Two Methods for Fitting a Rectangular Hyperbola to Data from Several Animals

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(Received 23 April 1976)

Two methods are described for fitting the Michaelis–Menten equation to sets of data with a common Michaelis constant but different maximum velocities. One of them uses the method of least squares, and the other is based on the direct plot of Eisenthal & Cornish-Bowden [*Biochem. J.* (1974) 139, 715–720].

A statistical problem often encountered by biochemists is that of fitting a rectangular hyperbola to experimental data: for instance, to estimate the Michaelis–Menten constants of an enzyme-catalysed reaction (K_m and V), or the parameters defining the binding of a ligand to a protein (K_d , the dissociation constant, and N , the maximum number of mol of ligand bound per mol of protein). There are several ways of solving this problem, and their relative merits have been examined in some detail (e.g. Endrenyi & Kwong, 1972; Atkins & Nimmo, 1975).

In some experiments enough data can only be obtained by using batches of enzyme or binding protein that were prepared from different animals and analysed on different days. For example, the yield of active protein per animal may be low or (especially if equilibrium dialysis is being used) there may only be enough apparatus for four or five observations to be made per day. In these circumstances a reasonable assumption might be that in the different batches the nature of the protein being studied is always the same, but that its concentration is not, i.e. that K_m or K_d is constant, whereas V or N varies from batch to batch. Consequently the statistical problem, say for the enzyme kinetic experiment, is to fit to the i sets of data (one set per batch) the equation:

$$v_{ij} = \frac{V_i \cdot s_{ij}}{K_m + s_{ij}} \quad (1)$$

$$\sum_i \frac{\sum_j w_{ij} \cdot \frac{v_{ij} \cdot s_{ij}}{K_m + s_{ij}}}{\sum_j w_{ij} \cdot \frac{s_{ij}^2}{(K_m + s_{ij})^2}} \left(\sum_j w_{ij} \cdot \frac{v_{ij} \cdot s_{ij}}{(K_m + s_{ij})^2} - \frac{\sum_j w_{ij} \cdot \frac{v_{ij} \cdot s_{ij}}{K_m + s_{ij}}}{\sum_j w_{ij} \cdot \frac{s_{ij}^2}{(K_m + s_{ij})^2}} \cdot \sum_j w_{ij} \cdot \frac{s_{ij}^2}{(K_m + s_{ij})^3} \right) = 0 \quad (4)$$

where (s_{ij}, v_{ij}) is the j th observation of substrate concentration and initial velocity made on the i th batch of enzyme. Two of the ways in which this may be done are described below.

Theory and Methods

Atkins & Nimmo (1975) decided that K_m and V were best estimated from data with the usual sorts of random experimental error either by a least-squares method, such as that of Wilkinson (1961), or by the direct plot devised by Eisenthal & Cornish-Bowden (1974). Consequently these two methods have been adapted to the present problem.

Least-squares method

The sum-of-squares (SS) function is:

$$SS = \sum_i \sum_j w_{ij} \left(v_{ij} - \frac{V_i \cdot s_{ij}}{K_m + s_{ij}} \right)^2 \quad (2)$$

where w_{ij} is the appropriate weighting factor.

The least-squares solution is found by partially differentiating eqn. (2) with respect to K_m and V_i . . . V_1 , setting the partial differentials to zero, and solving the $(i+1)$ simultaneous equations thus generated. The V values are given by equations of the type:

$$V_i = \left(\sum_j w_{ij} \cdot \frac{v_{ij} \cdot s_{ij}}{K_m + s_{ij}} \right) / \left(\sum_j w_{ij} \cdot \frac{s_{ij}^2}{(K_m + s_{ij})^2} \right) \quad (3)$$

and K_m is given by the non-linear equation:

Eqn. (4) can only be solved for K_m by iterative techniques, like the one attributed to Davidenko (Broyden, 1969). Once K_m has been found the various values of V can be calculated from eqn. (3).

Fig. 1. Least-squares fit of Michaelis-Menten equation to enzymic data for three different individuals

Initial velocities were determined [by the method of Ellman *et al.* (1961) as described in Newman (1974)] for the hydrolysis of acetylthiocholine by acetylcholine hydrolase prepared from the erythrocytes of three different donors (O, ●, Δ). Eqn. (1) was fitted to the data by the least-squares method (see the text). The parameter estimates (with those given by the direct plot in parentheses) are: $K_m = 88.3$ (86.3) $\mu\text{mol/l}$; $V_1 = 1.53$ (1.60), $V_2 = 1.38$ (1.35), $V_3 = 1.27$ (1.22) fmol/min per cell.

A computer program to fit eqn. (1) to several sets of data in this way was written in the Edinburgh language IMP. The non-linear equation was solved by using the Edinburgh Regional Computing library program Imp Davden, which is based on Davidenko's method.

Direct-plot method

Each of the i sets of data (containing j observations) is analysed independently to give $\frac{1}{2}j(j-1)$ estimates of K_m and V_i , exactly as described for a single set of data by Eisenthal & Cornish-Bowden (1974). All the estimates of K_m are then pooled and ranked in order of increasing magnitude; their median (not mean) is taken as the best estimate of K_m (if the number of estimates is even, the arithmetic mean of the two median values is taken). The best estimate of each V_i

is equated to the median value of the $\frac{1}{2}j(j-1)$ estimates of it.

Results and Conclusions

Both of the methods described above have been used to fit eqn. (1) to data obtained by Newman (1974) for the hydrolysis of acetylthiocholine by human erythrocyte acetylcholine hydrolase (EC 3.1.1.7). Initial velocities were measured at four substrate concentrations by using enzyme prepared from erythrocytes given by three different individuals. Fig. 1 shows the fit given by the least-squares method, and the parameter estimates derived from it. The direct plot gave similar results. Both methods have also been used to analyse the sort of binding data described by Strange *et al.* (1976).

The kinds of situation for which the method described here are intended were outlined in the introduction. If the experimental error in the dependent variable can be said to be normally distributed and of known variance, the least-squares method is the one to use. On the other hand, if the nature of the error is not known the direct plot is the method of choice, as it is likely to be the more robust (Cornish-Bowden & Eisenthal, 1974). It should also be noted that these methods cannot wholly compensate for poor experimental design, for example, when one batch of enzyme is studied solely at low concentrations of substrate and another at high ones.

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