ENZYMIC PARAMETERS: MEASUREMENT OF V AND K_m

HYUN-JAE LEE AND IRWIN B. WILSON

Department of Chemistry, University of Colorado, Boulder, Colo. 80302 (U.S.A.)

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SUMMARY

A slight modification of the Lineweaver–Burk equation (and other variants) in which the form is retained but the instantaneous velocity is replaced by the negative change in substrate concentration divided by the time and the initial substrate concentration is replaced by the arithmetic average substrate concentration during the time interval, allows this linear reciprocal form to be used with little error even when as much as 50% of the substrate is consumed. This result is in sharp contrast to the prevailing belief that the initial velocities and initial substrate concentrations must be used.

The kinetic behavior of the majority of enzymes follows the Michaelis–Menten form:

$$v = -\frac{d[S]}{dt} = \frac{V}{1 + \frac{K_m}{[S]}}$$ (1)

where v is the instantaneous velocity at a time t when the substrate concentration is $[S]$. This same form is predicted by many quite complicated enzyme mechanisms although the physical interpretation of the constants may, in different cases, be very different. The usual way of evaluating V and K_m is to form the double reciprocal[1].

$$\frac{1}{v} = \frac{1}{V} + \frac{K_m}{V} \cdot \frac{1}{[S]}$$ (2)

or a variant form[2,3] and measure the velocity at several different initial 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 experimental meaning of initial velocity. Clearly a velocity calculated on the basis of a change of 1 or 2% in substrate concentration during the time interval could be taken as an 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 1 or 2% and procedures must be used which involve the integrated form of Eqn. 1, namely

$$V_t = [S_0] - [S_t] + K_m \ln \frac{[S_0]}{[S_t]}$$ (3)

where $[S_0]$ is the substrate concentration at zero time and $[S_t]$ the concentration after the time interval t (ref. 7).

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% of the substrate is utilized during the time interval.

The best we can do in estimating v is to form the quotient, $([S_0]-[S_t])/t = \bar{v}$. If $[S_0] - [S_t] = \triangle[S]$ is an appreciable part of $[S_0]$, it is clear that it would be unwise to use either $[S_0]$ in Eqn. 2 or $[S_t]$ at the end of the time interval. Some intermediate value is suggested. This intuitive procedure is given mathematical support by the Theorem of the Mean[8] which states that for $[S] = f(t)$ some value of $[S]$ exists in the interval $[S_0] - [S_t]$ where $d[S]/dt$ does equal $([S_0] - [S_t])/t$. If we could always select this value of $[S]$, or a sufficient approximation to it, we should be able to always use Eqn. 2, in an exact manner.

We shall show that the arithmetic mean value of $[S]$, $[\bar{S}] = \frac{1}{2}([S_0] + [S_t])$ is a good approximation to the correct value of $[S]$ for Eqn. 2, and its use introduces negligible error even when 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. This result is in sharp contrast to the prevailing belief that "initial velocities" must be measured in order to use the double reciprocal equation.

The integrated Michaelis–Menten equation, Eqn. 3, can be put in the form

$$\frac{1}{\bar{v}} = \frac{1}{V} + \frac{K_m}{V} \cdot \frac{1}{[S_0] - [S_t]} \ln \frac{[S_0]}{[S_t]}$$ (4)

The proposed modification of the Lineweaver–Burk equation is

$$\frac{1}{\bar{v}} = \frac{1}{V} + \frac{K_m}{V} \cdot \frac{1}{[\bar{S}]}$$ (5)

where $\bar{v} = ([S_0] - [S_t])/t$ and $[\bar{S}] = ([S_0] + [S_t])/2$.
The question is "how closely does $1/[\bar{S}]$ approximate to

$$\frac{1}{[S_0] - [S_t]} \ln \frac{[S_0]}{[S_t]} ?"$$

Let

$$\frac{[\bar{S}]}{[S_0] - [S_t]} \ln \frac{[S_0]}{[S_t]} = 1 + \alpha$$ (6)

where α is the relative error involved in using $1/[\bar{S}]$ rather than

$$\frac{1}{[S_0] - [S_t]} \ln \frac{[S_0]}{[S_t]}.$$

Substitution from Eqn. 6 into Eqn. 4 yields

$$\frac{1}{\bar{v}} = \frac{1}{V} + \frac{K_m}{V}(1 + \alpha) \frac{1}{[\bar{S}]}$$ (7)

Values of α were calculated from Eqn. 6 for different extents of reaction in percent,

$$\left[1 - \frac{[S_t]}{[S_0]}\right] \times 100$$

and the results are plotted in Fig. 1. The calculation indicates that the relative error, α, is less than 1% even when the reaction has gone to the extent of 30%. The relative error is 2% at 40% extent of reaction and <4% at 50% extent of reaction.

