Enzymes: Basic Concepts & Kinetics

Based on
Chapter 8, Biochemistry, by Berg, Tymoczko, Stryer
(from a set of slides on Chapter 8 from 2007)
What is a protein

- Building block of proteins are amino acids

- Amino acids are linked using peptide bonds

- Amino acid sequences and their interaction determine shape of folded protein

https://www.nature.com/scitable/topicpage/protein-structure-14122136
What is an enzyme?

- Nearly all known enzymes are proteins (long chain of 100+ amino acids)
- Some amino acids (residues) form one or more active sites
- Speeds the rate of a biochemical reaction by binding with substrate
Enzyme structure

- **Active site** is a region along the chain that:
  - a 3-D cleft or crevice
  - contains amino acid residues that participate directly in making/braking of bonds
  - occupies a small percentage of the total surface area of an enzyme
    - Enzymes are big molecules
    - Amino acids not participating in active site provide a scaffold
  - **Specificity of binding** depends on defined arrangements in active site
    - Substrate must have matching shape to fit into the site
    - But enzymes are flexible and shapes an be modified by binding of a substrate
  - binds substrates, cofactors, and coenzymes
Enzyme/Substrate complex

Active sites and substrate specificity

Induced Fit

Co-factors & co-enzymes

- Co-factor is a non protein molecule which carries out chemical reaction that cannot be performed by the standard 20 amino acids
- Typically metals or small organic molecules called co-enzymes
- Co-enzymes can be either tightly bound (prosthetic group) or loosely bound to the enzyme
Enzymes (E) catalyze reactions with substrate S, and product P

- Substrate (S)
  - The reactant in biochemical reaction is termed as S.

- When a substrate binds to an enzyme (E) it forms an enzyme-substrate complex (ES)

- Product (P)
  - The product is formed as an outcome of the reaction

- Reactions can be reversible
  - Which is the reactant and product side?
Enzyme Nomenclature

- Enzyme is often named according to the name of the substrate it catalyzes
  - Add a –ase
  - Examples
    - Substrate: lactose, enzyme: lactase
    - Substrate: maltose, enzyme: maltase
How are enzymes related to systems biology?

- We model cells:
  - How do enzyme properties contribute to cellular behavior?
  - Link reaction properties to pathways to cellular behavior

- Let’s learn about:
  - Thermodynamics provide information about the spontaneity of the reaction
  - Enzyme kinetics allow us to analyze rate of reactions
Free-energy change of a reaction ($\Delta G$)

- Free energy (G) or Gibbs free energy is a thermodynamic property that is a measure of useful energy, or the energy that is capable of doing work.

Why do we care:

1. The free-energy difference ($\Delta G$, free-energy change of a reaction) between products and reactants determines if a reaction will occur spontaneously.
2. The energy required to initiate the conversion of reactants into products ($\Delta G^{\ddagger}$, free energy of activation) determines the reaction rate.
1. Standard Free-energy change of a reaction

- Consider the reaction: $A + B \rightleftharpoons C + D$

- $\Delta G$ for this reaction is calculated as:

$$
\Delta G = \Delta G^\circ + RT \ln \frac{[C][D]}{[A][B]}
$$

where:
- $\Delta G^\circ$ standard free-energy change (all reactants are at 1.0 M; if at pH 7, then use $\Delta G^\circ'$)
- $R$ gas constant
- $T$ is absolute temp
- $[X]$ molar concentration of reactant $X$

To calculate $\Delta G^\circ$, assume equilibrium (no net change in reactant/product; reaction has stopped):

$$
\Delta G^\circ = -RT \ln \frac{[C][D]}{[A][B]}
$$

**KEY IDEA:** Whether the $\Delta G$ for a reaction is larger, smaller, or the same as $\Delta G^\circ$ depends on the concentrations of the reactants and products
2. Enzymes accelerate reactions by facilitating the formation of the transition state

- Consider the intermediate state between the end points of a reaction:
  \[ S \rightarrow X^{\ddagger} \rightarrow P \]
  
- The double dagger denotes the transition state.

