ANALYSIS OF KINETIC DATA OF ALLOSTERIC ENZYMES BY A LINEAR PLOT

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1. Introduction

The use of the cooperative model proposed by Monod, Wyman and Changeux (M-W-C model [1]) permits a quantitative description of the kinetic data of allosteric enzymes. However, the unequivocal distinction from other similar mechanisms [2,3] is impossible with classical kinetic treatments, as for example in measurements of initial velocity as a function of substrate and effector concentrations. Recently, Kirschner et al. [4], using relaxation methods, found that the M-W-C model is valid for the binding of NAD to glyceraldehyde-3-phosphate dehydrogenase.

In general the determination of kinetic constants of allosteric enzymes is difficult. The Hill plot [5] which is frequently used yields coefficients which are interpreted as a measure of the cooperativity of single effectors. However, this method fails to give the allosteric constant L of the M-W-C model and the true number of protomers. The method described by Frieden [6] is more generally applicable but permits the kinetic parameters to be established only by extrapolation or by comparing experimental values with theoretical curves.

In the present paper a linear graph is proposed, which permits estimation of the kinetic parameters independently of each other for the special case of exclusive ligand binding ($c = 0$) of the M-W-C model [1].

2. Theory of the method

According to the model the saturation function for the substrate is given by:

$$\bar{Y}_S = \frac{\alpha(1+\alpha)^{n-1}}{L' + (1+\alpha)^n}, \qquad (1)$$

where $\bar{Y}_S = v/V_{max}$; $\alpha = S/K_S$; n = number of protomers; $c = K_S/K_T = 0$; $L' = L(1+\beta)^n/(1+\gamma)^n$; $\beta = I/K_I$; $\gamma = A/K_A$; S, I, A = concentrations of substrate, inhibitor, and activator; and K_S, K_I, K_A = microscopic dissociation constants of the enzyme complexes, respectively.

After conversion of equation (1) to the reciprocal form

$$\frac{1}{\bar{Y}_S} = \frac{L' + (1+\alpha)^n}{\alpha(1+\alpha)^{n-1}} \qquad (2)$$

followed by division by $(1+\alpha)^{n-1}$ and transformation, the following equations are obtained:

$$\frac{1}{\bar{Y}_S} = \frac{1}{\alpha}\left(\frac{L'}{(1+\alpha)^{n-1}} + 1 + \alpha\right) \qquad (3)$$

and

$$\frac{\alpha}{\bar{Y}_S} - \alpha - 1 = \frac{L'}{(1+\alpha)^{n-1}}. \qquad (4)$$

Taking logarithms of equation (4),

$$\log\left(\frac{\alpha}{\bar{Y}_S} - \alpha - 1\right) = \log L' - (n-1)\log(1+\alpha) \qquad (5)$$

is obtained. It is evident that the graph of $\log[(\alpha/\bar{Y}_S) - \alpha - 1]$ versus $\log(1+\alpha)$ gives a straight line with a slope of $-(n-1)$.

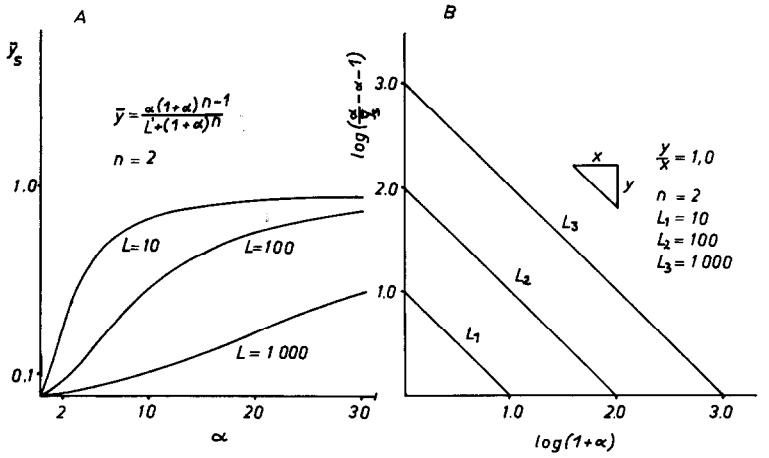

Fig. 1. Theoretical curves of the saturation function $\bar{Y}_S$ [equation (1)] drawn to various values of the constant L, with $n = 2$ for a dimer. (A) The usual representation $\bar{Y}_S = v/V_{max}$ versus α. (B) The proposed plot $\log[(\alpha/\bar{Y}_S) - \alpha - 1]$ versus $\log(1+\alpha)$ according to equation (5).

