The 'Double' Michaelis-Menten Equation: Estimation of Parameters

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Sum of Two Michaelis-Menten Terms, Integrated Rate Equation, Parameter Estimation

When the concentration dependence of an enzymic reaction or a transport process can be described by the sum of two Michaelis-Menten terms, reliable data that relate reaction rate and substrate concentration can be obtained even when as much as 70 per cent of substrate was consumed during the assay. Each data pair consists of the average reaction rate during an assay and the concentration where the instantaneous reaction rate was equal to the average rate. Although that concentration cannot be computed exactly (as it depends on the four kinetic parameters), it may be computed in a good approximation as if the reaction followed the simple Michaelis-Menten relationship. The relative error in the approximated concentration for $1 \leqq K_2/K_1 \leqq 10^5$ and $10^{-2} \leqq V_2/V_1 \leqq 10^2$ did not exceed 5 per cent up to 50 per cent of substrate consumption, and did not exceed 10 per cent up to 70 per cent of substrate consumption.

Introduction

In general, initial reaction rates, or uptake rates, are measured at various substrate concentrations to determine the Michaelis constant (K) and the maximal reaction rate (V) of an enzyme preparation or a transport process. In some cases it may be convenient, or even necessary to measure an average reaction rate over a time interval in which the substrate concentration had decreased considerably. If during the assay the substrate concentration decreased from S_0 at zero time to S_1 at time t_1, the average reaction rate is given by $(S_0 - S_1)/t_1$. According to the Theorem of the Mean some value of S exists in the interval where the instantaneous reaction rate is equal to the average rate. If the process obeys the Michaelis-Menten equation, the value of that concentration equals $(S_0 - S_1)/\ln(S_0/S_1)$, as can be derived from the integrated form of that equation [1, 2].

In some enzymological studies [3, 4] and in many transport studies [5, 6] it has been found that the concentration dependence of the reaction rate can be described by the sum of two Michaelis-Menten terms. In such a system one can again ask for the concentration where the instantaneous reaction rate equals the average rate over a time interval. This problem is considerably more complicated than the single-reaction case (see Appendix). When the double reaction is treated as if it were a single reaction, however, it can be demonstrated that the resulting error is small for quite a large range of values of the four parameters involved, K_1, K_2, V_1, and V_2. In addition it is shown that the estimation of these parameters is hardly affected by this procedure.

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Results and Discussion

In case of two, simultaneously occurring Michaelis-Menten reactions, the concentration, S_x, where the instantaneous reaction rate equals the average rate over a time interval $(0, t_1)$ depends on S_0 and S_1, but also on the kinetic parameters K_1, K_2, V_1, and V_2 [see Appendix, Eqn. (7)]. As the latter parameters are unknown one has to find a good approximation which is independent of these parameters.

When S_x is calculated as if we were dealing with a single reaction,

$$S_x \approx \tilde{S}_x = (S_0 - S_1)/\ln(S_0/S_1)$$

the relative error, ϱ, in $\tilde{S}_x$ may be defined by

$$\varrho = (S_x/\tilde{S}_x) - 1 .$$

Since $\tilde{S}_x/S_0$ depends on S_1/S_0, and since S_x/S_0 is a function of the ratios S_1/S_0, S_0/K_1, K_2/K_1, and V_2/V_1 [see Appendix, Eqn. (8)] it follows that ϱ is a function of these ratios as well. Accordingly, the

Table I. Values of the ratios K_2/K_1 and V_2/V_1 in some enzyme preparations and transport functions.

	Biological material	K_2/K_1	V_2/V_1	Ref.
Enzyme				
Ornithine decarboxylase	*Physarum polycephalum*	130	10	[4]
3':5'-cyclic nucleotide phosphodiesterase	rat liver	130	60	[3]
Transport function				
K^+	barley roots	540	1	[9]
D-glucose	hamster small intestine	20	1	[10]
D-glucose	*Neocosmospora vasinfecta*	130	1	[11]
D-galactose	rat intestine	10	2	[12]
L-leucine	cultured mouse cells	10	3	[13]
L-leucine	duckweed	330	5	[14]
L-histidine	*Salmonella typhimurium*	650	5	[15]
L-histidine	baker's yeast	20	3	[16]
L-histidine	S37 ascites tumor cells	70	6	[17]

relative error ϱ in $\tilde{S}_x$ was computed for various values of these ratios. Some realistic values of K_2/K_1 and V_2/V_1 are listed in Table I.

