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Enzyme Catalysis :—

A very important type of homogenous catalysis includes reactions catalysed 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 catalyse a specific reaction

e.g, the enzyme diastase produced in the germinated seeds of barley converts starch into maltose sugar.

$$2(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\text{Diastase}} nC_{12}H_{22}O_{11}$$

Starch — Maltose

Another enzyme, maltase converts maltose into glucose.

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

Zymase, produced by living yeast cells, converts glucose into ethyl alcohol.

$$C_6H_{12}O_6 \xrightarrow{\text{Zymase}} 2C_2H_5OH + 2CO_2$$

Mechanism and kinetics of Enzyme Catalysed Reactions :-

In 1913, biochemist L. Michaelis and Mary Menten proposed a mechanism for the kinetics of enzyme-catalysed reactions as follows:

Step I :- Formation of the Enzyme-Substrate complex.

The reaction between enzyme E and substrate S to form complex ES:

$$E + S \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} ES \quad \text{(fast)} \quad -(1)$$

This rexn. is very fast and the equilibrium is rapidly established

Step II :- $ES \underset{k_2'}{\overset{k_2}{\rightleftharpoons}} E + P$ (slow) $-(2)$

The complex then dissociates forming one or more products P and regenerating the enzyme. (ES is enzyme substrate complex). In the overall reaction $S \longrightarrow P$, the enzyme is consumed in Step I 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 $[E] \ll [S]$, so that $[ES] \ll [S]$.

Hence, we can use the steady state app. for the intermediate, ES.

Acc. to the slow rate determining step, the rate of reaction is given by,

$$r = -\frac{d[S]}{dt} = +\frac{d[P]}{dt} = k_2[ES] \quad \text{---} (3)$$

Using steady state app. for ES, we ~~have~~ that is the rate of change of conc. of ES with time must be equal to zero. But the rate of change of concentration of ES with time will be given by

$\frac{d[ES]}{dt}$ = Rate of formation of ES from E and S − Rate of decomposition of ES into E and P

$$= k_1[E][S] - k_1'[ES] - k_2[ES] \quad \text{—(4)}$$

For steady state app., $\frac{d[ES]}{dt} = 0$, Hence,

$$k_1[E][S] - k_1'[ES] - k_2[ES] = 0$$

or $$[ES][k_1' + k_2] = k_1[E][S]$$

$$[ES] = \frac{k_1}{k_1' + k_2}[E][S] \quad \text{—(5)}$$

This expression gives steady state concentration [ES].

putting $\frac{k_1' + k_2}{k_1} = k_m$ called Michaelis constant.

expression (5) can be written as

$$[ES] = \frac{[E][S]}{k_m} \quad \text{—(6)}$$

since [ES] is a steady state, we must have

Rate of disappearance of S = Rate of disappearance of ES

i.e $$-\frac{d[S]}{dt} = k_2[ES] \quad \text{—(7)}$$

But rate of disapp. of S means the rate of the enzyme catalysed reaction, usually represented by v (called steady state velocity). Hence

$$v = -\frac{d[S]}{dt} = k_2[ES] \quad \text{—(8)}$$

Substituting the value of [ES] from eq. (6) in eq. (8), we get

$$v = -\frac{d[S]}{dt} = k_2[ES] = k_2\frac{[E][S]}{k_m} \quad \text{—(9)}$$

The eq. (9) is, however, not useful in this form since it includes the concentration of free enzyme [E] whereas the experimentally measurable quantity is $[E_0]$, the total concentration of the enzyme, i.e free as well as combined in the complex ES. But we have

$[E_0] = [E] + [ES]$ so that

$[E] = [E_0] - [ES]$

Substituting this value in eq. (6), we get

$$[ES] = \frac{\{[E_0] - [ES]\}[S]}{k_m}$$

or $k_m[ES] = [E_0][S] - [ES][S]$

or $k_m[ES] + [ES][S] = [E_0][S]$

or $[ES]\{k_m + [S]\} = [E_0][S]$

$$[ES] = \frac{[E_0][S]}{k_m + [S]} \quad \text{—(10)}$$

Sub. this value in eq. (8), we get

$$v = \frac{-d[S]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_m + [S]} \quad \text{—(11)}$$

