MATHEMATICAL MODELING OF BIOCHEMICAL PROCESS

CONSIDERATION OF THE CONTRIBUTION OF CHEMICAL (NON-ENZYMATIC) CONVERSION OF SUBSTRATE IN THE GENERAL MECHANISM OF ENZYME REACTION

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When enzyme-catalyzed reactions are studied, it is necessary to take into account the contribution of the chemical (non-enzymatic) conversion of the substrate to the product, which is carried out together with the enzyme-catalyzed conversion of the substrate. It is generally believed that the difference of the product concentration that was formed in the presence of the enzyme and in its absence (during the same time interval) is the concentration of the product that was formed directly in the enzyme-catalyzed reaction, i.e. that there is additivity of the product concentrations at each time point. In this paper, we have analyzed when there is additivity and how to correctly take into account the contribution of chemical (non-catalytic) substrate conversion when the enzyme-catalyzed reactions are investigated. We have shown that the additivity of product concentrations and initial rates is observed only for a period when the product concentration increases linearly with time. The longer the reaction proceeds the more the deviation from the additivity. Under equilibrium condition, there is no additivity of equilibrium product concentrations but under conditions of detailed balance the equilibrium product concentration of the overall reaction, including the enzyme-catalyzed and chemical (non-enzymatic) conversion of the substrate, is also at the same time the equilibrium concentration of the product of the enzyme-catalyzed conversion of the substrate.

K e y w o r d s: kinetics of the enzyme-catalyzed reactions, initial rate, equilibrium state, cyclic reactions, detailed balance, cyclic equilibrium, catalysis, rate constant, equilibrium constant, dissociation constant.
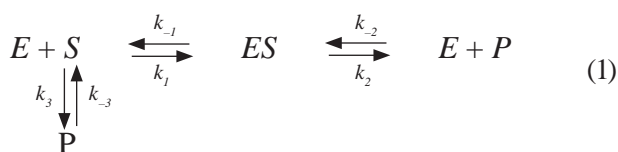
W hen investigating catalytic (including enzyme-catalyzed) reactions, as a rule, only the pathway of catalytic conversion of initial substances into reaction products is considered neglecting the pathway of chemical (non-catalytic) conversion of the substrate in the reaction mechanism [1, 2]. This can be explained by the obvious fact that catalysts (enzymes) significantly increase the rates of the chemical processes, so calculating the rate of the catalytic process can neglect the rate of the chemical (non-catalytic) process. It is believed that the catalyst must accelerate both the forward and reverse reactions equally and therefore can not affect the equilibrium state of a reaction, i.e. addition catalyst to the reaction medium does not lead to a change in the thermodynamic equilibrium constant of the reaction [3]. Thus the state of thermodynamic equilibrium is independent of the path by which the state has been reached.
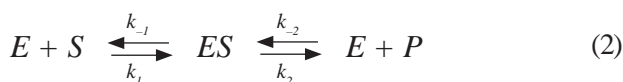
However, it is not always possible to neglect the rate of the non-catalytic reaction in comparison with the catalytic reaction. Due to the fact that the chemical reaction proceeds simultaneously with the catalytic reaction, and it is impossible to avoid the

influence of the chemical (non-catalytic) reaction during the investigation of the catalytic reaction, in experimental studies, in particular enzyme-catalyzed reactions, the contribution of the non-catalytic conversion of the substrate is always taken into account. Since the chemical (non-catalytic) reaction taking place in the absence of the enzyme can be investigated independently, control experiments are performed to take into account its contribution to the amount of the reaction product formed. The difference in product concentrations that was formed in the presence of the enzyme and in its absence (during the same time interval) is taken as the concentration of the product that was formed directly in the enzyme-catalyzed reaction [4], i.e. it is believed that there is additivity of the concentrations of the reaction product at any given time period.

Using a simple mechanism (1) as an example we will investigate the validity and correctness of such actions, i.e. is it possible to calculate the product concentration of the enzyme-catalyzed conversion of substrate as the difference in the concentrations of the product of the overall reaction (including chemical and enzyme-catalyzed reactions) and the chemical conversion of substrate.

$$E + S \underset{k_1}{\overset{k_{-1}}{\rightleftarrows}} ES \underset{k_2}{\overset{k_{-2}}{\rightleftarrows}} E + P \qquad (1)$$
$$\underset{P}{\overset{k_3 \, \downarrow\uparrow \, k_{-3}}{}}$$

The mechanism (1) is the simplest scheme that takes into account the possibility of conversion of substrate simultaneously in two directions: along the reaction pathway of the enzyme-catalyzed conversion of the substrate into the product (ECC)

$$E + S \underset{k_1}{\overset{k_{-1}}{\rightleftarrows}} ES \underset{k_2}{\overset{k_{-2}}{\rightleftarrows}} E + P \qquad (2)$$

and along the reaction pathway of the chemical (non-enzymatic) conversion of the substrate into the product (ChC)

$$S \underset{k_3}{\overset{k_{-3}}{\rightleftarrows}} P \qquad (3)$$

In the mechanisms (1)-(3) the following notation is used: E – enzyme, S – substrate, ES – enzyme-substrate complex, P – product of chemical and enzyme-catalyzed conversion of substrate S, k_1 and k_2 – rate constants of forward reactions of the first and second steps of overall reaction (reaction pathway of the enzyme-catalyzed conversion

of substrate into product), $M^{-1}s^{-1}$; k_{-1} and k_{-2} – the rate constants of the reverse reactions of the first and second steps of overall reaction (reaction pathway of the enzyme-catalyzed conversion of substrate into product), s^{-1}; k_3 and k_{-3} – rate constants of the forward and reverse reactions of the third steps of overall reaction (reaction pathway of the chemical conversion of substrate into product), s^{-1}.

