# NEARLY IDEAL BIOCHEMICAL RELAXATIONAL GENERATOR BASED ON COVALENT MODIFICATION OF ENZYME

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UDC 577.150.2

A mathematical model is analyzed describing the dynamics of the open reaction $\rightarrow S_1 \rightarrow S_2 \rightarrow$, catalyzed by the enzyme E(A, B), the two forms of which A and B are subjected to covalent modification in the cycle $A \xrightarrow{E_A} B \xrightarrow{E_B} A$ with the involvement of the modifier enzymes $E_A$ and $E_B$. It is assumed in the analysis that B form is catalytically inactive, that the reagents $S_1$ and $S_2$ inhibit the inactivating enzyme $E_A$ (competitively or noncompetitively with respect to one another), and that the enzymes A, $E_A$, and $E_B$ may be saturated by their substrates. It is shown that the reaction $\rightarrow S_1 \rightarrow S_2$ under definite conditions represents a nearly ideal generator of relaxational autooscillations or a trigger. Asymptotic formulas are derived for the amplitude and period of oscillations of the variables determining these quantities with a relative error on the order of several percentages. On the basis of the introduced criterion of autogenerator quality the studied reaction is compared with biochemical relaxational autogenerators based on the direct allosteric regulation of oligomer enzymes E(R, T). This comparison demonstrated the substantial advantage of a regulator equation based on nonequilibrium cyclic transitions A⇌B. compared with equilibrium transitions of the conformers R⇌T of the allosteric oligomer E(R, T).

In recent years the mechanisms of the cyclic covalent modification of enzymes [1-4] have attracted special interest. This is because the activities of enzymes occupying strategic positions in cellular metabolism, as a rule, are controlled by such mechanisms specifically and not by the simple equilibrium binding of allosteric ligands with target enzymes [1-4]. This is explained by the fact that the cycle of covalent modification due to the loss of energy in the recirculation of the ortho form of the enzyme in the ortho → meta → ortho cycle is capable of providing for a substantially greater amplification of the signal than the mechanism of equilibrium binding [3-9]. This is apparently why weak signals received from the environment by the receptors of cell membranes are amplified by cascades of covalent modification [1-4].

It may be assumed that the function of amplifying weak signals received by receptors and circulated in negative feed-back loops providing for cellular homeostasis is not the sole function in cellular organization. Theoretical papers [10-14] have examined different variants of models describing the dynamics of a system containing cycles of the covalent modification of enzymes. It was shown that oscillations [12, 13] and trigger phenomena [10, 11, 14] can arise in such systems. Insofar as similar nonlinear phenomena are observed in biochemical systems without involvement of cycles of covalent modification, it is of interest to determine the advantages a biochemical system acquires upon the transition from direct allosteric regulation to homologous regulation mediated by covalent modification.

The present paper investigates a mathematical model describing the dynamics of an open reaction, whose enzyme E(A, B) undergoes a cyclic (A⇌B) covalent modification.

## KINETIC MODEL

We shall examine an open enzymatic reaction

Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino, Moscow Oblast. Translated from Molekulyarnaya Biologiya, Vol. 20, No. 6, pp. 1550-1561, 1986. Original article submitted February 12, 1986.

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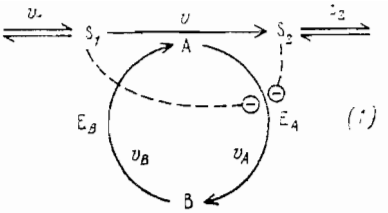



(1)

in which the transformation of substrate $S_1$ into product $S_2$ is catalyzed by the two forms A and B of the enzyme E(A, B), while the interconversion of forms A and B is catalyzed by the enzymes of covalent modification $E_B$ and $E_A$ (scheme 1). We make the following assumptions.

1. **Form B is catalytically inactive.**

2. $S_1$ and $S_2$ are inhibitors of enzyme $E_A$; moreover, $S_1$ and $S_2$ compete with one another but not with A.

3. The total concentration of enzyme E is at least an order of magnitude less than the concentrations of reagents $S_1$ and $S_2$, while the total concentrations of $E_A$ and $E_B$ are at least an order of magnitude lower than the total concentration of enzyme E.

