Enzymes

Presented By

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Catalytic Proteins 4: Enzymes
Enzyme Inhibition
Enzyme inhibition is one way of regulating enzyme activity.

Most therapeutic drugs function by inhibition of a specific enzyme.

In the body, some of the processes controlled by enzyme inhibition are blood coagulation, blood clot dissolution (fibrinolysis) and inflammatory reactions.

Enzyme inhibitors are Reversible or Irreversible
Enzyme Inhibition means decreasing or cessation in the enzyme activity.

The inhibitor is the substance that decreases or abolishes the rate of enzyme action.

According to the similarity between the inhibitor and the substrate, enzyme inhibition is either:

- Competitive inhibition: Reversible
- Non-competitive inhibition: Irreversible
I. **Competitive inhibition**

- There is **structural similarity** between the inhibitor and substrate.
- The **inhibitor and the substrate compete** with each other to bind to the same **catalytic site** of the enzyme.
- The inhibition is **reversible**.
- It can be **relieved by increasing substrate concentration**.
- It does **not affect Vmax. It increases Km**.
1. Inhibitor compete with substrate
2. Structurally is similar to the substrate
3. Inhibition is reversible
4. Inhibitor does not bind with E-S complex
5. Inhibition is relieved in excess substrate concentration
6. Inhibitor with enzyme forms enzyme inhibitor complex (E-I)
Competitive Inhibition

presented as:

\[ E + S \rightleftharpoons E\cdot S \rightarrow E + P \]

I resembles S

I binds at active site reversibly

\( E\cdot I \) cannot bind to S so no reaction
Competitive Inhibitor

- Substrate
- Products
- Inhibitor

Competition depends upon concentration

C. Ophardt, c. 2003
Molecular interpretation for competitive inhibition

♦ competitive inhibitor binds to same site as the substrate (competes).

♦ its structure usually resembles substrate.

♦ While the inhibitor is bound, the enzyme cannot bind substrate and no reaction possible.

♦ Many pharmaceutical agents are competitive inhibitors so are many toxic substances.
In competitive inhibition, can always add enough [S] to overcome inhibition. ⇒ same $V_{\text{max}}$
The graph shows the relationship between the rate of a reaction ($v$) and the concentration of substrate ([S]). The maximum rate ($V_{\text{max}}$) is indicated by $V_{\text{max}(i)}$. The red line represents the uninhibited reaction, while the purple line represents the inhibited reaction. The $K_m$ and $K_{m(i)}$ are the Michaelis constants for the uninhibited and inhibited reaction, respectively.
with competitive inhibitor
Examples and Clinical Use:

1- Malonate blocks the action of Succinate dehydrogenase which catalyzes the transformations of succinate to fumarate.
The formulae of malonic and succinic acids show the **structural similarity** between them.
Succinate and Malonate are 2 structural analogs.
enzyme (e.g. succinate dehydrogenase)
substrate (e.g. succinate)
competitive inhibitor (e.g. malonate)
2. *Sulfonamides*: are structural analogs of p-aminobenzoic acid *used by bacteria for folic acid synthesis*.

- Folic acid is essential for growth and multiplication.

Hence the sulfa drugs as bacteriostatic.
3. *Dicumarol* is structurally similar to vitamin K and can act as anticoagulant by competitive inhibition of vitamin K activity.
1. The substrate binds to the active site of the enzyme.
2. The enzyme and substrate interact, resulting in the formation of products of enzyme activity.
3. A drug with similarities in shape to the substrate binds to the active site on the enzyme, blocking its activity.
<table>
<thead>
<tr>
<th>Enzymatic process</th>
<th>Substrate</th>
<th>Inhibitor</th>
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</thead>
<tbody>
<tr>
<td>Succinate dehydrogenase</td>
<td>Succinic acid</td>
<td>Malonic acid</td>
</tr>
<tr>
<td>Folic acid synthesis in bacteria</td>
<td>Para aminobenzoic acid (PABA)</td>
<td>Sulfanilamide</td>
</tr>
<tr>
<td>Prothrombin synthesis</td>
<td>Vitamin K</td>
<td>Dicumarol</td>
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<tr>
<td>Carbonic anhydrase</td>
<td>Carbonic acid</td>
<td>Acetazolamide (diamox)</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Xanthine</td>
<td>Allopurinol (zyloric)</td>
</tr>
<tr>
<td>Choline esterase</td>
<td>Acetyl choline</td>
<td>physostigmine</td>
</tr>
</tbody>
</table>
Enzyme behavior in present of competitive inhibitor:

- \( V_{\text{max}} \) is unchanged.