The reactions proceeding by the mechanism (1) are described by the following system of differential equations:

$$\frac{d[E]}{dt} = -k_1[E][S] + k_{-1}[ES] + k_2[ES] -$$
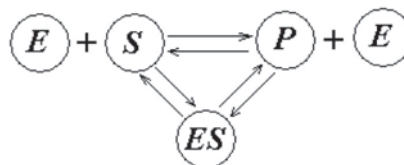$$- k_{-2}[E][P] = -W_1 + W_2 \qquad (4)$$

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] +$$
$$+ k_{-2}[E][P] = W_1 - W_2 \qquad (5)$$

$$\frac{d[P]}{dt} = -k_{-2}[E][P] + k_2[ES] - k_{-3}[P] +$$
$$+ k_3[S] = W_2 + W_3 \qquad (6)$$

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES] + k_{-3}[P] -$$
$$- k_3[S] = -W_1 - W_3 \qquad (7)$$

where W_n is the rate of the n-th step, i.e. the difference between the forward and reverse reaction rates of the n-th step.

Analyzing this system of differential equations, it can be established that in an equilibrium state (when all derivatives are zero) $W_1 = W_2 = -W_3$. This indicates that the reactions occurring by this mechanism are cyclic and the mechanism (1) can be represented as a cycle,

although separately taken ChC and ECC proceeding according to the mechanisms (3) and (2) respectively, are acyclic reactions and obey the principle of detailed balance.

It is believed that all chemical and catalytic reactions should proceed in accordance with the principle of detailed balance, i.e. in the state of thermodynamic equilibrium the rate of forward reac-

tion must be equal to the rate of the reverse reaction within each step [5, 6].

Most chemical reactions are acyclic: the rate constants of these reactions can have an arbitrary value and they always proceed in accordance with the principle of detailed balance. Catalytic (enzyme-catalyzed) reactions are cyclic, although many of them, like acyclic ones, also obey the principle of detailed balance. However, there are such cyclic reactions in which the principle of detailed balance can theoretically be violated and a cyclic equilibrium can be established. These reactions will be called true cyclic reactions [7].

In 1901 Wegscheider [8] analyzing the mechanisms of true cyclic reactions found that in a state of cyclic equilibrium the thermodynamic equilibrium constants of reaction or individual steps (calculated from equilibrium concentrations) do not coincide in magnitude with the corresponding kinetic equilibrium constants (calculated from the ratio of the rate constants of forward and reverse reactions). This discrepancy was called the "Wegscheider's paradox" [6].

Since it is assumed that the equilibrium constants must always remain unchanged (because they are related to the standard Gibbs energy ΔG^0), in order to avoid the manifestation of the Wegscheider's paradox, it is necessary the true cyclic reactions obey the principle of detailed balance [6].

As a result, the rate constants of true cyclic reactions cannot be arbitrary but must obey the Wegscheider condition: the product of rate constants in the clockwise direction must be equal to the product of the rate constants in the counter-clockwise direction (or, in other words, the product of the equilibrium constants of all steps in a cycle must be equal to one) [9, 10]. The fulfillment of the principle of detailed balance ensures that the thermodynamic equilibrium constant of the reaction will be equal to its kinetic equilibrium constant [6]. When the principle of detailed balance in the true cyclic reactions is violated, the cyclic equilibrium is reached. In this case, the rate constants do not obey the Wegscheider condition and can take any values, and also the thermodynamic and kinetic equilibrium constants of the corresponding steps do not coincide.

Thus under the condition of detailed balance the equilibrium constant of the reaction must always remain unchanged: it must not depend on either the mechanism (i.e. the number and sequence of steps necessary to convert the reactants to the products)

or the presence of other reactions that can simultaneously occur in the reaction system, neither from the initial concentrations of the reactants nor from the presence of a catalyst (enzyme) and/or other compounds in the reaction system, including those that can interact with reactants (for example, inhibitors, activators, ions of metals and hydrogen).

As can be seen, the reactions proceeding by the mechanism (1) are true cyclic reactions and they can reach a cyclic equilibrium when the reaction rates of all steps are not equal to zero but have the same value for all steps ($W_1 = W_2 = -W_3$).

