Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2022

Lecture 15:

Using Trypsin to Make Insulin

and

Introduction to Enzyme Inhibitors

1 March 2022

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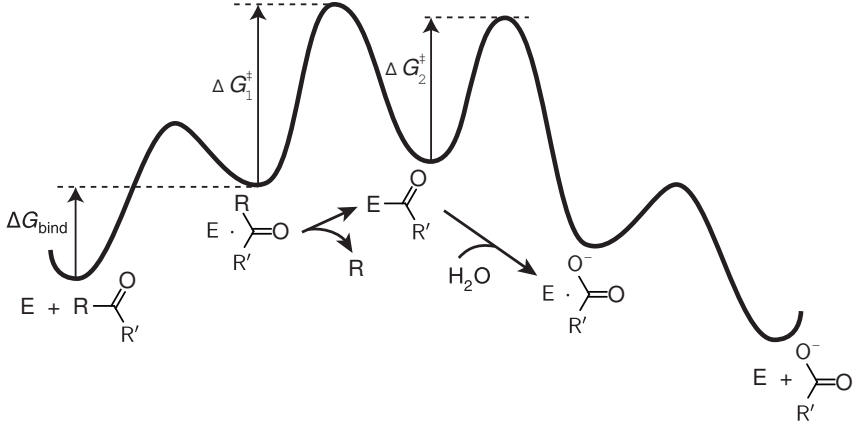
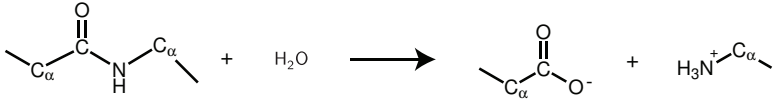
A More Complete Energy Profile for Serine Proteases



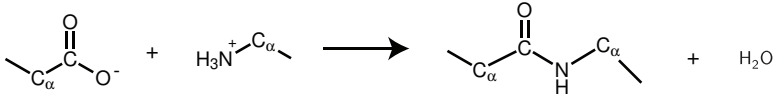
- Thermodynamics requires that enzymes catalyze reactions in both directions.
- Why don't we see reverse reaction with proteases?
- Could we make the reverse reaction more favorable?

Could We Make Peptide Bonds with a Protease?

- "Forward" reaction:

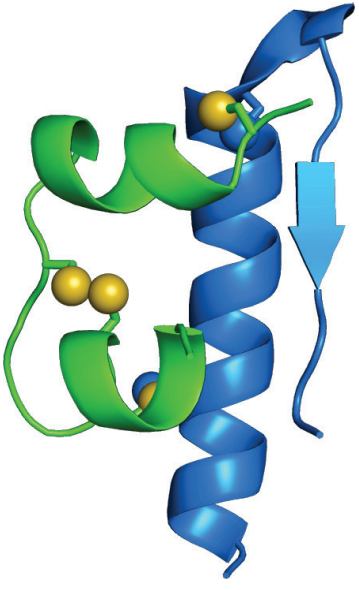


- "Reverse" reaction:



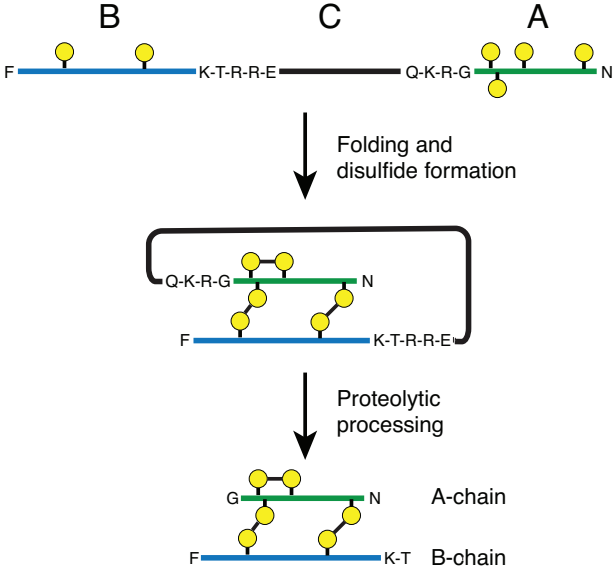
- How could we make peptide synthesis favorable?
  - Lower the product concentrations (relative to reactants) for the synthesis reaction.
  - Activate the carboxyl group.