- \( K_m \) is increased, more substrate is needed to keep the reaction going at \( \frac{1}{2} V_{\text{max}} \).
Michaelis Menten hyperbolic plot:

- The initial velocity \((V_i)\) of the enzyme reaction rises more slowly when competitive inhibitor is present, but eventually reaches normal \(V_{max}\) when \([S]\) is very high.
In competitive inhibition, can always add enough [S] to overcome inhibition.

\[ \Rightarrow \text{same } V_{\text{max}} \]
- Inhibitor

+ Inhibitor

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

\[
K_m,\text{app}
\]

\[
K_m
\]

[Substrate]
Lineweaver-Burk plot:

- It shows a **steeper slope** to the line when a competitive inhibitor is present.

- The **series of lines pivot on the they intercept**, since **Vmax is not changed** for competitive inhibition.

- The **X-intercept becomes smaller** as **Km increases** in competitive inhibition.
**Competitive inhibition**

Double reciprocal plot

- **Same** $1/v$ intercept, **same** $V_{max}$
- **Different slopes**, competitive

Inhibition changes apparent $K_m$

- **Note:** inhibition line always **above** no inhibition.
\[ \frac{1}{v} = \frac{K_{\text{m,app}}}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}} \]
Figure 8-20
Biochemistry, Sixth Edition
© 2007 W.H. Freeman and Company
Competitive inhibition: $K_m$ INCREASED, $V_{max}$ NO CHANGE
Got any Questions ?!
II- Non-competitive inhibition

Non-competitive inhibition may be:

- Specific.
- Non-specific.
Non-specific non-competitive inhibition

As enzymes are **proteins** in nature, any factor that causes **protein denaturation** will inhibit enzyme activity e.g.

- **Strong acids,**
- **Strong alkalis,**
- **Severe agitation**
- **Repeated freezing and thawing.**
Specific non-competitive inhibition

- There is **no structural similarity** between the inhibitor and the substrate.
- The **inhibitor does not bind to the catalytic site** as the substrate but **it binds to another site**.
- It can **bind enzyme or to enzyme substrate complex**.
- The inhibition is **irreversible. It cannot be relieved by increasing substrate concentration**.
- It **decreases Vmax. It does not affect Km**.
(c) A noncompetitive inhibitor binds to the enzyme at a location away from the active site, but alters the conformation of the enzyme so that the active site is no longer fully functional.

Not resemble substrate
Binds to site other than active site
Can bind with ES complex
Usually It is irreversible
Increasing substrate concentration not abolish inhibition
Irreversible Noncompetitive inhibitors

A noncompetitive inhibitor binding to both free enzyme and enzyme-substrate complex.
Noncompetitive Inhibition

$E + S \rightleftharpoons E\cdot S \rightarrow E + P$

$E\cdot I \rightleftharpoons E\cdot S\cdot I$

$E\cdot I$ and $E\cdot S\cdot I$ not productive, depletes

$E$ and $E\cdot S$
Noncompetitive Inhibitor

substrate

products

substrate

Noncompetitive inhibitor - permanently bonded

inhibitor

C. Ophardt, c. 2003
Non- Competitive Inhibition

- [I] and [S] may combine at different sites of the enzyme, so formation of both [EI] and [EIS] complexes is possible.

- Since [EIS] may break down to form product at a slower rate than [ES] complex, the reaction may be slowed but not stopped. Irreversible non competitive inhibition decreases Vmax but does not affect Km.
Noncompetitive Inhibition

\[
E + S \rightleftharpoons E \cdot S \rightarrow E + P
\]

\[
\begin{align*}
+ & + \\
\downarrow & \downarrow
\end{align*}
\]

\[
E \cdot I \leftarrow E \cdot S \cdot I
\]

\[
\begin{align*}
\uparrow & \uparrow \\
\uparrow & \uparrow
\end{align*}
\]

E\cdot I and E\cdot S\cdot I not productive, depletes E and E\cdot S
Molecular Interpretation:

- Inhibitor binds the enzyme somewhere different from where the substrate binds.
- So the inhibitor does not care whether substrate is bound or not.
- Inhibitor changes the conformation of the enzyme at the active site so no reaction is possible with inhibitor bound.

E•I and E•S•I not productive
Non-competitive inhibition may be caused by:

- Inhibition of sulphahydryl group.
- Inhibition of cofactors.
- Inhibition of specific ion activator.
A) Inhibition of Sulphahydryl (-SH) group

- Many enzymes depend on free sulphahydryl group for its activity.
- Inhibition of this group leads to loss of the enzyme activity.
Sulphahydryl group can be inhibited by:

1- Oxidizing agents as potassium ferricyanide

2 E-SH → E-S-S-E

Potassium Ferricyanide  |  Potassium Ferrocyanide

Where E-SH is an enzyme containing free sulphahydryl group.
2- Alkylation agents as iodoacetic acid (I-CH₂-COOH) and iodoacetamide

\[
\begin{align*}
E-SH & \quad \rightarrow \quad E-S-\text{CH}_2-\text{COOH} \\
\text{I-CH}_2-\text{COOH} & \quad \rightarrow \quad \text{HI (iodic acid)}
\end{align*}
\]
3- Effect of heavy metals:

