SUPPORTING INFORMATION

Quasi-Equilibrium Assumption for Arbitrary Mechanism of Enzymatic Reaction. Criteria for Existence of Equilibrium Segments

{running title} EQUILIBRIUM SEGMENTS IN ENZYMATIC REACTION MECHANISM

P. V. Vrzheshch[1,2]

[1]Department of Bioengineering and Bioinformatics, Lomonosov Moscow State University, 119992 Moscow, Russia; fax: (495) 939-4218; E-mail: peter@genebee.msu.ru

[2]International Biotechnological Center, Lomonosov Moscow State University, 119992 Moscow, Russia; fax: (495) 939-5022

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Abstract—The possible application of the quasi-equilibrium assumption for arbitrary mechanism of enzymatic reaction is considered. It is shown at what ratios of kinetic constants a segment consisting of two, three, and four intermediates may be considered as an equilibrium one. Expressions for evaluation of accuracy of distribution of intermediate concentrations inside the equilibrium segment and accuracy of determination of intermediate concentrations inside and outside the equilibrium segment as a function of the ratio of kinetic constants are derived. A method for determination of the limitations on the ratio of rate constants in a case of equilibrium segment of arbitrary structure is suggested.

Key words: enzymatic kinetics, quasi-equilibrium assumption, accuracy of assumption, Cha method, rapid equilibrium, steady-state kinetics, graph method

The quasi-equilibrium assumption in enzymatic catalysis and calculation procedure for kinetic schemes of enzymatic reactions in the quasi-equilibrium assumption suggested by Cha [1] are widely used. However, application of the quasi-equilibrium assumption for calculation of the steady-state schemes of enzymatic reactions crucially depends on a proper choice of an equilibrium segment, that is, a segment in which distribution of intermediate concentrations is supposed to be almost the same as that at the state of equilibrium. The Cha method for calculation of schemes of enzymatic reactions under conditions of the quasi-equilibrium assumption is not proved in the general case, the general criteria for existence of equilibrium segments are absent, and this raises discussions on the subject and doubts of correctness of the Cha method and in applicability of the quasi-equilibrium assumption at all [2-6].

Quantitative conditions for existence of a quasi-equilibrium segment consisting of two or three intermediates in a general scheme of ordered enzymatic reaction were determined earlier [7]. Cases of existence of equilibrium segments of two and three intermediates were considered in [8] for a general mechanism of ordered single-substrate reactions, the necessary and sufficient conditions for application of the quasi-equilibrium assumption were found, and accuracy of this assumption in the considered cases was numerically evaluated. Accuracy of the quasi-equilibrium assumption for the ordered bisubstrate reactions was evaluated in [9], limitations on its application were revealed, and the origin of errors was analyzed when graphical interpretations were used for discrimination of the mechanism of bisubstrate enzymatic reactions.

The necessary and sufficient conditions for application of the quasi-equilibrium assumption and the Cha method are substantiated in this work by direct proof for an arbitrary mechanism of enzymatic reaction for equilibrium segments consisting of two, three, and four intermediates. A method for determination of limitations on the ratios of rate constants for an equilibrium segment of arbitrary structure is also suggested.

RESULTS

Generally a kinetic scheme of a chemical reaction catalyzed by enzyme E

$$\alpha_1 \mathrm{S}_1 + \alpha_2 \mathrm{S}_2 + \quad \ldots \quad \rightleftarrows \pi_1 \mathrm{P}_1 + \pi_2 \mathrm{P}_2 + \quad \ldots \qquad (1)$$

may be written as follows:

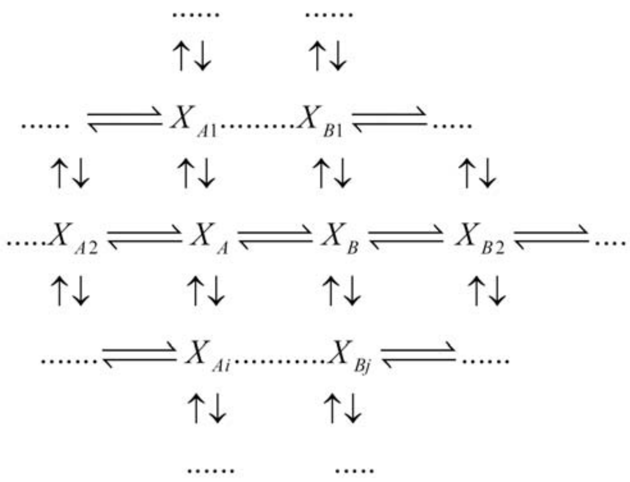
$$X_i \underset{k_{j,i}}{\overset{k_{i,j}}{\rightleftarrows}} X_j, \tag{2}$$

