Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2017

Lecture 14:

A Bit More on K_m, k_{cat} and Energy Profiles

and

Introduction to Reversible Enzyme Inhibitors

23 February 2017

University of Utah
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Public Service Announcement

Science Employer Event

Thursday, 23 February 2017

- Panel: 3:00 PM - 4:00 PM

  ASB 220

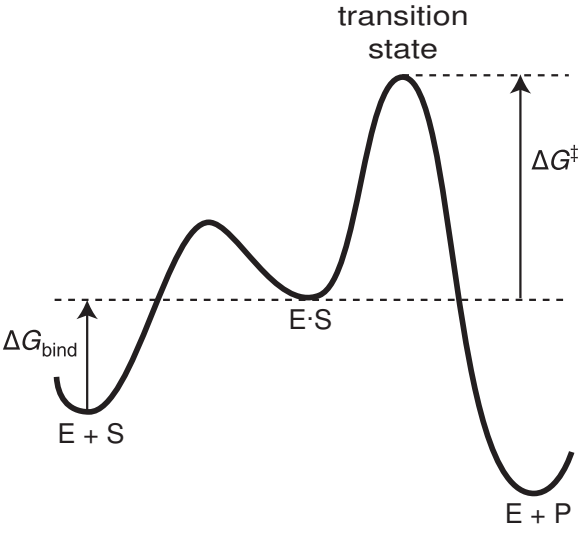
  Representatives from:
  - Zions Bank
  - Myriad Genetics
  - ThermoFisher Scientific
  - Qualtrics
  - College of Science

- Networking: 4:00 PM - 5:00 PM

  ASB Lobby

  Representatives from:
  - Biofire Defense
  - Utah State Parks
  - Goldman Sachs
  - Edwards LifeSciences

Energy Profile for an Enzyme-Catalyzed Reaction



- Free-energy change for binding:

$$\Delta G_{\text{bind}} = RT \ln K_{\text{d}}$$

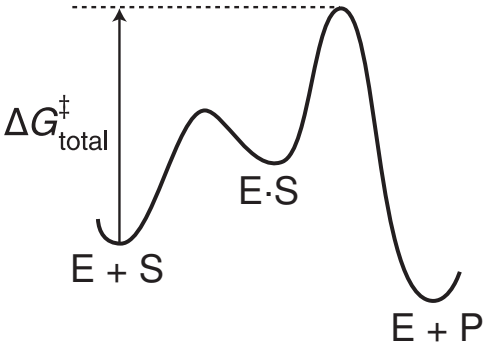
R = Gas constant
T = Temperature

- Free-energy change from E·S complex to transition state:

$$\Delta G^{\ddagger} = RT \ln \left(\frac{k_{\text{b}} T}{k_{\text{cat}} h} \right)$$

k_{b} = Boltzmann constant
h = Planck constant

The Significance of k_{cat}/K_m



- Free-energy difference between E + S and the transition state:

$$\Delta G^{\ddagger}_{total} = \underbrace{RT \ln\left(\frac{k_b T}{h}\right)}_{\text{Constant}} + RT \ln\left(\frac{K_d}{k_{cat}}\right)$$

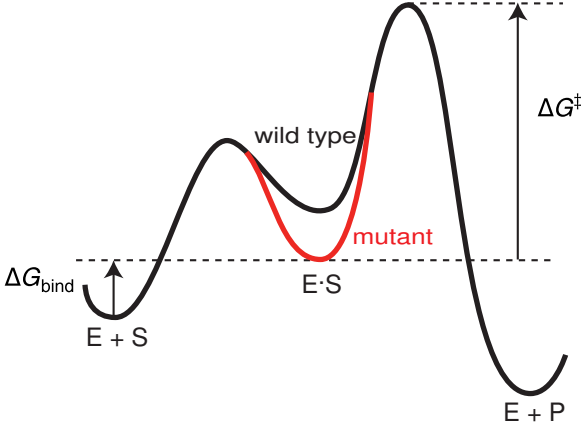
- If $k_2 \gg k_{cat}$, $K_d \approx K_m$:

$$\Delta G^{\ddagger}_{total} = C - RT \ln\left(\frac{k_{cat}}{K_m}\right)$$

- The ratio k_{cat}/K_m reflects the free energy difference between E + S and the transition state.
- k_{cat}/K_m is commonly interpreted as a measure of enzymatic efficiency.
- Catalytic efficiency is favored by a large value of k_{cat} and a small value of K_m.

