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Quasi-Equilibrium Assumption in Enzyme Kinetics. Necessary and Sufficient Conditions and Accuracy of Its Application for Single-Substrate Reactions

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Abstract—Steady-state kinetics of compulsory-ordered single-substrate irreversible and reversible enzyme reactions with two, three, and arbitrary number of intermediates were observed. Necessary and sufficient conditions for application of the quasi-equilibrium assumption and restrictions of this assumption were found in cases of two and three intermediates in the equilibrium segment. For all cases, accuracy of the quasi-equilibrium assumption was evaluated.

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The quasi-equilibrium assumption in enzyme kinetics is a widespread approach that implies that some intermediate enzyme forms in the mechanism of the enzyme reaction are considered to be in a rapidly established equilibrium state, and this state is practically not subjected to the disturbing effect of the reaction catalyzed by the enzyme. The other intermediates are considered to be in the steady state. The quasi-equilibrium assumption is very popular in enzyme kinetics [1-3]. Strictly speaking, the history of enzyme kinetics began with the use of this assumption, because the classical Michaelis–Menten equation was first derived using the conception of equilibrium between free enzyme and enzyme–substrate complex in the mechanism of catalysis [4].

Combined character of the quasi-equilibrium assumption as a superposition of a steady state of the system, when intermediates supposed to be in the rapid equilibrium state behave themselves as an independent equilibrium subsystem, was reflected in the general method for computation of quasi-equilibrium systems derived by Cha [5]. According to this method, each group of intermediates in the mechanism of the enzyme reaction that are supposed to be in the rapid equilibrium state (so-called equilibrium segment) is considered as one intermediate, and each rate constant of the elementary reaction transferring intermediate outside the segment is multiplied by a coefficient equal to the concentration fraction of this intermediate in its equilibrium segment. The rest of the mechanism of the enzyme reaction remains unchanged. For this reduced scheme, the kinetic equation in steady-state assumption is derived in an ordinary way or using the King–Altman method [6] (to simplify the procedure), either case using the graphical method (modification suggested by Volkenstein and Goldstein [7]). It is also possible to derive kinetic equations for enzyme reactions with the quasi-equilibrium assumption using computer programs [8, 9].

The quasi-equilibrium assumption is considered to be useful when pH dependence of enzyme reactions is studied [10, 11] because rapid equilibrium can occur in the case of ionization reactions due to high proton transfer rates in aqueous solutions. The quasi-equilibrium assumption is usually applied when the effect of reversible modifiers on catalytic characteristics of single-substrate enzymes is described [12, 13].

It should be noted that wide use of the quasi-equilibrium assumption in enzyme kinetics is mostly due to relative simplicity (as compared with the steady-state assumption) of the resulting kinetic equations and also their easy derivation [2, 5, 14]. However, an adequate

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critical analysis of criteria and application limits as well as evaluation of the accuracy of the quasi-equilibrium assumption is absent from the literature. Possible application of the Cha method for computation of enzyme reaction schemes with the quasi-equilibrium assumption is not justified in general, and there are no clear criteria allowing consideration of the suggested segment of a kinetic scheme as an equilibrium one. This raises doubts about the correctness of the Cha method and general applicability of the quasi-equilibrium assumption [15-19]. Unjustified application of the quasi-equilibrium method often results in serious errors in interpretation of kinetic schemes of enzyme reactions.

In this work, criteria for application of the quasi-equilibrium assumption for steady-state systems have been developed for single-substrate enzyme reactions, and quantitative evaluation of accuracy of this assumption is given.

RESULTS AND DISCUSSION

In future discussion, we shall use the term "quasi-equilibrium assumption", although some authors use terms "equilibrium assumption" [5, 15] or "rapid-equilibrium assumption" [9, 12]. In general, all three terms are related with the same suggestion that the ratio of intermediate concentrations in the equilibrium segment negligibly depends on whether the enzyme reaction proceeds or not. The essence of the quasi-equilibrium assumption is that the ratio of intermediate concentrations in the equilibrium segment is accepted as equal to that when the rate of the enzyme reaction in zero. It is assumed that the system in which the enzyme reaction proceeds is in a steady state in relation to concentrations of the enzyme intermediates. To avoid detailed discussion of conditions for establishment of a steady state (for contemporary discussion, see [20]), let us limit ourselves by theoretical possibility for establishing a steady state under conditions for maintenance of constant values of substrate and product concentrations during the enzyme reaction; this will be sufficient for the goal of this study.

