The Computation of Hyperbolic Dependences in Enzyme Kinetics

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(Received 29 January 1976)

A computer program aimed at analysing results following Michaelis–Menten kinetics can be used unmodified in the treatment of other kinetic results provided that the kinetic equations in these cases can be written in the form of the Michaelis–Menten equation. A list is presented of the parameters to be set instead of substrate concentration and reaction rate, and of constants replacing K_m and V , if such a program is applied in analysing enzyme inhibitions, activations and pH-dependences.

Although the theory of the simplest enzyme kinetic case, namely Michaelis–Menten kinetics, was developed more than 60 years ago, its analysis has been repeatedly studied up to very recent years. Eisenthal & Cornish-Bowden (1974) and de Miguel Merino (1974) proposed a method of analysing enzyme kinetic results, in which the constants K_m and V can be derived from the hyperbolic expression of the Michaelis–Menten equation. In addition, Eisenthal & Cornish-Bowden (1974) pointed out that this method of ‘direct linear plots’ is also applicable to other cases in which a hyperbolic relationship exists between a measured response and a controlled variable; inhibition, activation and multiple-substrate reactions are enumerated as examples by these authors.

The idea of estimating the values of K_m and V by the direct linear plot method can be applied also to a simple computer program, in which K_m and V (K_{IJ} and V_{IJ}) are first calculated for each pair of measurement points (s_i, v_i) and (s_j, v_j). The calculation of K_{IJ} is based on the normal Michaelis–Menten equations:

$$v_i = \frac{V}{1 + K_m/s_i} \quad \text{and} \quad v_j = \frac{V}{1 + K_m/s_j}$$

in which V will be set equal and K_m (K_{IJ}) will then be solved. The solutions are:

$$K_{IJ} = \frac{v_j - v_i}{v_i/s_i - v_j/s_j} \quad \text{and} \quad V_{IJ} = \frac{s_i - s_j}{s_i/v_i - s_j/v_j}$$

According to Cornish-Bowden & Eisenthal (1974) the median of the K_{IJ} values (or V_{IJ} values) represents the final K_m (V), and the median values are then chosen by the computer. In addition to K_m and V , the same computer program can be used unmodified in calculations of other constants in enzyme kinetics, if the kinetic equations can be written in the form of the normal Michaelis–Menten equation. The purpose of this present paper is to list the parameters to be substituted for s or v , and the derived constants replacing K_m or V , if the program is used to calculate inhibition, activation or ionization constants.

Theory

Table 1 presents the treatment of the most familiar cases of enzyme inhibition, activation and pH-dependences. The inhibitions can be described by the following scheme:

and it gives a rate equation (Cornish-Bowden, 1974):

$$v = \frac{V}{1 + \frac{i}{K'_i} + \frac{K_m}{s} \left(1 + \frac{i}{K_i}\right)}$$

The special cases of inhibition can be derived by setting, in case 1, $K'_i = K_i$, in case 2, $K'_i \rightarrow \infty$, and in case 3, $K_i \rightarrow \infty$. The activation situations dealt with in Table 1 were based on the scheme:

and described by the rate equations:

$$v = \frac{V/(1 + K'_a/a)}{1 + \frac{K_m}{s} \cdot \frac{(1 + a/K_a)}{(1 + a/K'_a)}} = \frac{V/(1 + K'_a/a)}{1 + \frac{K'_m}{s} \cdot \frac{(1 + K_a/a)}{(1 + K'_a/a)}}$$

The special cases of activation can be derived as in the inhibition cases, by setting, in case 1, $K'_a = K_a$ (A and S bound independently), in case 2, $K'_a = 0$ (ES non-existent, or A always bound before S), and in case 3, $K_a \rightarrow \infty$ (EA non-existent, or S bound before A). The cases of pH-dependence may be assumed to represent special cases of inhibition or activation with H^+ ion acting as an inhibitor or an activator. The four most

Table 1. Application of a computer program for K_m and V to various kinetic cases

Determination	Equation	Measurement	In input		In results	
			Instead of s	Instead of v	Instead of K_m	Instead of V
K_m and V	$v = \frac{V}{1 + K_m/s}$	$v = f(s)$	s	v	K_m	V
Inhibitions						
Case 1, non-competitive	$v = \frac{V/(1 + K_m/s)}{1 + \frac{1/K_1}{1 + 1/i}}$	$v = f(i)$	$1/i$	v	$1/K_1$	$\frac{V}{1 + K_m/s}$
Case 2, competitive*	$v = \frac{V/(1 + K_m/s)}{1 + \frac{1/(1 + s/K_m) K_1}{1/i}}$	$v = f(i)$	$1/i$	v	$\frac{1}{(1 + s/K_m) K_1} = 1/K_1^{app.}$	$\frac{V}{1 + K_m/s}$
	$1/K_1^{app.} = \frac{1/K_1}{1 + \frac{1/K_m}{1/s}}$	$K_1^{app.} = f(s)$	$1/s$	$1/K_1^{app.}$	$1/K_m$	$1/K_1$
Case 3, uncompetitive*	$v = \frac{V/(1 + K_m/s)}{1 + \frac{1/(1 + K_m/s) K_1'}{1/i}}$	$v = f(i)$	$1/i$	v	$\frac{1}{(1 + K_m/s) K_1'} = 1/K_1'^{app.}$	$\frac{V}{1 + K_m/s}$
	$1/K_1'^{app.} = \frac{1/K_1'}{1 + K_m/s}$	$K_m'^{app.} = f(s)$	s	$1/K_1'^{app.}$	K_m	$1/K_1'$
Activations						
Case 1	$v = \frac{V/(1 + K_m/s)}{1 + K_a/a}$	$v = f(a)$	a	v	$K_a' = K_a$	$\frac{V}{1 + K_m/s}$
Case 2†	$v = \frac{V/(1 + K_m/s)}{1 + \frac{K_a/(1 + s/K_m')}{a}}$	$v = f(a)$	a	v	$K_a/(1 + s/K_m') = K_a'^{app.}$	$\frac{V}{1 + K_m'/s}$
	$K_a'^{app.} = \frac{K_a}{1 + \frac{1/K_m}{1/s}}$	$K_a'^{app.} = f(s)$	$1/s$	$K_a'^{app.}$	$1/K_m'$	K_a
Case 3†	$v = \frac{V}{1 + \frac{K_a'(1 + K_m/s)}{a}}$	$v = f(a)$	a	v	$K_a'(1 + K_m/s) = K_a'^{app.}$	V
	$1/K_a'^{app.} = \frac{1/K_a'}{1 + K_m/s}$	$K_a'^{app.} = f(s)$	s	$1/K_a'^{app.}$	K_m	$1/K_a'$

