IIT Guwahati Lecture 22

Course BT 631

Protein Structure, Function and Crystallography

Prof. Arun Goyal

Dept. of Biosciences and Bioengineering

Kinetics of Unireactant Enzymes

Enzyme reactions with a single substrate and a single product, are called uni-reactions. $A \rightarrow P$

A bi-reactant, bi-product reaction may be illustrated as $A + B \rightarrow P + Q$

The first general rate equation for reaction involving unireactant enzymes was derived in 1903 by Victor Henri.

Henri's equation accounted for the observation that the initial rate of a reaction was directly proportional to the concentration of enzyme preparation, but increased in a nonlinear manner with increasing substrate concentration up to a limiting maximum rate.

Victor Henri
French Russian
1872-1940

Kinetics of Unireactant Enzymes

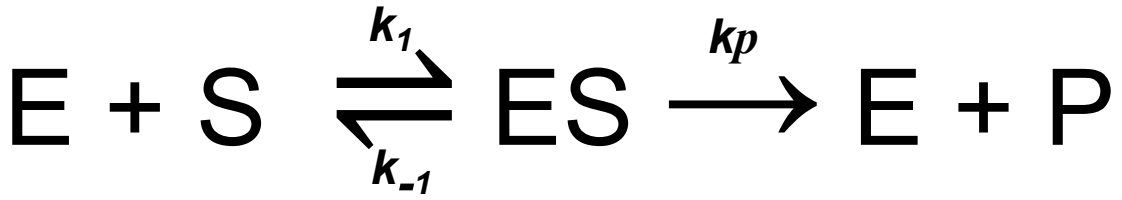
The derivation of Henri's equation was based upon the assumption that

1. The enzyme is a catalyst (proposed in 1835 to 1837 by Berzelius).
2. The enzyme and substrate react rapidly to form an enzyme-substrate complex (proposed in 1902 by Brown).
3. Only a single substrate and a single enzyme-substrate complex are involved and the enzyme-substrate complex breaks down directly to form a free enzyme and product.
4. Enzyme, substrate and the enzyme-substrate complex are at equilibrium; that is, the rate at which ES dissociates to E+S is much faster than the rate at which ES breaks down to form E+P.
5. The substrate concentration is very much larger than the enzyme concentration so that the formation of an ES complex does not alter the substrate concentration.
6. The overall rate of the reaction is limited by the breakdown of the ES complex to form free enzyme and product.
7. The velocity is measured during the very early stages of the reaction so that the reverse reaction is insignificant.

Kinetics of Unireactant Enzymes

The assumption that only the early components of the reaction are at equilibrium is called the quasi-equilibrium or rapid equilibrium assumption.

The overall reaction was visualized as:



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Kinetics of Unireactant Enzymes

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{kp} E + P$$

The Henri's equation is

$$v = \frac{K[S]}{1 + \frac{[S]}{K_S}} \qquad \text{-(i)}$$

Where, v = initial velocity (the velocity, d[P]/dt or – d[S]/dt) at given substrate concentration [S].

[S] = a fixed substrate concentration

In practice, v can be taken as Δ[P]/Δt or – Δ[S]/Δt provided the appearance of P is linear with time for the duration of the assay and no more than 5% of the original [S] is utilized.

k_p = Rate constant for the breakdown of ES to E + P

K_s = the Dissociation constant of the ES complex

$K_s = k_{-1}/k_1 = [E][S]/[ES]$ -(ii)

K = a constant characteristic of the particular enzyme preparation

$K = k_p [E]_t/K_s$, -(iii)

Where, $[E]_t$ is the total concentration of the enzyme, $[E]_t = [E] + [ES]$

Kinetics of Unireactant Enzymes

Ten years later in 1913, Michaelis and Menten confirmed Henri's experimental work and presented a slightly modified version of the rate equation, by putting Equation *(iii)* into *(i)*

$$v = \frac{kp \quad [E]_t[S]}{K_S + [S]} \qquad \text{-}(iv)$$

$$\text{If } v = k_p\,[\text{ES}] \qquad \text{-}(v)$$

then, k_p $[E]_t$ can be taken as V_{max} as maximum velocity would be observed when all Enzyme $[E]_t$ is present as [ES].

