# REVERSIBLE REGULATORY ENZYMATIC REACTIONS

## A THEORETICAL ANALYSIS

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UDC 577.150.2

A mathematical model describing the dependence of the rate of the reversible enzymatic reaction $S_1 \overset{E(R,T)}{\rightleftharpoons} S_2$ on the concentrations of the substrates $S_1$ and $S_2$ and their analog A, which is capable of binding reversibly to the active sites of the oligomeric enzyme E(R, T), has been derived and analyzed. The ranges of values of the parameters at which the model obtained describes different types of kinetic curves, viz., sigmoidal curves, curves with one maximum, and curves with two extrema, have been evaluated. The limits of the values of the parameters at which isosteric activation of the enzyme by the product $S_2$ or its analog A is possible in the reactions $S_1 \overset{E(R,T)}{\rightarrow} S_2$ and $S_1 \overset{E(R,T)}{\rightleftharpoons} S_2$ have been determined. An explanation for two phenomena that have been observed in experiments with oligomeric enzymes, viz., unidirectional effect of isosteric modifiers on the rates of the forward and reverse conversions and the occurrence of a reversible reaction in the thermodynamically unfavorable direction, has been given in terms of the model. It has been shown that the results obtained are also valid for multisubstrate reactions catalyzed by the enzyme E(R, T).

The theoretical analysis of the kinetics of the action of oligomeric regulatory enzymes has heretofore generally been restricted to an examination of the simplest models, viz., one-substrate irreversible reactions. In particular, these models include those of Monod, Wyman, and Changeux [1], Koshland [2], and Frieden and Kurganov [3, 4], which have been widely used for the theoretical interpretation of data from enzymological experiments with oligomeric enzymes.

However, the overwhelming majority of regulated enzymatic reactions have more complex mechanisms, i.e., they usually have several substrates and products and are frequently reversible.

In order to account for a number of features of the kinetics of key biochemical conversions, as well as to analyze the mechanisms of the regulation of cellular metabolism, we need mathematical models which take into account the multisubstrate nature and reversibility of real enzymatic reactions. We have previously applied the Monod–Wyman–Changeux model (the MWC model) [1] to a reversible reaction of the type $S_1 \rightleftharpoons S_2$ [5], and this model and the Frieden–Kurganov model [3, 4] have been generalized to the case of an enzymatic reaction with an arbitrary number of reactants and an arbitrary mechanism of interaction between the reactants and the active sites of the oligomers [6]. However, the kinetic features of the reactions described by such models have heretofore scarcely been investigated.

In the present work we investigated the Monod–Wyman–Changeux model [1] in the case of the reversible enzymatic reaction $S_1 \overset{E(R,T),A}{\rightleftharpoons} S_2$ under isosteric and allosteric regulation. All the results of the analysis are valid for multisubstrate reactions of the type $\sum_i S_i \overset{E(R,T)}{\rightleftharpoons} \sum_j S_j$ under the condition that the interaction between the substrates and the active sites of the enzymes is fast. An analysis of the model makes it possible to account for the features of the kinetic behavior of reversible reactions that are important for the regulation of metabolism, such as the unidirectional effect of modifiers on the rates of the forward and reverse reactions and the occurrence of the reaction in the thermodynamically unfavorable direction.

Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino, Moscow Region. Translated from Molekulyarnaya Biologiya, Vol. 12, No. 5, pp. 1139-1151, September-October, 1978. Original article submitted March 14, 1978.



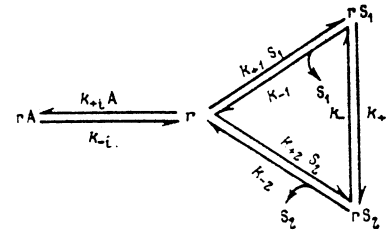
0026-8933/78/1205-0878$07.50 

## DESCRIPTION OF THE MODEL

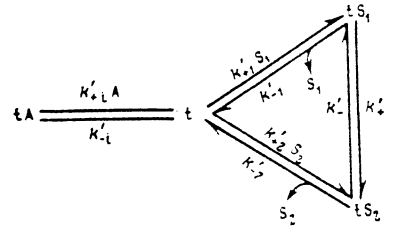
Let us first examine the one-substrate reversible reaction

$$S_1 \overset{E(R,T),A}{\rightleftharpoons} S_2, \tag{1}$$

which takes place in the presence of the isosteric effector A and is catalyzed by the oligomeric enzyme E(R, T), the latter satisfying the requirements of the model in [1]. Let the catalysis of the conversions in (1) by the single active sites r and t in the conformations R and T be described by the following graphs:




(2)




(3)

Then, according to our data from [6], the rate of reaction (1) under the assumptions made regarding the enzyme is described by the following expression:

$$v = \frac{V_+ S_1/K_1 - V_- S_2/K_2}{\Delta} \times \frac{1 + \dfrac{V'_+ K_1}{V_+ K'_1} L \left(\dfrac{\Delta'}{\Delta}\right)^{n-1}}{1 + L\left(\dfrac{\Delta'}{\Delta}\right)^{n}}, \tag{4}$$

where

$$\Delta = 1 + \frac{S_1}{K_1} + \frac{S_2}{K_2}\frac{A}{K_A}, \quad \Delta' = 1 + \frac{S_1}{K'_1} + \frac{S_2}{K'_2} + \frac{A}{K'_A} \tag{5}$$

are the normalized determinants [7] of graphs (2) and (3); $V_+$, $V_-$, and $V_+'$ are the maximum rates of reactions (2) and (3), $K_1$, $K_2$, $K_1'$, and $K_2'$ are the Michaelis constants for $S_1$ and $S_2$, $K_A$ and $K_A'$ are the inhibition constants in reactions (2) and (3), and L is the allosteric constant [1].

