Enzyme inhibitors

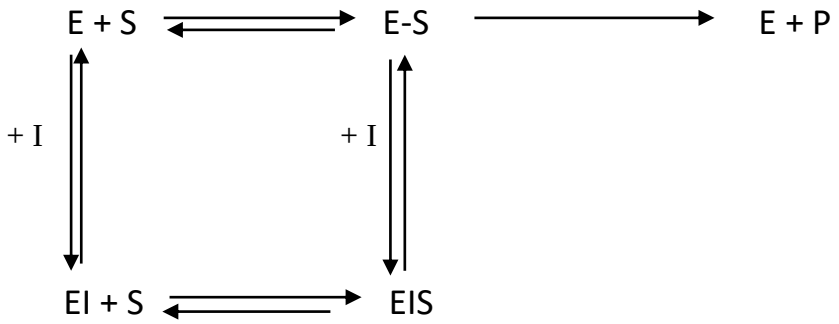
1. Introduction :

In addition to their substrates, enzymes can bind substances that they are unable to catalyze. These substances are often inhibitors. So an inhibitor :

1. Reduces or decreases the speed of the reaction catalyzed by the enzyme on a substrate ;
2. Is not transformed by the enzyme.

Some inhibitions result from the reversible binding of an inhibitor to an enzyme. Others are the result of irreversible binding of compounds to the enzyme.

General presentation :



2. Types of reversible inhibition of enzymatic reactions :

Inhibitors are classified according to their binding site on the enzyme and their impact on kinetic constants. There are four classes :

- Competitive inhibitors.
- Non-competitive inhibitors.
- Uncompetitive inhibitors.
- Mixed inhibitors.

2. 1. Competitive inhibition :

A competitive inhibitor binds only to the free enzyme at the substrate binding site (catalytic site), with its binding and that of the substrate being mutually exclusive. In this case, the inhibitor competes with the substrate for the same binding site.

Factors that enable various compounds to function as competitive inhibitors :

- ✓ The inhibitor is similar to the substrate in terms of its shape and the spatial arrangement of its polar functions ;
- ✓ The inhibitor meets a number of criteria that allow it to fit into the active site by forming the same type of association (hydrogen bonding, ionic bridges, hydrophobic interactions, etc.) as the substrate ;
- ✓ The inhibitor shows an analogy with the first transition state (a transition state can correspond to a fleeting, unstable state that can be reproduced in a stable form in another molecule).

Two balanced reactions occur simultaneously :

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\text{-}S \xrightarrow{k_2} E + P$$

$[E]\,[S] / [ES] = K_M$ from which **$K_M\,[ES] = [E]\,[S]$ .....(1)**

$$E + I \underset{k_{-3}}{\overset{K_3}{\rightleftharpoons}} E\text{-}I$$

According to the second equilibrium, we have : $k_3\,[E]\,[I] = k_{-3}\,[EI]$

From this : **$[E]\,[I] / [EI] = k_{-3} / k_3 = K_I$** it is ***the inhibition constant***

Consequently, equilibria can be displaced by an excess of substrate or an excess of inhibitor. Therefore, to completely displace the inhibitor from its combination with the enzyme, it is sufficient to add a large excess of substrate.

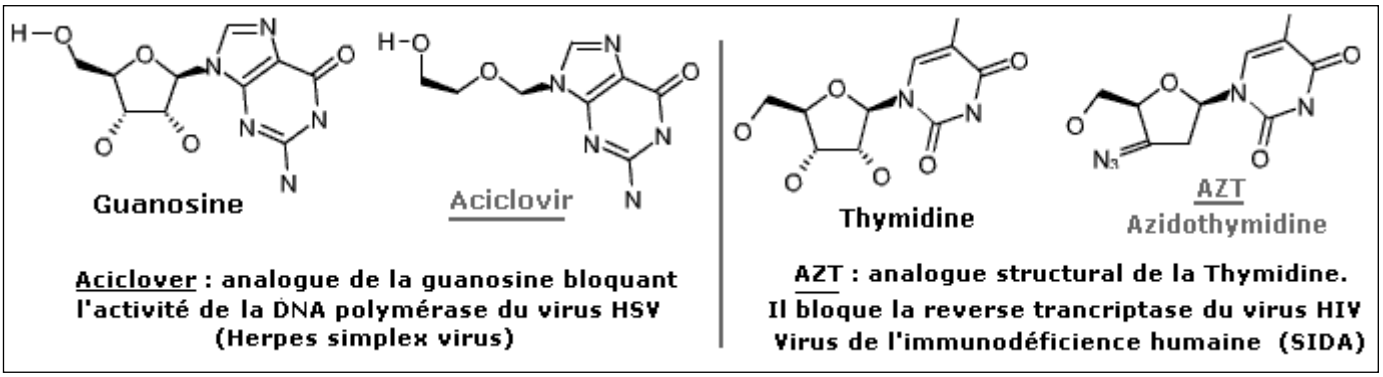
The Michaelis-Menten equation becomes :

$$\mathbf{v = v_m\,[S] / [K_M\,(1 + [I] / K_I) + [S]]}$$

We can see that the maximum speed does not change. However, K_M increases by a factor equal to $(1 + [I] / K_I)$. This means that more substrate is needed to obtain $v_m / 2$.