From equations *(iv)* and *(v)*

$$v = \frac{Vmax \quad [S]}{K_S + [S]}$$

This gives a familiar Michaelis Menten equation.

As pointed by a number of people it would be appropriate to refer to the equation as Henri-Michaelis Menten Equation.

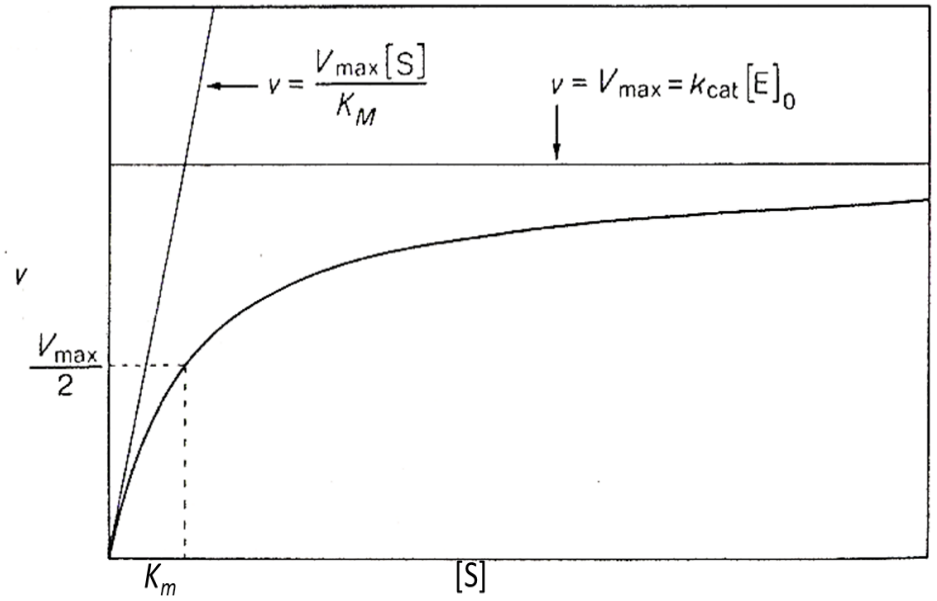
Kinetics of Unireactant Enzymes

Michaelis-Menten equation

It is found experimentally, that *v* is directly proportional to the concentration of enzyme, $[E]_0$.

However, *v* generally follows saturation kinetics with respect to the concentration of substrate, [S].

At sufficiently low [S], *v* increases linearly with [S], but as [S] is increased, this relationship begins to break down and *v* increases less rapidly than [S] until, at sufficiently high or saturating [S], *v* tends towards a limiting value that is termed maximum velocity, V_{max}.



Kinetics of Unireactant Enzymes

This is expressed quantitatively in the Michaelis-Menten equation, the basic equation of enzyme kinetics:

$$v = \frac{k_{cat}[E]_o[S]}{K_M + [S]}$$

k_{cat} **is the turnover number**, the number of times each enzyme site converts substrate to product per unit time.

Where,

$$k_{cat}[E]_o = V_{max}$$

Michaelis-Menten equation

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

K_m **(Michaelis constant)**

Kinetics of Unireactant Enzymes

Michaelis-Menten equation:

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

1. At very low substrate concentration [S] << K_M,

   $v = V_{max}[S]/K_M$ that is the rate is directly proportional to [S].

2. At high substrate concentration, [S]>> K_M, {[S]/[S] cancels}

   $v = V_{max}$, that is the rate is maximal and independent of [S]

3. When substrate concentration [S] = K_M, then v = ½ V_{max} $\{V_{max}[S]/2[S]\}$

Kinetics of Unireactant Enzymes

Further analysis reveals the physical meaning of K_m is :

- ***K_m is the concentration of substrate at which the reaction velocity is half of its maximal value.***
- ***A low value for K_m indicates a high affinity of the enzyme for its substrate*.**