Rates of reactions catalysed by a dimeric enzyme

Effects of the reaction scheme and the kinetic parameters on co-operativity

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For the reaction $S \rightleftharpoons P$ catalysed by a dimeric enzyme, the reaction schemes are considered on the basis of the KNF model. For each of the ten possible schemes, the rate equation is derived on the basis of the combined steady-state and rapid-equilibrium assumptions. The curves of the plots of initial velocity v versus the substrate concentration $[S]$ and the Hill coefficients h calculated from the rate equations depend strongly on the reaction scheme and the parameter X_1 . This parameter is defined by $\log(K_{s2}/K_{s1})$ and is a measure of the relative affinities of the first and second protomers for the substrate. When $X_1 < 0$, v - $[S]$ curves for some schemes exhibit negative co-operativity ($h < 1.0$) and v - $[S]$ curves for other schemes are similar to that of the Michaelis–Menten scheme, indicating that, even if there is interaction between the distinct protomers, sigmoidal rate behaviour is not necessarily observed. When $X_1 > 0$, all the reaction schemes except one, which shows substrate-inhibition kinetic behaviour, exhibit sigmoidal kinetic behaviour ($h > 1.0$), and at the limit of $X_1 \gg 0$ the Hill coefficients attain the maximum possible value of 2.0. Furthermore, we have found that, even if $X_1 = 0$, the v - $[S]$ curve for almost all the schemes considered in the present work does not necessarily agree with that for the Michaelis–Menten scheme. This means that the deviation of the v - $[S]$ curve from a hyperbola can be observed even if there is no interaction between the distinct protomers.

INTRODUCTION

Many enzymes are oligomers consisting of two or more protomers or subunits. These enzymes exhibit sigmoidal curves of plots of initial velocity versus substrate concentration (v - $[S]$ curves) that are different from that for the Michaelis–Menten scheme. They are generally classified as allosteric enzymes, and are physiologically very important because of their catalysis and metabolic regulation. The unique features of these enzymes have been well explained by the models proposed by Monod *et al.* (1965) (the MWC model) and Koshland *et al.* (1966) (the KNF model). Both models assumed that the co-operativity of the distinct protomers originates with the conformation change, which occurs in a different fashion in the two models. In these analyses, only sigmoidal ligand-saturation curves were generated on the basis of very simple equilibrium reactions in which a single kind of ligand binds to the enzymes. However, sigmoidal v - $[S]$ curves can also be obtained with additional assumptions (Frieden, 1967). There has been no systematic investigation concerning how the rate equations can be derived for the multiple substrates–multiple products/oligomeric enzyme system and how the reaction scheme, the rate and the equilibrium constants affect the co-operativity. Therefore, as a first step, the present work was undertaken to clarify the effects of the kinetic scheme and the rate and the equilibrium constants of the single substrate–single product reaction catalysed by a dimeric enzyme consisting of two identical protomers on the co-operativity or the v - $[S]$ curves on the basis of the KNF model.

ANALYSIS

General reaction scheme and its reaction rate

For a single substrate–single product/dimeric enzyme system, the general reaction scheme can be expressed by Scheme I, where E is a dimeric enzyme consisting of two identical protomers, each of which has one binding site for S or P, and S and P are the substrate and product respectively. ES and EP are complexes in which one of the protomers is bound to S and to P respectively. ESS, ESP and EPP are complexes in which both the protomers are bound to S and S, to S and P and to P and P respectively. The reaction scheme expressed by Scheme I includes all the possible steps, reaction steps and binding or dissociation steps of substrate and product, and consists of the following ten steps:

Scheme I

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where k_{+1} – k_{-10} are the rate constants. In this reaction scheme there are six enzyme forms, i.e. one free enzyme and five enzyme complexes. The rate equation for Scheme I can be derived by the use of a computer program developed by the present authors, which is an advanced version of the previous program (Ishikawa *et al.*, 1988). When the steady-state assumption is applied to all the enzyme forms, the rate equation is derived as:

$$\frac{v}{[E]_0} = \frac{\alpha_1[S] + \alpha_2[S]^2 + \alpha_3[P] + \alpha_4[P]^2 + \alpha_5[S][P] + \alpha_6[S]^3 + \alpha_7[S]^2[P] + \alpha_8[S][P]^2 + \alpha_9[P]^3}{\beta_0 + \beta_1[S] + \beta_2[S]^2 + \beta_3[P] + \beta_4[P]^2 + \beta_5[S][P] + \beta_6[S]^3 + \beta_7[S]^2[P] + \beta_8[S][P]^2 + \beta_9[P]^3} \quad (11)$$