- The difference in free energy between the transition state and the substrate is called the **Gibbs free energy of activation** or simply the activation energy, symbolized by \( \Delta G^\ddagger \).

- Enzymes lower the activation energy, and equilibrium between \( S \) and \( P \) is achieved sooner (amount of product is the same).

*Figure 8.2 Enzymes accelerate the reaction rate. The same equilibrium point is reached but much more quickly in the presence of an enzyme.*

*Figure 8.3 Enzymes decrease the activation energy. Enzymes accelerate reactions by decreasing \( \Delta G^\ddagger \), the free energy of activation.*
Key Ideas to remember

- A reaction can take place spontaneously only if $\Delta G$ is negative. Such reactions are said to be exergonic.

- A reaction cannot take place spontaneously if $\Delta G$ is positive. An input of free energy is required to drive such a reaction. These reactions are termed endergonic.

- If $\Delta G$ is zero, system is at equilibrium

- The $\Delta G$ of a reaction is independent of the path (or molecular mechanism) of the transformation.

- The reaction rate is a function of the the free energy of activation ($\Delta G^\ddagger$), which is largely unrelated to the $\Delta G$ of the reaction.
Kinetic Analysis of Enzymes

Why study enzyme kinetics:
- Allow studying complex dynamic enzyme behavior
- Systematic evaluation of enzyme function

Our questions:
- How does the activity of an enzyme vary with substrate concentrations and why?
- What are kinetic parameters, $k_{cat}$, $V_m$, and $K_m$, and what do they tell us about the enzymatic reaction?
Concentrations and rates

- The (molar) concentration of B, or molarity, is given by:
  
  \[ [B] = \text{moles of } B / \text{liters} = \text{molarity} = \text{mol/L (or mol/m}^3) \]

- The rate of product formation P from a substrate S can be expressed as:
  
  \[
  \frac{d[P]}{dt} = k [S]
  \]

  Reaction rate usually has the units of \( \text{mol} / (L \cdot \text{S}) \)
Enzyme Kinetics

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

- \( E \) = enzyme concentration
- \( S \) = Substrate concentration
- \( ES \) = Enzyme-substrate complex concentration
- \( P \) = product concentration
- \( k_1 \) = rate constant for formation of ES from \( E + S \)
- \( k_{-1} \) = rate constant for decomposition of ES to \( E + S \)
- \( k_2 \) = rate constant for decomposition of ES to \( E + P \)

Assumptions:
- Only ES exists; no EP
- \( P \rightarrow S \) is negligible
- \([S] \gg [E]\)
- Rate of ES formation = Rate of ES decomposition

Figure from Tony Carruthers
Michaelis-Menton Derivation

1. The overall rate of product formation: \( v_o = k_2 [ES] \)
2. Rate of formation of [ES]: \( v_f = k_1 [E][S] \)
3. Rate of decomposition of [ES]:
   \[
   v_d = k_{-1} [ES] + k_2 [ES]
   \]
4. Rate of ES formation = Rate of ES decomposition (steady state)
5. So: \( k_1 [E][S] = k_{-1} [ES] + k_2 [ES] \)
Michaelis-Menton Derivation

6. In solving for [ES], use the enzyme balance to eliminate [E].

\[ E_T = [E] + [ES] \]

7. \[ k_1 (E_T - [ES])[S] = k_{-1}[ES] + k_2 [ES] \]

\[ k_1 E_T[S] - k_1[ES][S] = k_{-1}[ES] + k_2 [ES] \]

8. Rearrange and combine [ES] terms:

\[ k_1 E_T[S] = (k_{-1} + k_2 + k_1 [S])[ES] \]

9. Solve for \[ [ES] = \frac{k_1 E_T[S]}{(k_{-1} + k_2 + k_1 [S])} \]
10. Divide through by $k_1$:  
$$[ES] = \frac{E_T[S]}{(k_{-1} + k_2)/k_1 + [S]}$$

