BiCh110 2017

Enzymes: catalysis and regulation (II)

Representation of Kinetic Data

\[ k_{\text{cat}} = \frac{k_c a t}{K_M + [S]} \]

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

What happens to these graphs if:

- \( K_M \) increases 5-fold;
- \( k_{\text{cat}} \) increases 5-fold;
- both \( k_{\text{cat}} \) and \( K_M \) increased 5-fold;
What we will discuss about enzymes:

• Free energy diagrams and transition state theory
• Catalytic strategies of enzymes
• Michaelis-Menten equation (steady-state kinetics)
• Enzyme inhibitors
• Enzyme regulation

Competitive Inhibition: I binds to E but not ES

\[ \frac{[E]}{[E] + [I]} = \frac{E}{1 + \frac{[I]}{K_i}} \]

\[ \frac{[ES]}{[ES]} = \frac{E[S]}{[S] + K_m(l + [I])} \]

\[ \text{Increased } K_m \]

No change in \( V_{max} \)
Noncompetitive inhibition: both $S$ and $I$ bind to $E$

$$\frac{V}{V_{\text{max}}} = \frac{[S]}{K_S + [S]}$$

Decrease in $k_{\text{cat}}$
No change in $K_M$

Mechanism-based inhibitors:
A case study of the Proteasome

Uncompetitive inhibition: $I$ binds to ES

$$\frac{V}{V_{\text{max}}} = \frac{[S]}{K_M + [I]/K_i}$$

Both $K_M$ and $V_{\text{max}}$ change
No change in $k_{\text{cat}}/K_m$

Uncompetitive Inhibitor
Core particle: enclosed proteolytic compartment
Regulatory particle
- recognizes proteolytic tags
- controls access to CP
- unfolds target protein (requires ATP)
- translocates protein to CP (requires ATP)
The proteasome is an N-terminal Threonine protease

![Peptide aldehyde inhibitor (LLnL-al)](image)

Inhibition of all active sites is needed to block proteasome activity

<table>
<thead>
<tr>
<th>Sites inactivated</th>
<th>Inhibitor</th>
<th>Protein substrate</th>
<th>Ovalbumin</th>
<th>Histones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymotrypsin-like</td>
<td>NLVA</td>
<td>11 ± 8</td>
<td>35 ± 2</td>
<td></td>
</tr>
<tr>
<td>Cysteine-like</td>
<td>Ac-AlaL-Ala</td>
<td>9 ± 7</td>
<td>19 ± 8</td>
<td></td>
</tr>
<tr>
<td>Trypsin-like</td>
<td>Leupeptin</td>
<td>14 ± 1</td>
<td>30 ± 13</td>
<td></td>
</tr>
</tbody>
</table>

![β-ring β-ring](image)

Dipeptide Boronic acid inhibitors

Bortezomib (Velcade)

- empty p-orbital on Boron readily accepts nucleophilic attack by -OH

Highly potent inhibitors of Proteasome

<table>
<thead>
<tr>
<th>X</th>
<th>K_i [μM]</th>
<th>X</th>
<th>K_i [μM]</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Inhibitor 1" /></td>
<td>1.06</td>
<td><img src="image" alt="Inhibitor 2" /></td>
<td>0.42</td>
</tr>
</tbody>
</table>
What we will discuss about enzymes:

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Major Regulatory Strategies:

- Allostery and cooperativity
- Isozymes
- Covalent Modifications
  - Kinases and phosphatases (reversible)
  - Ubiquitylation, glycosylation (Irreversible)
  - Proteolytic Activation (Irreversible)
- Enzymatic cascades
- Control of Transcription

Crystal structure of Bortezomib with yeast 20S

Allostery: the basis of enzymatic control

From the Greek: allos = ‘other’
stereos = ‘solid’ or ‘space’

Action at a distance

Examples of allostery:
- enzyme cooperativity
- induced fit
- feedback inhibition
- oligomerization
- gating