We introduce the dimensionless concentrations

$$\sigma_1 = \frac{S_1}{K_1}, \quad \sigma_2 = \frac{S_2}{K_2}, \quad \alpha = \frac{A}{K_A} \tag{6}$$

and the parameters

$$c_1 = \frac{K_1}{K'_1}, \quad c_2 = \frac{K_2}{K'_2}, \quad c_\alpha = \frac{K_A}{K'_A},$$
$$a = c_1 \frac{V'_+}{V_+}, \quad \varkappa = \frac{V_-}{V_+} \tag{7}$$

We shall subsequently assume that the maximum rate of the catalysis of the forward direction of the reaction by the enzyme in the T conformation does not exceed the maximum rate of the catalysis by the R conformation, i.e., $V_+' \leq V_+$. This places the following restriction on the parameter $a$:

$$a = c_1 \frac{V'_+}{V_+} \leqslant c_1. \tag{8}$$

With the consideration of expressions (6) and (7), Eq. (4) becomes:

$$v = \frac{v}{V_+} = \frac{\sigma_1 - \varkappa\sigma_2}{1+\sigma_1+\sigma_2+\alpha} \times \frac{1+aLq^{n-1}}{1+Lq^n}, \tag{9}$$

$$q = (1 + c_1\sigma_1 + c_2\sigma_2 + c_\alpha\alpha)/(1+\sigma_1+\sigma_2+\alpha).$$

We shall now consider the reversible conversion of the s substrates $S_i$ into the p products $S_j$ under catalysis by the enzyme E(R, T):

$$\sum_{i=1}^{s} S_i \xrightarrow{E(R,T),A} \sum_{j=s+1}^{N} S_j, \tag{10}$$

where N = s + p is the number of reactants.

If the binding of the substrates and the products $S_g$ to the active sites of the enzyme is far more rapid than the catalytic conversion, according to our data [6], the rate of reaction (10) is equal to

$$v = \frac{V_+ \prod_{i=1}^{s} S_i/K_i - V_- \prod_{j=s+1}^{N} S_j/K_j}{1+\Phi(S_1, \ldots, S_N) + A/K_A} \times \frac{1+\frac{V'_+}{V_+}\prod_{i=1}^{s}\frac{K_i}{K'_i}Lq^{n-1}}{1+Lq^n}, \tag{11}$$

where

$$q = \frac{[r]}{[t]} = \left(\frac{e_0K_A\prod_{i=1}^{s}K_i}{\Delta}\right)\Big/\left(\frac{e_0K'_A\prod_{i=1}^{s}K'_i}{\Delta'}\right) = \frac{1+\Phi'(S_1, \ldots, S_N)+A/K'_A}{1+\Phi(S_1, \ldots, S_N)+A/K_A} = \frac{1+\theta'+q'S_1+\psi'S_N+A/K'_A}{1+\theta+qS_1+\psi S_N+A/K_A}. \tag{12}$$

Here $\theta$, $\theta'$, $\varphi$, $\varphi'$, $\psi$, and $\psi'$ are functions of the variables $S_2, \ldots, S_{N-1}$. We fix the concentration of all the substrates except $S_1$ and of all the products except $S_N$ and introduce the following parameters:

$$\bar{V}_+ = V_+\prod_{i=2}^{s} S_i/K_i, \quad \bar{\varkappa} = \frac{V_-}{V_+}\prod_{j=s+1}^{N-1} S_j/K_j, \quad \bar{a} = \frac{V'_+(1+\theta)}{V_+(1+\theta')}\prod_{i=1}^{s}\frac{K_i}{K'_i},$$

$$\bar{L} = L\left(\frac{1+\theta'}{1+\theta}\right)^n, \quad \bar{c}_1 = \frac{\varphi'(1+\theta)}{\varphi(1+\theta')}, \quad \bar{c}_N = \frac{\psi'(1+\theta)}{\psi(1+\theta')},$$

$$\bar{c}_\alpha = \frac{K_A(1+\theta)}{K'_A(1+\theta')} \tag{13}$$

and the dimensionless variables

$$\sigma_1 = S_1\frac{\varphi}{1+\theta}, \quad \sigma_N = S_N\frac{\psi}{1+\theta}, \quad \alpha = \frac{A}{K_A(1+\theta)}. \tag{14}$$

With the consideration of Eqs. (13) and (14) expression (11) becomes

$$v = \bar{V}_+\frac{\sigma_1 - \bar{\varkappa}\sigma_N}{1+\sigma_1+\sigma_N+\alpha} \times \frac{1+\bar{a}\bar{L}q^{n-1}}{1+\bar{L}q^n},$$

$$q = (1 + \bar{c}_1\sigma_1 + \bar{c}_N\sigma_N + \bar{c}_\alpha\alpha)/(1+\sigma_1+\sigma_N+\alpha). \tag{15}$$

The form of the dependence $v(\sigma_1, \sigma_N, \alpha)$ (15) is similar to expression (9) for the rate of reaction (1); however, unlike the parameters $a$, $\varkappa$, c, L, V, and K, which are constants in Eq. (9), the parameters $\bar{a}$, $\bar{\varkappa}$, $\bar{c}$, $\bar{L}$, $\bar{V}$, and $\bar{K}$ in Eq. (15) are functions of the "frozen" concentrations of substrates $S_2, \ldots, S_{N-1}$. We note that while in Eq. (9) the parameter $a$ takes on values in the range $0 \le a \le c_1$, in Eq. (15) the range is $0 \le a \le +\infty$.

