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Closed-Form Solution of the Reduced Haldane Equation for Enzyme Kinetics with Strong Substrate Inhibition

Marko Goličnik

Institute of Biochemistry, Faculty of Medicine, University of Ljubljana,

Vrazov trg 2, 1000 Ljubljana, Slovenia

marko.golicnik@mf.uni-lj.si

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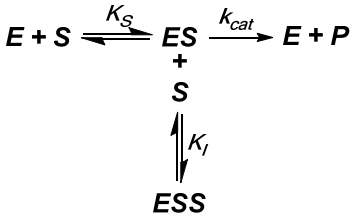
Abstract

The Haldane equation has been widely used to describe enzyme kinetics of inhibitory substrates, although its use is limited because the integrated form cannot be expressed in an explicit closed-form reformulation of the time-dependent solution. However, the standard three-parameter Haldane rate equation must be *per se* reduced to describe the kinetics of enzyme-catalyzed reactions that refers to a strongly inhibiting substrate, while the analysis of initial rates instead of complete progress curves over time leads to an additional loss of information relating to the reaction kinetics. Thus, I present an explicit solution of the parameterized two-parameter Haldane equation which is expressed in terms of the Lambert W function, as the most elegant and ideal simplification of the evaluation of kinetics parameters can be performed from progress curves.

1. Introduction

The Haldane reaction model (*see Scheme 1*) is widely used in biochemistry to describe enzyme substrate inhibition processes in solution [1],



Scheme 1

and enzyme activities are typically characterized in terms of initial rates that are determined at various substrate concentrations and analyzed with the three-parameter Haldane rate equation as:

$$v = \frac{V \cdot [S]}{[S] + K_S + [S]^2/K_I} = \frac{V}{1 + K_S/[S] + [S]/K_I} \tag{1}$$

where v, $[S]$, V, K_S and K_I represent the rate of reaction, the substrate concentration, the limiting rate, and the dissociation constants for productive ES and unproductive ESS complexes, respectively. When $K_S/[S] + [S]/K_I >> 1$ for all the substrate concentration values that may have been used for the experiments, an optimization is not possible in order to obtain the parameters V, K_S, and K_I with reasonable accuracy from the experimental points. A typical case is the presence of strong substrate inhibition when the concentration of complex ES is negligible with respect to the sum of the concentrations of free E and complex ESS, and consequently the reduced version of two-parameter Haldane equation is more appropriate for the fitting to the experimental data:

$$v = \frac{V \cdot [S]}{K_S + [S]^2/K_I} = \frac{V \cdot K_I \cdot [S]}{K_S \cdot K_I + [S]^2} = \frac{k_1 \cdot [S]}{k_2 + [S]^2} \tag{2}$$

It is obvious that fitted parameters k_1 (= $V \cdot K_I$) and k_2 (= $K_S \cdot K_I$) in Eq. (2) are not intrinsic constants of the standard Haldane equation, but are implicit functions of the three constants in that equation which cannot be separated.

Although the usual experimental practice is to follow enzyme reaction; i.e. concentrations over an extended period of time, many biochemists cannot explain the observations in terms of time-dependent solutions of integrated rate equations as the resulting solutions are frequently implicit in the substrate concentration. Hence, they avoid this problem by correct adaptation and analysis of the experiment, i.e. differentiation of the progress-curve data into rates. However, by their very nature, the initial rate methods make use of only a small portion of the information in any progress curve [2]. Thus, attempts have been made to obtain enzyme kinetics parameters by fitting the computed curves over the entire concentration-time courses through numerical integration [3-5] or algebraic solutions to the rate equations [6-9]. As large discrepancies have been recognized among the data from the computer programs that apply the numerical integration approaches [10], I believe that algebraic integration approaches, which are based on available exact solutions to the particular rate equations, provide an improvement that fully deserve the attention. When a closed-form solution to integrated rate equation exists, it greatly simplifies and facilitates the nonlinear evaluation of the kinetics parameters, as the intense numerical computations applied to solve the rate equation are replaced with an explicit time-dependent mathematical formula for concentrations. Therefore, the objective of this study was to find explicit solutions to two-parameter Haldane Eq. (2) that will enable the direct analyses of raw time-concentration data of strongly substrate inhibition reaction systems by using a spreadsheet nonlinear regression curve-fitting program.