Heavy metal ions as **mercury** \((\text{Hg}^{++})\) and **lead** \((\text{Pb}^{++})\) block **sulphahydryl** group of enzymes forming **mercaptides**.

\[
2 \text{E-SH} + \text{Pb}^{++} \rightarrow \text{E-S-Pb-E-S}
\]
B) Inhibition of cofactors

The inhibitors block an active group in coenzymes or the prosthetic group:
1- Coenzyme inhibition: e.g. hydrazine and hydroxylamine block the aldehyde group in the pyridoxal phosphate, which is a coenzyme needed for transmination, decarboxylation and desulphhydration of amino acids.
2- Inhibitors of prosthetic group: e.g. carbon monoxide (CO), cyanide and bisulphate block the iron in the haem which is the prosthetic group of cytochrome oxidase enzyme.
C) Inhibition of metal ion activator

- Removal of calcium ions from blood prevents its coagulation.

- Ca^{++} is needed to activate thrombokinase enzyme which converts the inactivate prothrombin to active thrombin that causes blood clotting.
Enzyme behavior in presence of non-competitive inhibitor:

- \( V_{\text{max}} \) is decreased.
- \( K_m \) remains unchanged.
Michaelis Menten hyperbolic plot:

- It shows that the initial velocity \((V_i)\) of the enzyme reaction *rises more slowly* when non-competitive inhibitor is present, and levels off at *reduced Vmax*. 
Reaction Rate vs. [Substrate]

- Inhibitor
- $V_{\text{max}}$
- $V_{\text{max,app}}$
- $\frac{1}{2} V_{\text{max}}$
- $\frac{1}{2} V_{\text{max,app}}$

+ Inhibitor
- $K_m$
- $K_{m,\text{app}}$

Inhibitor Effects:

- Inhibitor reduces the maximal reaction rate ($V_{\text{max}}$) to $V_{\text{max,app}}$.
- The Michaelis constant ($K_m$) increases to $K_{m,\text{app}}$. 
Lineweaver-Burk plot:

- It shows that the series of lines pivot on the negative X intercept, since Km is unchanged for non-competitive inhibition.

- Y-intercept and slope increase due to the reciprocal dependence on Vmax, which decreases.
Noncompetitive Inhibition

different slopes, different $1/v$ intercepts.
\[ \frac{1}{v} = \frac{K_m}{V_{\text{max, app}}} \frac{1}{[S]} + \frac{1}{V_{\text{max, app}}} \]
Non competitive inhibition: Km no change, V-max decreased
Got any Questions ?!
The Effects of Inhibitors on Enzyme Kinetics

The distinction between competitive and non-competitive enzyme inhibition can be determined by plotting enzyme activity with and without the inhibitor present.
Enzyme behavior in present of competitive inhibitor:

- $V_{\text{max}}$ is unchanged.

- $K_m$ is increased, more substrate is needed to keep the reaction going at $\frac{1}{2} V_{\text{max}}$. 
Inhibitor effects on enzyme activity:

- Inhibitor

$V_{\text{max}}$

$K_m$

$K_{m,\text{app}}$

[Substrate]

Reaction Rate
Enzyme behavior in presence of non-competitive inhibitor:

- $V_{\text{max}}$ is decreased.
- $K_m$ remains unchanged.
Reaction Rate

\[ V_{\text{max}} \]

\[ V_{\text{max,app}} \]

\[ \frac{1}{2} V_{\text{max}} \]

\[ \frac{1}{2} V_{\text{max,app}} \]

[Substrate]

\[ K_m \]

\[ K_{m,\text{app}} \]

- Inhibitor

+ Inhibitor

Reaction Rate

- Inhibitor

+ Inhibitor

V_{\text{max,app}}
Don’t be inhibited!
Ask Questions!
1- .............. occurs when the inhibitory chemical, which has no similarity to the substrate, binds to the enzyme other than at the active site.

(A) Noncompetitive Inhibition
(B) Competitive Inhibition
(C) Un-catalysed reaction
(D) All A, B and C

2- Km values are not altered by which type of inhibitor

a) Competitive inhibitors
b) Non competitive inhibitors
c) Uncompetitive inhibitors
d) All of these
1- The inhibition of succinate dehydrogenase by malonate is an example for

(A) Noncompetitive Inhibition
(B) Competitive Inhibition
(C) Feedback inhibition
(D) None of these

2- The noncompetitive inhibitor

a) Increases $V_{max}$ and $K_m$
b) Decreases $V_{max}$
c) Increases $K_m$ without affecting $V_{max}$
d) Decreases $K_m$ and $V_{max}$
Activity

- Compare between competitive and non-competitive enzyme inhibition.
- Discuss with 3 examples competitive inhibition
- Enumerate 3 methods of non-competitive inhibition
- Discuss in short the effect of inhibitors on enzyme kinetics.
Thank you