Thus, the analysis of the kinetic properties of the multisubstrate reaction under the assumptions made (rapid addition of the reactants to the active sites and constancy of all except two selected concentrations) can be reduced to an analysis of the properties of the model of reaction (1).

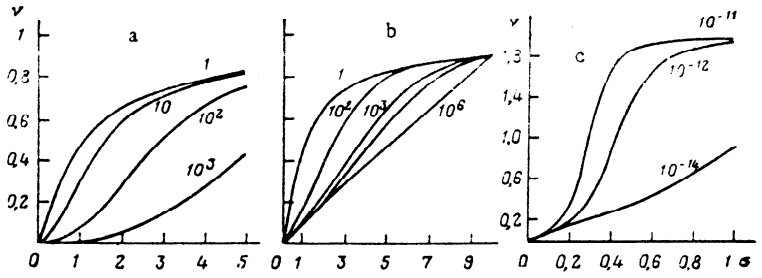



Fig. 1. Substrate activation of the rate of reaction (1). Here $\nu$ is the dimensionless reaction rate described by Eq. (16), and $\sigma$ is the dimensionless substrate concentration. The curves were plotted for various values of L (numbers on the curves) and n = 4 (a, b), 10 (c); $a$ = 0 (a), 0.1 (b), and 200 (c); and c = 0.01 (a, b) and 100 (c).

## ANALYSIS OF THE MODEL

Let us first examine Eq. (9) in the special case $\sigma_2 = \alpha = 0$, in which it describes the MWC model [1]:

$$\nu = \frac{v}{V_+} = \frac{\sigma_1}{1+\sigma_1} \times \frac{1+aL\left(\frac{1+c_1\sigma_1}{1+\sigma_1}\right)^{n-1}}{1+L\left(\frac{1+c_1\sigma_1}{1+\sigma_1}\right)^{n}}. \tag{16}$$

The analysis of Eq. (16) in [8-12] showed that, depending on the values of the parameters, this model can describe substrate activation [8, 9], substrate inhibition of the reaction rate [10], kinetic curves with an intermediate plateau [11, 12], and simple saturation of the enzyme with the substrate.

The range of values of the parameters in which the plots of $\nu(\sigma_1)$ have a sigmoidal initial segment can be evaluated from the inequality

$$\left.\frac{\partial^2\nu}{\partial\sigma_1^2}\right|_{\sigma_1\to 0} > 0. \tag{17}$$

This inequality leads to the following three systems of necessary conditions for the existence of substrate activation: 1) if $a = 0$, then

$$n \geqslant 2,\ c_1 < \frac{n-1}{n},\ L > \frac{1}{n-1-nc_1}; \tag{18}$$

2) if $0 < a < A$, then

$$n \geqslant 2,\ c_1 < \frac{n-1}{n},\ L_1 < L < L_2;$$

3) if $a > A$, then

$$n \geqslant 2,\ c_1 > \frac{n}{n-1},\ L_1 < L < L_2.$$

Here we have used the notation

$$A = \left(\frac{(1-c_1)\sqrt{n(n-1)} - \sqrt{c_1}}{n-(n-1)c_1}\right)^2,\ L_1 = \frac{B - \sqrt{B^2-4ac_1}}{2ac_1},$$

$$L_2 = \frac{B + \sqrt{B^2-4ac_1}}{2ac_1},\ B = (n-1)(1+ac_1) - n(a+c_1). \tag{19}$$

The necessary condition for the existence of substrate inhibition is defined by the inequality

$$\left.\frac{\partial\nu}{\partial\sigma_1}\right|_{\sigma_1\to\infty} < 0, \tag{20}$$

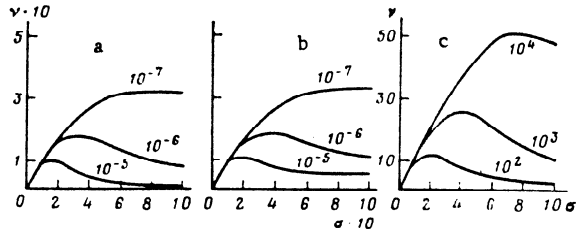



Fig. 2. Substrate inhibition of the reaction rate described by Eq. (16) for n = 4 and $a$ = 0, c = 100 (a), $a$ = 4, c = 100 (b), and $a$ = 1, c = 0.01 (c). The numbers on the curves are the values of L.