This result is of fundamental importance. It indicates that a detailed balance can be violated not only in a limited number of catalytic (enzyme-catalyzed) reactions, but absolutely in all such reactions, since it is impossible to isolate the pathway of catalytic conversion of substrate from a chemical (non-catalytic) one: the chemical conversion of substrate is necessarily carried out at a greater or lesser rate. Therefore, despite the dominant point of view that the principle of detailed balance cannot be violated, it is necessary to carefully consider this possibility since there are also strong reasons to believe that a cyclic equilibrium can be realized [7]. In this connection, we will consider both possibilities: a special case where cyclic reaction obeys the principle of detailed balance, and the general case including the possibility of reaching cyclic equilibrium.

The equilibrium state

We will analyze whether it is necessary to subtract the product concentration of the ChC from the product concentration formed in the overall reaction proceeding by mechanism (1) to determine the fraction of the ECC in it, if an equilibrium state is studied when all three reactions (1-3) have reached equilibrium (the time for which each reaction can reach equilibrium can vary significantly).

To derive an equation for calculating the equilibrium concentration of a product in the case of the ECC proceeding by the mechanism (2), it is necessary to solve a system of two equations (taking into account the fact that in the equilibrium state the rates of both steps are zero):

$$k_1([E]_0 - [ES])([S]_0 - [ES] - [P]) - k_{-1}[ES] = 0 \qquad (8)$$
$$k_2[ES] - k_{-2}([E]_0 - [ES])[P] = 0 \qquad (9)$$

The solution is a quadratic equation which can be represented in the form:

$$^{eq}[P]_{enz}[E]_0 = \left(K_2 + {}^{eq}[P]_{enz} \right)\left\{ [S]_0 - \right.$$

$$\left. - {}^{eq}[P]_{enz}\frac{(K+1)}{K} \right\} \qquad (10)$$

where: $[E]_0$ and $[S]_0$ are the initial concentrations of the enzyme and substrate respectively, M; $^{eq}[P]_{enz}$ is the equilibrium concentration of the product of the enzyme-catalyzed conversion of the substrate, M; $K = \dfrac{k_1 k_2}{k_{-1}k_{-2}}$ and $K_2 = \dfrac{k_2}{k_{-2}}$ are the equilibrium constants of the enzyme-catalyzed conversion of substrate proceeding by the mechanism (2) and the second step of this reaction, respectively.

In the case of the overall reaction (1) which obeys the principle of detailed balance it is necessary to solve a system of three equations:

$$k_1([E]_0 - [ES])([S]_0 - [ES] - [P]) - k_{-1}[ES] = 0 \qquad (11)$$
$$k_2[ES] - k_{-2}([E]_0 - [ES])[P] = 0 \qquad (12)$$
$$k_3([S]_0 - [ES] - [P]) - k_{-3}[P] = 0 \qquad (13)$$

However, under detailed balance condition one of the equations is superfluous since there are two unknown variables ($[P]$ and $[ES]$) and three equations. Therefore it is obvious that when choosing the first two equations, the solution will be identical to the solution obtained for the ECC (equation (10)). It is quite clear that if we choose another pair of equations, the solution is the same.

Thus, under detailed balance condition, the equilibrium product concentration of the ECC proceeding by the mechanism (2) is always equal to the equilibrium product concentration of the overall reaction (1). Therefore, if the investigated reaction proceeding by the mechanism (1) reached equilibrium, the obtained value of the equilibrium product concentration is at the same time the equilibrium product concentration of the ECC. As it can be seen, the addition of the ChC pathway to the ECC pathway does not affect the equilibrium product concentration.

Under detailed balance condition, as it was said before, the thermodynamic equilibrium constant (K) of substrate conversion into the product remains unchanged for all three mechanisms:

$$\frac{^{eq}[P]}{^{eq}[S]} = K = \frac{k_1 k_2}{k_{-1}k_{-2}} = \frac{k_3}{k_{-3}} \qquad (14)$$

For the ChC pathway we can write:

$$K = \frac{^{eq}[P]_{chem}}{^{eq}[S]_{chem}} = \frac{^{eq}[P]_{chem}}{[S]_0 - {}^{eq}[P]_{chem}} \qquad (15)$$

from which it follows that the equilibrium product concentration is:

$$^{eq}[P]_{chem} = \frac{K[S]_0}{K+1}. \qquad (16)$$

For the ECC pathway and the overall reaction (1) the thermodynamic equilibrium constant can be expressed in the same way:

$$K = \frac{^{eq}[P]_{enz}}{^{eq}[S]_{enz}} = \frac{^{eq}[P]_{enz}}{[S]_0 - {}^{eq}[ES]_{enz} - {}^{eq}[P]_{enz}};$$

$$K = \frac{^{eq}[P]_{tot}}{^{eq}[S]_{tot}} = \frac{^{eq}[P]_{tot}}{[S]_0 - {}^{eq}[ES]_{tot} - {}^{eq}[P]_{tot}} \qquad (17)$$

It is seen that if the equality $^{eq}[P]_{tot} = {}^{eq}[P]_{enz}$ is satisfied, the equality $^{eq}[ES]_{tot} = {}^{eq}[ES]_{enz}$ is also observed. The equilibrium product concentration formed both along the ECC pathway and in the overall reaction is always less than along the ChC pathway, since a part of the substrate binds to the enzyme in the enzyme-substrate complex and does not convert into product:

$$^{eq}[P]_{tot} = \frac{K\left([S]_0 - {}^{eq}[ES]\right)}{K+1} = {}^{eq}[P]_{enz} \qquad (18)$$

