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Steady-State Kinetics of Bifunctional Enzymes. Taking into Account Kinetic Hierarchy of Fast and Slow Catalytic Cycles in a Generalized Model

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Abstract—A steady-state approximation of the generalized two-dimensional model of a bifunctional enzyme catalyzing independent proceeding of two one-pathway reactions is considered in a case of mutual influence of the active sites. Coexistence of fast and slow catalytic cycles in the reaction mechanism is analyzed. Conditions when the hierarchy of fast and slow catalytic cycles allows simplification of a two-dimensional model and its reduction to the one-dimensional cyclic schemes were determined. Kinetic equations describing these simplified schemes are presented.

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Bifunctional (or polyfunctional) enzymes seem to appear in evolution by the combination of genes encoding enzymes tightly bound functionally [1, 2]. Bifunctional enzymes and bifunctional enzyme complexes contain a minimum of two active sites and usually catalyze two consecutive reactions [3, 4]. These features potentially allow manifestation of some new specific properties of bifunctional enzymes. First, the possible mobility of an intermediate (a product of the first reaction and at the same time, substrate of the second reaction) between two active sites without its appearance in solution [4-6]. Second, the state of the active site of the first reaction may influence kinetic properties of the active site of the second reaction, and vice versa, the state of the active site of the second reaction may influence kinetic properties of the active site of the first reaction [3, 4]. For thymidylate synthase–dihydrofolate reductase from *Leishmania major* [3], thymidylate synthase–dihydrofolate reductase from *Toxoplasma gondii* [7], tryptophan synthase from *Salmonella typhimurium* [8], and dimethylglycine oxidase from *Arthrobacter globiformis* [9], the effect of intermediate mobility was found, whereas for such enzymes as thymidylate synthase–dihydrofolate reductase from *Cryptosporidium hominis* [10] and acetyl transferase–uridyl transferase from *Escherichia coli* [11], intermediate mobility is not observed. For thymidylate synthase–dihydrofolate reductase from *L. major* [3], carbamoyl phosphate synthase from *E. coli* [12], and glutamine phosphoribosylpyrophosphate amidotransferase from *E. coli* [13], the effect of one reaction proceeding on kinetics of another reaction is observed, whereas for enzymes thymidylate synthase–dihydrofolate reductase from *C. hominis* [10] and *T. gondii* [14], such effect is not found. A report about prevailing conversion of endogenous intermediate (prostaglandin G_2) by microsomal bifunctional enzyme prostaglandin-H-synthase and the absence of this effect in a case of purified enzyme is also worth noting [15].

The above-mentioned features of kinetic behavior of bifunctional enzymes should have an adequate kinetic description, and numerous attempts to do this have been made. The values of lag period and intermediate concentrations during consecutive reactions catalyzed by bifunctional enzymes and discrimination of mechanisms including mobility of intermediate between active sites have been accounted for [16, 17]. Action of bifunctional enzyme thymidylate synthase–dihydrofolate reductase in a case when proceeding of one reaction influences kinetics of another one has been kinetically described [3, 14, 18]. Attempts to describe kinetic features of bifunctional

enzyme prostaglandin-H-synthase have been made [19, 20]. It should be noted that in all these cases, the fact that two reactions catalyzed by bifunctional enzyme proceed simultaneously and independently from each other was not accounted for, but "one-dimensional" modified kinetic models were considered. Such consideration is absolutely inadequate, because the presence of two active sites provides independent proceeding of two reactions; this requires for simultaneous accounting for the state of both active sites and thus, intermediates in kinetic schemes should be characterized by two indexes (one index for each active site). Accounting for independent change of two indexes transforms kinetic schemes into two-dimensional ones. Earlier [21], we considered a steady-state approximation for generalized models of a multisubstrate bifunctional enzyme including a two-dimensional scheme. It was shown that expressions for the rate of enzymatic reactions in this case are complex dependencies on concentrations of substrates and products of the catalyzed reactions; this should result in significant deviation from widespread hyperbolic dependencies like the Michaelis–Menten equation. A problem of overcomplicated kinetic expressions is common for enzymatic kinetics [22-24]. As shown by practice, account for hierarchy of the rates of certain reactions in some cases allows significant simplification of the system of equations and obtaining kinetic expressions conforming well experimental data. This is related with a quasi-steady-state approximation widespread in chemical kinetics (so-called Bodenstein steady-state principle) justified by the Tikhonov theorem [25] and also with a quasi-equilibrium approximation in enzymatic kinetics [24].

The goal of this study was to consider a generalized kinetic two-dimensional model of bifunctional enzyme [21] and to find conditions when hierarchy of fast and slow catalytic cycles allows reduction of a two-dimensional model to one-dimensional cyclic schemes that have been analyzed [26-29].