For any mechanism of enzyme reaction considered in this work, let us take dependences of intermediate concentrations and the reaction rates on concentrations of participants of the enzyme reaction (substrates, products, modifiers, etc.) under conditions for steady-state establishment via all intermediate enzyme forms as exact decisions. Let us also take relative deviations of concentrations of enzyme intermediates and of the reaction rate obtained by the quasi-equilibrium assumption from corresponding values obtained by the exact (steady-state) decision as a quantitative evaluation of accuracy of the quasi-equilibrium assumption.

Three-stage enzyme reaction. This simplest scheme of the reaction catalyzed by enzyme E, in which one molecule of substrate S is converted into one molecule of product P, looks as follows:

$$\mathrm{E}\underset{k_{-1}}{\overset{k_1[\mathrm{S}]}{\rightleftarrows}}\mathrm{ES}\underset{k_{-2}}{\overset{k_2}{\rightleftarrows}}\mathrm{EP}\underset{k_{-3}[\mathrm{P}]}{\overset{k_3}{\rightleftarrows}}(\mathrm{E}). \tag{1}$$

For this scheme and its modifications, let us analyze cases when fragments

$$\mathrm{EP}\underset{k_{-3}[\mathrm{P}]}{\overset{k_3}{\rightleftarrows}}\mathrm{E}\underset{k_{-1}}{\overset{k_1[\mathrm{S}]}{\rightleftarrows}}\mathrm{ES} \text{ and } \mathrm{E}\underset{k_{-1}}{\overset{k_1[\mathrm{S}]}{\rightleftarrows}}\mathrm{ES}$$

are suggested as an equilibrium segment and also the case where EP intermediate form is omitted (often discussed in the literature).

For mechanism (1), the rate of enzyme reaction in a steady state (v^{ss}) is:

$$v^{ss} = e_0\frac{k_1k_2k_3[\mathrm{S}] - k_{-1}k_{-2}k_{-3}[\mathrm{P}]}{k_1k_2[\mathrm{S}] + k_2k_{-3}[\mathrm{P}] + k_{-1}k_{-3}[\mathrm{P}] + k_2k_3 + k_{-1}k_3 + k_{-1}k_{-2} + k_1k_3[\mathrm{S}] + k_1k_{-2}[\mathrm{S}] + k_{-2}k_{-3}[\mathrm{P}]}, \tag{2}$$

where e_0 is the total enzyme concentration (net concentration of all its intermediate forms). From here on, superscript "ss" means steady state. From (2) it is obvious that the rate is positive when substrate (S) concentration decreases and product (P) concentration increases.

Steady-state concentrations of intermediate enzyme forms are:

$$[\mathrm{E}]^{ss} = e_0\frac{k_2k_3 + k_{-1}k_3 + k_{-1}k_{-2}}{k_1k_2[\mathrm{S}] + k_2k_{-3}[\mathrm{P}] + k_{-1}k_{-3}[\mathrm{P}] + k_2k_3 + k_{-1}k_3 + k_{-1}k_{-2} + k_1k_3[\mathrm{S}] + k_1k_{-2}[\mathrm{S}] + k_{-2}k_{-3}[\mathrm{P}]}, \tag{3}$$

$$[\mathrm{ES}]^{ss} = e_0\frac{k_1k_3[\mathrm{S}] + k_1k_{-2}[\mathrm{S}] + k_{-2}k_{-3}[\mathrm{P}]}{k_1k_2[\mathrm{S}] + k_2k_{-3}[\mathrm{P}] + k_{-1}k_{-3}[\mathrm{P}] + k_2k_3 + k_{-1}k_3 + k_{-1}k_{-2} + k_1k_3[\mathrm{S}] + k_1k_{-2}[\mathrm{S}] + k_{-2}k_{-3}[\mathrm{P}]}, \tag{4}$$

