Enzymes III
Dr. Kevin Ahern
Enzyme Inhibition
Enzyme Inhibition

Competitive Inhibitor Resembles Natural Substrate and Competes with it for Binding to the Active Site
Enzyme Inhibition

Normal Substrate for Dihydrofolate Reductase
Enzyme Inhibition

Normal Substrate for Dihydrofolate Reductase

Competitive Inhibitor of Dihydrofolate Reductase

Methotrexate

Dihydrofolate
Enzyme Inhibition

At Low [S], Competitive Inhibitor
Very Effective - $K_m$ Increases
At Low [S], Competitive Inhibitor
Very Effective - $K_m$ Increases

Competitive Inhibitor Less Effective
as [S] Increases - $V_{\text{max}}$ Does Not Change
Enzyme Inhibition

At Low [S], Competitive Inhibitor Very Effective - $K_m$ Increases

Competitive Inhibitor Less Effective as [S] Increases - $V_{max}$ Does Not Change
Enzyme Inhibition

At Low [S], Competitive Inhibitor Very Effective - $K_m$ Increases

Competitive Inhibitor Less Effective as [S] Increases - $V_{max}$ Does Not Change
Enzyme Inhibition

Lineweaver Burk Plot: Competitive Inhibition

-1/Km  -1/Km(inhib)
Enzyme Inhibition

Lineweaver Burk Plot: Competitive Inhibition

$1/V_{max}$ Unchanged
Enzyme Inhibition

1/$V_{\text{max}}$ Unchanged

-1/$K_m$ Increases
(= $K_m$ Increases)
Enzyme Inhibition

- Normal Substrate
- Noncompetitive Inhibitor
Non-Competitive Inhibitors Do Not Resemble the Substrate and Do Not Compete With it for the Active Site.
Non-Competitive Inhibitors Do Not Resemble the Substrate and Do Not Compete With it for the Active Site.

Instead, They Affect Enzymes by Binding a Different Location on the Enzyme
Enzyme Inhibition

\[ \frac{V_{\text{max}}}{2} \quad \frac{V_{\text{max}}}{2i} \]

Uninhibited

Non-Competitively Inhibited
Enzyme Inhibition

- Noncompetitive Inhibitors Cannot be Out-Competed by Substrate,
Enzyme Inhibition

- Noncompetitive Inhibitors Cannot be Out-Competed by Substrate,
- Inhibit a Fixed Amount of Enzyme.
Enzyme Inhibition

- Noncompetitive Inhibitors Cannot be Out-Competed by Substrate,
- Inhibit a Fixed Amount of Enzyme.
- $V_{\text{max}}$ Varies With the Amount of Enzyme,
Noncompetitive Inhibitors Cannot be Out-Competed by Substrate,
Inhibit a Fixed Amount of Enzyme.
$V_{\text{max}}$ Varies With the Amount of Enzyme,
$V_{\text{max}}$ Decreases for a Non-Competitive Inhibitor
Enzyme Inhibition

- Noncompetitive Inhibitors Cannot be Out-Competed by Substrate,
- Inhibit a Fixed Amount of Enzyme,
- $V_{\text{max}}$ Varies With the Amount of Enzyme,
- $V_{\text{max}}$ Decreases for a Non-Competitive Inhibitor
Enzyme Inhibition

$K_m$ is Not Affected by Non-Competitive Inhibition

\[
\frac{V_{\text{max}}}{2} \leq \frac{V_{\text{max}}}{2i}
\]

Uninhibited

Non-Competitively Inhibited
Enzyme Inhibition

Lineweaver Burk Plot: Noncompetitive Inhibition

- $1/K_m$
- $1/V_o$
- $1/V_{max}$
- $1/V_{max(\text{inhib})}$
- $1/[S]$
Enzyme Inhibition

Lineweaver Burk Plot: Noncompetitive Inhibition

Same Values of $-1/K_m$
Enzyme Inhibition

Lineweaver Burk Plot: Noncompetitive Inhibition

1/V\textsubscript{max} Increases (=V\textsubscript{max} Decreases)

Same Values of -1/K\textsubscript{m}
Suicide Inhibition
Suicide Inhibition

Credit: https://en.wikipedia.org/wiki/User:Mstrother
Penicillin Covalently Binds to Active Site of Enzyme Needed for Making Bacterial Cell Walls

Credit: https://en.wikipedia.org/wiki/User:Mstrother
Enzyme Regulation
Enzyme Regulation

Allosterism - binding of a small molecule to an enzyme affects enzyme activity
Enzyme Regulation

Allosterism - binding of a small molecule to an enzyme affects enzyme activity
Homotropic effector - A substrate for the enzyme
Enzyme Regulation

Allosterism - binding of a small molecule to an enzyme affects enzyme activity
Homotropic effector - A substrate for the enzyme
Heterotropic effector - A non-substrate
Models of Allosterism
Models of Allosterism

