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Paper 05: Molecular Enzymology and Protein Engineering

Module No. 10: Determination of kinetics parameters using Lineweaver-Burk, Eadie-Hofstee plot, Scatchard plot

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Objective:

The kinetics parameters such as Km, Vmax, Kcat and Kcat/Km are important to understand the enzyme. These kinetics parameters are determined by the various methods. The objective of this module is to discuss the different methods that are used for the determination of the kinetic parameters. This module is divided into following sections-

1. Introduction
2. Methods used for the kinetics parameter determination:
    - 2.1 Lineweaver-Burk plot
    - 2.2 Hanes Woolf plot
    - 2.3 Eadie-Hofstee plot
    - 2.4 Scatchard plot
3. Summary
4. Questions
5. Resources and suggested reading

1. Introduction:

Enzyme enhances the rate of enzyme-catalyzed reaction. Kinetics parameters play very significant role in the enzyme kinetics. There are following parameters, which are used to explain the characteristics of the enzymes namely, Michaelis-Menten

constant or K_m, maximum velocity or V_{max}, turnover number or catalytic constant or K_{cat} and efficiency constant or specificity constant or K_{cat}/K_m. Km gives information about the binding affinity of the substrate with the enzyme, V_{max} gives information about maximum velocity of the reaction, K_{cat} gives information about rate of product appearance and K_{cat}/k_m gives information about overall efficiency of the reaction. Because the v_o versus [s] curve in hyperbolic hence it is difficult to determine V_{max} and therefore difficult to determine K_m which is [S] at half V_{max}. To facilitate the determination of the kinetic constant, the data are usually plotted in one of the linear forms described in this module. Hence it is important to determine the kinetics parameters. In the present module, we have discussed about the different methods used to the determination of the kinetics parameters.

2. **Methods for kinetics parameter determination:**

There are following methods that are used in the determination of the kinetics parameters-Lineweaver-Burk plot, Eddie-Hofstee plot, Scatchard plot and Hanes-Woolf plot and Dixon plot. Each plot will be explained one by one.

2.1 Lineweaver-Burk plot-

This is a graphical representation of the Lineweaver-Burk equation described by Hans Lineweaver and Dean Burk in 1934. This plot is also known as double reciprocal plot because it is plot of two reciprocal values i.e 1/[S] and 1/[v_o]. The Lineweaver-Burk equation can be derived from the Michaelis-Menten constant by taking reciprocal of the Michaelis-Menten Equation.

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

Taking reciprocal of the above equation

$$\frac{1}{v_o} = \frac{K_m + [S]}{V_{max}.[S]}$$

$$\frac{1}{v_o} = \frac{K_m}{V_{max}.[S]} + \frac{[S]}{V_{max}.[S]}$$

The final equation of Lineweaver-Burk equation becomes-

$$\frac{1}{v_o} = \frac{K_m}{V_{max}} . \frac{1}{[S]} + \frac{1}{V_{max}}$$

By assuming it as a straight-line equation, the Lineweaver-Burk plot can be drawn where; X-axis is represented by 1/[S] while y-axis is represented by $1/v_o$.