Eqn. (11) has only 19 independent constants, α_j and β_j , which are functions of the 20 rate constants k_{+1} – k_{-10} . Each constant includes from eight to 146 terms, each of which consists of a product of the rate constants. The values of α_j and β_j can be determined by correlating the experimental reaction rates measured at various concentrations of the substrate, the product and the total enzyme to eqn. (11). Although it is possible to study theoretically the effect of any specific reaction step or its rate constant on the reaction rate, it is not possible to determine 20 rate constants from only 19 experimental values of α_j and β_j . Therefore, because it is important to study the effect of the specific reaction steps, of which the kinetic constants can be determined experimentally, on the overall reaction rate, we introduce some simplification techniques in order to reduce the number of kinetic constants to the same as or less than that of the experimentally obtainable α_j and β_j .

First, we assume that both the binding rates of the substrate to the enzyme and the dissociation rates of the product from the enzyme are much faster than that of a reaction in which the change in the molecular structure of the substrate or product occurs. Therefore the rapid-equilibrium assumption can be applied to the steps expressed by eqns. (1)–(6). The equilibrium constants are defined by:

$$K_1 = k_{+1}/k_{-1}, \quad K_2 = k_{+2}/k_{-2}, \quad K_3 = k_{+3}/k_{-3} \quad (12)–(14)$$

$$K_4 = k_{+4}/k_{-4}, \quad K_5 = k_{+5}/k_{-5}, \quad K_6 = k_{+6}/k_{-6} \quad (15)–(17)$$

When the rapid-equilibrium assumption is applied to the steps expressed by eqns. (1)–(6) and the steady-state assumption is applied to the steps expressed by eqns. (7)–(10), the rate equation can be derived as:

$$\frac{v}{[E]_0} = \frac{\alpha_1[S] + \alpha_2[S]^2 + \alpha_3[P] + \alpha_4[P]^2 + \alpha_5[S][P]}{\beta_0 + \beta_1[S] + \beta_2[S]^2 + \beta_3[P] + \beta_4[P]^2 + \beta_5[S][P]} \quad (18)$$

Eqn. (18) has only 11 independent constants, α_j and β_j , each of which is a function of some of the eight rate constants and the six equilibrium constants. These 14 kinetic constants can be determined from the experimental α_j and β_j values. The number of experimental data must be equal to or more than 14. Therefore, at this stage, the 11 experimental data of α_j and β_j that have now been obtained are still not enough to determine the 14 kinetic constants.

In the above, all the kinetic constants were regarded as being independent. However, we must take into consideration the relationship between the rate constants and the relationship between the equilibrium constants. As the enzyme consists of two identical protomers, it may be sufficient to consider only two sets of the kinetic constants: K_{S1} , K_{P1} , k_{t1} and k_{r1} for the steps in which the first protomer is concerned and K_{S2} , K_{P2} , k_{t2} and k_{r2} for the steps in which the second protomer is concerned. K_8 is an equilibrium constant of the step in which the substrate binds or

dissociates, K_p is an equilibrium constant of the step in which the product binds or dissociates, and k_t and k_r are the rate constants for the forward and the backward reactions of the slow reaction step respectively. The equilibrium and the rate constants mentioned above are related to these new equilibrium and rate constants as:

$$K_1 = 2K_{S1}, \quad K_2 = K_{S2}/2, \quad K_3 = K_{S2} \quad (19)–(21)$$

$$K_4 = 2K_{P1}, \quad K_5 = K_{P2}, \quad K_6 = K_{P2}/2 \quad (22)–(24)$$

$$k_{+7} = 2k_{t1}, \quad k_{+8} = k_{t1}, \quad k_{+9} = k_{t1}, \quad k_{+10} = k_{t2} \quad (25)–(28)$$

$$k_{-7} = k_{r1}, \quad k_{-8} = 2k_{r1}, \quad k_{-9} = k_{r1}, \quad k_{-10} = k_{r2} \quad (29)–(32)$$

As a result, the six equilibrium constants are expressed by the four equilibrium constants, K_{S1} , K_{S2} , K_{P1} and K_{P2} , and the eight rate constants are related to the four rate constants, k_{t1} , k_{t2} , k_{r1} and k_{r2} . Theoretically, these eight kinetic constants can be evaluated with 11 experimental data of α_j and β_j values.

