Enzyme Kinetics? Elementary, my dear ...

2. The Analysis and Significance of Kinetic Parameters

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Catalysis is essential to make many critical biochemical reactions proceed at useful rates under physiological conditions. We had earlier discussed in Part 1[1] the basic principles of enzyme catalysis and derived the Michaelis–Menten equation. In this article, the significance of kinetic parameters and analysis of kinetic data will be discussed.

Why should one determine K_m and V_{max}?

K_m, the Michaelis constant is a dynamic constant expressing the relationship between the actual steady-state concentrations rather than the equilibrium concentrations. *Table 1* shows the K_m values of some enzymes. K_m depends on the particular substrate used, pH, temperature and ionic strength. Observed values of K_m for different substrates and different enzymes vary widely; the smaller values are in the region of 10^{-7} M whereas poor substrates can have very high values. Typical values for physiological substrates are generally in the region of 10^{-3} M to 10^{-6} M.

Inspection of Michaelis–Menten equation shows that K_m is equivalent to the substrate concentration that yields half maximal velocity. If $v = V_{max}/2$ then,

$$\frac{Vmax}{2} = \frac{Vmax[S]}{K_m + [S]}.$$

Dividing both sides by V_{max} gives:

$$\frac{1}{2} = \frac{[S]}{K_m + [S]}$$

hence, $K_m = [S]$. Very often, K_m is assumed to be equal to the

[1] See *Resonance*, Vol.3, No.6, 1998.

Enzyme	Substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$)
Carbonic anhydrase	CO_2	8000	600,000	7.5×10^7
Chymotrypsin	Acetyl-L-tryptophanamide	5000	100	2×10^4
Penicillinase	Benzylpenicillin	50	2,000	4×10^7
Lysozyme	Hexa-N-acetyl glucosamine	6	0.5	8.3×10^4
Pyruvate decarboxylase	Pyruvate	400	–	–
	HCO_3^-	1000	–	–
	ATP	60		
*Eco*RV endonuclease	Plasmid DNA containing one *Eco*RV site	0.0005	0.015	3×10^7
*Hha*I DNA methylase	λ DNA *Bst*EII digest	0.06	0.22	3.7×10^6
*Eco*RV endonuclease	Oligonucleotide containing one *Eco*RV site	3.8	0.115	3.0×10^4

Table 1. Kinetic constants of some enzymes.

dissociation constant for the ES complex. This is true only if the rate constant for the formation of products, k_3 is significantly smaller than k_2. Consider a situation where k_2 is much greater than k_3. The dissociation of the ES complex to E and S is much faster than the formation of E and the product. Under these conditions $(k_2 >> k_3)$,

$$K_m = k_2/k_3.$$

The dissociation constant of the ES complex (K_{ES}) is

$$K_{ES} = \frac{[E][S]}{[ES]} = k_2 / k_1.$$

This means, K_m is equal to the dissociation constant of the ES complex. Under these conditions $(k_2 >> k_3)$, K_m is a measure of the strength of the ES complex – a high K_m means weak binding and a low K_m means strong binding. Most often, $k_3 >> k_2$, in which case K_m is not directly equivalent to a dissociation constant for ES. In any case K_m is the concentration of substrate at which half the active sites are filled.

The numerical value of K_m is of interest for several reasons,

(a) The K_m establishes an approximate value for the intracellular level of the substrate.

(b) As K_m is a constant for a given enzyme, its numerical value provides a means of comparing enzymes from different organisms or from different tissues of the same organism or from the same tissue at different stages of development.

(c) A ligand-induced change in the effective value of K_m is one way of regulating the activity of an enzyme. By measuring the effects of different compounds on K_m, it is possible to identify physiologically important inhibitors and activators.

The maximum velocity V_{max} is not by itself a very useful comparative parameter because of its dependence on enzyme concentration.

$$V_{max} = k_3 [E_t]$$

A more useful parameter is turnover number [2] or k_{cat}, which is equivalent to the rate constant k_3, for the breakdown of the ES complex to product when the enzyme is fully saturated with substrate. Since $V_{max}=k_3[E_t]$, the term V_{max} reveals the turnover number of an enzyme if the concentration of enzyme $[E_t]$ is known.

$$k_{cat}=V_{max}/[E_t].$$

k_{cat} is a first order rate constant and therefore will have units of reciprocal time. The turnover number is a measure of the maximum potential catalytic activity of an enzyme. The reciprocal of the turnover ($1/k_{cat}$) is the time taken for a single round of catalysis to occur when the enzyme is saturated with substrate. Turnover numbers vary widely, the highest value observed is for carbonic anhydrase (*Table 1*). This means that with a turnover number of 600,000/sec, a 10^{-6} M solution of carbonic anhydrase catalyses the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate. Each round of catalysis occurs in 1.7μ seconds

[2] The turnover number of an enzyme is the number of substrate molecules converted into product by an enzyme molecule in unit time when the enzyme is fully saturated with substrate.