2. Theory

2.1. The standard and parameterized Haldane equations

The mathematical formalism of the enzyme reaction model shown in Scheme 1 was initially described by Haldane [1] with three-parameter differential equation and initial condition as:

$$\frac{d[S]}{dt} = -\frac{V \cdot [S]}{[S] + K_S + [S]^2 / K_I}\,;\ [S](t=0) = [S]_0 \tag{3}$$

Eq. (3) can be readily integrated, but the solution can be expressed only in implicit form as:

$$V \cdot t = K_S \cdot \ln([S]_0 / [S]_t) + ([S]_0 - [S]_t) + \frac{1}{2 \cdot K_I} \cdot \left([S]_0^2 - [S]_t^2\right) \tag{4}$$

The latter equation cannot be reformulated as an explicit closed-form equation like the integrated Michaelis-Menten equation [7,8], and numerical root-solving techniques must be used to estimate the substrate concentration at each time-point from Eq. (4). Alternatively, kinetics parameters can be estimated from time-concentration data by using other nonconvential mathematical techniques; e.g. the Adomian decomposition method [11], which was examined recently to obtain approximate solutions to the Michaelis-Menten [12,13], standard Haldane [14] and Webb [15] rate equations.

Unfortunately, nonlinear numerical methods are sensitive to the initial conditions [16]. Sokol and Howell found this to be the case in using the Marquardt algorithm to fit the standard three-parameter Haldane rate Eq. (3) to data on phenol oxidation [17,18] where more than one local solution was found for the parameter values. The data were observed to fit better to two-parameter parameterized version of the Haldane model equation

$$\frac{d[S]}{dt} = -\frac{V \cdot K_I \cdot [S]}{K_S \cdot K_I + [S]^2} = -\frac{k_1 \cdot [S]}{k_2 + [S]^2}\,;\ [S](t=0) = [S]_0 \qquad (5)$$

This corresponds to the strong substrate inhibition situation where [ESS] >> [ES] and total enzyme concentration is divided mainly between the free form and the inhibited state, with ES complex present only in small amounts. In contrast to the classical Haldane Eq. (3), the Eq. (5) has an integrated solution which can be transformed into an explicit equation in terms of the Lambert W function as will be shown in this report.

2.2. The Lambert W(x) function and its approximations

The Lambert W(x) function is a mathematical function that has numerous well-documented applications in mathematics, physics and computer science [18]. Its definition is probably most easily understood by analogy with the inverse relationship between the exponential function and the natural logarithmic function ln(x), as shown in expression exp(y) = x. Given x, it is possible to write y = ln(x) to find y. The Lambert W(x) function works similarly, with the difference being the initial expression that connects x and y. W(x) is defined as the inverse of the function satisfying Eq. (6):

$$y \cdot \exp(y) = x \qquad (6)$$

i.e. the solution to Eq. (6) is expressed by the Lambert W(x) function as y = W(x), and therefore Eq. (6) is more frequently written in the form of Eq. (7):

$$W(x)\cdot\exp\left(W(x)\right)=x \tag{7}$$

The Lambert function has real values for $-\exp(-1) \le x < +\infty$, as shown in Figure 1. For the range $-\exp(-1) < x < 0$, W(x) is negative, and there are two solutions, although for $x > 0$, W(x) is positive and has a unique value. The function has a defined derivative for $x > -\exp(-1)$ that satisfies the following Eq. (8):

$$\frac{dW(x)}{dx}=\frac{W(x)}{x\cdot\left(1+W(x)\right)} \tag{8}$$

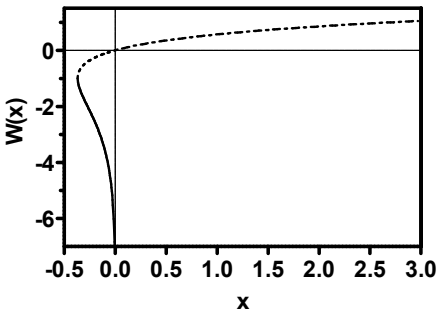


Figure 1. The three real branches of the Lambert W(x) function for the corresponding values of argument x. Region 1: (---) $x > 0$, region 2: (···) $-\exp(-1) < x < 0$ and $-1 < W(x) < 0$; region 3: (-) $-\exp(-1) < x < 0$ and $W(x) < -1$. Regions 1 and 2 divide the principle $W_0(x)$ branch as $W_0^+(x)$ and $W_0^-(x)$, respectively, but region 3 represents the lower $W_{-1}(x)$ branch.