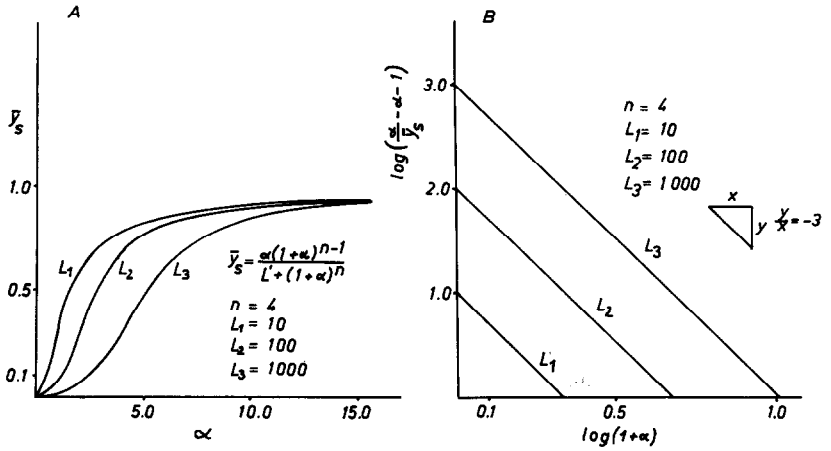

Fig. 2. Theoretical curves as described in fig. 1 except with $n = 4$ for a tetramer.

The intercept with the abscissa at $\log[(\alpha/\bar{Y}_S) - \alpha - 1] = 0$ yields $(\log L')/(n-1)$.

The value of L' is obtainable from the intercept on the ordinate, since it can be shown that this value ($\log(1+\alpha) = 0$) is numerically equal to the degree of interaction in terms of the M-W-C model.

A theoretical example is illustrated in fig. 1 for $n = 2$ and $L = 10$, 100 and 1000, respectively.

Fig. 1A shows the usual representation of $\bar{Y}_S = v/V_{max}$ versus α, while in fig. 1B the linear graph proposed here is shown. As expected, the slopes of the straight lines are -1, and $\log L' = 1.0$, 2.0 and 3.0, respectively. The same relation for $n = 4$ is illustrated in fig. 2. The slopes of the lines are -3, and L', calculated from the intercept with the vertical axis, equals 10, 100 and 1000, respectively.

Finding L' from the equation $L' = L(1+\beta)^n/(1+\gamma)^n$ not only permits a prediction of the effects of inhibitors and activators on substrate cooperativity, but also makes it possible to calculate the microscopic dissociation constants. An allosteric inhibitor increases the cooperativity of the substrate; L' is therefore

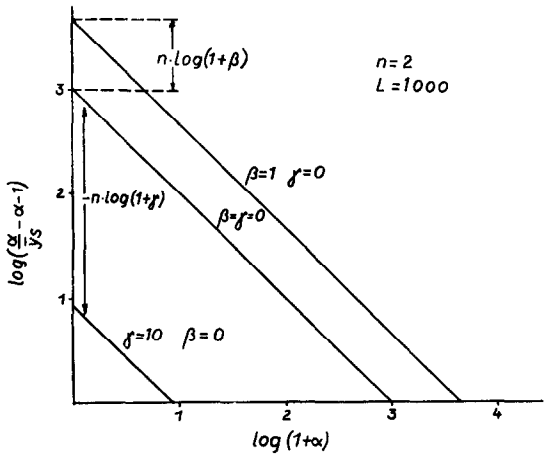

Fig. 3. Comparison of the plots log $[(\alpha/\bar{Y}_S)-\alpha-1]$ versus $\log(1+\alpha)$ for substrate ($\beta=\gamma=0$), inhibitor (β), and activator (α) (see text).

enlarged by the factor $(1+\beta)^n$. Since $\log L' = \log L + n\log(1+\beta) - n\log(1+\gamma)$, a straight line is obtained which is displaced above and parallel to the non-inhibited curve by an amount equal to $n\log(1+\beta)$ (fig. 3). By contrast, activators decrease the cooperativity of the substrate. Therefore the straight line in the linear graph undergoes an opposite displacement by an amount equal to $-n\log(1+\gamma)$ (fig. 3).

Since n is determined by the slope of the straight lines, and the concentrations of inhibitor or activator are known, K_I and K_A can be calculated from $n\log(1+\beta)$ and $n\log(1+\gamma)$, respectively. The graph requires knowledge of the value of α and therefore of K_S. When L is very small the kinetics obey the Michaelis-Menten relation; at $L = 0$, equation (1) simplifies to

$$v/V_{max} = \frac{\alpha}{1+\alpha} = \frac{S}{K_S + S}.$$

In these conditions K_S can be determined by the usual kinetic methods [7–9].