4. The rates of the individual stages of scheme (1) can be approximated by the following equations

$$v_1 = V_1 - k_1 S_1,\ v_2 = -V_2 + k_2 S_2,$$
$$v = kA\frac{S_1}{K_S + S_1},\quad v_B = V_B\frac{B}{K_B + B},$$
$$v_A = V_A\frac{A}{(K_A + A)\left(1 + \frac{S_1}{K_{i_1}} + \frac{S_2}{K_{i_2}}\right)}, \qquad (2)$$
$$A + B = E_0,$$

where $v_1$ and $v_2$ are the rates of exchange of $S_1$ and $S_2$ with the medium, v is the rate of the reaction $S_1 \xrightarrow{A} S_2$; $v_B$ and $v_A$ are the rates of the modification reactions $B \xrightarrow{E_B} A$ and $A \xrightarrow{E_A} B$; $E_0$ is the total concentration of enzyme E; $K_S$, $K_A$, and $K_B$ are the Michaelis constants of the enzymes A, E, and $E_A$; $K_{i1}$ and $K_{i2}$ are the inhibition constants of enzyme $E_A$; $K_1$ and $K_2$ are the rate constants of $\rightleftharpoons S_1$ and $S_2 \rightleftharpoons$; K is the rate constant of the reaction $S_1 \xrightarrow{A} S_2$.

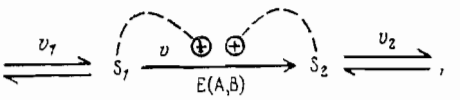
5. The enzymes A, $E_A$, and $E_B$ are easily saturated by their substrates. This means that $K_A/E_0 \ll 1$, $k_B/E_0 \ll 1$, $K_S/K_{i1} \ll 1$.

6. The catalytic activity of the enzymes $E_A$ and $E_B$ is of the same order of magnitude but is several orders of magnitude lower than the activity of enzyme A, i.e., $V_A \sim V_B \ll kE_0$.

7. The inhibition of $E_A$ by $S_1$ is weak, such that $K_{i_2}/K_{i_1} \ll 1$.

8. Reaction (1) occurs in medium of ideal mixing under isothermic conditions.

Provided assumptions 1 and 2 are met, reaction (1) is equivalent to the reaction




the substrate $S_1$ and product $S_2$ of which are indirect activators of enzyme E(A, B).

## MATHEMATICAL MODEL

Considering the assumptions made, the occurrence of reaction (1) in time is described by the system of equations

$$\left.\begin{aligned}\frac{dS_1}{dt} &= v_1 - v,\\ \frac{dS_2}{dt} &= v - v_2,\\ \frac{dA}{dt} &= v_B - v_A,\end{aligned}\right\}, \tag{3}$$

in which $S_1$, $S_2$, and A denote the concentrations of the corresponding substances.

To facilitate the analysis of system (3), we introduce the dimensionless variables and parameters:

$$\begin{gathered}\sigma_1 = \frac{S_1}{K_{i_1}}, \quad \sigma_2 = \frac{S_2}{K_{i_2}}, \quad \alpha = \frac{A}{E_0}, \quad \tau = \frac{kE_0 t}{K_{i_1}},\\ \nu = \frac{V}{kE_0}, \quad \nu_{1m} = \frac{V_1}{kE_0}, \quad \nu_{2m} = \frac{V_2}{kE_0}, \quad \varkappa_1 = \frac{k_1 K_{i_1}}{kE_0},\\ \varkappa_2 = \frac{k_2 K_{i_2}}{kE_0}, \quad \varkappa_A = \frac{k_A}{E_0}, \quad \varkappa_B = \frac{k_B}{E_0}, \quad \varkappa_S = \frac{k_S}{K_{i_1}},\\ \varepsilon_1 = \frac{K_{i_2}}{K_{i_1}}, \quad \varepsilon_2 = \frac{V_B}{kK_{i_1}}, \quad r = \frac{V_A}{V_B}.\end{gathered} \tag{4}$$

In the new variables, system (3) assumes the form

$$\left.\begin{aligned}\frac{d\sigma_1}{d\tau} &= \nu_{1m} - \varkappa_1\sigma_1 - \nu,\\ \varepsilon_1\frac{d\sigma_2}{d\tau} &= \nu - \varkappa_2\sigma_2 + \nu_{2m},\\ \varepsilon_2\frac{d\alpha}{d\tau} &= \frac{1-\alpha}{\varkappa_B + 1 - \alpha} - \frac{r\alpha}{(\varkappa_A + \alpha)(1 + \sigma_1 + \sigma_2)},\\ &\text{where } \nu = \frac{\alpha\sigma_1}{\varkappa_S + \sigma_1},\end{aligned}\right\} \tag{5}$$

$\nu$ is the dimensionless rate of the reaction $S_1 \to S_2$. According to the assumptions made, in model (5)

$$\varepsilon_1 \ll 1, \ \varepsilon_2 <<< 1, \ \varkappa_A, \ \varkappa_B, \ \varkappa_S \ll 1, \ r \sim 1. \tag{6}$$

When the first two inequalities of (6) are met, model (5) has a slow variable $\sigma_1$, a fast $\sigma_2$, and a super-fast $\alpha$. Thus, using the limit transition $\varepsilon_2 \to 0$, one can forego consideration of the behavior of model (5) at super-brief times $\tau \sim \varepsilon_2$. Established during these times is the quasi-steady state value $\alpha = \tilde{\alpha}$, determined by the root of the equation

$$\frac{1-\alpha}{\varkappa_B + 1 - \alpha} - \frac{r\alpha}{(\varkappa_A + \alpha)(1 + \sigma_1 + \sigma_2)} = 0. \tag{7}$$