This eq. is known as Michaelis Menten equation which contains measurable quantities, $[E_0]$ and $[S]$. Eq. (11) is usually written in terms of maximum velocity v_m of the enzyme-catalyzed reaction which is reached when $[S]$ becomes so large that $[S] >> k_m$ so that k_m can be neglected in comparison to $[S]$. Then eq (11) takes the form

$$v_m = \frac{k_2[E_0][S]}{[S]} \qquad \left[\because K_m << [S]\right].$$

$$v_m = k_2[E_0] \qquad \text{---(12)}$$

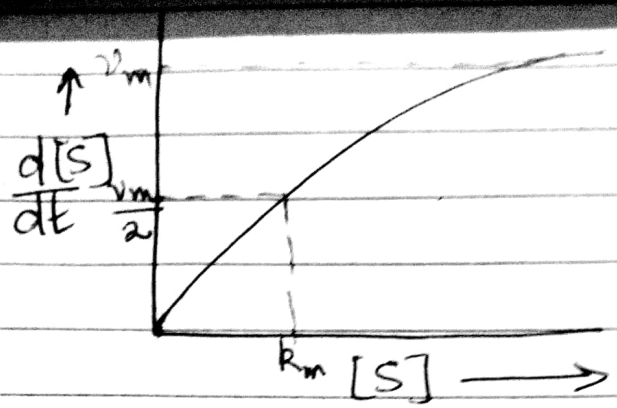
or $$\frac{v}{v_m} = \frac{k_2[E_0][S]}{K_m + [S]} \times \frac{1}{k_2[E_0]}$$

$$\frac{v}{v_m} = \frac{[S]}{K_m + [S]} \qquad \text{---(13)}$$

From the above eq., we find that when $[S] = K_m$, $\frac{v}{v_m} = \frac{1}{2}$ i.e $v = \frac{v_m}{2}$

Thus Michaelis constant is equal to that concentration of the substrate at which the rate of the enzyme catalysed reactions falls to one half of the maximum value. Thus the value of v_m and K_m can be obtained by plotting rate of the reaction (i.e steady state velocity, v) versus the concentration of the substrate S.

A graph of the type shown in the fig is obtained. The values of v_m and K_m can be obtd. as



Alternatively, to convert eq. (13) into a linear form, it may be written in the form

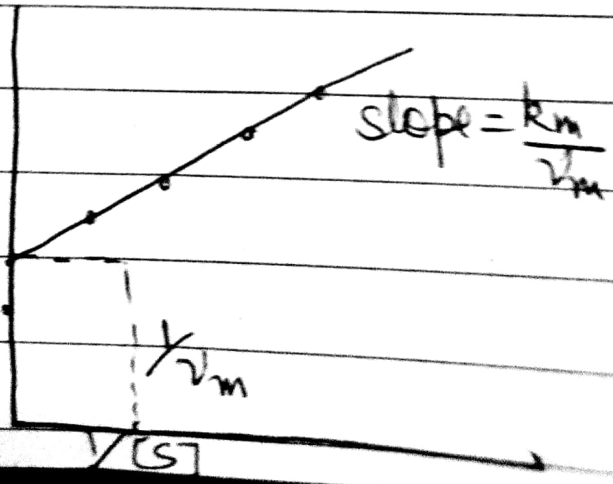
$$\frac{v_m}{v} = \frac{K_m + [S]}{[S]}$$

or $$\frac{v_m}{v} = \frac{K_m}{[S]} + 1$$

or $$\frac{1}{v} = \frac{K_m}{v_m [S]} + \frac{1}{v_m} \quad \text{—— (14)}$$

Thus a plot of $\frac{1}{v}$ versus $\frac{1}{[S]}$ will be a straight line with intercept $\frac{1}{v_m}$ and slope $= \frac{K_m}{v_m}$. The values of v_m & K_m can then be calculated.

Further, since $[E_0]$ is known, using eq (12), k_2 can be calculated. However k_1 and k_1' cannot be determined.



Order of Enzyme-Catalysed Reactions :-

(I) At low concentrations of the substrate, [S] can be neglected in comparison to K_m in eq. (II), we have

$$v = -\frac{d[S]}{dt} = \frac{k_2}{K_m}[E_0][S]$$

i.e $v = -\frac{d[S]}{dt} \propto [E_0][S]$ Hence it is a second order reaction.

(II) If the enzyme concentration is kept constant & the concentration of the substrate is varied, the reaction of the first order.

(III) If the substrate conc. is so high that $K_m << [S]$, eq. (II) reduces to

$$v = -\frac{d[S]}{dt} = k_2[E_0]$$ Thus the rate of reaction becomes independent of the conc. of substrate i.e it becomes zero order w.r.t substrate.

acid-base catalysis and enzyme catalysis are the most important.