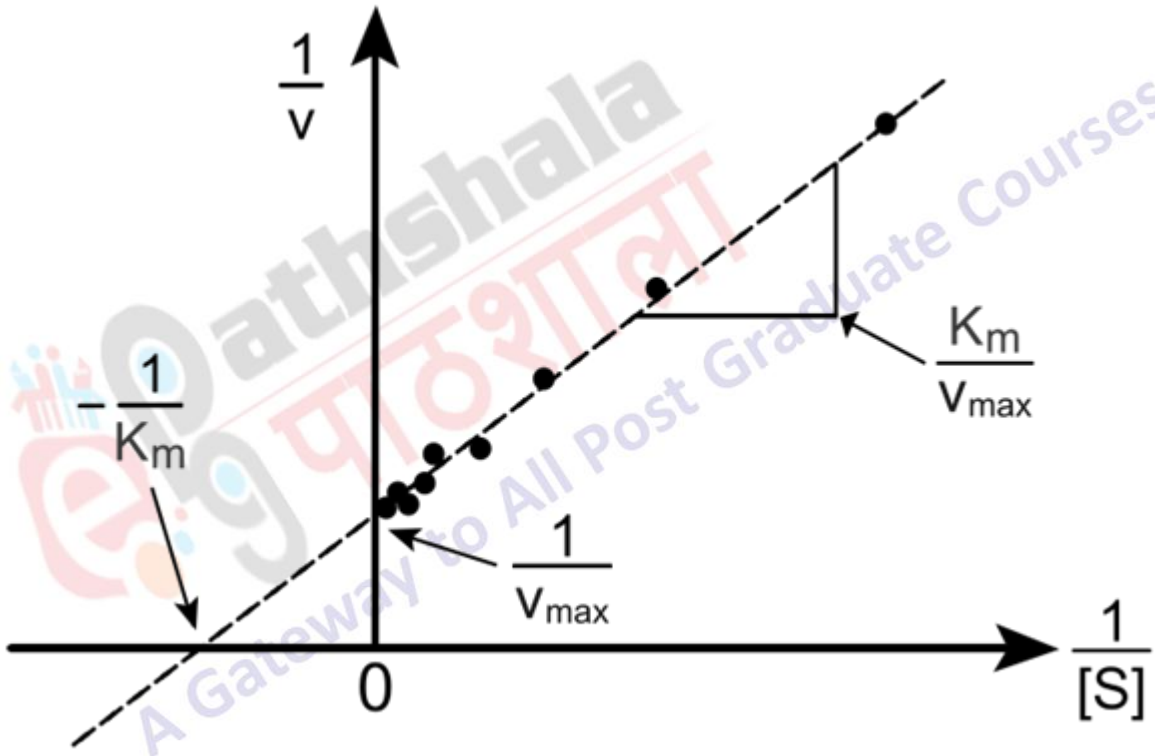

Figure1: Figure showing the Lineweaver-Burk plot

Lineweaver-Burk plot is widely used for the determination of the kinetic parameters. Y-axis intercept is represented by 1/Vmax while x-axis intercept is represented by the -1/Km hence the reciprocal of the y-axis intercept gives information about Vmax while reciprocal of the x-axis gives information about Km. The Kcat can be calculated if the concentration of the enzyme (Et) is known using the formula $K_{cat} = V_{max}/E_t$. Lineweaver-Burk plot is used to explain the type of inhibition such as competitive, non-competitive and mixed inhibition. Different types of inhibition have different type

of plot hence by comparing the Lineweaver-Burk in the presence and absence of inhibitor can be used to find mode of action of the inhibitor.

Lineweaver-Burk method has limitation, First, as y-axis is the reciprocal of the v_o hence if there is even a small mistake in the calculation of v_o, it get amplified. This is prominent at lower low [s]. Second, with the equal increment of [s] that yield equally spaced point on th basic v versus [s] plot (Michaelis-Menten plot), do not yield equally spaced points on the reciprocal plot (Lineweaver Plot). Hence, the reciprocal of the [s], clusters at the axis of the $1/v_o$ therefore the lower [S] i.e its reciprocal 1/[s] will be higher and have more effect on the plot. The concentration of the substrate chosen to generate reciprocal plot should be neighborhood or close to the Km value. When [s] chosen is much less than Km value, the velocity of the reaction is approximately proportional to the substrate concentration and rate of reaction is said to be first order. This result into curve will intercept both axes too close to the origin to allow either V_{max} or K_m to be determined accurately. When [s] is much greater than K_m value, the velocity is constant and equal to V_{max}. The rate of reaction is then independent of [s] and is said to be zero order. The curve will be essentially horizontal. This will allow V_{max} to determine but the slope of the line will be near zero therefore it is difficult to determine Km accurately.

2.2 Hanes Woolf plot

Rearrangement of Lineweaver Burk equation, Hanes Woolf plot can be derived.

$$\frac{1}{v_o} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

Multiplying both side of the equation by [S],

$$\frac{[S]}{v_o} = \frac{[S]K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{[S]}{V_{max}}$$

$$\frac{[S]}{v_o} = \frac{[S]K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{[S]}{V_{max}}$$

Cancelling the [S]

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

Rearranging the equation

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

or

$$\frac{[S]}{v_o} = \frac{1}{V_{max}} . [S] + \frac{K_m}{V_{max}}$$

This plot of $[S]/v_o$ versus [S] is known as Hanes Woolf plot and its Y-axis intercept represent as K_m/V_{max} while x-axis intercept as $-K_m$ when $[S]/v_o$ is equal to zero, and slope will represent the $1/V_{max}$. This plot removes the problem of Lineweaver Burk plot becauase of use of reverse value of [S]. If the substrate concentration range is very low compared to the Km, the plot will be nearly horizontal. If the substrate concentration range is very high compared to the Km, the plot will intersect the axes very close to origin.