RESULTS AND DISCUSSION

Description of two-dimensional kinetic model of a bifunctional enzyme. Let us generally consider the mechanism of action of a bifunctional enzyme [21]. Although being somewhat cumbersome, such consideration has its own advantages because it is impossible to say *a priori* what intermediate enzyme–substrate complexes are in the mechanism of action of a certain enzyme and what is their number.

Let us suggest that bifunctional enzyme E catalyzes two reactions, A and B:

$$S_1^A + S_2^A + ... \rightleftarrows P_1^A + P_2^A + ... ,$$

$$S_1^B + S_2^B + ... \rightleftarrows P_1^B + P_2^B + ... ,$$

where S_i^A and P_i^A are substrates and products of reaction A, and S_i^B and P_i^B are substrates and products of reaction B, respectively. One of the products of reaction A catalyzed by bifunctional enzyme E can be a substrate for reaction B. In this study, we consider a case when bifunctional enzyme E has two active sites (in which reactions A and B are catalyzed), and reactions A and B may proceed independently. Let us designate intermediate forms of bifunctional enzyme E appearing during catalysis as X_{ij}. In this symbol, the first and the second indexes are related with the state of the active sites of reactions A and B, respectively.

It is supposed that reactions A and B proceed via one-way mechanisms. In the absence of components of reaction B (that is, substrates and products), the mechanism of reaction A will look as follows:

$$X_{00} \underset{\beta_{00}^A}{\overset{\alpha_{00}^A}{\rightleftarrows}} X_{10} \underset{\beta_{10}^A}{\overset{\alpha_{10}^A}{\rightleftarrows}} X_{20} \underset{\beta_{20}^A}{\overset{\alpha_{20}^A}{\rightleftarrows}} .....$$

$$..... X_{i0} \underset{\beta_{i0}^A}{\overset{\alpha_{i0}^A}{\rightleftarrows}} ..... X_{m0} \underset{\beta_{m0}^A}{\overset{\alpha_{m0}^A}{\rightleftarrows}} (X_{00}) . \qquad (1)$$

In this expression the state of the active site of reaction B is indexed by 0 (its numerical value is insignificant; significant is the fact that this index does not change).

In the absence of the components of reaction A, the mechanism of reaction B will look as follows:

$$X_{00} \underset{\beta_{00}^B}{\overset{\alpha_{00}^B}{\rightleftarrows}} X_{01} \underset{\beta_{01}^B}{\overset{\alpha_{01}^B}{\rightleftarrows}} X_{02} \underset{\beta_{02}^B}{\overset{\alpha_{02}^B}{\rightleftarrows}} .....$$

$$..... X_{0j} \underset{\beta_{0j}^B}{\overset{\alpha_{0j}^B}{\rightleftarrows}} ..... X_{0n} \underset{\beta_{0n}^B}{\overset{\alpha_{0n}^B}{\rightleftarrows}} (X_{00}) . \qquad (2)$$

In this expression the unchanged state of the active site of reaction A is indexed by 0. If components of both reactions—A and B—are present, both reactions proceed simultaneously, and the general mechanism of the process will be described by a two-dimensional scheme:

$$\begin{array}{ccccccccccccc}
(X_{00}) & \rightleftarrows & (X_{01}) & \rightleftarrows & (X_{02}) & \rightleftarrows & ..... & (X_{0j}) & \rightleftarrows & ..... & (X_{0n}) & \rightleftarrows & (X_{00}) \\
\uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & & & \uparrow\downarrow & & & \uparrow\downarrow & & \uparrow\downarrow \\
X_{m0} & \rightleftarrows & X_{m1} & \rightleftarrows & X_{m2} & \rightleftarrows & ..... & X_{mj} & \rightleftarrows & ..... & X_{mn} & \rightleftarrows & (X_{m0}) \\
\uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & & & \uparrow\downarrow & & & \uparrow\downarrow & & \uparrow\downarrow \\
\multicolumn{13}{c}{.....} \\
\uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & & & \uparrow\downarrow & & & \uparrow\downarrow & & \uparrow\downarrow \\
X_{i0} & \rightleftarrows & X_{i1} & \rightleftarrows & X_{i2} & \rightleftarrows & ..... & X_{ij} & \rightleftarrows & ..... & X_{in} & \rightleftarrows & (X_{i0}) \\
\uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & & & \uparrow\downarrow & & & \uparrow\downarrow & & \uparrow\downarrow \\
\multicolumn{13}{c}{.....} \\
\uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & & & \uparrow\downarrow & & & \uparrow\downarrow & & \uparrow\downarrow \\
X_{10} & \rightleftarrows & X_{11} & \rightleftarrows & X_{12} & \rightleftarrows & ..... & X_{1j} & \rightleftarrows & ..... & X_{1n} & \rightleftarrows & (X_{10}) \\
\uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & & & \uparrow\downarrow & & & \uparrow\downarrow & & \uparrow\downarrow \\
X_{00} & \rightleftarrows & X_{01} & \rightleftarrows & X_{02} & \rightleftarrows & ..... & X_{0j} & \rightleftarrows & ..... & X_{0n} & \rightleftarrows & (X_{00})
\end{array} . \qquad (3)$$