Example of a competitive inhibitor :

This is the case for certain antiviral drugs such as :



2. 2. Non-competitive inhibition :

In this case, the inhibitor can bind to both the free enzyme and the E-S complex with different equilibrium constants. The inhibitor binds at a site different from the substrate binding site (catalytic site). The resulting conformational change decreases the catalytic power and makes electron exchanges in the E-S complex more difficult. In fact, the ternary EIS complex is inactive, unable to produce a product under any circumstances.

Therefore, we have the following equilibria :

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\text{-}S \xrightarrow{k_2} E + P \quad , \quad \mathbf{K_M\,[ES] = [E]\,[S] \ldots\ldots\ldots\ (1)}$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E\text{-}I \quad , \quad [E]\,[I] / [EI] = K_I \qquad \mathbf{[EI] = [E]\,[I] / K_I}$$

$$ES + I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} EIS \quad , \quad [ES]\,[I] / [EIS] = K_I \qquad \mathbf{[EIS] = [ES]\,[I] / K_I}$$

The Michaelis-Menten equation becomes :

$$\mathbf{v = v_m\,[S] / (1 + [I] / K_I)\,(K_M + [S])}$$

We observe that the Michaelis constant remains unchanged, while the maximum speed decreases by a factor of (1 + [I] /KI).

Example of a non-competitive inhibitor :

Various small-molecule ligands such as sulfonamides or benzodiazepines behave as non-competitive inhibitors of ɣ-secretase, which is involved in Alzheimer's disease, with formation of the ternary complex.

2. 3. Uncompetitive inhibition (by blocking an intermediate complex) :

The noncompetitive inhibitor binds only to the ES complex. Generally, the binding of the substrate to the enzyme causes a change in the conformation of the enzyme, thereby revealing a binding site for the inhibitor. The inhibitor, in turn, changes the conformation of the active site of the enzyme and prevents the reaction.

So, there are two equilibria :

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\text{-}S \xrightarrow{k_2} E + P \quad , \quad \mathbf{K_M\,[ES] = [E]\,[S] \ldots\ldots\ldots(1)}$$

$$ES + I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} EIS \quad , \quad [ES]\,[I] / [EIS] = K_I \qquad \mathbf{[EIS] = [ES]\,[I] / K_I}$$

The Michaelis-Menten becomes :

$$\mathbf{v = [v_m\,[S] / (1 + [I]/K_I)] / [K_M / (1 + [I]/K_I) + [S]]}$$

We observe that the two kinetic constants (K_M and v_m) decrease by a factor of (1 + [I] /K_I).

Example of an Uncompetitive inhibitor :

Lithium (Li^+) exerts an Uncompetitive inhibition on IMPase (inositol monophosphatase), which decreases the concentration of cytoplasmic inositol.

2. 4. Mixed inhibition :

In some cases, inhibition can be both competitive and non-competitive. It results in an increase in K_M and a decrease in v_m. Thus, the Michaelis-Menten equation becomes :

$$\mathbf{v = [v_m\,[S] / (1 + [I]/K_I)] / [K_M\,(1 + [I]/K_I) + [S]]}$$

Example of a mixed inhibitor :

This is the case with thio-oligosaccharide, a mixed cellulase inhibitor.

There are other types of inhibition :

- Inhibition by binding of the inhibitor to the substrate
- Inhibition by high substrate concentrations
- Inhibition by reaction products

3. Graphical representations :

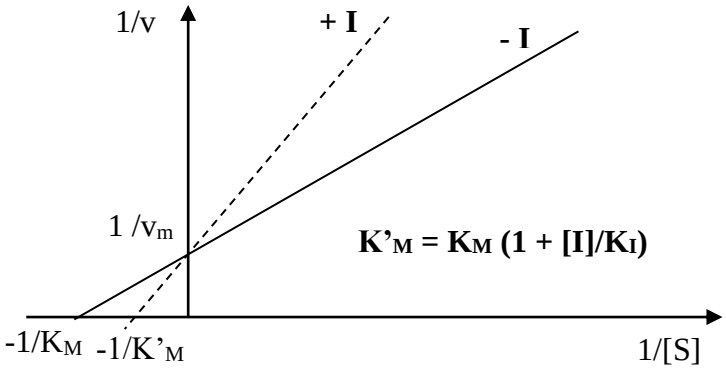
In general, graphical representations can be used to determine the type of inhibition, the kinetic constants of the enzyme, and the inhibition constant.