If equilibrium concentrations of $^{eq}[P]_{chem}$ and $^{eq}[P]_{tot}$ are known (for different initial substrate concentrations) all equilibrium constants (overall reaction, ChC and ECC pathways, and the first and the second steps of the ECC pathway) can be calculated without corresponding equilibrium substrate concentration values:

$$\frac{[E]_0}{^{eq}[P]_{chem} - {}^{eq}[P]_{tot}} = \frac{(K+1)}{K} +$$

$$+ \frac{K_2(K+1)}{K}\frac{1}{^{eq}[P]_{tot}} \qquad (19)$$

The result obtained ($^{eq}[P]_{tot} = {}^{eq}[P]_{enz}$) is an exact solution and it remains valid also for mechanisms with more complicated catalytic pathways (as follows from equations (17)-(18)), in which in addition to the enzyme-substrate complex other complexes may be present (we again remind that this is true only if the principle of detailed balance holds).

Thus, it is not necessary to subtract the equilibrium product concentration of the ChC from the equilibrium product concentration of the overall reaction in order to calculate the equilibrium product concentration of the ECC.

However, the reaction product may be present in the substrate (due to a slow chemical conversion or simply as contamination) or in other reagents. We denote this concentration $[P]_0$. This additional concentration of the product will not affect the reaction equilibrium constant (under detailed balance condition)

$$K = \frac{{}^{eq}[P]_{chem}}{[S]_0 + [P]_0 - {}^{eq}[P]_{chem}} =$$

$$= \frac{{}^{eq}[P]_{enz}}{[S]_0 + [P]_0 - {}^{eq}[ES]_{enz} - {}^{eq}[P]_{enz}} =$$

$$= \frac{{}^{eq}[P]_{tot}}{[S]_0 + [P]_0 - {}^{eq}[ES]_{tot} - {}^{eq}[P]_{tot}} \qquad (20)$$

but will affect the equilibrium product concentration,

$$^{eq}[P]_{chem} = \frac{K([S]_0 + [P]_0)}{K+1} ;$$

$$^{eq}[P]_{tot} = \frac{K([S]_0 + [P]_0 - {}^{eq}[ES])}{K+1} = {}^{eq}[P]_{enz} \qquad (21)$$

If it is necessary that the value of the equilibrium product concentration refers to the conditions when $[P]_0 = 0$, it is necessary to subtract from ${}^{eq}[P]_{tot}$ not $[P]_0$, but $\dfrac{K[P]_0}{K+1}$ (see equation (18)):

$$^{eq}[P]_{tot} - \frac{K[P]_0}{K+1} = \frac{K([S]_0 - {}^{eq}[ES]_{tot})}{K+1} \qquad (22)$$

This is especially important in cases where the equilibrium constant of the reaction is close to one or less. However, if $K \gg 1$, then it is practically possible to subtract $[P]_0$.

Although the exact solution shows that the equilibrium product concentration in the process of ChC is always greater than for the ECC, but this difference may be insignificant. From equation (9) it is possible to obtain an expression for the equilibrium concentration of the *ES* complex:

$$^{eq}[ES]_{tot} = \frac{k_{-2}[E]_0 {}^{eq}[P]_{tot}}{k_2 + k_{-2} {}^{eq}[P]_{tot}} \qquad (23)$$

After its substitution into equation (17), the equilibrium product concentration can be expressed as follows:

$$^{eq}[P]_{tot} = \frac{K[S]_0}{1 + K\left(1 + \dfrac{[E]_0}{K_2 + {}^{eq}[P]_{tot}}\right)} \qquad (24)$$

Comparing the equation obtained with the equation (16)

$$\frac{{}^{eq}[P]_{chem}}{{}^{eq}[P]_{tot}} = \frac{1 + K\left(1 + \dfrac{[E]_0}{K_2 + {}^{eq}[P]_{tot}}\right)}{1 + K} =$$

$$= 1 + \left(\frac{K}{1+K}\right)\frac{[E]_0}{\left(K_2 + {}^{eq}[P]_{tot}\right)} \qquad (25)$$

it can be seen that the smaller initial enzyme concentration is used for research, and the greater the equilibrium constant of the second step of the reaction K_2, and the smaller the equilibrium constant of reaction K, the less the equilibrium product concentration formed along the ChC pathway is different from the equilibrium product concentration in the overall reaction (or formed along the ECC pathway).

Assuming that $[E]_0 \ll [S]_0$ the next equation is obtained from equation (10) (see also equation (16)):

$$^{eq}[P]_{tot} = {}^{eq}[P]_{enz} = \frac{K[S]_0}{(K+1)} = {}^{eq}[P]_{chem} \qquad (26)$$

It also shows that under such conditions the equilibrium product concentration of the ChC is practically the same as the equilibrium product concentration in the overall reaction (or when the ECC is carried out).

