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The Quasi-Equilibrium Assumption for Bi-Bi Ordered Bisubstrate Enzymatic Reaction. How to Discriminate the Mechanism Correctly

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Abstract—Application of the quasi-equilibrium assumption for the steady-state kinetics of bisubstrate irreversible enzymatic reactions in the case of ordered binding of substrates (Bi-Bi ordered mechanism) is considered. The necessary and sufficient conditions for application of the quasi-equilibrium assumption have been found and accuracy of this assumption has been numerically evaluated. The limitations on application of the quasi-equilibrium assumption have been shown and errors of its application have been analyzed. It is shown that possible discrimination of substrate binding order using asymmetrical expressions grounded on the quasi-equilibrium assumption is inconsistent because such asymmetrical expressions arise from incorrect application of the quasi-equilibrium assumption. Moreover, it has been proved in the general case that mechanisms generating such substrate-asymmetrical expressions for the steady-state rate of enzymatic reaction do not exist. The error source when using graphical interpretation for discrimination of mechanisms of bisubstrate enzymatic reactions has been determined. The strategy to avoid such errors is pointed out.

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The quasi-equilibrium assumption in catalysis including enzymatic catalysis suggests that some reversible processes accompanying catalysis (dissociation of ligands, ionogenic groups, etc.) attain the equilibrium state, which is practically not displaced as a result of the catalyzed reaction [1]. In spite of the fact that the quasi-equilibrium assumption has been applied in enzymatic kinetics since 1913 [2], systematical analysis of the necessary and sufficient conditions for application of this assumption is absent from the literature. Although this problem is still considered [3-6], conditions for application of the quasi-equilibrium assumption adduced in these works cannot be accepted as adequate.

Most enzymatic reactions are bi- or multi-substrate ones, and in the vast majority of works mechanisms of such reactions are analyzed using the quasi-equilibrium assumption [7-9]. This is mainly due to the fact that if the manifold of pathways of multi-substrate enzymatic reactions is taken into account, expressions for the steady-state rate of reactions become more complicated due to high powers of concentrations of substrates, products, modifiers, and other reaction participants. Application of the quasi-equilibrium assumption in all such cases allows simplification of the expressions for the rate of the enzymatic reaction, which often can be reduced to equations of the Michaelis–Menten type [10]. However, use of the quasi-equilibrium assumption without proper substantiation can result (and results as will be shown below) in major errors.

For any enzymatic reaction, by decreasing total enzyme concentration it is possible to attain unlimited approximation of the experimental system to the steady state via all intermediate forms of the enzyme at almost constant concentrations of substrates, products, modifiers, and other reaction participants. Let us consider solution for such steady state to be exact, and the closer the suggested approximate solution to the exact one the higher is the accuracy of approximate solutions considered in this work.

In case of the quasi-equilibrium assumption it is necessary to determine an equilibrium segment or several

equilibrium segments: inside these segments the intermediate enzyme forms are considered to be practically in the equilibrium state, and in the rest of the mechanism the steady state is supposed to be established [1]. However, use of the quasi-equilibrium assumption was practically never established; this resulted in discussions and doubts whether the quasi-equilibrium assumption was applied properly [3, 4, 11-13].

Qualitative evaluation of conditions for existence of a quasi-equilibrium segment consisting of two and three intermediates in the general scheme of the ordered enzymatic reaction was fulfilled earlier [14]. For the general mechanism of the ordered single-substrate reactions, cases of equilibrium segments consisting of two and three intermediates are considered, the necessary and sufficient conditions for application of the quasi-equilibrium assumption are found, and accuracy of this assumption is numerically evaluated in [15] using the graph method [16].

In this work, using ordered bisubstrate reactions as an example, accuracy of the quasi-equilibrium assumption is determined, limitations on its application are shown, and errors resulting from graphic interpretations for establishment of mechanism of enzymatic reaction are analyzed.

RESULTS AND DISCUSSION

The simplest mechanism for the ordered bisubstrate enzymatic reaction with formation of a triple complex in the absence of reaction products is as follows:

$$E \underset{k_{-1}}{\overset{k_1[S_1]}{\rightleftarrows}} ES_1 \underset{k_{-2}}{\overset{k_2[S_2]}{\rightleftarrows}} ES_1S_2 \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} EP_1P_2 \xrightarrow{k_4^*} E+P_1+P_2, \quad (1)$$

where E is enzyme, S is substrate, P is product. The stage characterized by the rate constant k_4* accounts for a series of irreversible transformations accompanied by splitting out of the reaction product(s).

Let us designate the values corresponding to the exact steady-state solution by the superscript symbols "ss" and the values corresponding to solution in case of the quasi-equilibrium assumption by the superscript symbols "qe".

The steady-state solution for mechanism (1) is defined by expressions:

$$\frac{v^{ss}}{e_0} = \frac{D(EP_1P_2)k_4^*}{\sum_j D(X_j)}, \quad (2)$$

$$[X_i]^{ss} = \frac{e_0 D(X_i)}{\sum_j D(X_j)}, \quad (3)$$

where

$$D(E) = k_2[S_2]k_3k_4^* + k_{-1}k_3k_4^* + k_{-1}k_{-2}k_4^* + k_{-1}k_{-2}k_{-3},$$

$$D(ES_1) = k_1[S_1]k_3k_4^* + k_1[S_1]k_{-2}k_4^* + k_1[S_1]k_{-2}k_{-3},$$

$$D(ES_1S_2) = k_1[S_1]k_2[S_2]k_4^* + k_1[S_1]k_2[S_2]k_{-3},$$

$$D(EP_1P_2) = k_1[S_1]k_2[S_2]k_3. \quad (4)$$

From here on v is the rate of enzymatic reaction, e_o is total enzyme concentration (the sum of concentrations of all intermediate forms of the enzyme), X_i are intermediates.

