Clinical significance of enzyme assays

Enzymes are the preferred markers in various disease states such as myocardial infarction, jaundice, pancreatitis, cancer, neurodegenerative disorders, etc. They provide insight into the disease process by diagnosis, prognosis, and assessment of response therapy.

Enzyme assays are performed to serve two different purposes:

(i) to identify a special enzyme, to prove its presence or absence in a distinct specimen, like an organism or a tissue

(ii) to determine the amount of the enzyme in the sample.

How substrate binds to the enzyme's active side

Active side: - a region in the enzyme at which the substrate binds to the enzyme, it consist of amino acids residues which participate in the catalysis.

There are two theories to explain how the enzyme binds to substrate

1. "Lock and key" theory.

In this model, the substrate has a specific shape match the active site of enzyme, like a lock and key [figure (1)]. The limitation of this theory is the rigidity of active side related to the substrate. However, this theory is applied on the number of simple kinetics enzymes.

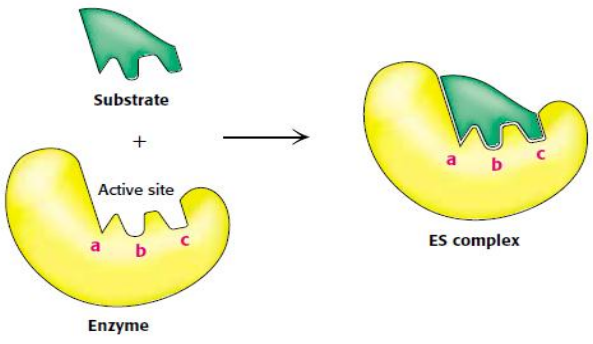


Figure (1) Lock-and-key model of enzyme–substrate binding. In this model, the active site of the unbound enzyme is complementary in shape to the substrate.

2. Induced-fit model.

In this model, the active site changes shape as it interacts with the substrate [figure (2)]. Once the substrate is fully locked in and in the exact position, the catalysis can begin. In essence, substrate binding alters the conformation of the protein, so that the protein and the substrate "fit" each other more precisely.

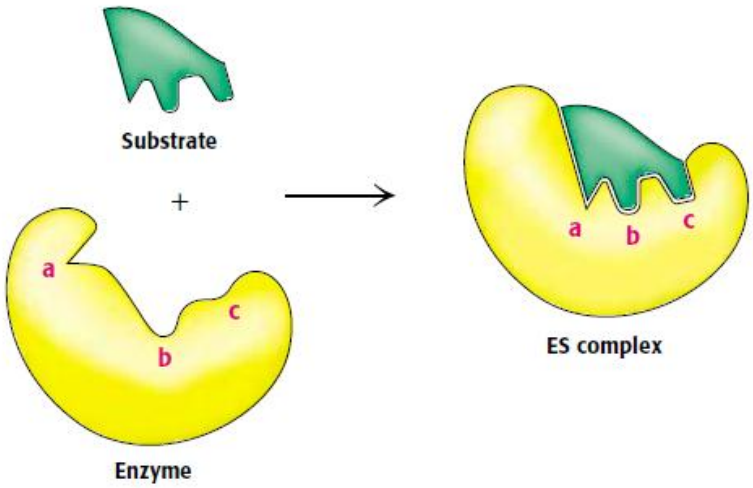


Figure (2) Induced-fit model of enzyme–substrate binding. In this model, the enzyme changes shape on substrate binding. The active site forms a shape complementary to the substrate only after the substrate has been bound

Michaelis-Menten equation

Michaelis and Menten suggests that the enzyme reversibly combines with its substrate to form an ES complex that subsequently yields product, regenerating the free enzyme.