2.3 Eadie-Hofstee Plot

Eadie-Hofstee Plot is also known as Woolf-Eadie-Augustinsson-Hofstee or Eadie-Augustinsson plot. This is a graphical representation of enzyme kinetics in which velocity is plotted as a function of the ratio between velocity and substrate concentration. This can be derived from the Michaelis-Menten equation.

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

Invert and multiply with Vmax

$$\frac{V_{max}}{v_o} = \frac{V_{max}(K_m + [S]}{V_{max} . [S]}$$

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

Rearrange (Multiply with v_0)

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

$$V_{max} = \frac{v_o K_m}{[S]} + v_0$$

The final Eadie-Hostee equation becomes-

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

If the above equation is straight line equation then plot can be drawn using $y = m.x + c$

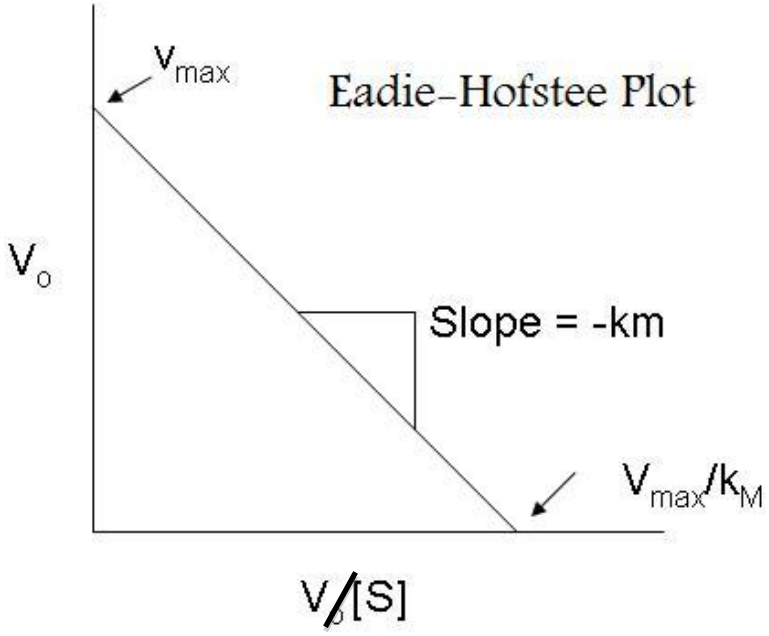

Figure 2: Figure showing the Eadie-Hofstee plot

This plot of v_o against ${}^{v_o}/_{[S]}$ is known as Eadie-Hofstee Plot and its Y-axis intercept represent as V_{max} while x-axis intercept as ${}^{V_{max}}/_{K_m}$ when v=0; and slope will be represented by -Km. This plot remove the problem of the using reverse value of v_o. If the substrate concentration range is very low compared to the Km, the plot will be extremely steep slope, approaching a vertical line intersects horizontal axis at ${}^{V_{max}}/_{K_m}$ (i.e the first order rate constant for reaction). If the substrate concentration

range is very high compared to the Km, the plot will be nearly horizontal at a height of Vmax above the v/[S] axis.

2.3 Eadie-Scatchard plot:

This equation can be derived by the Eadie-hofstee equation by dividing both the equation by Km.