Insulin: A Brief History

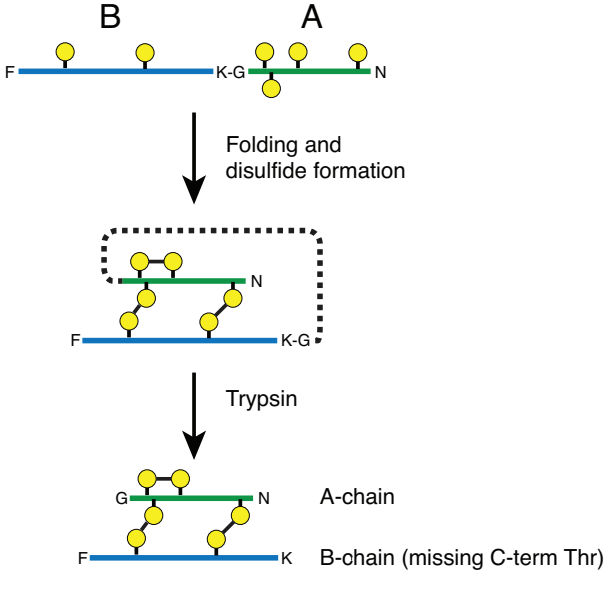


- 1921: Isolated by Frederick Banting and Charles Best.
- 1922: Used to treat diabetic patients.
- 1951-1953: Sequence determined by Fred Sanger.
- 1966: Chemical synthesis of active insulin at the Chinese Academy of Science.
- 1969: Three-dimensional structure determined by Dorothy Hodgkin.
- 1978: Production of human insulin in genetically engineered bacteria, by Genentech.

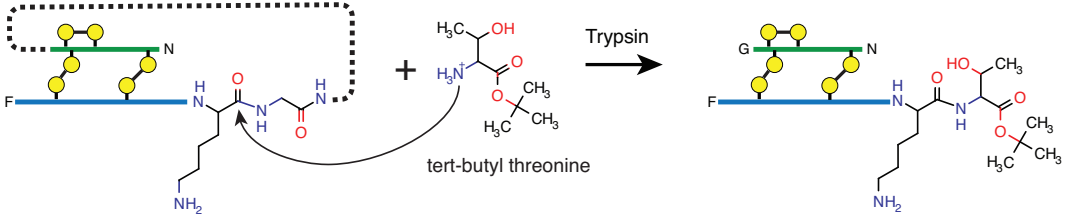
Natural Biosynthesis of Human Insulin



Production of Almost-human Insulin in Yeast



Transpeptidation of Insulin from Yeast



- Lys-Gly peptide bond is replaced with Lys-Thr bond.
- Solvent: 80% Dimethylformamide, 20% water.
- Tert-butyl ester is hydrolyzed with acid to leave carboxyl group.
- Other tert-butyl amino acids can be used for different terminal residues.

Warning!

Direction Change

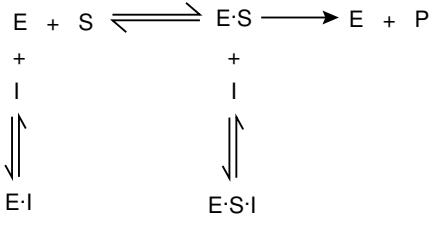
Enzyme Inhibitors

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 complex with enzyme
  2. Irreversible
     $E + I \rightarrow E\text{-}I$
     Usually form covalent bond with enzyme

Reversible Inhibitors

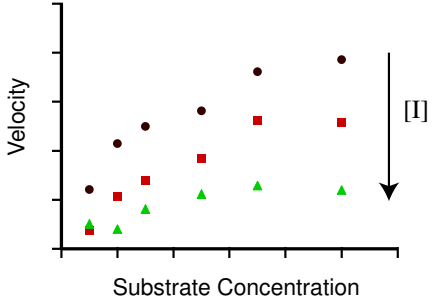
- Can bind to either free enzyme or enzyme-substrate complex



- Decrease the concentration of the 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.

