DESCRIPTION OF THE KINETICS OF TWO-SUBSTRATE REACTIONS OF THE TYPE $S_1 + S_2 \rightleftharpoons S_3 + S_4$ BY A GENERALIZED MONOD, WYMAN, AND CHANGEUX MODEL

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A series of methematical models, generalizations of the Monod, Wyman, and Changeux model, which describe the kinetics of two-substrate reactions of the type $S_1 + S_2 \overset{E(R, T)}{\rightleftharpoons} S_3 + S_4$, in which protomers of the oligomeric enzyme E(R, T) concerted conformational transitions of the $R_0 \rightleftharpoons T_0$, have been derived. Cases of random and ordered binding of substrates to the active sites and consequent ejection of the products by the active sites are examined. It is shown that depending on the values of the parameters determining the relative affinity of substrates for the active sites the conformations of R and T and the relative activities of these conformations, the models can describe isosteric substrate activation [sigmoidal nature of the curves $v(S_1)$ and $v(S_2)$], inhibition of the enzyme activity by an excess of the substrate, as well as the intermediate plateau on the curve v(S). The two reaction products can have both an activating and inhibiting isosteric effect on the enzyme. The relative merits and shortcomings of the two principal methods of determining the parameters of the models derived: Experimental kinetic and computer methods, based on the technique of optimization of the parameters of the models, are discussed. The second method is illustrated by fitting one of the models derived to experimental curves of substrate saturation of the human thrombocytic phosphofructokinase. The fitting showed that a good qualitative and quantitative description of the characteristic features of the kinetics of this enzyme — the sigmoidal nature of v (fructose-6-phosphate), coupled with substrate inhibition by the cofactor (ATP) — is possible without employing a hypothesis about allosteric substrate—enzyme interactions.

The key reactions of cell metabolism are catalyzed, as a rule, by oligomeric enzymes, whose activity is regulated not only by the main metabolites, but also by the reactants themselves. The majority of such reactions are multisubstrate reactions, which are frequently easily reversed. However, an analysis of the kinetics of regulatory reactions is limited to models of one-substrate irreversible reactions, such as the Monod, Wyman, and Changeux model (MWC model) [1], the Koshland model [2], and the Frieden—Kurganov model [3, 4]. Such a simplified examination of complex multicomponent reactions, not based on a preliminary analysis of a complete model of the reaction, first, makes an interpretation of the quantitative evaluations of the kinetics constants obtained difficult, since these "constants" are functions of the concentrations of substrates which have not been considered and, second, it inhibits the development of methods of computer analysis of the kinetics of complex reactions. Finally, a description of a complex reaction by a one-substrate model is frequently completely unacceptable in a theoretical examination of the behavior of enzymatic reactions in the multienzyme systems of the living cell.

Earlier [5] the one-substrate MWC and Frieden—Kurganov models were generalized for the case of an enzymatic reaction of arbitrary complexity. In the present work mathematical models of two-substrate reactions with different assumptions relative to the mechanism of the interaction of the substrates with the active sites of the enzyme are constructed on the basis of an algorithm that we derived [5], the main kinetic properties of these reactions are described, and different approaches to an evaluation of the parameters of the models are examined.

MODELS OF REVERSIBLE TWO-SUBSTRATE REACTIONS

Let us examine the quasisteady state of the reaction

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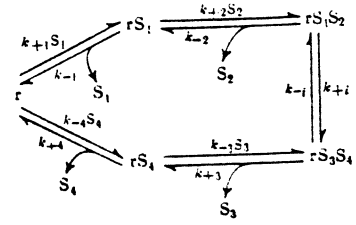
$$S_1 + S_2 \overset{E(R,T)}{\rightleftharpoons} S_3 + S_4, \tag{1}$$

catalyzed by the oligomeric enzyme E(R, T) consisting of n identical protomers. Let us assume that free protomers of the enzyme E are capable of performing concerted [1] conformational transitions:

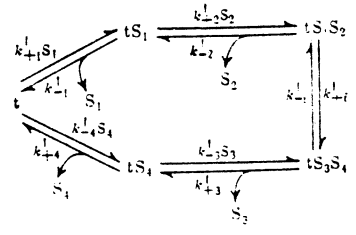
$$R_0 \underset{l_-}{\overset{l_+}{\rightleftharpoons}} T_0. \tag{2}$$

As follows from [5], to derive the equation of the initial quasisteady state of reaction (1) it is sufficient to examine separately reaction (2) and the interaction of the substrates with single active sites of protomers of conformations R and T. We shall derive mathematical models of reaction (1) under different assumptions about the mechanism of the reaction in a separate active site. We shall examine the following cases in accordance with Cleland's classification [6].