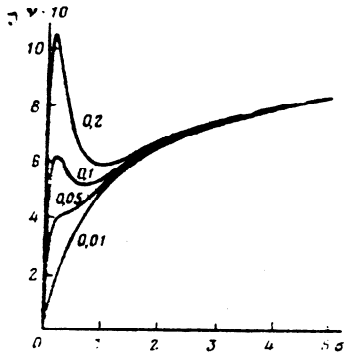



Fig. 3. Family of plots of $\nu(\sigma, L)$ with an intermediate plateau and two extrema constructed from Eq. (16) for n = 8, $a$ = 100, and c = 0.01. The numbers on the curves are the values of L.

which leads to the following three systems of conditions:

1) if $a_0 = 0$, then

$$n \geqslant 2,\ c_1 > \frac{n}{n-1},\ L > \frac{1}{c_1^{n-1}[c_1(n-1)-n]}, \tag{21}$$

2) if $0 < a < A$, then

$$n \geqslant 2,\ c_1 > \frac{1}{n-1},\ L_1 < L < L_2;$$

3) if $a > A$, then

$$n \geqslant 2,\ c_1 < \frac{n-1}{n},\ L_1 < L < L_2.$$

Here we have

$$A = \left(\frac{(c_1 - 1)\sqrt{c_1 n(n-1)} - c_1}{nc_1 - (n-1)}\right)^2,\ L_1 = \frac{B - \sqrt{B^2 - 4ac_1^2}}{2ac_1^n},\quad L_2 = \frac{B + \sqrt{B^2 - 4ac_1^2}}{2ac_1^n},\ B = (n-1)(a + c_1^2) - nc_1(1+a). \tag{22}$$

Figure 1 presents families of plots of $\nu(\sigma_1)$, which were constructed from Eq. (16) for different values of the allosteric function and the values of the other parameters taken from inequalities (18). The curves in Fig. 2 were plotted for values of the parameters taken from inequalities (21).

When the conditions

$$\frac{\partial v}{\partial \sigma_1} = 0,\ \frac{\partial v}{\partial \sigma_1}\Big|_{\sigma_1 \to \infty} > 0 \tag{23}$$

are fulfilled, a plot of $\nu(\sigma_1)$ described by Eq. (16) can have an intermediate plateau or two extrema. Examples of such curves are presented in Fig. 3.

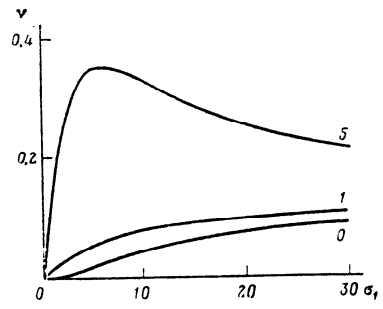



Fig. 4

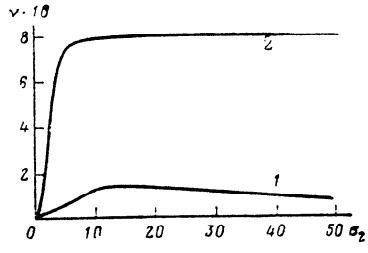



Fig. 5

Fig. 4. Variation of the nature of the kinetic plot of $\nu(\sigma_1)$ with variation of the concentration of the reaction product. The $\nu(\sigma_1, \sigma_2)$ curves were plotted with the aid of Eq. (24) for n = 8, $\alpha = 0$, $a = 0$, $L = 10^4$, $c_1 = 0.4$, and $c_2 = 0$. The numbers on the curves are the values of $\sigma_2$.

Fig. 5. Isosteric (1) and allosteric (2) activation of the reaction rate by the product. Curve 1 was plotted with the aid of Eq. (24) with n = 4, $a = 0$, L = 100, $c_1 = 1$, $c_2 = 10^{-4}$, $\alpha = 0$, and $\sigma_1 = 4$ and curve 2 was plotted with the aid of Eq. (16) with the allosteric function $L = L_0[(1 + c_a\sigma_2)(1 + \sigma_2)]^m$ for m = n = 4, $a = 0$, $L_0 = 100$, $c_1 = 1$, $c_a = 10^{-4}$, and $\sigma_1 = 4$.

Let us now consider Eq. (9) in another special case, viz., when the isomerization of the substrate–site complexes is irreversible. In this case, we have $\varkappa = 0$, and Eq. (9) becomes

$$\nu = \frac{\sigma_1}{1 + \sigma_1 + \sigma_2 + \alpha} \times \frac{1 + aLq^{n-1}}{1 + Lq^n}, \quad q = \frac{1 - c_1\sigma_1 + c_2\sigma_2 + c_\alpha\alpha}{1 + \sigma_1 + \sigma_2 + \alpha}. \tag{24}$$

With the aid of the replacement of the variable and of the parameters

$$\bar{\sigma}_1 = \frac{\sigma_1}{1 + \sigma_2 + \alpha}, \quad \bar{a} = a\frac{1 + \sigma_2 + \alpha}{1 + c_2\sigma_2 + c_\alpha\alpha}, \quad \bar{c}_1 = c_1\frac{1 + \sigma_2 + \alpha}{1 + c_2\sigma_2 + c_\alpha\alpha}, \quad \bar{L} = L\left(\frac{1 + c_2\sigma_2 + c_\alpha\alpha}{1 + \sigma_2 + \alpha}\right)^n \tag{25}$$

this variant of Eq. (9) reduces to Eq. (16):

$$\nu = \frac{\bar{\sigma}_1}{1 + \bar{\sigma}_1} \times \frac{1 + \bar{a}\bar{L}q^{n-1}}{1 + \bar{L}q^n}, \quad q = \frac{1 - \bar{c}_1\bar{\sigma}_1}{1 - \bar{\sigma}_1}. \tag{26}$$

