ENZYME CATALYSIS

A very important type of homogeneous catalysis includes reactions catalyzed by certain complex organic substances known as **enzymes.** Enzymes are proteins with high relative molar mass of the order of 10,000 or even more and are derived from living organisms. Each enzyme can catalyze a specific reaction. For instance, the enzyme diastase produced in the germinated barley seeds converts starch into maltose sugar.

Mechanism and Kinetics of Enzyme-catalyzed Reactions. In 1913, biochemists L. Michaelis and Miss Maud Menten proposed a mechanism for the kinetics of enzyme–catalyzed reactions which envisages the following steps :

Step 1. Formation of the Enzyme–Substrate Complex :

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{\text{(complex)}}{ES} \qquad \text{(fast)}$$

Step 2. Decomposition of the Complex : $ES \xrightarrow{k_2} P + E$ (slow)

where E is the (free) enzyme ; S is the substrate (*i.e.*, the reactant); ES is the enzyme–substrate complex and P is the product. In the overall reaction $S \longrightarrow P$, the enzyme is consumed in step 1 and regenerated in step 2.

The problem can be handled using either the equilibrium approximation or the steady state approximation. Experiment shows, however, that true equilibrium is not achieved in the fast step because the subsequent slow reaction is constantly removing the intermediate enzyme-substrate complex, ES. Generally, the enzyme concentration is far less than the substrate concentration, *i.e.*, [E] << [S], so that [ES] << [S]. Hence, we can use the steady state approximation for the intermediate, ES.

According to the slow rate-determining step, the rate of the reaction is given by

$$r = -[dS]/dt = +d[P]/dt = k_2[ES] \quad \ldots(15)$$

Using steady state approximation for ES, we have

$$d[ES]/dt = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0 \quad \ldots(16)$$

Now, [E] cannot be measured experimentally. The equilibrium between the free and the bound enzyme is given by the *enzyme conservation equation, viz.,*

$$[E]_0 = [E] + [ES] \quad \ldots(17)$$

where $[E]_0$ is the total enzyme concentration (which can be measured) ; [E] is the free enzyme concentration and [ES] is the bound (or reacted) enzyme concentration. Thus,

$$[E] = [E]_0 - [ES] \quad \ldots(18)$$

Substituting for [E] in Eq. 16,

$$d[ES]/dt = k_1\{[E]_0 - [ES]\}[S] - k_{-1}[ES] - k_2[ES] = 0 \quad \ldots(19)$$

Collecting terms and simplifying,

$$k_1[E]_0[S] = \{k_{-1} + k_2 + k_1[S]\}[ES] \quad \ldots(20)$$

$$[ES] = \frac{k_1[E]_0[S]}{k_{-1} + k_2 + k_1[S]} \quad \ldots(21)$$

Substituting for [ES] in Eq. 15,

$$r = \frac{k_1k_2[E]_0[S]}{k_{-1} + k_2 + k_1[S]} \quad \ldots(22)$$

Dividing the numerator and the denominator by k_1,

$$r = \frac{k_2[E]_0[S]}{(k_{-1} + k_2)/k_1 + [S]} = \frac{k_2[E]_0[S]}{K_m + [S]} \quad \ldots(23)$$

where the new constant K_m, called the **Michaelis constant,** is given by

$$K_m = (k_{-1} + k_2)/k_1 \quad \ldots(24)$$

Note that K_m is not an equilibrium constant.

Eq. 23 is known as the **Michaelis-Menten equation.**

Further simplification of Eq. 23 can be made. When all the enzyme has reacted with the substrate at high concentration, the reaction will be going at maximum rate. No free enzyme will remain so that $[E]_0$=[ES]. Hence, from Eq. 15,

$$r_{max} = V_{max} = k_2[E]_0 \quad \ldots(25)$$

where V_{max} is the maximum rate, using the notation of enzymology.

The Michaelis-Menten equation can now be written as

$$r = V_{max}[S]/(K_m + [S]) \quad \ldots(26)$$

Two cases arise :

(*a*) $K_m >> [S]$ so that [S] can be neglected in the denominator of Eq. 26, giving

$$r = V_{max}[S]/K_m = k'[S] \qquad \text{(first-order reaction)} \quad \ldots(27)$$

(*b*) $[S] >> K_m$ so that K_m can be neglected in the denominator, giving

$$r = V_{max} = \text{constant} \qquad \text{(zero-order reaction)} \quad \ldots(28)$$

These two cases are shown diagrammatically in Fig. 2.

Again, *if* $K_m = [S]$, $r = \frac{1}{2} V_{max}$ ...(29)

Thus, **Michaelis constant** is equal to that concentration of S at which the rate of formation of product is half the maximum rate obtained at high concentration of S.

The constant k_2 in Eq. 25 is called the **turnover number** of the enzyme. The turnover number is the number of molecules converted in unit time by one molecule of enzyme. Typical values of k_2 are 100 to 1,000 per second though they may be as large as 10^5 to 10^6 per second.

We would like to know the physical reason why the reaction rate of an enzyme-catalyzed reaction changes from first-order to zero-order as the substrate concentration is increased. The answer is that each *enzyme molecule has one or more 'active sites' at which the substrate must be bound in order that the catalytic action may occur.* At low substrate concentration, most of these active sites remain unoccupied at any time. As the substrate concentration is increased, the number of active sites which are occupied increases and hence the reaction rate also increases. However, at very high substrate concentration, virtually all the active sites are occupied at any time so that further increase in substrate concentration cannot further increase the formation of enzyme-substrate complex.