The limit transition $\varepsilon_2 \to 0$ reduces system (5) to a second order model

$$\left.\begin{aligned}\frac{d\sigma_1}{d\tau} &= \nu_{1m} - \varkappa_1\sigma_1 - \tilde{\nu},\\ \varepsilon_1\frac{d\sigma_2}{d\tau} &= \tilde{\nu} - \varkappa_2\sigma_2 + \nu_{2m},\end{aligned}\right\} \tag{8}$$

where

$$\tilde{\nu} = \frac{\tilde{\alpha}\sigma_1}{\varkappa_S + \sigma_1}, \tag{9}$$

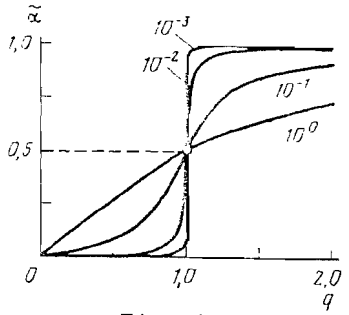



Fig. 1

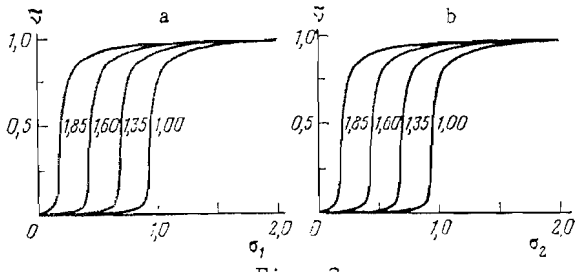



Fig. 2

Fig. 1. Dependence of relative quasi-steady state concentration $\tilde{\alpha}$ of active form A of enzyme E(A, B) upon function of relationship $q = (1 + \sigma_1 + \sigma_2)/r$, constructed from Eq. (11) for $r = 3$ and four values of relative Michaelis constants $\varkappa_A = \varkappa_B$, indicated in figure.

Fig. 2. Stepwise character of dependence of relative quasi-steady state rate of reaction $\tilde{\nu}$ upon concentration of substrate $\tilde{\sigma}_1$ at various concentrations of product (a) and upon concentration of product $\sigma_2$ at various values of $\sigma_1$ (b). Values of $\sigma_2$ (a) and $\sigma_1$ (b) indicated in figures. Curves constructed using Eqs. (9-11) for $\varkappa_A = \varkappa_B = \varkappa_S = 10^{-3}$, $r = 3$

$\tilde{\nu}$ is the quasi-steady state value of the rate $\nu$. Equation (7) determines the major nonlinearity of reaction (1), i.e., the dependence of $\tilde{\alpha}$ upon the relative activity of the enzymes $E_B$ and $E_A$, represented by the function

$$q = \frac{1 + \sigma_1 + \sigma_2}{r}. \tag{10}$$

A plot of this dependence, constructed from the inverse function $q(\tilde{\alpha})$,

$$q = \frac{\tilde{\alpha}}{\varkappa_A + \tilde{\alpha}}\left(1 + \frac{\varkappa_B}{1 - \alpha}\right), \tag{11}$$

is presented in Fig. 1. As apparent from the figure, a plot of the function $\tilde{\alpha}(q)$ at small $\varkappa_A$ and $\varkappa_B$ is of a stepwise character with a steepness

$$Sl_q^{\tilde{\alpha}} = \left(\frac{d\tilde{\alpha}}{dq}\right)_{q=q^*} \cong \frac{1}{\left(\sqrt[3]{\varkappa_A} + \sqrt[3]{\varkappa_B}\right)^3} \tag{12}$$

at the point of inflection with coordinates

$$q^* \cong \frac{1 + \varkappa_B + \sqrt[3]{\varkappa_B^2 \varkappa_A}}{1 + \varkappa_A + \sqrt[3]{\varkappa_A^2 \varkappa_B}}, \quad \alpha^* \cong \frac{1}{1 + \sqrt[3]{\varkappa_B/\varkappa_A}}. \tag{13, 14}$$

