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Parameter estimation using a direct solution of the integrated Michaelis-Menten equation

Chetan T. Goudar [a], Jagadeesh R. Sonnad [b], Ronald G. Duggleby [c,*]

[a] *School of Civil Engineering and Environmental Science, University of Oklahoma, Norman, OK 73019, USA*
[b] *Department of Radiological Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190, USA*
[c] *Centre for Protein Structure, Function and Engineering, Department of Biochemistry, University of Queensland, Brisbane, QLD 4072, Australia*

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Abstract

A novel method of estimating enzyme kinetic parameters is presented using the Lambert ω function coupled with non-linear regression. Explicit expressions for the substrate and product concentrations in the integrated Michaelis-Menten equation were obtained using the ω function which simplified kinetic parameter estimation as root-solving and numerical integration of the Michaelis-Menten equation were avoided. The ω function was highly accurate in describing the substrate and product concentrations in the integrated Michaelis-Menten equation with an accuracy of the order of 10^{-16} when double precision arithmetic was used. Progress curve data from five different experimental systems were used to demonstrate the suitability of the ω function for kinetic parameter estimation. In all cases, the kinetic parameters obtained using the ω function were almost identical to those obtained using the conventional root-solving technique. The availability of highly efficient algorithms makes the computation of ω simpler than root-solving or numerical integration. The accuracy and simplicity of the ω function approach make it an attractive alternative for parameter estimation in enzyme kinetics.

Keywords: Enzyme kinetics; ω function; Parameter estimation; Progress curve analysis

1. Introduction

Early work on the kinetics of enzyme-catalyzed reactions (e.g. [1]), drawing on similar studies of non-enzymic catalysis, involved measurements of the entire time course of the reaction. In principle, the variation of substrate ($[A]_t$) or product ($[P]_t$) concentration as a function of time (t) can provide information on the maximum velocity (V_m) and Michaelis constant (K_a) from a single progress curve. A change of emphasis away from progress curves was brought about by the seminal work of Michaelis and Menten [2] who focused attention on the variation of rate with substrate concentration (1).

$$d[A]_t/dt = -V_m[A]_t/(K_a + [A]_t) \quad (1)$$

The main difficulty in using progress curve analysis can be illustrated using the simplest case of a stable enzyme that catalyzes an irreversible one-substrate reaction for which the products are non-inhibitory.

* Corresponding author. Fax: +61 (7) 33654699; E-mail: duggleby@biosci.uq.edu.au

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The progress curve equation for this situation is given as Eq. 2.

$$V_m t = ([A]_0 - [A]_t) - K_a \ln([A]_t/[A]_0) \quad (2)$$

There are some obvious ways (see [3]) to use this equation to determine V_m and K_a; for example, plotting $([A]_0-[A]_t)/t$ versus $\ln([A]_t/[A]_0)/t$ will yield a straight line with a slope of K_a and an ordinate intercept of V_m. However, such an analysis ignores the reality that the variable that will have associated experimental error is $[A]_t$ so the linear transformation will distort these errors leading to possible bias in the estimates of V_m and K_a.

There are three main ways to overcome this difficulty. One, proposed by Cornish-Bowden [4] and adapted from his 'direct linear plot' [5], is to solve two simultaneous equations for every possible ($[A]_t$, t) pair. From the multiple values of V_m and K_a obtained, the median values can be calculated. This method has not been used widely and the author points out that its main value is to provide parameter estimates as vehicles for calculating initial velocities.

The second approach is to numerically integrate Eq. 1 using any of several algorithms for the numerical solution of differential equations. This method has been used [6,7] but it is computationally intensive and therefore relatively slow.

The third approach is to solve Eq. 2 for $[A]_t$ but until recently there was no general algebraic method for doing so. Consequently, numerical solutions were employed such as the Newton-Raphson method, first applied to this problem by Fernley [8]. Since then, a variety of other variations of root-finding methods having been applied to Eq. 2 (reviewed in [9]). In all cases, a series of successive approximations are made until a value of $[A]_t$ is found that comes close to satisfying Eq. 2; the methods only differ in how one approximation is calculated from its predecessor.