Let us assume that for mechanism (1) processes of addition and dissociation of substrates attain equilibrium that is not significantly shifted as a result of enzymatic reaction. In other words the quasi-equilibrium segment is as follows: $E \rightleftarrows ES_1 \rightleftarrows ES_1S_2$.

Based on these suppositions, solutions for mechanism (1) in the quasi-equilibrium assumption are as follows:

$$\frac{v^{qe}}{e_0} = \frac{Q(EP_1P_2)k_4^*}{\sum_j Q(X_j)}, \quad (5)$$

$$[X_i]^{qe} = \frac{e_0 Q(X_i)}{\sum_j Q(X_j)}, \quad (6)$$

where

$$Q(E) = k_{-1}k_{-2}k_{-3} + k_{-1}k_{-2}k_4^*,$$

$$Q(ES_1) = k_1[S_1]k_{-2}k_{-3} + k_1[S_1]k_{-2}k_4^*,$$

$$Q(ES_1S_2) = k_1[S_1]k_2[S_2]k_{-3} + k_1[S_1]k_2[S_2]k_4^*,$$

$$Q(EP_1P_2) = k_1[S_1]k_2[S_2]k_3. \quad (7)$$

Comparison of expressions (2)-(4) and (5)-(7) shows that for mechanism (1) the necessary and sufficient condition for realization of the quasi-equilibrium assumption is simultaneous fulfillment of conditions:

$$k_{-2} >> k_3, \quad (8)$$

$$k_{-1}k_{-2} >> k_2[S_2]k_3. \quad (9)$$

On fulfillment of these conditions $v^{qe} \approx v^{ss}$, $[E]^{qe} \approx [E]^{ss}$, $[ES_1]^{qe} \approx [ES_1]^{ss}$, $[ES_1S_2]^{qe} \approx [ES_1S_2]^{ss}$, $[EP_1P_2]^{qe} \approx [EP_1P_2]^{ss}$, and the ratios of steady-state concentrations of intermediates from the equilibrium segment approach the ratios of concentrations of these intermediates under the true equilibrium conditions. To evaluate accuracy of the quasi-equilibrium assumption, let us define the numerical characteristic of fulfillment of inequalities (8) and (9):

$$k_3/k_{-2} \leqslant \varepsilon, \quad (10)$$

$$k_2[S_2]k_3/k_{-1}k_{-2} \leqslant \varepsilon, \quad (11)$$

where the positive dimensionless parameter ε is equal to for now the maximal value of the left parts of inequalities (10) and (11) in the chosen fixed range of S_2 concentrations.

It can be easily shown that using the value of ε defined by inequalities (10) and (11), the following is true:

$$\frac{1}{1+2\varepsilon} v^{qe} < v^{ss} < v^{qe}, \quad (12)$$

$$\frac{[E]^{qe}}{1+2\varepsilon} < [E]^{ss} < [E]^{qe}(1+2\varepsilon), \quad (13)$$

$$\frac{[ES_1]^{qe}}{1+2\varepsilon} < [ES_1]^{ss} < [ES_1]^{qe}(1+\varepsilon), \quad (14)$$

$$\frac{[ES_1S_2]^{qe}}{1+2\varepsilon} < [ES_1S_2]^{ss} < [ES_1S_2]^{qe}, \quad (15)$$

$$\frac{[EP_1P_2]^{qe}}{1+2\varepsilon} < [EP_1P_2]^{ss} < [EP_1P_2]^{qe}. \quad (16)$$

The inequalities given above are strict due to the fact that physical values in the left parts of inequalities (10) and (11) expressed in real values cannot be strictly equal to one another. Taking the relative deviations of the values obtained by using the quasi-equilibrium assumption from their steady-state values as the numerical evaluation of accuracy of the quasi-equilibrium assumption, we obtain based on (12)-(16):

$$0 < \frac{v^{qe} - v^{ss}}{v^{ss}} < 2\varepsilon, \quad (17)$$

$$\left|\frac{[E]^{qe} - [E]^{ss}}{[E]^{ss}}\right| < 2\varepsilon, \quad (18)$$

$$\left|\frac{[ES_1]^{qe} - [ES_1]^{ss}}{[ES_1]^{ss}}\right| < 2\varepsilon, \quad (19)$$

$$0 < \frac{[ES_1S_2]^{qe} - [ES_1S_2]^{ss}}{[ES_1S_2]^{ss}} < 2\varepsilon, \quad (20)$$

$$0 < \frac{[EP_1P_2]^{qe} - [EP_1P_2]^{ss}}{[EP_1P_2]^{ss}} < 2\varepsilon. \quad (21)$$

Thus, accuracy of the quasi-equilibrium assumption depends on the value of ε: the lower is ε, the more exact is the assumption.

