Enzyme Inhibition

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 classified into:

1. Competitive inhibition
2. Noncompetitive inhibition

I. Competitive Inhibition

In this type of 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 V_max. It increases K_m.

<table>
<thead>
<tr>
<th>Enzymatic process</th>
<th>Substrate</th>
<th>Inhibitor</th>
</tr>
</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>
</tr>
<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>Phystostigmine</td>
</tr>
</tbody>
</table>

The formulae of malonic and succinic acids show the structural similarity between them.

\[
\text{Malonic Acid} \quad \text{Succinic Acid}
\]
II- Noncompetitive inhibition

Non-competitive inhibition may be specific or non-specific.

A- Specific noncompetitive inhibition

In this type of enzyme 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 to enzyme or to enzyme substrate complex
The inhibition is irreversible.
It cannot be relieved by increasing substrate concentration.
It decreases $V_{max}$.
It does not affect $K_m$.

Noncompetitive inhibition decreases $V_{max}$ but does not affect $K_m$

Noncompetitive inhibition may be caused by:
1. Inhibition of sulphahydryl group.
2. Inhibition of cofactors.
3. Inhibition of specific ion activator.

1)- 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:
   a- Oxidizing agents as potassium ferricyanide.

   $$2\ E-SH \rightarrow E-S-S-E$$

   Potassium ferricyanide  Potassium Ferrocyanide

   Where $E-SH$ is an enzyme containing free sulphahydryl group.

   b- Alkylating agents as iodoacetic acid (I-CH$_2$-COOH) and iodoacetamide

   $$E-SH \rightarrow E-S-CH_2-COOH$$

   I-CH$_2$-COOH  HI (iodic acid)

   Enzyme inhibition by iodoacetic acid
c- **Effect of heavy metals:** Heavy metal ions as mercury (Hg++) and Lead (Pb++) block sulphhydryl group of enzymes forming mercaptides.

\[ 2 \text{E-SH} + \text{Pb}^{++} \rightarrow \text{E-S-Pb-E-S} \]

2)- **Inhibition of cofactors**

The inhibitors block an active group in coenzymes or the prosthetic group:

a. **Coenzyme inhibition** e.g. hydrazine and hydroxylamine block the aldehyde group in the pyridoxal phosphate, which is a coenzyme needed for transamination, decarboxylation and desulfhydration of amino acids.

b. **Inhibitors of prosthetic group** e.g. carbon monoxide (CO), cyanide and bisulphate block the iron in the haeme which is the prosthetic group of cytochrome oxidase enzyme

3)- **Inhibition of metal ion activator**

Removal of calcium ions from blood prevents its coagulation as Ca++ is needed to activate thrombokinase enzyme which converts the inactivate prothrombin to active thrombin that causes blood clotting.

B- **Non-specific non-competitive inhibition**

As enzymes are protein in nature, any factor that causes protein denaturation will inhibit enzyme activity e.g. strong acids, strong alkalis severe agitation and repeated freezing and thawing.

**Enzyme Kinetics**

Kinetics are concerned with the rates of reactions. This is a very important matter for the living organism which maintains its steady state by adjusting reaction rates in response to the environment and to hormonal controls.

The study of the rate at which an enzyme acts is called enzyme kinetics.

**Michaelis-Menten hyperbolic plot**

Enzyme kinetics are studied by plotting the initial velocity \( (V_i) \) on the Y axis and the substrate concentration \([S]\) on the X axis, we find that:

- At low values of \([S]\), the initial velocity \((V_i)\) rises almost linearly with increasing \([S]\).
- A further increase in \([S]\) produces a less than proportional increase in reaction rate.
- Eventually, the enzyme becomes saturated with the substrate and the reaction attains its maximal velocity \((V_{max})\).
- Any further increase in \([S]\) does not affect the velocity of the reaction.
- The substrate concentration that produces \(1/2V_{max}\) is the Michaelis-Menten constant, \(K_m\).
- The produced curve attains the shape of a rectangular hyperbola. So the curve is called Michaelis-Menten hyperbolic curve.
- It is hard to draw accurately and hard to determine \(V_{max}\) and \(K_m\) precisely.
Lineweaver-Burk plot

Plotting the reciprocals of Vi and [S] (plotting 1/Vi on the Y axis and 1/[S] on the X axis) yields a "double-reciprocal" or Lineweaver-Burk plot.
- \( V_{\text{max}} \) is determined by the point where the line crosses the Y axis as the [S] is infinite at that point.
- \( K_m \) is easily determined from the intercept on the X axis.
- The curve is linear, so it is widely used as it is easy to draw and provides a more precise way to determine \( V_{\text{max}} \) and \( K_m \).