In 1997, Schnell and Mendoza [10] proposed a closed solution of Eq. 2, which is formulated here as Eq. 3. In this equation, ω represents Lambert's function defined as the solution to the transcendental equation given as Eq. 4.

$$[A]_t = K_a \omega[([A]_0/K_a)\exp([A]_0/K_a - V_m t/K_a)] \quad (3)$$

$$\omega(x)\exp(\omega(x)) = x \quad (4)$$

At first sight, this appears to offer no advantage since the problem now becomes one of finding a value of $\omega(x)$ that satisfies Eq. 4 for any given x. However, efficient and highly accurate algorithms have been published to solve Eq. 4 [11–13] making the use of Eq. 3 of practical value in analyzing progress curves of enzyme-catalyzed reactions.

Here we illustrate the procedure and apply it to the non-linear regression analysis of some experimental progress curves.

2. Theory

Given the implicit nature of Eq. 2, the substrate concentration at any given time cannot be directly estimated and has to be numerically approximated. However, this can be avoided if Eq. 2 can be rewritten such that the resulting expression is explicit in $[A]_t$. First, Eq. 2 is rearranged as:

$$[A]_t + K_a \ln([A]_t) = [A]_0 - V_m t + K_a \ln([A]_0) \quad (5)$$

Substituting $\phi = [A]_t/K_a$ in Eq. 5 and simplifying results in:

$$\phi + \ln \phi = [A]_0/K_a + \ln([A]_0/K_a) - V_m t/K_a \quad (6)$$

Eq. 6 is now of the form ω+ln ω and hence an expression for ϕ can be obtained from Eq. 4 as:

$$\phi = \omega\{([A]_0/K_a)\exp\{([A]_0 - V_m t)/K_a\}\} \quad (7)$$

Substituting for ϕ in terms of $[A]_t$ and K_a results in Eq. 3. If it is desired to calculate the product concentration, this may be obtained from Eq. 8.

$$[P]_t = [P]_0 + [A]_0 - K_a \omega\{([A]_0/K_a)\exp\{([A]_0 - V_m t)/K_a\}\} \quad (8)$$

It could be argued that Eq. 3 and Eq. 8 are of little relevance to most real systems as they apply only to enzymes that catalyze reactions in which a single substrate is converted irreversibly to a single, non-inhibitory product. The progress curves of real enzymes usually obey more complex equations than Eq. 2 but experimental conditions can sometimes be manipulated so that this is a very good approximation. Alternatively, Eq. 2 can often be applied provided it is acknowledged that for any given progress curve, V_m and K_a are apparent values (here denoted V_m' and K_a'). For example, a one-substrate,

one-product, reversible reaction can be described by a simple variation of Eq. 2 that leads to the closed form solution given as Eq. 9.

$$[A]_t = K_a'\omega[(\Delta[P]_\infty/K_a')\exp(\Delta[P]_\infty/K_a' - V_m't/K_a')] \quad (9)$$

where

$$\Delta[P]_\infty = (V_f[A]_0/K_f - V_r[P]_0/K_r)/(V_f/K_f + V_r/K_r)$$

$$V_m' = (V_f/K_f + V_r/K_r)/(1/K_f - 1/K_r)$$

$$K_m' = (1 + [A]_0/K_f + [P]_0/K_r)/(1/K_f - 1/K_r) - \Delta[P]_\infty$$

and V_f, K_f, V_r and K_r represent the maximum velocities and Michaelis constants in the forward and reverse directions, respectively.

The value of K_m' depends on the initial concentration of the substrate, and the product if any is added. Thus, by combining data from several progress curves obtained under different starting conditions it is possible to estimate all of the kinetic parameters.

An interesting feature of these relationships is that V_m' and K_m' can take negative values if $K_f > K_r$, although they must both have the same sign. This is not just an abstract possibility and later we will present data for two real examples. It will be evident from Eq. 9 that ω must also be negative and to understand the implications of this, we must examine the form of the ω function (Fig. 1).