Rate equation for each reaction scheme

The general reaction scheme shown above includes all the possible steps, reaction steps and binding and dissociation steps of substrate and product. However, from a practical viewpoint, some of them can be neglected. If some steps of the general reaction scheme (Scheme I) are neglected, it reduces to one of the ten schemes, Schemes 1–10 given in Table 1. In these Schemes some steps are assumed to be in equilibrium as mentioned above.

The rate equation was derived for each of Schemes 1–10. The rate equations can be expressed generally by eqn. (18). The constants α_j and β_j in the equation are functions of the kinetic constants: the rate constants k_{t1} , k_{t2} , k_{r1} and k_{r2} and the equilibrium constants K_{S1} , K_{S2} , K_{P1} and K_{P2} . Table 2 gives the α_1 – α_5 and β_0 – β_5 that appeared in the rate equation for each reaction scheme as functions of the kinetic constants. In Schemes 3, 5, 8 and 10 the following relation:

$$\frac{K_{S1}}{K_{S2}} = \frac{K_{P1}}{K_{P2}} \quad (33)$$

must hold. For all reaction schemes except Scheme 4, since the number of experimental data of α_j and β_j is equal to or more than that of the rate and equilibrium constants, they can be evaluated with the experimental α_j and β_j values. In the case of Scheme 4, the rate equation includes eight kinetic constants, but only seven values of α_j and β_j can be obtained by correlating the experimental reaction data. Therefore the kinetic constants cannot be determined by using only the seven values of α_j and β_j . These kinetic constants can be determined with the aid of the relation between the Hill coefficient h and parameter X_1 , which is mentioned in the Discussion section below, in addition to the experimental seven values of α_j and β_j .

When $[P] = 0$, the curves of the plots of initial velocity versus substrate concentration (v – $[S]$ curves) calculated from eqn. (18) with the constants α_1 , α_2 and β_0 – β_2 in Table 2 are generally different from that calculated from the Michaelis–Menten equation for an irreversible reaction, as is shown below. However, when $[P] = 0$ and $K_{S1} = K_{S2}$, the rate equation for Scheme 8 reduces to the Michaelis–Menten rate equation. Furthermore, when $[P] = 0$, $K_{S1} = K_{S2}$ and $k_{t1} = k_{t2}$, the rate equation for Scheme 6 also reduces to the Michaelis–Menten rate equation.

Table 1. Reaction schemes considered in the present work

In the following schemes $\rightleftharpoons$ expresses the reaction step where the steady-state assumption is applied, and $\longleftrightarrow$ expresses the reaction step where the rapid-equilibrium assumption is applied.

Table 2. Relationship between coefficients in eqn. (18) and rate and equilibrium constants in Schemes 1–10

Scheme 1

$$\alpha_1 = 0, \alpha_2 = 2K_{S1}K_{S2}k_{t2}, \alpha_3 = 0, \alpha_4 = -2K_{P1}K_{P2}k_{r2}, \alpha_5 = 0$$

$$\beta_0 = 1, \beta_1 = 2K_{S1}, \beta_2 = K_{S1}K_{S2}, \beta_3 = 2K_{P1}, \beta_4 = K_{P1}K_{P2}, \beta_5 = 0$$

Scheme 2

$$\alpha_1 = 0, \alpha_2 = 4K_{S1}K_{S2}k_{t1}^2, \alpha_3 = 0, \alpha_4 = -4K_{P1}K_{P2}k_{r1}^2, \alpha_5 = 0$$

$$\beta_0 = k_{t1} + k_{r1}, \beta_1 = 2K_{S1}(k_{t1} + k_{r1}), \beta_2 = K_{S1}K_{S2}(3k_{t1} + k_{r1}), \beta_3 = 2K_{P1}(k_{t1} + k_{r1}), \beta_4 = K_{P1}K_{P2}(k_{t1} + 3k_{r1}), \beta_5 = 0$$

