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THE USE OF STEADY-STATE RATE EQUATIONS TO ANALYSE PROGRESS CURVE DATA

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Summary

Analysis of progress curves for enzyme-catalyzed reactions has been made by using a procedure that does not require the derivation of complex integrated rate equations. The method involves conversion of progress curve data to reaction velocities that are then fitted to the appropriate differential rate equation. Application of the procedure to data obtained for the reaction catalyzed by aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1), showed that the resulting values for the kinetic parameters agreed well with those obtained by conventional progress curve analysis (Duggleby, R.G. and Morrison, J.F. (1978) Biochim. Biophys. Acta 526, 398—409).

Introduction

Previous reports from this laboratory [1,2] have demonstrated the value of using progress curve data for elucidating the kinetic mechanism of an enzyme-catalyzed reaction and for determining values of the kinetic parameters associated with the reactants. The principles involved in the analysis of progress curve data are straightforward, but the integrated rate equation for any particular kinetic mechanism is considerably more complex than the corresponding differential rate equation. Thus the algebraic manipulation required in connection with the preparation of a computer program for making a least-squares fit of progress curve data to an assumed integrated rate equation can be both complex and tedious.

It occurred to us that the analysis of progress curve data could be greatly simplified by transforming such data into steady-state velocity data which could be fitted to appropriate differential rate equations by procedures that are now very familiar to enzyme kineticists. This paper reports the successful application of the technique to progress curve data for the reaction catalyzed by aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1). The resulting values for the kinetic parameters are in good agreement with those determined by fitting the data to the appropriate integrated rate equation [2].

Theory

The progress curve for almost any irreversible, enzyme-catalyzed reaction involving one substrate and two products is described (cf. Appendix) by the general equation:

$$R_1 t = z + \frac{R_2 z^2}{2} - R_3 \ln\left(1 - \frac{z}{A_0}\right) \tag{1}$$

where z is the amount of product formed by reaction and A_0 is the initial concentration of substrate, while R_1, R_2 and R_3 are parameters which depend on the kinetic constants for, and initial concentrations of, other reaction components. Eqn. 1 also describes progress curves for two-substrate reversible reactions provided that the concentration of one substrate remains virtually constant either because it is present at a relatively high concentration [1] or through recycling of the corresponding product [2]. The data from individual progress curves may be fitted to Eqn. 1 using the PROCURA computer program and values, together with their standard errors, obtained for each of the R parameters. The velocity (v) at any point along the progress curve may then be calculated by using the differential form of Eqn. 1 which is given as Eqn. 2:

$$\frac{\mathrm{d}z}{\mathrm{d}t} = v = \frac{R_1}{1 + R_2 z + R_3/(A_0 - z)} \tag{2}$$

The variance of any velocity value is obtained from the relationship:

$$\mathrm{var}(v) = x^T \cdot [s^2 (X^T \cdot X)^{-1}] \cdot x \tag{3}$$

where $s^2(X^T \cdot X)^{-1}$ is the variance-covariance matrix, x is the vector of partial derivatives with respect to the parameters ($\mathrm{d}v/\mathrm{d}R$) evaluated at the chosen point and x^T is the transform of x.

The general procedure adopted for analysis of the progress curve data was to fit the data for each progress curve to Eqn. 1 and to use the resulting values for R_1, R_2 and R_3 to calculate velocities at three points along each curve. (A progress curve experiment would consist of several progress curves obtained at different concentrations of substrate and product, cf. Ref. 2). The points for calculation of the velocities corresponded to the first and last time interval at which a datum point was collected as well as the mean of these two time intervals. Weighting factors corresponding to the reciprocals of var(v) were calculated using Eqn. 3. They were then scaled so that the relative weights for the three velocities from a single progress curve were proportional to the weighting factors while the sum of the three weights for each of the progress curves was proportional to $1/A_0$. The concentration of substrate and product were calculated from their initial concentrations by addition or subtraction, as appropriate, of the amount of product formed by reaction at the selected time. Finally, the data were fitted to the appropriate rate equation using the method of Marquardt [3.] The fitting was performed by using a modification of the MARQ program which was written by L.W. Jackson and J.P. Chandler of Oklahoma State University and which was obtained as part of the STEPT package (QCPE 307) from the Quantum Chemistry Program Exchange at Indiana University. This is a general program for which the user need supply only the equation to which the data are to be fitted. The partial derivatives of the function with respect to each of the parameters are calculated by the program through the use of numerical methods of differentiation.