ACID-BASE CATALYSIS

Acid-base catalysis includes reactions in solution which are catalyzed by acids or bases or both. A reaction which is catalyzed by H^+(or H_3O^+) ions but not by other Bronsted acids (proton donors) is said to be *specifically proton-catalyzed.* Examples are solvolysis of esters, inversion of sugars and keto-enol transformation. On the other hand, a reaction that is catalyzed by any Bronsted acid, is an example of *general acid catalysis.* Similarly, a reaction catalyzed only by OH^- ions is said to be *specifically base-catalyzed* while a reaction catalyzed by any Bronsted base is an example of *general base catalysis.* The solvent water may act as a

Bronsted acid or a Bronsted base. There are also reactions such as the mutarotation of glucose which require the presence of both a proton-donor and a proton-acceptor. This is also an example of *acid-base catalysis*.

Kinetics of Acid-Base Catalysed Reactions. If the rate of disappearance of a substrate S is first-order with respect to S, then, $-d[S]/dt = k[S]$. However, in a buffer solution, the first-order rate constant k for a reaction may depend linearly on $[H^+]$, $[OH^-]$, $[HA]$ and $[A^-]$, where HA is the weak acid in the buffer and A^- is the corresponding conjugate base. Thus, we can write

$$k = k_0 + k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{HA}[HA] + k_{A^-}[A^-] \quad \ldots(1)$$

Here k_0 is the first-order rate constant for the uncatalyzed reaction and $k_{H^+}, k_{OH^-}, k_{HA}$ and k_{A^-} are the catalytic coefficients. These coefficients may be evaluated experimentally using different concentrations of these species. The reaction is said to be subjected to *specific hydrogen ion catalysis* if only the $k_{H^+}[H^+]$ term is important. If, however, the term $k_{HA}[HA]$ is important, then the reaction is said to be subjected to *general acid catalysis*. Similarly, if only the term $k_{OH^-}[OH^-]$ is important, the reaction is said to be subjected to *specific hydroxyl ion catalysis* and, if the term $k_{A^-}[A^-]$ is important, the reaction is said to be subjected to *general base catalysis*.

We are interested in knowing how the various terms in Eq. 1 arise. This will be illustrated by considering two types of catalytic mechanisms.

1. First mechanism. Here we assume that a proton is transferred from an acid AH^+ to the substrate S. The acid form of the substrate then reacts with a water molecule to form the product P :

$$S + AH^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SH^+ + A$$

$$SH^+ + H_2O \xrightarrow{k_2} P + H_3O^+$$

Applying *steady state approximation* (*s.s.a.*) for $[SH^+]$, we have

$$d[SH^+]/dt = 0 = k_1[S][AH^+] - k_{-1}[A][SH^+] - k_2[SH^+] \quad \ldots(2)$$

Since we work with very dilute solutions, the concentration of H_2O remains almost constant so that in Eq. 2, the last term on the right hand side is written as $k_2[SH^+]$ rather than $k_2[SH^+][H_2O]$.

Solving for $[SH^+]$, we find that

$$[SH^+] = \frac{k_1[S][AH^+]}{k_{-1}[A] + k_2}$$

Making use of Eq. 3, the rate of formation of the product is given by

$$\frac{d[P]}{dt} = k_2[SH^+] = \frac{k_1 k_2[S][AH^+]}{k_{-1}[A] + k_2}$$

If $k_2 \gg k_{-1}[A]$, then

$$\frac{d[P]}{dt} = k_1 [S] [AH^+] \qquad ...(5)$$

Thus, we may say that the reaction is *general acid catalyzed.*
If, however, $k_2 \ll k_{-1} [A]$, then

$$\frac{d[P]}{dt} = \frac{k_1 k_2 [S] [AH^+]}{k_{-1} [A]} = \left(\frac{k_1 k_2}{k_{-1} K}\right) [S][H^+] \qquad ...(6)$$

where $K = [A][H^+]/[AH^+]$. In this case we say that the reaction is *specifically hydrogen-ion catalyzed.*

2. Second mechanism. Here we assume that in the second step, the acid form of the substrate reacts with a base A instead of a water molecule :

$$S + AH^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SH^+ + A$$

$$SH^+ + A \xrightarrow{k_2} P + AH^+$$

Applying steady state approximation for $[SH^+]$, as before, we have

$$\frac{d[SH^+]}{dt} = 0 = k_1 [S] [AH^+] - k_{-1} [SH^+] [A] - k_2 [SH^+] [A] \qquad ...(7)$$

Solving for $[SH^+]$,

$$[SH^+] = \frac{k_1 [S] [AH^+]}{[k_{-1} + k_2] [A]} \qquad ...(8)$$

Making use of Eq. 8, the rate of formation of the product is given by

$$\frac{d[P]}{dt} = k_2 [SH^+][A] = \frac{k_1 k_2 [S][AH^+]}{k_{-1} + k_2} \qquad ...(9)$$

This is an example of *general acid catalysis.*