Scheme 3

$$\alpha_1 = 0, \alpha_2 = 2K_{S1}K_{S2}k_{t1}, \alpha_3 = 0, \alpha_4 = -2K_{P1}K_{P2}k_{r1}, \alpha_5 = 2K_{S2}K_{P1}(k_{t1} - k_{r1})$$

$$\beta_0 = 1, \beta_1 = 2K_{S1}, \beta_2 = K_{S1}K_{S2}, \beta_3 = 2K_{P1}, \beta_4 = K_{P1}K_{P2}, \beta_5 = 2K_{S2}K_{P1}$$

Scheme 4

$$\alpha_1 = 0, \alpha_2 = 2K_{S1}K_{S2}(2k_{t1}^2 + k_{t1}k_{t2} + k_{t2}k_{r1}), \alpha_3 = 0, \alpha_4 = -2K_{P1}K_{P2}(k_{t1}k_{r2} + 2k_{r1}^2 + k_{r1}k_{r2}), \alpha_5 = 0$$

$$\beta_0 = k_{t1} + k_{r1}, \beta_1 = 2K_{S1}(k_{t1} + k_{r1}), \beta_2 = K_{S1}K_{S2}(3k_{t1} + k_{r1}), \beta_3 = 2K_{P1}(k_{t1} + k_{r1}), \beta_4 = K_{P1}K_{P2}(k_{t1} + 3k_{r1}), \beta_5 = 0$$

Scheme 5

$$\alpha_1 = 0, \alpha_2 = 2K_{S1}K_{S2}(k_{t1} + k_{t2}), \alpha_3 = 0, \alpha_4 = -2K_{P1}K_{P2}(k_{r1} + k_{r2}), \alpha_5 = 2K_{S2}K_{P1}(k_{t1} - k_{r1})$$

$$\beta_0 = 1, \beta_1 = 2K_{S1}, \beta_2 = K_{S1}K_{S2}, \beta_3 = 2K_{P1}, \beta_4 = K_{P1}K_{P2}, \beta_5 = 2K_{S2}K_{P1}$$

Scheme 6

$$\alpha_1 = 2K_{S1}k_{t1}, \alpha_2 = 2K_{S1}K_{S2}k_{t2}, \alpha_3 = -2K_{P1}k_{r1}, \alpha_4 = -2K_{P1}K_{P2}k_{r2}, \alpha_5 = 0$$

$$\beta_0 = 1, \beta_1 = 2K_{S1}, \beta_2 = K_{S1}K_{S2}, \beta_3 = 2K_{P1}, \beta_4 = K_{P1}K_{P2}, \beta_5 = 0$$

Scheme 7

$$\alpha_1 = 2K_{S1}k_{t1}(k_{t1} + k_{r1}), \alpha_2 = 4K_{S1}K_{S2}k_{t1}^2, \alpha_3 = -2K_{P1}k_{r1}(k_{t1} + k_{r1}), \alpha_4 = -4K_{P1}K_{P2}k_{r1}^2, \alpha_5 = 0$$

$$\beta_0 = k_{t1} + k_{r1}, \beta_1 = 2K_{S1}(k_{t1} + k_{r1}), \beta_2 = K_{S1}K_{S2}(3k_{t1} + k_{r1}), \beta_3 = 2K_{P1}(k_{t1} + k_{r1}), \beta_4 = K_{P1}K_{P2}(k_{t1} + 3k_{r1}), \beta_5 = 0$$

Scheme 8

$$\alpha_1 = 2K_{S1}k_{t1}, \alpha_2 = 2K_{S1}K_{S2}k_{t1}, \alpha_3 = -2K_{P1}k_{r1}, \alpha_4 = -2K_{P1}K_{P2}k_{r1}, \alpha_5 = 2K_{S2}K_{P1}(k_{t1} - k_{r1})$$

$$\beta_0 = 1, \beta_1 = 2K_{S1}, \beta_2 = K_{S1}K_{S2}, \beta_3 = 2K_{P1}, \beta_4 = K_{P1}K_{P2}, \beta_5 = 2K_{S2}K_{P1}$$

Scheme 9

$$\alpha_1 = 2K_{S1}k_{t1}(k_{t1} + k_{r1}), \alpha_2 = 2K_{S1}K_{S2}(2k_{t1}^2 + k_{t1}k_{t2} + k_{t2}k_{r1}), \alpha_3 = -2K_{P1}k_{r1}(k_{t1} + k_{r1}), \alpha_4 = -2K_{P1}K_{P2}(k_{t1}k_{r2} + 2k_{r1}^2 + k_{r1}k_{r2}), \alpha_5 = 0$$

$$\beta_0 = k_{t1} + k_{r1}, \beta_1 = 2K_{S1}(k_{t1} + k_{r1}), \beta_2 = K_{S1}K_{S2}(3k_{t1} + k_{r1}), \beta_3 = 2K_{P1}(k_{t1} + k_{r1}), \beta_4 = K_{P1}K_{P2}(k_{t1} + 3k_{r1}), \beta_5 = 0$$