Equations (12-14) are calculated with consideration of the smallness of the relative Michaelis constants $\varkappa_A$ and $\varkappa_B$ according to assumption 5. Because of the stepwise character of the function $\tilde{\alpha}(q)$, the dependence of the rate $\tilde{\nu}$ upon $\sigma_1$ and $\sigma_2$ is also of a stepwise character (Fig. 2). The steepness of the plots of $\tilde{\nu}(\sigma_1)$ and $\tilde{\nu}(\sigma_2)$ at the points of inflection is determined by the expressions

$$Sl_{\sigma_1}^{\tilde{\nu}} \equiv \left(\frac{\partial \tilde{\nu}}{\partial \sigma_1}\right)_{\sigma_1 = \sigma_1^*} = \frac{\varkappa_S \tilde{\alpha}}{(\varkappa_S + \sigma_1)^2} + \frac{\sigma_1/r}{\varkappa_S + \sigma_1} Sl_q^{\tilde{\alpha}}, \tag{15}$$

$$Sl_{\sigma_2}^{\tilde{\nu}} \equiv \left(\frac{\partial \tilde{\nu}}{\partial \sigma_2}\right)_{\sigma_2 = \sigma_2^*} = \frac{\sigma_1/r}{\varkappa_S + \sigma_1} Sl_q^{\tilde{\alpha}}, \tag{16}$$

where when $\varkappa_S \ll \sigma_1$ (assumption 5)

$$Sl_{\sigma_1}^{\tilde{\nu}} \cong \frac{1}{r} Sl_q^{\tilde{\alpha}}, \quad Sl_{\sigma_2}^{\tilde{\nu}} \cong \frac{1}{r} Sl_q^{\tilde{\alpha}}. \tag{17, 18}$$

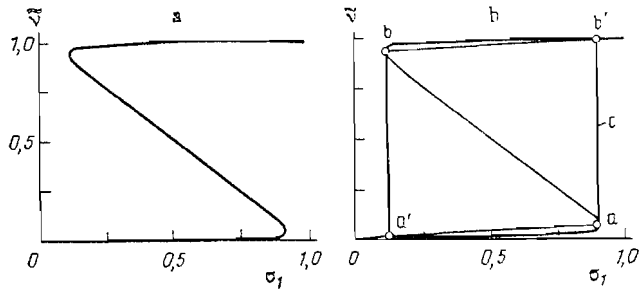



Fig. 3. a) Quasi-steady state input characteristics of reaction (1): dependence of dimensionless rate $\nu$ upon concentration of substrate $\sigma_1$ when conditions (23) are met at $\varkappa_A = \varkappa_B = \varkappa_S = 10^{-3}$, , r = 3, $\varkappa_2 = 1$, $\nu_{2m} = 1$. b) Discontinuous limit cycle C [heavy line (ab'ba')] encompassing hysteretic region of input characteristic curve $\nu(\sigma_1)$. a and b are points of discontinuity, while a' and b' are points of decline in the representative point $(\sigma_1, \nu)$. The regions ab' and ba' are jumps in rate, while the regions a'a and b'b are intervals of slow quasi-steady state drift in the representative point. The curve $\tilde{\tilde{\nu}}(\sigma_1)$ is constructed for values of parameters indicated for the left plot. Cycle C corresponds to the regimen of autooscillations in which $\varepsilon_2$ and $\varepsilon_1 \approx 0$.

The coordinates of the inflection points of the curves $\tilde{\nu}(\sigma_1)$ and $\tilde{\nu}(\sigma_2)$ are determined in terms of $q^*$ and $\alpha^*$ by the expressions

$$\sigma_1^* \cong rq^* - 1 - \sigma_2, \quad \tilde{\nu}^* \cong \tilde{\alpha}^*, \tag{19}$$

$$\sigma_2^* \cong rq^* - 1 - \sigma_1, \quad \tilde{\nu}^* \cong \tilde{\alpha}^*. \tag{20}$$

The linear shift to the left of the abscissa of the inflection point $\sigma_2^*$ of the plot of $\tilde{\nu}(\sigma_2)$ with increase in $\sigma_1$ with an unaltered ordinate of this point $\tilde{\nu}^*$ creates a linear dependence of the unstable quasi-steady state rate $\tilde{\tilde{\nu}}$ upon the concentration $\sigma_1$ (see Fig. 3a) and makes it possible to approximate with high precision model (8) with a much simpler piecewise linear model.

In spite of the exclusion of the super-fast variable $\alpha$, model (8) still contains the variable $\sigma_2$, the rate of change of which is $\sim 1/\varepsilon_1$ times greater than the rate of change of $\sigma_1$. This makes it possible to complete still another limit transition $\varepsilon_1 \to 0$, reducing it to a first order model

$$\frac{d\sigma_1}{d\tau} = \nu_{1m} - \varkappa_1\sigma_1 - \tilde{\tilde{\nu}}, \tag{21}$$

in which $\tilde{\tilde{\nu}}$ is the value of $\tilde{\tilde{\nu}}$ satisfying the condition of quasi-steady state for $\sigma_2$:

$$\varepsilon_1 \frac{d\sigma_2}{d\tau} = \tilde{\nu}(\sigma_1, \sigma_2) - \varkappa_2\sigma_2 + \nu_{2m} = 0. \tag{22}$$