where X_i ($i = 1, 2, \ldots, n$) are intermediates, S_1, S_2… – substrates, P_1, P_2… – products of chemical reaction, α_1, α_2… and π_1, π_2… – stoichiometric coefficients. Chemical conversions (2) may account for monomolecular reactions as well as bimolecular reactions between intermediates and molecules of substrates, products, modifiers, and other reaction participants. Being wholly stochastic by their nature, with increasing number of particles in the system processes (2) are in the limit described by equations of chemical kinetics; for monomolecular processes, $k_{i,j}$ values are rate constants of the first order reaction and for bimolecular processes, $k_{i,j}$ values are the products of corresponding rate constants of the second order by substrate (or another reaction participant) concentration. In turn, for any experimental system governed by mechanism (2), by decrease in total concentration of enzyme E it is possible to attain unlimited approximation to the steady state via all intermediate enzyme forms at practically constant initial concentrations of substrates, products, modifiers, and other reaction participants. A system of equations corresponding to such steady state of mechanism (2) can be solved using elements of the graph theory according to Volkenstein and Goldstein [10, 11]. Let us consider this solution as an exact one, and accuracy of approximate solutions considered in this work will be higher as closer the suggested approximate solution is to this exact solution.

In a case of the quasi-equilibrium assumption an equilibrium segment (or several equilibrium segments) should be determined; inside these segments intermediate forms of the enzyme are considered to be practically in the equilibrium state, in the remaining part of the mechanism the steady state is considered to be established. For complex mechanisms, for the quasi-equilibrium assumption kinetic schemes are calculated according to the Cha method [1]: the initial kinetic scheme of enzymatic reaction mechanism is replaced by the quasi-equilibrium kinetic scheme. In the latter a new intermediate corresponds to each equilibrium segment of the initial scheme and each rate constant of elementary reaction in the initial scheme transferring an intermediate of the equilibrium segment outside this segment is multiplied by a coefficient equal to the fraction of the above-mentioned intermediate in its equilibrium segment (in the equilibrium state). The remaining scheme of the enzymatic reaction mechanism remains unchanged, and a new quasi-equilibrium kinetic scheme is calculated in the steady-state assumption.

When analyzing kinetic mechanism of enzymatic reaction by the graph method [10, 11], the mechanism of enzymatic reaction (2) in the steady state is considered as a graph and each intermediate X_i (graph vertex) can be chosen as a base. A basal tree corresponding to it is a set of branches (chemical transformations) passing through all graph vertices and directed to the base. Branches of the basal tree do not form cycles. The value of the basal tree is a product of branch values (the rate constants of chemical transformations) forming it. The sum of values of all basal trees of intermediate X_i is a basal determinant designated as D_{Xi}. The ratio of steady-state concentrations of intermediates is equal to the ratio of their basal determinants.

Equilibrium segment consisting of two intermediates. Let us suppose that two intermediates of mechanism (2) designated as X_A and X_B can be considered as an equilibrium segment. Neither of them is a constituent of a dead-end complex. Along with X_B, intermediate X_A via corresponding branches is connected with some other intermediates: X_{A1}, X_{A2}, … X_{Ai} ($A_i \neq B$). Along with X_A, intermediate X_B via corresponding branches is connected with some other intermediates: X_{B1}, X_{B2}, … X_{Bj} ($B_j \neq A$). In this case mechanism (2) looks as follows:



(3)

Let us consider the basal determinant of base X_B (D_B). It should be noted that each basal tree belonging to some base (e.g. X_B) contains one and the only branch outgoing from another vertex (e.g. X_A). This makes it possible to write

$$D_B = (k_{A,B})^B + (k_{A,A1})^B + (k_{A,A2})^B + \ldots = (k_{A,B})^B + \sum_{Ai}(k_{A,Ai})^B \,, \tag{4}$$

where $(k_{A,B})^B$ is a sum of values of basal trees belonging to X_B and containing $k_{A,B}$, $(k_{A,A1})^B$ is a sum of values of basal trees belonging to X_B and containing $k_{A,A1}$ and so on.

In a case of the basal determinant of base X_A (D_A) analogous to D_B:

$$D_A = (k_{B,A})^A + (k_{B,B1})^A + (k_{B,B2})^A + \ldots = (k_{B,A})^A + \sum_{Bj} (k_{B,Bj})^A \, . \tag{5}$$

It can be easily determined that

$$\frac{(k_{A,B})^B}{k_{A,B}} = \frac{(k_{B,A})^A}{k_{B,A}} \, . \tag{6}$$

In fact, each of values ($\frac{(k_{A,B})^B}{k_{A,B}}$ and $\frac{(k_{B,A})^A}{k_{B,A}}$) is equal to a sum of values of all trees of graph (2); each of these trees is directed to X_A and/or to X_B and does not contain branches directed from X_A or X_B.

Moreover, comparison of basal trees belonging to X_B and containing $k_{A,B}$ with those belonging to X_B and containing $k_{A,Ai}$ shows that

$$\frac{(k_{A,B})^B}{k_{A,B}} > \frac{(k_{A,Ai})^B}{k_{A,Ai}} \tag{7}$$

for all A_i, and analogous reasoning about X_A allows writing

$$\frac{(k_{B,A})^A}{k_{B,A}} > \frac{(k_{B,Bj})^A}{k_{B,Bj}} \tag{8}$$

for all branches outgoing from X_B.