pH-dependences

K_m^{app} is dependent on pH (a)§
(inhibition, case 2)†

$$1/K_m^{app} = \frac{1/K_m}{1 + \frac{1/K_H}{1/H}} \quad 1/K_H \quad 1/K_m^{app} \quad 1/K_H \quad 1/K_m$$

K_m^{app} is dependent on pH (b)§
(activation, case 2)†

$$1/K_m^{app} = \frac{1/K_m}{1 + K_H/H} \quad K_H \quad 1/K_m^{app} \quad K_H \quad 1/K_m$$

V^{app} is dependent on pH (a)§
(inhibition, case 1)

$$V^{app} = \frac{V}{1 + \frac{1/K_H}{1/H}} \quad 1/H \quad V^{app} \quad 1/K_H \quad V$$

V^{app} is dependent on pH (b)§
(activation, case 1)

$$V^{app} = \frac{V}{1 + K_H/H} \quad H \quad V^{app} \quad K_H \quad V$$

K_1^{app} is dependent on pH (c)*§

$$1/K_1^{app} = \frac{1/K_1}{1 + \frac{1/K_H}{1/H}} \quad 1/H \quad 1/K_1^{app} \quad 1/K_H \quad 1/K_1$$

K_1^{app} is dependent on pH (d)*§

$$1/K_1^{app} = \frac{1/K_1}{1 + K_H/H} \quad H \quad 1/K_1^{app} \quad K_H \quad 1/K_1$$

* K_1 (K_1') is the real inhibition constant, independent of substrate concentration or pH; K_1^{app} ($K_1'^{app}$) is the corresponding changing constant.

† K_s (K_s') is the activation constant independent of substrate concentration; K_s^{app} ($K_s'^{app}$) is dependent on the substrate concentration.

‡ K_m is independent of pH; K_m^{app} is dependent on pH.

§ (a) The enzyme is active on the basic side of the pK value; (b) the enzyme is active on the acidic side of the pK value; (c) the inhibitor is effective on the basic side of the pK value; (d) the inhibitor is effective on the acidic side of the pK value.

Table 2. Values of K_i and pK for the pH-dependent inhibition of pantothenase

The rate of the pantothenase-catalysed reaction was measured at various pH values and in the presence of various inhibitor concentrations (R. K. Airas, unpublished work). The buffers were either 20mM-potassium phosphate or 10mM-potassium *N*-morpholinopropane-3-sulphonate (Mops). The values calculated by linear regression were obtained by R. K. Airas (unpublished work), and the other values were calculated as suggested in Table 1 with a Wang 2200 mini computer using a program for K_m and V .

Inhibitor	Buffer	Values by linear regression		Values by K_m program	
		pK	K_i (mM)	pK	K_i (mM)
Oxaloacetate	Phosphate	6.39	0.36	6.49	0.41
Oxaloacetate	Mops	6.38	0.38	6.59	0.52
Oxalate	Phosphate	6.40	0.35	6.52	0.42
Oxalate	Mops	6.10	0.20	6.11	0.20

important of six possible cases of pH-dependence are presented in Table 1, with two additional cases in which K_i^{app} is dependent on pH.

In many cases two successive computations of parameters are needed, as seen from Table 1. In competitive inhibition, for instance, the values of K_i^{app} are first calculated; in the secondary calculation these values are used to determine the substrate-independent K_i . Corresponding two stages of treatment are naturally needed if the constants are determined by manual plotting.

The hyperbolic form of the Michaelis-Menten equation can be regarded as a basic expression, and therefore only the computation based on the hyperbolic form has been dealt with here. The insertions listed in Table 1 are, however, valid also if other types of programs, aimed at the calculation of K_m and V , are used, for example programs based on any one of the three linear expressions of the Michaelis-Menten equation.

Example

An example of the use of the above procedure is taken from studies (R. K. Airas, unpublished work)

performed in this laboratory. Pantothenase from *Pseudomonas fluorescens* has an ionizable group at the binding site of a non-competitive type inhibitor, and the inhibitor is effective at the acidic side of the pK value, the last case in Table 1. The treatment of the results consisted of calculating the pH-dependent K_i^{app} values, and then the K_i and pK values by the same program. The results are shown in Table 2, together with some earlier results calculated by linear regression from the expressions $1/v = f(i)$ and $K_i^{app} = f(1/H)$. The pK values tend to be about 0.1 pH unit higher by the direct linear plot computation; also, the K_i values are somewhat higher.

References

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