Is a Low K_m Always Good?

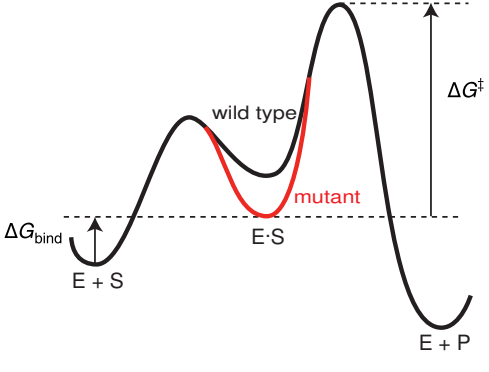
Suppose that we could design a mutant enzyme that forms a more stable complex with substrate.



This will lower K_m, but leave k_{cat}/K_m the same.

Clicker Question #1

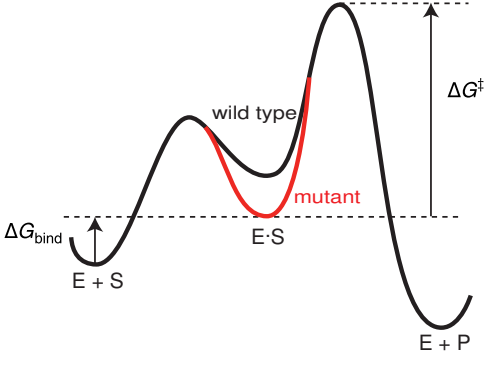
At **low** substrate concentration, will the velocity for the mutant enzyme be greater or less than that of the original enzyme?



1. Greater than the original enzyme
2. Less than the original enzyme
3. The same as the original enzyme

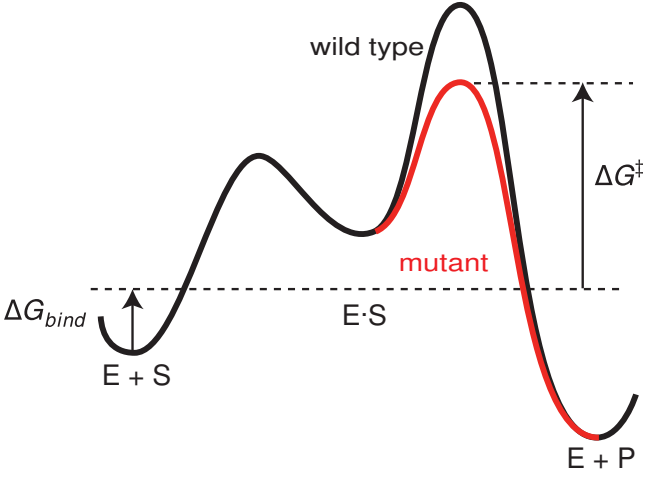
Clicker Question #2

At **high** substrate concentration, will the velocity for the mutant enzyme be greater or less than that of the original enzyme?



1. Greater than the original enzyme
2. Less than the original enzyme!
3. The same as the original enzyme

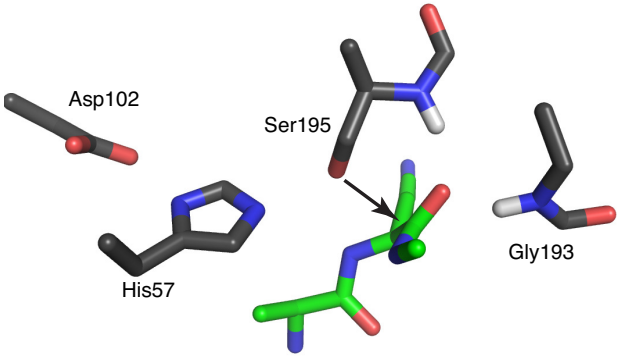
To Make a Better Enzyme, Stabilize the Transition State!



- Increased rate at all substrate concentrations.
- Easier said than done!