As follows from expressions (10) and (11), accuracy of application of the quasi-equilibrium assumption in this case depends not only on the ratio of reaction rate constants but also on the variable range of substrate S_2 concentrations. Let us limit the variable range of substrate S_2 concentrations in which application of the quasi-equilibrium assumption with the maximal accuracy is allowable using the fact that the left part of inequality (10) does not depend on substrate S_2 concentration. Let us designate the left part of inequality (10) as ε. In this case fulfillment of condition (11) is equal to fulfillment of condition:

$$[S_2] \leqslant k_{-1}/k_2. \quad (22)$$

If condition (22) is fulfilled, accuracy of the quasi-equilibrium assumption will be defined by inequality (10), and if substrate S_2 concentrations are outside the limits (22), accuracy of the quasi-equilibrium assumption will respectively decrease.

It should be mentioned that fulfillment of inequalities (10) and (11) is the necessary and sufficient condition for realization of the quasi-equilibrium assumption with the given accuracy for irreversible reaction (1) in case of the equilibrium segment $E \rightleftarrows ES_1 \rightleftarrows ES_1S_2$ being independent of the number of stages in this mechanism [15].

Ignoring conditions (10) and (11) can introduce large errors. The main danger is that substrate S_2 concentration is included in condition (11). In turn, it limits the allowed (in a case of the quasi-equilibrium assumption) range of substrate S_2 concentrations, and application of the quasi-equilibrium assumption under conditions of unlimited increase in concentration of this substrate (it is clearly demonstrated in graphical interpretations) results in incorrect conclusions. It is easy to check that steady-state solution (2) causes dependence of the reaction rate on substrate concentration in the double reciprocal coordinates (at various concentrations of the partner substrate) as a bundle of lines crossing in the second (third) quadrant (Fig. 1). Quasi-equilibrium solution (5) causes dependence of the reaction rate on substrate S_1 concentration in the double reciprocal coordinates as a bundle of lines crossing in the second quadrant and dependence of the reaction rate on substrate S_2 concentration in the double reciprocal coordinates as a bundle of lines crossing at the Y axis (Fig. 2). Thus it is desirable to use presentation in these coordinates for discrimination of substrate binding order in the mechanism of ordered bisubstrate enzymatic reaction (1): "if a plot in the double reciprocal coordinates shows that for one of substrates the lines cross at Y axis, this substrate is the second to be bound by the enzyme" [6, 17].

This approach for discrimination of mechanisms of bisubstrate enzymatic reactions is widely applied [18-30], but it cannot be accepted as a proper one. The quasi-equilibrium solution as assumption of an exact steady-state solution within the given error limits must properly

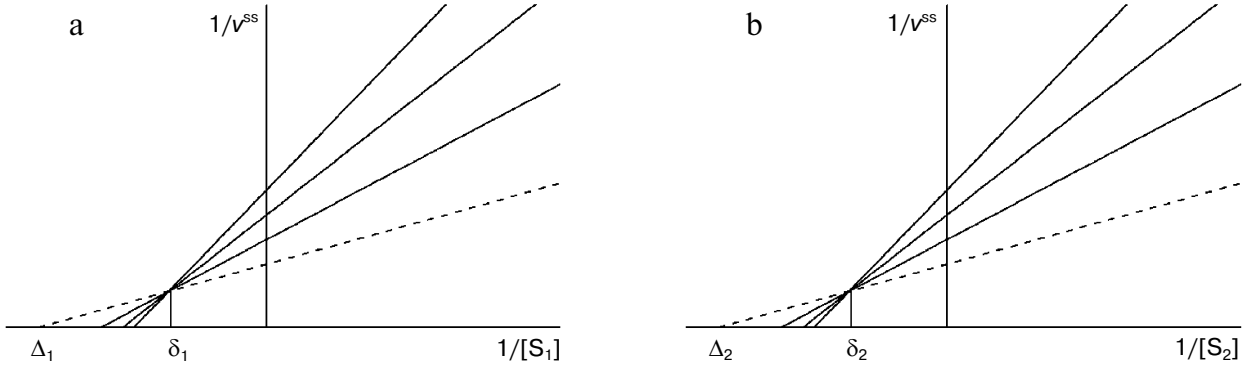

Fig. 1. Bisubstrate enzymatic reaction with ordered substrate binding (mechanism (1)). The steady-state assumption (Eqs. (2) and (23)). Dependence of the reaction rate on substrate concentration at the saturating concentration of the partner substrate is depicted by the dashed line.