$$[\mathrm{EP}]^{ss} = e_0\frac{k_1k_2[\mathrm{S}] + k_2k_{-3}[\mathrm{P}] + k_{-1}k_{-3}[\mathrm{P}]}{k_1k_2[\mathrm{S}] + k_2k_{-3}[\mathrm{P}] + k_{-1}k_{-3}[\mathrm{P}] + k_2k_3 + k_{-1}k_3 + k_{-1}k_{-2} + k_1k_3[\mathrm{S}] + k_1k_{-2}[\mathrm{S}] + k_{-2}k_{-3}[\mathrm{P}]}. \tag{5}$$

Equilibrium segment $\mathrm{EP}\underset{k_{-3}}{\overset{k_3}{\rightleftarrows}}\mathrm{E}\underset{k_{-1}}{\overset{k_1[\mathrm{S}]}{\rightleftarrows}}\mathrm{ES}$.

Let us suggest that in the course of the enzyme reaction (1) the ratios of intermediate enzyme forms EP, E, and ES negligibly differ from those attained under true equilibrium conditions:

$$\mathrm{EP}\underset{k_{-3}}{\overset{k_3}{\rightleftarrows}}\mathrm{E}\underset{k_{-1}}{\overset{k_1[\mathrm{S}]}{\rightleftarrows}}\mathrm{ES}, \tag{6}$$

that is, the quasi-equilibrium assumption is justified.

In this case the rate of the enzyme reaction and concentration of intermediates calculated using the quasi-equilibrium assumption are:

$$v^{qe} = e_0 \frac{k_1k_2k_3[S] - k_{-1}k_{-2}k_{-3}[P]}{k_1k_3[S] + k_{-1}k_3 + k_{-1}k_{-3}[P]}\ , \qquad (7)$$

$$[E]^{qe} = e_0 \frac{k_{-1}k_3}{k_1k_3[S] + k_{-1}k_3 + k_{-1}k_{-3}[P]}\ , \qquad (8)$$

$$[ES]^{qe} = e_0 \frac{k_1k_3[S]}{k_1k_3[S] + k_{-1}k_3 + k_{-1}k_{-3}[P]}\ , \qquad (9)$$

$$[EP]^{qe} = e_0 \frac{k_{-1}k_{-3}[P]}{k_1k_3[S] + k_{-1}k_3 + k_{-1}k_{-3}[P]}\ . \qquad (10)$$

From here on, superscript "qe" means the quasi-equilibrium assumption.

Comparison of expressions (2)-(5) and (7)-(10) shows that for mechanism (1) the necessary and sufficient condition for application of the quasi-equilibrium assumption in this case is simultaneous fulfillment of conditions (11)-(14):

$$k_{-1} >> k_2, \qquad (11)$$

$$k_3 >> k_{-2}, \qquad (12)$$

$$k_1k_2[S] << k_{-1}k_{-3}[P], \qquad (13)$$

$$k_{-2}k_{-3}[P] << k_1k_3[S]. \qquad (14)$$

If these conditions are fulfilled, $v^{qe} \approx v^{ss}$, $[E]^{qe} \approx [E]^{ss}$, $[ES]^{qe} \approx [ES]^{ss}$, $[EP]^{qe} \approx [EP]^{ss}$. However, the degree of accuracy with which these approximate equalities are fulfilled is of great importance. For quantitative evaluation of accuracy of the quasi-equilibrium assumption, let us use the fact that all values in inequalities (11)-(14) are positive and limited; thus, we can write obvious relationships not imposing any conditions:

$$\frac{k_2}{k_{-1}} \leqslant \varepsilon\ , \qquad (15)$$

$$\frac{k_{-2}}{k_3} \leqslant \varepsilon\ , \qquad (16)$$

$$\frac{k_1k_2[S]}{k_{-1}k_{-3}[P]} \leqslant \varepsilon\ , \qquad (17)$$

$$\frac{k_{-2}k_{-3}[P]}{k_1k_3[S]} \leqslant \varepsilon, \qquad (18)$$

where positive dimensionless parameter ε is equal to the maximal value of the left parts of inequalities (15)-(18) at the chosen limited range of S and P concentrations.

