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EVALUATION OF KINETIC PARAMETERS IN ENZYMIC NON-LINEAR PROGRESS REACTIONS

by

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ABSTRACT

A modified form of the integrated Michaelis-Menten equation would provide a useful means of evaluating enzyme kinetic parameters in nonlinear progress reaction with time. A slight modification of the Lineweaver-Burk form (and other variants) using for the velocity, the change in substrate concentration divided by time ($\bar{v}$), and for the substrate concentration, the arithmetic mean value for the time interval ($\bar{S}$), allows this linear reciprocal form to be used with negligible error even when as much as half of the substrate is utilized during the time interval.

INTRODUCTION

The kinetic behavior of the vast majority of enzymes follows the Michaelis-Menten equation for the mechanism of $E+S \rightleftharpoons ES \rightarrow E+P$:

$$v = -\frac{ds}{dt} = \frac{V_{max}}{1+(K_m/s)}$$

This same form is predicted by many quite complicated enzyme mechanisms although the physical interpretation of the kinetic constants may, in different case, be very different. The usual way of evaluating K_m and V_{max} is to form the double reciprocal (1) or a variant form (2, 3) and measure the initial velocity at different substrate concentrations. The comparative advantages of different modifications of the double reciprocal have been discussed at some length (4-6) but there has been little or no discussion of the practical exqerimental meaning of initial velocity. In general, initial velocities (steady state velocities) were measured by extrapolation to zero time to eliminate the effect of the products on the rate. There may, however, be some difficulties on making such extrapolation to zero time since initial velocity may decrease rather rapidly as the reaction goes even in the absence of product inhibition. In fact, there are many enzymic reactions the progress of which is non-linear with time. In those cases, most enzymatic reactions were allowed to more than 10 percent utilization of initial substrate concentration for the accuracy in the measurement. Clearly a velocity measured on the basis of a change of one or two percent in substrate concentration during the time interval could be taken as initial velocity, but it is not clear just how large a change in substrate concentration would be acceptable. In many cases, it is not practical to limit the change in substrate concentration to a few percent and procedures must be used which involve the integrated rate equation of the Michaelis-Menten form.

MODIFIED MICHAELIS-MENTEN EQUATION

The integrated rate equation for the single substrate enzyme reaction mechanism was obtained based on the assumption (7) that the time required to reach the steady state is negligible, $d(ES)/dt=0$, and $(S)\gg(E)^\circ$:

$$V_{max}t=\Delta S+K_m\ln\frac{S_o}{S_t} \qquad (1)$$

where S_o is initial substrate concentration, S_t the concentration after the time interval t, and $\Delta S=S_o-S_t$. Equation 1 may be rearranged into the double reciprocal form resembling that of Lineweaver-Burk (1);

$$\frac{1}{\bar{v}}=\frac{1}{V_{max}}+\frac{K_m}{V_{max}}\frac{1}{\Delta S}\ln\frac{S_o}{S_t} \qquad (2)$$

where $\bar{v}$ $(=\Delta S/t)$ is the mean velocity during the incubation time interval. Using Equation 2, a plot of the reciprocal mean velocity $1/\bar{v}$ versus $(1/\Delta S)\ln(S_o/S_t)$ would provide a useful method enabling to measure the same kinetic parameters as that obtained from the double reciprocal form of the original Michaelis- Menten equation in which initial velocity and initial substrate concentration should be employed.

With the substitution of the power series expansion to the logarithmic term in Equation 2,

$$\frac{1}{\bar{v}}=\frac{1}{V_{max}}+\frac{K_m}{V_{max}}\frac{1}{S_o}\sum_{n=1}^{\infty}\frac{1}{n}\left[\frac{\Delta S}{S_o}\right]^{n-1} \qquad (3)$$

where the limiting condition is $O\leq\Delta S/S_o<1$.

From Equation 3 it is evident that the mean velocity $\bar{v}$ can only be used with initial substrate concentration if ΔS is a very small part of S_o; i. e., when $\lim_{\Delta S\to 0}\frac{1}{n}\left(\frac{\Delta S}{S_o}\right)^{n-1}=1$, Equation 3 becomes to same as the reciprocal form of the Michaelis-Menten equation:

$$\lim_{\Delta S\to 0}\bar{v}=v_o$$

where v_o is initial velocity. As increase in value of ΔS, in other word, increase in percent reaction, the greater change in substrate concentration is expected during the time interval as shown in Fig. 1.