Transition State Stabilization in Serine Proteases

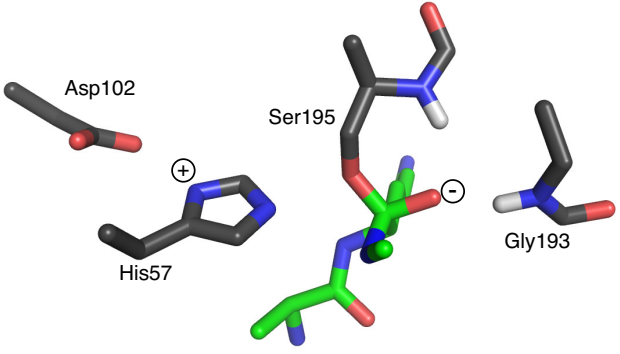
Enzyme-Substrate Complex Transition State



Substrate-enzyme model from structure of trypsin-BPTI complex (PDB entry 2FTL)

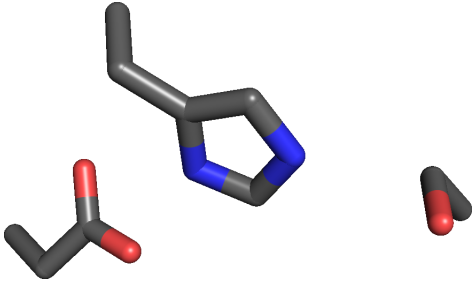
Transition State Stabilization in Serine Proteases

Enzyme-Substrate Complex Transition State

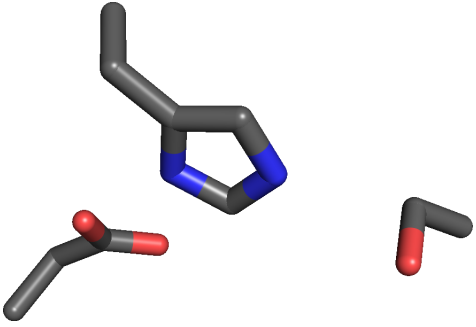


Transition state model from structure of trypsin with boronic inhibitor (PDB entry 1BZT)

Catalytic Triads in Two Serine Proteases



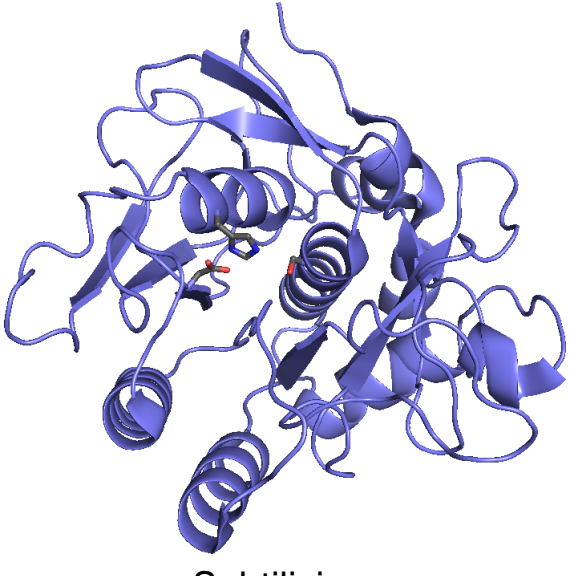
Trypsin



Subtilisin

Catalytic Triads in Two Serine Proteases

Trypsin

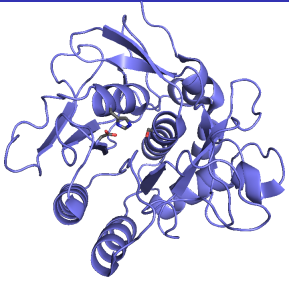


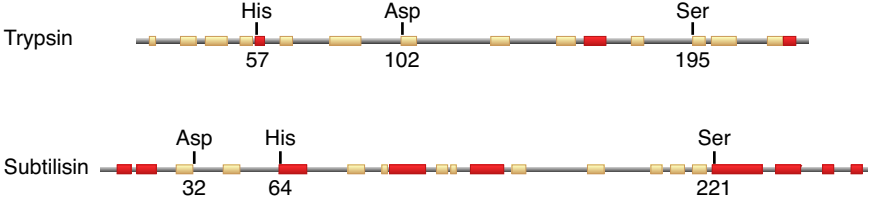
Subtilisin

Convergent Evolution of Serine Proteases

Trypsin

Subtilisin





Warning!

