BIOCHEMICAL LETTERS

A novel parameter estimation from the linearized Michaelis–Menten equation at low substrate concentrations

The $V_{\max}$ and K_m of enzymes which follow Michaelis–Menten kinetics can be determined by different kinds of linearization of the original equation (Hanes, 1932; Lineweaver & Burk, 1934; Eadie, 1942; Hofstee, 1952; Fajsz & Endrényi, 1974; Eisenthal & Cornish-Bowden, 1974; De Miguel Merino, 1974; Cornish-Bowden, 1981), or by non-linearized methods such as logarithmic plot, non-linear regression and progress curve analysis (Fernley, 1974; Nimmo & Atkins, 1974; Yun & Suelter, 1977; Halwachs, 1978; Keleti, 1981; Markus & Plessner, 1981; Markus *et al.*, 1981). I would like to suggest a novel method for the determination of $V_{\max}$ and K_m which may be useful if only low substrate concentrations can be used.

Let us start with the well-known Michaelis–Menten equation (Michaelis & Menten, 1913):

$$v_0 = V_{\max} [S] / (K_m + [S]) \quad (1)$$

where v_0 is the initial velocity, $V_{\max}$ is the maximum velocity, K_m is the Michaelis constant and $[S]$ is the concentration of the substrate.

From eqn. (1) by simple rearrangement we get

$$[S] = v_0 K_m / (V_{\max} - v_0) \quad (2)$$

Differentiating eqn. (2) according to v_0 :

$$d[S]/dv_0 = V_{\max} K_m / (V_{\max} - v_0)^2 \quad (3)$$

From eqn. (3)

$$dv_0/d[S] = (V_{\max} - v_0)^2 / V_{\max} K_m \quad (4)$$

If we use saturating concentration of S, $dv_0/d[S] = 0$ and $v_0^2 - 2V_{\max} v_0 + V_{\max}^2 = 0$, i.e. $v_{0(1,2)} = V_{\max}$. From eqn. (4)

$$(dv_0/d[S])^\dagger = (V_{\max}/K_m)^\dagger - v_0/(V_{\max} K_m)^\dagger \quad (5)$$

Eqn. (5) could be used for the determination of $V_{\max}$ and K_m either by measuring v_0 at different $[S]$ or by the numerical or graphical estimation of $dv_0/d[S]$ from a single progress curve. The plot of

$(dv_0/d[S])^\dagger$ versus v_0 gives a straight line with intercepts $(V_{\max}/K_m)^\dagger$ and $V_{\max}$ at the ordinate and abscissa, respectively, and with slope $-1/(V_{\max} K_m)^\dagger$. However, due to the difficulty of precise determination of $dv_0/d[S]$, this plot has no advantage over the usual ones. Nevertheless, we can solve eqn. (4) in another way:

$$dv_0/d[S] = V_{\max}/K_m - (v_0/K_m)(2 - v_0/V_{\max}) \quad (6)$$

and if $[S] \ll K_m$, then $v_0 \ll V_{\max}$ and $v_0/V_{\max} \ll 2$, i.e.

$$dv_0/d[S] \cong V_{\max}/K_m - (2v_0/K_m) \quad (7)$$

If $2v_0 \ll V_{\max}$, then $dv_0/d[S] = V_{\max}/K_m = \text{constant}$. Consequently, the lower and upper limits of v_0 which can be used for the determination of K_m and $V_{\max}$ lie between $V_{\max}/2$ (cf. eqn. 7) and $V_{\max}/5$ (cf. eqn. 6), respectively. It is diagnostic of the proper range that the plot $dv_0/d[S]$ versus v_0 is approximately linear.

Eqn. (7) permits one to determine $V_{\max}$ and K_m (Fig. 1) in the case when $[S] < K_m$. This was practically unfeasible up to now, since at low substrate concentrations the hyperbolic saturation curve degenerates due to the dependence of the duration of the pre-steady state on substrate concentration (Fischer & Keleti, 1975). This behaviour cancels the validity of any linearization at $[S] < K_m$, since the real $t = 0$ for the steady state becomes uncertain. However, if instead of the absolute values of $[S]$ and v_0 their relative changes are compared, this source of error is eliminated. Obviously, there remains the error of determination of $dv_0/d[S]$, which can be reduced by computer-facilitated numerical differentiation by progress curves instead of resorting to the usual saturation curve determination, which gives only the approximation $\Delta v_0/\Delta[S]$. Moreover, the accuracy of determination of $dv_0/d[S]$ is not less than that of v_0 at low $[S]$, where the duration of steady state is very short due to the rapid decrease in $[S]$ (Gutfreund, 1972; Fischer & Keleti, 1975). Eqn. (1) is not even valid at low $[S]$, since the underlying assumption is that $[S] = [S]_0$, where $[S]_0$ is the total substrate concentration at $t = 0$. However, in eqn. (7), the change

Fig. 1. Determination of V_{max} and K_m when $[S] < K_m$

of $[S]$ itself is taken into account to evaluate the kinetics.

In simulated experiments, if the Lineweaver-Burk plot was used to evaluate the constants from data obtained using $[S]$ only below K_m and if an error of $\pm 5\%$ for the measurement of v_0 was assumed, the error of V_{max} determination was $\pm 25\%$ and that of $K_m \pm 35\%$. With the method presented in Fig. 1, the errors are the same in the range of v_0 between $V_{max}/2$ and $V_{max}/3$, but decrease to $\pm 10\%$ for V_{max} and to $\pm 20\%$ for K_m in the range of v_0 between $V_{max}/3$ and $V_{max}/5$.

Consequently, if only poorly soluble substrate is available (e.g. substrates of membrane-embedded enzymes, natural substrates of proteolytic enzymes or amylase, etc.) or the use of high substrate

concentrations is to be avoided (inhibition or activation by excess of substrate, analysis of enzyme action at near physiological $[S]$, high price of the substrate, etc.) the above method may prove helpful.

T. KELETI

*Institute of Enzymology, Biological
Research Center, Hungarian Academy of Sciences,
Budapest, Hungary*

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