It is easy to show that if ε defined by relationships (15)-(18) is used, the following inequalities are justified:

$$\frac{k_{-2}k_{-3}[P]}{k_1k_3[S]} \leqslant \varepsilon\ , \qquad (19)$$

$$\frac{[E]^{qe}}{1+2\varepsilon} < [E]^{ss} < [E]^{qe}(1+2\varepsilon)\ , \qquad (20)$$

$$\frac{[ES]^{qe}}{1+2\varepsilon} < [ES]^{ss} < [ES]^{qe}(1+2\varepsilon)\ , \qquad (21)$$

$$\frac{[EP]^{qe}}{1+2\varepsilon} < [EP]^{ss} < [EP]^{qe}(1+2\varepsilon)\ . \qquad (22)$$

Expressions (19)-(22) are strict inequalities because physical values of the left parts of expressions (15)-(18) are real numbers and cannot be strictly equal to each other. Taking the relative deviations of values obtained by the quasi-equilibrium assumption from the steady-state values as the quantitative evaluation of accuracy of the quasi-equilibrium assumption and based on (19)-(22), we obtain:

$$\left|\frac{v^{qe} - v^{ss}}{v^{ss}}\right| < 2\varepsilon, \qquad (23)$$

$$\left|\frac{[E]^{qe} - [E]^{ss}}{[E]^{ss}}\right| < 2\varepsilon, \qquad (24)$$

$$\left|\frac{[ES]^{qe} - [ES]^{ss}}{[ES]^{ss}}\right| < 2\varepsilon, \qquad (25)$$

$$\left|\frac{[EP]^{qe} - [EP]^{ss}}{[EP]^{ss}}\right| < 2\varepsilon. \qquad (26)$$

As obvious from expressions (15)-(18), in this case accuracy of application of the quasi-equilibrium assumption depends not only on the ratio of rate constants, but also on the range in which substrate and product concentrations change. There is a correct question whether such substrate and product concentration range exists in which the quasi-equilibrium assumption can be used with sufficient accuracy.

To solve this question, let us use the fact that the left parts of inequalities (15) and (16) are independent of product and substrate concentrations. Let us assume that, e.g. the left part of inequality (16) (let us designate it ε) is greater than the left part of (15):

$$\frac{k_2}{k_{-1}} < \frac{k_{-2}}{k_3} = \varepsilon\ . \qquad (27)$$

It is easy to show that if substrate and product concentrations are varied in the range

$$K_{eq} \leqslant \frac{[P]}{[S]} \leqslant \frac{k_1}{k_{-3}}, \tag{28}$$

where K_{eq} is the equilibrium constant of the reaction considered:

$$K_{eq} = \frac{[P]_{eq}}{[S]_{eq}} = \frac{k_1 k_2 k_3}{k_{-1} k_{-2} k_{-3}}, \tag{29}$$

the accuracy of the quasi-equilibrium assumption (23)-(26) is defined by ε value, which is in turn defined by expression (27). From here on, subscript "eq" means true equilibrium conditions. When the ranges of substrate and product concentrations become wider than the limits given by (28), accuracy of the quasi-equilibrium assumption defined by inequalities (15)-(18) will be less than that defined by expression (27).

If the left part of (16) is greater than the left part of (15) (in turn let us designate the latter as ε),

$$\frac{k_{-2}}{k_3} < \frac{k_2}{k_{-1}} = \varepsilon, \tag{30}$$

and substrate and product concentrations are varied in the range

$$\frac{k_{-1}}{k_3} \leqslant \frac{[P]}{[S]} \leqslant K_{eq}, \tag{31}$$

the accuracy of the quasi-equilibrium assumption (23)-(26) is defined by ε value, which is in turn defined by (30). When the ranges of substrate and product concentrations become wider than the limits given by (31), the accuracy of the quasi-equilibrium assumption defined by inequalities (15)-(18) will be less than that defined by expression (30).