($1/k_{cat}$). In other words, each enzyme molecule can hydrate 10^5 molecules of CO_2 per second.

Another important parameter is the ratio of the turnover number to the Michaelis constant. The value k_{cat}/K_m is a second order rate constant for the reaction of enzyme and substrate to form products.

Consider the following reaction:

$$E+S <----> E+P$$

when $[S]>>K_m$, then,

$$v = \frac{-\mathrm{d}[S]}{\mathrm{d}t} = \frac{k_{cat}}{K_m}[E][S].$$

This ratio is referred to as specificity constant for an enzyme. For example, suppose an enzyme can catalyse a reaction with either of the two substrates A and B, then if the value of k_{cat_a}/K_{m_a} is greater than k_{cat_b}/K_{m_b}, then it follows that substrate A will be utilised at a greater rate i.e., the enzyme has a greater specificity for substrate A than for B. Since an enzyme and substrate cannot combine more rapidly than diffusion permits, there is an upper limit on enzyme catalysis. The value of k_{cat}/K_m cannot be greater than about $10^9 s^{-1}M^{-1}$. Some enzymes like carbonic anhydrase have values of k_{cat}/K_m that approach the diffusion limit, indicating extreme efficiency in binding substrate and in converting it to a product. The specificity constant is a useful parameter when one compares a wild-type enzyme with a mutant enzyme. This is all the more important especially when one is studying amino acid replacements at the active sites of enzymes by site-directed mutagenesis [3] *Therefore, high catalytic activity and high specificity for substrates, can be described kinetically by the constants* k_{cat} *and* k_{cat}/K_m.

[3] Site-directed mutagenesis is a procedure for producing a protein in which specific amino acid residues have been replaced by others at the DNA level.

Graphical Methods to Estimate K_m and V_{max}

As the v versus $[S]$ curve is a hyperbola (*Figure 3* in Part 1), it is

difficult to determine V_{max} and K_m. Many students assume that V_{max} can be estimated from a v versus S plot by finding the point at which v 'reaches' its limiting value. The main drawback lies in drawing a curve that flattens out too abruptly and then drawing an asymptote too close to the curve. An asymptote is a straight line approached by a given curve, as one of the variables in the equation of the curve approaches infinity. Enzyme kinetic data may be treated graphically in the same manner as that of ligand binding. Over the years many graphical and computational methods have been developed in an attempt to improve the accuracy with which the kinetic constants may be determined. The most widely used graphical method for the determination of K_m and V_{max} has been the Lineweaver–Burk plot, named after the two scientists who devised the plot. This is a double reciprocal plot, derived by taking reciprocals of both sides of the Michaelis–Menten equation (equation 4) to give

$$\frac{1}{v} = \frac{1}{[S]}\frac{K_m}{Vmax} + \frac{1}{Vmax} \quad (\text{cf} : y = mx + c) .$$

Hence, when $1/v$ is plotted against $1/[S]$ a straight line is obtained with a slope of K_m/V_{max} and an intercept of $1/V_{max}$ on the $1/v$ axis (*Figure 1*).

This plot does suffer from a very serious disadvantage that is often ignored. By taking reciprocals, greater significance is

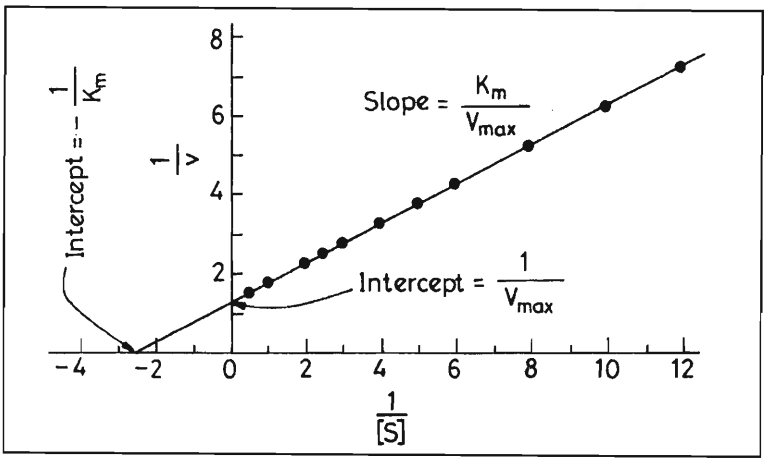

Figure 1. Double-reciprocal (1/v versus 1/[S]) Lineweaver–Burk plot.