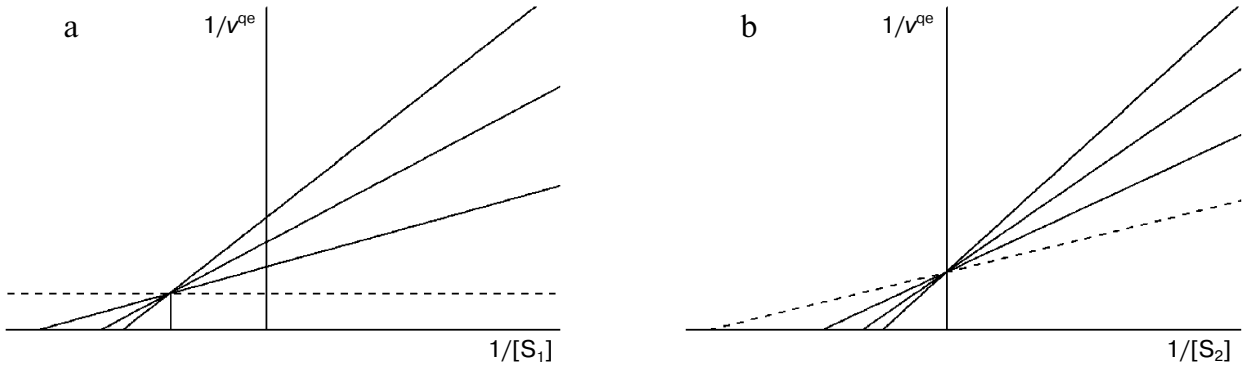

Fig. 2. Bisubstrate enzymatic reaction with ordered substrate binding (mechanism (1)). The quasi-equilibrium assumption (Eqs. (5) and (25)). Dependences of the reaction rate on concentration of substrates are presented without account for limitations on substrate S_2 concentration in accord with inequality (22). Dependence of the reaction rate on substrate concentration at the saturating concentration of the partner substrate is depicted by the dashed line.

account for regularities of the steady-state solution. If adequately used, the quasi-equilibrium assumption should not change the main features of steady-state solutions. In this case one can suggest that an intersection point on the Y axis in case of the quasi-equilibrium solution (Fig. 2) accounts for a limiting state in the process in which the steady-state solution (Fig. 1) demonstrates asymmetry on change of any parameters and approaches the solution presented in Fig. 2. However, it is easy to check that the steady-state solution (2) is completely symmetrical in relation to substrates S_1 and S_2, and no changes in the ratio of constants of mechanism (1) will result in asymmetry of the reaction rate dependences on substrate concentrations in double reciprocal coordinates.

To check this, let us consider Fig. 1. The steady-state solution (2) presented in Fig. 1 in general corresponds with expression:

$$\frac{e_0}{v^{ss}} = a + \frac{b}{[S_1]} + \frac{c}{[S_2]} + \frac{d}{[S_1][S_2]}, \qquad (23)$$

in which nonzero coefficients can be easily determined from formula (2). Let us take a ratio of abscissa of an intersection point of a bundle of lines (δ_1 in Fig. 1a and δ_2 in Fig. 1b) to the abscissa of an intersection point of a line corresponding to the highest (saturating) concentration of the partner substrate with the X axis (Δ_1 in Fig. 1a and Δ_2 in Fig. 1b) as the numerical value characterizing the degree of approximation of a intersection point of lines in the plot in double reciprocal coordinates to the Y axis. One can easily see that for any mechanism having dependences like (23) the following equalities are true:

$$\frac{\delta_1}{\Delta_1} = \frac{\delta_2}{\Delta_2} = \frac{bc}{ad}. \qquad (24)$$

It immediately follows that the degrees of approximation of an intersection point of lines in the plot in double reciprocal coordinates to the Y axis are equal for both substrates, that is dependences (23) are absolutely sym-

metrical in relation to both substrates. This means that no variation in kinetic constants of any enzymatic reaction mechanism giving dependences as (23) will result in asymmetrical changes in the plot in double reciprocal coordinates: if an intersection point approaches the Y axis for a one substrate, an analogous tendency will be observed also for another one.

Thus, under any conditions plots of Fig. 1 corresponding to the reaction mechanism (1) cannot be transformed into plots of Fig. 2. However, plots of Fig. 2 account for the quasi-equilibrium solution (5) for the same mechanism (1), which is described by expression

$$\frac{e_0}{v^{qe}} = a + \frac{c}{[S_2]} + \frac{d}{[S_1][S_2]} \qquad (25)$$

asymmetrical in relation to substrates.

The origin of the apparent contradiction is that the expression for the quasi-equilibrium assumption (5) approximates dependence (2) with the given accuracy only when conditions (10) and (11) are met. If ε value is fixed, plots of v^{qe} dependence on substrate concentrations will correspond with those depicted in Fig. 3 accounting for the fact that in this case the range of $[S_2]$ changes is limited: $[S_2] \le [S_2]_{max}$, where $[S_2]_{max} = k_{-1}/k_2$.

If conditions (10) and (11) are neglected, this results in significant errors. Thus, a bisubstrate enzymatic reaction with ordered binding of substrates was considered in [6] (constants are valued as in the cited reference):

$$E \underset{k_2}{\overset{k_1[S_1]}{\rightleftarrows}} ES_1 \underset{k_4}{\overset{k_3[S_2]}{\rightleftarrows}} ES_1S_2 \xrightarrow{k_5} E+P \qquad (26)$$

and it was postulated that rapid equilibrium will be attained when

$$k_2 >> k_5, \qquad k_4 \leqslant k_5. \qquad (27)$$

The expression for the reaction rate (26) obtained under the assumption of rapid equilibrium [6] gave plots analogous to those presented in Fig. 2 of this work, that is, dependences at high concentrations of substrate S_2 were interpreted incorrectly, although inequalities (27) do not formally restrict S_2 concentrations. The reason is that conditions for application of the quasi-equilibrium assumption (27) for mechanism (26) were postulated in [6] incorrectly and as shown in this work, conditions analogous to (8) and (9) will be proper in this case, that is,

$$k_4 >> k_5, \qquad (28)$$

$$k_2k_4 >> k_3[S_2]k_5. \qquad (29)$$

As follows from inequality (29), S_2 concentration in the case of the quasi-equilibrium assumption should be limited, and in the framework of the quasi-equilibrium assumption dependences must not be approximated on unlimited increase in S_2 concentrations.