The ω function consists of three regions. In the first (Fig. 1, broken line) both ω and x are positive, corresponding to positive values of V_m' and K_m'. However, in the second (solid line) and third (dotted line) regions, both ω and x are negative and there are two solutions on either side of $\omega = -1$, that is, values of ω which satisfy the function for any given x. Thus, we need to know which of these solutions is the appropriate one. Eq. 9 can be differentiated to obtain:

$$d[A]_t/dt = \{(\Delta[P]_\infty/\exp(\omega))\exp\{(\Delta[P]_\infty - V_m't)/K_a'\}\}$$
$$\{-V_m'/K_a'(1+\omega)\} \quad (10)$$

For reactions where the initial conditions are to the left of the equilibrium position, the change in the concentration of P to reach equilibrium ($\Delta[P]_\infty$) is positive and the first term in Eq. 10 is always positive irrespective of the values of V_m', K_a', t and ω. Thus, the second term has to be negative in order to be consistent with the fact that the substrate concentration declines with time. As V_m' and K_a' are either both positive or negative, this implies that (1+ω) must be greater than 0 and hence ω must be greater than −1. Thus the required solution is either from region 1 (Fig. 1, broken line) or region 2 (solid line) but never from region 3 (dotted line).

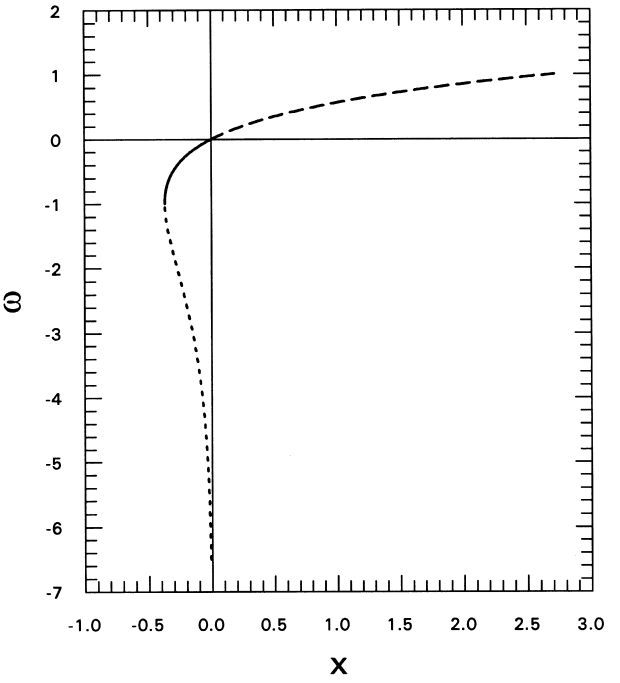

Fig. 1. Profile of the three real branches of the ω function for corresponding values of x. Region 1 (— — —) $x > 0$; region 2 (——) $-1/e < x < 0$ and $0 > \omega > -1$; region 3 (···) $-1/e < x < 0$ and $\omega < -1$.

3. Materials and methods

3.1. Experimental procedures

3.1.1. Pyruvate kinase

The progress curves for pyruvate kinase have been described previously [14]. Although the reaction catalyzed by pyruvate kinase has two substrates and two products, by appropriate selection of experimental conditions it behaves in progress curve experiments as if it were a single substrate, irreversible

reaction [15]. These conditions include coupling with lactate dehydrogenase to remove pyruvate and using a high MgADP concentration which remains effectively constant and also prevents product inhibition by accumulating MgATP. Reaction mixtures contained 5.6 mM ADP, 11 mM $MgCl_2$, 22 mM KCl, and 10 IU lactate dehydrogenase in 0.1 M Tris-HCl buffer, pH 8.0. Phospho*enol*pyruvate was used at concentrations between 93 and 185 μM and sufficient NADH was added to ensure that there was an excess over phospho*enol*pyruvate of between 60 and 120 μM. After equilibration at 30°C, the reaction was started by adding pyruvate kinase. After a set period of incubation, the reaction was stopped by adding 0.3 ml of 5% sodium dodecyl sulfate and the absorbance at 340 nm was determined. Ten identical reaction mixtures were prepared for each progress curve and the reaction stopped after different times.

