Biochimica et Biophysica Acta, 377 (1975) 197—202

BBA 67383

ACTIVE SITE DIRECTED EFFECTORS OF ALLOSTERIC ENZYMES

G.D. SMITH, D.V. ROBERTS and P.W. KUCHEL

Department of Physical Biochemistry, The John Curtin School of Medical Research, Australian National University, P.O. Box 334, Canberra City, A.C.T., 2601 (Australia)

(Received August 12 th, 1974)

Summary

This communication introduces the concept of an active site directed effector, in terms of the two state model of Monod et al. (Monod, J., Wyman, J. and Changeux, J.-P. (1965) J. Mol. Biol. 12, 88—118), a consideration made necessary by the observation that the activity of a number of enzymes of the control type is modulated by effector molecules whose structure is similar to that of the substrate. We present equations which describe the kinetic responses obtained in its absence; this seemingly paradoxical activation, at low [*S*], is not tions the *v* versus [*S*] plot obtained in the presence of the effector crosses that obtained in its absence; this seemingly paradoxial activation, at low [*S*], is not explainable by the other frequently used two state models (Monod, J., Wyman, J. and Changeux, J.-P. (1965) J. Mol. Biol. 12, 88—118; Rubin, M.M. and Changeux, J.-P. (1966) J. Mol. Biol. 21, 265—274; Frieden, C. (1967) J. Biol. Chem. 242, 4045—4052; Dalziel, K. (1968) FEBS Lett. 1, 346—348 and Nichol, L.W., O'Dea, K. and Baghurst, P.A. (1972) J. Theor. Biol. 34, 255—263). The model is discussed using examples taken from the literature and successfully used to reanalyse published data on the enzyme deoxythymidine diphosphate D-glucose pyrophosphorylase (Frieden, C. (1967) J. Biol. Chem. 242, 4045—4052).

Introduction

Homotropic and heterotropic control of the activity of a number of enzymes can be satisfactorily explained by theories in which substrates and effectors bind with different affinities to alternate isomeric [1—4] or polymeric [3,5] forms of an enzyme which coexist in rapid equilibrium. It is usually postulated that the effector molecules bind to sites on the enzyme which are distinct from the substrate binding (active) sites, and with certain enzymes this is undoubtedly the case [6]. However with other enzymes the similarity of the

chemical structures of the substrate and effector molecules suggests that both may compete for the same sites on the enzyme molecules, and the consequences of this behavior appear not to have been explicitly considered. We thus present equations which describe this competitive situation and which may be used to interpret experimental kinetic data. We have reinterpreted kinetic data [3,7] obtained with the enzyme deoxythymidine diphosphate D-glucose pyrophosphorylase, whose substrate and effector (metabolic end product inhibitor) are of similar chemical structure. Previously these results had been interpreted [3,7] in terms of separate binding sites for substrate and end product inhibitor, but their fit to the present theory shows that competition for the same sites is a plausible and simpler alternative. Finally, although the effector competes for the same sites as the substrate, the model is capable of predicting both inhibition and activation.

Theory

Consider an oligomeric enzyme which in solution exists in two forms, E_1 and E_2, in equilibrium,

$$m\,E_1 \rightleftharpoons E_2 \;;\; X = [E_2]/[E_1]^m \tag{1}$$

where X is the association equilibrium constant. When $m = 1$, Eqn 1 describes an isomerization, and when $m > 1$ a polymerization. Substrate (S) interacts with n_1 and n_2 catalytic sites on E_1 and E_2, with intrinsic dissociation constants $K_{1\,S}$ and $K_{2\,S}$, respectively. A ligand (L), acting as an active site competitor, binds at the same n_1 sites on E_1 and the same n_2 sites on E_2 with respective intrinsic dissociation constants, $K_{1\,L}$ and $K_{2\,L}$. The simplest scheme that will yield sigmoidal kinetics is one in which there are at least two catalytic sites on one or both forms of the enzyme [1]. In a solution of E, S and L there will exist unbound S and L and a series of complexes of $E_h S_i L_j$, where $0 \leqslant i + j \leqslant n_h$, $h = 1,2$.