To summarize, an approximate solution shows that the equilibrium product concentrations of all three reactions (if they obey the detailed balance principle) can be practically the same when using low concentrations of the enzyme ($[E]_0 \ll [S]_0$).

Based on the results of the analysis, the following conclusion can be drawn: when the investigations are carried out under conditions of detailed balance, there is no need to introduce any correction to exclude the effect of the ChC occurring together with the ECC: the equilibrium product concentration in the overall reaction proceeding by mechanism (1) is simultaneously equilibrium product concentration of the ECC.

As a result of the violation of the detailed balance principle, the equilibrium constant of the cyclic reaction can vary: it becomes dependent on the specific rate constants and often on the concentrations of the reacting substances. Let us check whether additivity is observed when a detailed balance is violated.

The equilibrium product concentration of the ECC (mechanism (2)) is expressed by the equation (10) which we derived earlier, and the equilibrium product concentration of the ChC (mechanism (3)) is expressed by equation (27)

$$^{eq}[P]_{chem} = \frac{k_3[S]_0}{k_3 + k_{-3}} \qquad (27)$$

To derive the equation for the equilibrium product concentration of the overall reaction proceeding by the mechanism (1), it is necessary to solve the system of equations:

$$k_1([E]_0 - [ES])([S]_0 - [ES] - [P]) -$$
$$- k_{-1}[ES] - k_2[ES] + k_{-2}([E]_0 - [ES])[P] = 0$$

$$k_2[ES] - k_{-2}([E]_0 - [ES])[P] + k_3([S]_0 -$$
$$- [ES] - [P]) - k_{-3}[P] = 0 \qquad (28)$$

As a result of the solution we obtain a cubic equation (see 29), where $K_m = \dfrac{k_{-1} + k_2}{k_1}$ is the Michaelis constant.

Obviously, in this case, additivity is not observed. Comparing equations (29) and (10), it becomes clear that the equilibrium product concentration of the ECC does not coincide with the equilibrium product concentration of the overall reaction, although the coincidence was observed under the conditions of detailed balance. Equation (29) turns into equation (10) only under the condition $k_3 = k_{-3} = 0$.

The initial rate

In contrast to the equilibrium state which allows us to obtain an exact solution, the solution is approximated when investigating the initial rate.

Therefore, this can lead to certain (sometimes significant) errors (which are difficult to control) in individual cases.

The absence of an exact solution is due to the fact that the initial rate is to a greater or lesser extent averaged over a certain interval of time. In addition, to find the initial reaction rate equation in the presence of the enzyme (reactions (1) and (2)), a quasi-steady state approximation is used. It is based on the fact that the enzyme-catalyzed reaction quickly passes into a state where the concentration of reaction intermediates remains practically unchanged for a relatively long period (or a change in their concentration is negligible compared to a change in the concentration of the reagents). However, it must be taken into account that in the case of ChC (reaction (3)) there is no stationary state, although it is possible to calculate the initial rate of such a reaction (i.e. the rate under conditions where the reaction product is practically absent). In addition, it is necessary to take into account that the steady state of the ECC and the overall reaction, when the product concentration increases linearly with time, may not coincide in time with the initial period of the ChC for which the initial rate is calculated.

The rate of ChC (v_{chem}) at the start of the reaction when the product concentration is negligible and therefore the rate of the reverse reaction can be neglected is:

$$v_{chem} = k_3[S]_0 \qquad (30)$$

As is known the initial rate of ECC (v_{enz}) proceeding by the mechanism (2) is described by the Michaelis-Menten equation (when $[E]_0 << [S]_0$) [1, 2, 4]:

$$v_{enz} = \frac{k_1 k_2 [E]_0 [S]_0}{k_2 + k_{-1} + k_1[S]_0} = \frac{k_2 [E]_0 [S]_0}{K_m + [S]_0} \qquad (31)$$

To derive the initial rate equation of the overall reaction (v_{tot}), which obeys the principle of detailed balance (under conditions when the product is formed in negligibly small quantities, i.e. $[P] = 0$), it is necessary to solve a system of two equations:

$$\left\{ [E]_0 \left(k_2 - k_3 \right) + k_3[S]_0 - {}^{eq}[P]_{tot} \left(k_3 + k_{-3} \right) \right\} \left\{ {}^{eq}[P]_{tot} \left(k_{-2}[E]_0 + k_3 + k_{-3} \right) - k_3[S]_0 \right\} =$$

$$= \left\{ [E]_0 \left(k_2 - k_3 \right) + k_3[S]_0 - {}^{eq}[P]_{tot} \left(k_3 + k_{-3} \right) \right\} \left[[S]_0 + \left(\frac{k_{-2}}{k_1} - 1 \right) {}^{eq}[P]_{tot} \right] \left(k_2 - k_3 + k_{-2} {}^{eq}[P]_{tot} \right) -$$

$$- \left\{ {}^{eq}[P]_{tot} \left(k_{-2}[E]_0 + k_3 + k_{-3} \right) - k_3[S]_0 \right\} \left(k_2 - k_3 + k_{-2} {}^{eq}[P]_{tot} \right) K_m \qquad (29)$$

$$k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0 \qquad (32)$$

$$k_2[ES] + k_3[S] = v_{tot} \qquad (33)$$

Assuming that $[E]_0 \ll [S]_0$ we obtain the initial rate equation in the following form:

$$v_{tot} = \frac{k_2[E]_0[S]_0}{K_m + [S]_0} + k_3[S]_0 =$$

$$= \frac{[S]_0 \left\{ k_2[E]_0 + k_3 \left([S]_0 + K_m \right) \right\}}{K_m + [S]_0} \qquad (34)$$