Comparison of Eqs. (4) and (5) with inequalities (7) and (8) gives

$$(k_{A,B})^B < D_B < (k_{A,B})^B (1 + \frac{\sum_{Ai} k_{A,Ai}}{k_{A,B}}) \, , \tag{9}$$

$$(k_{B,A})^A < D_A < (k_{B,A})^A \left(1 + \frac{\sum_{Bj} k_{B,Bj}}{k_{B,A}}\right). \tag{10}$$

Since all the rate constants $k_{i,j}$ in the given range of concentrations of enzymatic reaction participants are positive limited values, it can be written without the loss of generality:

$$\begin{array}{l} \dfrac{\sum_{Ai} k_{A,Ai}}{k_{A,B}} \le \varepsilon \\ \dfrac{\sum_{Bj} k_{B,Bj}}{k_{B,A}} \le \varepsilon \end{array}, \tag{11}$$

where ε > 0 and ε may be equal to the larger value of those two in the left parts of inequalities (11). In this case inequalities (9) and (10) can be rewritten as follows:

$$(k_{A,B})^B < D_B < (k_{A,B})^B (1 + \varepsilon), \tag{12}$$

$$(k_{B,A})^A < D_A < (k_{B,A})^A (1 + \varepsilon). \tag{13}$$

Combining inequalities (12-13) and taking into account (6), it can be shown that

$$\frac{k_{A,B}}{k_{B,A}} \frac{1}{1+\varepsilon} < \frac{D_B}{D_A} < \frac{k_{A,B}}{k_{B,A}} (1 + \varepsilon). \tag{14}$$

Accounting that in a case of true equilibrium between X_A and X_B their equilibrium concentrations $[X_A]^{eq}$ and $[X_B]^{eq}$ should correspond with the ratio $\frac{[X_B]^{eq}}{[X_A]^{eq}} = \frac{k_{A,B}}{k_{B,A}}$, inequality (14) takes the form:

$$\frac{[X_B]^{eq}}{[X_A]^{eq}} \frac{1}{1+\varepsilon} < \frac{[X_B]^{ss}}{[X_A]^{ss}} < \frac{[X_B]^{eq}}{[X_A]^{eq}} (1 + \varepsilon), \tag{15}$$

and if

$$\varepsilon \ll 1, \tag{16}$$

then

$$\frac{[X_B]^{eq}}{[X_A]^{eq}} \approx \frac{[X_B]^{ss}}{[X_A]^{ss}} . \tag{17}$$

The latter expression means that if condition (16) is fulfilled, intermediates X_A and X_B are in a state close to the equilibrium one and according to Cha [1], we can say that X_A and X_B compose an equilibrium segment $X_A \underset{k_{B,A}}{\overset{k_{A,B}}{\rightleftharpoons}} X_B$.

Let us consider an arbitrary vertex X_m ($m \neq A$, $m \neq B$) of graph (3) and its determinant (D_m). Since each basal tree belonging to base X_m contains one and the only branch outgoing from another vertex (X_A or X_B in this case), it can be written:

$$D_m = \sum_{Ai}(k_{B,A}, k_{A,Ai})^m \quad + \sum_{Bj}(k_{A,B}, k_{B,Bj})^m \quad + \sum_{Ai}\sum_{Bj}(k_{A,Ai}, k_{B,Bj})^m , \tag{18}$$

where $(k_{B,A}, k_{A,Ai})^m$ is a sum of values of basal trees belonging to X_m and containing $k_{B,A}$ and $k_{A,Ai}$, $(k_{A,B}, k_{B,Bj})^m$ is a sum of values of basal trees belonging to X_m and containing $k_{A,B}$ and $k_{B,Bj}$, $(k_{A,Ai}, k_{B,Bj})^m$ is a sum of values of basal trees belonging to X_m and containing $k_{A,Ai}$ and $k_{B,Bj}$.

Analysis of structure of basal trees belonging to X_m and containing possible combinations $k_{B,A}$, $k_{A,B}$, $k_{A,Ai}$, and $k_{B,Bj}$ indicates that for all A_i and B_j,

$$\begin{aligned} \frac{(k_{A,Ai}, k_{B,Bj})^m}{k_{A,Ai} k_{B,Bj}} &< \frac{(k_{B,A}, k_{A,Ai})^m}{k_{B,A} k_{A,Ai}} \\ \frac{(k_{A,Ai}, k_{B,Bj})^m}{k_{A,Ai} k_{B,Bj}} &< \frac{(k_{A,B}, k_{B,Bj})^m}{k_{A,B} k_{B,Bj}} \end{aligned} \tag{19}$$