Strategy for Analyzing Reversible Inhibition

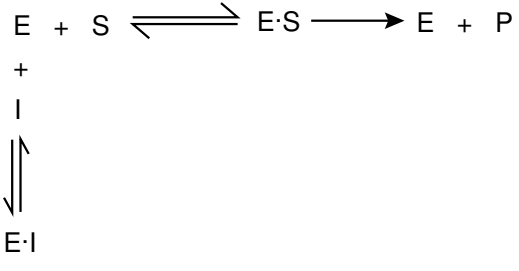
- Measure velocity versus [S] in the presence of different inhibitor concentrations



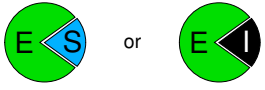
- Kinetics can still be fit to the Michaelis-Menten equation!
- Does the inhibitor change the apparent values of K_m, V_{max} or both?
- Experimental scatter can make it hard to tell!

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 #1:

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

A) Decrease V_{max}

B) Increase V_{max}

C) Decrease K_m

D) Increase K_m

E) Change V_{max} and 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:

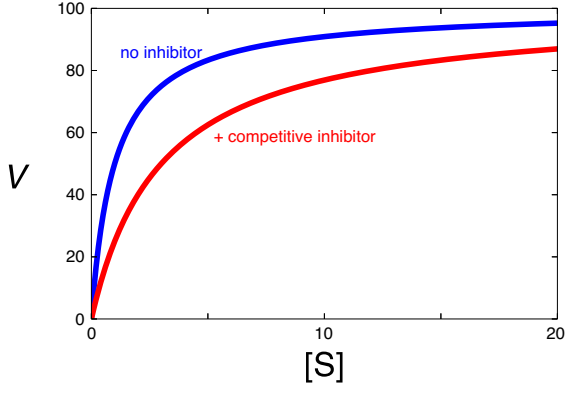
$$V = \frac{[S]V_{max}}{K_m\,(1 + [I]/K_i) + [S]}$$

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

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

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

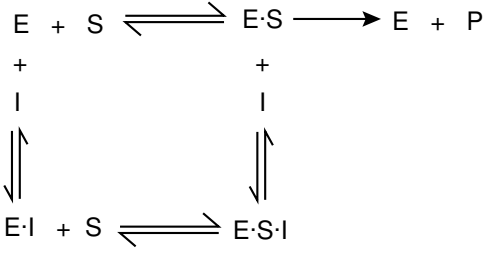
Competitive Inhibition



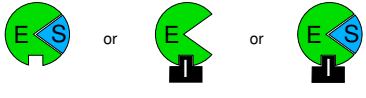
- Effect of inhibitor can be overcome at high substrate concentrations.
- Competitive inhibitor is most effective at low substrate concentrations.

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 #2:

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

A) Decrease V_{max}

B) Increase V_{max}

C) Decrease K_m

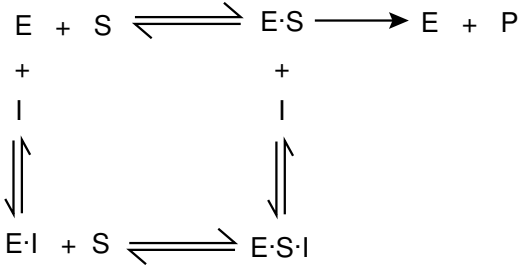
D) Increase K_m

E) Change V_{max} and 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 $\mathrm{E} \cdot \mathrm{S}$ are assumed to be equal:

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

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

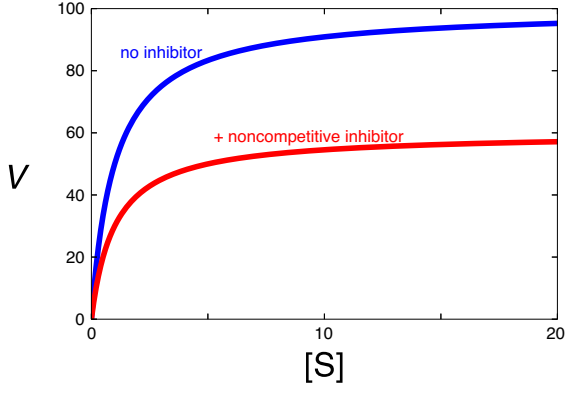
$$V = \frac{V_\mathrm{max}}{1 + [\mathrm{I}]/K_\mathrm{i}} \cdot \frac{[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

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

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

- Apparent V_max is decreased.
- Apparent K_m is unaffected.