It is supposed that as a result of a complete cycle in the vertical direction one turn of reaction A occurs and as a result of a complete cycle in the horizontal direction one turn of reaction B occurs. Movement towards increase in the lower index values is considered to be the reaction pathway. It is obvious that according to such definition, none of the rate constants α is equal to zero, whereas some of the rate constants β are. Designations of the rate constants of reaction (3) (taking intermediate form X_{ij} as an example) are as follows:

$$\begin{array}{ccc} & X_{i+1,j} & \\ \alpha_{ij}^{A} & \uparrow\downarrow\ \beta_{ij}^{A} & \\ & X_{ij} \underset{\beta_{ij}^{B}}{\overset{\alpha_{ij}^{B}}{\rightleftharpoons}} X_{i,j+1} . & \end{array} \qquad (4)$$

Let us consider kinetics of reactions proceeding via mechanism (3) in a case of steady state establishment with respect to the intermediate enzyme forms. Analysis of a steady state of kinetic scheme (3) can be significantly simplified by application of graph theory; the graph is a combination of point (vertices) and lines connecting them (branches) [30]. Here X_{ij} intermediates play the role of vertices, and each branch of the graph is an elementary chemical reaction, and numerical values equal to the rate constant of the first (pseudo-first) order for the corresponding chemical reaction is ascribed to this branch. In our case these are α_{ij}^{A}, β_{ij}^{A}, α_{ij}^{B}, and β_{ij}^{B}. The concept of base tree is inserted for analysis of kinetic schemes by the graph theory. For any arbitrary graph vertex X_{kl} (let us name it as base), the combination of branches passing through all graph vertices (excluding X_{kl}) and directed to the base composes a base tree. A base tree does not contain cycles. The value of the base tree is equal to a product of values of all branches composing this base tree. The base determinant is defined as a combination of all base trees of this base, and the value of base determinant (D_{kl}) is equal to the sum of values of all base trees of a given base X_{kl}. As follows from a definition of base tree, it has an important property: each base tree has exactly one branch exiting from each graph vertex (of course, excluding the vertex which is a base). A ratio of steady-state concentrations of any intermediate enzyme forms X_{kl} and X_{op} is equal to a ratio of the values of corresponding base determinants D_{kl} and D_{op} [30]:

$$[X]_{kl}/[X]_{op} = D_{kl}/D_{op}, \qquad (5)$$

and consequently, steady-state concentration of any intermediate enzyme form X_{kl} will be:

$$[X_{kl}] = E_0 \frac{D_{kl}}{\sum_i \sum_j D_{ij}}, \qquad (6)$$

where E_0 is a total enzyme concentration (sum of concentrations of all intermediate forms of enzyme E).

The steady-state rates of reactions A (v^A) and B (v^B) for mechanism (3) may be written as follows:

$$v^A = \sum_j (\alpha_{mj}^A [X_{mj}] - \beta_{mj}^A [X_{0j}]) , \qquad (7)$$

$$v^B = \sum_i (\alpha_{in}^B [X_{in}] - \beta_{in}^B [X_{i0}]) . \qquad (8)$$

Using the graph theory concepts and Eq. (6):

$$v^A = E_0 \frac{\sum_j (\alpha_{mj}^A D_{mj} - \beta_{mj}^A D_{0j})}{\sum_i \sum_j D_{ij}} , \qquad (9)$$

$$v^B = E_0 \frac{\sum_i (\alpha_{in}^B D_{in} - \beta_{in}^B D_{i0})}{\sum_i \sum_j D_{ij}} . \qquad (10)$$

Let substrate of reaction A (S^A) interact with all intermediate forms of enzyme E_{kj}, where k value is fixed ($0 \le k \le m$) and j varies in the range $0 \le j \le n$; let substrate of reaction B (S^B) interact with all intermediate forms of enzyme E_{il}, where l value is fixed ($0 \le l \le n$) and i varies in the range $0 \le i \le m$. This means that in the absence of components of reaction B, substrate of reaction A (S^A) once participates in catalytic cycle, interacting with the only intermediate enzyme form E_{k0}. In the presence of components of reaction B, substrate of reaction A (S^A) interacts with ($n + 1$) intermediate forms of the enzyme E_{kj} ($0 \le j \le n$) in a catalytic cycle. In the absence of components of reaction A, substrate of reaction B (S^B) once participates in a catalytic cycle, interacting with the only intermediate enzyme form E_{0l}. In the presence of components of reaction A, substrate of reaction B (S^B) interacts with ($m + 1$) intermediate forms of enzyme E_{il} ($0 \le i \le m$) in a catalytic cycle.