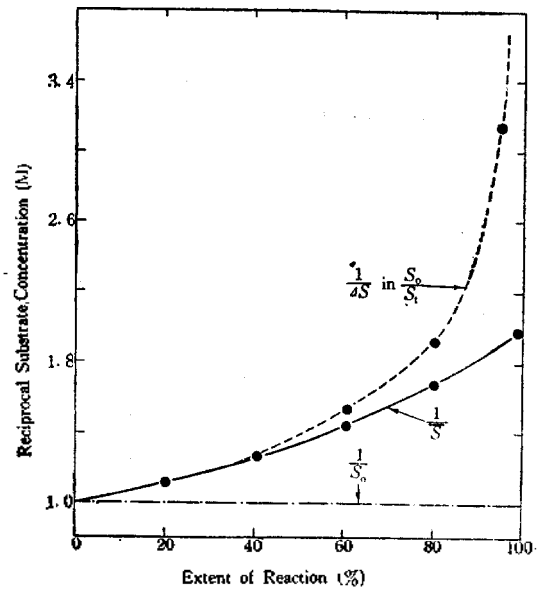

Fig. 1. Change of reciprocal substrate concentrations as a function of percent reaction.

When the velocity of reaction is measured as a mean value of concentration $\bar{v}$ during the time interval and is applied with initial substrate concentration to the double reciprocal form, a deviation α from Equation 2 can be calculated as:

$$(1+\alpha)\frac{1}{S_o}=\frac{1}{\Delta S}\ln\frac{S_o}{S_t}$$

By rearrangement with $\gamma=S_t/S_o$

$$\frac{\alpha}{1+\alpha}=1+\frac{1-\gamma}{\ln\gamma}$$

A variation in percent deviation, $100\alpha/(1+\alpha)$, at different extents of the enzyme reaction was shown in Fig. 2.

From this it is evident that for the evaluation of correct kinetic parameters the integrated rate equation (Equation 2) should be used whenever

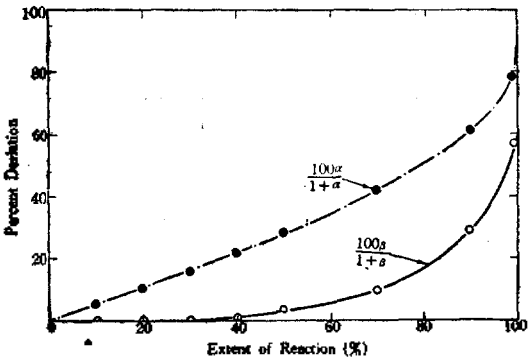

Fig. 2. Change in percent deviation of two double reciprocal forms at different percent reaction.

the enzyme reaction is allowed to utilize more than 10 percent of initial substrate concentration.

The gist of this paper is that a simple modification of the double reciprocal form enables that form to be retained even when as much as 50 percent, or more, of the substrate is utilized during time interval. The best we can do in estimating $\bar{v}$ is to form the quotent $(\Delta S/t)$, if ΔS as a positive number is an applicable part of S_o, and it is clear that it would be unwise to use either S_o or S_t against $\bar{v}$ for the double reciprocal plot of the Michaelis-Menten equation. Some intermediate value is suggested in this paper. This intuitive procedure is given mathematical support by the theorem of the Mean(9) which requires that some value of S exist in the interval of S_o and S_t where ds/dt does equal to $\Delta S/t$. If we could always select this value of S or a sufficient approximation to it, we should be able to always use the double reciprocal form of the Michaelis-Menten equation, in an exact manner:

$$\frac{1}{\bar{v}} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{S} \qquad (4)$$

where S is the arithmetic mean value of substrate during the time interval: $S=S_o-{}^1/_2\ \Delta S$.

We shall show that the arithmetic mean value of substrate S is good approximation of the correct S for Equation 4, and the use introduces negligible error even one third of the substrate is utilized in the time interval, and an acceptable error when as much as one half of the substrate is used up. The result is in sharp contrast to the prevailing belief that initial velocities must be measured in order to use the double reciprocal form of the Michaelis-Menten equation.