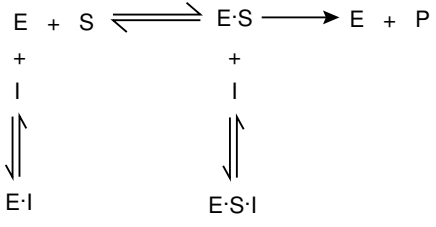
Direction Change

Enzyme Inhibitors

- Important aspects of enzyme inhibitors
  - Natural regulators of metabolism
  - Tools for studying enzyme mechanisms
  - Tools for studying other biological processes
  - Pharmaceuticals
- Major classes of inhibitors
  1. Reversible
     $E + I \rightleftharpoons E{\cdot}I$
     Usually form non-covalent interaction with enzyme
  2. Irreversible
     $E + I \rightarrow E\text{-}I$
     Usually form covalent bond with enzyme

Reversible Inhibitors

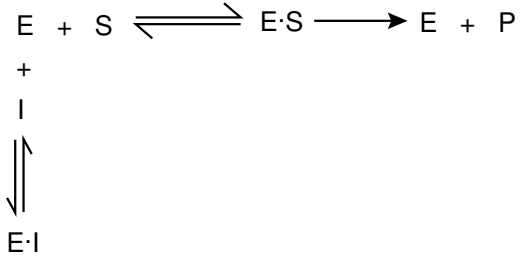
- Can bind to either free enzyme or enzyme-substrate complex



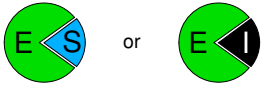
- Classification of reversible inhibitors:
  - Competitive: Inhibitor binds only to free enzyme
  - Noncompetitive: Inhibitor binds equally well to E and $E \cdot S$
  - Uncompetitive: Inhibitor binds only to $E \cdot S$
  - Mixed inhibition: Inhibitor binds to E and $E \cdot S$, but with different affinities.
- Different classes have different kinetic properties.

Competitive Inhibition

- Inhibitor binds only to free enzyme



- Competitive inhibitors often mimic the substrate.



- Inhibitor disfavors formation of the $E \cdot S$ complex.

Clicker Question #3:

How will a competitive inhibitor affect the Michaelis-Menten parameters?

1. Decrease V_{max}
2. Increase V_{max}
3. Decrease K_m
4. Increase K_m
5. Change V_{max} and K_m
6. Change neither V_{max} nor K_m

All answers count (for today)!

The Inhibition Constant, K_i, for a Competitive Inhibitor

- Characterized by an equilibrium dissociation constant:

$$K_i = \frac{[I][E]}{[E \cdot I]}$$

- Modified Michaelis-Menten equation in the presence of a competitive inhibitor:

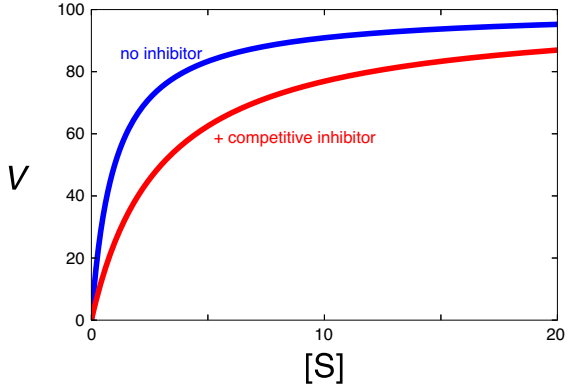
$$V = \frac{[S]\,V_{max}}{K_m\,(1 + [I]/K_i) + [S]}$$

$$V = \frac{[S]\,V_{max}}{K'_m + [S]}$$

$$K'_m = K_m\,(1 + [I]/K_i)$$

- Apparent K_m is increased: Higher substrate concentrations are required to approach saturation.
- Apparent V_{max} is unaffected.