Ordered Bi Bi Mechanism. We shall represent the interactions of the substrates and products with active sites r and t of conformations R and T by the following diagrams:



(3)



(4)

The rate of reaction (1), catalyzed by a single active site r according to mechanism (3), is determined in accordance with the data of Volkenstein and Goldstein [7] by the equation

$$f = \frac{-V_+ S_1 S_2 / K_{d_1} K_{m_2} - V_- S_3 S_4 / K_{m_3} K_{d_4}}{\Delta}, \tag{5}$$

where

$$\Delta = 1 + \frac{S_1}{K_{d_1}} + \frac{K_{m_1}}{K_{d_1}} \frac{S_2}{K_{m_2}} + \frac{S_1 S_2}{K_{d_1} K_{m_2}} + \frac{S_4}{K_{d_4}} + \frac{K_{m_4}}{K_{d_4}} \frac{S_3}{K_{m_3}} + \frac{S_3 S_4}{K_{m_3} K_{d_4}} + \frac{K_{d_1}}{K_{m_1}} \frac{S_2 S_4}{K_{m_2} K_{d_4}} + \frac{K_{d_4}}{K_{m_4}} \frac{S_1 S_3}{K_{d_1} K_{m_3}} + \frac{k_{+i} + k_{-i}}{k_{-i}} \left(\frac{K_{m_2} K_{m_4}}{K_{d_2} K_{d_4}} \cdot \frac{S_1 S_2 S_3}{K_{d_1} K_{m_2} K_{m_3}} + \frac{K_{m_1} K_{m_2}}{K_{d_1} K_{d_3}} \frac{S_2 S_3 S_4}{K_{m_2} K_{m_3} K_{d_4}} \right),$$

$$V_+ = \lim_{\substack{S_1, S_2 \to \infty \\ S_3, S_4 = 0}} f = \frac{k_{-i} k_{+3} n e_0}{k_{-i} k_{+4} + k_{+i} k_{+4} + k_{+3} k_{+4} + k_{+i} k_{+3}},$$

$$V_- = -\lim_{\substack{S_1 = S_2 = 0 \\ S_3, S_4 \to \infty}} f = \frac{k_{-i} k_{-2} n e_0}{k_{-i} k_{-1} + k_{-i} k_{-2} + k_{-1} k_{-2} + k_{+i} k_{-1}},$$

$$K_{m_1} = \lim_{\substack{S_3 = S_4 = 0 \\ S_2 \to \infty}} [S_1]_{0.5} = \frac{k_{-i} k_{+3} k_{+4}}{k_{+1} (k_{-i} k_{+4} + k_{+i} k_{+3} + k_{+i} k_{+4} + k_{+3} k_{+4})},$$

$$K_{m_2} = \lim_{\substack{S_3 = S_4 = 0, \\ S_1 \to \infty}} [S_2]_{0.5} = \frac{k_{+4} (k_{-i} k_{-2} + k_{+i} k_{+3} + k_{-2} k_{+3})}{k_{+2} (k_{-i} k_{+4} + k_{+i} k_{+4} + k_{+i} k_{+3} + k_{+3} k_{+4})},$$

$$K_{d_1} = k_{-1}/k_{+1}, \quad K_{d_2} = k_{-2}/k_{+2},$$

$$K_{m_3} = \lim_{\substack{S_1 = S_2 = 0 \\ S_4 \to \infty}} [S_3]_{0.5} = \frac{k_{-1} (k_{-i} k_{-2} + k_{+i} k_{+3} + k_{-2} k_{+3})}{k_{-3} (k_{-i} k_{-1} + k_{-i} k_{-2} + k_{+i} k_{-1} + k_{-1} k_{-2})},$$

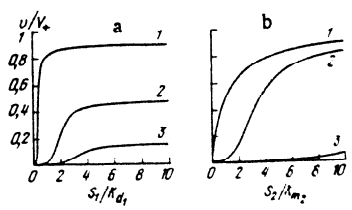


Fig. 1

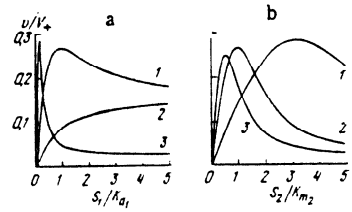


Fig. 2

Fig. 1. Cooperative dependence of rate of two-substrate reaction with rapid successive binding of substrates S_1 and S_2 to the active sites of the enzyme [model (16)] on substrate concentration. Values of parameters of model (16): n = 6, $a = 0$, $L = 10^4$, $K_{d_1}/K'_{d_1} = K_{m_2}/K'_{m_2} = 10^{-4}$. S_2/K_{m_2} (a) and S_1/K_{d_1} (b): 10 (1), 1 (2), and 0.2 (3).