General solution of the problem of asymmetrical expressions. As shown above, expression (23) for the steady-state rate of bisubstrate enzymatic reactions is strictly symmetrical in relation to concentrations of substrates, and no changes in the values of reaction rate constants will result in asymmetrical dependences typical of Fig. 2. Thus, dependences governed by equation (25) cannot be related with mechanism (1).

Since experimentally obtained dependences like (25) are often described [18-30], a more general question arises. The latter is outside the framework of application of the quasi-equilibrium assumption as well as the framework of discussion of the ordered bisubstrate reaction mechanisms: do mechanisms of enzymatic reactions leading to expressions like (25) for the steady-state rate exist at all? There is a simple answer on this question: no such mechanisms exist.

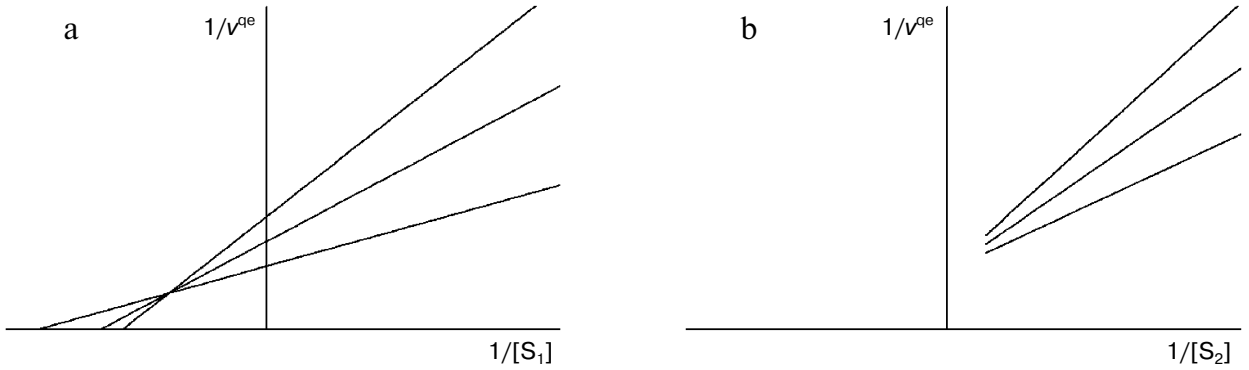

Fig. 3. Bisubstrate enzymatic reaction with ordered binding of substrates (mechanism (1)), the quasi-equilibrium assumption (Eqs. (5) and (25)). The reaction rates versus concentrations of substrates accounting for limitations on S_2 concentration in accord with inequality (22) are presented.

To prove this statement, let us consider an arbitrary mechanism of an enzymatic reaction including the following fragments:

$$\begin{array}{l} \ldots\ X_1 \xrightarrow{k_1[S_1]} \ldots \\ \qquad\vdots \\ \ldots\ YP \xrightarrow{k_P} Y + P \ldots . \end{array} \qquad (30)$$

Mechanism (30) accounts for the fact that substrate S_1 interacts with the intermediate form of enzyme X_1 (in general view – with several intermediate enzyme forms, a case of interaction with one is considered for clearness) and there is only one point for exit of product P. There are no other restrictions of the enzymatic reaction mechanism. At zero P concentration the steady-state rate of reaction (30) can be presented as follows using the graph method [16]:

$$\frac{e_0}{v^{ss}} = \sum_i T_i \,/\, k_p \sum_j T_j^{YP}, \qquad (31)$$

where ΣT_i is a sum of all basic trees of all vertices of graph (30) and ΣT_j^{YP} is a sum of basic trees of the YP vertex.

Let us take an arbitrary tree T_j^{YP} and add a branch resulting in reaction product P (the value of this branch is k_P). It is easy to check that this results in a new tree with a single cycle. Branch $k_1[S_1]$ is obligatory in the body of this cycle. If this branch is removed, we obtain a base tree of X_1 vertex equal to $T_j^{YP}k_P/k_1[S_1]$. This tree is one of the members of the sum in numerator of expression (31). Then the numerator of expression (31) will be as follows:

$$\sum_i T_i = \sum_j T_j^{YP} k_P / k_1[S_1] + \sum_m T_m,$$

where ΣT_m is the sum of all other trees. Expression (31) is rearranged as follows:

$$\frac{e_0}{v^{ss}} = 1/k_1[S_1] + \sum_m T_m/k_p \sum_j T_j^{YP} = 1/k_1[S_1] + R, \qquad (32)$$

where R is a rational fractional function with positive coefficients, that is, $R > 0$. Result analogous to (32) can be easily obtained in a case of several intermediate forms of enzyme with which substrate S_1 interacts as well as in a case of several points for exit of product P in the general model (30).

Thus, it is shown that for an arbitrary mechanism of enzymatic reaction, the expression for the reciprocal rate as related to concentration of any substrate S_1 is like a sum of a positive rational function and $1/k_1[S_1]$. Consequently, there is no mechanism in which the expression for the reciprocal rate does not have a sum member with reciprocal substrate concentration like $1/k_1[S_1]$, and existence of asymmetrical expression like (25) for the steady-state kinetics of enzymatic reactions is in principle impossible.