Transforming x in W(x) to $x = \alpha\cdot\exp(\alpha - \beta\cdot t)$ shows that

$$\frac{dW\left[\alpha\cdot\exp(\alpha-\beta\cdot t)\right]}{dt}=-\frac{\beta\cdot W\left[\alpha\cdot\exp(\alpha-\beta\cdot t)\right]}{1+W\left[\alpha\cdot\exp(\alpha-\beta\cdot t)\right]} \tag{9}$$

or equivalently that

$$\varphi(t)=W\left[\alpha\cdot\exp(\alpha-\beta\cdot t)\right] \tag{10}$$

represents a solution for φ in the differential equation with initial conditions:

$$\frac{d\varphi}{dt} = -\frac{\beta \cdot \varphi}{1+\varphi};\ \varphi(t=0) = \alpha \tag{11}$$

Many equations that involve exponentials or logarithms can be solved using the W(x) function. The general strategy is to move all of the instances of the unknown to one side of the equation and to make it look like Eq. (7), at which point the W(x) function provides the value of the variable in y. However, the solution needs to be acquired computationally when fitting explicit Eq. (10) to data; here, evaluating W(x) is the main problem, as it is not an implemented function in most nonlinear regression computer programs.

Simple, yet accurate, approximations of W(x) that can be useful for quickly generating results are based on the principle that W(x) can be expressed by a composite of natural logarithms [18]. For x > 0 and W(x) > 0, we can take the natural logarithm of Eq. (7) and rearrange it to Eq. (12):

$$W(x) = \ln(x) - \ln(W(x)) \tag{12}$$

It is clear from Eq. (12) that a possible analytical expression for W(x) shows a degree of self-similarity. Unrolling this self-similarity as a recursive relationship, the curious mathematical formula for W(x) shown by Eq. (13) can be obtained:

$$W(x) = \ln(x) - \ln\left(\ln(x) - \ln\left(\ln(x) - \cdots\right)\right), \tag{13}$$

or in the shorthand for a continued logarithm, we can arrive at Eq. (14):

$$W(x) = \ln\left(x / \ln\left(x / \ln\left(x / \cdots\right)\right)\right) \tag{14}$$

Barry et al. [18] reduced this form to a sequence of approximations, with the second term of this sequence shown by Eq. (15):

$$W(x) \approx \ln\left(\frac{1.2 \cdot x}{\ln\left(\frac{2.4 \cdot x}{\ln(1+2.4 \cdot x)}\right)}\right) \tag{15}$$

The maximum relative error computed for Eq. (15) is 2.4%. Linear interpolation between the first two approximation terms of produces a significant improvement [19], which is given by Eq. (16), as:

$$W(x) \approx 1.45869 \cdot \ln\left(\frac{1.2 \cdot x}{\ln\left(\frac{2.4 \cdot x}{\ln(1+2.4 \cdot x)}\right)}\right) - 0.45869 \cdot \ln\left(\frac{2 \cdot x}{\ln(1+2 \cdot x)}\right) \tag{16}$$

For Eq. (16), the maximum relative error is only 0.2% as shown in Fig. 2.

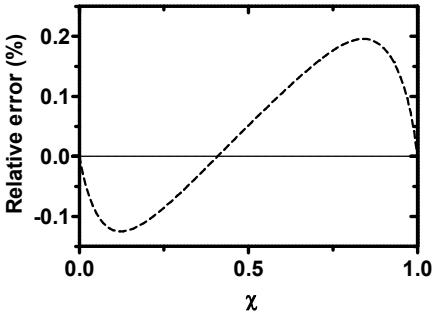


Figure 2. Relative error of the approximation of the Lambert W function given by Eq. (16) for x > 0 on the exponential χ-scale. The infinite x range interval (0,∞) has been mapped onto the interval (0,1) with the aid of the exponential χ-scale $\chi = 1 - 1/\ln(x + \exp(1))$.