In order to study the dependence of the A and B reaction rate on substrate concentrations, let us consider a special case when reactions A and B proceed irreversibly. For example, the absence of reverse reaction is possible at the absence of one of the products of direct reaction in the reaction mixture. Irreversibility allows for equality to zero of at least one reverse rate constant in the enzymatic mechanism.

For definiteness sake, let $\beta_{mj}^A = 0$ for all $0 \le j \le n$ and $\beta_{in}^B = 0$ for all $0 \le i \le m$. In this case, in the absence of reaction B components, dependence of the steady-state rate of enzymatic reaction A (v^A) on concentration S^A is a simple hyperbolic function:

$$v^A = a^A [S^A]/(b^A + [S^A]), \qquad (11)$$

and in the absence of reaction A components, dependence of the steady-state rate of enzymatic reaction B (v^B) on concentration S^B is also a simple hyperbolic function:

$$v^B = a^B[S^B]/(b^B + [S^B]). \quad (12)$$

This is true because in the absence of the alternative reaction components, reactions A and B have one-way mechanisms of an irreversible enzymatic reaction [28].

When all components of reactions A and B are present in the reaction mixture simultaneously, it is worthwhile to consider two alternative cases.

First case. Proceeding of one reaction does not change the values of kinetic constants of another reaction, that is, for reaction A the following is correct

$$\alpha^A_{li} = \alpha^A_{lj},\ \beta^A_{li} = \beta^A_{lj} \quad (13)$$

for all $0 \le l \le m$, $0 \le i \le n$, $0 \le j \le n$, and for reaction B the following is correct

$$\alpha^B_{il} = \alpha^B_{jl},\ \beta^B_{il} = \beta^B_{jl} \quad (14)$$

for all $0 \le l \le n$, $0 \le i \le m$, $0 \le j \le m$.

In this case, reactions A and B proceed strictly independently, and their kinetics is formally described by Eqs. (1) and (2), dependencies of v^A and v^B on substrate concentrations $[S^A]$ and $[S^B]$ are presented by Eqs. (11) and (12), respectively, and v^A is independent of $[S^B]$ and v^B is independent of $[S^A]$.

Second case. Proceeding of one reaction changes kinetic constants of another reaction, that is, conditions (13) and (14) are violated. In this case, dependencies of v^A and v^B on substrate concentrations $[S^A]$ and $[S^B]$ are fractionally-rational functions:

$$v^A = \frac{c_1[S^A] + c_2[S^A]^2 + \ldots + c_{n+1}[S^A]^{n+1}}{d_0 + d_1[S^A] + d_2[S^A]^2 + \ldots + d_{n+1}[S^A]^{n+1}}, \quad (15)$$

$$v^B = \frac{e_0 + e_1[S^A] + e_2[S^A]^2 + \ldots + e_{n+1}[S^A]^{n+1}}{d_0 + d_1[S^A] + d_2[S^A]^2 + \ldots + d_{n+1}[S^A]^{n+1}}, \quad (16)$$

$$v^B = \frac{f_1[S^B] + f_2[S^B]^2 + \ldots + f_{m+1}[S^B]^{m+1}}{g_0 + g_1[S^B] + g_2[S^B]^2 + \ldots + g_{m+1}[S^B]^{m+1}}, \quad (17)$$

$$v^A = \frac{h_0 + h_1[S^B] + h_2[S^B]^2 + \ldots + h_{m+1}[S^B]^{m+1}}{g_0 + g_1[S^B] + g_2[S^B]^2 + \ldots + g_{m+1}[S^B]^{m+1}}. \quad (18)$$

In Eqs. (15)-(18), positive parameters c_i ($1 \le i \le (n + 1)$), d_i, e_i ($0 \le i \le (n + 1)$) depend on concentration $[S^B]$ and positive parameters f_i ($1 \le i \le (m + 1)$), g_i, h_i ($0 \le i \le (m + 1)$) depend on concentration $[S^A]$.

Thus, realization of the second case manifests itself in deviation from hyperbolic dependence of enzymatic rate in case of the presence of components of an alternative reaction in the reaction mixture and also in dependence of the reaction rate on concentration of substrate of an alternative reaction.

