Factors affecting enzymic activity

Major factors responsible to affect the enzymic activity are the following:

1. Concentration of substrate
2. Concentration of enzyme
3. Concentration of reaction products
4. Effect of pH
5. Effect of temperature and
6. Effect of time

Concentration of Substrate (the reaction velocity increases)

It is sometimes found that as the substrate concentration is increased the reaction velocity increases, but then falls at high substrate concentrations. This is know

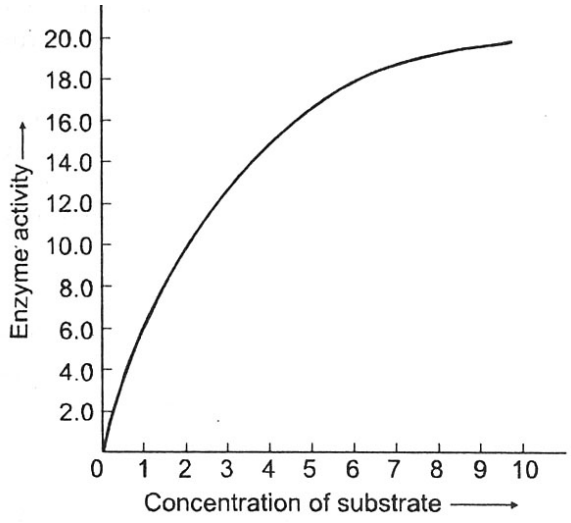


Fig. 11.7 : Effect of Substrate concerntration on enzyme activity

Liver alcohol dehydrogenase, which has a mechanism in which NAD* binds first, is inhibited by high concentrations of the second substrate, alcohol.

Concentration of enzyme

Within fairly wide limits the speed of an enzymatic reaction is proportional to the enzyme concentration. This can be shown to hold for many enzyme systems, provided interfering conditions do not develop and the substrate concentration is maintained constant. **Fig (11.8)** Effect of enzyme concenteration on enzyme activity.

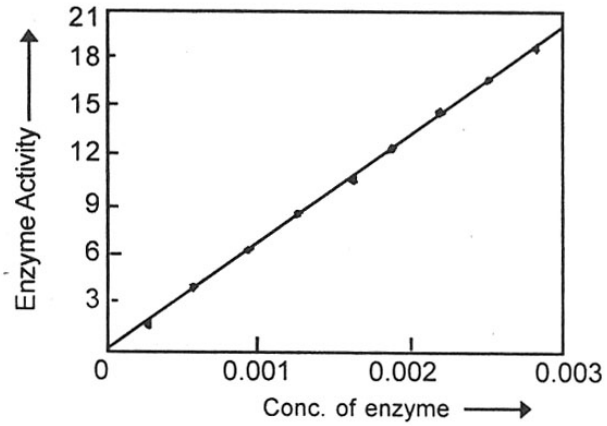


Fig. 11.8 : Effect or enzyme concentration on enzyme activity

Concentration of reaction products

An enzyme-product complex is part of the reaction sequence of enzyme-catalysed reactions. In the presence of large concentrations of product, this complex is dominant and product inhibition is observed. This is one of the reasons why the velocity of the reaction falls as the equilibrium is approached and it is of physiological importance in controlling pathways by negative feedback. In single-product enzymes as shown below in equation, addition of the product causes reversal of the reaction, but if there are two or more products, both are needed for reversal to occur, so inhibition of the forward reaction by each can be studied separately.

$$\overset{K_1}{\rightleftharpoons} \qquad k_3$$

$$E+S \underset{k_2}{\longrightarrow} ES \longrightarrow E+P$$

Usually each product is competitive with the substrate from which it is derived,

Enzyme kinetics and K_m value : The enzyme (E) and substrate (S) combine with each other to form an unstable enzyme-substrate complex (ES) for the formation of product (P).

$$E+S \underset{k_2}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E+P$$

Here k_1, k_2 and k_3 represent the velocity constants for respective reactions, as indicated by arrows.

K_m, the Michaelis-Menten constant (or Brig's and Haldane's constant) is given by the formula

$$K_m = \frac{K_2 + k_3}{K_1}$$

The following equation is obtained after suitable algebraic manipulation.

$$V = \frac{V_{max}[S]}{K_m + [S]} \qquad equation\ (1)$$

Where V = Measured velocity,

V_{max} = Maximum velocity,

S = Substrate concentration,

K_m = Michaelis - Menten constant,

Let us assume that the measured velocity (V) is equal to ½V_{max}. Then the equation (1) may be substituted as follows

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

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

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

$$K_m = [S]$$

K stands for a constant and m stands for Michaelis (in K_m).

K_m or the Michaelis-Menten constant is defined as the substrate concentration (expressed in moles/lit) to produce half-maximum velocity in an enzyme catalysed reaction. It indicates that half of the enzyme molecules (i.e. 50%) are bound with the substrate with the substrate molecules when the substrate concentration equals the K_m value.