As a consequence, when for an arbitrary bisubstrate enzymatic reaction the rate dependences on concentrations of substrates are linearized in double reciprocal coordinates, dependences (23) with nonzero b and c coefficients are obtained, that is, expressions are completely symmetrical in relation to substrates.

Visualization problem on discrimination of enzymatic reaction mechanisms. Using Figs. 4-7, let us illustrate the general practice [6, 18-30] in analysis of bisubstrate reaction mechanisms from the data on steady-state kinetics. The first stage is drawing a plot of the rate of enzymatic reaction versus concentration of one of substrates (at various concentrations of the partner substrate) in double reciprocal coordinates (Figs. 4 and 6). If these dependences are linear, plots of the slope (angular coefficient)

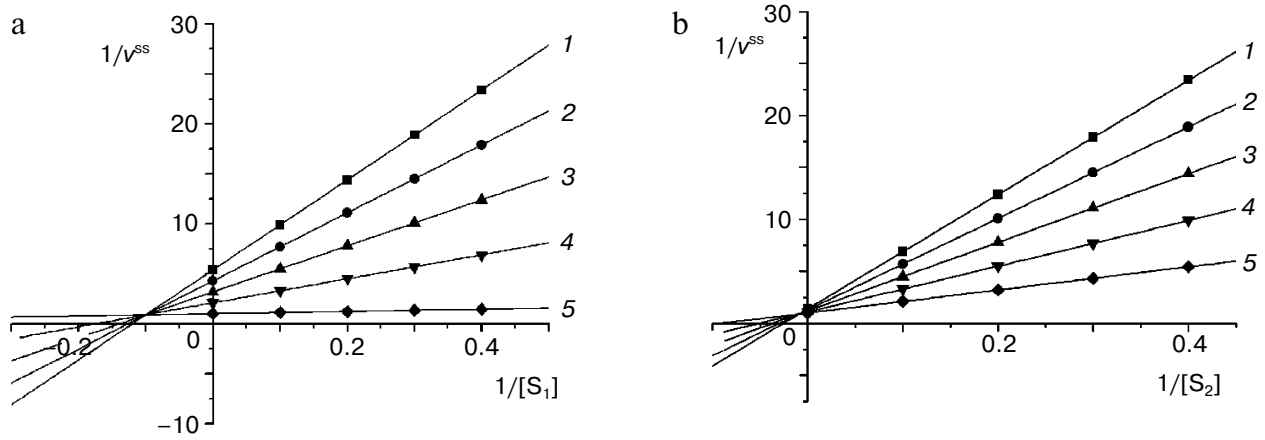

Fig. 4. Bisubstrate enzymatic reaction with ordered substrate binding (mechanism (26)). The steady-state assumption, $k_1 = 1$, $k_2 = 10$, $k_3 = 1$, $k_4 = 10$, $k_5 = 1$. a) $[S_2]$: *1*) 2.5; *2*) 3.33; *3*) 5; *4*) 10; *5*) 1000. b) $[S_1]$: *1*) 2.5; *2*) 3.33; *3*) 5; *4*) 10; *5*) 1000.

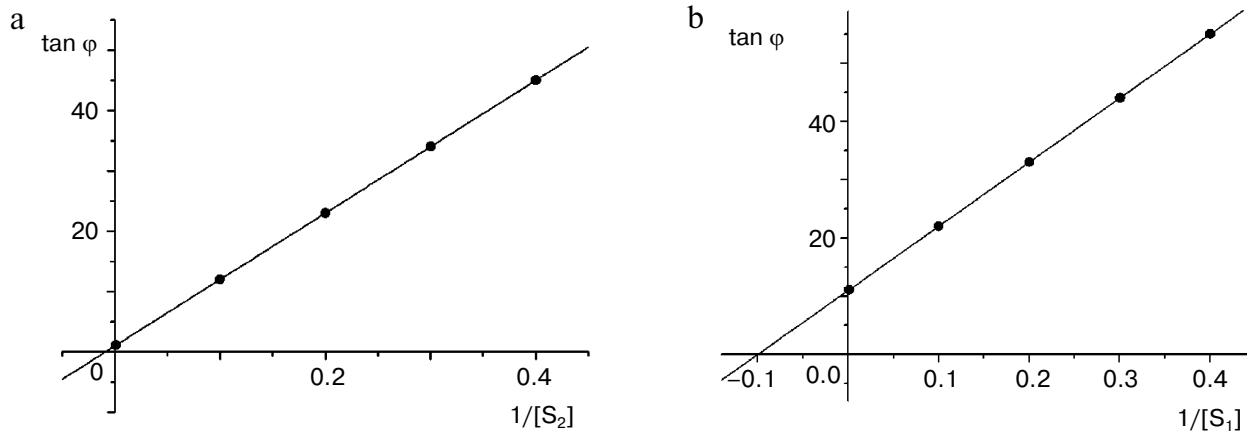

Fig. 5. Slope of lines in Fig. 4 versus reciprocal concentration of partner substrate.