Equation (18) together with inequalities (11) and (19) gives

$$\sum_{Ai}(k_{B,A}, k_{A,Ai})^m \quad + \sum_{Bj}(k_{A,B}, k_{B,Bj})^m < D_m < [\sum_{Ai}(k_{B,A}, k_{A,Ai})^m \quad + \sum_{Bj}(k_{A,B}, k_{B,Bj})^m](1 + \varepsilon/2) . \tag{20}$$

Using the obvious ratios

$$\sum_{Ai}(k_{B,A},k_{A,Ai})^m=(k_{B,A})^m\,,$$

$$\sum_{Bj}(k_{A,B},k_{B,Bj})^m=(k_{A,B})^m\,,$$

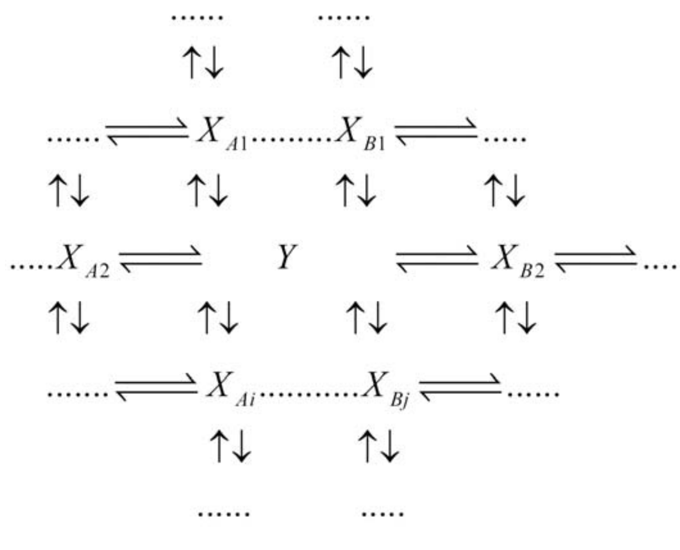
inequality (20) can be rewritten as follows:

$$(k_{B,A})^m+(k_{A,B})^m<D_m<[(k_{B,A})^m+(k_{A,B})^m](1+\varepsilon/2)\,. \tag{21}$$

Inequalities (12), (13), and (21) indicate that if condition (16) is fulfilled, graph (3) can be approximated by a new graph in which all basal trees belonging to X_A contain branch $k_{B,A}$, all basal trees belonging to X_B contain branch $k_{A,B}$, and all basal trees belonging to an arbitrary base contain either branch $k_{A,B}$ or branch $k_{B,A}$ and thus they sequentially pass through both intermediates X_A and X_B. This allows transformation of the initial graph (3) into graph (22) in which an equilibrium segment

$$X_A \underset{k_{B,A}}{\overset{k_{A,B}}{\rightleftharpoons}} X_B$$

is replaced by one vertex designated as Y



(22)

According to the Cha method [1], each rate constant for reaction $Y\rightarrow X_{Ai}$ in graph (22) is equal to

$$k_{A,Ai}\frac{k_{B,A}}{k_{A,B}+k_{B,A}}$$

and each rate constant for reaction $Y \rightarrow X_{Bj}$ in graph (22) is equal to

$$k_{B,Bj}\frac{k_{A,B}}{k_{A,B}+k_{B,A}}.$$

All other rate constants in graph (22) are equal to the corresponding rate constants in graph (3). Let us calculate graph (22) in accord with the steady-state assumption. To avoid confusion, let us designate base determinants for any vertex X_i of graph (22) with the upper asterisk (*): D_i*. Graph (22) is a quasi-equilibrium assumption of graph (3), so we designate the concentration of any intermediate X_i of graph (22) with the upper index *qe*: $[X_i]^{qe}$, by contrast to concentrations of graph (3) intermediates, which we shall designate with the upper index *ss*: $[X_i]^{ss}$.

Let us consider intermediates X_m ($m \neq A$, B), X_A, and X_B (graph (3)) and intermediates X_m and Y (graph (22)). It can easily be shown that

$$D_m^* = \frac{(k_{B,A})^m + (k_{A,B})^m}{k_{B,A}+k_{A,B}}, \tag{23}$$

$$D_Y^* = \frac{(k_{A,B})^B}{k_{A,B}} = \frac{(k_{B,A})^A}{k_{B,A}}. \tag{24}$$

Expressions for $[X_m]^{qe}$ (graph (22)) and expressions for $[X_m]^{ss}$ (graph (3)) look as follows:

$$[X_m]^{qe} = E_0 \frac{D_m^*}{D_Y^* + \sum\limits_{i\neq A,B} D_i^*}, \tag{25}$$

$$[X_m]^{ss} = E_0 \frac{D_m}{D_A + D_B + \sum\limits_{i\neq A,B} D_i}, \tag{26}$$

where E_o is the total enzyme concentration. Taking into account (23) and (24),

$$[X_m]^{qe} = E_0 \frac{(k_{B,A})^m + (k_{A,B})^m}{(k_{B,A})^A + (k_{A,B})^B + \sum\limits_{i \neq A,B} (k_{B,A})^i + (k_{A,B})^i} . \tag{27}$$

