Report

THE ANALYSIS OF KINETIC DATA IN BIOCHEMISTRY. A CRITICAL EVALUATION OF METHODS.

M. MARKUS and B. HESS*
Max-Planck-Institut für Ernährungsphysiologie, 4600 Dortmund, Rheinlanddamm 201, Federal Republic of Germany

and

J. H. OTTAWAY
Department of Biochemistry, University of Edinburgh, Medical School, Teviot Place, Edinburgh, EH8 9AG, Scotland

and

A. CORNISH-BOWDEN
Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham, B15 2TT, England

Received 10 December 1975

A workshop on 'The analysis of data' was held in Edinburgh during the Joint Meeting of The Biochemical Society, the Gesellschaft für Biologische Chemie and the Schweizerische Gesellschaft für Biochemie (September 9–12, 1975). This workshop was organized by B. Hess, Max-Planck-Institute, Dortmund, and J. H. Ottaway, University of Edinburgh. Most of the material contained in the individual papers has been reported elsewhere [1–5], or will be reported in the Transactions of The Biochemical Society so this report will be confined to the general discussion, which was lead by B. Hess and was based on a paper prepared by M. Markus. The aim was to proceed systematically through the many methods that have been proposed for analysis of enzyme kinetic data, to delineate the special advantages and disadvantages of each. We hope that the following tabulation, distilled from that discussion, will be helpful as a guide for choosing between methods.

List A compares measuring methods (progress curves and initial velocity measurements) and list B evaluation methods (linear and non-linear methods).

The linear methods listed apply only to reactions whose rate effectively depends on a single substrate. Because of space consideration we often give only hints in this list; details can be found in the references. Fromm [6] has discussed some of these topics in a recent book. The advantages of a method may only appear in the lists as disadvantages of the alternative method, and vice versa. We exclude a comparison of different non-linear optimization techniques (see [7–9]), weighting methods (see [10-12]) and test of goodness of fits (see [13,14]).

Symbols used: v: reaction rate, s: substrate concentration, K_M: Michaelis constant, V_{max}: maximal rate, $K_{0.5}$: half saturation concentration, n_H: Hill coefficient, p: product concentration, p_∞: product concentration at equilibrium, t: time, i: inhibitor concentration, K_i: dissociation constant of the EI (enzyme–inhibitor) complex, K_i': dissociation constant of the EIS (enzyme–inhibitor–substrate) complex, K_R, K_T: intrinsic dissociation constants of the substrate and an R and T protomer, respectively, L': allosteric constant, n: number of protomers.

*To whom correspondence should be addressed

A. Comparison of initial rate measurements and progress curves

1. *Initial rate measurements*

Advantages: Easier to handle if no computer is available.

Disadvantages: The beginning of a progress curve, from which the initial rate is determined, is often ill-defined.

Waste of the information contained in the non-linear part of the progress curve.

2. *Progress curves*

Advantages: Much less measuring time; more information can be obtained from each experiment [15,16].

Disadvantages: Integration of the rate law necessary for fitting (fits to progress curves without integration, taking the tangents, have been shown to lead to wrong weighting [17]).

Reversibility of the reactions, loss of activity of the enzyme and formation or consumption of an effector may have to be taken into account.

Results are sensitive to errors in total elapsed time and initial concentrations.

B. Comparison of evaluation methods

1. *Linear plots*

1.1. Plots of $1/v$ versus $1/s$, s/v versus s and v versus v/s [18]

Advantages: If the process can be described by an equation of the form of the Michaelis–Menten equation (possibly with apparent K_M and V_{max}), these plots give the ordinate intercepts $1/V_{max}$, K_M/V_{max} and V_{max}, respectively, and the abcissa intercepts $-1/K_M$, $-K_M$ and V_{max}/K_M, respectively.

Easy and well known.

$1/v$ versus $1/s$ separates the variables v and s.

Deviations from a straight line can be used to diagnose mechanistic variants [19–21].

Disadvantages: Only for initial rate measurements.

No separation of v and s in the last two plots.

Misleading display: results obtained by more exact methods do not in general appear to be correct when presented with these plots [22].

Information provided about the presence of poor observations can be wrong [23].