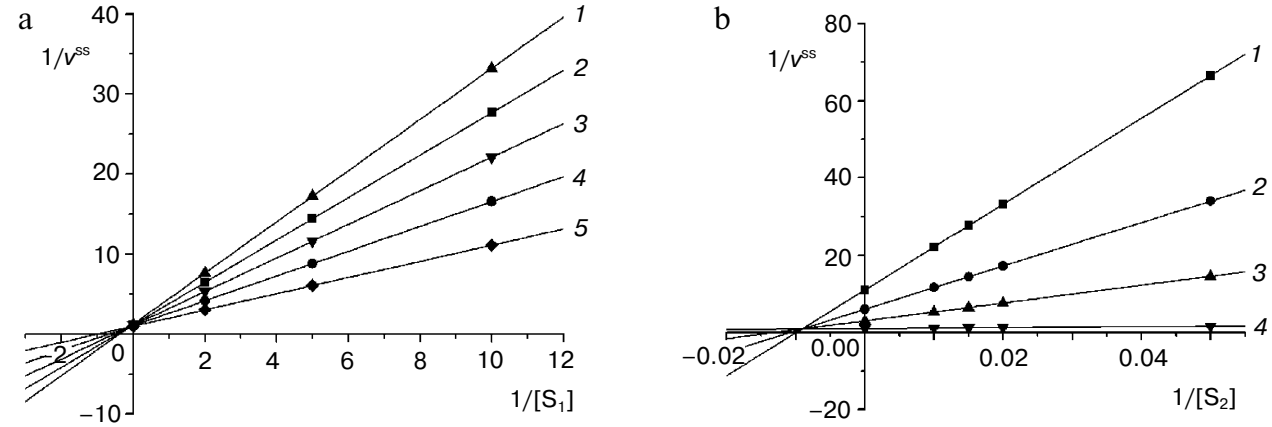

Fig. 6. Bisubstrate enzymatic reaction with ordered substrate binding (mechanism (26)). The steady-state assumption, $k_1 = 1$, $k_2 = 10$, $k_3 = 1$, $k_4 = 10$, $k_5 = 1$. a) $[S_2]$: *1*) 50; *2*) 66.67; *3*) 100; *4*) 200; *5*) 10000. b) $[S_1]$: *1*) 0.1; *2*) 0.2; *3*) 0.5; *4*) 100.

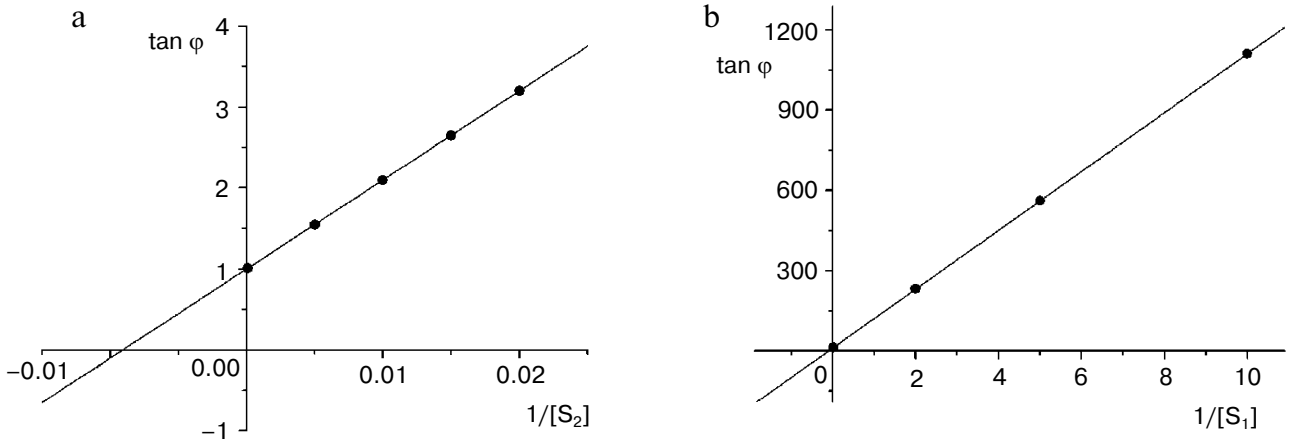

Fig. 7. Slope of lines in Fig. 6 versus reciprocal concentration of partner substrate.