This equation is equivalent to the system

$$\left.\begin{aligned} &q\frac{1-\alpha}{\varkappa_A + 1 - \alpha} - \frac{\alpha}{\varkappa_A + \alpha} = 0, \\ &\nu - \varkappa_2\sigma_2 + \nu_{2m} = 0, \\ &\frac{\alpha\sigma_1}{\varkappa_S + \sigma_1} - \nu = 0, \end{aligned}\right\} \tag{23}$$

which when $\sigma_1$ = const may have from one to three positive roots determining the values of $\tilde{\tilde{\nu}}$. Thanks to this, the quasi-steady state input characteristic of reaction (1) – the dependence of $\tilde{\tilde{\nu}}$ upon $\sigma_1$ – is of a hysteretic character (Fig. 3a). This nonlinearity of the function $\tilde{\tilde{\nu}}(\sigma_1)$ has the result that under specific conditions (Fig. 4) relaxational autooscillations appear in reaction (1) (Fig. 5). When $\varepsilon_2 \ll \varepsilon_1 \ll 1$ such oscillations in the plane $(\sigma_1, \nu)$ correspond to the discontinuous limit cycle C, shown in Fig. 3b (cycle ab'ba').

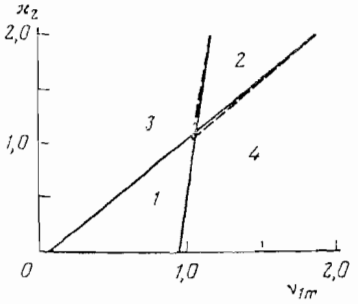



Fig. 4

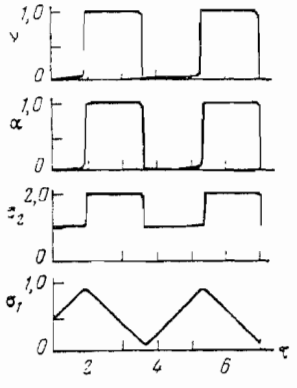



Fig. 5

Fig. 4. Parametric portrait of model (5). In region 1 reaction (1) has a single unstable steady state and a single discontinuous limit cycle. In region 2 reaction (1) represents a trigger with two stable steady states. In regions 3 and 4 the reaction has a single stable state in which $\nu = 0$ (region 3) or $\nu \approx 1$ (region 4).

Fig. 5. Autooscillations of variables of model (5) at parameter values corresponding to region 1 in Fig. 4. Curves obtained by numerical integration (5) by Calahan method of fourth order precision [15] with precision of integration at step $\tau = 10^{-4}$, $\varepsilon_1 = \varepsilon_2 = 10^{-3}$, $\varkappa_A = \varkappa_B = 10^{-3}$, $\varkappa_1 = 0$, $\nu_{1m} = 0.5$, $\varkappa_2 = \nu_{2m} = 1$, $r = 3$.

Thus, depending upon the desired precision of description of the behavior at super-brief and brief times, reaction (1) can be represented either by the initial third order model (5), by a second order model (8), or, finally, by a first order model (21). This latter model makes it possible to obtain approximate analytical expressions for the amplitudes and period of relaxational oscillation of variables.

## APPROXIMATE FORMULAS FOR AMPLITUDES OF RELAXATIONAL OSCILLATIONS

Model (21) still contains two small parameters $\varkappa_A$ and $\varkappa_B$, upon which the solution of system (23) and the form of the characteristic curve $\nu(\sigma_1)$ depend (Fig. 3a). The smallness of $\varkappa_A$ and $\varkappa_B$ makes it possible to obtain approximate expressions for the coordinates of the extrema of the characteristic curve $\tilde{\nu}(\sigma_1)$ (points a and b in Fig. 3b) and for the coupled coordinates of the so-called "decline points" (points a' and b' in Fig. 3b).

Regions of rapid movement ab' and ba' along the limit cycle C surrounding the hysteretic region of the characteristic curve $\tilde{\nu}(\sigma_1)$ terminate at the decline points.

We note that function (11) is the product of two hyperbolic functions such that

$$q \cong \frac{\alpha(1+\varkappa_B)}{\varkappa_A+\alpha} \text{ when } \alpha \to 0, \tag{24}$$

$$q \cong \frac{1+\varkappa_B-\alpha}{(1+\varkappa_A)(1-\alpha)} \text{ when } \alpha \to 1. \tag{25}$$

From which there follow the two asymptotics for $\tilde{\alpha}$:

$$\tilde{\alpha} = \frac{q\varkappa_A}{1+\varkappa_B-q}, \quad \tilde{\alpha} \to 0, \tag{26}$$

$$\tilde{\alpha} = \frac{1+\varkappa_B-(1-\varkappa_A)q}{1-(1+\varkappa_A)q}, \quad \tilde{\alpha} \to 1. \tag{27}$$

