BHARATHIDASAN UNIVERSITY

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Programme: M.Sc., Biochemistry

Course Title : Enzymology

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Unit-2

Enzyme kinetics

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Michaelis-Menten Kinetics

Enzyme-catalyzed reaction is as follows:

$$E+S \rightarrow k1[ES] \rightarrow k2E+P \quad (1)$$

To understand Michaelis-Menten Kinetics, we will use the general enzyme reaction scheme shown below

$$E+S \rightarrow k1[ES] \rightarrow k2E+P \quad (2)$$

$$E+S \leftarrow k3[ES] \leftarrow k4E+P \quad (3)$$

Substrate Complex

$E+S \rightarrow k1ESE+S \rightarrow k1ES \quad vo=k1[E][S]$

$ES \rightarrow k2E+SES \rightarrow k2E+S \quad vo=k2[ES]$

$ES \rightarrow k3E+PES \rightarrow k3E+P \quad vo=k3[ES]$

$E+P \rightarrow k4ESE+P \rightarrow k4ES \quad vo=k4[E][P]=0$

Method 1: The Rapid Equilibrium Approximation

E, S, and the ES complex can equilibrate very rapidly. The instantaneous velocity is the catalytic rate that is equal to the product of ES concentration and k_2 the catalytic rate constant.

$vo=k2[E-S]$ (4)

The total enzyme concentration (E_T) is equal to the concentration of free enzyme E (E_F) plus the concentration of the bound enzyme in ES complex:

$[E]T=[EF]+[ES]$ (5)

$Ks=k2k1=[E][S] / [ES]$ (6)

$Ks([Eo]-[ES])[S] / [ES]$ (7)

$[ES]=[Eo][S]Ks+[S]$ (8)

$vo=(dPdt)o=k3[ES]$ (9)

$vo=(dPdt)o=k3[Eo][S] / Ks+[S]$ (10)

At high substrate concentrations, $[S]>>Ks[S]>>Ks$ we get:

$vo=(dPdt)o=k3[Eo]=Vmax$ **(1)**

Method 2: The Steady-State Approximation

The rates of formation and breakdown of the E - S complex are given in terms of known quantities:

- The rate of formation of E-S = k1[E][S]k1[E][S]

(with the assumption that [P] =0)

- The rate of breakdown of E-S = k2[ES]+k3[ES]=(K2+K3)[ES]k2[ES]+k3[ES]=(K2+K3)[ES]

At steady state,

d[ES]dt=k1[E][S]+k2[ES]+k3[ES]=0 (11)

Therefore, rate of formation of E-S is equal to the rate of breakdown of E-S

So,

k1[E][S]=(k2+k3)[ES] (12)

Dividing through by k1:

[E][S]=(k2+k3) / k1[E−S] (13)

Substituting (k2+k3) / k1with kM:

[E][S]=KM[ES]

KM=breakdown[ES] / formation[ES] (14)

Substituting [EF] with [ET]-[ES]: ET = [ES] + [EF]

$$([ET] - [ES])\,[S] = kM\,[ES]$$

$$[ET].[S] - [ES].[S] = kM\,[ES]$$

$$[ET].[S] = [ES].[S] + kM\,[ES]$$

$$[ET].[S] = [ES].([S] + KM)$$

Solving for [ES]:

$$[ES] = ([ET][S]) / ([S]+KM)$$

The rate equation from the rate limiting step is:

$Vo = dP / dt = k2[ES]$

Multiplying both sides of the equation by k2:

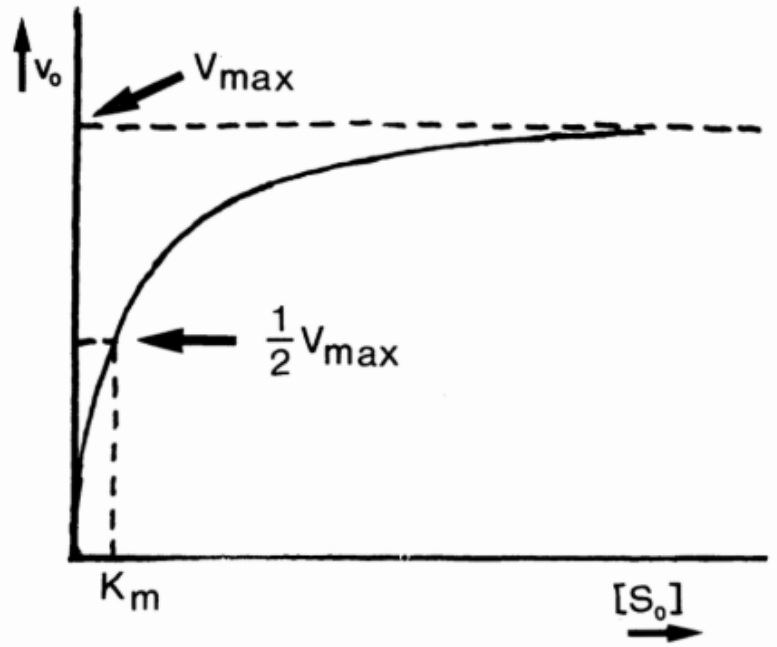
$k2[ES]=k2(([ET][S]) / (KM+[S])$ (15)

$Vo=k2(([ET][S]) / (KM+[S])$ (16)

When S>>KM, vo is approximately equal to k2[ET].