Scheme 10

$$\alpha_1 = 2K_{S1}k_{t1}, \alpha_2 = 2K_{S1}K_{S2}(k_{t1} + k_{t2}), \alpha_3 = -2K_{P1}k_{r1}, \alpha_4 = -2K_{P1}K_{P2}(k_{r1} + k_{r2}), \alpha_5 = 2K_{S2}K_{P1}(k_{t1} - k_{r1})$$

$$\beta_0 = 1, \beta_1 = 2K_{S1}, \beta_2 = K_{S1}K_{S2}, \beta_3 = 2K_{P1}, \beta_4 = K_{P1}K_{P2}, \beta_5 = 2K_{S2}K_{P1}$$

DISCUSSION

Dependence of v -[S] curves on the reaction scheme and kinetic constants

The effects of the reaction scheme and the rate and the equilibrium constants on the v -[S] curve were studied by using eqn. (18) and the constants α_i and β_i in Table 2. In the calculations we assumed that $[P] = 0$ and $V_{\max} = \alpha_2[E]_0/\beta_2$. The equilibrium constant K_{S1} was fixed at a constant value of 50 M^{-1} . The effects of the other kinetic constants were examined by means of the dimensionless parameters X_1 , X_2 and X_3 defined by:

$$X_1 = \log(K_{S2}/K_{S1}) \quad (34)$$

$$X_2 = k_{t2}/k_{t1} \quad (35)$$

$$X_3 = k_{r1}/k_{r1} \quad (36)$$

Figs. 1–3 show typical v -[S] curves for Schemes 2, 3, 7 and 8. In these Figures the values of the parameter X_1 are taken as -2 , 0 and $+3$, and the values of the parameters X_2 and X_3 are both kept constant at 0.1 and 0 respectively. These figures suggest that the feature of the v -[S] curve depends on both the reaction scheme and the kinetic constants. However, to evaluate quantitatively the effects of these factors on the v -[S] curves or on the degree of deviation of the v -[S] curves from those of the Michaelis–Menten reaction, some supplementary tools are required. The degree of deviation of the v -[S] curves from those of Michaelis–Menten reaction or apparent co-operativity can be quantitatively evaluated by the slopes of the Hill plot (the Hill coefficient h) (Hill, 1910) or the co-operativity index R_x (Taketa & Pogell, 1965). In the present work, the Hill coefficient h was adopted as an index of the apparent co-operativity.

Figs. 4–6 show the Hill plot, where the values of $\log[v/(V_{\max} - v)]$ calculated from the reaction rates shown in Figs. 1–3

Fig. 1. Curves of plots of initial velocity versus substrate concentration for Schemes 2, 3, 7 and 8, calculated by using the parameter values $X_1 = -2$, $X_2 = 0.1$ and $X_3 = 0$

The relation $V_{\max} = \alpha_2[E]_0/\beta_2$ was assumed. Numbers 2, 3, 7 and 8 in the Figure indicate the lines for Schemes 2, 3, 7 and 8 respectively.

are plotted against $\log[S]$. The substrate concentration ranges between $[S]_{0.1}$ and $[S]_{0.9}$, which are the substrate concentrations where the rates are equal to $0.1 \times V_{\max}$ and $0.9 \times V_{\max}$ respectively. At the nine substrate concentrations that divide the range between $\log[S]_{0.1}$ and $\log[S]_{0.9}$ into eight identical intervals, the values of $\log[v/(V_{\max} - v)]$ were calculated. From the slopes of the straight lines correlating the plots, the Hill coefficients h can be determined. As shown in Figs. 4–6, the slopes of the Hill plot or the Hill coefficients depend strongly on the reaction scheme and the value of the parameter X_1 .