placed on the rates obtained at low substrate concentrations and it is these that are most likely to be subject to greatest experimental error. The capacity of the double-reciprocal plot to 'launder' poor data i.e., to provide a visual effect of scatter (but not in reality) probably accounts for its extraordinary popularity with biochemists. It is therefore not recommended as a method for estimating K_m and V_{max}. However, by using suitable weightage, the problems with the double-reciprocal plot can be overcome but often a best fit line appears to the eye to fit badly. The Lineweaver–Burk plot is not the only linear transformation of the basic velocity equation. Indeed, under some circumstances one of the other linear plots may be more suitable or may yield more reliable estimates of the kinetic constants (see *Suggested Reading*).

A plot for obtaining the kinetic constants that has the advantage of simplicity as well as being statistically acceptable is the direct linear plot (see *Suggested Reading*). This method treats V_{max} and K_m as variables and v and $[S]$ as experimentally determined constants according to the rearranged Michaelis–Menten equation:

$$V_{max}/v - K_m/[S] = 1.$$

A plot is constructed with the x-axis labelled K_m and the y-axis labelled V_{max}. For each experimental result, a straight line is drawn from a value of $-[S]$ on the x-axis to the value v, on the y-axis. This line is extended into the first quadrant of the graph and represents a series of values of V_{max}/K_m that would produce a rate v_1 at substrate concentration $[S_1]$. Similarly, lines are drawn for a set of data $[S_2, S_3, \ldots, S_n]$, $v_2, v_3, \ldots, v_n$. The lines should all intersect at a single point with coordinates of K_m and V_{max} which represent the only values of K_m and V_{max} that satisfy all of the sets of data (*Figure 2*). In practice, there is likely to be more than one intersecting point (*Figure 2*, inset). When there are multiple intersections, the best estimates of the true values of K_m and V_{max} are determined by the coordinates of the *median* intersection point. The most obvious advantage of this plot is that the original form of it requires no calculations at all. This allows it to

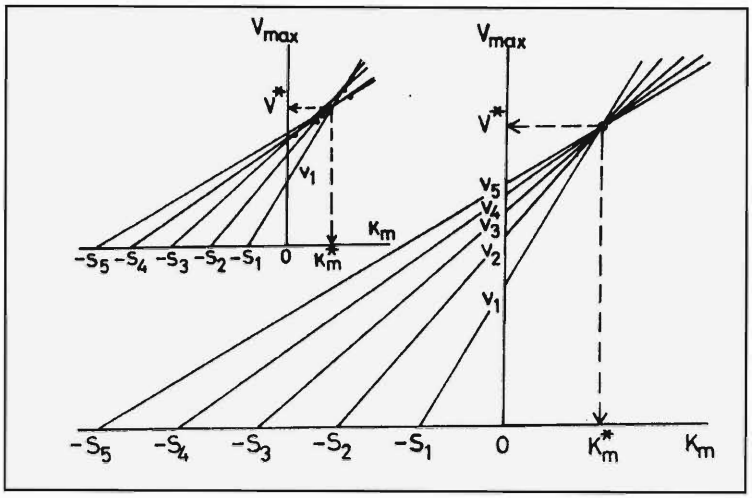

Figure 2. Direct linear plot of V_{max} against K_m. Each line represents one data point and is drawn with an intercept of –S on the x-axis. In an idealised situation, all the lines intersect at a unique point whose coordinates yield the values of K_m and V_{max} that fit the data. Inset shows more realistic data with experimental errors causing the unique point to breakdown into a family of points. The best estimate of K_m and V_{max} can be taken as the medians (middle values) of the two series.

be used very easily in the laboratory while the experiments are proceeding, so that one has an immediate visual idea of the likely parameter values and of design needed for defining them accurately. These features make it more suitable for use in the laboratory for actual analysis of data than for presenting the results in the literature.

Suggested Reading

[1] I H Segel. Enzymes in *Biochemical Calculations*. John Wiley and Sons Publishers. 208–237,1976.

[2] A C Bowden. Introduction to Enzyme Kinetics in *Fundamentals of Enzyme Kinetics*.Portland Press. U.K. Publishers. 19–34, 1995.

[3] Smith and Wood. Enzyme Kinetics in *Biological Molecules*. Chapman and Hall Publishers. 83–94, 1995.

[4] L Stryer. Enzymes: Basic Concepts and Kinetics in *Biochemistry*. W.H. Freeman and Company Publications. 181–195, 1995.

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