Results

The value of the procedure for analyzing progress curve data, as outlined in 'Theory', was tested by comparing the results so obtained with those obtained by use of the integrated form of the same rate equation. For this purpose, use was made of three sets of data for the asparate aminotransferase reaction that had been analyzed previously by fitting to the integrated form of Eqn. 4. Velocity data were calculated as described in 'Theory' and then fitted to Eqn. 4.

$$v = \frac{V}{1 + \frac{K_{\mathrm{oaa}}}{[\mathrm{oaa}]} + \frac{K_{\mathrm{Glu}}}{[\mathrm{Glu}]}\left[1 + \frac{[\mathrm{Asp}]}{K_{\mathrm{i(asp)}}}\left(1 + \frac{K_{\mathrm{i(oaa)}}}{[\mathrm{oaa}]}\right)\right]} \tag{4}$$

In this equation, oaa, Glu and Asp represent the concentrations of oxaloacetate, glutamate and aspartate, respectively; K_{oaa} and K_{Glu} denote Michaelis constants for oxaloacetate and glutamate, respectively; $K_{\mathrm{i(oaa)}}$ and $K_{\mathrm{i(Asp)}}$

TABLE I

KINETIC CONSTANTS ASSOCIATED WITH ASPARTATE AMINOTRANSFERASE

Three separate progress curve experiments were performed as described previously [2]. The data from each progress curve were compressed into three (velocity, concentraion data points as described in Theory and the compressed data for each experiment were fitted to Eqn. 4. Weighting factors were determined as described by Eqn. 3 and scaled as elaborated in the text. Values (θ) and standard errors (S.E. (θ)) for each of the five kinetic parameters which result from this analysis were used to calculate weighted means ($\overline{\theta}$) from the relationship $\overline{\theta} = \Sigma w\theta/\Sigma w$, where $w = (1/\mathrm{S.E.}\ (\theta))^2$.

Kinetic constant	Value ± S.E.			Weighted mean	
	Exp. 1	Exp.2	Exp. 3	This analysis	Previous work [2]
V(U/mg)	720 ± 31	790 ± 48	782 ± 43	751	744
K_{oaa} (μM)	35.8 ± 2.9	44.6 ± 3.6	46.0 ± 3.3	41.4	43.0
$K_{\mathrm{i(oaa)}}$ (μM)	28.1 ± 9.3	22.0 ± 5.1	21.7 ± 4.2	22.5	25.3
K_{Glu} (mM)	7.19 ± 0.50	10.23 ± 1.04	11.08 ± 1.00	8.31	8.98
$K_{\mathrm{i(Asp)}}$ (mM)	3.96 ± 0.50	4.09 ± 0.39	4.13 ± 0.34	4.08	3.94

represent inhibition constants for oxaloacetate and aspartate, respectively; V denotes the maximum velocity of the reaction. The values obtained for the kinetic parameters, together with values for their standard errors, are listed in Table I. There was good agreement between the values obtained in the three experiments and further, the weighted mean value for each parameter was similar to that obtained by conventional progress curve analysis.

Discussion

Progress curve studies have many advantages over steady-state velocity studies for kinetic investigations of enzyme-catalyzed reactions when the enzyme and reactants are stable under the experimental conditions [2]. However, the algebra associated with the analysis of progress curve data by non-linear regression is sufficiently complex to make the procedure appear less attractive. The differential rate equation for a particular kinetic mechanism must be integrated with respect to time and the resulting equation then differentiated with respect to each of the parameters so as to obtain the partial derivatives. Further, since integrated equations do not, in general, lead to explicit solutions for the amount of product (z) formed as a function of time, this quantity must be calculated by an iterative root-finding method such as the Newton-Raphson procedure. Application of this procedure requires that the integrated rate equation be differentiated with respect to z. Although a set of rules has been elaborated to simplify the algebraic manipulations [1], the task of deriving the required equations for the analysis of data conforming to various kinetic mechanisms still remains quite formidable.