One can also write the corresponding saturation functions for allosteric activators or inhibitors:

$$\bar{Y}_A = \frac{V_a}{V_{a_{max}}} = \frac{\gamma(1+\gamma)^{n-1}}{L' + (1+\gamma)^n} \quad (6)$$

$$\bar{Y}_I = \frac{\beta(1+\beta)^{n-1}}{L' + (1+\beta)^n}. \quad (7)$$

It is clear from this that a linear graph can be drawn which permits the determination of n and L' for activators and inhibitors.

3. Application of method

Three examples will be described here to show the applicability of the method. The first utilizes kinetic

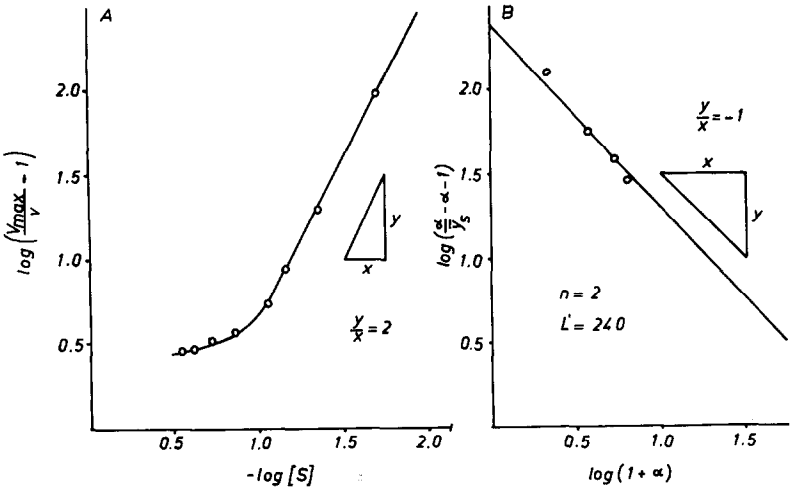

Fig. 4. Experimental data for the Mg^{2+}-dependent inorganic pyrophosphatase plotted as $\log(V_{max}/v - 1)$ versus $-\log(S)$ [9], compared with the plot $\log[(\alpha/\bar{Y}_S - \alpha - 1)$ versus $\log(1+\alpha)$. In the assay $MgPP_i^{2-}$ was the substrate with $[Mg^{2+}]/[PP_i^{4-}]$ ratios of 0.5:1.

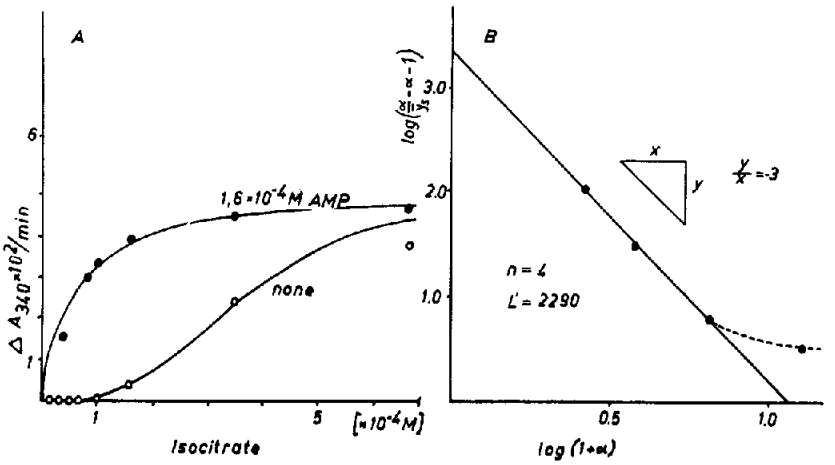

Fig. 5. Experimental data for the enzyme NAD-isocitrate dehydrogenase from yeast. Left side, results (points) obtained by Hataway and Atkinson [11]. Solid lines represent the recalculated curves according to equation (1) (lower curve), and $v/V_{max} = \alpha/(1+\alpha)$ (upper curve), respectively. L' and n were taken from the plot on the right side, V_{max} and K_m were calculated from the original values (points) measured in the presence of 1.6×10^{-4} M AMP. Right side, plot of log $[(\alpha/\bar{Y}_S)-\alpha-1]$ versus $\log(1+\alpha)$ with data calculated from results (points) on the left side.

data obtained from the Mg^{2+}-dependent inorganic pyrophosphatase (EC 3.6.1.1) of mouse liver cytoplasm. In a previous paper we found that this enzyme shows allosteric properties of a K-system with homotropic and heterotropic effects [10]. Examination of the data of the Hill plot for substrate and comparison with the new plot clearly shows that the data on the straight line in the former fit well on the straight line in the latter (fig. 4).

From the graph in fig. 4 we have calculated $n = 2$ and $L' = 240$. The parameters $V_{max} = 3.7$ μmoles $\times$ min^{-1} $\times$ mg protein^{-1}, and $K_S = 1.6 \times 10^{-5}$ M, which are necessary for constructing the plot, were calculated from the part of the curve with a Hill coefficient $\bar{n} = 1$.