Ignoring $\varkappa_B \ll 1$ in the expression for $\tilde{\nu}$, Eq. (9), we obtain the two asymptotics of the variand of Eq. (22)

$$\frac{(1+\sigma_1+\sigma_2)\varkappa_A}{r(1+\varkappa_B)-1-\sigma_1-\sigma_2} - \varkappa_2\sigma_2 + \nu_{2m} \cong 0, \ \tilde{\alpha} \to 0, \tag{28}$$

$$\frac{r(1+\varkappa_B)-(1+\varkappa_A)(1+\sigma_1+\sigma_2)}{r-(1+\varkappa_A)(1+\sigma_1+\sigma_2)} - \varkappa_2\sigma_2 + \nu_{2m} \cong 0, \ \tilde{\alpha} \to 1. \tag{29}$$

The solution of these quadratic equations for $\sigma_2$ makes it possible to determine approximate expressions for the values of the variables $\sigma_1$, $\sigma_2$, and $\tilde{\nu}$ at the four points of the cycle a, b', b, and a' shown in Fig. 3b:

$$\sigma_{2a} = \frac{r\varkappa_B' - 1}{2} + \frac{\nu_{2m} - \varkappa_A}{2\varkappa_2} - \frac{\sigma_{1a}}{2}, \tag{30}$$

$$\sigma_{1a} = \frac{\varkappa_A - \nu_{2m}}{\varkappa_2} + r\varkappa_B' - 1 - 2\sqrt{\frac{r\varkappa_A\varkappa_B'}{\varkappa_2}}, \tag{31}$$

$$\tilde{\nu}_a = \varkappa_2\sigma_{2a} - \nu_{2m}, \tag{32}$$

$$\sigma_{2b} = \frac{1 + \nu_{2m}}{2\varkappa_2} - \frac{1 + \sigma_{1b} - r/\varkappa_A'}{2}, \tag{33}$$

$$\sigma_{1b} = \frac{r}{\varkappa_A'} - 1 - \frac{1 + \nu_{2m}}{\varkappa_2} + \sqrt{\frac{\varkappa_B r}{\varkappa_2\varkappa_A'}}, \tag{34}$$

$$\tilde{\nu}_b = \varkappa_2\sigma_{2b} - \nu_{2m}, \tag{35}$$

$$\sigma_{2a'} = \frac{r\varkappa_B' - 1}{2} + \frac{\nu_{2m} - \varkappa_A}{2\varkappa_2} - \frac{\sigma_{1b}}{2} - \frac{R_a}{2\varkappa_2}, \tag{36}$$

$$\tilde{\nu}_{a'} = \varkappa_2\sigma_{2a'} - \nu_{2m}, \tag{37}$$

$$\sigma_{2b'} = \frac{1 - \nu_{2m}}{2\varkappa_2} - \frac{1 + \sigma_{1a} - r/\varkappa_A'}{2} + \frac{R_b}{2\varkappa_2}. \tag{38}$$

$$\tilde{\nu}_{b'} = \varkappa_2\sigma_{2b'} - \nu_{2m}, \tag{39}$$

where

$$\varkappa_B' = 1 + \varkappa_B, \quad \varkappa_A' = 1 - \varkappa_A,$$

$$R_b = 1 - \sqrt{[\nu_{2m} - 1 + \varkappa_2(1 + \sigma_{1a} - r\varkappa_A')]^2 - 4r\varkappa_B\varkappa_2},$$

$$R_a = 1 - \sqrt{[\varkappa_A - \nu_{2m} - \varkappa_2(r\varkappa_B' - 1 - \sigma_{1b})]^2 - 4\varkappa_2[\nu_{2m}(r\varkappa_B' - 1 - \sigma_{1b}) + \varkappa_A(1 + \sigma_{1b})]}.$$

The coordinates of points a and b are determined from the conditions of the multiplicity of the roots of Eqs. (28) and (29). The amplitudes of the oscillations of the variables are calculated from the obtained coordinates

$$A_{\sigma_1} = \sigma_{1a} - \sigma_{1b}, \tag{40}$$

$$A_{\sigma_2} = \sigma_{2b'} - \sigma_{2a'}, \tag{41}$$

$$A_\nu = \tilde{\nu}_{b'} - \tilde{\nu}_{a'}. \tag{42}$$

## ASYMPTOTIC FORMULAS FOR THE OSCILLATION PERIOD

As apparent from Fig. 3b, cords bb' and a'a run very close to analogous contracted regions of slow drift in the characteristic curve $\tilde{\nu}(\sigma_1)$. This makes it possible to approximate these regions with the cords and to reduce the problem of calculating the period of oscillations $\tau_0$ in model (21) to the integral

$$\tau_0 = \int_C \frac{d\sigma_1}{\nu_{1m} - \varkappa_1\sigma_1 - \tilde{\nu}(\sigma_1)}, \tag{43}$$

taken from the closed contour C, formed by the rectangle ab'ba' (Fig. 3b). Omitting the intermediate calculations, we obtain the following expression for the period