Fig. 2. Inhibitory effect of substrates S_1 and S_2 on rate of their utilization, described by model (16) at n = 6, $a = 0$, $L = 10^{-10}$, $K_{d_1}/K'_{d_1} = K'_{m_2}/K'_{m_1} = 10$. S_2/K_{m_2} (a) and S_1/K_{d_1} (b): 0.2 (1), 1 (2), and 5 (3).

$$K_{m_4} = \lim_{\substack{S_1=S_2=0 \\ S_3\to\infty}} [S_4]_{0.5} = \frac{k_{-i}k_{-1}k_{-2}}{k_{-4}(k_{-i}k_{-1} + k_{-i}k_{-2} + k_{+i}k_{-1} + k_{-1}k_{-2})}, \tag{6}$$

$$K_{d_3} = k_{+3}/k_{-3}, \quad K_{d_4} = k_{+4}/k_{-4},$$

e_0 is the total concentration of enzyme E(R, T).

The concentration of the free active site r in mechanism (3) is equal to

$$[r] = \frac{1 + \frac{K_{m_1}}{K_{d_1}}\frac{S_2}{K_{m_2}} + \frac{K_{m_4}}{K_{d_4}}\frac{S_3}{K_{m_3}}}{\Delta} ne_0. \tag{7}$$

The mechanism of the functioning of active site t (4) differs only in the designation of the rate constants and therefore by analogy with derived Eqs. (5)-(7) the equation for the rate of catalysis in active site t can be written as follows:

$$f' = \frac{V'_+ S_1 S_2 / K'_{d_1} K'_{m_2} - V'_- S_3 S_4 / K'_{m_3} K'_{d_4}}{\Delta'}, \tag{8}$$

where

$$\Delta' = 1 + \frac{S_1}{K'_{d_1}} + \frac{K'_{m_1}}{K'_{d_1}}\frac{S_2}{K'_{m_2}} + \frac{S_1 S_2}{K'_{d_1} K'_{m_2}} + \frac{S_4}{K'_{d_4}} + \frac{K'_{m_4}}{K'_{d_4}}\frac{S_3}{K'_{m_3}}$$
$$+ \frac{S_3 S_4}{K'_{m_3} K'_{d_4}} + \frac{K'_{d_1} S_2 S_4}{K'_{m_1} K'_{m_2} K'_{d_4}} + \frac{K'_{d_4} S_1 S_3}{K'_{m_4} K'_{d_1} K'_{m_3}} + \frac{k'_{+i} + k'_{-i}}{k'_{-i}}$$
$$\times \left(\frac{K'_{m_2} K'_{m_4}}{K'_{d_2} K'_{d_4}} \frac{S_1 S_2 S_3}{K'_{d_1} K'_{m_2} K'_{m_3}} + \frac{K'_{m_1} K'_{m_3}}{K'_{d_1} K'_{d_3}} \frac{S_2 S_3 S_4}{K'_{m_4} K'_{m_3} K'_{d_4}} \right). \tag{9}$$

The equation for the concentration of free active site t takes the form

$$[t] = \frac{1 + \frac{K'_{m_1}}{K'_{d_1}}\frac{S_2}{K'_{m_2}} + \frac{K'_{m_4}}{K'_{d_4}}\frac{S_3}{K'_{m_3}}}{\Delta'} ne_0. \tag{10}$$

The parameters of Eqs. (8)-(10) with a prime are determined in exactly the same way as the corresponding parameters of Eqs. (5)-(7) without a prime.

Supposing that catalytic sites r and t do not change the position of the thermodynamic equilibrium of reaction (1), we shall assume that the following conditions are simultaneously satisfied

$$\begin{cases} f = 0, \\ f' = 0. \end{cases} \tag{11}$$

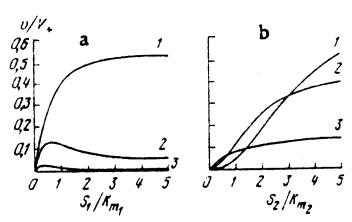


Fig. 3

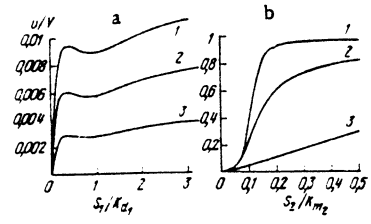


Fig. 4

Fig. 3. Inhibitory effect of substrate S_1 and activating effect of substrate S_2 on rate of reaction (1) described by model (25) at $n = 4$, $\alpha = 0$, $L = 10^{-3}$, $K_{m_1}/K'_{m_1} = 0.1$, $K_{m_2}/K'_{m_2} = 20$. S_2/K_{m_2} (a) and S_1/K_{m_1} (b): 5 (1), 1 (2), and 0.2 (3).

Fig. 4. Family of kinetic curves $v(S_1)$ with two extrema (a) and family of sigmoidal dependences of $v(S_2)$ (b), plotted according to model (16) at $n = 8$, $\alpha = 10$, $L = 1$, $K_{d_1}/K'_{d_1} = 0.01$, $K_{m_2}/K'_{m_2} = 10^3$. $S_2/K_{m_2} = 0.15$ (1), 0.01 (2), and 0.005 (3) (a), $S_1/K_{d_1} = 10$ (1), 1 (2), and 0.1 (3) (b).