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

where

S is the substrate

E is the enzyme

ES is the enzyme–substrate complex

P is the product

k1, k-1, and K2 are rate constants

The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration:

$$v_o = \frac{V_{max}\,[s]}{k_m + [s]} \ldots\ldots (1)$$

where v_o = initial reaction velocity

V_{max} = maximal velocity

Km = Michaelis constant = (k-1 + k2)/k1

[S] = substrate concentration

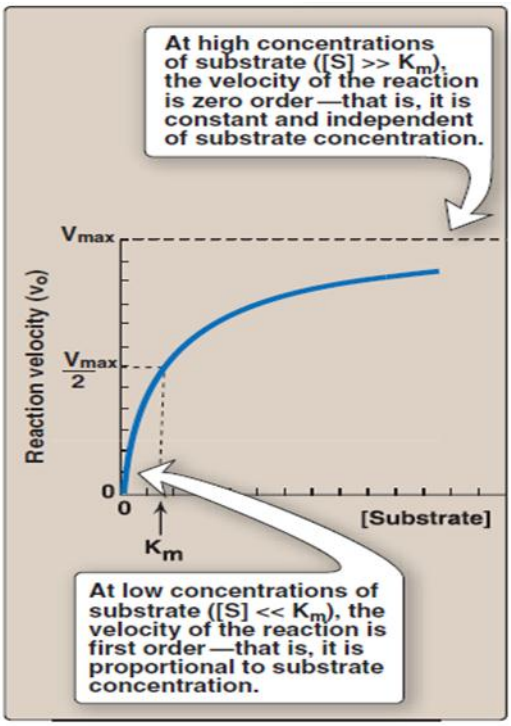
Important conclusions about Michaelis-Menten kinetics

I- Relationship of velocity to enzyme concentration:

The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations. For example, if the enzyme concentration is halved, the initial rate of the reaction (v_o), as well as V_{max}, are reduced to half.

II- Order of reaction:

When [S] is much less than Km, the velocity of the reaction is approximately proportional to the substrate concentration (Figure 3). The rate of reaction is then said to be first order with respect to substrate. When [S] is much greater than Km, the velocity is constant and equal to V_{max}. The rate of reaction is then independent of substrate concentration, and is said to be zero order with respect to substrate concentration.



III- Characteristics of K_m: -

K_m is the substrate concentration at which the reaction velocity equal to (1/2 V_{max}), it indicates how efficiently an enzyme selects its substrate and converts it to product. *K_m is often used as a measure of an enzyme's affinity for a substrate*. Let us assume that the measured velocity (v_o) is equal to 1/2 V_{max}. Then the equation (1) may be substituted as follows:

$$\frac{1}{2}V_{max} = \frac{V_{max}[S]}{K_m + [S]}$$

$$K_m + [S] = \frac{2V_{max}[S]}{V_{max}}$$

$$K_m + [S] = 2[S]$$

$$K_m = [S]$$

A low Km value indicates a strong affinity between enzyme and substrate figure (4), whereas **a high Km value** indicates a weak affinity between them. For majority of enzymes, the Km values are in the range

of 10^{-5} to 10^{-2} moles. It may however, be noted that Km is **not dependent on the concentration of enzyme**.

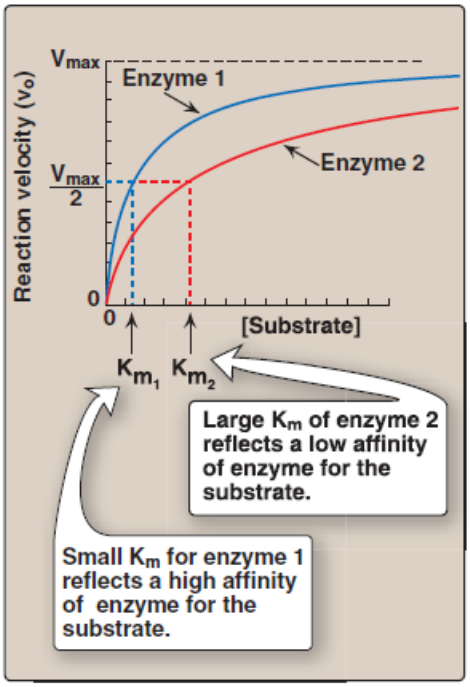


Figure (4) Effect of substrate concentration on reaction velocities for two enzymes:
enzyme 1 with a small Km, and
enzyme 2 with a large Km.