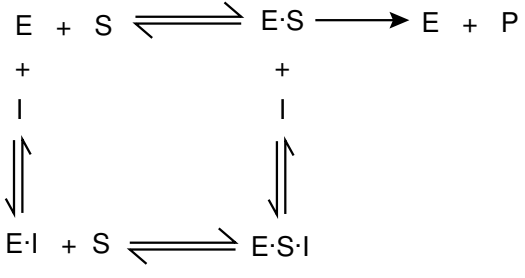
Competitive Inhibition



- Effect of inhibitor can be overcome at high substrate concentrations.
- Competitive inhibitor is most effective at low substrate concentrations.

Noncompetitive Inhibition

- Inhibitor binds equally to free enzyme and enzyme-substrate complex



- Noncompetitive inhibitors bind independently of the substrate.

- Bound inhibitor blocks catalytic step.

Clicker Question #4:

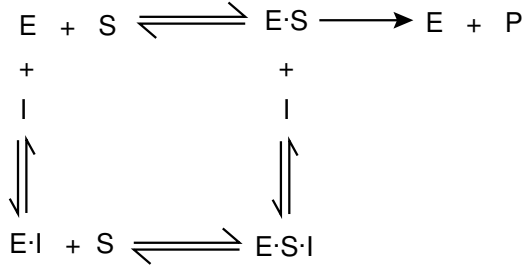
How will a noncompetitive inhibitor affect the apparent Michaelis-Menten parameters?

1. Decrease V_{max}
2. Increase V_{max}
3. Decrease K_m
4. Increase K_m
5. Change V_{max} and K_m
6. Change neither V_{max} nor K_m

All answers count (for now).

Noncompetitive Inhibition

- Inhibitor binds equally to free enzyme and enzyme-substrate complex



- Inhibitor effectively lowers concentration of both E and E·S

Inhibition Constant for a Noncompetitive Inhibitor

- Dissociation constants for E and $E \cdot S$ are assumed to be equal:

$$K_i = \frac{[I][E]}{[E \cdot I]} = \frac{[I][E \cdot S]}{[E \cdot S \cdot I]}$$

- Modified Michaelis-Menten equation in the presence of a noncompetitive inhibitor:

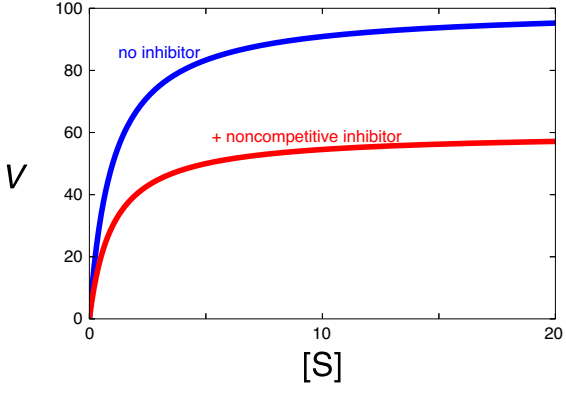
$$V = \frac{V_{max}}{1 + [I]/K_i} \cdot \frac{[S]}{K_m + [S]}$$

$$V = \frac{[S] V'_{max}}{K_m + [S]}$$

$$V'_{max} = V_{max} / (1 + [I]/K_i)$$

- Apparent V_{max} is decreased.
- Apparent K_m is unaffected.

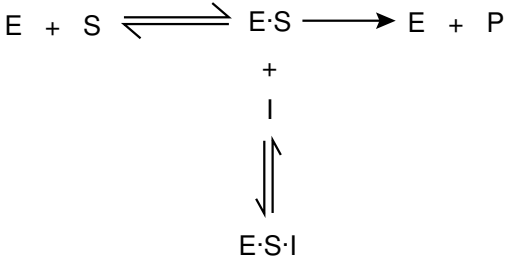
Noncompetitive Inhibition



- Noncompetitive inhibitors are effective at all substrate concentrations.

Uncompetitive Inhibition

- Inhibitor binds only to the enzyme-substrate complex



- Inhibitor requires substrate for binding, but ternary complex is inactive.

Clicker Question #5:

How will an uncompetitive inhibitor affect the apparent Michaelis-Menten parameters?