The result obtained shows that under conditions of detailed balance there is additivity of initial rates:

$$v_{tot} = v_{enz} + v_{chem} \qquad (35)$$

In conditions where the initial rates are additive, the concentration of the product must also be additive.

The results of the calculations show that at the start of the reaction (when the product concentration increases linearly with time in the reactions proceeding by mechanisms (1)–(3)) instantaneous rates (w), initial rates (v), as well as product concentrations $[P]$ are additive with respect to time. We note that the accuracy of the coincidence of the difference in the product concentrations of the overall reaction and the ChC with the product concentration of the ECC is approximately two times better than in the case of instantaneous rates (as was observed with all our calculations). Since the initial rate is averaged over several first points, the accuracy of the coincidence of the initial rates corresponds to the accuracy of the coincidence of the instantaneous rates approximately in the middle of the interval that was chosen for calculating the initial rate.

For example consider the reaction with the parameters: $k_1 = 160$; $k_{-1} = 40$; $k_2 = 75$; $k_{-2} = 100$; $k_3 = 3 \cdot 10^{-2}$; $k_{-3} = 1 \cdot 10^{-2}$; $[S]_0 = 1$ M; $[E]_0 = 1 \cdot 10^{-3}$ M; $K = 3$; $K_m = 0.72$. If we find the product concentration of the ECC by subtracting the product concentration of the ChC from the product concentration of the overall reaction, then at 10 seconds the calculated value ($[P]_{tot} - [P]_{chem} = 2.320 \cdot 10^{-1}$ M) can be one third smaller than the true value ($[P]_{enz} = 3.409 \cdot 10^{-1}$ M), in the case of instantaneous rate – by two thirds ($w_{tot} - w_{chem} = 8.277 \cdot 10^{-3}$ and $w_{enz} = 2.570 \cdot 10^{-2}$ Ms^{-1}, respectively), and in the case of the initial rate calculated from the first ten points, which were measured after 1 second – by a quarter ($v_{tot} - v_{chem} = 2.683 \cdot 10^{-2}$ and $v_{enz} = 3.599 \cdot 10^{-2}$ Ms^{-1}, respectively) although the time dependences of the product concentration ap-pear practically linear for all mechanisms (the root-mean-square deviation for the line constructed from the first ten points is 0.992 for the ChC, 0.986 for the ECC, 0.951 for the overall reaction).

However, it must be taken into account that additivity is observed only at the start of the reaction and in a relatively narrow time interval which is determined by the parameters of the reaction. Therefore, in the same time interval additivity can be observed in one reaction significantly better than in the other and the longer the reaction proceeds the greater the deviation from additivity. For example consider another reaction with the parameters: $k_1 = 1 \cdot 10^5$; $k_{-1} = 80$; $k_2 = 40$; $k_{-2} = 2000$; $k_3 = 5 \cdot 10^{-4}$; $k_{-3} = 2 \cdot 10^{-5}$; $[S]_0 = 1$; $[E]_0 = 1 \cdot 10^{-4}$; $K = 25$; $K_m = 1.2 \cdot 10^{-3}$. Unlike the previous reaction, the value calculated from the difference in the product concentrations of the overall reaction and the ChC at 10 seconds ($[P]_{tot} - [P]_{chem} = 3.980 \cdot 10^{-2}$ M) is only 0.28% lower than the true value ($[P]_{enz} = 3.990 \cdot 10^{-2}$ M), in the case of instantaneous rate – by 0.55% ($w_{tot} - w_{chem} = 3.963 \cdot 10^{-3}$ and $w_{enz} = 3.985 \cdot 10^{-3}$ Ms^{-1}, respectively), and in the case of the initial rate calculated by the first ten points which were measured after 1 second – by 0.22% ($v_{tot} - v_{chem} = 3.983 \cdot 10^{-3}$ and $v_{enz} = 3.991 \cdot 10^{-3}$ Ms^{-1}, respectively).

The portion of the progress curve between the initial linear part and the equilibrium

As was shown above the departure from the initial linear part of the progress curve causes a deviation from the additivity of the product concentrations and the longer the reaction proceeds the greater the deviation from the additivity.

If we analyze how the difference $[P]_{tot} - [P]_{enz}$ changes over time (under conditions of detailed balance) we can see that it first increases and then begins to decrease and becomes equal to zero in equilibrium. At the same time, $[P]_{chem}$ (which, if additivity is observed, must be equal to the difference $[P]_{tot} - [P]_{enz}$) increases with time and reaches the value $^{eq}[P]_{chem}$ in the equilibrium state. This can be regarded as the fact that nowhere else, besides the initial linear part of the progress curve, the additivity of the product concentration is observed.