The approach outlined in the present report circumvents the algebraic difficulties that arise with the use of particular integrated rate equations while it retains the advantages associated with the collection of progress curve data. Thus progress curve data have been converted to steady-state velocity data that can be fitted to differential rate equations by standard procedures. It has been pointed out previously [2] that the data from a single progress curve will show correlations and that this property must be compensated for, through the use of a data compression technique. Such a procedure involves the fitting to Eqn. 1 of data from a single progress curve and the subsequent calculation of three idealized (z, t) data points which contain all the essential information about the shape of the curve. The same general method has been applied in the present study, but instead of calculating idealized (z, t) data points which are then fitted to an integrated rate equation, idealized (velocity, concentration) points have been calculated in a similar manner and fitted to a differential rate equation.

It would be expected that the new procedure and conventional progress curve analysis would yield similar values for the kinetic parameters and this expectation was fulfilled (Table I). The new procedure must be considered as the method of choice because of the relative simplicity of the analysis. Progress curve data can be readily analyzed by means of the PROCURA program to give parameter values for use in connection with the calculation of steady-state velocity data. The latter can then be fitted to the appropriate differential equation(s) by well established procedures. If the MARQ or a similar program is used, the need to derive partial derivatives is avoided as only the differential rate equation has to be supplied. It should be noted that the program of Cleland [4] for analysis of competitive inhibition data can be used as the means of analyzing calculated velocity data for a Uni-Uni reaction. However, none of his other programs has been designed to analyze velocity data that are obtained in the presence of two or more substrates and products. In the present work, no difficulty was encountered in fitting data to Eqn. 4 which involves the concentrations of two substrates and one product and which requires five parameters to be estimated. The same general approach can be used in connection with model discrimination [2].

There is some similarity between the approach used in the present procedure and that developed by Bizzozero et al. [5] for studying enzyme kinetic mechanisms through the use of progress curve data. These authors calculated rates at 20—30 reference points along a progress curve by fitting the data from small sections of the curve to a low-order polynomial in time. The use of a polynomial to give an approximate description of a limited section of a progress curve is certainly less satisfactory than the use of an equation that exactly describes the complete progress curve. Further, the calculation and analysis of 20—30 velocity data points from a single curve implies that the information content of the curve is high. However, examination of Eqn. 1 reveals that a progress curve for an irreversible reaction can contain only sufficient information for three data points when the correlations within a curve are taken into account [2].

Appendix

The rate equation for an irreversible enzyme-catalyzed reaction involving one substrate and two products is of a form which is no more complex than Eqn. 5, where ϕ_0—ϕ_5 are collections

$$v = \frac{E_t}{\phi_0 + \phi_1 P + \phi_2/A + \phi_3 P/A + \phi_4 Q/A + \phi_5 PQ/A} \tag{5}$$

of rate constants. Provided that the enzyme is stable under the conditions chosen for assay, then the rate at the point in a progress curve where an amount z of reaction has occurred may be found by substituting $A = A_0 - z$, $P = P_0 + z$ and $Q = Q_0 + z$. Upon rearrangement we get Eqn. 6.

$$\begin{aligned} E_t/v = {} & \phi_0 + \phi_1 P_0 + \phi_1 z + [\phi_2 + \phi_3 P_0 + \phi_4 Q_0 + \phi_5 P_0 Q_0]/(A_0 - z) \\ & + [\phi_3 + \phi_4 + \phi_5(P_0 + Q_0)]z/(A_0 - z) + \phi_5 z^2/(A_0 - z) \end{aligned} \tag{6}$$

Using the equalities:

$$\frac{z}{A_0 - z} = \frac{A_0}{A_0 - z} - 1 \tag{7}$$

and

$$\frac{z^2}{A_0 - z} = \frac{A_0^2}{A_0 - z} - A_0 - z \tag{8}$$

We may rewrite Eqn. 6 to give Eqn. 9

$$E_t/v = \gamma_1 + \gamma_2 z + \gamma_3/(A_0 - z) \qquad (9)$$

where

$$\gamma_1 = \phi_0 + P_0(\phi_1 - \phi_5) - \phi_3 - \phi_4 - \phi_5(A_0 + Q_0)$$

$$\gamma_2 = \phi_1 - \phi_5$$

$$\gamma_3 = \phi_2 + \phi_3(A_0 + P_0) + \phi_4(A_0 + Q_0) + \phi_5(A_0 + P_0)(A_0 + Q_0)$$

Finally, division by γ_1 gives an equation of the form of Eqn. 2 where $R_1 = E_t/\gamma_1$, $R_2 = \gamma_2/\gamma_1$ and $R_3 = \gamma_3/\gamma_1$. Integration of Eqn. 2 then yields Eqn. 1.

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