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

Dividing by Km

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

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

$$\frac{v_o}{[S]} = -\frac{1}{K_m} \cdot v_o + \frac{V_{max}}{K_m}$$

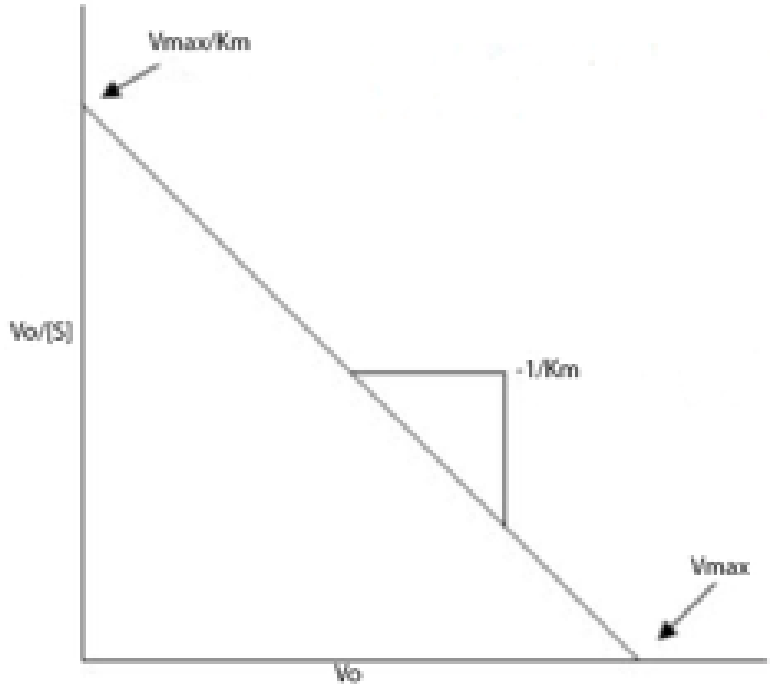

Figure 3: Figure showing the Eadie-Scatchard plot

A plot of $v_o/[S]$ against v_o is known as **Eadie-Scatchard plot** and its Y-axis intercept represent as V_{max}/K_m while x-axis intercept as V_{max} and slope will represent the -1/Km. This plot remove the problem of the using reverse value of v_o.

Eadie-Scatchard equation has been developed to calculate the interaction affinity of a biomolecule with its ligands. The interaction of biomolecule with ligands will be will be studied by equilibrium dialysis. The binding of substrates, inhibitors and activator to the enzyme can be studied by equilibrium dialysis if no catalytic reaction takes places. This is usually takes place for the bi-substrate or multi-substrate reaction if only one substrate is present. The equilibrium binding data are usually analyzed by Scatchard plot. The modified Scatchard equation becomes-

$$\frac{[S]_b}{[S]_f} = -\frac{1}{K_s}[S]_b + \frac{n[E]_t}{K_s}$$

Where $[S]_b = [\square S]$ concentration of bound ligand= concentration of occupied site, $[S]_f$= concentration of free ligand which indicating free substrate, $[E]_t$= total enzyme concentration, $n[E]_t$= total concentration of ligand binding sites, n= the number of identical and independent ligand binding site per molecule of enzyme and Ks= intrinsic substrate dissociation constant at a site. Scatchard plot is a plot of the ratio of concentration of bound ligand to the free ligand versus bound ligand concentration. This plot gives slope of –1/Ks, Binding affinity constant is inverse to the dissociation constant (Ks); intercept at X-axis is $n[E]_t$ or total concentration of ligand binding sites or Bmax (maximum binding). If the binding data is not straight line then binding is cooperative and ligand bound to biomolecule is not allowed achieving equilibrium. If the molar concentration of enzyme is known then another equation can be derived by dividing above equation with molar concentration of the enzyme i.e [Et].

$$\frac{[S]_b}{[S]_f[E]_t} = -\frac{1}{K_s}\frac{[S]_b}{[E]_t} + \frac{n[E]_t}{K_s[E]_t}$$

$$\frac{[S]_b}{[S]_f[E]_t} = -\frac{1}{K_s}\frac{[S]_b}{[E]_t} + \frac{n}{K_s}$$

A plot of $\frac{[S]_b}{[S]_f[E]_t}$ (i.e moles of ligands bound per molecule of enzyme divided by the concentration of free substrate) versus $\frac{[S]_b}{[E]_t}$ (i.e moles of ligand bound per mole of enzyme) is a linear with slope of -1/Ks. The intercept on the vertical Y-axis gives n/Ks. The intercept on the horizontal X-axis gives n, the number of ligand binding sites per molecule of enzyme. If the enzyme posses multiple independent binding site with different affinities for the ligand, the plot will be curved.

3. Summary:

In the present module, we have discussed about different methods used for the determination of kinetics parameters. Here, we have discussed the derivation of the different equation. We have also highlighted the significance and limitation of the different plot. Lineweaver Burk plot are most used plot for the calculation of the mode of enzyme inhibition as well as dissociation constant of the enzyme inhibition. Limitation of the Lineweaver-Burk has also been discussed. Edie Hofstee plot, Hanes Woolf plot and Scatchard plot are also used which further remove the shortcoming of the Lineweaver-Burk plot. The present module will explain how we can calculate the different kinetic parameters. In this module the use of modified Scatchard plot to calculate the affinity of the biomolecule with the ligand has been also explained. In the modified Scatchard plot, we have seen that number of ligand binding sites per molecule of enzyme as well as binding affinity of the enzyme with ligand can be calculated if the ligand is not converted into any product.