Bifunctional enzymes usually catalyze complex multisubstrate reactions whose mechanisms involve a large number of intermediate enzyme forms [4, 6]. Owing to this, dependencies of the rates on substrate concentrations (Eqs. (15)-(18)) should be complex fractionally-rational functions with high m and n values. But dependencies obtained experimentally are much simpler and are often described by the Michaelis–Menten equation even for those bifunctional enzymes whose kinetics is characterized by mutual influence of reactions [13] (that is, the above considered second case is realized). Here we come up against a common problem of kinetics, including enzymatic kinetics: theoretical dependencies are very complicated, and practice requires reasonable simplification. As mentioned above, significant progress may be achieved accounting for hierarchy of the rates of elementary reactions comprising a reaction mechanism (quasi-steady-state approximation in chemical kinetics, quasi-equilibrium approximation in enzymatic kinetics). It is worthwhile to analyze whether hierarchy of the rates of elementary reactions in the mechanism of action of bifunctional enzyme may allow simplification of kinetic schemes.

Accounting for hierarchy of fast and slow cycles. Let one of the cycles of reaction B presented by a fragment of scheme (3) be fast:

$$\begin{array}{ccccccc} \uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & & \\ X_{q0} & \underset{\beta^B_{q0}}{\overset{\alpha^B_{q0}}{\rightleftharpoons}} & X_{q1} & \underset{\beta^B_{q1}}{\overset{\alpha^B_{q1}}{\rightleftharpoons}} & X_{q2} & \underset{\beta^B_{q2}}{\overset{\alpha^B_{q2}}{\rightleftharpoons}} & \ldots\ldots \\ \uparrow\downarrow & & \uparrow\downarrow & & \uparrow\downarrow & & \end{array}$$

$$\begin{array}{ccccc} \uparrow\downarrow & & \uparrow\downarrow & & \\ \ldots\ldots X_{qj} & \underset{\beta^B_{qj}}{\overset{\alpha^B_{qj}}{\rightleftharpoons}} & \ldots\ldots X_{qn} & \underset{\beta^B_{qn}}{\overset{\alpha^B_{qn}}{\rightleftharpoons}} & (X_{q0}). \\ \uparrow\downarrow & & \uparrow\downarrow & & \end{array} \quad (19)$$

The assumption that reactions in this cycle are fast in relation to the other reactions and that the ratio of concentrations of intermediates in a fast cycle does not depend on concentrations of alternative reaction components is quite justified, although qualitative and intuitive. Analysis of Eqs. (9) and (10) shows that the following strict and quantitative definitions of "fast" and "reversibly-fast" cycles are productive.

Cycle (19) is "fast" if for positive but as small as possible value of ε the following set of inequalities is true for all $0 \le j \le n$:

$$\frac{(\alpha^A_{qj} + \beta^A_{q-1,j})}{\alpha^B_{qj}} \le \varepsilon ,$$

$$\frac{(\alpha^A_{qj} + \beta^A_{q-1,j})\beta^B_{qj}}{\alpha^B_{qj}\alpha^B_{q,j+1}} \le \varepsilon ,$$

$$\cdots\cdots\cdots\cdots$$

$$\frac{(\alpha^A_{qj} + \beta^A_{q-1,j})\beta^B_{qj}\beta^B_{q,j+1}\ldots\beta^B_{q,j+k-2}}{\alpha^B_{qj}\alpha^B_{q,j+1}\ldots\alpha^B_{q,j+k-1}} \le \varepsilon ,$$

$$\cdots\cdots\cdots\cdots$$

$$\frac{(\alpha^A_{qj} + \beta^A_{q-1,j})\beta^B_{qj}\beta^B_{q,j+1}\ldots\beta^B_{q,j-3}}{\alpha^B_{qj}\alpha^B_{q,j+1}\ldots\alpha^B_{q,j-2}} \le \varepsilon , \quad (20)$$

where $1 \le k \le n$, and since the second subscript index changes from 0 to n, the following is true: $j + k = j + k - n - 1$, if $j + k > n$.