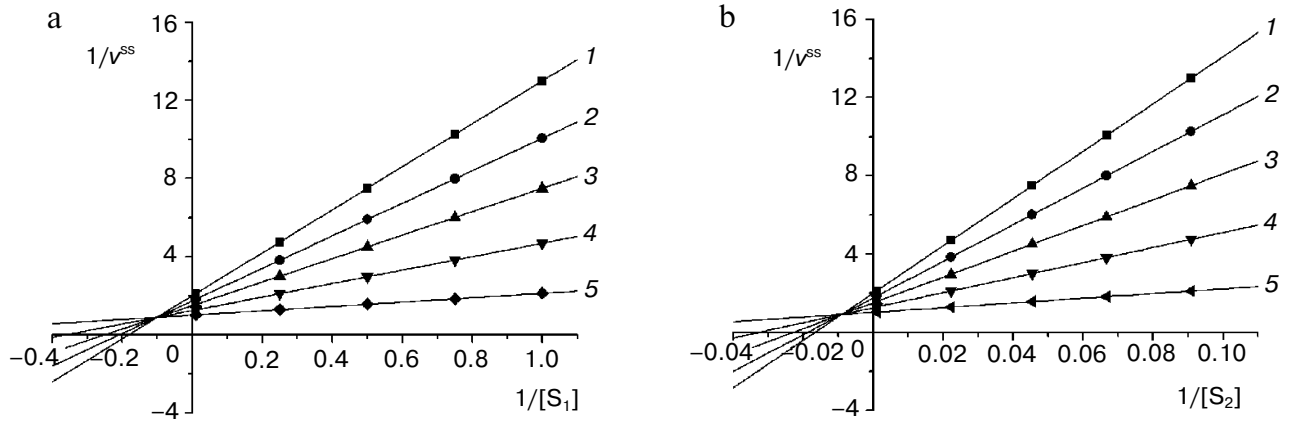

Fig. 8. Bisubstrate enzymatic reaction with ordered substrate binding (mechanism (26)). The steady-state assumption, $k_1 = 1$, $k_2 = 10$, $k_3 = 1$, $k_4 = 10$, $k_5 = 1$. a) $[S_2]$: *1*) 11; *2*) 15; *3*) 22; *4*) 45; *5*) 1000. b) $[S_1]$: *1*) 1; *2*) 1.33; *3*) 2; *4*) 4; *5*) 100.

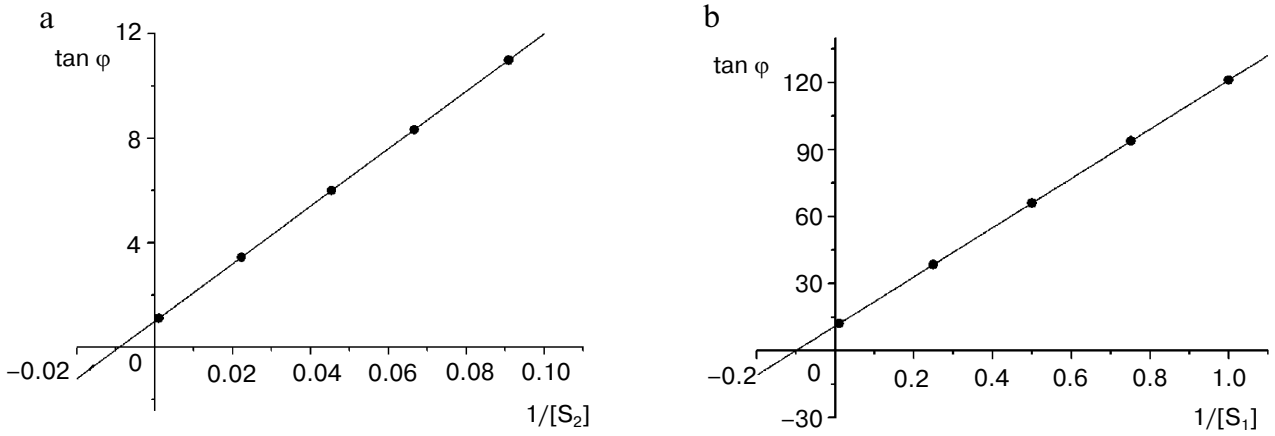

Fig. 9. Slopes of lines in Fig. 8 versus reciprocal concentration of partner substrate.

versus the reciprocal concentration of the partner substrate are drawn (Figs. 5 and 7).

The reaction mechanism is considered to have ordered substrate binding and its kinetics is described in the quasi-equilibrium assumption, if for one substrate, there is an intersection point of lines in the second quadrant of the plot in double reciprocal coordinates, and dependence of their slopes on the reciprocal concentration of the partner substrate is linear and crosses the origin, and for the other substrate, an intersection point of lines is on the Y axis of the plot in the double reciprocal coordinates and dependence of their slopes on the reciprocal concentration of the partner substrate is linear and intercepts a positive portion of the Y axis. The order of substrate binding is considered to be as follows: for the case depicted in Figs. 4 and 5, substrate S_1 is the first to be bound and for the case depicted in Figs. 6 and 7, substrate S_2 is the first.

However, as shown above, such linear dependences should be completely symmetrical, because there are no mechanisms for enzymatic reactions showing dependences (25). An apparent contradiction with the results described above is simply solved: plots depicted in Figs. 4-7 originate from one and the same mechanism (26) with one and the same set of constants, and conclusions about asymmetry of these plots are based on optical illusion.

The matter is that in Figs. 4-7 the data are presented in different ranges of substrate concentrations. As a result completely symmetrical dependences seem to be asymmetrical. There are no formal objections to the chosen range of concentrations in the case of Figs. 4 and 5 as well as in case of Figs. 6 and 7: the rate values in these ranges change from several percent to the maximal values. It should be noted that change in dependence mode on change in the range of used concentrations was found in [31] while analyzing experimental data. It seems like in [18-30] experimental data were presented in the ranges when dependences seemed to be asymmetrical.

To avoid such misunderstandings, the following algorithm for choosing concentration ranges should be adopted when experiments are planned and results are presented: at saturating concentration of the substrate (S_2) the rate dependence on S_1 concentration is plotted in double reciprocal coordinates and the intersection point of the line with the X axis is determined. The abscissa of the intersection point will give the minimal S_1 concentration in the planned concentration range. The minimal S_2 concentration in the planned concentration range is found in analogous way. Presentation of experimental dependences in the so found concentration range will not mislead. The rate dependences on concentrations of substrates for the same mechanism (26) with the same set of rate constants but in concentration range chosen in accord with the above described algorithm are presented in Figs. 8 and 9. As a result in properly chosen concentration ranges symmetrical expressions give rise to symmetrical plots.

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