The intrinsic dissociation constants for each ligand can be expressed as

$$K_{hS} = [E_h S_{i-1} L_j]\,[S]/[E_h S_i L_j] \;;\; h = 1,2 \tag{2a}$$

$$K_{hL} = [E_h S_i L_{j-1}]\,[L]/[E_h S_i L_j] \;;\; h = 1,2. \tag{2b}$$

with a statistical weighting factor (combination) given by ref. 8

$$w_{ij} = n_h!/i!j!\,(n_h - i - j)! \;\;;\; h = 1,2 \tag{3}$$

The binding of both substrate and ligand can be expressed as a molar binding function [9],

$$r_S = ([S_0] - [S])/[E_0] \tag{4a}$$

$$r_L = ([L_0] - [L])/[E_0] \tag{4b}$$

where $[S]$ and $[L]$ are the molar concentrations of unbound substrate and ligand, respectively, and the subscript 0 refers to the total base—molar concen-

trations of the particular species. It can be shown [8] that

$$r_S = \frac{n_1[E_1]\frac{[S]}{K_{1S}}\left(1+\frac{[S]}{K_{1S}}+\frac{[L]}{K_{1L}}\right)^{n_1-1} + n_2[E_2]\frac{[S]}{K_{2S}}\left(1+\frac{[S]}{K_{2S}}+\frac{[L]}{K_{2L}}\right)^{n_2-1}}{[E_0]} \quad (5)$$

where: $[E_0] = [E_1]\left(1+\frac{[S]}{K_{1S}}+\frac{[L]}{K_{1L}}\right)^{n_1} + m[E_2]\left(1+\frac{[S]}{K_{2S}}+\frac{[L]}{K_{2L}}\right)^{n_2}$ (6)

The binding equation for L is entirely analogous to that written for S in Eqn 5.

The product (P) can only be formed by the breakdown of those complexes $E_hS_iL_j$ that contain S. If it is assumed that the rate of breakdown to product of these complexes is the rate-determining step and is governed by unique intrinsic catalytic constants, k_1 for E_1 and k_2 for E_2 then, as Dalziel [4] has shown, binding equations (e.g. Eqn 5) can be directly transposed to kinetic expressions for the overall initial velocity of the reaction where K_{1S} and K_{2S} are now defined as microscopic Michaelis constants. Thus, since the numerator of Eqn 5 is the total concentration of all enzyme–substrate complexes, the overall reaction velocity is given by

$$v_0 = n_1k_1[E_1]\frac{[S]}{K_{1S}}\left(1+\frac{[S]}{K_{1S}}+\frac{[L]}{K_{1L}}\right)^{n_1-1} + n_2k_2[E_2]\frac{[S]}{K_{2S}}\left(1+\frac{[S]}{K_{2S}}+\frac{[L]}{K_{2L}}\right)^{n_2-1} \quad (7a)$$

hence

$$v_0 = \frac{V_1\frac{[S]}{K_{1S}}\left(1+\frac{[S]}{K_{1S}}+\frac{[L]}{K_{1L}}\right)^{n_1-1} + V_2X[E_1]^{m-1}\frac{[S]}{K_{2S}}\left(1+\frac{[S]}{K_{2S}}+\frac{[L]}{K_{2L}}\right)^{n_2-1}}{\left(1+\frac{[S]}{K_{1S}}+\frac{[L]}{K_{1L}}\right)^{n_1} + mX[E_1]^{m-1}\left(1+\frac{[S]}{K_{2S}}+\frac{[L]}{K_{2L}}\right)^{n_2}} \quad (7b)$$

where $V_h = n_hk_h[E_0]$, $h = 1,2$.

It may be noted that with $X = 0$, Eqn 7 reduces to

$$v_0 = \frac{V_1[S]}{K_{1S}\left(1+\frac{[L]}{K_{1L}}\right)+[S]} \quad (8)$$

which is the equation describing competitive inhibition of a simple Michaelis–Menten type enzyme.

Results and Discussion

For simplicity we will consider only isomerization cases ($m = 1$) where also $n_1 = n_2 = n$ and $k_1 = k_2$ so that $V_1 = V_2 = V_m$. For this case Eqn 7b

becomes

$$v_0 = \frac{V_m \left\{ \frac{[S]}{K_{1S}} \left(1 + \frac{[S]}{K_{1S}} + \frac{[L]}{K_{1L}}\right)^{n-1} + X\frac{[S]}{K_{2S}} \left(1 + \frac{[S]}{K_{2S}} + \frac{[L]}{K_{2L}}\right)^{n-1} \right\}}{\left(1 + \frac{[S]}{K_{1S}} + \frac{[L]}{K_{1L}}\right)^{n} + X\left(1 + \frac{[S]}{K_{2S}} + \frac{[L]}{K_{2L}}\right)^{n}} \qquad (9)$$

