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Topic : Enzyme kinetics

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Enzyme kinetics :- The speed of Enzyme catalyzed reaction is controlled by a no. of factors. And these are called Enzyme kinetics.

I Substrate Concentration : In the year 1903 Brown & Henri proposed that Enzyme (E) reacts reversibly with substrate forming enzyme-substrate complex (ES complex). Later, in 1913, Michaelis and Menten proposed an equation based on the effect of an enzyme on a single substrate. This was extended by G.E. Briggs and J.B.S. Haldane. which forms the basic principle underlying enzyme kinetics. According to this theory, the substrate combines with enzyme to form Enzyme-substrate Complex or ES Complex that breaks down into products setting the enzyme free. Both the reactions are reversible.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \quad \text{---------- (I)}$$

$$ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P \quad \text{---------- (II)}$$

k_1 and k_2 as well as k_3 and k_4 are reaction rate constants of forward and backward (reversible) reaction; respectively.

According to Michaelis and Menten, following assumptions are required for the reaction to continue.

A. The substrate concentration [S] is far greater than enzyme concentration [E] so that the amount of bound substrate is quite small.

B. The concentration of enzyme-substrate complex [ES] remains constant (steady state) because the rate of formation of [ES] from E and S equals the rate of breakdown of [ES] to E and P.

C. Reaction velocity is measured only as initial velocity that is just after the enzyme is mixed with substrate, and concentration of product is too small. Hence the rate of reversible reaction is ignored

On the basis of above assumptions at a given time t, the rate of formation of [ES] from E and S will be —

$$\frac{d[ES]}{dt} = k_1([E]_T - [ES])[S] \quad \text{--- (II)}$$

$\frac{d[ES]}{dt}$ = Rate of formation of [ES] at time t.

[E] = Total Enzyme Concentration
[ES] = Concentration of ES Complex.
[E] − [ES] = Concentration of free enzyme.
Likewise, the rate of breakdown of [ES] will be: $\frac{-d[ES]}{dt} = k_2[ES] + k_3[ES]$ ---- IV

As in a steady state of [ES], the rate of formation of [ES] is equal to its breakdown, hence

$k_1([E] - [ES])[S] = k_2[ES] + k_3[ES]$ --- V

$k_1([E] - [ES])[S] = (k_2 + k_3)[ES]$ (V)

i.e. $k_1([E] - [ES])[S] = (k_2 + k_3)[ES]$

i.e. $\frac{[E] - [ES][S]}{[ES]} = \frac{k_2 + k_3}{k_1} = k_m$ --- VI

$\frac{k_2 + k_3}{k_1}$ can be replaced by a single constant k_m named Michaelis-Menten constant.

Hence, $\frac{([E] - [ES])[S]}{[ES]} = k_m$

or, $\frac{[E][S] - [ES][S]}{[ES]} = k_m$

or, $\frac{[E][S]}{[ES]} - [S] = k_m$

or, $\frac{[E][S]}{[ES]} = k_m + [S]$

or, $[ES] = \frac{[E][S]}{k_m + [S]}$ --- VII

Since, the initial velocity (V) of enzymatic reaction is proportional to the conc.

of ES Complex.

$$V_1 = K_3[ES] \quad \text{---} \quad \text{VIII}$$

However, if the substrate concentration is very high compared to the enzyme concentration, all the enzyme will be present as [ES] and enzymatic reaction will be maximum (V_{max}).

$$V_{max} = K_m[E] \quad \text{---} \quad \text{IX}$$

Now, by substituting [ES] of Equn. VIII by its value from Equation VII we get

$$V_1 = K_3 \frac{[E][S]}{K_m + [S]} \quad \text{---} \quad \text{X}$$

Now, on dividing V_1 of Equation X by V_{max} of Equation IX, we get

$$\frac{V_1}{V_{max}} = \frac{K_3[E][S]}{K_m + [S]} \Big/ K_3[E]$$

$$i.e. \quad \frac{V_1}{V_{max}} = \frac{[E][S]}{K_m + [S]} \Big/ [E] = \frac{[S]}{K_m + [S]}$$

$$i.e. \quad V_1 = \frac{V_{max}[S]}{K_m + [S]} \quad \text{---} \quad \text{XI}$$

This is Michaelis-Menten Equation establishing the relationship of reaction velocity with substrate concentration. The equation also establishes the relationship of K_m with substrate concentration but shows no relationship

with enzyme concentration.

1. When K_m is too small to be considered it can be dropped from the equation resulting in

$$V_1 = \frac{V_{max}[S]}{[S]}$$

i.e. $V_1 = V_{max}$

This reflects high affinity of enzyme for substrate.

2. When K_m is equal to the substrate concentration, the reaction velocity is equal to $\frac{1}{2} V_{max}$

$$V_1 = \frac{V_{max}[S]}{K_m + [S]}$$

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

$$= \frac{V_{max}}{2}$$

Thus, one can evaluate K_m by estimating substrate concentration when initial velocity is half maximal.

3. Conversely to above, when K_m is very high compared to $[S]$, the value of $[S]$ can be dropped from the equation resulting in -

$$V = \frac{V_{max}[S]}{K_m \times [S]} = \frac{V_{max}}{K_m}$$

Concentration of Enzyme: Under optimal conditions of enzymatic reaction, rate of reaction

is proportional to enzyme concentration. However, at equilibrium, the velocity of forward and backward reactions is equal and net reaction velocity is 'nil'; Concentration of ES complex remains constant. Depicting both the forward and backward reaction in the following —

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P$$

we get. $E+S \xrightarrow{k_1} ES$ and $ES \xrightarrow{k_2} E+P$.

Rate of formation of ES from E+S is forward reaction $= k_1[E][S]$

Rate of formation of ES from E+P is backward reaction $= k_4[E][P]$.

At equilibrium $k_1[E][S] = k_4[E][P]$.

$$\text{Therefore, } \frac{k_1}{k_4} = \frac{[E][P]}{[E][S]} = \frac{[P]}{[S]}$$

$$K_{eq} = \frac{[P]}{[S]}$$

Thus, we find that in above overall reaction the term enzyme [E] get cancelled, showing thereby that enzyme concentration doesn't have any effect on equilibrium constant, though it does affect the reaction velocity. Equilibrium constant in a reaction, therefore, remains unchanged irrespective of the enzyme action.

(iii) Effect of Temperature :- Temperature increases kinetic energy of reacting molecules to over-

come the energy barrier of the reaction. Therefore, with increase in temperature, there is an increase in reaction velocity till maximum velocity is reached. Such a temperature where the enzyme attains optical activity is called <u>temperature optimum</u> of the enzyme. Further, increase in temp. beyond a limit brings decline in enzyme activity.

change in reaction velocity due to increase in temperature by 10°c is called <u>temperature coefficient</u> (Q_{10}). Generally, with increase or decrease in temp. by 10°C (Q_{10}) the reaction velocity gets doubled or halved. ($Q_{10} = 2$ or $\frac{1}{2}$)

IV <u>Effect of pH</u> :- Enzymes as a whole or their catalytic sites are in charged (Ionic) state. They can thus accept or donate protons. Any change in pH surrounding the enzyme will bring about change in the net charge on the enzyme and its catalytic site. This will thus affect enzyme activity. Obviously, there is a pH where enzyme activity is maximum, called <u>pH optimum</u> of the enzyme. Extreme change in pH will induce loss of enzyme activity. Most enzymes have pH optima near physiological pH of 6-7.