Fig. 1 shows, for $K_2/K_1 = 100$ and $V_2/V_1 = 10$, the increase in the error in $\tilde{S}_x$ as the reaction proceeds. At various initial substrate concentrations the error is smaller than 4 per cent up to a substrate consumption of 70 per cent ($S_1/S_0 = 0.3$). Fig. 1 also indicates that ϱ strongly depends on the initial substrate concentration. The error is largest when S_0/K_1 has some value between 1 and K_2/K_1, thus when S_0 has some value between K_1 and K_2. This is depicted more clearly in Fig. 2, where ϱ is presented as a function of S_0/K_1 at several values of K_2/K_1 and V_2/V_1, and a substrate consumption of 70 per cent.

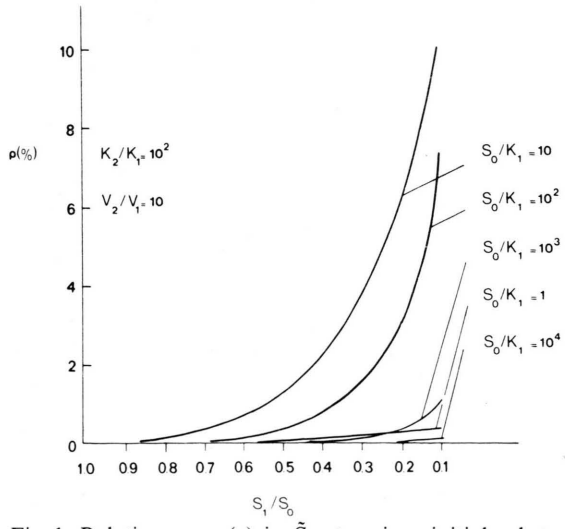

Fig. 1. Relative error (ϱ) in $\tilde{S}_x$ at various initial substrate concentration S (S_0/K_1) as a function of the progress of the reaction (S_1/S_0).

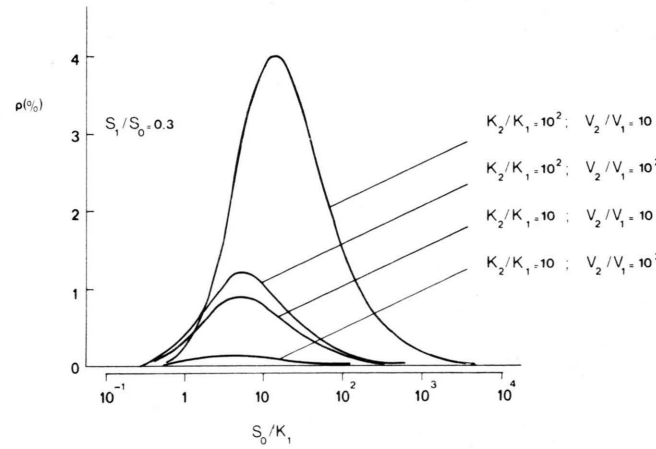

Fig. 2. Relative error (ϱ) in $\tilde{S}_x$ as a function of the initial substrate concentration (S_0/K_1) at 70 per cent substrate consumption ($S_1/S_0 = 0.3$) and various values of K_2/K_1 and V_2/V_1.

A detailed presentation of the dependence of ϱ on K_2/K_1 and V_2/V_1 at various initial substrate concentrations and 70 per cent substrate consumption is given in Fig. 3.

We investigated to what extent the values of the kinetic parameters to be estimated may be affected by the use of the approximate value $\tilde{S}_x$. As can be seen in Table II (data set (a)), deviations in the estimated parameters were less than 2 per cent in most cases, and never were larger than 5 per cent.