So, the problem of substrate and enzyme concentrations range, which allows use of the quasi-equilibrium assumption with sufficient accuracy, has positive decision: if the concentration range is defined by inequalities (28) or (31), accuracy is defined by inequalities (15) and (16), respectively; they are independent of concentrations.

Equilibrium segment $E \underset{k_{-1}}{\overset{k_1[S]}{\rightleftarrows}} ES$.

Suppose that in the course of enzyme reaction (1) the concentration ratio of only two intermediate enzyme forms (E and ES) negligibly differs from the concentration ratio of these forms under true equilibrium conditions:

$$E \underset{k_{-1}}{\overset{k_1[S]}{\rightleftarrows}} ES, \tag{32}$$

that is, the quasi-equilibrium condition is justified. Such case may be easily modeled if in the case considered above the ranges of substrate and product concentrations are significantly shifted towards decrease in [P]; this will result in violation of condition (17).

For reaction (1) the rate of the enzyme reaction and concentrations of intermediates calculated if equilibrium (32) is supposed to be established will be as follows:

$$v^{qe} = e_0 \frac{k_1 k_2 k_3 [S] - k_{-1} k_{-2} k_{-3} [P]}{\begin{array}{l} k_1 k_2 [S] + k_{-1} k_{-3} [P] + k_{-1} k_{-2} + k_1 k_{-2} [S] + \\ + k_{-1} k_3 + k_1 k_3 [S] \end{array}}, \tag{33}$$

$$[E]^{qe} = e_0 \frac{k_{-1} k_{-2} + k_{-1} k_3}{\begin{array}{l} k_1 k_2 [S] + k_{-1} k_{-3} [P] + k_{-1} k_{-2} + k_1 k_{-2} [S] + \\ + k_{-1} k_3 + k_1 k_3 [S] \end{array}}, \tag{34}$$

$$[ES]^{qe} = e_0 \frac{k_1 k_{-2} [S] + k_1 k_3 [S]}{\begin{array}{l} k_1 k_2 [S] + k_{-1} k_{-3} [P] + k_{-1} k_{-2} + k_1 k_{-2} [S] + \\ + k_{-1} k_3 + k_1 k_3 [S] \end{array}}, \tag{35}$$

$$[EP]^{qe} = e_0 \frac{k_1 k_2 [S] + k_{-1} k_{-3} [P]}{\begin{array}{l} k_1 k_2 [S] + k_{-1} k_{-3} [P] + k_{-1} k_{-2} + k_1 k_{-2} [S] + \\ + k_{-1} k_3 + k_1 k_3 [S] \end{array}}. \tag{36}$$

Comparison of expressions (2)-(5) and (33)-(36) shows that for mechanism (1), the necessary and sufficient condition for application of the quasi-equilibrium assumption is simultaneous fulfillment of conditions (11) and $k_{-3}[P] << k_1[S]$, if E and ES enzyme forms are supposed to be in equilibrium. Using quantitative characteristics of these relationships ((15) and $\frac{k_{-3}[P]}{k_1[S]} \leqslant \varepsilon$), we obtain:

$$\left| \frac{v^{qe} - v^{ss}}{v^{ss}} \right| < \varepsilon, \tag{37}$$

$$\left| \frac{[E]^{qe} - [E]^{ss}}{[E]^{ss}} \right| < \varepsilon, \tag{38}$$

$$\left| \frac{[ES]^{qe} - [ES]^{ss}}{[ES]^{ss}} \right| < \varepsilon, \tag{39}$$

$$\left| \frac{[EP]^{qe} - [EP]^{ss}}{[EP]^{ss}} \right| < \varepsilon, \tag{40}$$

that is, in this case the quasi-equilibrium assumption can be applied when only the two above-mentioned conditions are fulfilled, and accuracy of this assumption may be quantitatively evaluated by ε value.