When the [S] great, most of the enzyme is found in the bound state ([ES]) and Vo = Vmax

- We can then substitue $k_2[E_T]$ with $V_{max}$to get the **Michaelis Menten Kinetic Equation:**
- v_o =(vmax[S])(KM+[S])
- **Reaction Order Note**
- When [S]<<KM,
- v=Vmax[S]KM
- This means that the rate and the substrate concentration are directly proportional to each other. The reaction is first-order kinetics.
- When [S]>>KM,
- v=Vmax(17)
- This means that the rate is equal to the maximum velocity and is independent of the substrate concentration. The reaction is zero-order kinetics.
- Then, at
- v=Vmax2, KM=[S]
- V =Vmax[S] / KM+[S] (18)



Graph of v_0 against $[S_0]$ at constant $[E_0]$ for a single-substrate enzyme-catalysed reaction, from the Michaelis–Menten equation.

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- **Lineweaver-Burk Plot**

- For example, by taking the reciprocal of the Michaelis Menten Kinetics Equation, we can obtain the Lineweaver-Burk double reciprocal plot:

$$Vo = (V_{max}[S]) / (K_m + [S])$$

$$1 / v = (K_m + [S]) / V_{max}[S]$$

$$1 / v = (K_m \cdot V_{max}).(1/[S]) + 1 / V_{max}$$

- Apply this to equation for a straight line y=mx+by=mx+b and we have:

$$y = 1/v$$

$$x = 1/[S]$$

$$m = \text{slope} = K_m V_{max}$$

$$b = y\text{−intercept} = 1 / V_{max}$$

When we plot y =1 / v versus x=1 / [S], we obtain a straight line.

$$x\text{−intercept} = -1 / K_m$$

$$y\text{−intercept} = 1 / V_{max}$$

$$\text{slope} = K_m V_{max}$$

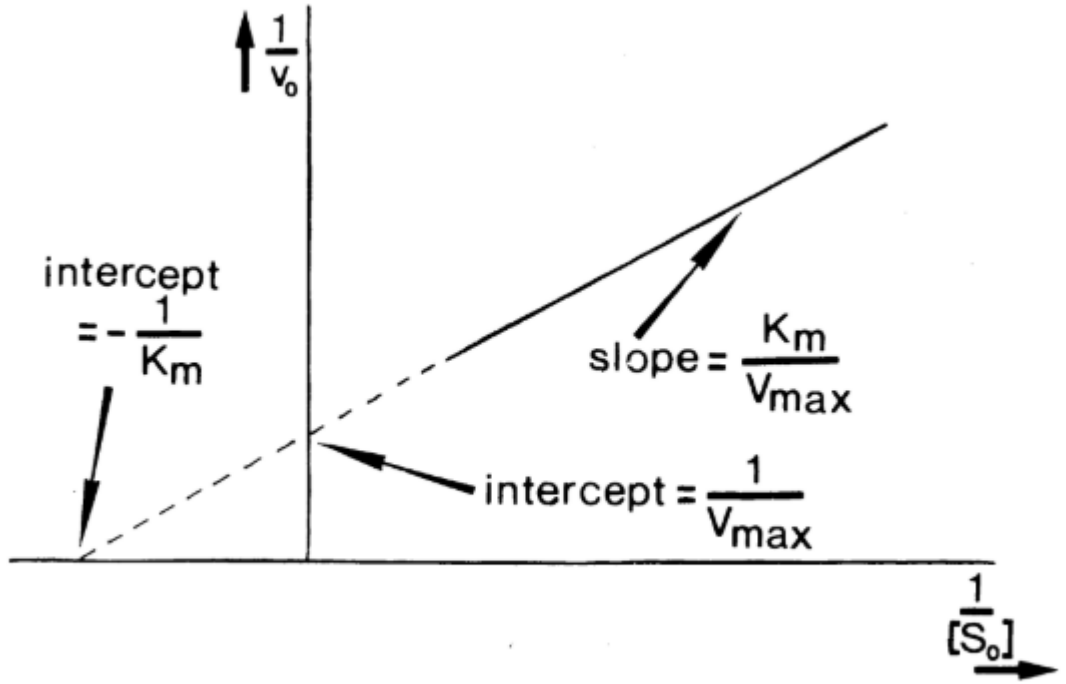

Fig. 7.2 — The Lineweaver–Burk plot.