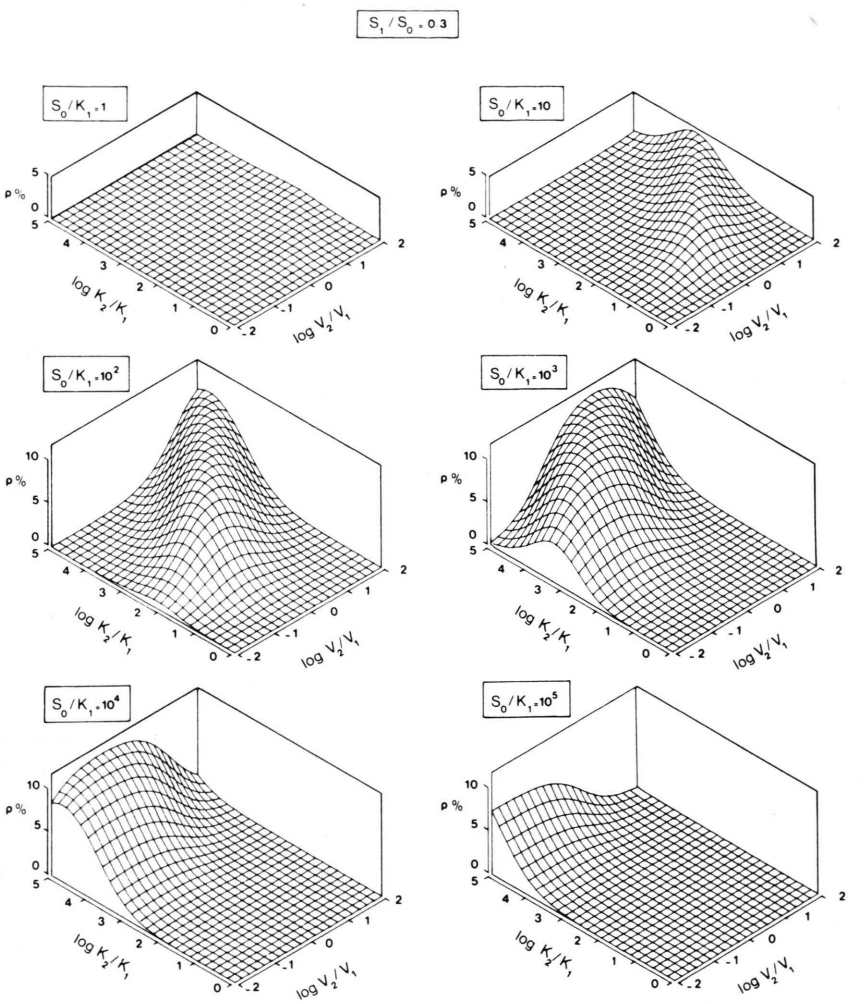

Fig. 3. Relative error (ϱ) in $\tilde{S}_x$ at 70 per cent substrate consumption ($S_1/S_0 = 0.3$) and various values of S_0/K_1, as a function of K_2/K_1 and V_2/V_1.

Parameter estimation may be disturbed, however, by 'experimental' error in the reaction rates. For example, a 5 per cent 'experimental' error resulted in poor estimation of the parameters K_1 and V_1 when $K_2/K_1 = 10$ and $V_2/V_1 = 10$ or $V_2/V_1 = 100$, or when $K_2/K_1 = 100$ and $V_2/V_1 = 100$. This was found for the data set of average reaction rates ($\bar{r}$) and the matching approximated values of substrate concentrations ($\tilde{S}_x$), as well as for the data set of initial substrate concentrations and reaction rates (Table II, data sets (b) and (c), respectively). The poor estimation of parameter-values in these cases, therefore, is not due to the use of $\tilde{S}_x$. As has been pointed out by Gross et al. [7] it results from the limitations of kinetic analysis at certain constellations of K_2/K_1, V_2/V_1, and the relative error in the data.

We conclude that the procedure described in this paper may be safely used to collect data from the determination of the parameters of the 'double' Michaelis-Menten equation. The restriction to be made is that during the assay the relationship between concentration and reaction rate should not change. Thus, enzyme reactions should be practically irreversible, and product inhibition or progressive denaturation of the enzyme should not occur. Similarly, in uptake experiments efflux should be negligible, and the influx may not be affected by transinhibition during the assay.

Table II. Deviations in the kinetic parameters due to the use of the approximate value $\tilde{S}_x = (S_0 - S_1)/\ln(S_0/S_1)$. The sum of two Michaelis-Menten terms was fitted to sets of simulated data, essentially as described by Cleland [8]. Each set consists of 25 data pairs; initial concentrations were spaced geometrically between $0.1\,K_1$ and $10\,K_2$. For each set of parameter-values, three data sets were composed: (a) Data were computed for 70 per cent substrate consumption. Reaction rates were equal to $\bar{r} = 0.7\,S_0/t$ (t being computed from Eqn. (3)), and substrate concentrations were equal to $\tilde{S}_x = (S_0 - S_1)/\ln(S_0/S_1)$; (b) data were obtained as in (a), but in addition a 5 per cent random error [*cf.* reference 18] was assigned to the values of the reaction rates; (c) data set of substrate concentrations and reaction rates at $t = 0$, with a 5 per cent random error in the reaction rates.