Therefore, Eq. (24) can describe the same types of plots of $\nu(\bar{\sigma}_1)$ as Eq. (16). Therefore, relationships of types (18) and (21) can now be described for the parameters $\bar{a}$, $\bar{c}_1$, and $\bar{L}$ in Eq. (26) in order to determine the conditions for the existence, respectively, of substrate activation and of substrate inhibition in the model described by Eq. (24). However, our attention should be turned to the dependence of the parameters $\bar{a}$, $\bar{c}_1$, and $\bar{L}$ on the "frozen" concentrations $\sigma_2$ and $\alpha$. Because of this dependence of the shape of the kinetic plots of $\nu(\sigma_1)$, Eqs. (24) can be altered by varying the values of $\sigma_2$ and $\alpha$. For example, in the case of $\alpha = 0$ and

$$n \geqslant 2, \; a = 0, \; c_2 < c_1 < \frac{n-1}{n}, \; L > \frac{1}{n - 1 - nc_1} \tag{27}$$

the plot of $\nu(\sigma_1)$ is sigmoidal when $\sigma_2 = 0$, has a maximum when

$$\sigma_2 > \sigma_2^* = \frac{n - c_1(n-1)}{c_1(n-1) - c_2 n}, \tag{28}$$

and does not have extrema or segments with positive curvature when $0 < \sigma_2 < \sigma_2^*$ (Fig. 4). Similar changes in the shape of the curve can be caused by changing the concentration of the isosteric substrate analog or by varying the concentration of a fixed substrate in a multisubstrate reaction.

From Fig. 4 it is seen that when the product concentration $\sigma_2$ is increased, the reaction rate defined by Eq. (24) increases sharply. Such product activation [13], which is purely isosteric, is a special case of the well known isosteric activation of oligomeric enzymes by substrate analogs [14].

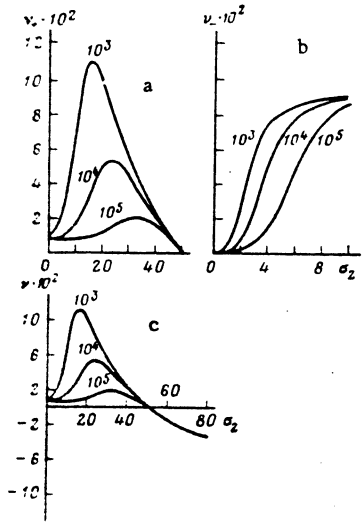



Fig. 6. Different sensitivities of the rate of the forward reaction $\nu_+$ (a), the reverse reaction $\nu_-$, and the rate $\nu = \nu_+ - \nu_-$ of the overall reaction (c) to the changes in the values of L (the numbers on the curves) with various concentrations of the activator product $\sigma_2$. The rates $\nu_+$, $\nu_-$, and $\nu$ were plotted with the aid of Eqs. (31)-(33) with n = 6, $\varkappa = 0.1$, $a = 0.01$, $c_1 = 1$, $c_2 = 10^{-4}$, and $\sigma = 5$ (a), 0 (b), and 5 (c).

The conditions for isosteric activation of the reaction rate by small concentrations of the product can be determined from the inequality

$$\left.\frac{\partial v}{\partial \sigma_2}\right|_{\sigma_2 \to 0} > 0. \tag{29}$$

In the case of $a = 0$ and $\sigma_1 \ll 1$, this inequality leads to the condition

$$n \geqslant 2,\ c_2 < \frac{n-1}{n},\ L > \frac{1}{n-1-c_2 n}. \tag{30}$$

When conditions (30) are fulfilled, the product has a greater affinity for the active R conformation than for the T conformation. Since the conformational changes of the protomers have a concerted character, the addition of $S_2$ to some of the active sites of the oligomer hold the remaining unoccupied active sites in the R conformation. This results in the progressive displacement of the $R \rightleftharpoons T$ equilibrium to the left and the simultaneous filling of the active sites not occupied by the substrate. The former process causes an increase in the reaction rate, and the latter process causes competitive inhibition of the activity of the enzyme. Just these two processes account for the biphasic nature of the dependence of the reaction rate $\nu$ on the concentration of the product. Figure 5 presents two plots of $\nu(\sigma_2)$, which were constructed with the aid of Eq. (24) with $\alpha < 0$ for the cases of isosteric and allosteric activation by the product. As we see from the figure, the reaction rate in the case of isosteric activation, even at the optimum concentration of the product, is only a small fraction of the maximum rate. Conversely, in the case of allosteric activation, the enzyme can be activated almost completely. Moreover, isosteric activation is described by a curve with a maximum, and purely allosteric activation is described by a curve with saturation. Precisely the same differences exist between the iso- and allosteric activation of the enzyme by the modifier A.