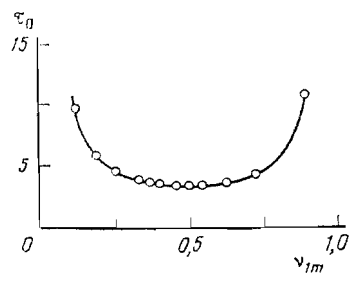



Fig. 6

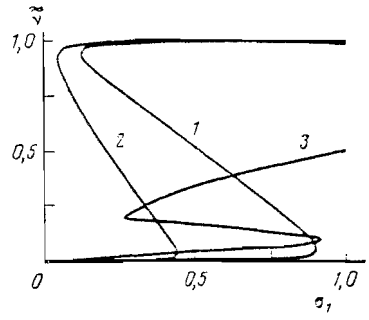



Fig. 7

Fig. 6. Comparison of oscillation period $\tau_0$ obtained by direct integration of model (5) (circles) with period calculated from asymptotic Eq. (4).

Fig. 7. Quasi-steady state input characteristics of reaction $\tilde{\tilde{\nu}}(\sigma_1)$ constructed for initial model (1) (curve 1), for noncompetitive inhibition of enzyme $E_A$ by reagents $S_1$ and $S_2$ (curve 2), and in absence of inhibition of $E_A$ by substrate $S_1$ (curve 3). Curves constructed by numerical solution of system (23) in which q was defined by Eqs. (10), (51), and (52) – curves 1, 2, and 3, respectively. Parameter values: $\varkappa_A=\varkappa_B=\varkappa_S=10^{-3}$, $r=3$ .

$$\tau_0=\ln\left[\left(\frac{\sigma_{1b}+\dfrac{a_1-\nu_{1m}}{\alpha_1}}{\sigma_{1a}+\dfrac{a_1-\nu_{1m}}{\alpha_1}}\right)^{\frac{1}{\alpha_1}}\left(\frac{\sigma_{1a}-\dfrac{a_2-\nu_{1m}}{\alpha_2}}{\sigma_{1b}-\dfrac{a_2-\nu_{1m}}{\alpha_2}}\right)^{\frac{1}{\alpha_2}}\right], \tag{44}$$

where

$$\alpha_1=\frac{\tilde{\tilde{\nu}}_b-\tilde{\tilde{\nu}}_a}{\sigma_{1b}-\sigma_{1a}}-\varkappa_1,\quad \alpha_2=\frac{\tilde{\tilde{\nu}}_b-\tilde{\tilde{\nu}}_a}{\sigma_{1b}-\sigma_{1a}},$$
$$a_1=\tilde{\tilde{\nu}}_a-\alpha_1\sigma_{1a},\quad a_2=\tilde{\tilde{\nu}}_a-\alpha_2\sigma_{1a}.$$

To estimate the precision with which Eq. (43) defines the period of relaxational fluctuations of the initial model (5), the period of the established fluctuations in this model at various values of the parameters $\nu_{1m}$ and $\varkappa_2$ was compared with the period calculated from Eq. (44). To do this model (5) was integrated numerically by Calahan's fourth order method [15] for the solution of rigorous systems of differential equations. The selection of this method was conditioned by the fact that the rigidity of model (5) at the parameter values used in the computational experiments comprised ~$10^5$. The stepwise relative error of integration was taken as $10^{-4}$. The period was calculated in the course of integration with the same error. The results of the comparison of the quantities obtained by the numerical integration of model (5) and by Eq. (44) are presented in Fig. 6 and in Table 1.

It follows from this comparison that the asymptotic Eq. (44) at small values of the parameters $\varepsilon_1$, $\varepsilon_2$, $\varkappa_A$, $\varkappa_B$ and $\varkappa_S$ gives $\tau_0$ values somewhat smaller (by several percentages) compared with those obtained by the direct integration of model (5).

In the special case of $\varkappa_1=0$ instead of the cumbersome Eq. (44) it is more convenient to use the much simpler equation

$$\tau_0=\frac{A_{\sigma_1}A_\nu}{(\tilde{\tilde{\nu}}_b-\nu_{1m})(\nu_{1m}-\tilde{\tilde{\nu}}_a)}. \tag{45}$$

This equation, obtained by approximation of the regions of slow movement of the characteristic curve $\tilde{\tilde{\nu}}(\sigma_1)$ by the lines $\tilde{\tilde{\nu}}=\tilde{\tilde{\nu}}_a(\sigma_1\leq\sigma_{1b})$ and $\tilde{\tilde{\nu}}=\tilde{\tilde{\nu}}_b(\sigma_1\geqslant\sigma_{1b})$, has about the same precision

