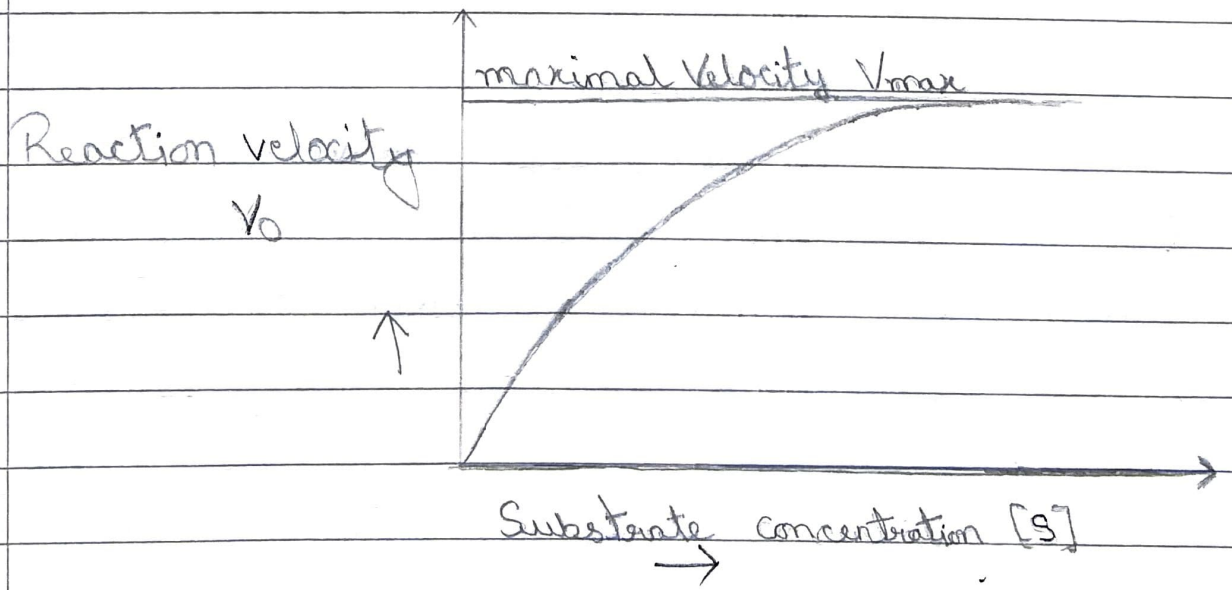
Enzyme Kinetics

In order to study the behaviour of enzyme, we need to study the rate at which these enzymes catalyze any rxn.

We study the rate of rxn by plotting the curve for reaction velocity versus substrate concentration.



This curve shows that initially the velocity increases proportionally to substrate concentration but as we increase the concentration of substrate the velocity approaches the Vmax asymptotically ie it never crosses Vmax.

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \underset{K_{-2}}{\overset{K_2}{\rightleftharpoons}} E + P$$

This equation describes the rxn once equilibrium has been established throughout.

To simplify we assume that we are at the begining. So Velocity = V_0, since at begining product formation is very low so it's reverse reaction ie K-2 is negligible.

So eqn becomes :- $E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P$

Rate law for the reaction $ES \xrightarrow{K_2} E + P$ is given by

$V_0 = K_2[ES]$ - (1)

Rate law for formation of $ES = K_1[E][S]$ - (2)

Rate law for dissociation of ES is given as

$K_{-1}[ES] + K_2[ES]$ - (3)

Steady state assumption

Assuming the steady state condition, the conc^n of Enzyme substrate complex will remain constant. This means the rate of formation of ES is the same as the rate of dissociation of ES.