Finally, let us consider the original model (9), which for the further discussion is conveniently represented in the form of the difference between the rates of the forward and reverse reactions:

$$\nu = \nu_+ - \nu_- \tag{31}$$

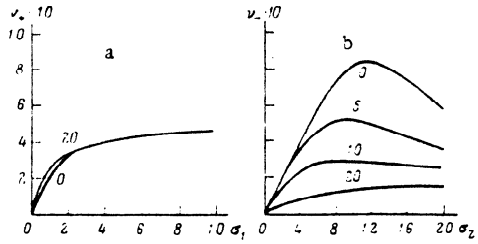



Fig. 7. Unidirectional effect of an isosteric modifier ($\alpha$) on the rates of the forward (a) and reverse (b) reactions. The families $\nu_+(\sigma_1, \alpha)$ and $\nu_-(\sigma_2, \alpha)$ were plotted with the aid of Eqs. (32)-(33) with n = 4, $\varkappa = 0.2$, $c_\alpha = 0$, $c_1 = 1$, $c_2 = 10^{-4}$, $a = 0.5$, $L = 5 \cdot 10^4$, and $\sigma_2 = 0$ (a) and $\sigma_1 = 0$ (b). The numbers on the curves are the values of $\alpha$.

where

$$\nu_+ = \frac{\sigma_1}{1+\sigma_1+\sigma_2+\alpha} \times \frac{1+aLq^{n-1}}{1+Lq^n}, \tag{32}$$

$$\nu_- = \frac{\varkappa\sigma_2}{1+\sigma_1+\sigma_2+\alpha} \times \frac{1+aLq^{n-1}}{1+Lq^n}, \quad q = \frac{1+c_1\sigma_1+c_2\sigma_2+c_\alpha\alpha}{1+\sigma_1+\sigma_2+\alpha}. \tag{33}$$

The rate $\nu$ can be either positive (when $\sigma_1 > \varkappa\sigma_2$) or negative (when $\sigma_1 < \varkappa\sigma_2$).

The equation for the rate of the forward reaction (32) coincides with Eq. (24), which we have already considered. Hence it follows that the forward reaction may be isosterically activated by small concentrations of the product and is always competitively inhibited by large concentrations of the product. It is clear that the existence of the product–activation effect in the present case depends on the value of the parameter $\varkappa$. From inequality (29), which is applicable to model (9), we can obtain the following conditions for the existence of isosteric product activation with small values of $\sigma_1$ and $\sigma_2$, $\alpha = 0$, and $a = 0$:

$$n \geqslant 2 + \varkappa, \quad c_2 < \frac{n-1-\varkappa}{n+\varkappa}, \tag{34}$$

$$L > \frac{1+3\varkappa}{n-1-c_2 n-\varkappa(2+c_2)}, \quad \sigma_1 > \frac{\varkappa}{n-1-c_2 n-\varkappa(1+c_2)}.$$

If we set $\varkappa = 0$ in Eq. (34), the system will define the conditions for the substrate activation of reaction (1) when it proceeds in the reverse direction, i.e., the condition for a sigmoidal dependence of the $\nu(\sigma_2)$ curve when $\sigma_1 = \alpha = 0$. Hence we have the following simple rule for reversible reactions catalyzed by oligomeric enzymes: A substrate which activates its own consumption can activate its formation when the reaction is reversed.

Figure 6 presents families of plots of $\nu_+(\sigma_2, L)$, $\nu_-(\sigma_2, L)$, and $\nu(\sigma_2, L)$, which were constructed with the aid of Eqs. (32), (39), and (31). As we see from the figure, the product ($\sigma_2$) in small concentrations activates both its own formation (a) and its consumption (b). Large concentrations of the product inhibit the forward reaction, and when $\sigma_2 > \sigma_1/\varkappa$, the rate changes sign (Fig. 6c).

Figure 6 clearly displays the different sensitivities of the rates of the forward and reverse conversions (1) to changes in the concentration of the allosteric modifiers described by the function L. For example, the forward reaction is practically insensitive to changes in L over a broad range (Fig. 6a), if the concentration of the product $\sigma_2$ is small. However, when the concentration of the product is increased ($\sigma_2 \sim 10$), i.e., in the phase of product activation, the forward reaction becomes very sensitive to changes in L. At the same time, the reverse reaction is sensitive to changes in L at small values of $\sigma_2$ and weakly dependent on L at large values of $\sigma_2$ (Fig. 6b). Thus, with fixed ratios between the parameters and the concentrations $\sigma_1$ and $\sigma_2$ changes in the concentrations of the allosteric modifiers can have an asymmetric, practically unidirectional effect on the rates of the forward and reverse reactions.

Isosteric modifiers can also have a unidirectional effect on reaction (1). Figure 7 shows plots of the dependence of the rates of the forward and reverse reactions in (1) on the concentrations of the substrates for various values of the concentration of an isosteric modifier ($\alpha$). As we see from the figure, the rate $\nu_+$ of the

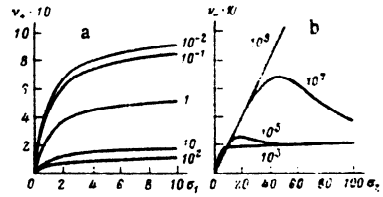



Fig. 8

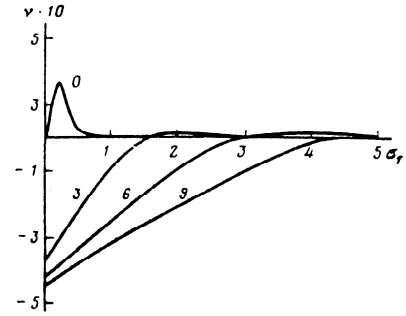


Fig. 9

Fig. 8. Asymmetric effect of an allosteric modifier on the rates of the forward (a) and reverse (b) reactions. The families of plots of $\nu_+(\sigma_1, L)$ and $\nu_-(\sigma_2, L)$ were constructed with the aid of Eqs. (32)-(33) for $\alpha = 0$, $n = 4$, $\varkappa = 0.2$, $c = 1$, $c_2 = 10^{-4}$, $a = 0.1$, and $\sigma_2 = 0$ (a) and $\sigma_1 = 0$ (b). The numbers on the curves are the values of L.