11. Defined Michaelis constant:  
$$K_M = \frac{(k_{-1} + k_2)}{k_1}$$


13. Then substitute $[ES]$ into $v_o = k_2 [ES]$ from step 1 and replace $k_2 E_T$ with $V_{max}$ to give:
Michaelis-Menton Plot

Relates reaction velocity and substrate concentration

\[ v_o = \frac{V_{\text{max}}[S]}{K_M + [S]} \]
The Michaelis-Menton Equation

Define Michaelis constant:

\[ K_M = \frac{(k_{-1} + k_2)}{k_1} \]

It quantifies the instability of the ES complex.

The overall velocity of an enzyme-catalyzed reaction is given by rate of conversion of ES to E + P.

\[ v_o = k_2[ES] = k_{cat}[ES] \]
Write the rate of change of $S$:
\[
d[S]/dt = -k_1[E][S] + k_{-1}[ES]
\]

What are units of $k_1$ and $k_{-1}$?
- $k_1$ has units of concentration$^{-1}$ time$^{-1}$
- $k_{-1}$ has units of time$^{-1}$

Similarly, $k_2$ has units of time$^{-1}$

$K_M = (k_{-1} + k_2) / k_1$ will have units of concentration
In an enzyme catalyzed reaction, the overall rate of product formation is  \( v = k_{\text{cat}} [ES] \).

If all of the enzyme molecules are complexed with substrate (excess \([S]\)) then the maximum velocity occurs and  \( V_{\text{max}} = k_{\text{cat}} E_T \) where \( k_{\text{cat}} \) is the overall reaction rate constant.

This can also be written as  \( k_{\text{cat}} = \frac{V_{\text{max}}}{E_T} \).

\( k_{\text{cat}} \) is called the turnover number (TON).
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Turnover number (per second)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonic anhydrase</td>
<td>600,000</td>
</tr>
<tr>
<td>3-Ketosteroid isomerase</td>
<td>280,000</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>25,000</td>
</tr>
<tr>
<td>Penicillinase</td>
<td>2,000</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1,000</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>100</td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>15</td>
</tr>
<tr>
<td>Tryptophan synthetase</td>
<td>2</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0.5</td>
</tr>
</tbody>
</table>
• $K_M$ has two meanings:

1. $K_M = [S]$ when $v_o = 1/2 \max$ $V$

$K_M$ provides a measure of the substrate concentration required for significant catalysis to take place

2. $K_M$ is related to the rate constants of the individual steps:

$$K_M = (k_{-1} + k_2) / k_1$$

$K_M \approx k_{-1} / k_1 = K_s$ (the enzyme-substrate dissociation constant) when $k_2$ is small ($<<$ either $k_1$ or $k_{-1}$).

$K_M$ then measures strength of ES complex: Generally, the lower the numerical value of $K_M$, the tighter the substrate binding.

$K_M$ is used as a measure of the affinity of E for S when $k_{-1} >> k_2$
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$K_M$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymotrypsin</td>
<td>Acetyl-(\text{L})-tryptophanamide</td>
<td>5000</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Hexa-N-acetylglucosamine</td>
<td>6</td>
</tr>
<tr>
<td>(\beta)-Galactosidase</td>
<td>Lactose</td>
<td>4000</td>
</tr>
<tr>
<td>Threonine deaminase</td>
<td>Threonine</td>
<td>5000</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>(\text{CO}_2)</td>
<td>8000</td>
</tr>
<tr>
<td>Penicillinase</td>
<td>Benzylpenicillin</td>
<td>50</td>
</tr>
<tr>
<td>Pyruvate carboxylase</td>
<td>Pyruvate</td>
<td>400</td>
</tr>
<tr>
<td>Arginine-t(\text{tRNA}) synthetase</td>
<td>(\text{HCO}_3^-)</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Arginine</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(\text{tRNA})</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>300</td>
</tr>
</tbody>
</table>
$k_{\text{cat}}/K_M$

$k_{\text{cat}}/K_M$ is taken to be a measure of the catalytic efficiency of an enzyme because it takes into account rate of catalysis ($k_{\text{cat}}$) and strength of E-S interaction ($K_M$).