Hence we obtain the relation between the parameters:

$$\frac{V_- K_{d_1} K_{m_2}}{V_+ K_{m_3} K_{d_4}} = \frac{V'_- K'_{d} K'_{m_2}}{V'_+ K'_{m_3} K'_{d_4}}. \tag{12}$$

According to the algorithm that we derived [5], the rate of reaction (1) can be expressed by Eqs. (5)-(7) and (8)-(10) in the following way:

$$v = f \frac{1 + \frac{f'}{f} L([r]/[t])^n}{1 + L([r]/[t])^n}. \tag{13}$$

Substituting the corresponding equations for f, f', [r] and [t] in Eq. (13), we obtain the equation for the rate of a reaction proceeding by an ordered bi bi mechanism:

$$v = \frac{V_+ \cdot \frac{S_1 S_2}{K_{d_1} K_{m_2}} - V_- \cdot \frac{S_3 S_4}{K_{m_3} K_{d_4}}}{\Delta} \cdot \frac{1 - \alpha L q^n (\Delta'/\Delta)^{n-1}}{1 - L q^n (\Delta'/\Delta)^n},$$

$$q = \frac{1 + \frac{K_{m_1}}{K_{d_1}} \frac{S_2}{K_{m_2}} + \frac{K_{m_4}}{K_{d_4}} \frac{S_3}{K_{m_3}}}{1 + \frac{K'_{m_1}}{K'_{d_1}} \frac{S_2}{K'_{m_2}} + \frac{K'_{m_4}}{K'_{d_4}} \frac{S_3}{K'_{m_3}}}, \quad \alpha = V'_- K_{d_1} K_{m_2} / V_+ K'_{d_1} K'_{m_2}, \tag{14}$$

where $L = l_+/l_-$ is the equilibrium constant of conformational transitions (2), which depends on the concentrations of the allosteric effectors. If the stage of the catalytic conversion $rS_1S_2 \rightleftarrows rS_3S_4$ is the limiting step in reactions (3) and (4), Eq. (14) is simplified. In this case $K_{m_1} = K_{m_4} = K'_{m_1} = K'_{m_4} = 0$ and Eq. (14) is reduced to the form

$$v = V_+ \cdot \frac{S_1 S_2 / K_{d_1} K_{m_2} - \varkappa S_3 S_4 / K_{m_3} K_{d_4}}{\Delta} \cdot \frac{1 + \alpha L (\Delta'/\Delta)^{n-1}}{1 + L (\Delta'/\Delta)^n},$$

$$\Delta = 1 + \frac{S_1}{K_{d_1}} \left(1 + \frac{S_2}{K_{m_2}}\right) + \frac{S_4}{K_{d_4}} \left(1 + \frac{S_3}{K_{m_3}}\right), \tag{15}$$

$$\Delta' = 1 + \frac{S_1}{K'_{d_1}} \left(1 + \frac{S_2}{K'_{m_2}}\right) + \frac{S_4}{K'_{d_4}} \left(1 + \frac{S_3}{K'_{m_3}}\right), \quad \varkappa = V_-/V_+.$$

Kinetic experiments are usually conducted in the absence of products, i. e., at $S_3 = S_4 = 0$.

Let us examine mathematical model (15) of reaction (1) for a rapid equilibrium ordered bi mechanism.

The equation for the reaction rate in this case is obtained from Eq. (15) by substituting $S_3 = S_4 = 0$:

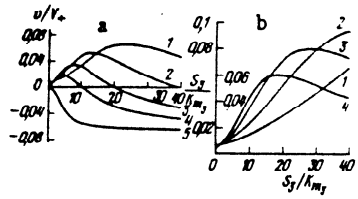


Fig. 5. Autocatalytic effect of low concentrations of product S_3 in the presence in the medium of the second product S_4 (a) and in its absence (b). The families were plotted according to model (23) at n = 4, $\varkappa = 0.1$, $a = 0$, L = 100, $K_{m_1}/K'_{m_1} = K_{m_2}/K'_{m_2} = K_{m_4}/K'_{m_4} = 1$, $K_{m_3}/K'_{m_3} = 10^{-4}$ and $S_4 = 2K_{m_4}$ (a), $S_4 = 0$ (b), $S_1/K_{m_1} = 8$ (1), 4 (2), 2 (3), 1 (4), 0 (5).

$$v = V_+ \cdot \frac{S_1 S_2 / K_{d_1} K_{m_2}}{\Delta} \cdot \frac{1 + aL\,(\Delta'/\Delta)^{n-1}}{1 + L\,(\Delta'/\Delta)^n},$$
$$\Delta = 1 + \frac{S_1}{K_{d_1}}\left(1 + \frac{S_2}{K_{m_2}}\right), \quad \Delta' = 1 + \frac{S_1}{K'_{d_1}}\left(1 + \frac{S_2}{K'_{m_2}}\right). \tag{16}$$