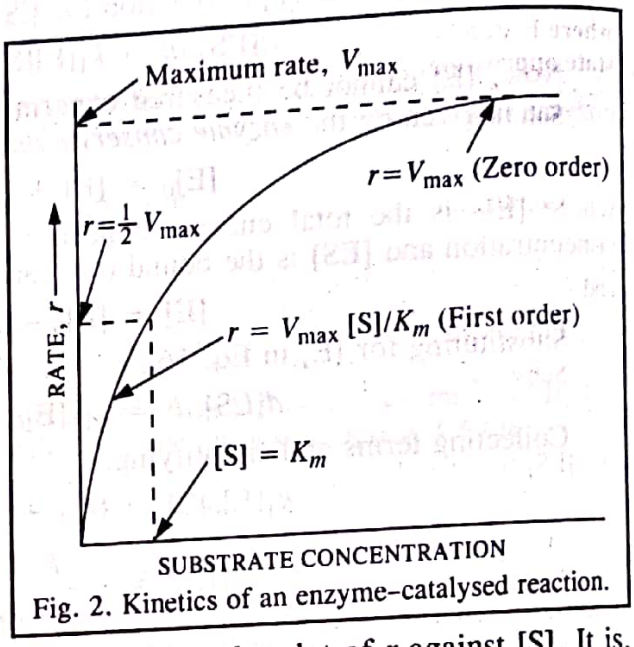

Fig. 2. Kinetics of an enzyme-catalysed reaction.

It is rather difficult to determine V_{max} (and hence K_m) directly from the plot of r against [S]. It is, however, possible to rearrange Eq. 26 so as to permit some alternative plots for easy determination of V_{max}. Two of the best known methods which make use of the re-arranged equations are as follows.

1. The Lineweaver-Burk method. This method uses the rearranged equation

$$1/r = K_m/([S]\ V_{max}) + 1/V_{max} \quad ...(30)$$

A plot of $1/r$ against $1/[S]$ gives a straight line whose intercepts on the x-axis and y-axis are $(-1/K_m)$ and $1/V_{max}$, respectively and whose slope is (K_m/V_{max}), as shown in Fig. 3.

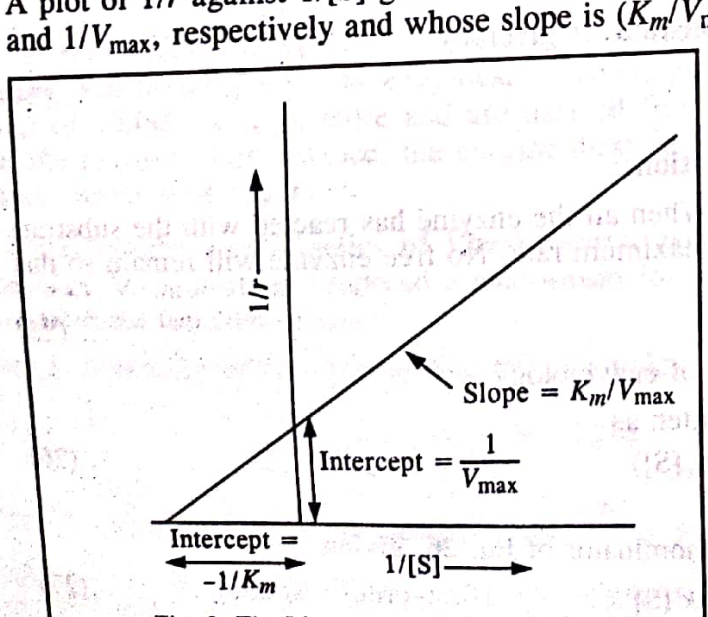

Fig. 3. The Lineweaver-Burk method of plotting enzyme kinetic data.

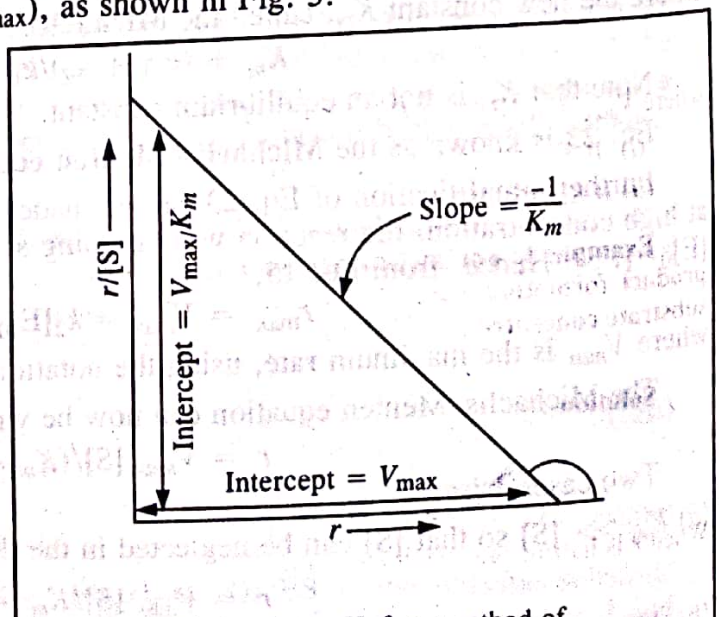

Fig. 4. Eadie-Hofstee method of plotting enzyme kinetic data.