Application of inequalities (12), (13), and (21) and equalities (26) and (27) results in inequality

$$\frac{1}{1+\varepsilon}[X_m]^{qe} < [X_m]^{ss} < [X_m]^{qe}(1+\varepsilon/2) , \tag{28}$$

which can be transformed into a simpler form:

$$\frac{|[X_m]^{qe} - [X_m]^{ss}|}{[X_m]^{ss}} < \varepsilon . \tag{29}$$

Concentration of intermediate Y normalized to the fraction of intermediate X_A in the equilibrium segment is the concentration of intermediate X_A calculated in the quasi-equilibrium assumption, $[X_A]^{qe}$:

$$[X_A]^{qe} = [Y]^{qe} \frac{k_{B,A}}{k_{B,A} + k_{A,B}} . \tag{30}$$

Analogously for $[X_B]^{qe}$:

$$[X_B]^{qe} = [Y]^{qe} \frac{k_{A,B}}{k_{B,A} + k_{A,B}} . \tag{31}$$

Using (12), (13), (21), (23), and (24), it can easily be shown that

$$\frac{|[X_A]^{qe} - [X_A]^{ss}|}{[X_A]^{ss}} < \varepsilon , \tag{32}$$

$$\frac{|[X_B]^{qe} - [X_B]^{ss}|}{[X_B]^{ss}} < \varepsilon . \tag{33}$$

So, if condition (16) is fulfilled for mechanism (3) under the steady-state conditions:

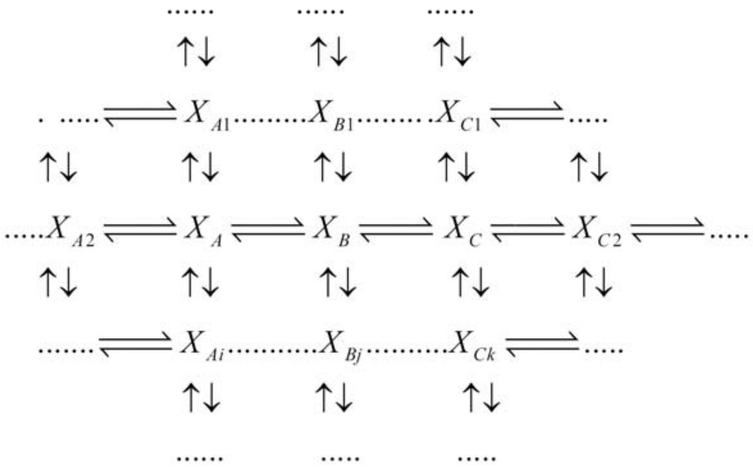
1. Intermediates X_A and X_B are practically in the equilibrium state.

2. A quasi-equilibrium graph (22) constructed according to the Cha method [1] correctly represents the properties of initial graph (3).

3. Accuracy of evaluation of concentrations on application of the quasi-equilibrium assumption is equal to ε (limitation to the ratios of rate constants in expression (11)) at all ε values.

As follows from the aforesaid, fulfillment of condition (16) is sufficient for application of the quasi-equilibrium assumption (22) of mechanism (3). It can easily be shown (e.g. for particular cases) that fulfillment of condition (16) is also a necessary condition for application of the quasi-equilibrium assumption.

Equilibrium segment consisting of three intermediates. Let us suppose that three intermediates of mechanism (2) designated as X_A, X_B, and X_C at scheme (34) may be considered as an equilibrium segment. Analogous to the previous section, let us define conditions for existence of such equilibrium segment and accuracy of the quasi-equilibrium assumption in this case.



(34)

It can easily be shown that fulfillment of condition (16) is the necessary and sufficient criteria for (a) establishment of the real equilibrium between X_A, X_B, and X_C in (34) and (b) possible transformation of graph (34) into graph (22) in which Y now means the equilibrium segment $X_A \underset{k_{B,A}}{\overset{k_{A,B}}{\rightleftharpoons}} X_B \underset{k_{C,B}}{\overset{k_{B,C}}{\rightleftharpoons}} X_C$ and the values of branches outgoing from the equilibrium segment of graph (22) are multiplied by a coefficient equal to a fraction of corresponding intermediate in the equilibrium segment. However, in case of graph (34) the value of ε accounts for limitations (35):

$$\frac{\sum_{Ai} k_{A,Ai}}{k_{A,B}} \le \varepsilon, \quad \frac{\sum_{Bj} k_{B,Bj}}{k_{B,A}} \le \varepsilon, \quad \frac{\sum_{Bj} k_{B,Bj}}{k_{B,C}} \le \varepsilon, \quad \frac{\sum_{Ck} k_{C,Ck}}{k_{C,B}} \le \varepsilon,$$
$$\frac{k_{B,A} \sum_{Ai} k_{A,Ai}}{k_{A,B} k_{B,C}} \le \varepsilon, \quad \frac{k_{B,C} \sum_{Ck} k_{C,Ck}}{k_{C,B} k_{B,A}} \le \varepsilon. \tag{35}$$