Figs. 1 and 4 show that when $X_1 = -2$ ($K_{S2} = K_{S1}/100$) the curves for Schemes 7 and 8 exhibit negative co-operativity (the

Fig. 2. Curves of plots of initial velocity versus substrate concentration for Schemes 2, 3, 7 and 8, calculated by using the parameter values $X_1 = 0$, $X_2 = 0.1$ and $X_3 = 0$

The relation $V_{\max} = \alpha_2[E]_0/\beta_2$ was assumed. Numbers 2, 3, 7 and 8 in the Figure indicate the lines for Schemes 2, 3, 7 and 8 respectively.

Fig. 3. Curves of plots of initial velocity versus substrate concentration for Schemes 2, 3, 7 and 8, calculated by using the parameter values $X_1 = 3$, $X_2 = 0.1$ and $X_3 = 0$

The relation $V_{\max} = \alpha_2[E]_0/\beta_2$ was assumed. Numbers 2, 3, 7 and 8 in the Figure indicate the lines for Schemes 2, 3, 7 and 8 respectively.

values of the Hill coefficient h are less than 1.0) and both the curves for Schemes 2 and 3 are similar to that of the Michaelis-Menten scheme, which exhibits a hyperbolic v -[S] curve ($h = 1.0$). The latter indicates that, even if there is interaction between the distinct protomers, sigmoidal rate behaviour is not necessarily observed.

Figs. 2 and 5 show that when $X_1 = 0$ ($K_{s2} = K_{s1}$), i.e. the affinity of the second protomer towards the substrate is identical with that of the first protomer towards the substrate, only the curve for Scheme 8 coincides with that for the Michaelis-Menten reaction. The curves for Schemes 2, 3 and 7 exhibit positive co-operativity ($h > 1.0$). Therefore deviation of the v -[S] curves from those of the Michaelis-Menten reaction does not necessarily mean the existence of interaction between the distinct protomers.

Figs. 3 and 6 show that when $X_1 = 3$ ($K_{s2} = 1000 K_{s1}$) all the v -[S] curves are sigmoid and exhibit positive co-operativity. The slopes of the Hill plot are almost equal to 2.0.

For each of the other reaction schemes except for Scheme 6, the v -[S] curve is almost similar to one of those for Schemes 2, 3, 7 and 8 under the conditions similar to those in Figs. 1-3. The reaction that obeys Scheme 6 exhibits substrate inhibition, as shown in Fig. 7.

As can be seen in the above Figures, the feature of the v -[S] curve or the degree of apparent homotropic co-operativity

Fig. 4. Hill plots for Schemes 2, 3, 7 and 8, calculated from the curves in Fig. 1 in which the parameter values were $X_1 = -2$, $X_2 = 0.1$ and $X_3 = 0$

Numbers 2, 3, 7 and 8 in the Figure indicate the lines for Schemes 2 (○), 3 (△), 7 (●) and 8 (▲) respectively.

Fig. 5. Hill plots for Schemes 2, 3, 7 and 8, calculated from the curves in Fig. 2 in which the parameter values were $X_1 = 0$, $X_2 = 0.1$ and $X_3 = 0$

Numbers 2, 3, 7 and 8 in the Figure indicate the lines for Scheme 2 (○), 3 (△), 7 (●) and 8 (▲) respectively.

Fig. 6. Hill plots for Schemes 2, 3, 7 and 8, calculated from the curves in Fig. 3 in which the parameter values were $X_1 = 3$, $X_2 = 0.1$ and $X_3 = 0$

Numbers 2, 3, 7 and 8 in the Figure indicate the lines for Scheme 2 (○), 3 (△), 7 (●) and 8 (▲) respectively. The data for Schemes 2 and 7 or those for Schemes 3 and 8 are plotted alternately, because the data for the two schemes fall on almost the same points.

Fig. 7. Curve of plots of initial velocity versus substrate concentration for Scheme 6, calculated by using the parameter values $X_1 = 0$, $X_2 = 0.1$ and $X_3 = 0$

The relation $V_{\max} = \alpha_2[E]_0/\beta_2$ was assumed.

Fig. 8. Effects of the X_1 value on the Hill coefficients for all the schemes considered except Scheme 6

The parameter values were $X_2 = 0.1$ and $X_3 = 0$. Numbers 1–5 and 7–10 in the Figure indicate the lines for Schemes 1–5 and 7–10 respectively.

depends strongly on the reaction scheme and the kinetic constants. In the present work these effects on the co-operativity were further studied quantitatively by using the Hill coefficient h .