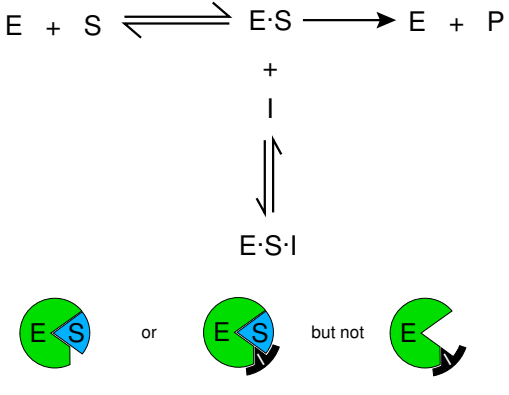
Noncompetitive Inhibition



- 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 #3:

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

A) Decrease V_{max}

B) Increase V_{max}

C) Decrease K_m

D) Increase K_m

E) Change V_{max} and 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:

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

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

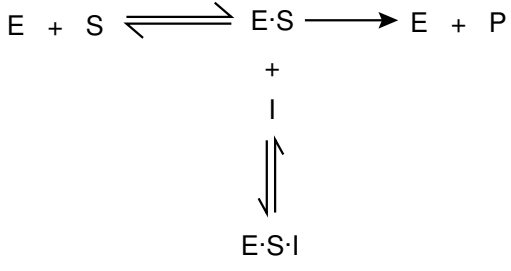
$$V'_\mathrm{max} = V_\mathrm{max}/\left(1+[\mathrm{I}]/K_\mathrm{i}\right)$$

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

- 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

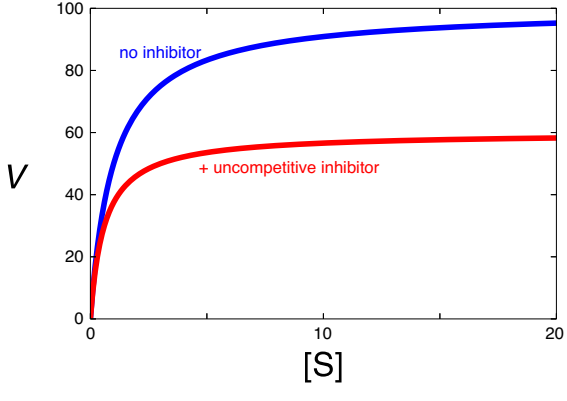
- Saturation occurs at *lower* substrate concentration!
- V_{max}/K_m is unaffected.
- Velocity at low substrate concentrations is unaffected.

$$[S] \ll K_m$$

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

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

Uncompetitive Inhibition



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

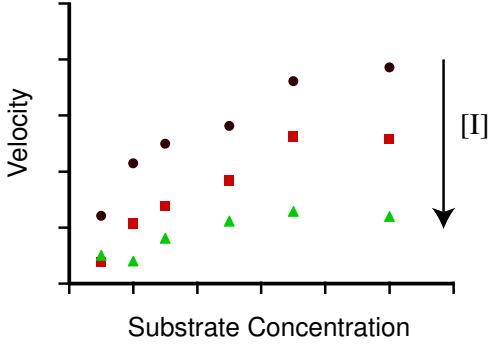
Why Bother Classifying Inhibitors?

1. Establishes 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.

Strategy for Analyzing Reversible Inhibition

- Measure velocity versus [S] in the presence of different inhibitor concentrations



- Does the inhibitor change the apparent values of K_m, V_{max} or both?
- Experimental scatter can make it hard to tell!