Winitzki [20] provided another simple analytic function that can approximate W(x) with a maximum relative error of less than 2%, as shown in Eq. (17):

$$W(x) \approx \ln(1+x) \cdot \left(1 - \frac{\ln(1+\ln(1+x))}{2+\ln(1+x)}\right) \tag{17}$$

All of these three equations, as Eqs. (15), (16) and (17), are easily computable approximations for W(x), with argument-x-dependent relative errors.

2.3. The exact solution to the parameterized two-parameters Haldane rate equation

First, redefine substrate concentration [S] in terms of a function σ such that $[S] = (k_2)^{1/2}\cdot\sigma$. Substitution into Eq. (5) gives

$$\frac{d\sigma}{dt} = -\frac{k_1\cdot\sigma}{k_2\cdot\left(1+\sigma^2\right)}, \qquad \sigma_0 = [S]_0/\sqrt{k_2} \tag{18}$$

Now considering $\sigma = (\varphi)^{1/2}$, where $\varphi(t)$ satisfies Eq. (11), the chain rule gives

$$\frac{d\sigma}{dt} = \frac{d\sigma}{d\varphi}\cdot\frac{d\varphi}{dt} = \frac{1}{2\cdot\sqrt{\varphi}}\cdot\left(-\frac{\beta\cdot\varphi}{1+\varphi}\right) = -\frac{\beta\cdot\sigma^2}{2\cdot\sigma\cdot\left(1+\sigma^2\right)} = -\frac{\beta\cdot\sigma}{2\cdot\left(1+\sigma^2\right)} \tag{19}$$

which matches Eq. (18) for $\beta = 2\cdot k_1/k_2$. Because $[S] = (k_2)^{1/2}\cdot\sigma = (k_2\cdot\varphi)^{1/2}$ and $\varphi(t) = W[\alpha\cdot\exp(\alpha - \beta\cdot t)]$ it follows that substrate concentration can be expressed as

$$[S](t) = \sqrt{k_2\cdot W\left[\alpha\cdot\exp\left(\alpha - 2\cdot\left(k_1/k_2\right)\cdot t\right)\right]} \tag{20}$$

The initial condition $[S](t = 0) = [S]_0$ that determines α implies

$$[S]_0 = \sqrt{k_2\cdot W\left[\alpha\cdot\exp(\alpha)\right]} = \sqrt{k_2\cdot\alpha} \tag{21}$$

and

$$\alpha = [S]_0^2/k_2 \tag{22}$$

Putting Eqs. (20-22) together gives

$$[S](t) = \sqrt{k_2\cdot W\left[\left([S]_0^2/k_2\right)\cdot\exp\left(\left([S]_0^2 - 2\cdot k_1\cdot t\right)/k_2\right)\right]} \tag{23}$$

Transforming x in Eq. (8) to the argument of the Lambert W(x) function of Eq. (23) shows again that Eq. (23) truly represents a solution for the differential equation given in Eq. (5). The derivative of the substrate concentration over time gives Eq. (24)

$$\frac{d[S]}{dt} = \sqrt{k_2}\cdot\frac{d\sqrt{W(x(t))}}{dt} = \sqrt{k_2}\cdot\frac{1}{2\cdot\sqrt{W(x(t))}}\cdot\frac{dW(x(t))}{dx}\cdot\frac{dx(t)}{dt} \tag{24}$$