Fig. 9. Occurrence of reversible reaction (1) in the thermodynamically unfavorable direction. The family of plots of $\nu(\sigma_1, \sigma_2)$ was constructed with the aid of Eq. (31) for $\alpha = 0$, $n = 10$, $\varkappa = 0.5$, $a = 0$, $c_1 = 20$, $c_2 = 0$, and $L = 1.5 \cdot 10^{-8}$. The numbers on the curves are the values of $\sigma_2$.

forward reaction is practically insensitive to changes in the concentration of the modifier $\alpha$, while the rate $\nu_-$ of the reverse reaction is strongly inhibited by $\alpha$. This feature of the behavior of reaction (1) is due to the fact that the R conformation is more active in the catalysis of the forward reaction, and the T conformation is more active in the catalysis of the reverse reaction. In fact, the valid relationships are

$$V_+/V'_+ = a'c_1, \; V'_-/V_- = a/c_2, \tag{35}$$

which, because of the independence of $c_1$ and $c_2$, can yield the inequalities

$$V_+ > V'_+, \; V'_- > V_-. \tag{36}$$

In the case under consideration (Fig. 7), the modifier A shifts the R $\rightleftharpoons$ T equilibrium in the direction of R; therefore, an increase in the concentration of the modifier has an inhibiting effect on the reverse reaction, and the rate of the forward reaction can be activated by it or be insensitive to it.

The difference between the catalytic efficiencies of the R and T conformations in the forward and reverse directions makes it possible to selectively regulate the rates of the forward and reverse reactions both by isosteric and by allosteric modifiers. Figure 8 presents two families of plots of $\nu_+(\sigma_1)_{\sigma_2=0}$ and $\nu_-(\sigma_2)_{\sigma_1=0}$, which were constructed with the aid of Eqs. (32) and (33) with different values of the allosteric function L. From this figure it is seen that at the small values of L at which the enzyme is mainly in the R conformation, the forward reaction is predominant. However, an increase in the concentration of the allosteric regulators inhibits the forward reaction and activates the reverse reaction. At large values of L the reverse reaction is predominant.

The asymmetric effect of regulatory metabolites on the rates of the forward and reverse reactions can be utilized in cellular metabolism for the suppression of the thermodynamically favorable conversions and the activation of the thermodynamically unfavorable conversions that are useful to the cell. An analysis of Eq. (9) reveals that such selective regulation of a reversible reaction can be realized by different methods, viz., isosteric inhibition of one of the reactions by the modifier A (Fig. 7), allosteric regulation by the modifier A (Fig. 8), and isosteric or allosteric regulation of the R $\rightleftharpoons$ T equilibrium by the substrates and products of the reaction being regulated.

The last method is illustrated in Fig. 9, which presents a family of plots of $\nu(\sigma_1)$, which were calculated with the aid of Eq. (9) for various concentrations of the product. In this model example we have considered a decameric enzyme ($n = 10$), which catalyzes a readily reversible reaction ($\varkappa = 0.5$). The T conformation of this enzyme is inactive ($a = 0$), but it has a significantly greater affinity toward the substrate $S_1$ than does the

R conformation ($c_1 = 20$). At the same time, in the T conformation the enzyme scarcely finds the product $S_2$ ($c_2 = 0$). Because of these special features, the thermodynamically favorable ($\varkappa < 1$) forward reaction is so strongly inhibited by the substrate that when $\sigma_2 = 0$ and $\sigma_1 > 1$, the forward conversion $S_1 \rightarrow S_2$ scarcely proceeds owing to the fact that the enzyme is "locked" in the inactive T conformation. Since $S_2$, the substrate of the reverse reaction, binds only to the active R conformation, as its concentration is increased, the conversion $S_2 \rightarrow S_1$ proceeds freely.

## DISCUSSION

As we see from the analysis made, the model described by Eq. (9) is characterized by a great variety of kinetic properties. The original model of Monod, Wyman, and Changeux [1] accounts for the substrate activation and substrate inhibition of the reaction rate, as well as the appearance of an intermediate plateau and two extrema on the kinetic curves. When the isosteric interactions in the active sites of the enzyme are taken into account, Eq. (24) describes the activating effect of substrate analogs and, in particular, of the reaction product, whose regulatory role becomes understood when open-chain enzyme systems are considered.