1. Decrease V_{max}
2. Increase V_{max}
3. Decrease K_m
4. Increase K_m
5. Change V_{max} and K_m
6. Change neither V_{max} nor K_m

All answers count (for now).

Inhibition Constant for an Uncompetitive Inhibitor

- Dissociation constant:

$$K_\mathrm{i} = \frac{[\mathrm{I}][\mathrm{E}\cdot\mathrm{S}]}{[\mathrm{E}\cdot\mathrm{S}\cdot\mathrm{I}]}$$

- Modified Michaelis-Menten equation in the presence of an uncompetitive inhibitor:

$$V = \frac{V_\mathrm{max}}{1+[\mathrm{I}]/K_\mathrm{i}} \cdot \frac{[\mathrm{S}]}{K_\mathrm{m}/(1+[\mathrm{I}]/K_\mathrm{i})+[\mathrm{S}]} = \frac{[\mathrm{S}]\,V_\mathrm{max}}{K_\mathrm{m}+[\mathrm{S}](1+[\mathrm{I}]/K_\mathrm{i})}$$

$$V = \frac{[\mathrm{S}]\,V'_\mathrm{max}}{K'_\mathrm{m}+[\mathrm{S}]}$$

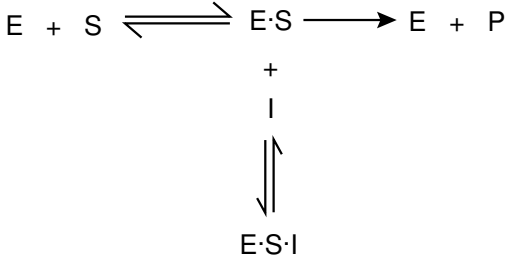
$$V'_\mathrm{max} = V_\mathrm{max}/\,(1+[\mathrm{I}]/K_\mathrm{i})$$

$$K'_\mathrm{m} = K_\mathrm{m}/\,(1+[\mathrm{I}]/K_\mathrm{i})$$

- Apparent V_max and K_m are both decreased.

Uncompetitive Inhibition

- Inhibitor binds only to the enzyme-substrate complex



- K_m is decreased because inhibitor shifts equilibrium towards favoring E-S complex.
- V_{max} is decreased because E-I-S complex can't undergo catalytic step.

Consequences of Lowered Apparent K_m for Uncompetitive Inhibition

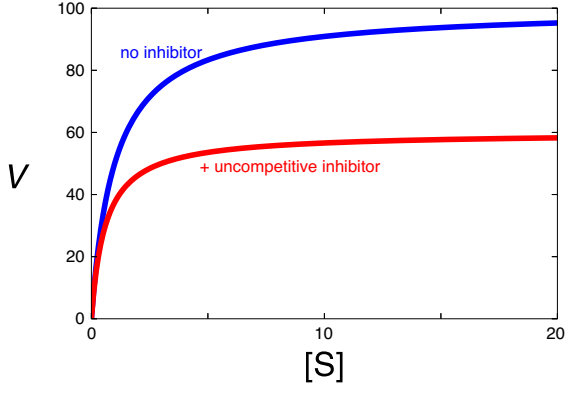
- Saturation occurs at *lower* substrate concentration!
- V_{max}/K_m is unaffected.
- Velocity at low substrate concentrations is unaffected.

$$[S] \ll K_m$$

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

$$V \approx \frac{[S]V_{max}}{K_m}$$

Uncompetitive Inhibition



- Uncompetitive inhibitors are effective at high, but not low, substrate concentrations.

Why Bother Classifying Inhibitors?

1. Extablishes a mental framework for thinking about mechanisms and kinetic effects.
2. Kinetic properties of inhibitors can help identify which species the inhibitor interacts with. May help in characterizing active site.
3. Different inhibition mechanisms dictate whether an inhibitor will be most effective at high or low substrate concentrations.
   - Competitive inhibitors are most effective when $[S] < K_m$
   - Uncompetitive inhibitors are most effective when $[S] > K_m$
   - Noncompetitive inhibitors are effective at all substrate concentrations.

   May be important for design of inhibitors for specific purposes or conditions.

More on analyzing experimental data next week.