The MWC model [1] of a one-substrate irreversible reaction is frequently used in a theoretical description of the kinetics of multisubstrate reactions catalyzed by oligomeric enzymes [8]. In this case it is implied that when the concentrations of all but one of the substrates are "frozen," the model of a multisubstrate reaction is reduced to the previously described model [1]:

$$v = \overline{V}\,\frac{S/\overline{K}}{1 + S/\overline{K}} \cdot \frac{1 + \overline{a}\overline{L}\,((1 + S/\overline{K}')/(1 + S/\overline{K}))^{n-1}}{1 + \overline{L}\,((1 + S/\overline{K}')/(1 + S/\overline{K}))^n}. \tag{17}$$

As is shown below, the model of a multisubstrate reaction can be reduced to model (17), in which $\overline{V}$, $\overline{K}$, $\overline{K}'$, $\overline{a}$, and $\overline{L}$ are functions of the concentrations of the frozen substrates only in the case of rapid establishment of equilibrium between the substrates and enzyme. Thus, when the concentration of S_1 is "frozen," model (16) is reduced to model (17), whose parameters are connected with the parameters V_+, K_{d_1}, K_{m_2}, K'_{d_1}, K'_{m_2}, a, and L of model (16) and the concentration of S_1 by the following relations:

$$\overline{K} = K_{m_2}\,\frac{1 + S_1/K_{d_1}}{S_1/K_{d_1}}, \quad \overline{K}' = K'_{m_2}\,\frac{1 + S_1/K'_{d_1}}{S_1/K'_{d_1}}, \quad \overline{V} = V_+,$$
$$\overline{a} = a\,\frac{1 + S_1/K_{d_1}}{1 + S_1/K'_{d_1}}, \quad \overline{L} = L\left(\frac{1 + S_1/K'_{d_1}}{1 + S_1/K_{d_1}}\right)^n. \tag{18}$$

When the concentration of the second substrate S_2 in model (16) is "frozen" the parameters of models (16) and (17) are connected by the relations

$$\overline{K} = \frac{K_{d_1}}{1 + S_2/K_{m_2}}, \quad \overline{K}' = \frac{K'_{d_1}}{1 + S_2/K'_{m_2}}, \quad \overline{a} = a,\ \overline{L} = L,\ \overline{V} = V_+\,\frac{S_2/K_{m_2}}{1 + S_2/K_{m_2}}. \tag{19}$$

As is seen from relations (18) and (19), the quantitative evaluations of constants $\overline{K}$ and $\overline{K}'$, obtained in a one-substrate approach to the kinetics of multisubstrate reactions, can depend in a rather complex way on the concentration of the fixed substrate, giving the true value either at $S_1 \to \infty$ [Eq. (18)] or at $S_2 \to 0$ [Eq. (19)]. Therefore when model (17) is used in place of model (16) it must be kept in mind that the values of $\overline{K}$ and $\overline{K}'$ obtained give only the apparent values of the dissociation constants, which can be nonphysiological and not comparable with the corresponding values obtained under other conditions.

Let us examine model (14) at $S_3 = S_4 = 0$. Substituting $S_3 = S_4 = 0$ in Eq. (14) we obtain the equation of the steady-state rate of reaction (1) in the absence of products:

$$v = V_+ \cdot \frac{S_1 S_2 / K_{d_1} K_{m_2}}{\Delta} \cdot \frac{1 + aLq^n\,(\Delta'/\Delta)^{n-1}}{1 + Lq^n\,(\Delta'/\Delta)^n}, \tag{20}$$

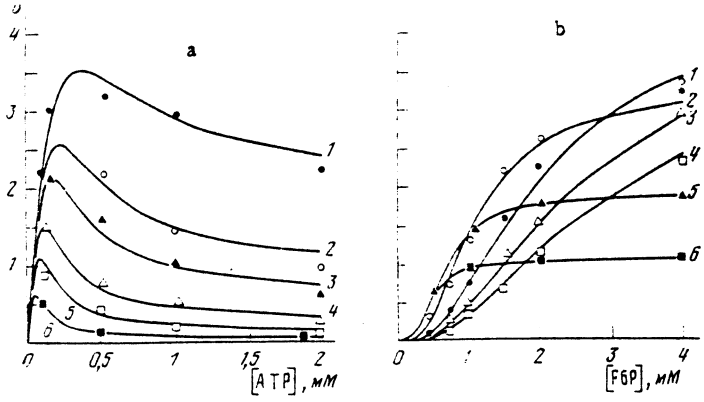


Fig. 6. Description of experimental data on kinetics of phosphofructokinase reaction [14] by model (25) at the following values of the parameters: n = 4, V = 10.9 arb. units, α = 0, L = 1.57, K and K' for ATP are equal to 0.36 and 0.056 mM and for fructose-6-phosphate (F6P) 10^{-2} and 1.35 mM, respectively. a) [F6P], mM: 4 (1), 2 (2), 1.5 (3), 1 (4), 0.75 (5), and 0.5 (6); b) [ATP], mM; 0.48 (1), 0.18 (2), 0.98 (3), 1.98 (4), 0.08 (5), and 0.04 (6).