This assumption gives us the equation :-

$$K_1[E][S] = K_{-1}[ES] + K_2[ES]$$

$$K_1[E][S] = [ES](K_{-1} + K_2)$$

$$\frac{[E][S]}{[ES]} = \frac{K_{-1} + K_2}{K_1} = K_m \leftarrow \boxed{\text{Michealis Constant}}$$

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$$[ES] = \frac{[E][S]}{K_m} \quad - (4)$$

At the begining when $t = 0$

$$[S]_{total} = [S] + [ES] \simeq [S]$$

$$[E]_{total} = [E] + [ES]$$

$$[E] = [E]_{total} - [ES] \quad - (5)$$

Using the value of $[E]$ from (5) in (4)

$$[ES] = \frac{([E]_{total} - [ES])[S]}{K_m}$$

$$K_m [ES] = [E]_{total}[S] - [ES][S]$$

$$K_m [ES] + [ES][S] = [E]_{total}[S]$$

$$[ES](K_m + [S]) = [E]_{total}[S]$$

$$[ES] = \frac{[E]_{total}[S]}{K_m + [S]}$$ using this value of $[ES]$ in (1)

$$V_o = \frac{K_2 [E]_{total}[S]}{K_m + [S]} \quad - (6)$$

→ Recall that rxn reaches a max^m velocity (V_{max}) when all enzyme active sites are occupied i.e

$V_{max} = K_2 [ES]_{max}$

$V_{max} = K_2 [E]_{total}$ since now $[ES] = [E]_{total}$

$[E]_{total} = \frac{V_{max}}{K_2}$ putting this value of $[E]_{total}$ in (6)

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$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$ Michealis - Menten equation

When $V_o = \frac{V_{max}}{2}$ then $K_m = [S]$

→ K_m is that substrate concentration at which velocity of the enzymatic rxn is exactly half of it's maximum velocity. K_m has unit of concentration

→ In other way K_m represents the [S] at which half of the enzymes active sites are filled by substrate molecules.

In conditions where $K_2 << K_{-1}$ then K_m describes the dissociation constant.

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES$$

$$K_1 [E][S] = K_{-1}[ES]$$

$$\frac{[E][S]}{[ES]} = \frac{K_{-1}}{K_1} = K_{eq}$$

When K_2 is rate limiting then $K_2 \ll K_{-1}$ & so K_m reduces to $\frac{K_{-1}}{K_1}$, which is defined as dissociation constant of ES complex.

$$K_m = K_{eq}$$

In this condition Km represents a measure of the affinity of the enzyme for it's substrate in the ES complex.

→ If Km is large the enzyme binds weakly to the substrate & there is a high probability that the substrate will dissociate from the active site of enzyme.

→ If the Km is small, the enzyme binds tightly to the substrate & will tend to remain bound to the active site.

3 step reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \overset{k_3}{\rightleftharpoons} E + P$$

The rate limiting step is such rk³ is $EP \overset{k_3}{\rightleftharpoons} E+P$

thus $V_{max} = k_3[E]_{total}$

But for 2 step rk³ $V_{max} = k_2[E]_{total}$

∴ A generalized term is used for the rate limiting rate constant ie **Kcat**

So, $V_{max} = K_{cat}[E]_{total}$

hence $V_0 = \dfrac{K_{cat}[E]_{total}[S]}{K_m + [S]}$

Kcat has unit of reciprocal time (sec^{-1}). It is also called as turnover number.

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Turnover number of an enzyme is the number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.
The term Kcat represents the kinetic efficiency of the enzyme.

→ To compare the catalytic efficiencies of different enzymes or the turnover of different substrates by the same enzyme, comparison of the ratio Kcat/Km for the two reactions can be done.

When [S] << Km, Michealis-Menten eqn reduces to:-

$$V_o = \frac{K_{cat}}{K_m} [E]_{total} [S]$$

Thus the ratio Kcat/Km is equivalent to the rate constant for the reaction between the free enzyme & the free substrate ie it is the rate constant for the conversion of E+S to E+P.

Kcat/Km ratio called specificity constant is often thought of as a measure of enzyme efficiency.

Units to express enzyme activity

→ **Enzyme Unit (U)**

The amount of enzyme causing transformation of 1 umole of substrate per minute under optimal conditions of measurement.

1 enzyme unit = 1 umole. min^{-1}

→ **Katal (Kat)**

It is the accepted SI unit of enzyme activity. One Katal is that amount of enzyme that catalyzes transformation of 1 mole of substrate per second.

1 Katal = 1 mol sec^{-1}