3.1.2. Prephenate dehydratase and acid phosphatase

These two enzymes catalyze irreversible reactions showing significant product inhibition. The data are as described by Duggleby and Morrison [16]. Prephenate dehydratase was assayed at 37°C and pH 7.5 in a mixed buffer containing 0.1 M imidazole, 0.054 M *N*-tris[hydroxymethyl]methyl-2-ethanesulfonic acid, 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol and 0.1 mg/ml bovine serum albumin. Prephenate was added at one of five concentrations over the range 0.225–2.7 mM and, after initiation of the reaction by enzyme addition, nine sequential samples were removed at intervals. The reaction was stopped by immediate addition of NaOH at a final concentration of 0.7 M and the product (phenylpyruvate) was estimated from its absorbance at 320 nm. Acid phosphatase was assayed at 25°C and pH 7.2 in 0.1 M 3-[*N*-morpholino]propanesulfonic acid/0.1 M NaCl using 0.988–2.964 mM *p*-nitrophenyl phosphate as the substrate. All reactions contained small amounts of phosphate that contaminates the substrate (approx. 1%) and some reactions contained additional phosphate at concentrations up to 0.7292 mM. The reaction was initiated by enzyme addition and the formation of the *p*-nitrophenolate ion was followed at 478 nm. The data consist of eight progress curves each containing between 24 and 36 measurements.

3.1.3. Fumarase

Fumarase catalyzes a readily reversible reaction with an equilibrium constant of approx. 4 in the direction fumarate → malate. The progress curves were collected at 30°C and pH 7.5 in a mixed buffer of 50 mM Tris/acetate and 25 mM sodium phosphate, as described by Duggleby and Nash [17]. Reactions were started by adding the enzyme to solutions of fumarate (10 curves containing 0.050–0.744 mM) or malate (nine curves containing 0.199–2.472 mM). The reaction was followed by monitoring the change in absorbance at 240 nm due to fumarate, collecting 17–19 observations for each progress curve.

3.2. Data analysis

3.2.1. ω function

An algorithm for computing ω for positive values of x was first proposed by Fritsch et al. [11]. However, as noted earlier, negative values of x can arise experimentally. More recently, Barry et al. [12,13] have obtained a more general solution for all values of x that will yield real values of ω (i.e. $x \geq -1/e$) and we have used this algorithm. The FORTRAN source code was obtained from the Netlib web site (URL http://www.netlib.org/toms/743).

3.2.2. Non-linear regression

Data analysis was performed using the BASIC general non-linear regression program, DNRP53 [18] or MATLAB 5.0. In both cases, the theoretical product formation data were calculated from the closed form solution of the integrated Michaelis-Menten equation (9) and the resulting weighted sum of squares of the differences between the theoretical and experimental product concentrations was minimized by the Levenberg-Marquardt method [19].

4. Results

The accuracy of the ω function in describing the product concentration was tested as follows. Assuming $[A]_0 = K_a = 1$ and $V_m = 1$, 999 values of t were computed using Eq. 2 with $[P]_t = ([A]_0 - [A]_t)$ varying from 0.001 $[A]_0$ to 0.999 $[A]_0$ in steps of 0.001. These t values were subsequently used in Eq. 8 to compute