where

$$\Delta = 1 + S_1/K_{d_1} + K_{m_1}S_2/K_{d_1}K_{m_2} - S_1S_2/K_{d_1}K_{m_2},$$
$$\Delta' = 1 + S_1/K'_{d_1} + K'_{m_1}S_2/K'_{d_1}K'_{m_2} - S_1S_2/K'_{d_1}K'_{m_2}, \qquad (21)$$
$$q = (1 + K_{m_1}S_2/K_{d_1}K_{m_2})/(1 + K'_{m_1}S_2/K'_{d}K'_{m_2}).$$

When the concentration of substrate S_1 is "frozen" model (20) is reduced to the one-substrate model (17) with the following relations between the parameters of the models and the concentrations of S_1 and S_2:

$$\bar{K} = K_{m_2}\frac{1 + S_1/K_{d_1}}{K_{m_1}/K_{d_1} + S_1/K_{d_1}}, \quad \bar{K}' = K'_{m_2}\frac{1 + S_1/K'_{d_1}}{K'_{m_1}/K'_{d_1} + S_1/K'_{d_1}},$$
$$\bar{V} = V_+\frac{S_1/K_{d_1}}{K_{m_1}/K_{d_1} + S_1/K_{d_1}}, \quad \bar{\alpha} = \alpha\frac{1 - S_1/K_{d_1}}{1 + S_1/K'_{d_1}}, \qquad (22)$$
$$\bar{L} = L\left(\frac{1 + S_1/K'_{d_1}}{1 + S_1/K_{d_1}}\right)^n\left(\frac{1 - K_{m_1}S_2/K_{d_1}K_{m_2}}{1 - K'_{m_1}S_2/K'_{d_1}K'_{m_2}}\right)^n.$$

As is seen from Eqs. (22), the allosteric function in the given case turns out to be the function of the concentration of the variable substrate S_2, i. e., model (17) describes isosteric and apparent allosteric interactions of substrate S_2 with enzyme E(R, T), although model (20) was constructed considering only isosteric interactions in the active sites of the enzyme. Thus, the description of a multisubstrate reaction by a one-substrate model can lead to an erroneous conclusion about the reaction mechanism.

We shall present without derivation the equation of the rate of reaction (1) for the mechanism of random binding of substrates to the active sites. We shall examine the following special cases.

1. Rapid equilibrium random bi bi. The rate equation takes the form:

$$v = V_+\frac{S_1S_2/K_{m_1}K_{m_2} - \varkappa S_3S_4/K_{m_3}K_{m_4}}{\Delta}\cdot\frac{1 + aL(\Delta'/\Delta)^{n-1}}{1 + L(\Delta'/\Delta)^n}, \qquad (23)$$

where

$$\Delta = \left(1 + \frac{S_1}{K_{m_1}}\right)\left(1 + \frac{S_2}{K_{m_2}}\right) + \frac{S_3}{K_{m_2}} + \frac{S_4}{K_{m_4}}\left(1 + \frac{S_3}{K_{m_3}}\right),$$
$$\Delta' = \left(1 + \frac{S_1}{K'_{m_1}}\right)\left(1 + \frac{S_2}{K'_{m_2}}\right) + \frac{S_3}{K'_{m_2}} + \frac{S_4}{K'_{m_4}}\left(1 + \frac{S_3}{K'_{m_3}}\right), \qquad (24)$$
$$\varkappa = V_-/V_+, \quad a = V'_+K_{m_1}K_{m_2}/V_+K'_{m_1}K'_{m_2}.$$

2. Rapid equilibrium random bi. The equation for the reaction rate is obtained from Eq. (23) by substituting $S_3 = S_4 = 0$:

$$v = V_+ \frac{S_1 S_2 / K_{m_1} K_{m_2}}{\Delta} \frac{1 + aL(\Delta'/\Delta)^{n-1}}{1 + L(\Delta'/\Delta)^n}, \quad (25)$$

$$\Delta = \left(1 + \frac{S_1}{K_{m_1}}\right)\left(1 + \frac{S_2}{K_{m_2}}\right), \quad \Delta' = \left(1 + \frac{S_1}{K'_{m_1}}\right)\left(1 + \frac{S_2}{K'_{m_2}}\right).$$

"Freezing" of the concentrations of S_1 or S_2 leads to analytically identical dependences of $\bar{K}$, $\bar{K}'$, $\bar{V}$, $\bar{a}$, and $\bar{L}$ on the concentration of the fixed substrate:

$$\bar{K} = K_m, \bar{K}' = K'_m, \bar{V} = V_+ \frac{S_f/K_m}{1 + S_f/K_m}, \quad (26)$$

$$\bar{a} = a \frac{1 + S_f/K_m}{1 + S_f/K'_m}, \quad \bar{L} = L\left(\frac{1 + S_f/K'_m}{1 + S_f/K_m}\right)^n,$$

where S_f is the concentration of the fixed substrate.