Variances should be transformed with the parameters for determining the weighting factors used in a fit [24,25]. This transformation is usually not done because it complicates the analysis. A test for bias and standard deviations of the parameters [26] showed that the results from all these plots, without transformation of variances, are not as satisfactory as those obtained with the direct linear plot [22] and a non-linear optimization [25]. (The worst results were obtained with $1/v$ versus $1/s$, as expected from [25,27,28]).

1.2. Plots of $\log [v/(V_{max}-v)]$ versus $\log(s)$ ('Hill plot'), s^{n_H}/v versus s^{n_H}, $1/v$ versus $1/s^{n_H}$ and v versus v/s^{n_H}

Advantages: If the process can be described by the Hill equation [29] these plots give ordinate intercepts,

$$-n_H \cdot \log K_{0.5},\ -1/V_{max},\ K_{0.5}^{n_H}/V_{max} \text{ and } V_{max},$$

respectively, and abcissa intercepts,

$$\log K_{0.5},\ -1/K_{0.5}^{n_H},\ -K_{0.5}^{n_H} \text{ and } V_{max}/K_{0.5}^{n_H},$$

respectively.

Separation of the variables v and s in the first and third plots.

Parameters in the model of Monod et al. [30] can be calculated from the results of these plots [31,32]. The first plot permits the determination of the average interaction energy involved in co-operative binding [33].

Combination of the first and second plots by Wieker et al. [34] leads for large number of points and small errors [35] to results as good as those obtained from complicated non-linear optimizations.

The shape of the first plot gives information about ratios of binding constants in Adair's model [1].

Disadvantages: Only for initial velocity measurements.

For the first plot, V_{max} has to be known in advance.

For the second, third and fourth plot, n_H has to be known in advance.

No separation of the variables v and s in the second and fourth plot.

The first plot, being a log–log plot, is quite insentitive to deviations.

Variances should be transformed as in 1.1.

1.3. Plot of p/t versus $(1/t)\cdot\ln[p_\infty/(p_\infty-p)]$ [36]

Advantages: If a process can be described by an equation of the form of the integrated Michaelis–Menten equation [37] (possibly with apparent K_M and V_{max}), this plot gives the ordinate intercept V_{max} and the abcissa intercept V_{max}/K_M. The plot is applicable to reversible reactions [38].

The plot serves as basis for the method of Foster and Niemann [39,40] for the analysis of competitive inhibition.

Disadvantages: Only for progress curves.

No separation of the variables p and t.

A test for bias and standard deviations of parameters [41] showed that the results obtained by Atkins and Nimmo [16] using this method were not as satisfactory as those with the non-linear optimization method of Fernley [42].

Parameter estimates are very sensitive to small errors in p, t and p_∞. [43–45].

Variances should be transformed as in 1.1.

1.4. Direct linear plot [22,23,46]. (Each measurement is represented by a straight line with ordinate intercept v and abcissa intercept $-s$)

Advantages: If the process can be described by an equation of the form of the Michaelis–Menten equation (possibly with apparent K_M and V_{max}), then the intersection of the lines gives directly K_M (abcissa) and V_{max} (ordinate).

Very simple (transformations not required), fast experimental design, direct indication of parameter errors, easy diagnosis of inhibition type. [22]

Very insensitive to outliers. More independent of correct weighting and of the assumption of a normal distribution than the least-squares method. [23]

A test for bias and standard deviations of parameters [26] gave better results than with the three linear transformations in 1.1 and the methods of de Miguel Merino [46] and Cohen [47]. It also gave better results than Wilkinson's method [25] for constant absolute error with 10% outliers and constant relative error with or without 10% outliers.

Analysis of processes that can be described by an equation of the form of the integrated Michaelis–Menten equation are possible when each measurement is represented by a straight line with ordinate intercept p/t and abcissa intercept $-p/\ln[p_\infty/p_\infty-p)]$.
The lines intersect again at the point (K_M, V_{max}); this method permits the determination of the initial velocity from progress curves and is less sensitive to errors in p_∞ as other methods. [48]

Disadvantages: Only small number of measurements can be displayed on one figure.

Method of Wilkinson [25] gave better results in a test for bias and standard deviation of parameters [26] than this method, for constant absolute error without outliers.

When used to estimate initial velocities [48], the time zero should be known accurately.

1.5. Plot of Filmer et al. [49]. Given the reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \xrightarrow{k_2} C + X$$

$$C \xrightarrow{k_3} E + D,$$

it is shown in [49] that $k_2=C_1(k_{-1}/k_1)+C_2$, where C_1 and C_2 are functions only of the initial conditions and the measured quantity X. As in the direct linear plot, one straight line is plotted for each measurement.