Inserting the derivative of x(t) over time, which is given in Eq. (25):

$$\frac{dx(t)}{dt} = -\frac{2 \cdot k_1}{k_2} \cdot x(t) \tag{25}$$

into Eq. (24) results in Eq. (26), the parameterized two-parameter Haldane equation (Eq. (5)):

$$\frac{d[S]}{dt} = -\frac{2 \cdot k_1 \cdot \sqrt{k_2}}{2 \cdot k_2 \cdot \sqrt{W(x(t))}} \cdot \frac{W(x(t)) \cdot x(t)}{x(t) \cdot (1 + W(x(t)))} = -\frac{k_1}{\sqrt{k_2 \cdot W(x(t))}} \cdot \frac{W(x(t))}{(1 + W(x(t)))} = -\frac{k_1}{[S]} \cdot \frac{[S]^2 / k_2}{\left(1 + [S]^2 / k_2\right)} =$$
$$= -\frac{k_1 \cdot [S]}{k_2 + [S]^2} \tag{26}$$

The parameterized two-parameter Haldane rate equation (Eq. 2) presents the maximum rate for $[S] = (k_2)^{1/2}$, and Fig. 3 shows the typical shape of progress curves obtained by the equation of the form of Eq. (23) and evaluated with the use of the Matlab *lambertw* built-in function. It is obvious that the inflection points at $[S] = (k_2)^{1/2}$ are more vivid for systems with lower K_I values (i.e. with strong substrate inhibition).

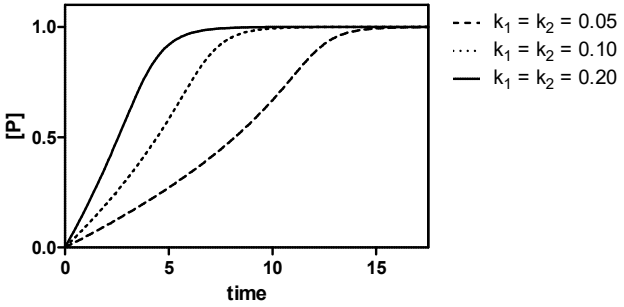


Figure 3. Simulated curves for normalized product concentration; i.e. $[P] = 1 - [S]/[S]_0$ obtained from Eq. 23 with the following parameters k_1 (= $V \cdot K_I$) and k_2 (= $K_S \cdot K_I$). It is evident that the inhibition constant K_I equivalently influences on both parameters k_1 and k_2.

However, applying the closed-form solution provides an excellent approach for progress-curve analysis of enzyme reaction systems that follow Haldane kinetics with strong substrate inhibition. This approach is based on the exact equation, although the direct calculation of equations that

involve the Lambert W function can be performed only by software with built-in W code (e.g. Mathematica, Matlab, Maple). Despite an accuracy of approximation function of around 0.2% only, which is in the range of usual experimental error, it appears that the approximation Eq. (16) can serve as simple analytical tool that can excellently substitute the Lambert W function in Eq. (23) as shown in Fig. (4). Thus, there are also no technical limits for direct fitting of this model equation to progress curves by any nonlinear regression curve-fitting software tools.

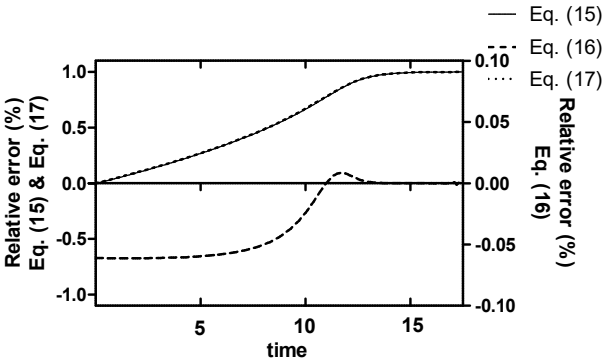


Figure 4. Relative errors to a simulated curve from Fig. 3 (for the parameters $k_1 = k_2 = 0.05$) obtained with the approximations of the Lambert W function given by Eqs. (15–17).

3. Conclusions

The present report describes the exact and efficient progress-curve analysis of enzyme reactions within the Haldane (strong) substrate inhibition framework. A practical point concerning the use of exact Eq. (23) and approximation Eq. (16) is the easy and accurate computation of the solutions to the parameterized two-parameter Haldane Eq. (5) using any optional software. Hence, it is possible to elegantly construct the time-concentration curves during any time interval, and the latter can be used to simulate or fit experimental data also without relying on highly specialized algorithms and technical software, but simply by encoding them in standard computer programs.

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