The phenomenon of the activation of the rate of an enzymatic reaction by the products, substrate analogs, or other modifiers is encountered fairly frequently in enzyme kinetics. If the modifier A is not a participant in the reaction, we can easily determine the type of activation: In the case of isosteric activation, the dependence of the rate of the reaction on the concentration of the modifier A has the form of a curve with a maximum, and in the case of allosteric activation, it is a curve with saturation. If the modifier A is a participant in the reaction, the dependence v([A]) can have a maximum in the case of both allosteric and isosteric interactions of A with E(R, T). The analytical conditions elucidated in the present study or the existence of the effects of the activation and inhibition of the reaction rate by substrates, products, and their analogs can be utilized to evaluate the initial values and limits of the variation of the parameters of the model for a quantitative description of experimental data by optimization techniques.

The most interesting features of model (9) of reversible reaction (1) are the selective unidirectional effect of iso- and allosteric regulators on the rates of the forward and reverse reactions and the ability of an oligomer to preferentially catalyze reaction (1) in the thermodynamically unfavorable direction. At once we note that Eq. (9) was derived with the observance of the principle of detailed balance; therefore, both the selective influence of modifiers and the catalysis of the thermodynamically unfavorable direction of the reaction are in complete agreement with the laws of chemical thermodynamics. Like any classical catalyst, the oligomer E(R, T) does not alter the position of the equilibrium of reaction (1) as a result of the $R \rightleftharpoons T$ transitions; however, these transitions can sharply alter the rate at which equilibrium is achieved. It is the modulation of the rate of attaining equilibrium which accounts for the unidirectional effect of modifiers and the "antithermodynamic" behavior of the enzyme E(R, T).

The unidirectional effect of iso- and allosteric modifiers on the forward and reverse directions of a reversible biochemical conversion was first described by Le John et al. [16-22] in the case of NAD-dependent glutamate dehydrogenase (E.C.1.4.1.2) from Thiobacillus novellus and Blastocladiella emersonii. This result was subsequently confirmed by Sanner [23, 24], as well as by Hooper et al [25] in the case of NADP-dependent glutamate dehydrogenase (E.C.1.4.1.3) from Tetrahymena. The reversible reaction between $\alpha$-ketoglutarate and glutamate, which is catalyzed by these enzymes, connects two metabolic pathways, viz., a biosynthetic pathway, whose substrate is glutamate, and the tricarboxylic acid cycle, in which $\alpha$-ketoglutarate is a participant. The role of the modifiers in this case is to switch the activity of the glutamate dehydrogenase reaction from the biosynthetic pathway to the catabolic pathway and vice versa, dependent on the state of the metabolism. In fact, citrate, isocitrate, and other intermediates of the Krebs cycle inhibit the rate of the oxidative deamination of glutamate without influencing the rate of the reductive amination [16-22], as illustrated in Fig. 7. As a result of such regulation, the diversion of intermediates of the Krebs cycle to biosynthesis is possible.

The allosteric modifier AMP, which is an indicator of the energy state of the metabolism, activates the deamination of glutamate and inhibits the amination of $\alpha$-ketoglutarate in Thiobacillus [16], i.e., it switches the metabolism from biosynthesis, which consumes ATP, to the production of ATP. A mechanism for such alternative regulation is shown in Fig. 8.

The ability predicted by Eq. (9) of oligomeric enzymes to catalyze readily reversible reactions predominantly in one direction, which is sometimes opposite to the favorable direction from the thermodynamic point of view, is closely related to the unidirectional effect of modifiers. Perhaps the most striking evidence

in support of this possibility is the existence in the cell of various enzymes which selectively catalyze the reverse reactions of reversible conversions. For example, the biosynthesis of Pseudomonas fluorescens ornithine carbamoyltransferase (E.C. 2.1.3.3), which has a molecular weight of 100,000 and four protomers [26], catalyzes the thermodynamically unfavorable synthesis of citrulline from ornithine and carbamyl phosphate:

$$\text{Ornithine} + \text{carbamyl phosphate} \rightleftharpoons \text{citrulline} + H_3PO_4. \quad (37)$$

A catabolic ornithine carbamoyltransferase, which is a considerably heavier protein with a molecular weight of 420,000 and eight identical protomers and catalyzes reaction (37) in the reverse direction, is synthesized in the same cells [26-29].

Two types of formate kinase (E.C.2.7.2.6) are synthesized in Clostridium cylindrosporum cells [30]. One of them (the anabolic enzyme) preferentially catalyzes the synthesis of formyl phosphate, which is the thermodynamically less favorable direction of the conversion

$$\text{Formate} + \text{ATP} \rightleftharpoons \text{formyl phosphate} + \text{ADP}. \quad (38)$$

The other (the catabolic) formate kinase catalyzes only the thermodynamically favorable phosphorylation of ADP at the expense of formyl phosphate. Besides those cited there is a whole series of enzymes which are capable of selectively catalyzing one of the directions of reversible biochemical reaction, depending on the metabolic state of the cells [31].

Thus, the model described by Eq. (9) of reversible reaction (1) makes it possible to account for a number of important properties of key biochemical conversions. All these properties, i.e., the different forms of the nonhyperbolic dependence of the rate on the concentrations of the reactants and the modifiers, the unidirectional action of the modifiers, and the selective catalysis of one of the directions of a reversible reaction, are possible in this model only when $n \geq 2$ and $L \neq 0$, $1/L \neq 0$, i.e., are associated with the oligomeric nature and conformational mobility of the structure of the enzyme, being more strongly manifested the greater is the molecular weight of the oligomer and the stronger is the cooperative nature of the $R \rightleftharpoons T$ transitions.

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