Sequential Model
Models of Allosterism

Sequential Model
Models of Allosterism

Sequential Model

Cause/Effect between binding of substrate/effector and enzyme change to T or R state
Models of Allosterism
Concerted Model of Catalysis

Models of Allosterism

Subunit in T-State
Models of Allosterism

Subunit in T-State

Subunit in R-State
Concerted Model of Catalysis

Subunit in T-State

Subunit in R-State

Binding of Ligand Converts Subunit Into R-State and Induces Neighbors to do Same
Concerted Model of Catalysis

Subunit in T-State

Subunit in R-State

Binding of Ligand Converts Subunit Into R-State and Induces Neighbors to do Same

Sequential Model

Models of Allosterism
Concerted Model of Catalysis

Subunit in T-State

Subunit in R-State

Binding of Ligand Converts Subunit Into R-State and Induces Neighbors to do Same

Sequential Model

Models of Allosterism

Cause/Effect between binding of substrate/effect and enzyme change to T or R state
Models of Allosterism
Models of Allosterism

Concerted (MWC) Model
Models of Allosterism

Concerted (MWC) Model

T-State \rightleftharpoons R-State
Models of Allosterism

Concerted (MWC) Model

Enzyme flips as a complex independently of binding of effector
Effector “locks” enzyme in T or R state
Models of Allosterism
Models of Allosterism

Morpheein Model
Models of Allosterism

Morpheein Model

Dissociated Monomers Can Bind Each Other, but Only in the Same State

T-State ↔ 4 ↔ 4 ↔ R-State
Enzymes
• EC Classification

- EC 1, Oxidoreductases: oxidation/reduction reaction catalysis
- EC 2, Transferases: transfer a functional group (e.g. a methyl or phosphate group)
- EC 3, Hydrolases: hydrolysis of bonds
- EC 4, Lyases: non-hydrolytic non-oxidative breaking of bonds
- EC 5, Isomerases: catalyze isomerization changes within a single molecule
- EC 6, Ligases: join two molecules by making covalent bonds.
Enzymes

- Oxidoreductases
Enzymes
• Oxidoreductases

• EC 1, Oxidoreductases: oxidation/reduction reaction catalysis
Enzymes
  • Oxidoreductases

Malate Dehydrogenase

  • EC 1, Oxidoreductases: oxidation/reduction reaction catalysis
Enzymes

- Oxidoreductases

**Concerted Model of Catalysis**

\[ \text{EC 1, Oxidoreductases: oxidation/reduction reaction catalysis} \]

\[
\text{Malate Dehydrogenase} \\
\text{HO} - \text{H} - \text{H} - \text{COOH} + \text{NAD}^+ \overset{\leftrightarrow}{\longrightarrow} \text{H} - \text{H} - \text{COOH} + \text{NADH} + \text{H}^+ 
\]

- EC 1, Oxidoreductases: oxidation/reduction reaction catalysis
**Enzymes**

- Oxidoreductases

**Concerted Model of Catalysis**

EC 1, Oxidoreductases: oxidation/reduction reaction catalysis

\[
\text{Malate Dehydrogenase} \quad \text{HO} - \text{H} - \text{H} - \text{COOH} + \text{NAD}^+ \quad \text{<==>} \quad \text{COOH} - \text{H} - \text{H} - \text{COOH} + \text{NADH} + \text{H}^+ 
\]

EC 1, Oxidoreductases: oxidation/reduction reaction catalysis
Enzymes
• Transferases
Enzymes

- Transferases
  
  - EC 2, Transferases: transfer a functional group (e.g. a methyl or phosphate group)
Enzymes

• Transferases

Hexokinase

• EC 2, Transferases: transfer a functional group (e.g. a methyl or phosphate group)
Enzymes

- Transferases

Hexokinase

- EC 2, Transferases: transfer a functional group (e.g. a methyl or phosphate group)
Enzymes

- Transferases

Hexokinase

- EC 2, Transferases: transfer a functional group (e.g. a methyl or phosphate group)
Enzymes
• Hydrolases
Enzymes
- Hydrolases

- EC 3, Hydrolases: hydrolysis of bonds
Enzymes

• Hydrolases

Proteases

• EC 3, Hydrolases: hydrolysis of bonds
Enzymes
- Hydrolases

Proteases
- EC 3, Hydrolases: hydrolysis of bonds
Enzymes

- Hydrolases

Proteases

- EC 3, Hydrolases: hydrolysis of bonds
Enzymes
  • Hydrolases

Proteases
  • EC 3, Hydrolases: hydrolysis of bonds
Enzymes
• Lyases
Enzymes
- Lyases