Fig. 8 shows the values of the Hill coefficient h for all the reaction schemes except Scheme 6 as a plot of h versus X_1 . The Hill coefficients for nine reaction schemes can be correlated by four lines, indicating that the nine reaction schemes can be divided into four groups. Each of the following groups of the schemes gives a similar v -[S] curve: Schemes 2 and 4, Schemes 1, 3 and 5, Schemes 7 and 9 and Schemes 8 and 10. Comparison of the coefficients α_i and β_j in the rate equations in Table 2 supports the above grouping of the reaction schemes. As shown in Fig. 8, the Hill coefficient attains the maximum value of 2.0 for any scheme when X_1 is large. When X_1 is small, the Hill coefficients

Table 3. Effects of the values of X_1 and X_2 on the Hill coefficients for Schemes 9 and 10

The value of X_3 was kept constant at 0.

Scheme	X_2	$X_1 \dots$	Hill coefficient h						
			-2	-1	0	+1	+3	+5	+7
9	0.01		0.574	0.852	1.25	1.65	1.96	2.00	2.00
	0.1		0.557	0.835	1.25	1.65	1.96	2.00	2.00
	1		0.514	0.786	1.23	1.65	1.96	2.00	2.00
	10		0.732	0.956	1.28	1.66	1.96	2.00	2.00
10	0.01		0.431	0.636	1.00	1.47	1.93	1.99	2.00
	0.1		0.432	0.638	1.00	1.47	1.93	1.99	2.00
	1		0.485	0.707	1.05	1.48	1.93	1.99	2.00
	10		0.912	0.970	1.15	1.49	1.93	1.99	2.00

Table 4. Effects of the values of X_1 and X_3 on the Hill coefficients for Schemes 2, 4, 7 and 9

The value of X_2 was kept constant at 0.1.

Scheme	X_3	$X_1 \dots$	Hill coefficient h						
			-2	-1	0	+1	+3	+5	+7
2	0		1.01	1.08	1.31	1.66	1.96	2.00	2.00
	0.5		1.01	1.07	1.28	1.62	1.95	2.00	2.00
	1.0		1.01	1.06	1.26	1.60	1.95	1.99	2.00
4	0		1.01	1.08	1.31	1.66	1.96	2.00	2.00
	0.5		1.01	1.07	1.28	1.62	1.95	2.00	2.00
	1.0		1.01	1.06	1.26	1.60	1.95	1.99	2.00
7	0		0.577	0.855	1.25	1.65	1.96	2.00	2.00
	0.5		0.711	0.928	1.24	1.62	1.95	2.00	2.00
	1.0		1.01	1.06	1.26	1.60	1.95	1.99	2.00
9	0		0.557	0.835	1.25	1.65	1.96	2.00	2.00
	0.5		0.608	0.866	1.22	1.62	1.95	2.00	2.00
	1.0		0.797	0.953	1.23	1.60	1.95	1.99	2.00

for Schemes 1–5 approach 1.0 with a decrease in X_1 , whereas those for Schemes 7–10 decrease with the X_1 value and become less than 1.0. When $X_1 < 0$, Schemes 1–5 or Schemes 7–10 show similar v -[S] curves. However, when $X_1 > 0$, Schemes 2 and 4 and Schemes 7 and 9 show similar v -[S] curves, and Schemes 1, 3 and 5 and Schemes 8 and 10 also show similar v -[S] curves. These results mean that when $X_1 < 0$, the reaction step expressed by eqn. (9) considerably affects the v -[S] curves and therefore widens the range of the h values. Furthermore, when $X_1 > 0$, the binding or dissociation steps expressed by eqns. (3) and (5) greatly affect the v -[S] curves. Fig. 8 also shows that, when $X_1 = 0$, only Schemes 8 and 10 give $h = 1.0$, which is specific to the Michaelis–Menten scheme. This means that the deviation of the v -[S] curve from a hyperbola specific to the Michaelis–Menten scheme can be observed even if there is no interaction between the distinct protomers. Therefore deviation of the v -[S] curve from a hyperbola does not necessarily indicate the existence of interaction between the distinct protomers, as mentioned above.