Cycle (19) is "reversibly-fast" if along with a set of inequalities (20), the following inequalities are also satisfied:

$$\frac{(\alpha^A_{qj} + \beta^A_{q-1,j})\alpha^B_{q,j-1}\alpha^B_{q,j-2}\ldots\alpha^B_{q,j+2}}{\beta^B_{q,j+1}\beta^B_{q,j+2}\ldots\beta^B_{q,j-1}} \le \varepsilon ,$$

$$\frac{(\alpha^A_{qj} + \beta^A_{q-1,j})\alpha^B_{q,j-1}\alpha^B_{q,j-2}\ldots\alpha^B_{q,j+3}}{\beta^B_{q,j+2}\beta^B_{q,j+3}\ldots\beta^B_{q,j-1}} \le \varepsilon ,$$

$$\cdots\cdots\cdots\cdots$$

$$\frac{(\alpha^A_{qj} + \beta^A_{q-1,j})\alpha^B_{q,j-1}\alpha^B_{q,j-2}\ldots\alpha^B_{q,j+k+1}}{\beta^B_{q,j+k}\beta^B_{q,j+k+1}\ldots\beta^B_{q,j-1}} \le \varepsilon ,$$

$$\cdots\cdots\cdots\cdots$$

$$\frac{(\alpha^A_{qj} + \beta^A_{q-1,j})}{\beta^B_{q,j-1}} \le \varepsilon , \quad (21)$$

where j, k, and ε have the same sense as in (20).

The value of ε obviously is a quantitative criterion for a "fast" cycle, and the smaller the value of ε, the "faster" is the cycle.

For sake of convenience of further discussion, let us consider a new separate cycle B_q (22): it can be formally obtained from cycle (19) by removal of all branches belonging to reaction A:

$$X_{q0} \underset{\beta^B_{q0}}{\overset{\alpha^B_{q0}}{\rightleftarrows}} X_{q1} \underset{\beta^B_{q1}}{\overset{\alpha^B_{q1}}{\rightleftarrows}} X_{q2} \underset{\beta^B_{q2}}{\overset{\alpha^B_{q2}}{\rightleftarrows}} \ldots\ldots$$

$$\ldots\ldots X_{qr} \underset{\beta^B_{qr}}{\overset{\alpha^B_{qr}}{\rightleftarrows}} \ldots\ldots X_{qn} \underset{\beta^B_{qn}}{\overset{\alpha^B_{qn}}{\rightleftarrows}} (X_{q0}) . \quad (22)$$

Cycle B_q (22) is a scheme of one-way reaction B catalyzed by enzyme E, in which the state of the active site of reaction A corresponds with index q. We shall designate as $\{B_{qr}\}$ any totality of the branches of cycle B_q (22) forming a base tree X_{qr} vertex in cycle B_q. As is obvious, several different base trees of vertex X_{qr} in scheme (22) correspond with one and the same designation $\{B_{qr}\}$.

Let us consider a base determinant (D_{ij}) of arbitrary intermediate form X_{ij} of the initial mechanism (3). The base determinant contains two types of trees of base X_{ij}. Trees of base X_{ij} including any $\{B_{qr}\}$ ($0 \le r \le n$) belong to the first type; trees of base X_{ij}, which do not include any $\{B_{qr}\}$ ($0 \le r \le n$), belong to the second type. That is why D_{ij} is equal to:

$$D_{ij} = (\{\bar{B}_{qr}\})_{ij} + \sum_r (\{B_{qr}\})_{ij} , \quad (23)$$

where $(\{B_{qr}\})_{ij}$ is the value of all trees of base X_{ij} including any $\{B_{qr}\}$ ($0 \le r \le n$) and $(\{\bar{B}_{qr}\})_{ij}$ is a value of all trees of base X_{ij} that do not include any $\{B_{qr}\}$ ($0 \le r \le n$).

It is possible to show that for a fast cycle (19), that is, when inequalities (20) are satisfied, as well as for a reversibly-fast cycle (19), that is, when inequalities (20) and (21) are satisfied, the following is true:

$$\sum_r (\{B_{qr}\})_{ij} < D_{ij} < \sum_r (\{B_{qr}\})_{ij}(1+\delta) , \quad (24)$$

where

$$\delta = P_1\varepsilon + P_2\varepsilon^2 + \ldots + P_n\varepsilon^n, \quad (25)$$

coefficients P_1, P_2,..., P_n of Eq. (25) are positive and depend only on n, namely:

$$P_1 = (n^2 + n)/2 \quad (26)$$

for a fast cycle (19) (inequalities (20) are satisfied) and

$$P_1 = (n^2 + n)/4 \quad (27)$$

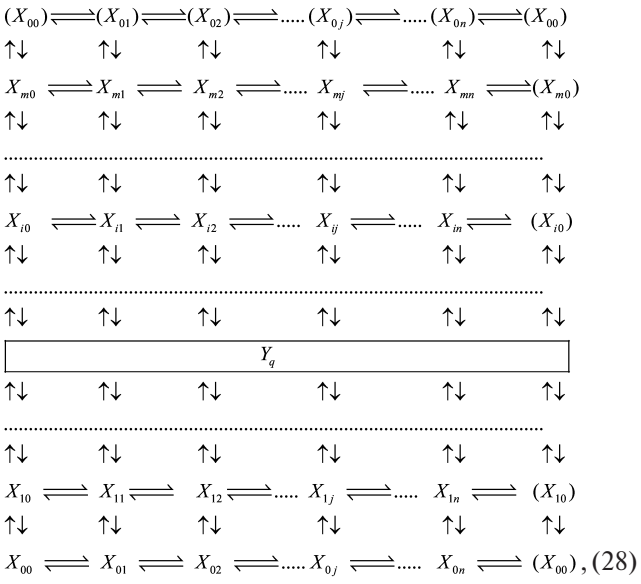
for a reversibly-fast cycle (19) (inequalities (20) and (21) are satisfied).

Satisfaction of inequality (24) means that if ε tends to zero, the ratio of steady-state concentrations of the intermediate enzyme forms X_{qj} ($0 \le j \le n$) in mechanism

(3) tends to the ratio of steady-state concentrations of the intermediate enzyme forms X_{qj} ($0 \le j \le n$) in cycle B_q (22), and the value of the base determinant of an arbitrary intermediate form X_{ij} of mechanism (3) tends to a value:

$$\sum_r (\{B_{qr}\})_{ij} .$$

In turn, this allows using a simple scheme (28) instead of scheme (3) when describing reaction A:



, (28)

in which cycle (19) is changed for one intermediate compound Y_q; the rest of the structure of graph (3) remains unchanged, excluding the fact that the values of each branch exiting from Y_q are multiplied by f_{qj} coefficients equal to the fraction of that intermediate form X_{qj} in a steady state of a separate cycle B_q (22), from which they exit in the initial graph (3):

$$f_{qj} = \frac{[X_{qj}]}{\sum_r [X_{qr}]}, \quad (29)$$

where $[X_{qr}]$ are steady-state concentrations calculated for a separate cycle B_q (22). Formulae for calculation of steady-state concentrations for separate cycles of B_q type (22) are given in [26-28].

Let us designate as $[X_{ij}]^*$ the steady-state concentrations of intermediate enzyme forms X_{ij} calculated for the modified scheme (28). It is easy to show that if (24) is fulfilled, the following conditions are satisfied:

$$\frac{|[X_{ij}]^* - [X_{ij}]|}{[X_{ij}]} < \delta, \quad (30)$$

where δ is defined by Eqs. (25)-(27), $0 \le i \le m$, $0 \le j \le n$, and for $i = q$, $[X_{qj}]^*$ values have the following sense:

$$[X_{qj}]^* = f_{qj}[Y_q], \quad (31)$$

where f_{qj} values are defined in (29).

When ε tends to zero, the high powers in Eq. (25) can be neglected, and evaluation (32) becomes practically important:

$$\delta = P_1 \varepsilon, \quad (32)$$

where P_1 is defined by Eqs. (26) and (27).

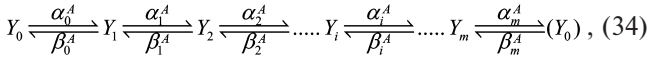
Next, let us assume that two cycles of reaction B in scheme (3) are simultaneously fast (or simultaneously reversibly-fast), that is, for them Eqs. like (20) or (20) and (21) are fulfilled. Then, repeating the considerations above, it is possible to modify the initial scheme (3) for description of reaction A by changing every fast cycle of reaction B for one intermediate form using the above-described algorithm.

In this case, the approximation accuracy is equal to $(1 + \delta)^2 - 1$, and at ε tending to zero, evaluation (33) becomes practically important:

$$\frac{|[X_{ij}]^{**} - [X_{ij}]|}{[X_{ij}]} < 2\delta, \quad (33)$$

where $[X_{ij}]^{**}$ are steady-state concentrations of intermediate enzyme forms X_{ij} calculated for a new modified scheme. Evaluation (32) is also correct.

In the case when all cycles of reaction B in scheme (3) are simultaneously fast (or simultaneously reversibly-fast), for description of reaction A the initial scheme (3) will look as follows:



, (34)

where

$$\alpha_i^A = \sum_j \alpha_{ij}^A f_{ij} , \quad (35)$$

$$\beta_i^A = \sum_j \beta_{ij}^A f_{i+1,j} \quad (36)$$

and f_{ij} values are defined analogously to (29).

Thus, if reaction B proceeds more rapidly, a two-dimensional initial scheme (3) for description of reaction A is reduced to a one-dimensional scheme (34); in the latter, the dependence of the rate of reaction A on concentrations of substrates and products of reaction B is expressed by Eqs. (35) and (36).

