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ENZYME INHIBITION BY EXCESS SUBSTRATE

EXPLOITATION OF KINETIC DATA

I. LINEAR METHOD

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SUMMARY

A reduced form of the Michaelis–Menten equation for enzyme inhibition by excess substrate is described. An iterative procedure permits the precise determination of the optimal concentration. This value is needed to bring the equation to its reduced form. Finally, an application of this technique to experimental data (hydrolysis of acetylcholine by acetylcholinesterase, EC 3.1.17) is illustrated.

INTRODUCTION

Some enzymes are inhibited by excess substrate and among them the most studied has certainly been acetylcholinesterase. However, other enzymes, such as isocitrase[1] were recently reported to present this type of inhibition.

It does not appear that specific methods for the exploitation of experimental data of inhibition by excess substrate have been reported; most authors use extrapolation methods which are actually trial and error techniques and therefore do not allow a realistic estimate of the reliability of the thermodynamic parameters of the reaction.

Within the framework of the MICHAELIS–MENTEN theory of enzymic reactions, an inhibition by excess substrate is represented by the equation:

$$E + S \rightleftharpoons ES \tag{1a}$$

$$ES + S \rightleftharpoons ES_2 \tag{1b}$$

$$ES \quad \rightarrow E + P \tag{1c}$$

where the different symbols have their conventional meaning.

The kinetic equation corresponding to the chemical Eqns. 1a–c with HALDANE's[2] terminology, is

$$\frac{v}{ke} = \frac{1}{1 + \frac{K_1}{x} + \frac{x}{K_2}} \tag{2}$$

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where v is the initial velocity, K_1 and K_2 the Michaelis constants corresponding to Reactions 1a and 1b, k the velocity constant of Reaction 1c (generally assumed to be slow), e the concentration of the enzyme and x the concentration of the substrate.

Eqn. 2 is more generally written, in modern notation[3], as

$$\frac{v_{\max}}{v} = 1 + \frac{K_M}{s} + \frac{s}{K_{SS}} \tag{3}$$

with

$$v_{\max} \equiv ke,\ s \equiv x,\ K_M \equiv K_1,\ K_{SS} \equiv K_2$$

It will be noticed that $v_{\max}$ is not the optimal velocity of the system but the optimal velocity which the system would possess if there were no inhibition.

The purpose of this paper is to describe an efficient technique for obtaining from a set of experimental data (v as a function of s) the values of $v_{\max}$, K_M and K_{SS}, and to present examples of application. It will be shown that the determination of these parameters can be made with accuracy when the optimal concentration is known; the latter can be computed from the experimental data by way of a highly exploitative technique.

THEORY

$1/v$ considered as a function of s is minimal when s has the value

$$s_0 = \sqrt{K_M K_{SS}}$$

By the algebraic substitution

$$s = a s_0$$

Eqn. 3 takes the reduced form

$$\frac{v_{\max}}{v} = \sqrt{\frac{K_M}{K_{SS}}}\left(a + \frac{1}{a}\right) + 1 \tag{4}$$

Therefore a plot of $1/v$ *versus* $(a + 1/a)$ will give a straight line, the slope of which will be $\frac{1}{v_{\max}}\sqrt{\frac{K_M}{K_{SS}}}$ and the intercept $1/v_{\max}$. Such a representation can be easily worked out either by a graphical method or by a least-squares method.

However, the success of this method is largely dependant on the precision with which the optimal concentration is known. It is possible to use an iterative method of computation to estimate the best values of the parameters s_0, K_M, K_{SS}, and $v_{\max}$.

Let us consider the substitution

$$s = a(s_0 + \varepsilon)$$

It follows:

$$\frac{1}{v} = \frac{1}{v_{\max}}\left\{1 + \frac{1}{a}\sqrt{\frac{K_M}{K_{SS}}}\frac{1}{\left(a + \frac{\varepsilon}{\sqrt{K_M K_{SS}}}\right)} + a\sqrt{\frac{K_M}{K_{SS}}} + \frac{a\varepsilon}{K_{SS}}\right\} \tag{5}$$