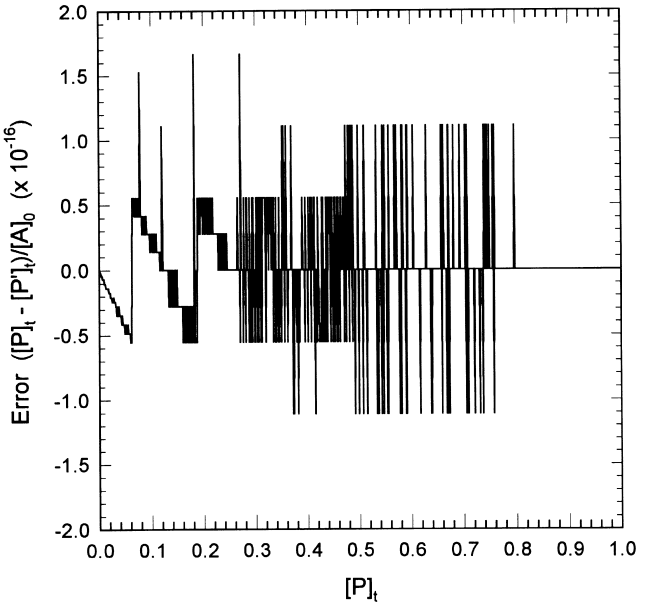

Fig. 2. Plot of the relative error in product concentration versus actual product concentration. $[P]_t$ denotes the actual product concentration while $[P']_t$ is the product concentration computed using the ω function.

the values of the product concentration (denoted $[P']_t$) using the ω function. From these two sets of product concentration data, the relative error was computed. A plot of the relative error versus actual product concentration is shown in Fig. 2. The ω function was able to accurately predict the product concentration in Eq. 8 and the maximum error was of the order of 10^{-10} ppm. Given that experimental determination of $[P]_t$ is unlikely to be accurate to much more than 100 ppm, the inaccuracies in estimating the ω function are of no practical consequence.

Table 1
Comparison of kinetic parameters for pyruvate kinase obtained using the Lambert ω function and root-solving using the Newton-Raphson method[a]

[PEP] (μM)	V_m (U/mg)	K_a (μM)
93.0	307.9 ± 25.5	44.29 ± 8.17
	(307.9 ± 25.5)	(44.29 ± 8.17)
122.2	388.9 ± 16.4	39.90 ± 4.60
	(388.9 ± 16.4)	(39.90 ± 4.60)
142.1	293.3 ± 7.6	41.86 ± 3.16
	(293.3 ± 7.5)	(41.85 ± 3.15)
157.7	324.0 ± 20.6	43.63 ± 8.41
	(324.0 ± 20.6)	(43.64 ± 8.41)
185.9	383.6 ± 16.9	39.55 ± 6.26
	(383.5 ± 16.9)	(39.54 ± 6.25)

[a]Numbers in parentheses denote parameter estimates obtained by root-solving using the Newton-Raphson method. Each curve consisted of 10 experimental points.

The use of the ω function for estimating V_m and K_a in the integrated Michaelis-Menten equation was demonstrated using progress curve data from five experimental systems. Pyruvate kinase represents a simple test of the procedure because, under the conditions employed, each progress curve obeys Eq. 2 and the values of V_m and K_a are true values, independent of the substrate concentration. Prephenate dehydratase and acid phosphatase represent the next level of complexity; each reaction is irreversible but shows product inhibition. The two differ in the

Table 2
Comparison of kinetic parameters obtained using the Lambert ω function and root-solving using the Newton-Raphson method[a]

Experiment	No. of data points	V_f (U/mg)	V_r (U/mg)	K_f (mM)	K_r (mM)
Prephenate dehydratase	45	18.01 ± 0.19	N/A	0.4718 ± 0.0082	4.590 ± 0.618
		(18.0 ± 0.2)		(0.472 ± 0.008)	(4.59 ± 0.62)
Acid phosphatase	224	117.0 ± 8.3	N/A	2.116 ± 0.201	0.2624 ± 0.0109
		(117 ± 8)		(2.12 ± 0.20)	(0.262 ± 0.011)
Fumarase (fumarate as substrate)	183	531.4 ± 19.0	347.8 ± 136.6	0.3113 ± 0.0176	0.8243 ± 0.2451
		(531.5 ± 19.0)	(347.4 ± 136.6)	(0.3114 ± 0.0177)	(0.8237 ± 0.2454)
Fumarase (malate as substrate)	163	592.1 ± 49.9	457.2 ± 145.2	1.221 ± 0.147	0.2702 ± 0.0739
		(591.8 ± 50.0)	(457.5 ± 146.7)	(1.221 ± 0.149)	(0.2704 ± 0.0746)

[a]Numbers in parentheses denote parameter estimates obtained by root-solving using the Newton-Raphson method, as published previously for prephenate dehydratase [16], acid phosphatase [16] and fumarase [17]. V_f, K_f, V_r and K_r represent the maximum velocity and Michaelis constant in the forward and reverse directions. For irreversible reactions, V_r is not relevant while V_f, K_f and K_r, respectively, correspond to V_m, K_a and the product inhibition constant K_p.