Advantages: The intersection of the straight lines leads to k_2 and k_{-1}/k_1.

No steady-state hypothesis necessary.

Direct indication of parameter errors.

Analysis correct at any measuring time.

Disadvantages: Only for equations of the form above

Tedious calculations involved.

Only small number of measurements can be displayed on one figure.

1.6. Plots obtained by combining pairs of measurements (s_i, v_i), (s_j, v_j).

1.6.1. Plot of

$(v_j s_i - v_i s_j)/s_j(v_i - v_j)$ versus s_i [46,50],

$v_i v_j(s_j - s_i)$ versus $(v_i s_j - v_j s_i)$ [50]

and other plots that can be obtained by transformations of these [50].

Advantages: If the process can be described by an equation of the form of the Michaelis–Menten equation (possibly with apparent K_M and V_{max}), then the first plot above gives a straight line through the origin with slope $1/K_M$ and the second plot one with slope V_{max}. Standard deviations of parameters are often lower than with non-linear optimization when the number of points is small (5 or 10) in a test performed in [50].

Disadvantages: Only for initial velocity measurements.

No separation of the variables v and s.

Bias in parameter estimates usually higher than with non-linear optimization in a test performed in [50].

In a test performed in [26], the first plot usually gave a bias and always gave higher standard deviations of parameter estimates than the s/v versus s plot, the v versus v/s plot, the direct linear plot [22], the method of Cohen [47], and the method of Wilkinson [25].

1.6.2. Plot of v_j versus v_i, where $s_j=\alpha s_i$ with constant α [51]

Advantages: If the process can be described by the Hill equation, then v_j versus v_i behaves like v versus s in the Michaelis–Menten equation with apparent Michaelis constant and saturation rate given by $V_{max}/(\alpha^{n_H}-1)$ and $(\alpha^{n_H}\cdot V_{max})/(\alpha^{n_H}-1)$, respectively.

Linearization of this plot (for instance with methods 1.1.) leads to V_{max} and n_H. The slope of $1/v_j$ versus $1/v_i$ is equal to $1/\alpha^{n_H}$. If n_H is not constant, then this slope yields a continuous measure of n_H at all concentrations.

Previous knowledge of V_{max} or n_H is not necessary.

Disadvantages: Only for initial rate measurements.

No separation of the variables v and s.

A test performed in [51] gave higher bias and higher standard deviations of parameters using this method than with the Hill plot or with non-linear optimization.

1.7 Special plots for systems with inhibitor

1.7.1. Plot of $1/v$ versus i ('Dixon plot') [52]

Advantages: If the process can be described by an equation of the form,

$$v = V_{max}s/[K_M(1+i/K_i)+s(1+i/K'_i)], \quad (1)$$

then the straight lines for different s intersect at $i=-K_i$ and $1/v=[1-(K_i/K'_i)]/V_{max}$, thus determining the constant K_i.

Disadvantages: Only for initial rate measurements.

Does not always distinguish between mixed and competitive inhibition.

Variances should be transformed as in 1.1.

1.7.2. Plot of s/v versus i [53]

Advantages: If the process can be described by an equation of the form (1), then the straight lines for different s intersect at $i=-K'_i$ and $s/v = K_M\ [1-(K'_i/K_i)]/V_{max}$ thus determining the constant K'_i.

Disadvantages: Only for initial rate measurements.

Does not always distinguish between mixed and uncompetitive inhibition.

Variances should be transformed as in 1.1.

1.8. Plot of

$$\log\left[\frac{v(1+\alpha)-\alpha V_{max}}{c\alpha V_{max}-v(1+c\alpha)}\right] \text{versus } \log\left[\frac{1+c\alpha}{1+\alpha}\right],$$

where $\alpha=s/K_R$, $c=K_R/K_T$

(see [54]. For $c=0$ see [55])

Advantages: If the process can be described by the equation of Monod et al. [30], then the ordinate intercept is equal to $\log L'$ and the abcissa intercept is equal to $-\log L'/(n-1)$.

Changes in L' due to effectors can easily be seen as parallel displacements of the plots.

Disadvantages: K_R, K_T and V_{max} have to be known in advance.