3. 1. Lineweaver-Burk representation :

If we trace the Lineweaver-Burk curve of an enzyme activity, in the absence and presence of an inhibitor, we obtain the following graphs :

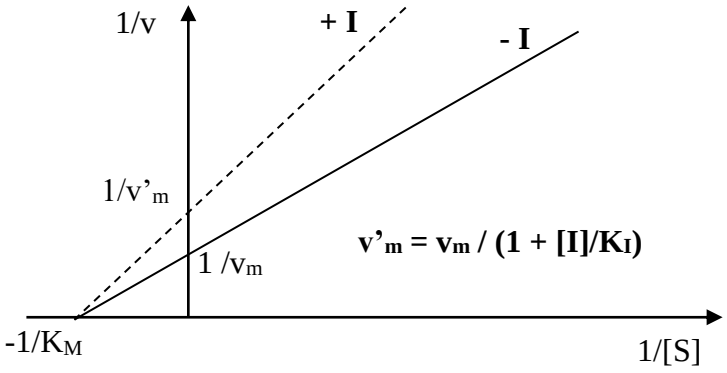
3. 1. 1. Competitive inhibition :

The line obtained in the presence of the inhibitor intersects the y-axis at the same point as the curve in the absence of the inhibitor. However, it intersects the x-axis at a point closer to 0 than the point (-1/K_M).



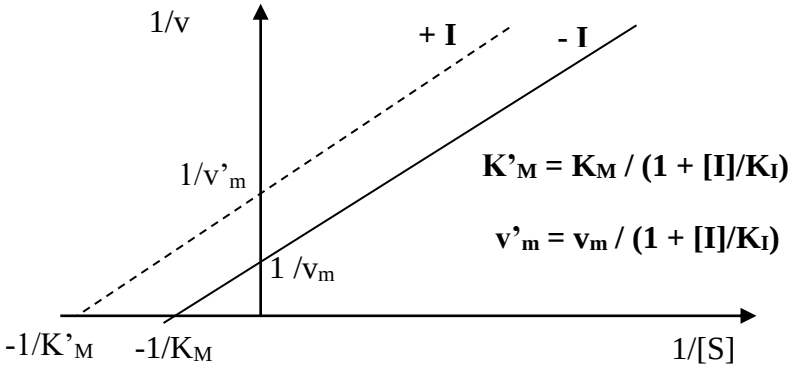
3. 1. 2. Non-competitive inhibition :

In the presence of the inhibitor, the curve intersects the y-axis higher than in the absence of the inhibitor and the x-axis at the same point ($-1/K_M$).



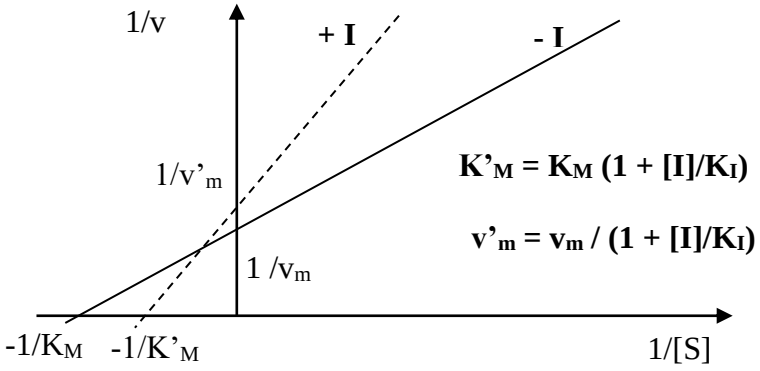
3. 1. 3. Uncompetitive inhibition :

In this case, the inhibition curve is parallel to that obtained in the absence of the inhibitor.



3. 1. 4. Mixed inhibition :

The Lineweaver-Burk line does not pass through either the point ($-1/K_M$) or the point ($1/v_m$).



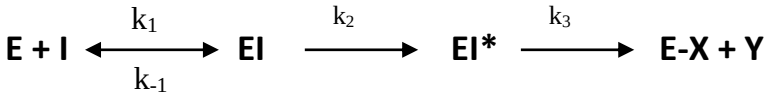
4. Irreversible inhibition :

There are many irreversible covalent inhibitors, which often make it possible to :

- Neutralize an enzyme whose effects we want to eliminate permanently.
- Mask the active site, which makes it possible to identify the chemical group that reacted with the inhibitor after denaturation and hydrolysis of the enzyme.

Suicide inhibitor :

A ligand that binds to the active site of an enzyme and forms a stable covalent bond there, thereby permanently inactivating the protein, is often called a “suicide” inhibitor.



The enzyme recognizes the inhibitor as its substrate and begins the process of modifying it. This is followed by a step during which the modified inhibitor becomes highly reactive and binds very stably to the enzyme. The enzyme thus contributes to its own irreversible inactivation, hence the name “suicide” inhibition. The inhibitor can bind to the enzyme's catalytic site or elsewhere.

These inhibitors are highly sought after in pharmacological applications and for the design of pesticides and herbicides,…

Example :

Allopurinol is a potent xanthine oxidase (XO) inhibitor used in the treatment of the gout disease.