Hemoglobin >> Myoglobin \( \times 4 \)

\[
\text{Mb}(O_2) + O_2 \xrightarrow{K} \text{Mb(O}_2\text{)} + O_2
\]

\[
Y = \frac{[\text{MbO}_2]}{[\text{Mb}] + [\text{MbO}_2]}
\]

Fraction of ligand bound

Substituting first eq. into second:

\[
Y = \frac{[O_2]}{[O_2] + K} \quad \Rightarrow \quad Y = \frac{pO_2}{pO_2 + K}
\]

Compare \( O_2 \) binding of Myoglobin and Hemoglobin
Compare O$_2$ binding of Myoglobin and Hemoglobin

Hemoglobin displays a Sigmoidal O$_2$ binding Curve (S-Curve):

\[
1 - \frac{1}{K} \left( \frac{P_O_2}{P_{50}} \right)^n = \frac{Y}{Y + 1}
\]

(Hi this rearranges to)

\[
\log \frac{Y}{1 - Y} = n \log[S] - \log K
\]

Hill coefficient

Cooperative ligand binding generates hypersensitivity in biology

\[
\log \frac{Y}{1 - Y} = n \log[S] - \log K
\]

Cooperativity and the Bohr effect explains oxygen pickup and release by Hemoglobin

Log (pO$_2$) vs. Hemoglobin at pH 7.6 (lung) and pH 7.2 (tissue)
Coupled Equilibria

Equilibrium is pathway-independent.

Therefore $1 \rightarrow 2 \rightarrow 3$ equals $1 \rightarrow 4 \rightarrow 3$.

$K_s' / K_s = K_s' / K_s = \text{const.}$

Reciprocity:
- binding of substrate promotes conformational change
- conformational change promotes substrate binding

Mechanism of cooperativity in Hemoglobin

Structural basis for cooperative oxygen binding to Hemoglobin
Example 2: ATCase
catalyzes commitment step in pyrimidine synthesis

- Everything that makes it through this step will eventually end up as C, T or U.
- It is the optimum opportunity for feedback regulation.

Allosteric regulation in ATCase
ATCase displays:
- Sigmoidal kinetics
- Multimeric enzyme with Cooperative binding of S
- Feedback inhibition by CTP
- allosteric (regulatory) sites on separate polypeptide chains

Molecular basis of regulation in ATCase

The c1 catalytic subunit catalyzes reaction.
The r2 regulatory subunit binds CTP.
CTP effects feedback regulation by favoring ATCase in the T state

Regulation by isozymes: lactate dehydrogenase

Increase in serum levels of the H₄ relative to the H₃M isozyme is an indicator of a recent heart attack.

Covalent modification on proteins

<table>
<thead>
<tr>
<th>TABLE 10.1 Common covalent modifications of protein activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modification</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>Phosphorylation</td>
</tr>
<tr>
<td>Acetylation</td>
</tr>
<tr>
<td>Myristoylation</td>
</tr>
<tr>
<td>ADP ribosylation</td>
</tr>
<tr>
<td>Farnesylation</td>
</tr>
<tr>
<td>γ-Carboxylation</td>
</tr>
<tr>
<td>Sulfation</td>
</tr>
<tr>
<td>Ubiquitination</td>
</tr>
</tbody>
</table>
Proteolytic activation of zymogens

<table>
<thead>
<tr>
<th>Site of synthesis</th>
<th>Zymogen</th>
<th>Active enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>Pepsinogen</td>
<td>Pepsin</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Chymotrypsinogen</td>
<td>Chymotrypsin</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Trypsinogen</td>
<td>Trypsin</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Preprorenninpeptidase</td>
<td>Carboxypeptidase</td>
</tr>
</tbody>
</table>

- The pancreas releases about 1.5 L of enzyme juice each day!
- These dense (protein shows up dense on EM) zymogen granules are released into the duodenum where a cascade of activation is initiated by enteropeptidase:

Enzymatic cascade to generate Fibrin during blood clotting

MAP Kinase signaling: combining covalent modification with enzyme cascade