It is not worth considering separately a case of quasi-equilibrium between E and EP because scheme (1) is symmetrical in relation to S and P.

If product concentration is supposed to be zero, the reaction mechanism looks as follows:

$$E \xrightleftharpoons[k_{-1}]{k_1[S]} ES \xrightleftharpoons[k_{-2}]{k_2} EP \xrightarrow{k_3} (E)\,. \qquad (41)$$

Inequality (11) will be a condition for establishment of quasi-equilibrium in segment

$$E \xrightleftharpoons[k_{-1}]{k_1[S]} ES\,.$$

Accuracy of the quasi-equilibrium assumption in this case will be determined by inequality (37)-(40), where ε is defined by inequality (15).

Scheme (41) is often reduced to a two-stage scheme of irreversible enzyme reaction:

$$E \xrightleftharpoons[k_{-1}]{k_1[S]} ES \xrightarrow{k_2} (E)$$

In this case, the only necessary and sufficient condition for application of the quasi-equilibrium assumption for equilibrium segment

$$E \xrightleftharpoons[k_{-1}]{k_1[S]} ES$$

in inequality (11). Accuracy of the quasi-equilibrium assumption will be determined by inequalities (37)-(39), where ε is defined by inequality (15).

A general scheme of a multi-stage enzyme reaction. Earlier we suggested that conversion of a substrate molecule to product molecule in the composition of the enzyme–substrate complex is a one-stage process. However, this is not at all obvious; moreover, it is not so if the number of substrates is more than one. A key question is how the increased number of reaction stages will influence applicability of the quasi-equilibrium assumption and the accuracy attained by this assumption. Suppose that the mechanism of a single-substrate enzyme reaction includes an uncertain number of stages (n); the reaction scheme looks as follows:

$$E \xrightleftharpoons[k_{-1}]{k_1[S]} ES \xrightleftharpoons[k_{-2}]{k_2} E_3 \xrightleftharpoons[k_{-3}]{k_3} \ldots$$

$$\ldots E_{n-1} \xrightleftharpoons[k_{-(n-1)}]{k_{n-1}} EP \xrightleftharpoons[k_{-n}[P]]{k_n} (E)\,, \qquad (42)$$

where E is an intermediate enzyme form which interacts with substrate (so-called free enzyme form) and ES and EP are enzyme–substrate and enzyme–product complexes, respectively. For scheme (42), we obtained [21-23] expressions for the rate of the enzyme reaction and concentrations of intermediates at steady state convenient for analysis:

$$[E_i]^{ss} = e_0 \frac{\sum\limits_j b_{ij}}{\sum\limits_i \sum\limits_j b_{ij}}\,, \qquad (43)$$

$$v^{ss} = e_0 \frac{\alpha_1\alpha_2\alpha_3 \ldots \alpha_n - \beta_1\beta_2\beta_3 \ldots \beta_n}{\sum\limits_i \sum\limits_j b_{ij}}\,, \qquad (44)$$

$$b_{ij} = \alpha_1\alpha_2 \ldots \alpha_{i-1}\beta_i\beta_{i+1} \ldots \beta_{j-1} 1 \alpha_{j+1} \ldots \alpha_n \quad (i<j)\,, \qquad (45)$$

$$b_{ij} = \alpha_1\alpha_2 \ldots \alpha_{i-1} 1 \alpha_{i+1} \ldots \alpha_n \quad (i=j)\,, \qquad (46)$$

$$b_{ij} = \beta_1\beta_2 \ldots \beta_{j-1} 1 \alpha_{j+1} \ldots \alpha_{i-1}\beta_i \ldots \beta_n \quad (i>j)\,, \qquad (47)$$

where $\alpha_1 = k_1[S],\ \beta_1 = k_{-1},\ \alpha_n = k_n,$

$$\beta_n = k_{-n}[P],\ \ \alpha_i = k_i,\ \beta_i = k_{-i} \qquad (48)$$

for $i = 2, 3, \ldots n-1$.

