LEAST SQUARES ESTIMATION OF MICHAELIS–MENTEN PARAMETERS BY A FIBONACCI SEARCH METHOD

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1. Introduction

In fitting the Michaelis–Menten equation directly to enzyme kinetic data by the method of least squares with the assumption of uniform weights for the velocities, the object is to find the values of the Michaelis constant (K_M) and the maximum velocity (V) which minimize the sum of squared error (SSE):

$$\mathrm{SSE} = \sum_i [v_i - Vs_i/(K_M + s_i)]^2, \quad (1)$$

where the v_i and s_i are the velocities and substrate concentrations, respectively, and the summation runs over the number of data points. The least squares solution satisfies the conditions (or normal equations):

$$\partial\,\mathrm{SSE}/\partial\,K_M = 0 \quad (2a)$$

$$\partial\,\mathrm{SSE}/\partial\,V = 0 \quad (2b)$$

Since the parameter K_M occurs nonlinearly in the Michaelis-Menten equation, the normal eqs. (2a) and (2b) are nonlinear in this parameter. It is standard practice to bypass nonlinear normal equations [1]. In fitting the Michaelis–Menten equation the recommended procedure has been to fit iteratively the approximation to the equation obtained by taking the linear part of a Taylor series expansion about K_M [2,3]. This requires starting values for K_M and V. We propose here an alternative optimization procedure which does not depend on the provision of initial estimates of the Michaelis–Menten parameters. The method takes advantage of the fact that the sum of squared error (1) has only one independent variable, the Michaelis constant, and optimization of the Michaelis constant optimizes both Michaelis–Menten parameters.

Since V is a linear parameter in the Michaelis–Menten equation, it can be eliminated from eq. (1) by means of condition (2b). This yields:

$$\mathrm{SSE} = \sum_i [v_i - V^* s_i/(K_M + s_i)]^2, \quad (3)$$

where

$$V^* = \sum_i v_i s_i/(K_M + s_i) \Big/ \sum_i [s_i/(K_M + s_i)]^2.$$

Eq. (3) can be made the objective function of a unidimensional search for the minimum of a unimodal function with one independent variable. This is conveniently done by the Fibonacci method. We have evaluated this method on simulated and real data.

2. Methods

The Fibonacci search was carried out by the simplified method of Kiefer [4,5]. The search was conducted between zero and the highest substrate concentration in the data. The size of the final interval of uncertainty was 1×10^{-5}. The estimates of the Michaelis–Menten parameters were compared with those obtained by the Taylor series linearization procedure of Wilkinson [2]. Here the iterations were stopped when the change in K_M became less than 1×10^{-5}.

For simulated data the true values of K_M and V were taken as unity. Velocities were computed for ten evenly spaced substrate concentrations in the

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range 0.5 K_M to 5 K_M. Normally distributed errors were superposed either with a constant variance of 0.01, or a coefficient of variation of 10% at each data point. The errors were simulated by generating pseudo-random numbers in the range 0 to 1. These were converted into normal deviates by the method of Box and Muller [6] and scaled as appropriate.

Real data were obtained on lactate dehydrogenase (EC 1.1.27) in cytosol preparations of fish muscle. The enzyme was assayed according to Kornberg [7]. The initial velocities were measured at 6 or 7 pyruvate concentrations in the range 0.13 mM to 2.66 mM. The velocities were referred to total protein in the preparations as determined by the Lowry method [8].

All computations were performed by means of a Hewlett-Packard 9100B Programmable Calculator equipped with a 9101A Extended Memory. The pseudo-random number generator was a standard Hewlett-Packard programme (part no. 09100-70816). Other programmes were specially written and are available on request to the authors.

3. Results

In 100 simulated experiments with each type of error the Fibonacci search found the same values of the Michaelis–Menten parameters as the Taylor series linearization method. With constant error, a mean value of 1.11 ± 0.51 (S.D.) was determined for K_M and mean value of 1.03 ± 0.14 (S.D.) was determined for V. With proportional error, a mean value of 1.07 ± 0.29 (S.D.) was determined for K_M and a mean value of 1.02 ± 0.09 (S.D.) was determined for V.

The values of the parameters found for fish muscle lactate dehydrogenase are shown in table 1. The Lineweaver–Burk estimates were the initial estimates for the procedure of Wilkinson [2]. In all cases the Fibonacci search found the same values of the Michaelis–Menten parameters as the Taylor series linearization and iteration.

Table 1
Least squares estimates of Michaelis–Menten parameters for fish muscle lactate dehydrogenase

Enzyme source	Estimation method			
	Weighted Lineweaver–Burk[a]		Wilkinson[b] *and* Fibonacci search	
	K_M	$10^2 V$	K_M	$10^2 V$
Bogue white muscle	0.69	2.28	0.8 ± 0.19	2.40 ± 0.30
Bogue red muscle	0.52	0.08	0.53 ± 0.07	0.08 ± 0.01
Dolphin fish white muscle	0.64	1.32	0.69 ± 0.21	1.33 ± 0.21
Dolphin fish red muscle	0.62	1.20	0.65 ± 0.11	1.22 ± 0.10
Grey mullet white muscle	0.76	2.6	0.85 ± 0.17	2.72 ± 0.3
Grey mullet red muscle	0.67	1.23	0.73 ± 0.08	1.26 ± 0.06

[a] See [2].
[b] Standard errors are given for the Wilkinson method (see [2]). In the Fibonacci search for K_M the size of the final interval of uncertainty was 1×10^{-5}.
K_M values refer to millimolar substrate concentration; V values refer to micromoles pyruvate converted per min per mg total protein in the preparations

4. Discussion

As Gardiner and Ottaway [9] have pointed out the problem of obtaining the best estimates of the Michaelis–Menten parameters, from a set of experimental data, is one of permanent interest to enzymologists. The Fibonacci search technique described here has some advantages as a least squares method.

The Taylor series linearization method for nonlinear least squares generally converges, but it can sometimes diverge increasing the sum of squared error instead of decreasing it [1]. The method requires initial parameter estimates. In the procedure of Wilkinson [2] the initial estimates are obtained by a weighted least squares fit of the Lineweaver–Burk form of the Michaelis–Menten equation ($1/v$ as a function of $1/s$) to the data. This particular device can be a drawback in automatic computation. In the Lineweaver–Burk equation the intercept occurs in the denominator of the expressions for V and K_M, and it is possible for the intercept occasionally to be close the zero so that absurdly large, positive or negative estimates of the Michaelis–Menton parameters are obtained. The same argument applies to the Hanes–Woolf linearization of the Michaelis–Menton equation (s/v as a function of s) where the slope occurs in the denominator of the expressions for V and K_M. However, in the Eadie–Hofstee linearization (v as a function of v/s) the slope of the line is $-K_M$ and the intercept is V so that absurdly large estimates of V and K_M are never found [10].

The Fibonacci search technique proposed here finds the same minimum for the sum of squared error as the Taylor series linearization method. The search is easily implemented. It does not require initial estimates of the Michaelis–Menten parameters. The search for the optimum value of the Michaelis constant can be made routinely between zero and the highest substrate concentration in the data, assuming that the range of substrate concentrations has bracketed the K_M. The size of the initial interval for the search can also be reduced by inspecting a plot of initial velocity against substrate concentration.

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