Isoenzymes

Isoenzymes (also called isozymes) are enzymes that catalyze the same reaction (act on the same substrate), but they differ in their physical and chemical properties which include (the structure, immunological properties, pH optimum, K_m and V_{max} values), also they possess different amino acid composition. For this reason, isoenzymes may contain different numbers of charged amino acids and may, therefore, be separated from each other by electrophoresis.

ENZYME INHIBITION

Enzyme inhibitor is defined as a substance which binds with the enzyme and brings about a **decrease in catalytic activity** of that enzyme. The

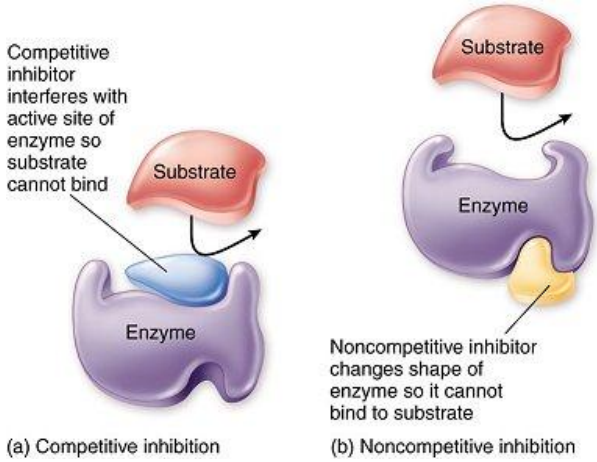
inhibitor may be organic or inorganic in nature. There are three broad categories of enzyme inhibition .

1. Reversible inhibition.
2. Irreversible inhibition.
3. Allosteric inhibition

1. Reversible inhibition

The inhibitor binds non-covalently with enzyme and the enzyme inhibition can be reversed if the inhibitor is removed. The reversible inhibition is further sub-divided into

I. Competitive inhibition
II. Non-competitive inhibition
III. Uncompetitive inhibitors



I. Competitive inhibition :

Competitive inhibitors compete with the substrate at the active site, raising Km (the michaelis-menten constant). However, Vmax remains

unaltered since the reaction may still be completed with sufficient substrate concentration.

The degree of inhibition depends on the relative concentrations of the substrate and the inhibitor.

Example:
Enzyme : Succinate Dehydrogenase
Substrate : Succinate
Inhibitors : Malonate, Glutarate, Oxalateetc.

II. Non-competitive inhibition :

Non-competitive inhibitors attach to another site on the enzyme and hence reduce Vmax. KM value, on the other hand, remains unaltered.

The degree of inhibition is not reversed by increasing the substrate concentration.

Example:
Metal ion-requiring enzymes can be inhibited by chelating agents like EDTA.

III. Uncompetitive inhibitors

Uncompetitive inhibition occurs when the inhibitor attaches to the enzyme-substrate complex after the substrate has bound and stops the reaction from taking place.

The degree of inhibition may increase when the substrate concentration is increased.

Example:
Rare in one-substrate reaction; But common in Bi-substrate reaction.

2. Irreversible inhibition

The inhibitors bind covalently with the enzymes and inactivate them, which is irreversible. These inhibitors are usually toxic substances that poison enzymes.

The **penicillin antibiotics** act as irreversible inhibitors of serine – containing enzymes, and block the bacterial cell wall synthesis.

Suicide inhibition

Suicide inhibition is a specialized form of irreversible inhibition A good example of suicide inhibition is **allopurinol** used in the treatment of gout, an inhibitor of xanthine oxidase, gets converted to alloxanthine, a more effective inhibitor of this enzyme.

3. Allosteric inhibition

The details of this type of inhibition are given under allosteric regulation as a part of the regulation of enzyme activity in the living system.