As is seen from Eq. (26), in the given special case of rapid random binding of substrates S_1 and S_2 to the active sites of the enzyme the one-substrate approach to an evaluation of the kinetic constants gives the true values of K_m and K'_m and the apparent values of $\bar{V}$, $\bar{a}$, and $\bar{L}$, in contrast to Eqs. (18), (19), and (22).

It is seen from the examples presented that a one-substrate description of the kinetics of two-substrate reactions without a preliminary analysis of the complete model can lead to an erroneous conclusion both about the mechanism of the reaction and about the values of the kinetic constants.

PROPERTIES OF MODELS OF TWO-SUBSTRATE REACTIONS

The main properties of the MWC model of irreversible one-substrate reactions are activation and inhibition of the reaction rate by the substrate, product, and their analogs, due to both isosteric and allosteric interactions of these compounds with the enzyme [8, 10-12]. Since the models of two-substrate reactions can be reduced to an isosteric or allosteric one-substrate MWC model, analogous properties are also characteristic of two-substrate (and more complex) reactions. If the model of a two-substrate reaction with "freezing" of the concentration of one of the substrates can be reduced to the isosteric one-substrate model (17), evaluations of the regions of values of the parameters at which model (17) describes different types of kinetic curves [9] can be used in a quantitative description of two-substrate reactions. However, in the case of two-substrate reactions the boundaries of these regions depend not only on the characteristic values of the kinetic constants, but also on the concentration of the fixed substrate. Thus, one of the regions of values of the parameters of model (17) at which substrate activation of the reaction rate is observed is determined by the inequalities [9]:

$$\begin{cases} n \geqslant 2, \\ \bar{c} = \bar{K}/\bar{K}' < (n-1)/n, \\ \bar{a} < \bar{c}, \\ \dfrac{1}{n(1-\bar{c}) - 1} < \bar{L} < \dfrac{n-1}{\bar{a}\bar{c}^{n-1}}, \end{cases} \quad (27)$$

where $\bar{K}$, $\bar{K}'$, $\bar{a}$, and $\bar{L}$ are functions of the concentration of the frozen substrate, determined by the relations (18), (19), (22), and (26). A change in the concentration of the fixed substrate leads to a disturbance in relations (27) and, consequently, to a change in the type of the kinetic curves. Substrate activation of the reaction rate by both substrates is shown in Fig. 1. The families of curves $v(S_1)$ and $v(S_2)$ have been plotted from model (16) at $K_{m_1}/K'_{m_1} = K_{d_1}/K'_{d_1} < 1$ and $L \gg 1$. One of the conditions of substrate inhibition of the reaction rate, which is described by model (17), is determined by the inequalities [9]

$$\begin{cases} n \geqslant 2, \\ \bar{c} = \bar{K}/\bar{K} > n/(n-1), \\ \bar{a} < \bar{c}, \\ \dfrac{1}{\bar{c}^{n-1}(\bar{c} - n/(n-1))} < \bar{L}. \end{cases} \quad (28)$$

Families of curves $v(S_1, S_2)$ with an inhibitory effect of both substrates, plotted according to model (16) at $K_{d_1}/K'_{d_1} = K_{m_2}/K'_{m_2} > 1$ and $L \ll 1$ are presented in Fig. 2.

If conditions (27) can be satisfied when one of the substrates in model (16) is fixed and conditions (28) can be satisfied when the other substrate is fixed, activation of the reaction rate by one of the substrates and inhibition by the other is possible (Fig. 3). The families presented in this figure were plotted according to model (25) with $K_{m_1}/K'_{m_1} > 1$ and $K_{m_2}/K_{m_2} < 1$.

Finally, at $\bar{c} < 1$ and $\bar{a} > \bar{c}$ in model (17) kinetic curves with an intermediate plateau are possible. Families of curves with two extrema, plotted according to model (16), are presented in Fig. 4.

As in the case of one-substrate reversible reactions [9-11], low concentrations of the products of two-substrate reactions can have an activating effect on the rate of the forward reaction. For multisubstrate reactions described by the MWC model, the rule derived for reactions of type $S_1 \overset{E(R,\ T)}{\rightleftharpoons} S_2$ holds [9-11]: The substrate whose utilization is of a cooperative nature, becomes an activating product upon reversal of the reaction. In contrast to activation of the reaction rate by allosteric effectors, activation by the product is replaced by inhibition upon an increase in the concentration of the products, first, as a result of competition with the substrates for the active sites of the enzyme and, second, owing to the change in the direction of the reaction. The effect of the products on the rate of the forward reaction is illustrated in Fig. 5 by families of kinetic curves $v(S_3)$ constructed according to model (23). At $S_4 = 0$ product S_3 has an effect on the reaction rate analogous to the effect of analogs of the substrate on the rate of a one-substrate reaction [9, 12]. At $S_4 \neq 0$ the inhibitory effect becomes more pronounced owing to the change in the direction of the reaction (Fig. 5a).