In the next example we utilize data obtained from the yeast NAD-isocitrate dehydrogenase (EC 1.1.1.41) characterized by Hataway and Atkinson [11].

The results are shown in fig. 5. From the Michaelis hyperbola (fig. 5A) measured in the presence of activator AMP we have calculated $V_{max} = 5.12$ (A_{340} $\times$ 10^2/min), and $K_m = 6 \times 10^{-5}$ M. Using these data in our plot we obtained a straight line of slope -3, corresponding to $n = 4$, and an intercept on the ordinate of 3.38, corresponding to $L' = 2290$. Calculation of equation (1) with these parameters yields curves (fig. 5A, solid lines) which fit the experimental points fairly well. Only the final experimental value (fig. 5A) deviates from the calculated sigmoidal shaped curve.

In the last example we took the α and $\bar{Y}$ values given in fig. 7 in the paper of Madsen and Shechosky [12], about muscle glycogen phosphorylase (EC 2.4.1.1). With these data the linear graph gave three straight lines (fig. 6) with slopes equal to -1, corresponding to $n = 2$. From the intercepts on the vertical axis values of $L' = 115$, 43 and 13, were calculated, corresponding to inhibitor concentrations of 15, 10.2 and 6.4 mM ATP, respectively. The values thus obtained are quite similar to the constants $L = 93$, 38 and 12, which Madsen and Shechosky used for fitting their experimental data.

It should be emphasized that errors may be introduced in the process of transfer of data from the original paper. These errors affect the calculations and might be responsible for the deviations of the measured points from linearity in the sensitive range of the plot (low and high values of $\log(1+\alpha)$). On the other hand, systematic errors due to the assumptions made, cannot be excluded, i.e. the possibility that $c > 0$.

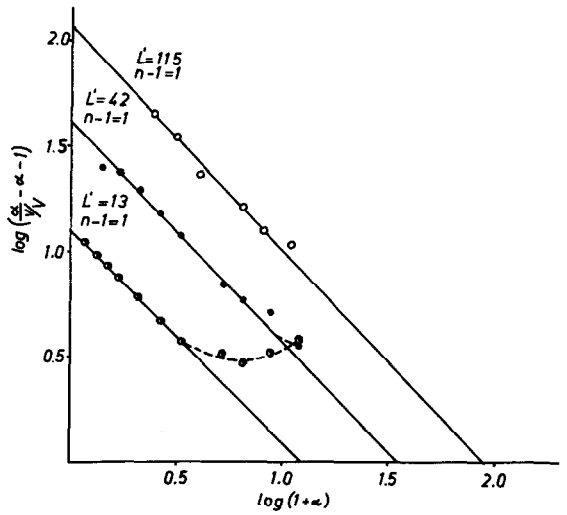

Fig. 6. Plot of log $[(\alpha/\bar{Y}_S - \alpha - 1]$ versus $\log(1+\alpha)$. Experimental data for muscle phosphorylase *b* from Madsen and Shechosky [12]. Assay conditions: 1 mM AMP plus (○) 6.2 mM ATP, (●) 10.2 mM ATP, (◉) 15 mM ATP, respectively.

4. Discussion

It is evident from the derivation that the graph proposed here is restricted to allosteric enzymes with simple exclusive ligand binding ($c = 0$). (For the general assumptions and restrictions of the model see references [1] and [6]).

However, it seems to us that the graph does not permit the distinction to be made from other possible models. As Koshland [3] has shown for hemoglobin, several different models may fit experimental saturation curves fairly well.

Moreover, the construction of the plot requires the value for K_S. Therefore, one needs effectors which shift the kinetics from sigmoidal to hyperbolic (Michaelis-Menten behaviour, $L = 0$). As the estimate of K_S may be subject to certain errors, the assay must be sensitive over a wide range of substrate concentrations, and artefacts must be ruled out.

On the other hand, the graph yields straight lines, which permit the determination of n and L' (or L) even by least square methods. It is evident that homotropic and heterotropic effects of substrate, inhibitor, and activator can be interpreted in terms of the M-W-C model.

Allosteric enzymes with non-exclusive ligand binding ($c > 0$) do not give straight lines, but show sigmoidal curves. The slopes of their tangents drawn at the inflection points did not correspond to $(n-1)$. In addition the slopes change with varying values of L, which does not happen when $c = 0$. In the latter case, the slopes of the straight lines are always integers and remain constant with changing L. To completely rule out non-exclusive binding, one would have to investigate several sets of data obtained under conditions in which different values of L' obtain.

The possible application of the graph to cases with non-exclusive ligand binding are at present being investigated.

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