At a time when the ChC proceeding by the mechanism (3) approaches equilibrium, the difference $[P]_{tot} - [P]_{chem}$ becomes negative since $^{eq}[P]_{chem}$ it is always greater than $^{eq}[P]_{tot}$ (under conditions of detailed balance). It is obvious that additivity is not observed since the product concentration of the ECC can not be negative.

If under conditions of detailed balance the product concentration of the overall reaction is always higher than for the ChC and ECC, then if the detailed balance is violated it is possible that in a certain time interval the maximum product concentration will be observed for ECC or ChC but not in the overall reaction.

The initial rate of reaction which does not obey the principle of detailed balance

Let us analyze whether the initial rates are additive under conditions when the reaction that proceeds by the mechanism (1) does not obey the principle of detailed balance.

We derive the instantaneous rate equation for the overall reaction w_{tot} that proceeds by the mechanism (1). To do this, we find the steady-state concentration of the enzyme-substrate complex from the equation

$$k_1([E]_0 - [ES])[S] - k_{-1}[ES] - k_2[ES] +$$
$$+ k_{-2}([E]_0 - [ES])[P] = 0 \qquad (36)$$

and substitute it into instantaneous rate equation

$$w_{tot} = k_2[ES] - k_{-2}([E]_0 - [ES])[P] +$$
$$+ k_3[S] - k_{-3}[P] \qquad (37)$$

As a result, we obtain the following equation:

$$w_{tot} = [E]_0 \left(\frac{k_1 k_2 [S]_{tot} - k_{-1} k_{-2} [P]_{tot}}{k_1 \left([S]_{tot} + K_m \right) + k_{-2}[P]_{tot}} \right) +$$

$$+ k_3[S]_{tot} - k_{-3}[P]_{tot} \qquad (38)$$

Equation (38) is satisfied not only for reactions reaching a detailed equilibrium, but also for reactions reaching cyclic equilibrium, i.e. is valid in the general case.

The instantaneous rate of the ECC w_{enz} is expressed by the equation:

$$w_{enz} = [E]_0 \left(\frac{k_1 k_2 [S]_{enz} - k_{-1} k_{-2} [P]_{enz}}{k_1 \left([S]_{enz} + K_m \right) + k_{-2}[P]_{enz}} \right) \quad (39)$$

and for the ChC the equation for the instantaneous rate w_{chem} has the form:

$$w_{chem} = k_3[S]_{chem} - k_{-3}[P]_{chem} \qquad (40)$$

If in this equation the current substrate concentration is expressed through the initial one, one can see that for the ChC the dependence of the instantaneous rate on the current product concentration is linear throughout the reaction:

$$w_{chem} = k_3[S]_0 - (k_{-3} + k_3)[P]_{chem} \qquad (41)$$

For the reactions proceeding by the mechanisms (1) and (2), this property is not typical.

As can be seen at the start of the reaction (when $[P] = 0$), equation (38) becomes the initial rate equation (34) obtained earlier for the overall reaction which obeys the principle of detailed balance. Therefore it can be concluded that both under conditions of detailed balance and in case of its violation, initial rates and accordingly the product concentrations are additive at the start of the reaction. This conclusion remains valid also when the reactions of chemical and/or enzyme-catalyzed conversion of the substrate, and accordingly the overall reaction, are irreversible (i.e. when $k_{-2} = 0$ and/or $k_{-3} = 0$).

As shown by the calculations performed if the principle of detailed balance is obeyed (when the equilibrium constants of the ChC and ECC are the same) the conversion of the substrate along the chemical pathway is much faster than along the enzyme-catalyzed pathway if the values of the rate constants and the concentrations of the initial substances are of the same order. If the enzyme-catalyzed reaction is carried out under conditions when the initial substrate concentration exceeds the enzyme concentration by 3-4 orders of magnitude, then in order that the initial rates of these reactions be close in magnitude, the rate constants of the chemical reaction should be 4-5 orders of magnitude lower than the rate constants of the enzyme-catalyzed reaction. The same conclusion can be reached by analyzing equation (37). Since the substrate and product concentrations are much larger than the enzyme and the enzyme-substrate complex concentrations, in the case of equal-order rate constants the first two terms which represent the rate of ECC are negligibly small compared to the last two representing the rate of ChC ($k_3[S] > k_2[ES]$, $k_{-3}[P] > k_{-2}[E][P]$).

If in the experiment it is established that the reaction with participation of the enzyme has practically ended and the product concentration remains unchanged, and during the same period of time the chemical transformation of substrate led to the formation of a product in quantity much lower than in the enzyme-catalyzed reaction, then under conditions of detailed balance this is possible only in one case: when the ChC proceeds very far from equilibrium, practically at the initial linear part of the progress curve. If during this time the chemical reaction reached equilibrium, its equilibrium product

concentration would exceed the equilibrium product concentration of the reaction with the participation of the enzyme.