No separation of the variables v and s.

Being a log–log plot, it is quite insensitive to deviations.

Variances should be transformed as in 1.1.

2. *Non-linear methods*

2.1. Non-linear plots: v versus $\log(s)$ [56] and v versus s

Advantages: Simple

Separation of the variables v and s

Useful for first decisions: for instance, if there is sigmoidicity, if a suitable measurement interval has been chosen, etc.

Disadvantages: Curved plots.

Difficult to find the asymptotes accurately.

Difficult to detect deviations from a model.

2.2. Iterative non-linear optimization (see, for instance : [2,7–9,24,25,27,42,57,58,59]

Advantages: More parameters can be determined simultaneously than in linear plots, where at most two parameters can be determined from each plot.

Less restriction in the choice of controlled variables than in linear plots. When one uses linear methods, there is the temptation to restrict the collection of data to regions, where a linear relationship holds.

Usually lower standard deviations and less bias in parameter estimates than with linear methods [26,41,51].

Applicable to all types of rate laws. Can use information coming from initial rate measurements or from progress curves.

No transformation of variables and corresponding transformation of variances necessary.

Disadvantages: Provisional estimates of parameters are often necessary before starting iteration.

Computer necessary.

Variances and covariances of the parameters calculated from the inverse of the normal matrix are only correct if the non-linearity is not important in the range of standard deviations.

Effects not taken into account in the model, leading to unsatisfactory fits, are not so easy to recognize as in linear methods (for instance, different types of inhibition can more easily be recognized with linear plots).

Acknowledgements

We would like to thank the participants to the meeting for their engagement in the discussions; especially we thank Drs A. R. Curtis, E. M. Chance, H. N. Fernley and G. L. Atkins.

References

[1] Cornish-Bowden, A. and Koshland, D. E. Jr (1975) J. Mol. Biol. 95, 201–212.
[2] Curtis, A. R. and Chance, E. M. (1974) Atomic Energy Research Establishment (Harwell, Didcot, Oxfordshire OX11 ORA, England), report R. 7345, H.M.S.O.
[3] Curtis, A. R. (1975) Atomic Energy Research Establishment (Harwell, Didcot, Oxfordshire OX11 ORA, England), internal report C.S.S. 17.
[4] Schoot Uiterkamp, A. J. M., van der Deen, H., Berenken, H. J. C. and Boas, J. F. (1974) Biochim. Biophys. Acta 372, 407–425.
[5] Boiteux, A., Goldbeter, A. and Hess, B. (1975) Proc. Nat. Acad. Sci. 72, 3829–3833.
[6] Fromm, H. J. (1975) Initial Rate Enzyme Kinetics; Springer-Verlag, Berlin, Heidelberg, New York.
[7] Amadori, R., Mika, K. and v. Studnitz, I. (1976) Jül. Report, in the press, KFA Jülich, 516 Jülich, West-Germany.
[8] Beveridge, G. S. G. and Schechter, R. S. (1970) Optimization: Theory and Practice, McGraw-Hill.
[9] NAG Library Manual Mark 2, Section EO4, Nottingham Algorithms Group (1973).
[10] Ottaway, J. H. (1973) Biochem. J. 134, 729–736.
[11] Atkins, G. L. (1974) Biochem. J. 138, 125–127.
[12] Storer, A. C., Darlison, M. G. and Cornish-Bowden, A. (1975) Biochem. J. 151, 361–367.
[13] Reich, J. G., Wangermann, G., Falck, M. and Rohde, K. (1972) Eur. J. Biochem. 26, 368–379.
[14] Bártfai, T. and Mannervik, B. (1972) FEBS Lett. 26, 252–256.
[15] Markus, M. and Plesser, Th. (1976) Biochem. Soc. Trans., in the press.
[16] Atkins, G. L. and Nimmo, I. A. (1973) Biochem. J. 135, 779–784.
[17] Cornish-Bowden, A. (1972) Biochem. J. 130, 637–639.
[18] Woolf, B. quoted in Haldane, J. B. S. and Stern, K. G. (1932) Allgemeine Chemie der Enzyme, Dresden and Leipzig, Steinkopff Verlag, p. 119.
[19] Wharton, C. W., Cornish-Bowden, A., Brocklehurst, K. and Crook, E. M. (1974) Biochem. J. 141, 365–381.
[20] Bardsley, W. G. and Childs, R. E. (1975) Biochem. J. 149, 313–328.
[21] Frieden, C. (1967) J. Biol. Chem. 242, 4045–4052.
[22] Eisenthal, R. and Cornish-Bowden, A. (1974) Biochem. J. 139, 715–720.
[23] Cornish-Bowden, A. and Eisenthal, R. (1974) Biochem. J. 139, 721–730.
[24] Cleland, W. W. (1967) Advan. Enzymology 29, 1–32.
[25] Wilkinson, G. N. (1961) Biochem. J. 80, 324–332.
[26] Atkins, G. L. and Nimmo, I. A. (1975) Biochem. J. 149, 775–777.
[27] Johansen, G. and Lumry, R. (1961) Compt. Rend. Trav. Lab. Carlsberg 32, 185–214.
[28] Dowd, J. E. and Riggs, D. S. (1965) J. Biol. Chem. 240, 863–869.