In the case when $\varepsilon < s_0$, Eqn. 5 is not very much different from:

$$\frac{1}{v} = \frac{1}{v_{\max}} \left\{ 1 + \frac{1}{a} \sqrt{\frac{K_{\mathrm{M}}}{K_{\mathrm{SS}}}} \left(1 - \frac{\varepsilon}{\sqrt{K_{\mathrm{M}} K_{\mathrm{SS}}}} \right) + a \sqrt{\frac{K_{\mathrm{M}}}{K_{\mathrm{SS}}}} + \frac{a\varepsilon}{K_{\mathrm{SS}}} \right\}$$

and finally:

$$\frac{1}{v} = \frac{1}{v_{\max}} + \frac{1}{v_{\max}} \sqrt{\frac{K_{\mathrm{M}}}{K_{\mathrm{SS}}}} \left(a + \frac{1}{a} \right) + \frac{\varepsilon}{K_{\mathrm{SS}} v_{\max}} \left(a - \frac{1}{a} \right) \tag{6}$$

Eqn. 6 which becomes identical to Eqn. 4 when $\varepsilon = 0$, can be interpreted as a multiple linear regression of the dependant variable $1/v$ on the two independant variables $a + 1/a$ and $a - 1/a$. By solving Eqn. 6 an estimation of ε can be made and therefore a better estimation of s_0 can be obtained; the process can be repeated until two successive values of s_0 so obtained agree.

The computation can be made using standard techniques[4]. However, it is possible to greatly simplify the calculations; in the first steps of the computation, the main concern is obtaining the optimal concentration while the determination of the other parameters, especially the equilibrium constants, can be left for the last step.

Let us define:

$$p_1 = \sum \frac{1}{v} \left[\left(a + \frac{1}{a} \right) - \overline{\left(a + \frac{1}{a} \right)} \right]$$

$$p_2 = \sum \frac{1}{v} \left[\left(a - \frac{1}{a} \right) - \overline{\left(a - \frac{1}{a} \right)} \right]$$

$$t_{11} = \sum \left[\left(a + \frac{1}{a} \right) - \overline{\left(a + \frac{1}{a} \right)} \right]^2$$

$$t_{22} = \sum \left[\left(a - \frac{1}{a} \right) - \overline{\left(a - \frac{1}{a} \right)} \right]^2$$

$$t_{12} = \sum \left[\left(a + \frac{1}{a} \right) - \overline{\left(a + \frac{1}{a} \right)} \right] \left[\left(a - \frac{1}{a} \right) - \overline{\left(a - \frac{1}{a} \right)} \right]$$

Then

$$\frac{1}{v_{\max}} \sqrt{\frac{K_{\mathrm{M}}}{K_{\mathrm{SS}}}} = p_1 t^{11} + p_2 t^{12}$$

$$\frac{\varepsilon}{K_{\mathrm{SS}} v_{\max}} = p_1 t^{12} + p_2 t^{22}$$

where the t^{hi} are the elements of T^{-1}, the inverse of the matrix defined by

$$T = \begin{bmatrix} t_{11} & t_{12} \\ t_{12} & t_{22} \end{bmatrix}$$

We have

$$T^{-1} = \frac{1}{\det (T)} \begin{bmatrix} t_{22} & -t_{12} \\ -t_{12} & t_{11} \end{bmatrix}$$

Therefore

$$\frac{1}{v_{\max}} \sqrt{\frac{K_M}{K_{SS}}} = \frac{p_1 t_{22} - p_2 t_{12}}{\det (T)}$$

and

$$\frac{\varepsilon}{v_{\max} K_{SS}} = \frac{-p_1 t_{12} + p_2 t_{11}}{\det (T)}$$

It follows:

$$\frac{\dfrac{\varepsilon}{v_{\max} K_{SS}}}{\dfrac{1}{v_{\max}} \sqrt{\dfrac{K_M}{K_{SS}}}} = \frac{\varepsilon}{\sqrt{K_M K_{SS}}} = \frac{-p_1 t_{12} + p_2 t_{11}}{p_1 t_{22} - p_2 t_{12}}$$

or

$$\varepsilon = s_0 \frac{-p_1 t_{12} + p_2 t_{11}}{p_1 t_{22} - p_2 t_{12}}$$

Theoretically, at the last stage of computation, the different parameters can be obtained by a direct fitting to Eqn. 4, which enables one to obtain* the "best" values of $\frac{1}{v_{\max}}$ and $\frac{1}{v_{\max}} \sqrt{\frac{K_M}{K_{SS}}}$ finally $s_0 = \sqrt{K_M K_{SS}}$ is used with these relationships to obtain the constants K_M and K_{SS}. In practice, however, it is advisable to fit Eqn. 6; by this procedure, among other advantages, a check of the corrections of the added value of s_0 is provided.