The Effects of Inhibitors on enzyme kinetics

The distinction between competitive and noncompetitive enzyme inhibition can be determined by plotting enzyme activity with and without the inhibitor present.

Enzyme behavior in presence of competitive inhibitor:
- \( V_{\text{max}} \) is unchanged
- \( K_m \) is increased, more substrate is needed to keep the reaction going at 1/2 \( V_{\text{max}} \)
- \textit{Michaelis Menten hyperbolic plot} shows that the initial velocity (\( v_i \)) of the enzyme reaction rises more slowly when competitive inhibitor is present, but eventually reaches normal \( V_{\text{max}} \) when [S] is very high.
- \textit{Lineweaver-Burk plot} shows a steeper slope to the line when a competitive inhibitor is present. The series of lines pivot on the y intercept, since \( V_{\text{max}} \) is not changed for competitive inhibition. The X-intercept becomes smaller as \( K_m \) increases in competitive inhibition.

Enzyme behavior in presence of non-competitive inhibitor:
- \( V_{\text{max}} \) is decreased
- \( K_m \) remains unchanged
- \textit{Michaelis Menten hyperbolic plot} shows the initial velocity (\( v_i \)) of the enzyme reaction rises more slowly when noncompetitive inhibitor is present and levels off at reduced \( V_{\text{max}} \)
- \textit{Lineweaver-Burk plot} shows that the series of lines pivot on the negative X intercept, since \( K_m \) is unchanged for non-competitive inhibition. Y-intercept and slope increase due to the reciprocal dependence on \( V_{\text{max}} \), which decreases.
Enzyme units

The amount of enzyme present or used in a process is difficult to determine in absolute terms (e.g. grams), as its purity is often low and a portion of the enzyme may be in an inactive, or partially active, state. For these reasons, enzymes are usually measured in terms of activity rather than weight. The term activity means the number of micromoles of substrate converted to product per unit of time.

Many ways are used to measure enzyme activity:

1- The activity unit (U), which also is called the international unit (IU), is defined as the amount of the enzyme that catalyses the conversion of one micromole of substrate to product in one minute under standard conditions. Standard conditions refer to optimal conditions, especially with regard to pH, ionic strength, temperature, substrate concentration and the presence and concentration of cofactors.

2- The katal Unit (kat) is defined as the amount which will catalyse the transformation of one mole of substrate per second (kat = 60,000,000 IU). It is an impracticable unit and has not yet received widespread acceptance.

3- Volume activity is the enzyme Activity in international units per ml of extract (the solution in which the enzyme is present). Its unit is IU/ml.

4- The specific activity is used to monitor the purity of an enzyme during a purification procedure.

Specific activity of an enzyme in extract equals volume activity (measured in IU/ml) divided by the protein concentration in the same extract (measured in mg/ml). The unit of specific activity is IU/mg.

5- Catalytic Efficiency (Kcat) is a direct measure of the catalytic activity under optimum conditions (i.e. fully saturated enzyme).

It is also called the turnover number as it equals the number of substrate molecules that are turned over (converted to product) in one second when the enzyme acts at optimum conditions (i.e. at its maximum velocity, Vmax). The unit of Kcat is the number of substrate molecules turned over per enzyme molecule per second.

6- Specificity constant equals the catalytic efficiency of the enzyme (kcat) divided by its Michaelis constant (Km). It provides a direct measures of enzyme efficiency and specificity of different enzymes.

7- Sometimes arbitrary (non-standard) units are used to measure enzyme activity.