Accuracy of approximation in this case is equal to $(1 + \delta)^n - 1$, and if ε tends to zero, evaluation (37) becomes practically important:

$$\frac{|[X_{ij}]^{***} - [X_{ij}]|}{[X_{ij}]} < n\delta, \quad (37)$$

where $[X_{ij}]^{***}$ values have the following sense:

$$[X_{ij}]^{***} = f_{ij}[Y_i]. \quad (38)$$

Evaluation (32) is also correct.

So, if reaction B proceeds significantly faster than reaction A (that is, relations like (20) or (20) and (21) are fulfilled for all $m + 1$ cycles of reaction B, kinetics of reaction A will be described by one cycle (34) in which the effect of reaction B components is accounted for in Eqs. (35) and (36).

It should be noted that dependence of the reaction rate (34) on concentrations of substrates of reaction A will be the same as for the rate of reaction A in the absence of components of reaction B, that is, for mechanism (1), and bifunctional character of enzyme E will manifest itself for reaction A only depending on the rate constants of reaction A (α_i^A and β_i^A) on concentration of components of reaction B.

Using results of [27], we shall present the dependence of the rate of reaction A for mechanism (34) in an explicit form as a function of the rate constants α_i^A and β_i^A:

$$v^A = E_0 \frac{\alpha_0^A \alpha_1^A \alpha_2^A \ldots \alpha_m^A - \beta_0^A \beta_1^A \beta_2^A \ldots \beta_m^A}{\sum_i \sum_j b_{ij}}, \quad (39)$$

where

$$b_{ij} = \alpha_0^A \alpha_1^A \alpha_2^A \ldots \alpha_{i-1}^A \beta_i^A \beta_{i+1}^A \ldots \beta_{j-1}^A 1 \alpha_{j+1}^A \ldots \alpha_m^A \quad (i < j),$$
$$b_{ij} = \alpha_0^A \alpha_1^A \alpha_2^A \ldots \alpha_{i-1}^A 1 \alpha_{i+1}^A \ldots \alpha_m^A \quad (i = j),$$
$$b_{ij} = \beta_0^A \beta_1^A \beta_2^A \ldots \beta_{j-1}^A 1 \alpha_{j+1}^A \ldots \alpha_{i-1}^A \beta_i^A \beta_{i+1}^A \ldots \beta_m^A \quad (i > j). \quad (40)$$

The values of α_i^A and β_i^A as functions of elementary constants of the initial mechanism (3) are defined in Eqs. (35) and (36).

In a case when all cycles of reaction B in scheme (3) are fast (or all are reversibly-fast), mechanism (3) for description of reaction B will include $m + 1$ independent cycles, and the rate of reaction B will be described by:

$$v^B = E_0 \sum_t e_t \frac{\alpha_{t0}^B \alpha_{t1}^B \alpha_{t2}^B \ldots \alpha_{tn}^B - \beta_{t0}^B \beta_{t1}^B \beta_{t2}^B \ldots \beta_{tn}^B}{\sum_i \sum_j b_{ij}^t}, \quad (41)$$

where

$$b_{ij}^t = \alpha_{t0}^B \alpha_{t1}^B \alpha_{t2}^B \ldots \alpha_{t,i-1}^B \beta_{ti}^B \beta_{t,i+1}^B \ldots \beta_{t,j-1}^B 1 \alpha_{t,j+1}^B \ldots \alpha_{tm}^B \quad (i < j),$$
$$b_{ij}^t = \alpha_{t0}^B \alpha_{t1}^B \alpha_{t2}^B \ldots \alpha_{t,i-1}^B 1 \alpha_{t,i+1}^B \ldots \alpha_{tm}^B \quad (i = j),$$
$$b_{ij}^t = \beta_{t0}^B \beta_{t1}^B \beta_{t2}^B \ldots \beta_{t,j-1}^B 1 \alpha_{t,j+1}^B \ldots \alpha_{t,i-1}^B \beta_{ti}^B \beta_{t,i+1}^B \ldots \beta_{tm}^B \quad (i > j), \quad (42)$$

and

$$e_t = \frac{\sum_j b_{tj}}{\sum_i \sum_j b_{ij}}, \quad (43)$$

where b_{ij} are defined by Eq. (40) and t varies from 0 to m.

So, if reaction B proceeds significantly faster than reaction A (that is, relations like (20) or (20) and (21) are fulfilled for all $m + 1$ cycles of reaction B), the general mechanism of a bifunctional enzyme is reduced to one cycle for "slow" reaction A and to several independent cycles for "fast" reaction B. For each of these reactions, the rate equations are obtained in an explicit form and a quantitative assay of this approximation is given.

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