The presence of the reaction product in the reagents

Let us also consider the case when a product is present in the initial reagents (in the concentration $[P]_0$). When deriving the equation for the initial rate under such conditions, it is no longer possible to neglect the reverse reactions of the second and third steps as was done in deriving equations (30), (31), and (34). In this case, we can use equations (38)–(40) in which the current concentration $[S]$ is expressed as follows: $[S]_0 + [P]_0 - [P]$ for the ChC, and $[S]_0 + [P]_0 - [ES] - [P]$ for both the overall reaction and the ECC.

Since the form of the equations remains unchanged, it can be concluded that additivity is also observed in the case where the product is present in the initial reagents at a concentration of $[P]_0$. In this case, the initial rate equation of ECC, under conditions when $[E]_0 \ll [S]_0$ and the product concentration varies little, has the following form:

$$v_{enz} = \frac{k_2[E]_0 \left([S]_0 - \dfrac{[P]_0}{K} \right)}{K_m + [S]_0 + \dfrac{k_{-2}}{k_1}[P]_0} \tag{42}$$

If it is necessary to find the initial rate of ECC under conditions when $[P]_0 = 0$, it is better to proceed as follows: write equation (42) in the form

$$v_{enz}[S]_0 = -(K_m)\, v_{enz} - \left(\frac{k_{-2}}{k_1} \right) v_{enz}[P]_0 +$$
$$+ (k_2)[E]_0[S]_0 - \left(\frac{k_2}{K} \right)[E]_0[P]_0$$

and find all the coefficients written in parentheses; of these coefficients all the rate constants can be calculated and then the initial rate of the ECC can be calculated from equation (31) without taking into account $[P]_0$. Note that in order to determine all four coefficients it is necessary that the product concentration $[P]_0$ and the substrate concentration $[S]_0$ vary independently of each other.

Conclusions

As a result of the conducted studies, it was shown that the additivity of product concentrations and initial rates is observed (both in conditions of detailed balance and when it is violated) only during the reaction period, when a linear increase of the product concentration over time is observed. Under these conditions, additivity is also satisfied in the presence of the reaction product in the reagents. However, the longer the reaction proceeds the greater the deviation from the additivity of both product concentrations and initial rates.

Under equilibrium conditions (both detailed and cyclic) there is no additivity of the equilibrium product concentrations, but under conditions of detailed balance the equilibrium product concentration of the overall reaction is also simultaneously the equilibrium product concentration of the enzyme-catalyzed conversion of the substrate.

Thus, for enzyme-catalyzed reactions in which a sufficiently large amount of the reaction product is formed in the control (i.e. when the chemical transformation of the substrate occurs at a relatively high rate), it is possible to accurately determine the concentration of the product of the enzyme-catalyzed conversion of the substrate at only two points: in the initial period of the reaction, when the product concentration increases linearly with time (as the difference in the concentration of the product formed in the presence of the enzyme and in the control), and also in an equilibrium state where the equilibrium product concentration of the overall reaction is simultaneously the equilibrium product concentration of the enzyme-catalyzed conversion of the substrate (only in conditions when the principle of detailed balance holds). Between these points (i.e. actually throughout the entire reaction pathway) the error in determining the product concentration of the enzyme-catalyzed conversion of the substrate, as the difference in concentrations of the product that is formed in the presence of the enzyme and in the control, can be quite large.

ВРАХУВАННЯ ВНЕСКУ ХІМІЧНОГО (НЕЕНЗИМАТИЧНОГО) ПЕРЕТВОРЕННЯ СУБСТРАТУ В ЗАГАЛЬНИЙ МЕХАНІЗМ ЕНЗИМАТИЧНОЇ РЕАКЦІЇ

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Під час проведення досліджень ензиматичних реакцій необхідно враховувати внесок хімічного перетворення субстрату на продукт, яке відбувається разом з ензиматичним перетворенням. Зазвичай вважають, що різниця концентрацій продукту, що утворився в присутності ензиму та за його відсутності (впродовж одного й того ж інтервалу часу), є концентрацією продукту, що утворився безпосередньо в ензиматичній реакції, тобто, що існує адитивність концентрацій продукту реакції в кожний момент часу. У цій статті проаналізовано коли існує адитивність і як правильно враховувати внесок некаталітичного перетворення субстрату під час проведення досліджень ензиматичних реакцій. Показано, що адитивність концентрацій продукту і початкових швидкостей існує лише в період, коли спостерігається лінійне зростання концентрації продукту в часі. Чим довше перебігає реакція, тим більше порушується адитивність. В умовах рівноваги адитивність рівноважних концентрацій продукту відсутня, проте в умовах детального балансу рівноважна концентрація продукту узагальненої реакції, що включає ензиматичне і хімічне перетворення субстрату, одночасно є також і рівноважною концентрацією продукту ензиматичного перетворення.

К л ю ч о в і   с л о в а: кінетика ензиматичних реакцій, початкова швидкість, рівноважний стан реакції, циклічні реакції, детальний баланс, циклічна рівновага, каталіз, константа швидкості, константа рівноваги, константа дисоціації.

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