EXAMPLE

As an example, this technique will be applied to the analysis of the hydrolysis of acetylcholine by Helix blood. The experimental data used are presented in Table I.

TABLE I

HYDROLYSIS OF ACETYLCHOLINE CHLORIDE BY HELIX BLOOD

Experimental data from AUGUSTINSSON[5]. The data, in arbitrary units, have been corrected for non-enzymic hydrolysis.

s	v
14.8	120
49.3	356
148	419
493	413
1479	324
4928	194

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* $\frac{1}{v_{\max}}$ is obtained from $\overline{\left(\frac{1}{v}\right)} = \frac{1}{v_{\max}} + \frac{p_1 t_{22} - p_2 t_{12}}{\det (T)} \overline{\left(a + \frac{1}{a}\right)} + \frac{-p_1 t_{12} + p_2 t_{11}}{\det (T)} \overline{\left(a - \frac{1}{a}\right)}$

TABLE II

ITERATIVE STEPS IN THE EXPLOITATION OF DATA OF TABLE I

In the column "s_0" are shown the adopted values for the fitting of Eqn. 6 and in the column "ε" the value of the corrective term deduced at the end of each step.

s_0	ε
150	−106
150 + 106 = 256	−84
256 + 84 = 340	−19
340 + 19 = 359	−0.5
359 + 0.5 = 359.5	0

If $s_0 = 150$ is chosen as a starting value, a value $\varepsilon = -106$ is deduced. Then a better value of s_0 is $150 - (-106) = 256$. The successive steps of the iteration are shown in Table II. It will be noticed that the convergence of the process is rapid but successive values of s_0 do not bracket the "best value" $s_0 = 359.5$.

The three following relations are then obtained:

$$v_{\text{max}} = 606; \quad \sqrt{\frac{K_M}{K_{SS}}} = 0.1576; \quad \sqrt{K_M K_{SS}} = 359.5$$

and the rate constants are (expressed in the arbitrary units of AUGUSTINSSON[5]):

$$K_M = 56.66; \quad K_{SS} = 2281$$

It will be noticed that, in practice, a starting value better than 150 could easily be obtained from a plot of *v versus s* or from a plot of *v versus* −log *s*. It is obvious that the better the starting value, the smaller the number of iterations required. However, the fitting of Eqn. 6 provides (i) another check of the fact that the value $s_0 = 359.5$ is the best value (in the numerical example here discussed $1/(v_{\text{max}}K_{SS})$ is of the order of $2 \cdot 10^{-8}$) and (ii) provides a better estimate of $1/v_{\text{max}}$ (here the difference between the estimates of $1/v_{\text{max}}$ obtained from Eqn. 4 and from Eqn. 6) is $6.6 \cdot 10^{-4}$, *i.e.* nearly negligible.

In certain cases, the successive values of s_0 obtained by the iterative process bracket the best value, *i.e.* the successive values of ε are alternatively positive and negative. In that case it could be advantageous to shorten the interval, taking for the next approximation of s_0 the middle of the bracketed interval, following a classical technique of numerical analysis.

DISCUSSION

The method which is proposed here has the advantage of computational simplicity because of the possibility of reduction of Eqn. 2 to Eqn. 4. Furthermore, it shares, with all iterative methods, the advantage of being self-correcting, at least within a reasonable range (the range defined by $\varepsilon < s_0$): if at any step of the computation an error is made, its influence is equivalent to a new start with a new value of s_0. The computation itself is easily performed with any desk calculator.

It will be noticed, however, that a certain doubt persists with respect to the validity of the use of a crude least-square method. The fitting of Eqn. 6 with the formula proposed implicitly assumes that the variance of $1/v$ is constant at all points

$$\left[\left(a+\frac{1}{a}\right), \left(a-\frac{1}{a}\right)\right]$$

Also, as in the case of the numerical example worked out here, the different concentrations have been prepared by dilution so that the errors of concentration are very likely not randomly distributed.

A straightforward least-squares method, even if not ideally suited, has been used, and appears to furnish sound results. As a matter of fact, it has been observed that this is often the case in statistics.

The use of the linear method seems appropriate either when the optimal concentration must be determined with accuracy or when this concentration is known precisely enough: in the latter case, the number of iterations necessary will obviously be decreased. It will be noticed that the fiducial limits of the different parameters can be determined by means of an adaptation of conventional techniques. However, a plot of $1/v$ *versus* $a + 1/a$ will in many cases be sufficient to estimate the reliability of the data and of the results.

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