The above results were obtained under the conditions where $X_2 = 0.1$ and $X_3 = 0$. Here, we study how these two parameters affect the Hill coefficients. Schemes 4, 5, 6, 9, and 10 all include the reaction step $ESS \rightleftharpoons EPP$ expressed by eqn. (10) and therefore in general their reaction rates depend on the values of X_2 ($= k_{12}/k_{11}$). However, the Hill coefficients of Schemes 4 and 5 are both independent of X_2 . The Hill coefficients of Schemes 9 and

10 do depend on the value of X_2 . Table 3 shows the effect of parameter X_2 on the Hill coefficient for Schemes 9 and 10. In these schemes the values of h for $X_2 > 1.0$ differ from those for $X_2 < 1$ in the lower X_1 region ($X_1 < 0$). Under some conditions Scheme 6 no longer exhibits the substrate-inhibition behaviour. When the value of X_2 is much less than 1.0 or the value of X_1 is much larger than 1.0, the effect of the X_2 value on the Hill coefficients of all schemes except Scheme 6 can be neglected.

In Schemes 2, 4, 7 and 9 the rate equations are functions of the rate constant k_{r1} in addition to K_{s1} , K_{s2} , k_{t1} and k_{t2} , even if $[P] = 0$. Therefore the effect of parameter X_3 on the Hill coefficient is examined in Table 4, in which the h values are tabulated as functions of X_1 and X_3 . The Table shows that the Hill coefficients for Schemes 2 and 4 are only a little dependent on the X_3 value. However, the Hill coefficients for Schemes 7 and 9 do depend on the X_3 value. The Hill coefficient for Scheme 7 approaches those for Schemes 2 and 4 with increase in the X_3 value.

Fig. 8 can be used together with some other information to estimate the reaction scheme for a given substrate/enzyme system. Needless to say, it will only be possible to identify the correct reaction scheme if a large amount of accurate reaction rate data is available.

Substrate inhibition scheme

As Fig. 7 shows, the reaction that obeys Scheme 6 exhibits substrate-inhibition kinetic behaviour. The reason for this occurrence of substrate inhibition is as follows. The conversion of complex ES, which is produced by combining S and E, into complex EP occurs at a rate with rate constant k_{t1} and this EP rapidly dissociates to produce P. The ES also combines rapidly with S to become ESS, but the conversion of this complex into EPP and therefore the production of P from the EPP is rather slow when X_2 is small, i.e. $k_{t2} \ll k_{t1}$. Therefore, when the substrate concentration is high, the concentration of the complex ESS becomes higher and as a result the rate of production of P is decreased.

Concluding remarks

We have shown on the basis of the KNF model that the feature of the v -[S] curve of the reaction $S \rightleftharpoons P$ catalysed by the dimeric enzyme consisting of two identical protomers depends strongly on the reaction scheme and the parameter X_1 . The parameter X_1 is a measure of the relative affinities of the first and second protomers for the substrate. When $X_1 < 0$, the v -[S] curves for some schemes exhibit negative co-operativity ($h < 1.0$)

and those for the others are similar to that of the Michaelis-Menten scheme. The latter indicates that, even if there is interaction between the distinct protomers, sigmoidal rate behaviour is not necessarily observed. As far as we know, there has been no previous report on this point. When $X_1 > 0$, all the reaction schemes except Scheme 6 exhibit sigmoidal kinetic behaviour ($h > 1.0$), and at the limit of $X_1 \gg 0$ the Hill coefficients attain the maximum possible value of 2.0. Furthermore, we have found that, even if $X_1 = 0$, the v -[S] curve for almost all the schemes considered in the present work does not necessarily agree with that of the Michaelis-Menten scheme. This means that the deviation of the v -[S] curve from a hyperbola specific to the Michaelis-Menten scheme can be observed even if there is no interaction between the distinct protomers. Similar findings have already been reported by several investigators for monomeric enzymes (Sweeny & Fisher, 1963; Ferdinand, 1966; Griffin & Brand, 1968; Bardsley *et al.*, 1980; Pettersson, 1986*a,b*). For example, Pettersson (1986*a,b*) showed that the kinetic co-operativity of hexokinase type L₁ can be well explained by an ordered ternary-complex mechanism in which the leading substrate participates in formation of the abortive complex, and that the sigmoidal rate behaviour of glucokinase can be demonstrated by a random ternary-complex mechanism even if one of the alternative pathways for formation of the ternary complex contributes negligibly to the net reaction flow.

In the present work analyses were based on the KNF model. Further work based on the MWC model will be required to understand fully the effects of the reaction scheme on the reactions catalysed by oligomeric enzymes.

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