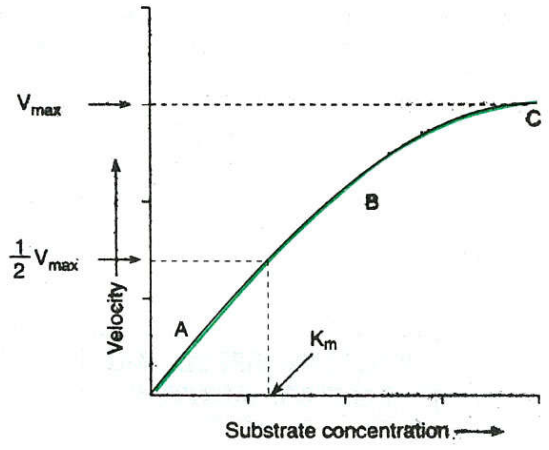


Fig. 6.2 : Effect of substrate concentration on enzyme velocity (A-linear; B-curve; C-almost unchanged).

K_m value is a constant and a characteristic feature of a given enzyme. It is a representative for measuring the strength of ES complex. A low K_m value indicates a strong affinity between enzyme and substrate, whereas a high K_m value reflects a weak affinity between them. For majority of enzymes, the K_m values are in the range of 10^{-5} to 10^{-2} moles.

Effect of pH

Each enzyme has a pH optimum- i.e., a H^+ concentration at which the enzyme reacts at maximum speed. Very slight changes toward either side of the pH optimum may result in profound alterations of reaction rates.

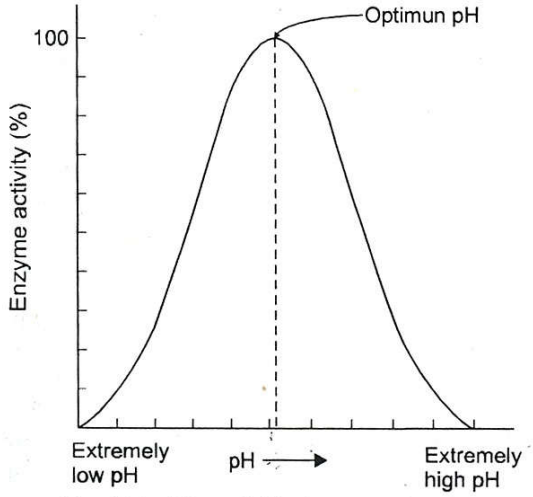


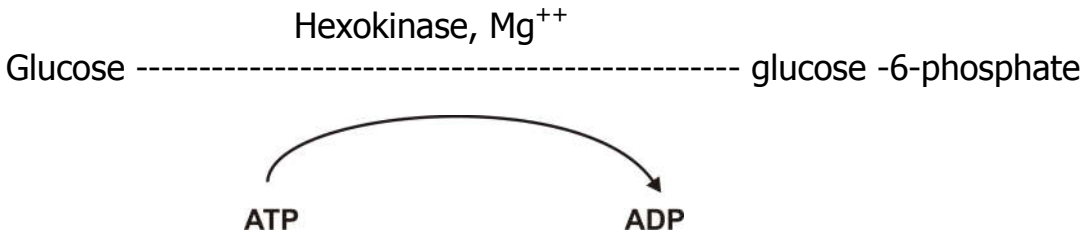
Fig. 11.9 : Effect of pH on enzyme activity

Effect of temperature

Chemical reactions, both catalyzed and noncatalyzed proceed at a faster rate as the reaction temperature is increased. This is true of enzymatically catalyzed reactions in general only up to about 50ºC. Above this temperature, heat inactivation of enzymes becomes a more important factor than the increased reaction rate and in all but a few exceptional cases, the speed of reaction slows and ceases around 70º to 80ºC.

The optimum temperature of an enzyme is that temperature at which the greatest amount of substrate is changed in unit time.

Hexokinase reaction is an example of product inhibition. In this reaction, ADP is competitive with ATP, and glucose-6-phosphate is competitive with glucose.



g-6P may also act as a product inhibitor if it is not removed rapidly.

Effect of time

Scientist Gortner points out that the element of time is generally not given sufficient consideration in discussing factors that affect the rate of enzyme action. He states there cannot be an optimum hydrogen temperature independent of time. He indicates further that the optimum temperature of many concentration enzymes from warm blooded animals is approximately 37ºC only if time is measured in hours. It has been established that the time element is important in defining other conditions which regulate the rate of enzyme action.

Effect of light and radiation

Exposure of enzymes to ultraviolet, beta, gamma and X-rays inactivates certain enzymes due to the formation of peroxides. e.g. Uvrays inhibit salivary amylase activity.