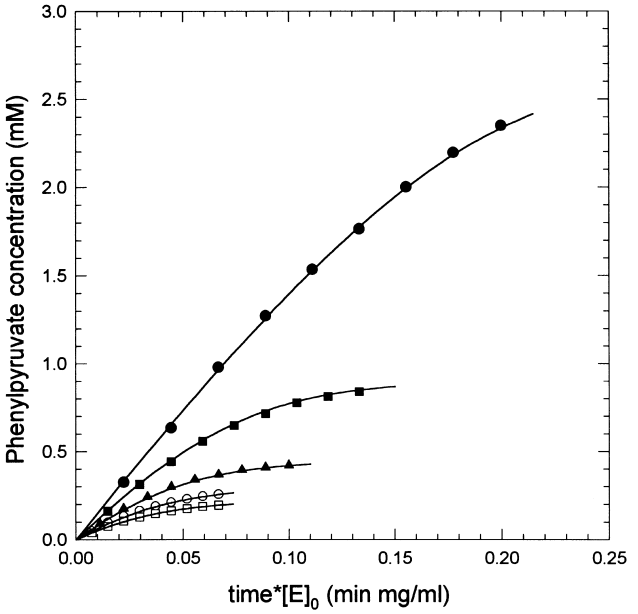

Fig. 3. Time profile for phenylpyruvate concentration. The points represent experimental data while the lines are theoretical concentrations obtained from the integrated Michaelis-Menten equation using the ω function and the parameter values shown in Table 2. The initial substrate concentrations are as follows: ●, 2.7 mM; ■, 0.9 mM; ▲, 0.45 mM; ○, 0.3 mM; □, 0.225 mM.

strength of this inhibition; for prephenate dehydratase, it is relatively weak but for acid phosphatase the reverse is true leading to negative values for V_m' and K_a'. The final two sets of data are for fumarase, which catalyzes a reversible reaction. Depending on whether fumarate or malate is used as the substrate, V_m' and K_a' are either both positive, or both negative, respectively.

The results of parameter estimation are summarized in Tables 1 and 2 along with those obtained previously using a numerical approximation for the product concentration by the Newton-Raphson method. Parameter estimates obtained from both methods were almost identical confirming the suitability of the ω function for kinetic parameter estimation. Representative progress curves for prephenate dehydratase are shown in Fig. 3. Experimental product formation data were accurately described by the integrated Michaelis-Menten equation with the kinetic parameters shown in Table 2.

5. Discussion

Non-linear least squares analysis of progress curve data is a convenient method of estimating the associated kinetic parameters. However, given the implicit nature of the integrated Michaelis-Menten equation, this approach is not straightforward and the theoretical substrate or product concentration has to be approximated numerically. The use of the ω function makes it possible to obtain explicit solutions for the substrate and product concentrations thereby presenting an attractive technique for parameter estimation. Moreover, algorithms for the evaluation of ω are simple and fast [11–13] as compared to root-finding procedures where several iterations are necessary to find the solution.

In the present study, we have shown that the ω function works well when the apparent values of V_m and K_a are either both positive (pyruvate kinase, prephenate dehydratase, or fumarase with fumarate as substrate) or negative (acid phosphatase and fumarase with malate as substrate). This is important because when K_a' is negative, the argument of ω (designated as x) in Eq. 7 becomes negative.

The Lambert ω function offers a convenient method of explicitly expressing the substrate and product concentrations in the integrated Michaelis-Menten equation. This simplifies kinetic parameter estimation as root-finding and differential equation solving are avoided. Moreover, highly efficient algorithms are available for computing ω which further add to the suitability of this method for progress curve analysis of enzyme kinetic data.

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