1.5 Enzyme Catalysis

Enzymes are complex protein molecules with three –dimensional structure. These are responsible for catalyzing the chemical reactions in living organisms. The diameters of the enzyme molecules fall in the range of 10-100 nm. Enzymes are often present in colloidal state and are extremely specific in their catalytic functions. Various enzyme –catalysed reactions are known. Some important examples are:

(i) Urease, an enzyme that catalyses the hydrolysis of urea but has no effect on the hydrolysis of substituted urea, e.g, methyl urea.

$$NH_2CONH_2 + H_2O \xrightarrow{\text{urease}} 2\,NH_3 + CO_2$$

(ii) Peptide, glycyl-L-glutamyl –L-tyrosine is hydrolysed by an enzyme known as pepsin.

(iii)Hydrolysis of starch into maltose by diastase

$$2(C_6H_{10}O_6)_n + n\,H_2O \xrightarrow{\text{diastase}} nC_{12}H_{22}O_{11}$$

Starch — Maltose

(iv)Conversion of glucose into ethanol by zymase present in yeast.

$$C_6H_{12}O_6 + H_2O \xrightarrow{\text{zymase}} 2C_2H_5OH + 2\,CO_2$$

(v) Conversion of maltose into glucose by maltase

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{maltase}} 2C_6H_{12}O_6$$

Almost all enzymes fall into one of two classes, the hydrolytic enzymes and the oxidation – reduction enzymes. The hydrolytic enzymes appear to be complex acid-base catalysis which accelerate the ionic reactions mainly due to the transfer of hydrogen ions. The oxidation-reduction enzymes catalyse electron transfer perhaps through the information of an intermediate radical.

Mechanism of Enzyme Reactions

The mechanism if an enzyme reaction was proposed by Michaelis and Menten and can be represented in the following manner: Let E represent the enzyme and S the substrate, then the overall reaction is

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

It is to be noted that in the formation of the product P, the enzyme does not undergo any change. The rate of formation of the product depends on the concentration of enzyme. In the above scheme ES denotes the intermediate between the enzyme and the substrate which decomposes into the product with a first order rate constant k_2. The rate of formation of the product is given by

$$dP/dt=k_2[ES] \qquad \text{…….. (1.5.1)}$$

The Steady-State equation is

$$k_1[E][S]-k_{-1}[ES]-k_2[ES]=0 \qquad \text{…….. (1.5.2)}$$

In studies of enzyme reaction the molar concentration of substrate is usually very much greater than that of the enzyme; only a small proportion of the substrate therefore is bound to the enzyme. The total concentration of enzyme, $[E]_0$, is equal to the concentration of free enzyme,[E],plus the concentration of complex,[ES]:

$$[E]_0=[E]+[ES] \qquad \text{……..(1.5.3)}$$

Elimination of [E] between these two equations gives

$$k_1([E]_0-[ES])[S] -(k_{-1}-k_2)[ES]=0 \qquad \text{…… …….. (1.5.4)}$$

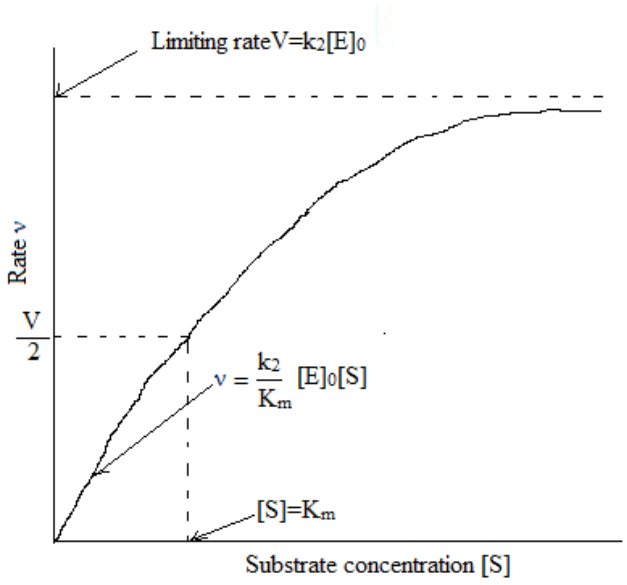

Figure 3: Variation of rate with substrate concentration for an enzyme-catalysed reaction obeying the Michaelis Menten equation

And therefore

$$[ES]=\frac{k_1[E]_0[S]}{k_{-1}+k_2+k_1[S]} \qquad \ldots\ldots\ldots\ldots\ldots\ldots(1.5.5)$$

The rate of the reaction is

$$v=k_2[ES]=\frac{k_1 k_2 [E]_0[S]}{k_{-1}+k_2+k_1[S]} \qquad \ldots\ldots\ldots\ldots\ldots\ldots(1.5.6)$$

$$=\frac{k_2 [E]_0[S]}{(k_{-1}+k_2)/k_1+[S]} \qquad \ldots\ldots\ldots\ldots\ldots\ldots(1.5.7)$$

$$=\frac{k_2 [E]_0[S]}{K_m -+[S]} \qquad \ldots\ldots\ldots\ldots\ldots\ldots(1.5.8)$$

In this equation K_m equals to $(k_{-1}+k_2)/k_1$ is known as the Michaelis constant and equation of the form of eq. (1.5.8) is referred as Michaelis-Menten equation.