Concentrations of intermediates of the equilibrium segment calculated in the quasi-equilibrium assumption are defined by expressions:

$$[X_A]^{qe} = [Y]^{qe} \frac{k_{B,A} k_{C,B}}{k_{B,A} k_{C,B} + k_{A,B} k_{C,B} + k_{A,B} k_{B,C}}, \tag{36}$$

$$[X_B]^{qe} = [Y]^{qe} \frac{k_{A,B} k_{C,B}}{k_{B,A} k_{C,B} + k_{A,B} k_{C,B} + k_{A,B} k_{B,C}}, \tag{37}$$

$$[X_C]^{qe} = [Y]^{qe} \frac{k_{A,B} k_{B,C}}{k_{B,A} k_{C,B} + k_{A,B} k_{C,B} + k_{A,B} k_{B,C}}. \tag{38}$$

Using inequalities (35), it is possible to compare the steady-state concentrations of intermediates X_m ($m \neq A, B, C$), X_A, X_B and X_C, ($[X_m]^{ss}$, $[X_A]^{ss}$, $[X_B]^{ss}$, and $[X_C]^{ss}$) obtained for graph (34) with the quasi-equilibrium concentrations ($[X_m]^{qe}$, $[X_A]^{qe}$, $[X_B]^{qe}$, and $[X_C]^{qe}$) obtained for graph (22) accounting for expressions (36)-(38):

$$\frac{|[X_m]^{qe} - [X_m]^{ss}|}{[X_A]^{ss}} < 3\varepsilon + \varepsilon^2, \tag{39}$$

$$\frac{|[X_A]^{qe} - [X_A]^{ss}|}{[X_A]^{ss}} < 3\varepsilon + \varepsilon^2, \tag{40}$$

$$\frac{|[X_B]^{qe} - [X_B]^{ss}|}{[X_B]^{ss}} < 3\varepsilon + \varepsilon^2, \tag{41}$$

$$\frac{|[X_C]^{qe} - [X_C]^{ss}|}{[X_C]^{ss}} < 3\varepsilon + \varepsilon^2. \tag{42}$$

Thus, for mechanism (34), fulfillment of (16) and (35) under the steady-state conditions is the sufficient condition for application of the quasi-equilibrium assumption, accuracy of application of the quasi-equilibrium assumption in this case is equal to $3\varepsilon + \varepsilon^2$. It can easily be shown (e.g. for particular cases [9]) that fulfillment of conditions (16) and (35) is also the necessary condition for application of the quasi-equilibrium assumption in this case.

Equilibrium segment consisting of four intermediates (open segment). Let us suppose that four intermediates of mechanism (2) designated as X_A, X_B, X_C, and X_D can be considered as an equilibrium segment $X_A \underset{k_{B,A}}{\overset{k_{A,B}}{\rightleftharpoons}} X_B \underset{k_{C,B}}{\overset{k_{B,C}}{\rightleftharpoons}} X_C \underset{k_{D,C}}{\overset{k_{C,D}}{\rightleftharpoons}} X_D$. Analogous to the previous section, let us define conditions for existence of such equilibrium segment and accuracy of the quasi-equilibrium assumption in this case.

Application of the quasi-equilibrium assumption in this case is possible if conditions (16) and (43) are fulfilled:

$$\begin{gathered}
\frac{\sum\limits_{Ai} k_{A,Ai}}{k_{A,B}} \le \varepsilon, \quad \frac{\sum\limits_{Bj} k_{B,Bj}}{k_{B,A}} \le \varepsilon, \quad \frac{\sum\limits_{Bj} k_{B,Bj}}{k_{B,C}} \le \varepsilon, \quad \frac{\sum\limits_{Ck} k_{C,Ck}}{k_{C,B}} \le \varepsilon, \quad \frac{\sum\limits_{Ck} k_{C,Ck}}{k_{C,D}} \le \varepsilon, \quad \frac{\sum\limits_{Dl} k_{D,Dl}}{k_{D,C}} \le \varepsilon, \\
\frac{k_{B,A}\sum\limits_{Ai} k_{A,Ai}}{k_{A,B}k_{B,C}} \le \varepsilon, \quad \frac{k_{C,B}\sum\limits_{Bj} k_{B,Bj}}{k_{B,C}k_{C,D}} \le \varepsilon, \quad \frac{k_{B,C}\sum\limits_{Ck} k_{C,Ck}}{k_{C,B}k_{B,A}} \le \varepsilon, \quad \frac{k_{C,D}\sum\limits_{Dl} k_{D,Dl}}{k_{D,C}k_{C,B}} \le \varepsilon, \\
\frac{k_{B,A}k_{C,B}\sum\limits_{Ai} k_{A,Ai}}{k_{A,B}k_{B,C}k_{C,D}} \le \varepsilon, \quad \frac{k_{B,C}k_{C,D}\sum\limits_{Dl} k_{D,Dl}}{k_{B,A}k_{C,B}k_{D,C}} \le \varepsilon
\end{gathered} \tag{43}$$