[29] Hill, A. V. (1910) J. Physiol. 40, iv–vii.
[30] Monod, J., Wyman, J. and Changeux, J.-P. (1965) J. Mol. Biol. 12, 88–118.
[31] Blangy, D., Buc, H. and Monod, J. (1968) J. Mol. Biol. 31, 13.
[32] Johannes, K. J. and Hess, B. (1973) J. Mol. Biol. 76, 181–205.
[33] Wyman, J. (1967) J. Am. Chem. Soc. 89, 2202.
[34] Wieker, H.-J., Johannes, K.-J. and Hess, B. (1970) FEBS Lett. 8, 178–175.
[35] Atkins, G. L. (1973) Eur. J. Biochem. 33, 175–180.
[36] Walker, A. C. and Schmidt, C. L. A. (1944) Arch. Biochem. 5, 445–467.
[37] Henri, V. (1902) C. R. Acad. Sci., Paris 135, 916.
[38] Laidler, K. J. and Bunting, P. S. (1973) The Chemical Kinetics of Enzyme Action, 2nd Edn. p. 167–169 Clarendon Press, Oxford.
[39] Foster, R. J. and Niemann, C. (1953) Proc. Nat. Acad. Sci. USA 39, 999.
[40] Foster, R. J. and Niemann, C. (1955) J. Am. Chem. Soc. 77, 1886.
[41] Nimmo, I. A. and Atkins, G. L. (1974) Biochem. J. 141, 913–914.
[42] Fernley, H. N. (1974) Eur. J. Biochem. 43, 337–378.
[43] Newman, P. F. J., Atkins, G. L. and Nimmo, I. A. (1974) Biochem. J. 143, 779–781.
[44] Fleisher, G. A. (1953) J. Am. Chem. Soc. 75, 4487–4490.
[45] Klyosov, A. A. and Berezin, I. V. (1972) Biokhimiya 37, 170–183. (Engl. transl. 141–151).
[46] de Miguel Merino, F. (1974) Biochem. J. 143, 93–95.
[47] Cohen, S. R. (1968) Anal Biochem. 22, 549–552.
[48] Cornish-Bowden, A. (1975) Biochem. J. 149, 305–312.
[49] Filmer, D. L., Cannon, J. R. and Reiss, N. (1967) J. Theor. Biol. 16, 280–293.
[50] Fajszi, Cs. and Endrenyi, L. (1974) FEBS Lett. 44, 240–246.
[51] Endrenyi, L., Fajszi, C. and Kwong, F. H. F. (1975) Eur. J. Biochem. 51, 317–328.
[52] Dixon, M. (1953) Biochem. J. 55, 170–171.
[53] Cornish-Bowden, A. (1974) Biochem. J. 137, 143–144.
[54] Endrenyi, L., Chan, M. S. and Wong, J. T.-F. (1971) Canad. J. Biochem. 49, 581.
[55] Horn, A. and Börnig, H. (1969) FEBS Lett. 3, 325–329.
[56] Michaelis, L. and Menten, M. L. (1913) Biochem. Z. 49, 333–369.
[57] Chandler, J. P., Hill, D. E. and Spivey, H. O. (1972) Computers and Biomedical Research 5, 515–534.
[58] Powell, M. J. D. (1965) Computer Journal 7, 303–307.
[59] Chance, E. M., Hess, B., Plesser, Th. and Wurster, B. (1975) Eur. J. Biochem. 50, 419–424.