Lecture 18: Introduction to Inhibitors.

Goals:
- Distinguish between types of inhibitors (competitive, mixed, covalent)
- Key properties of competitive inhibitors.
- Effect of competitive inhibitors on steady-state kinetics.
- Measurement of $K_i = K_D$ for competitive inhibitors.

Studies on Inhibitors are useful for:
1. Mechanistic studies to learn about how enzymes interact with their substrates.
2. Understanding the role of inhibitors in enzyme regulation.
3. Drugs if they inhibit aberrant biochemical reactions:
   - penicillin, ampicillin, etc. interfere with the synthesis of bacterial cell walls
4. Understanding the role of biological toxins:
   - Amino acid analogs - useful herbicides (i.e. roundup)
   - Insecticides - chemicals targeted for insect nervous system.

Types of Inhibitors:
1. Competitive – inhibitor blocks substrate, reversibly
2. Mixed type (allostERIC) – inhibitor causes allosteric change, reversibly.
3. Covalent/Suicide – inhibitor covalently modifies enzyme, usually in active site.

Competitive Inhibition
1. Inhibitor binds to the same site on the enzyme as the substrate.
2. Inhibitor ONLY binds to the free enzyme.
3. Inhibitor usually is structurally very similar to the substrate. Example: succinate is the normal substrate for the enzyme succinate dehydrogenase. Malonate is an effective competitive inhibitor of this enzyme.
4. The inhibitor can’t undergo the chemical reaction.

Effect of competitive inhibitor on kinetics: A competitive inhibitor reduces the amount of [E] by the formation of [EI] complex. The inhibitor cannot affect the [ES] complex after it has formed since the inhibitor can no longer bind. How will high concentrations of substrate affect the inhibition?

$E + S \rightleftharpoons (ES) \rightarrow EP$

$I$

$K_D = \frac{(E)}{(EI)}$

$K_i = \frac{(E)(I)}{(EI)}$
There are two consequences of a competitive inhibitor binding on the kinetics of the enzyme:

1. \( V_{\text{MAX}} \) is unchanged: At high levels of substrate all of the inhibitor can be displaced by substrate, and \( E_{\text{TOT}} = [ES] \). \( V_{\text{MAX}} = k_{\text{CAT}}[ES] = k_{\text{CAT}}[E_{\text{TOT}}] \).

2. The observed \( K_M \) is increased: It requires more substrate to reach 1/2 maximal velocity because some of the enzyme is complexed with inhibitor. The change in \( K_M \) can be used to determine how well the inhibitor binds to the free enzyme.

**Measuring Inhibitor-Enzyme Affinity (\( K_i \))**

1. Measure initial velocity for different \([S]\), in the absence of the inhibitor.
2. Measure initial velocity for different \([S]\), in the presence of a known and fixed concentration of inhibitor.
3. Both data sets are plotted on a double reciprocal plot.
4. Ratio of the slopes gives \( \alpha \) (degree of inhibition).
5. \( K_i = [I]/(\alpha - 1) \).

**Example:**

<table>
<thead>
<tr>
<th>[S] mM</th>
<th>( v_i )</th>
<th>( v_i )</th>
<th>1/[S]</th>
<th>1/v</th>
<th>1/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.7</td>
<td>9.1</td>
<td>1.0</td>
<td>0.060</td>
<td>0.110</td>
</tr>
<tr>
<td>5</td>
<td>50.0</td>
<td>33.3</td>
<td>0.2</td>
<td>0.020</td>
<td>0.030</td>
</tr>
<tr>
<td>10</td>
<td>66.7</td>
<td>50.0</td>
<td>0.1</td>
<td>0.015</td>
<td>0.020</td>
</tr>
</tbody>
</table>

\[ y = 0.1x + 0.01 \]

\[ y = 0.05x + 0.01 \]

\[ \alpha = 0.1 \]

\[ 0.05 = 2 \]

\[ \frac{0.1}{0.05} = 2 \]

\[ \alpha = \frac{9}{0.05} = 2 \]

\[ K_i = \frac{10 \text{ mM}}{2-1} = 10 \text{ mM} = K_d \]

**Inhibitor Present**

\[ v = \frac{V_{\text{MAX}} \alpha K_M}{[S]} + \frac{1}{V_{\text{MAX}}} \]

\[ \frac{1}{v} = \frac{1}{V_{\text{MAX}}} \frac{1}{[S]} + \frac{1}{V_{\text{MAX}}} \]

\( \alpha = \text{ratio of slopes} \)

\( (+/\text{no inh}) \)

\[ K_i = [I]/(\alpha - 1) \]

inhibitor can go to \( \frac{1}{2} \) sat

\( \text{Eng} \) (absence of sub)