The various types of behaviour which may be observed with systems described by Eqn 9 are best illustrated with numerical examples considered in relation to experimental results. When S binds preferentially to E_1, and E_2 predominates initially (large X), the kinetic plot obtained in the absence of L is sigmoidal in form, as shown by Curve a in Fig. 1. If L also binds preferentially to E_1 then its addition results in behaviour as shown by Curve c, in which activation is observed at low $[S]$ and inhibition at high $[S]$. The cross-over of Curves a and c in this case is a characteristic of the competitive model described by Eqn 9, although analysis of this equation shows that the presence or absence of cross-over is dependent on the magnitudes of the various parameters; it can be shown mathematically that such a cross-over is never exhibited by the alternate effector site model, where the basic equation is of different form (cf. Eqn 2a of ref. 3) and its observation excludes the latter model. In contrast, if L were to bind preferentially to E_2, inhibition over the entire range of $[S]$ would be observed as shown in Curve b of Fig. 1. Both types of kinetic behaviour have been observed with the enzyme deoxycytidylate aminohydrolase (Fig. 5 of ref. 10) which is activated (at low substrate concentration) by deoxycytidine triphosphate and inhibited by deoxythymidine triphosphate. The authors [10] considered the binding of these effectors in terms of separate sites but in view of their structural similarity to the substrate, deoxycytidine monophosphate, and the kinetic similarity to that of Fig. 1, the competitive model described by Eqn 9 seems equally plausible.

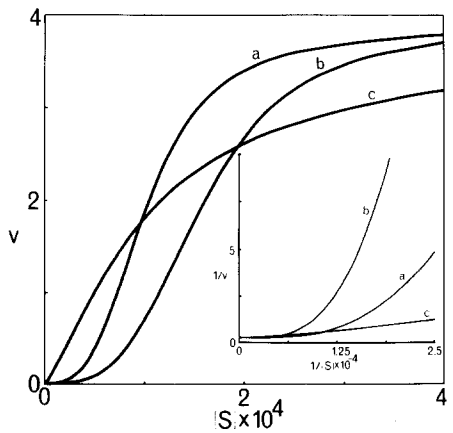

Fig. 1. Plots of initial velocity versus substrate concentration and corresponding double reciprocal plots (inset) calculated with Eqn 9 for an enzyme with four catalytic sites (n = 4) on each of two alternative conformations in equilibrium, and with the following parameters: V_m = 4, $K_{1S} = 2 \cdot 10^{-5}$, $K_{2S} = 2 \cdot 10^{-2}$, $X = 1 \cdot 10^3$. The additional parameters for each curve are: Curve a, $[L] = 0$; Curve b, $K_{1L} = 2 \cdot 10^{-3}$, $K_{2L} = 2 \cdot 10^{-4}$, $[L] = 1 \cdot 10^{-4}$ M; Curve c, $K_{1L} = 2 \cdot 10^{-5}$, $K_{2L} = 2 \cdot 10^{-2}$, $[L] = 8 \cdot 10^{-5}$ M.

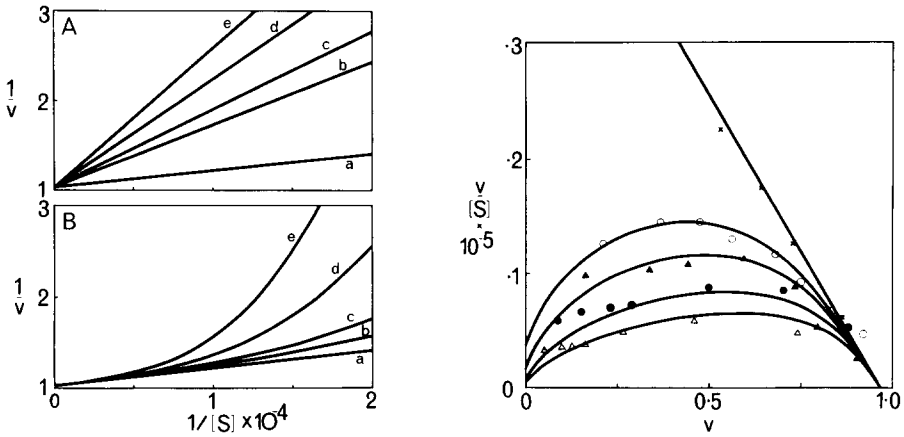

Fig. 2. Double reciprocal plots calculated with Eqn 9 and the following values of the parameters: $n = 4$, $V_m = 0.967$, $X = 0.1$, $K_{1S} = 1.8 \cdot 10^{-5}$, $K_{2S} = 6 \cdot 10^{-4}$. A, $K_{1L} = 1.1 \cdot 10^{-5}$, $K_{2L} = 3.67 \cdot 10^{-4}$ and B, $K_{1L} = 3.67 \cdot 10^{-4}$, $K_{2L} = 1.1 \cdot 10^{-5}$. The molar concentrations of unbound effector, $[L]$, are 0 (a), $3 \cdot 10^{-5}$ M (b), $4 \cdot 10^{-5}$ M (c), $6 \cdot 10^{-5}$ M (d) and $8 \cdot 10^{-5}$ M (e).

