Enzyme Kinetics

• Most Reactions involve multiple substrates.
  • There are three different ways that the binding substrates can occur.
    • Ordered sequential
    • Random sequential
    • Double displacement (Ping Pong)
  • Determining the binding order can tell you something about the mechanism of the reaction.

Enzyme Kinetics

• Ordered sequential

Enzyme Kinetics

• Random sequential
**Enzyme Kinetics**

- Double displacement (Ping Pong)

![Chem 452, Lecture 4 - Enzymes](image1)

- Not all enzyme obey the Michaelis-Menten model.

![Chem 452, Lecture 4 - Enzymes](image2)

- The behavior is often seen with allosterically regulated enzymes.

![Chem 452, Lecture 4 - Enzymes](image3)

- The behavior is often seen with allosterically regulated enzymes.
Enzyme Kinetics

- The behavior is often seen with **allosterically regulated** enzymes.

![Graph showing enzyme kinetics](image)

Enzyme Inhibition

- The inhibition of enzyme activity can be physiological or not.
- It can be reversible or irreversible.
- Many drugs, pesticides and herbicides operate by inhibiting enzyme activity.

Enzyme Inhibition

- Irreversible inhibition, while not usually physiological, can be used as a tool to study an enzyme.
  - Catalytic groups at the active site are often more reactive than groups elsewhere on the enzyme.

Enzyme Inhibition

- Irreversible inhibition, while not usually physiological, can be used as a tool to study an enzyme.
  - DPPF is a powerful nerve gas toxin.
Enzyme Inhibition

- Irreversible inhibition, while not usually physiological, can be used as a tool to study an enzyme.
  - Catalytic groups at the active site are often more reactive than groups elsewhere on the enzyme.

Bromoacetol phosphate is an affinity label which mimics the natural substrate for the enzyme triosephosphate isomerase.

Suicide inhibitors of monoamine oxidase (MAO)
Enzyme Inhibition

- Irreversible inhibition, while not usually physiological, can be used as a tool to study an enzyme.
  - Catalytic groups at the active site are often more reactive than groups elsewhere on the enzyme.

Enzyme Inhibition

- Reversible inhibition comes in three different forms.
  - Competitive
  - Noncompetitive
  - Uncompetitive
- Enzyme kinetics can be used to distinguish between these.

Enzyme Inhibition

- Competitive Inhibition

Substrate

Competitive inhibitor

Enzyme
Enzyme Inhibition

* Competitive Inhibition

\[ K_{\text{M app}} = K_M \left(1 + \frac{[I]}{K_I}\right) \]

Enzyme Inhibition

* Noncompetitive Inhibition
Enzyme Inhibition

Noncompetitive Inhibition

\[ V_{\text{app}} = \frac{V_{\text{max}}}{1 + \frac{I}{K_I}} \]

Enzyme Inhibition

Uncompetitive Inhibition

Substrate

Enzyme

Uncompetitive inhibitor

Enzyme Inhibition

Uncompetitive Inhibition

\[ V_{\text{app}} = \frac{V_{\text{max}}}{1 + \frac{I}{K_I}} \]
**Problem**

C) Ibuprofen is an inhibitor of the enzyme prostaglandin endoperoxide synthase. By inhibiting the synthesis of prostaglandins, ibuprofen reduces both inflammation and pain. Using the data below, determine the type of inhibition that ibuprofen exerts on prostaglandin endoperoxide synthase.

<table>
<thead>
<tr>
<th>[S] (mM)</th>
<th>$v_o$ (mM/min)</th>
<th>$v_o$ (mM/min) /w Ibuprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>23.5</td>
<td>16.67</td>
</tr>
<tr>
<td>1</td>
<td>32.2</td>
<td>25.25</td>
</tr>
<tr>
<td>1.5</td>
<td>36.9</td>
<td>30.49</td>
</tr>
<tr>
<td>2.5</td>
<td>41.8</td>
<td>37.04</td>
</tr>
<tr>
<td>3.5</td>
<td>44</td>
<td>38.91</td>
</tr>
</tbody>
</table>

**Enzyme Inhibition**

- Some inhibitors are transition state analogues instead of substrate analogues.

**Thermodynamics**

"I think that enzymes are molecules that are complementary in structure to the activated complexes of the reactions that they catalyze, that is, to the molecular configuration that is intermediate between the reacting substance and the products of reaction for these catalyzed processes. The attraction of the enzyme molecule for the activated complex would thus lead to a decrease in its energy and hence to the decrease in the energy of activation of the reaction and to the increase in the rate of the reaction."

- Linus Pauling (Nature 161 (1948):707-709)
Synthetic Enzymes

Antibody enzymes (Abzymes)
- Antibodies raised to transitions state analogues exhibit enzymatic activity

Antibodies raised to this compound have ferrochelatase activity (~2,500 x the uncatalyzed reaction)

Enzyme Classification

Enzymes are classified based on the types of reactions they catalyze

<table>
<thead>
<tr>
<th>Class</th>
<th>Type of reaction</th>
<th>Example</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidoreductase</td>
<td>Oxidation-reduction</td>
<td>Lactate dehydrogenase</td>
<td>16</td>
</tr>
<tr>
<td>Transferase</td>
<td>Group transfer</td>
<td>Nicotinamide dehydrogenase</td>
<td>9</td>
</tr>
<tr>
<td>Hydrolase</td>
<td>Hydrolysis reactions (transfer of functional groups to water)</td>
<td>Chymotrypsin</td>
<td>9</td>
</tr>
<tr>
<td>Lyase</td>
<td>Addition or removal of groups to form double bonds</td>
<td>Lactase</td>
<td>17</td>
</tr>
<tr>
<td>Isomerases</td>
<td>Isomerization (intramolecular)</td>
<td>Triose phosphate isomerase</td>
<td>16</td>
</tr>
<tr>
<td>Ligases</td>
<td>Ligation of two subunits at the expense of ATP hydrolysis</td>
<td>Adenylate dipyr-mutase</td>
<td>30</td>
</tr>
</tbody>
</table>

Called Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)
http://www.chem.qmul.ac.uk/iubmb/enzyme/

Next up

- Catalytic Strategies (Chapter 9)
  - Protease reaction (Hydrolysis rxn)
  - Carbonic anhydrase (Hydration rxn)
  - Restriction endonuclease (Hydrolysis rxn)
  - Myosin ATPase