></td>
<td>(\text{Fumarate})</td>
<td>(2.5 \times 10^3)</td>
</tr>
<tr>
<td>Carbonic anhydrase (bovine)</td>
<td>(\text{CO}_2)</td>
<td>(8 \times 10^4)</td>
</tr>
<tr>
<td></td>
<td>(\text{HCO}_3^-)</td>
<td>(3 \times 10^4)</td>
</tr>
</tbody>
</table>

*Typically, turnover number \(\sim 10^3 \ S^{-1}\) within a factor of 10.
Two Intermediate Complexes

The reaction catalyzed by catalase is essentially irreversible. Therefore, it is not necessary to worry about $EP$. Most enzymatic reactions are readily reversible, so an enzyme-product complex can often be detected.

**Good Example**

\[
\begin{align*}
\text{fumarate} & \quad \text{L-malate} \\
\begin{array}{c}
\text{H} \quad \text{COO}^- \\
\text{C} \quad \text{||} \\
\text{H} \quad \text{C} \\
\text{OOC}^- \quad \text{H}
\end{array} & \quad \begin{array}{c}
\text{C} \\
\text{HO} \quad \text{C} \\
\text{COO}^- \\
\text{OOC}^- \quad \text{H}
\end{array}
\end{align*}
\]

Fumarase

\[
E + S \xrightleftharpoons[k_1]{k_4} ES \xrightleftharpoons[k_2]{k_1} EP \xrightleftharpoons[k_3]{k_2} E + P
\]

Equilibrium 20% 80% at room temperature

So in this case, you cannot ignore $EP$! More complete treatment leads to the following results:

**1. Forward Reaction:** $[P]_0 = 0$

\[
v_F = \left( \frac{d[P]}{dt} \right)_0 = k_3 [EP]
\]

\[
v_F = \frac{k_2 k_3 E_0 [S]}{k_2 + k_{-2} + k_3 \left( \frac{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3}{k_1 (k_2 + k_{-2} + k_3)} + [S] \right)}
\]

which can be reduced to

\[
v_F = \frac{V_F[S]}{K_M^F + [S]}
\]

with \( V_F = \frac{k_2 k_3 E_0}{k_2 + k_{-2} + k_3} \) and \( K_M^F = \frac{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3}{k_1 (k_2 + k_{-2} + k_3)} \)
(2) **Reverse Reaction:** \([S]_0 = 0^1\)

\[
v_R = \left( \frac{d[S]}{dt} \right)_0 = k_{-1}[ES] = \frac{V_R[P]}{K^R_M + [P]}
\]

with \(V_R = \frac{k_{-1}k_2E_0}{k_{-1} + k_2 + k_{-2}}\) and \(K^R_M = \frac{k_{-1}k_{-2} + k_{-2}k_3 + k_2k_3}{k_{-3}(k_{-1} + k_2 + k_{-2})}\)

(3) **Net Velocity**

\[
\]

Note that at equilibrium, \(v = 0\) and hence

\[
V_F K^R_M [S]_{eq} = V_R K^F_M [P]_{eq}
\]

and

\[
K = \frac{[P]_{eq}}{[S]_{eq}} = \frac{V_F K^R_M}{V_R K^F_M}
\]

There are too many kinetic constants here to be sorted out by steady state kinetics.

We must appeal to the methods of rapid kinetics.

---