- EC 4, Lyases: non-hydrolytic non-oxidative breaking of bonds
Enzymes

• Lyases

Isocitrate Lyase

• EC 4, Lyases: non-hydrolytic non-oxidative breaking of bonds
Enzymes
• Lyases

Isocitrate Lyase

• EC 4, Lyases: non-hydrolytic non-oxidative breaking of bonds
Enzymes

• **Lyases**

Isocitrate Lyase

• **EC 4, Lyases: non-hydrolytic non-oxidative breaking of bonds**
Enzymes

- Lyases

Isocitrate Lyase

- EC 4, Lyases: non-hydrolytic non-oxidative breaking of bonds
Enzymes

- Isomerases
Enzymes

- Isomerases

- EC 5, Isomerases: catalyze isomerization changes within a single molecule
Enzymes

• Isomerases

Phosphoglucoisomerase

• EC 5, Isomerases: catalyze isomerization changes within a single molecule
**Enzymes**
- Isomerases

- EC 5, Isomerases: catalyze isomerization changes within a single molecule

**Phosphoglucoisomerase**

\[
\text{Glucose-6-Phosphate} \quad \leftrightarrow \quad \text{Fructose-6-Phosphate}
\]
Enzymes

- Ligases
Enzymes
- Ligases

- EC 6, Ligases: join two molecules by making covalent bonds.
Enzymes

- Ligases

Citrulline + Aspartate + ATP ⇌ Argininosuccinate + AMP + 2P"
Enzymes
• Ligases

Citrulline + Aspartate + ATP $\rightleftharpoons$ Argininosuccinate + AMP + 2P$_i$

Argininosuccinate Synthetase

• EC 6, Ligases: join two molecules by making covalent bonds.
Enzymes

• Ligases

Citrulline + Aspartate + ATP $\leftrightarrow$ Argininosuccinate + AMP + 2P_i

Argininosuccinate Synthetase

• EC 6, Ligases: join two molecules by making covalent bonds.
Enzymes

- Ligases

Citrulline + Aspartate + ATP $\leftrightarrow$ Argininosuccinate + AMP + 2P_i

Argininosuccinate Synthetase

- EC 6, Ligases: join two molecules by making covalent bonds.
Enzymes
• Ligases

Citrulline + Aspartate + ATP ⇌ Argininosuccinate + AMP + 2P_i

Argininosuccinate Synthetase
• EC 6, Ligases: join two molecules by making covalent bonds.
Enzymes

- Ligases

EC 6, Ligases: join two molecules by making covalent bonds.

Citrulline + Aspartate + ATP $\iff$ Argininosuccinate + AMP + 2P$_i$

Argininosuccinate Synthetase
Enzymes
• Ligases

Citrulline + Aspartate + ATP $\leftrightarrow$ Argininosuccinate + AMP + 2P_i

Argininosuccinate Synthetase

• EC 6, Ligases: join two molecules by making covalent bonds.
Metabolic Melody
Catalyze
(To the tune of "Close to You")
Copyright Kevin Ahern
Catalyze
(To the tune of "Close to You")
Copyright Kevin Ahern

My enzymes
Truly are inclined
To convert
Things they bind
Turn the key
Covalently
Cat-a-lyze

How do cells
Regulate these roles?
    Allo-ster
    -ic controls

Two forms, see
States R and T
Mod-u-late

Competing inhibition keeps
The substrates from the active site
They raise Km, but leave Vmax and shirk
While the non-competers bind elsewhere
And lift the plot made on Lineweaver-Burk

Other ways
Enzymes can be blocked
When things bind
Then get locked
Stuck not free
Tied to the key
Su-i-cide
Catalyze
(To the tune of "Close to You")
Copyright Kevin Ahern

My enzymes
Truly are inclined
To convert
Things they bind
Turn the key
Covalently
Cat-a-lyze

How do cells
Regulate these roles?
Allo-ster
-ic controls

Two forms, see
States R and T
Mod-u-late

Competing inhibition keeps
The substrates from the active site
They raise Km, but leave Vmax and shirk
While the non-competers bind elsewhere
And lift the plot made on Lineweaver-Burk

Other ways
Enzymes can be blocked
When things bind
Then get locked
Stuck not free
Tied to the key
Su-i-cide

Penicillin’s action stops
Peptidoglycan cross-links in
Bacterial cell walls in awesome ways
Beta lactam ring’s reactive site
Starts bonding with D-D-transpeptidase

So there are
Several enzyme states
Counteract
-ing substrates

Now you see
Blocking the key
Regulates
Cat-a-lysts
Have to be controlled
Some get slowed
Put on hold

It's sublime
How the enzymes
(slow) Cat-a-lyze

ahhhhhhhhhhhhhhhhhhh - cat-a-lyze

ahhhhhhhhhhhhhhhhhhhhhhhhh - cat-a-lyze

ahhhhhhhhhhhhhhhhhhhhhhhhh - cat-a-lyze