True parameter-values					Estimated parameter-values			
K_1	K_2	V_1	V_2		K_1	K_2	V_1	V_2
1	10	1	10	(a)	1.02	9.89	1.01	9.97
				(b)	0.64	8.48	0.53	10.2
				(c)	0.77	9.37	0.71	10.3
1	10	1	100	(a)	1.04	9.99	1.02	99.9
				(b)	1.06	11.1	1.25	104
				(c)	2.04	11.6	3.43	101
1	100	1	10	(a)	1.02	96.9	1.01	9.91
				(b)	0.97	101	0.94	9.86
				(c)	0.97	108	0.95	9.96
1	100	1	100	(a)	1.05	99.4	1.04	99.8
				(b)	0.55	89.3	0.60	95.8
				(c)	0.53	93.0	0.62	97.8
1	1000	1	10	(a)	1.01	955	1.01	9.78
				(b)	1.04	974	1.03	10.0
				(c)	1.02	1003	1.01	10.1
1	1000	1	100	(a)	1.03	983	1.03	99.4
				(b)	1.03	1030	1.06	101
				(c)	1.00	1029	1.04	100

Appendix

In case of two, simultaneously operating, Michaelis-Menten processes, the rate of change of the substrate concentration S is described by the equation

$$v = -\frac{dS}{dt} = V_1 S/(K_1 + S) + V_2 S/(K_2 + S). \quad (1)$$

This can be rewritten as

$$\frac{1}{v} = -\frac{dt}{dS} = \alpha + \beta/S + \gamma/(\delta + S) \quad (2)$$

where α, β, γ, δ are abbreviations for various combinations of K_1, K_2, V_1, and V_2:

$$\alpha = 1/(V_1 + V_2)$$

$$\beta = K_1 K_2/(K_1 V_2 + K_2 V_1)$$

$$\gamma = V_1 V_2 (K_1 - K_2)^2/((V_1 + V_2)^2 (K_1 V_2 + K_2 V_1))$$

$$\delta = (K_1 V_2 + K_2 V_1)/(V_1 + V_2).$$

The differential equation (2) can easily be solved, resulting in an expression describing t as a function of S (the more straightforward way of expressing S in terms of t being impossible in this case):

$$t = \alpha(S_0 - S) + \beta \ln(S_0/S) + \gamma \ln((\delta + S_0)/(\delta + S)) \quad (3)$$

where S_0, as usual, is the substrate concentration at $t = 0$. The average rate of change over the time interval $(0, t_1)$ is

$$\bar{r} = (S_0 - S_1)/t_1. \quad (4)$$

By means of Eqn. (3) t_1 can be expressed in S_1, resulting in

$$\frac{1}{\bar{r}} = t_1/(S_0 - S_1) = \alpha + (\beta/(S_0 - S_1)) \ln(S_0/S_1) + (\gamma/(S_0 - S_1)) \ln((\delta + S_0)/(\delta + S_1)). \quad (5)$$

Some time between 0 and t_1 there must be an instant x and a corresponding substrate concentration S_x at which the rate of change r_x equals the average rate of change $\bar{r}$. S_x can be solved from the equation $r_x = \bar{r}$, or, equivalently,

$$\frac{1}{r_x} = \frac{1}{\bar{r}}. \quad (6)$$

Equation (6) can be solved by inserting Eqn. (2) and Eqn. (5). This results in a quadratic equation in S_x:

$$\frac{1}{(S_0 - S_1)}\left(\beta \ln \frac{S_0}{S_1} + \gamma \ln \frac{\delta + S_0}{\delta + S_1}\right) S_x^2 \tag{7}$$
$$+ \left\{\frac{\delta}{S_0 - S_1}\left(\beta \ln \frac{S_0}{S_1} + \gamma \ln \frac{\delta + S_0}{\delta + S_1}\right) - \beta - \gamma\right\} S_x - \beta\delta = 0.$$

Analysis of the coefficients shows that there is always precisely one positive – in order words: physically meaningful – solution for S_x. Equation (7) can be rearranged in a quadratic equation in S_x/S_0:

$$\frac{A(\varphi + 1)}{(1 - \varepsilon)(\varphi + \varkappa)}\left(\frac{S_x}{S_0}\right)^2 \tag{8}$$
$$+ \left\{\frac{A}{\zeta(1 - \varepsilon)} - \frac{(\varphi\varkappa + 1)}{(\varphi + 1)}\right\}\left(\frac{S_x}{S_0}\right) - \frac{\varkappa}{\zeta} = 0$$

where $\varepsilon = S_1/S_0$; $\zeta = S_0/K_1$; $\varkappa = K_2/K_1$; $\varphi = V_2/V_1$ and

$$A = -\varkappa \ln \varepsilon + \frac{\varphi(1 - \varkappa)^2}{(\varphi + 1)^2} \ln \frac{\varphi + \varkappa + \zeta(\varphi + 1)}{\varphi + \varkappa + \varepsilon\zeta(\varphi + 1)}.$$

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