Accuracy of evaluation of intermediate concentrations on application of the quasi-equilibrium assumption in this case will be defined by expressions:

$$\frac{|[X_m]^{qe} - [X_m]^{ss}|}{[X_A]^{ss}} < 6\varepsilon + 5\varepsilon^2 + \varepsilon^3, \tag{44}$$

$$\frac{|[X_A]^{qe} - [X_A]^{ss}|}{[X_A]^{ss}} < 6\varepsilon + 5\varepsilon^2 + \varepsilon^3, \tag{45}$$

$$\frac{|[X_B]^{qe} - [X_B]^{ss}|}{[X_B]^{ss}} < 6\varepsilon + 5\varepsilon^2 + \varepsilon^3, \tag{46}$$

$$\frac{|[X_C]^{qe} - [X_C]^{ss}|}{[X_C]^{ss}} < 6\varepsilon + 5\varepsilon^2 + \varepsilon^3, \tag{47}$$

$$\frac{|[X_D]^{qe} - [X_D]^{ss}|}{[X_D]^{ss}} < 6\varepsilon + 5\varepsilon^2 + \varepsilon^3. \tag{48}$$

Equilibrium segment consisting of four intermediates (closed segment). If the equilibrium segment of mechanism (2) looks like (49),

$$\begin{tikzcd}
X_A \arrow[r, shift left, "k_{A,B}"] \arrow[d, shift left, "k_{A,D}"] & X_B \arrow[l, shift left, "k_{B,A}"] \arrow[d, shift left, "k_{B,C}"] \\
X_D \arrow[r, shift left, "k_{D,C}"] \arrow[u, shift left, "k_{D,A}"] & X_C \arrow[l, shift left, "k_{C,D}"] \arrow[u, shift left, "k_{C,B}"]
\end{tikzcd} \tag{49}$$

application of the quasi-equilibrium assumption in this case is possible on fulfillment of conditions (16) and (50):

$$\begin{gathered}
\frac{\sum\limits_{Ai} k_{A,Ai}}{k_{A,B}} \le \varepsilon, \quad \frac{\sum\limits_{Ai} k_{A,Ai}}{k_{A,D}} \le \varepsilon, \quad \frac{\sum\limits_{Bj} k_{B,Bj}}{k_{B,A}} \le \varepsilon, \quad \frac{\sum\limits_{Bj} k_{B,Bj}}{k_{B,C}} \le \varepsilon, \\
\frac{\sum\limits_{Ck} k_{C,Ck}}{k_{C,B}} \le \varepsilon, \quad \frac{\sum\limits_{Ck} k_{C,Ck}}{k_{C,D}} \le \varepsilon, \quad \frac{\sum\limits_{Dl} k_{D,Dl}}{k_{D,A}} \le \varepsilon, \\
\frac{k_{D,A}\sum\limits_{Ai} k_{A,Ai}}{k_{A,D}k_{D,C}} \le \varepsilon, \quad \frac{k_{A,B}\sum\limits_{Bj} k_{B,Bj}}{k_{B,A}k_{A,D}} \le \varepsilon, \quad \frac{k_{B,C}\sum\limits_{Ck} k_{C,Ck}}{k_{C,B}k_{B,A}} \le \varepsilon
\end{gathered} \tag{50}$$

Accuracy of evaluation of intermediate concentrations on application of the quasi-equilibrium assumption if the principle of detailed equilibrium is obeyed in this case will be defined by expressions:

$$\frac{|[X_m]^{qe} - [X_m]^{ss}|}{[X_A]^{ss}} < \frac{7}{2}\varepsilon + \frac{11}{6}\varepsilon^2 + \frac{1}{4}\varepsilon^3, \tag{51}$$

$$\frac{|[X_A]^{qe} - [X_A]^{ss}|}{[X_A]^{ss}} < \frac{7}{2}\varepsilon + \frac{11}{6}\varepsilon^2 + \frac{1}{4}\varepsilon^3, \tag{52}$$

$$\frac{|[X_B]^{qe} - [X_B]^{ss}|}{[X_B]^{ss}} < \frac{7}{2}\varepsilon + \frac{11}{6}\varepsilon^2 + \frac{1}{4}\varepsilon^3, \tag{53}$$

$$\frac{|[X_C]^{qe} - [X_C]^{ss}|}{[X_C]^{ss}} < \frac{7}{2}\varepsilon + \frac{11}{6}\varepsilon^2 + \frac{1}{4}\varepsilon^3, \tag{54}$$

$$\frac{|[X_D]^{qe} - [X_D]^{ss}|}{[X_D]^{ss}} < \frac{7}{2}\varepsilon + \frac{11}{6}\varepsilon^2 + \frac{1}{4}\varepsilon^3. \tag{55}$$

Equilibrium segment in the general case. It is impossible to derive the expression for accuracy of the quasi-equilibrium assumption in the case of an arbitrary equilibrium segment in the context of this work, but it is possible to suggest a method for evaluation of the necessary limitations on the ratio of rate constants in the general case. Let us consider an equilibrium segment of arbitrary structure. To evaluate the necessary limitations, the following procedure should be used.