Eqn. 7 which is exact indicates that if the approximate Eqn. 5 is used, the value obtained for K_m will be high by the error α. Thus if the extent of the reaction is 50%, K_m will be high by 4%.

The common practice is to use Eqn. 2 with $v = \triangle[S]/t$ and $[S] = [S_0]$. Whether this is good practice depends upon how closely $1/[S_0]$ approximates $1/[S_0] - [S_t]$ ln $[S_0]/[S_t]$, the approximation is poor! Even when the reaction has proceeded to only 10% the error in using $[S_0]$ already exceeds 5% (Fig. 1). These results indicate
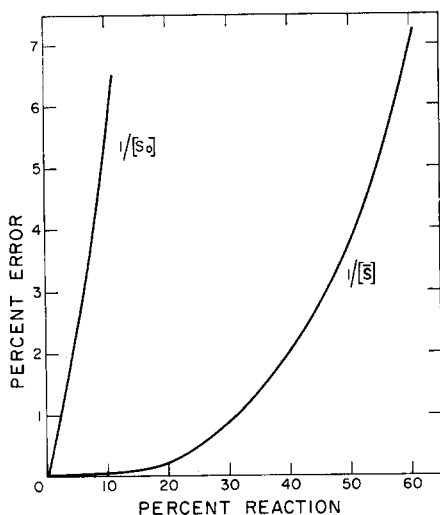

Fig. 1. Percent errors at different extents of reaction. Percent errors, 100α, were calculated from Eqn. 6 for different extents of reaction,

$$\left[1 - \frac{[S_t]}{[S_0]}\right] \times 100$$

that the use of Eqn. 2 with initial velocities estimated by $\triangle[S]/t$ and the initial substrate concentrations must be restricted to extents of reaction that are less than 10% and preferably less than 5%.

The use of average substrate concentrations enables the investigator to retain the Lineweaver–Burk form even when quite substantial extents of reaction have occurred. In discussing this question we have assumed that there is no product inhibition. This is a question that is easily answered experimentally and is in any case, one that must be answered before any equation can be used. However, it is more likely to be significant when large extents of reaction are involved. Similarly,

the reaction must not be significantly reversible. This is required also in the case of the Michaelis–Menten or Lineweaver–Burk equation. However, reversibility is more likely to be a significant factor when large extents of reaction are involved.

In experimental work with angiotensin converting enzyme, it has not been feasible for us to restrict the extent of reaction to much less than 40%. However, identical values for K_m ($4.2 \cdot 10^{-5}$ M) were obtained whether Eqn. 4 or Eqn. 5 was used[9,10].

Our conclusion that the Lineweaver–Burk plot can be used for relatively large extents of reaction, if the average value of $[S]$, $[\bar{S}]$ is used, extends to all the variants of this plot including those for reactions occurring in the presence of inhibitors.

REFERENCES

1 H. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.
2 C. S. HANES, *Biochem. J.*, 26 (1932) 1406.
3 B. H. J. HOFSTEE, *J. Biol. Chem.*, 179 (633) 1949.
4 B. H. J. HOFSTEE, *Nature*, 184 (1959) 1296.
5 M. DIXON AND E. C. WEBB, *Nature*, 184 (1959) 1298.
6 P. STUTTS AND I. FRIDOVICH, *Science*, 149 (1965) 447.
7 V. HENRI, C. R. *Acad. Sci. Paris*, 135 (1902) 916.
8 I. S. SOKOLNIKOFF, *Adv. Calculus*, McGraw Hill, New York and London, 1939.
9 H-J. LEE, J. N. LaRUE AND I. B. WILSON, *Biochim. Biophys. Acta*, 235 (1971) 521.
10 H-J. LEE, J. N. LaRUE AND I. B. WILSON, *Arch. Biochem. Biophys.*, 142 (1971) 548.