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- **Eadie-Hofstee Plot:**

- Another way to calculate these values (K_m, V_{max}) and represent enzyme kinetics:

$$v_o = (V_{max}[S])/(K_m + [S])$$

$$v_o (K_m + [S]) = V_{max}[S]$$

$$v_o K_m + v_o[S] = V_{max} [S]$$

$$v_o [S] = - v_o K_m + V_{max} [S]$$

- Dividing through by [S]

$$v_o = - v_o K_m / [S] + V_{max}$$

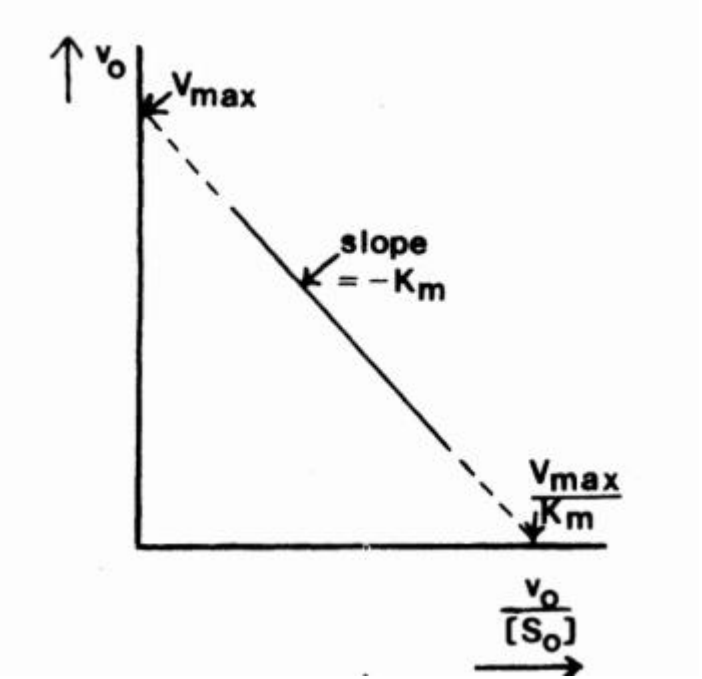

Fig. 3 Eadie- Hofstee Plot
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Enzyme activity is influenced by various factors, which can affect the rate at which enzymes catalyze reactions. Here are the key factors:

1. Temperature

Optimal Temperature: Enzymes have an optimum temperature at which they function most efficiently. For most human enzymes, this is around 37°C.

High Temperature: Excessive heat can denature enzymes by breaking their structural bonds, rendering them inactive.

Low Temperature: Reduces kinetic energy, slowing down molecular interactions and enzyme activity.

2. pH Levels

Each enzyme has an optimal pH at which it is most active. For example:

Pepsin (in the stomach) has an optimum pH of ~2.
Amylase (in saliva) works best around pH 7.
Trypsin (in the intestine) has an optimum pH of ~8.

Deviation from the optimal pH can lead to denaturation or reduced enzyme activity.

3. Substrate Concentration

Low Substrate Concentration: Enzyme activity increases with substrate concentration due to more frequent collisions between enzyme and substrate.

Saturation Point: At high substrate levels, enzyme activity reaches a maximum as all active sites are occupied.

4. Enzyme Concentration

Increasing enzyme concentration (while keeping substrate constant) generally increases reaction rate, provided substrates are available in excess.

5. Presence of Inhibitors

Competitive Inhibitors: Bind to the active site, competing with the substrate and reducing enzyme activity.

Non-Competitive Inhibitors: Bind to a site other than the active site, altering enzyme structure and reducing activity.

6. Presence of Activators

Certain ions or molecules (e.g., metal ions like Mg^{2+} or Ca^{2+}) can enhance enzyme activity by stabilizing the enzyme-substrate complex or modifying the enzyme's structure.

7. Coenzymes and Cofactors

Coenzymes (organic molecules like vitamins) and cofactors (inorganic ions) are essential for the catalytic activity of some enzymes.

8. Time

The longer an enzyme-substrate mixture is incubated under optimal conditions, the greater the amount of product formed, until substrate depletion occurs.

9. Pressure

For enzymes functioning under extreme conditions (e.g., deep-sea organisms), pressure can influence activity.

10. Allosteric Regulation

Allosteric enzymes have regulatory sites. Binding of effectors (activators or inhibitors) at these sites can modulate enzyme activity.

11. Environmental Factors

Physical and chemical changes in the environment (e.g., salinity, oxidative stress) can impact enzyme structure and function.

Single Displacement and Double Displacement reactions

Feature	Single Displacement	Double Displacement
Reactants	One element and one compound	Two compounds
Products	One element and one compound	Two new compounds
Type of Exchange	One element replaces another	Ions are exchanged between compounds
Example Reaction	$Zn+HCl \rightarrow ZnCl_2+H_2$	$BaCl_2+Na_2SO_4 \rightarrow BaSO_4\downarrow+2NaCl$

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