Let us suppose that two intermediates, X_i and X_j, belong to this equilibrium segment, and there are branches (at least one branch) outgoing from X_i and directed outside the segment. There is at least one pathway connecting X_i and X_j and located inside the equilibrium segment. Let us go from X_i to X_j via this pathway. A product of branch values on this pathway we shall designate as “denominator”. Then let us recede from X_j on one stage back within the closed pathway and go to X_i via the same pathway. A product of branch values on this pathway (the number of branches is one less than that on the preceding pathway) we shall designate as “nominator”. The sum of values of all branches outgoing from X_i and directed outside the equilibrium segment we shall designate as “sum”. Condition for application of the quasi-equilibrium assumption in the case of an arbitrary segment is fulfillment of (16) where ε is defined by expression

$$\text{sum}\ \frac{\text{nominator}}{\text{denominator}} \le \varepsilon \tag{56}$$

for all X_i and X_j belonging to the equilibrium segment and for all possible pathways between them inside the equilibrium segment. Accuracy of application of the quasi-equilibrium assumption can be derived for each particular case.

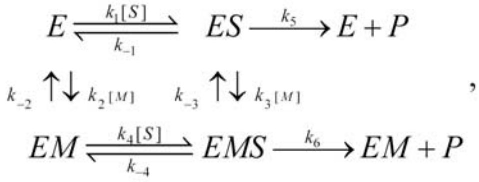
DISCUSSION

In this work an arbitrary mechanism of enzymatic reaction containing an equilibrium segment or two, three, and four intermediates is considered using the graph method. Expressions for determination of accuracy of distribution of intermediate concentrations inside the equilibrium segment and accuracy of determination of intermediate concentrations inside and outside the equilibrium segment as a function of the ratio of kinetic constants on application of the quasi-equilibrium assumption are derived. Conditions with which arbitrary equilibrium segment should comply are derived by the induction method. However, accuracy of application of the quasi-equilibrium assumption in a case of an arbitrary equilibrium segment and accuracy of determination of the rate of enzymatic reaction can be evaluated separately in each case.

To determine such parameters in a general view, other (deductive) approaches are required.

In this work application of the quasi-equilibrium assumption was analyzed for an arbitrary mechanism of enzymatic reaction. When the arbitrary mechanism is adapted to a certain case, conditions for application of the quasi-equilibrium assumption generally become simpler.

For example, for the Botts–Morales mechanism [12], possible application of the equilibrium assumption was discussed in [3, 4, 13]:



, (57)

and which is a special case of mechanism (2) with equilibrium segment (49), conditions for application of the quasi-equilibrium assumption for a segment of four intermediates (50) degenerate to two necessary and sufficient conditions:

$$\frac{k_5}{k_{-1}} \le \varepsilon, \quad \frac{k_6}{k_{-4}} \le \varepsilon \,. \tag{58}$$

In this case accuracy of application of the quasi-equilibrium assumption on fulfillment of conditions (58) is defined by the following expressions:

$$\begin{aligned}
&\frac{1}{1+4\varepsilon+\varepsilon^2}[E]^{qe} < [E]^{ss} < (1+4\varepsilon+\varepsilon^2)[E]^{qe} \\
&\frac{1}{1+\varepsilon}[ES]^{qe} < [ES]^{ss} < (1+4\varepsilon+\varepsilon^2)[ES]^{qe} \\
&\frac{1}{1+\varepsilon}[EMS]^{qe} < [EMS]^{ss} < (1+4\varepsilon+\varepsilon^2)[EMS]^{qe} \\
&\frac{1}{1+4\varepsilon+\varepsilon^2}[EM]^{qe} < [EM]^{ss} < (1+4\varepsilon+\varepsilon^2)[EM]^{qe} \\
&\frac{1}{1+\varepsilon}v^{qe} < v^{ss} < (1+4\varepsilon+\varepsilon^2)v^{qe}
\end{aligned} \tag{59}$$

where v^{ss} is the steady-state rate for mechanism (57) and v^{qe} is the rate in the case of the quasi-equilibrium assumption for mechanism (57). It should be mentioned that mechanism (57) is a significant simplification of the minimally required scheme of enzymatic reaction, which should contain intermediates *EP* and *EMP* along with intermediates *ES* and *EMS*. Such intermediates may be ignored in kinetic mechanism if kinetic behavior of this mechanism does not change as a result. It can be easily shown that the presence of intermediates *EP* and *EMP* significantly changes kinetic behavior of mechanism (57) in the sense that conditions for application of the quasi-equilibrium assumption are complicated as compared with (58). This indicates that caution is required when applying the simplified kinetic schemes and that it is useful to analyze general schemes.

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