Rewriting $k_{\text{cat}}/K_M$ in terms of the kinetic constants gives:

$$
\frac{k_{\text{cat}}}{K_M} = \frac{k_1k_2}{k_{-1} + k_2}
$$

So, when $k_2$ is small, the denominator becomes $k_{-1}$ and $k_{\text{cat}}/K_M$ is small. \(\rightarrow\) continued
\[ \frac{k_{\text{cat}}}{K_M} = \frac{k_1 k_2}{K_M (k_{-1} + k_2)} \]

And where \( k_2 \) is large, the denominator becomes \( k_2 \)
and \( \frac{k_{\text{cat}}}{K_M} \) is limited by the value of \( k_1 \) or
formation of the ES complex. This formation is in
turn limited by the rate of diffusion of S into the
active site of E. So, the maximum value for this
second-order rate constant \( (\frac{k_{\text{cat}}}{K_M}) \) is the rate of
diffusion \( (~10^9 \text{ sec}^{-1} \text{ M}^{-1}) \).

If \( \frac{k_{\text{cat}}}{K_M} \) is at the upper limit \( (10^8 \text{ or } 10^9) \), the enzyme
has attained kinetic perfection – every interaction
between substrate and enzyme is productive...
### Table 8.6 Substrate preferences of chymotrypsin

<table>
<thead>
<tr>
<th>Amino acid in ester</th>
<th>Amino acid side chain</th>
<th>$k_{\text{cat}}/K_M$ (s$^{-1}$ M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>$-\text{H}$</td>
<td>$1.3 \times 10^{-1}$</td>
</tr>
<tr>
<td>Valine</td>
<td>$-\text{CH}$</td>
<td>2.0</td>
</tr>
<tr>
<td>Norvaline</td>
<td>$-\text{CH}_2\text{CH}_3$</td>
<td>$3.6 \times 10^{2}$</td>
</tr>
<tr>
<td>Norleucine</td>
<td>$-\text{CH}_2\text{CH}_2\text{CH}_3$</td>
<td>$3.0 \times 10^{3}$</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>![Phenylalanine structure]</td>
<td>$1.0 \times 10^{5}$</td>
</tr>
</tbody>
</table>


### Table 8.7 Enzymes for which $k_{\text{cat}}/K_M$ is close to the diffusion-controlled rate of encounter

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k_{\text{cat}}/K_M$ (s$^{-1}$ M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td>$1.6 \times 10^{8}$</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>$8.3 \times 10^{7}$</td>
</tr>
<tr>
<td>Catalase</td>
<td>$4 \times 10^{7}$</td>
</tr>
<tr>
<td>Crotonase</td>
<td>$2.8 \times 10^{8}$</td>
</tr>
<tr>
<td>Fumarase</td>
<td>$1.6 \times 10^{8}$</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>$2.4 \times 10^{8}$</td>
</tr>
<tr>
<td>$\beta$-Lactamase</td>
<td>$1 \times 10^{8}$</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>$7 \times 10^{9}$</td>
</tr>
</tbody>
</table>

Summary

- Enzymes have active sites that allow substrate binding
- Gibbs Free Energy:
  - Spontaneity of the reaction is a function of \( \Delta G \), which is a function of reactant concentrations
- Enzymes lower the free energy of activation (\( \Delta G^\ddagger \)) as reaction proceeds
- Making simplifying assumptions, we derived the Michaelis-Menten equations
- We discussed \( K_m \), \( K_s \), \( K_{cat} \) and \( V_m \) discussed their biological implications
References

- Download PDF for biochem book
  https://archive.org/details/BiochemistryStryer7th

- More detailed slides on kinetics:
  - http://elte.prompt.hu/sites/default/files/tananyagok/IntroductionToPracticalBiochemistry/ch09s02.html