In a comparison of Equation 2 and 4, a deviation β between two forms can be expressed as:

$$(1+\beta)\frac{1}{\bar{S}} = \frac{1}{\Delta S} \ln \frac{S_o}{S_t}$$

By rearrangement into a fraction of deviation:

$$\frac{\beta}{1+\beta} = 1 - \frac{(1-\gamma)}{(1+\gamma)} \frac{2}{\ln\gamma}$$

where $\gamma = S_t/S_o$

The percent deviation $100\beta/(1+\beta)$ betwen Equation 2 and 4 were calculated and shown in Fig. 2. As a result, the arithmetic mean value of the substrate in the time interval (S) is clearly an attractive quantity to use of the attending error should be small up to 5 percent. This is in fact the case and an evaluation of the deviation between Equatin 2 and 4 shows that the error is less than 3.5 percent for utilization of up to 50 percent of the initial substrate concentration.

For most purposes the arithmetic mean value of substrate concentration is a good selection for the mean velocity in the time interval enabling to use the double reciprocial form, Equation 4.

APPLICATION TO ANGIOTENSIN-CONVERTING ENZYME REACTION

Angiotensin converting enzyme which was isolated from human plasma (10) catalyzes hydrolysis of angiotensin I, physiologically inactive

decapeptide, to the vasoconstrictive octapeptide, angiotensin II and the dipeptide, histidylleucine. The radioactive assay for the converting enzyme activity was proved as a simple and considerably sensitive method (10), but the system was not suitable to measure the initial velocities of the enzyme reaction except under the zero order kinetics. For the accuracy in the radioactivity measurement of enzyme activity, at least 10 percent utilization of initial substrate concentration is required.

In a typical kinetic experiment with human plasma angiotensin converting enzyme, the reaction was carried out at pH 7.5 using 0.05M Na-phosphate buffer at different substrate concentrations such that 20 to 40 percent of the substrates were utilized in 120 minutes, and the mean velocities in the time interval were calculated.

Three different plots of $1/S_o$, $1/S$, and $(1/S)$ ln (S_o/S_t) versus $1/\bar{v}$ were shown in Fig. 3, and the K_m values obtained from different plots were summarized in Table 1. Again, in conculusion, the arithmetic mean value of the substrate concentration and the mean velocity in the time interval are clearly useful quantities enabling to use the double reciprocal form for correct evaluation of enzyme kinetic parameters.

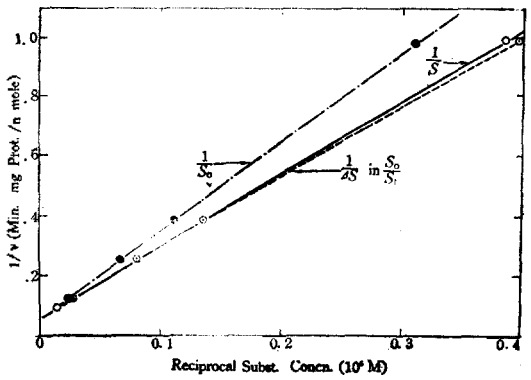

Fig. 3. **A typical double reciprocal plot employing three different terms of substrate concentration against the mean velocity.**

Table 1 The K_m Values Measured By Three Different Plots

Method of plot	K_m	Error
	(μM)	(%)
$\frac{1}{\bar{v}}$ vs. $\frac{1}{\Delta S} \ln \frac{S_o}{S_t}$	42	—
$\frac{1}{\bar{v}}$ vs. $\frac{1}{S}$	42	0
$\frac{1}{\bar{v}}$ vs. $\frac{1}{S_o}$	53	25

Three different plots gave the same V^*_{max} value of 17.5 n mole/min/mg drotein.

REFERENCES

1) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.* **56**, 658 (1934)
2) C. S. Hanes, *Biochem. J.* **26**, 1406 (1932)
3) B. H. J. Hofstee, *J. Biol. Chem.* **179**, 633 (1949); Nature, London, **184**, 1298 (1959)
4) M. Dixon and E. C. Webb, Nature, London **184**, 1296 (1959)
5) P. Stutts and I. Fridovich, *Science* **149**, 447 (1965)
6) A. C. Walker and C. L. A. Schmidt, *Arch. Biochem.* **5**, 445 (1944)
7) M. F. Morales and D. E. Goldman, *J. Am. Chem Soc.* **77**, 6069 (1955)
8) V. C. R. Henri, Acad. Sci., Paris **135**, 916 (1902)
9) I. S. Sokolnikoff, Adv. Calculus, McGraw Hill, N. Y. & London (1939)
10) H.-J. Lee, J. N. LaRue and I. B. Wilson, Mol. Pharmacol. (in press); Arch. Biochem. Biophys. (in press); Circulation Res. (in press)