Fig. 3. An interpretation of experimental data taken from Frieden [3] in terms of Eqn 9. The concentrations of the effector, [L] are: ×, none; ○, $3 \cdot 10^{-5}$ M; ▲, $4 \cdot 10^{-5}$ M; ●, $6 \cdot 10^{-5}$ M; △, $8 \cdot 10^{-5}$ M. Other parameters used for calculation of the solid lines are as given in Fig. 2B.

An alternative situation is where S binds preferentially to the predominant form E_1 (small X). In the absence of L the plot of v versus $[S]$ is essentially hyperbolic; i.e. essentially linear in double reciprocal form (Curves A in Fig. 2). If L also binds preferentially to E_1 the form of the plots resembles classical linear competitive inhibition (Fig. 2A). However, addition of L when L binds preferentially to E_2 results in plots of v versus $[S]$ which become increasingly sigmoidal as the concentration of effector is increased: this is illustrated in Fig. 2B by increasing curvature of the double reciprocal plots. For the enzyme deoxythymidine diphosphate D-glucose pyrophosphorylase [3,7] inhibition by the product of the reaction (deoxythymidine triphosphate) results in kinetic behaviour as depicted in Fig. 2A whereas inhibition by the end product of the metabolic pathway (deoxythymidine diphosphate L-rhamnose) is of the form depicted in Fig. 2B. Frieden [3] has interpreted the latter behaviour in terms of a model in which the inhibitor binds at sites other than the catalytic sites (Fig. 3 of ref. 3). The solid lines in Fig. 3, computed with Eqn 9 and the same ratios of K_{1S}/K_{2S} and K_{1L}/K_{2L} as employed by Frieden are seen to describe the experimental results equally as well and thus the present model is equally plausible.

The present theory can be extended to describe the antagonism between two or more effectors in the presence of substrate by the addition of terms $[M]/K_{hM}$, $[N]/K_{hN}$, $h = 1,2$ etc. inside the brackets in Eqns 7 and 9. Moreover, the conclusions drawn above are also applicable to enzymes which polymerize, their kinetics being distinguishable from those for an isomerizing enzyme by dependence of the kinetic behaviour on enzyme concentration. The model can also be extended to V systems [1], where the intrinsic catalytic constants, k_1 and k_2, for the complexes of E_1 and E_2 are different.

In conclusion, it is hoped that this theoretical treatment of the competitive model may assist the interpretation of kinetic results obtained with other enzymes, particularly those whose substrates and effectors have structural similarity, for example, phenylalanine ammonia lyase [11], phosphoenolpyruvate carboxylase [12], deoxythymidine kinase [13], NAD-isocitrate dehydrogenase [14] and the catalytic subunit of aspartate transcarbamylase [15].

Acknowledgement

We wish to thank Professor L.W. Nichol for valuable discussion and criticism of this manuscript.

References

1 Monod, J., Wyman, J. and Changeux, J.-P. (1965) J. Mol. Biol. 12, 88—118
2 Rubin, M.M. and Changeux, J.-P. (1966) J. Mol. Biol. 21, 265—274
3 Frieden, C. (1967) J. Biol. Chem. 242, 4045—4052
4 Dalziel, K. (1968) FEBS Lett. 1, 346—348
5 Nichol, L.W., O'Dea, K. and Baghurst, P.A. (1972) J. Theor. Biol. 34, 255—263
6 Gerhart, J.C. and Schachman, H.K. (1965) Biochemistry 4, 1054—1062
7 Melo, A. and Glaser, L. (1965) J. Biol. Chem. 240, 398—405
8 Nichol, L.W., Smith, G.D. and Ogston, A.G. (1969) Biochim. Biophys. Acta 184, 1—10
9 Klotz, I.M. (1953) The Proteins (Neurath, H. and Bailey, K., eds), Vol. 1B, 1st edn, pp. 727—806, Academic Press Inc., New York
10 Scarano, E., Geraci, G. and Rossi, M. (1967) Biochemistry 6, 192—201
11 Nari, J., Mouttet, C., Fouchier, F. and Ricard, J. (1974) Eur. J. Biochem. 41, 499—515
12 Izui, K. (1970) J. Biochem. Tokyo 68, 227—238
13 Okazaki, R. and Kornberg, A. (1964) J. Biol. Chem. 239, 275—284
14 Sanwal, B., Zink, M. and Stachow, C. (1964) J. Biol. Chem. 239, 1597—1603
15 Heyde, E. (1973) Biochim. Biophys. Acta 293, 351—358