Equilibrium segment $EP \xrightleftharpoons[k_{-n}[P]]{k_n} E \xrightleftharpoons[k_{-1}]{k_1[S]} ES$.

For calculations in the quasi-equilibrium assumption in this case, let us pull segment

$$EP \xrightleftharpoons[k_{-n}[P]]{k_n} E \xrightleftharpoons[k_{-1}]{k_1[S]} ES$$

of scheme (42) into point E_0 using Cha method [5]. Ascribing corresponding coefficients to the branches issuing out of point E_0, we obtain the quasi-equilibrium scheme (49):

$$E_0 \xrightleftharpoons[\beta_0]{\alpha_0} E_3 \xrightleftharpoons[\beta_3]{\alpha_3} \ldots E_{n-1} \xrightleftharpoons[\beta^*_{n-1}]{\alpha_{n-1}} (E_0)\,, \qquad (49)$$

where

$$\alpha_0 = \frac{\alpha_1\alpha_2\alpha_n}{\alpha_1\alpha_n + \beta_1\alpha_n + \beta_1\beta_n}\,, \qquad (50)$$

$$\beta_0 = \beta_2, \quad \beta^*_{n-1} = \frac{\beta_1\beta_{n-1}\beta_n}{\alpha_1\alpha_n + \beta_1\alpha_n + \beta_1\beta_n}\,. \qquad (51)$$

The other values have the same meaning.

Let us compute scheme (49) in the steady-state assumption; this will give us expressions for the quasi-equilibrium assumption of scheme (42):

$$[E_i]^{qe} = e_0 \frac{\sum\limits_j b^*_{ij}}{\sum\limits_i \sum\limits_j b^*_{ij}}\,, \qquad (52)$$

$$[E_1]^{qe} = [E]^{qe} = [E_0]^{qe} \frac{\beta_1\alpha_n}{\alpha_1\alpha_n + \beta_1\alpha_n + \beta_1\beta_n}\,, \qquad (53)$$

$$[E_2]^{qe} = [ES]^{qe} = [E_0]^{qe} \frac{\alpha_1\alpha_n}{\alpha_1\alpha_n + \beta_1\alpha_n + \beta_1\beta_n}\,, \qquad (54)$$

$$[E_n]^{qe} = [EP]^{qe} = [E_0]^{qe} \frac{\beta_1\beta_n}{\alpha_1\alpha_n + \beta_1\alpha_n + \beta_1\beta_n}\,, \qquad (55)$$

$$v^{qe} = e_0 \frac{\alpha_0\alpha_3\ldots\alpha_{n-1} - \beta_0\beta_3\ldots\beta^*_{n-1}}{\sum_i\sum_j b^*_{ij}} , \qquad (56)$$

$$b^*_{ij} = \alpha_0\alpha_3\cdots\alpha_{i-1}\beta_i\beta_{i+1}\cdots\beta_{j-1}1\alpha_{j+1}\cdots\alpha_{n-1} \quad (i<j) , \qquad (57)$$

$$b^*_{ij} = \alpha_0\alpha_3\cdots\alpha_{i-1}1\alpha_{i+1}\cdots\alpha_{n-1} \quad (i=j) , \qquad (58)$$

$$b^*_{ij} = \beta_0\beta_3\cdots\beta_{j-1}1\alpha_{j+1}\cdots\alpha_{i-1}\beta_i\cdots\beta^*_{n-1} \quad (i>j) , \qquad (59)$$

where $i = 0, 3, \ldots n-1$; $j = 0, 3, \ldots n-1$.