EVALUATION OF PARAMETERS OF MODEL FROM EXPERIMENTAL DATA

For models of single-site enzymes catalyzing multisubstrate reactions methods of determining the parameters of the models from families of hyperbolic curves have been developed [6]. For models of regulatory reactions the linear correspondences between the parameters of the models and the characteristic points on nonhyperbolic curves cannot be plotted [1-4]. The parameters of such models are evaluated by various methods: for example, by conducting kinetic experiments under extreme conditions and evaluating the parameters from the corresponding hyperbolic dependences or by solving an optimization problem, i. e., by finding the values of the parameters at which the model under consideration best describes the nonhyperbolic kinetic data available with the help of an electronic computer. Each of these methods has its own shortcomings.

Let us examine the first, experimental kinetic method, which was first used [8] in determining the parameters of the one-substrate MWC model. The method of finding the kinetic constants is similar in the case of a two-substrate reaction.

1. Conducting of kinetic experiments in the presence of an excess of an allosteric activator. Under these conditions the enzyme is present mainly in conformation R, therefore the curves obtained are hyperbolic, and model (25), for example, is reduced to the form

$$v_R = \lim_{L \to \infty} v = V_+ \frac{S_1 S_2 / K_{m_1} K_{m_2}}{(1 + S_1/K_{m_1})(1 + S_2/K_{m_2})}. \tag{29}$$

The values of K_{m_1}, K_{m_2}, and V_+ are easily determined from the kinetic data.

2. The values of K'_{m_1}, K'_{m_2}, and V'_+ are determined in much the same way from kinetic experiments at an excess of an allosteric inhibitor in accordance with the model

$$v_T = \lim_{L \to \infty} v = V'_+ \frac{S_1 S_2 / K'_{m_1} K'_{m_2}}{(1 + S_1/K'_{m_1})(1 + S_2/K'_{m_2})}. \tag{30}$$

If conformation T is catalytically inactive, then K'_{m_1} and K'_{m_2} are determined from data on the saturation of the enzyme by the substrates at an excess of an allosteric inhibitor.

3. The values of n and L are determined from the nonhyperbolic curves obtained at intermediate values of L. Model (25) can be presented in the form

$$\frac{v_R - v}{v - v_T} = L \left[\frac{(1 + S_1/K'_{m_1})(1 + S_2/K'_{m_2})}{(1 + S_1/K_{m_1})(1 + S_2/K_{m_2})} \right]^n = Lq^n \tag{31}$$

or

$$\log \frac{v_R - v}{v - v_T} = \log L + n \log q. \quad (32)$$

The value of n can be determined from Eq. (32) from the slope of the straight line $\{\log[(v_R - v)/(v - v_T)]; \log q\}$. The value of L can be calculated from Eq. (31). In plotting relation (32) the concentrations of substrates S_1 and S_2 at which the kinetic curve differs most from hyperbolic, i. e., in the region of the inflection in the case of substrate activation or on the segment of a drop in the function v (S_1, S_2) in the case of substrate inhibition, must be used.

4. The allosteric function L(M) (M is a modifier) is plotted from the equation

$$L(M) = \frac{v_R(S_1, S_2) - v(M, S_1, S_2)}{v(M, S_1, S_2) - v_T(S_1, S_2)} \cdot \frac{1}{q^n}. \quad (33)$$

The experimental kinetic method of evaluating the parameters enables the mechanism of the interaction of the substrates with the enzyme to be determined more precisely and, consequently, to refine the model of the process. The problem of determining the kinetic constants in such an approach has a single solution. However this method is very time-consuming and requires comprehensive kinetic information about the enzyme.

The second method of finding the parameters of the model is based on the solution of an optimization problem with the help of an electronic computer, i. e., on a search for the values of the parameters at which minimal deviation of the theoretical curve from the experimental points is achieved. Such a method of finding the parameters, generally speaking, does not require complete information about the enzyme or about its allosteric activators and inhibitors, although such information can often prove to be useful. In particular, at redundancy of the parameters of the model and a shortage of kinetic information no single solution of the problem is possible [13]. In this case additional data on the behavior of the reaction under extreme conditions are necessary.

In determining the parameters of the MWC model by optimization methods the region of the values of the parameters and their initial values can be determined from relations (27) and (28).

Kinetic data for phosphofructokinase from human thrombocytes are presented in Fig. 6 [14]. The unbroken curves were plotted according to model (25) at values of the parameters found by fitting model (25) to the experimental data [14] using a previously described algorithm [15]. As is seen from this figure, the experimental data on the kinetics of the phosphofructokinase reaction can be described satisfactorily by the simplest version of the model of a two-substrate reaction without employing the hypothesis that the substrates interact with the allosteric sites.

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