When [S] is sufficiently small, it may be neglected in the denominator in comparison with K_m

$$v=\frac{k_2}{K_m}[E]_0[S] \qquad \ldots\ldots\ldots\ldots\ldots..(1.5.9)$$

so that the kinetics are first order in substrate concentration. When, on the other hand,

$[S] >> K_m$,

$$v= k_2[E]_0 \qquad \ldots\ldots\ldots\ldots\ldots(1.5.10)$$

and the kinetics are zero order ; the enzyme is then saturated with substrate, and a further increase in [S] has no effect on the rate. Thus Eq.(1.5.8) is consistent with the behavior shown in fig. 3

Equation (1.5.10) can be rewritten as

$$v = \frac{V[S]}{K_m+[S]} \qquad \ldots\ldots\ldots\ldots\ldots(1.5.11)$$

Where V, equal to $k_2[E]_0$, is the limiting rate at high substrate concentration, it is often known as the maximal velocity. When [S] is equal to K_m, Eq. (1.5.11) becomes

$$v = \frac{V[S]}{[S]+[S]} = \frac{V}{2} \qquad \ldots\ldots\ldots\ldots\ldots..(1.5.12)$$

The relationship is illustrated in Fig. 3 . The Michaelis constant K_m can be determined from a plot of ν versus [S], by finding the concentration of substrate that gives one-half of the limiting rate. In practice, however, this procedure does not provide a very reliable value.

To see whether experimental data are consistent with eq. (1.5.11), the equation can be recast into a form that gives a linear plot. If reciprocals of Eq. (1.5.11) are taken the result is

$$\frac{1}{\nu} = \frac{K_m}{V[S]} + \frac{1}{V} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1.5.13)$$

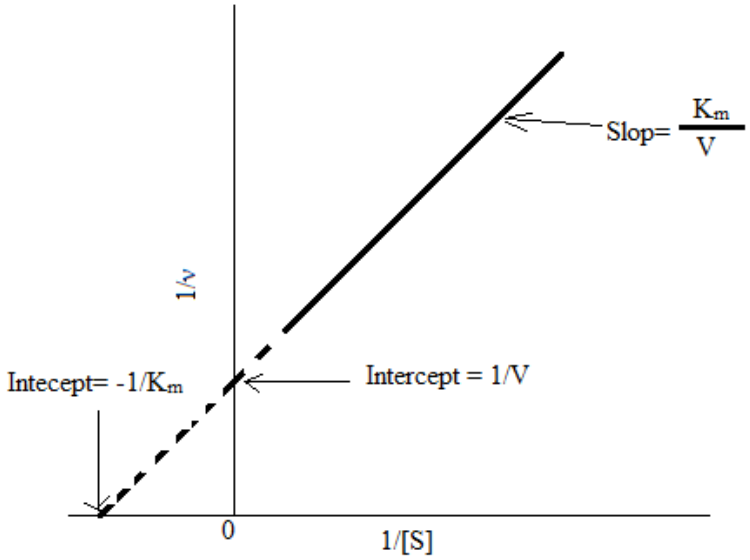

Figure 4: Lineweaver- Burk plot of 1/ ν versus 1/[S]

Therefore, a plot of 1/ν versus 1/[S] gives a straight line if Eq. (1.5.11) applies. This type of plot, suggested by Lineweaver and Burk, is shown schematically in Figure 4 which gives the intercepts and the slop. The parameters V and K_m can be derived from such a plot. If the enzyme concentration $[E]_0$ is known, k_2 also can be calculated, since according to this simple mechanism $V=k_2[E]_0$. However, the individual constants k_1 and k_{-1} cannot be obtained from studies of rate as a function of substrate concentration; the way they can be obtained from transient-phase studies is considered later.

****Turnover number :** In enzymology, turnover number (also termed k_{cat}) is defined as the maximum number of chemical conversions of substarte molecules per second that a single catalytic site will execute for a given enzyme concentration $[E_T]$. It can be calculated from the maximum reaction rate V_{max} and catalyst site concentration $[E_T]$ as follows

$$k_{cat} = V_{max} /[E_T]$$

For example, carbonic anhydrase has a turnover number of 400,000 to 6000,000 S^- , which means that each carbonic anhydrase molecule can produce up to 6000,000 molecules of product (bi carbonate ions) per second.

……******End*******……