Comparing expressions (52)-(59) and (43)-(48), it is easy to ascertain that if conditions:

$$\frac{\alpha_2}{\beta_1} \leqslant \varepsilon , \qquad (60)$$

$$\frac{\beta_{n-1}}{\alpha_n} \leqslant \varepsilon , \qquad (61)$$

$$\frac{\alpha_1\alpha_2}{\beta_1\beta_n} \leqslant \varepsilon , \qquad (62)$$

$$\frac{\beta_{n-1}\beta_n}{\alpha_1\alpha_n} \leqslant \varepsilon , \qquad (63)$$

equivalent to conditions (15)-(18) are fulfilled, the difference between the values for the steady-state assumption and those for the quasi-equilibrium assumption is determined by inequalities:

$$\left|\frac{v^{qe} - v^{ss}}{v^{ss}}\right| < 2\varepsilon , \qquad (64)$$

$$\left|\frac{[E]^{qe} - [E]^{ss}}{[E]^{ss}}\right| < 2\varepsilon , \qquad (65)$$

$$\left|\frac{[ES]^{qe} - [ES]^{ss}}{[ES]^{ss}}\right| < 2\varepsilon , \qquad (66)$$

$$\left|\frac{[EP]^{qe} - [EP]^{ss}}{[EP]^{ss}}\right| < 2\varepsilon , \qquad (67)$$

$$\left|\frac{[E_i]^{qe} - [E_i]^{ss}}{[E_i]^{ss}}\right| < 2\varepsilon , \qquad (68)$$

for $i = 3, 4, \ldots n-1$.

In other words, conditions for application of the quasi-equilibrium assumption and the resulting accuracy are independent of how many intermediates are in the enzyme reaction mechanism (42). Since scheme (42) was analyzed in general, conditions (60)-(63) govern conditions for application of the quasi-equilibrium assumption to any segment of three intermediates of general scheme (42), and expressions (64)-(68) allow evaluation of accuracy of this assumption.

It is easy to ensure that all the other manipulations of section "Three-stage enzyme reaction" are justified also in the case of scheme (42), in particular, the equilibrium segment

$$E \underset{k_{-1}}{\overset{k_1[S]}{\rightleftarrows}} ES$$

can exist in the framework of scheme (42) only if conditions (60) and

$$\frac{\alpha_1}{\beta_n} \leqslant \varepsilon \qquad (69)$$

are fulfilled.

In this case, the accuracy of the quasi-equilibrium assumption will be defined by inequalities:

$$\left|\frac{v^{qe} - v^{ss}}{v^{ss}}\right| < \varepsilon , \qquad (70)$$

$$\left|\frac{[E]^{qe} - [E]^{ss}}{[E]^{ss}}\right| < \varepsilon , \qquad (71)$$

$$\left|\frac{[ES]^{qe} - [ES]^{ss}}{[ES]^{ss}}\right| < \varepsilon , \qquad (72)$$

$$\left|\frac{[EP]^{qe} - [EP]^{ss}}{[EP]^{ss}}\right| < \varepsilon , \qquad (73)$$

$$\left|\frac{[E_i]^{qe} - [E_i]^{ss}}{[E_i]^{ss}}\right| < \varepsilon , \qquad (74)$$

for $i = 3, 4, \ldots n-1$.

Attempts to define criteria for the quasi-equilibrium assumption were made earlier. A rather evident condition (11) for the quasi-equilibrium assumption in the case of the simplest two-stage scheme of irreversible enzyme reaction is presented in [24]. Earlier we developed conditions for existence of a quasi-equilibrium segment consisting of two or three intermediates in a general scheme of compulsory-ordered enzyme reaction [22]. Successful attempts to justify criteria for application of the quasi-equilibrium assumption in enzyme catalysis thus seem to be exhausted.

An attempt to simplify complex first-order reactions by dividing into groups of intermediates based on discrimination of rapid and slow reactions was presented in [25]; however, a purely mental approach did not allow elucidation of adequate conditions for application of the quasi-equilibrium assumption. In [26], the absence of sufficient conditions for application of the quasi-equilibrium assumption in case of a compulsory-ordered two-substrate mechanism resulted in obviously wrong concentration dependences. Until now, the evaluation of the accuracy of the quasi-equilibrium assumption was absent from literature.

So, in this work we justified criteria for application of the quasi-equilibrium assumption in the case of single-substrate enzyme reactions and evaluated the accuracy of this assumption.

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