TABLE 1. Comparison of Experimental Values and Period of Oscillations ($\tau_{0N}$, $\tau_0$) of Model (5) Determined by Numerical Integration and by Asymptotic Formulas

| Value of parameter $\varkappa_2$ | Numerical integration of model (5) | | | Calculation from asymptotic formulas (31, 34, 44) | | | Relative error, $\delta$, % |
|---|---|---|---|---|---|---|---|
| | $\sigma_{1}$ max | $\sigma_{2}$ min | $\tau_{0N}$ | $\sigma_{1a}$ | $\sigma_{1b}$ | $\tau_0$ | |
| 1,0 | 0,909 | 0,1005 | 3,315 | 0,891 | 0,109 | 3,314 | —0,1 |
| 1,1 | 1,005 | 0,271 | 3,015 | 0,987 | 0,286 | 2,981 | —1,1 |
| 1,2 | 1,085 | 0,417 | 2,754 | 1,067 | 0,433 | 2,704 | —1,8 |
| 1,3 | 1,154 | 0,540 | 2,533 | 1,135 | 0,557 | 2,471 | —2,5 |
| 1,4 | 1,213 | 0,646 | 2,345 | 1,193 | 0,664 | 2,272 | —3,1 |
| 1,5 | 1,264 | 0,738 | 2,184 | 1,244 | 0,756 | 2,100 | —3,8 |
| 1,6 | 1,309 | 0,818 | 2,043 | 1,289 | 0,836 | 1,950 | —4,6 |
| 1,7 | 1,348 | 0,888 | 1,920 | 1,328 | 0,907 | 1,819 | —5,3 |

Note. Integration performed by Calahan's method of fourth order precision [15] with integration precision per step of $<<10^{-4}$ at $\varepsilon_1 = \varepsilon_2 = 10^{-3}$; $\varkappa_1 = 0$; $\nu_{1m} = 0.5$; $\varkappa_A = \varkappa_B = \varkappa_S = 10^{-3}$, $r = 3$, $\nu_{2m} = 1$.

as Eq. (44), so long as the small parameters of model (5) are in fact small. Equation (45) within the limit of $\varkappa_A \sim \varkappa_B \to 0$ assumes the very simple form

$$\tau_0 = \frac{1}{\varkappa_2 \nu_{1m}(1-\nu_{1m})}, \tag{46}$$

or in dimensional form

$$T_0 = \frac{K_{i_1}(kE_0)^2}{k_2 K_{i_2}(kE_0 - V_1)}. \tag{47}$$

This Eq. (46) also introduces a marked error, predicting (at the parameter values presented in Table 1) a roughly 20% larger period than the true period. However, such an error is more than compensated for by the great simplicity of Eq. (46), permitting the rapid estimation of the magnitude of $\tau_0$ or $T_0$.

## ASSESSMENT OF QUALITY OF ATUOGENERATOR

As apparent from the family of input characteristic curves $\tilde{\nu}(\sigma_1)$ in Fig. 3, the form of this characteristic curve is close to the ideal hysteretic nonlenearity used in electronic systems to generate relaxational oscillations and create trigger regimens.

In order to estimate the similarity of scheme (1) to the ideal relaxational autogenerator and to be able to compate it to other schemes, we introduce the coefficient of quality

$$K_Q = U_v U_s \tag{48}$$

as the product of the coefficient of instability ($U_V$) and the coefficient of substrate utilization ($U_S$), defined by the relationships

$$U_v = \Delta v / V, \quad U_s = A_s / S_{\max} \tag{49, 50}$$

Here $\Delta v$ is the interval of unstable values of the reaction rate, V is the maximum reaction rate, $A_S$ and $S_{max}$ are the amplitude and maximum of the substrate concentration in the established oscillatory regimen. By definition all coefficients are postiive and do not exceed unity. Only in the ideal case $K_Q = K_V = K_S = 1$.

Mechanism (1) at the small parameter values indicated in Fig. 3a has

$$U_v = \nu_b - \nu_a, \quad U_{\sigma_1} = \frac{\sigma_{1a} - \sigma_{1b}}{\sigma_{1a}}, \quad K_Q = U_v U_{\sigma_1} = 0{,}77.$$

Such high quality of scheme (1) is inaccessible for the homologous mechanism of direct allosteric activation of the enzyme F(R, T) by product [16] – theoretically it might be obtained only when the activating centers number ~500. A much lower quality of $K_Q$ = 0.23-0.3 is possible for the more widespread case of direct allosteric regulation of the tetrameric enzyme with four or eight activating centers.

For a comparison of scheme (1) with its possible variants, Fig. 7 presents in addition to the characteristic curve $\tilde{\nu}(\sigma_1)$ of this scheme (curve 1) the analogous characteristic curves for its two modifications (curves 2 and 3).

The characteristic curve presented by curve 2 in Fig. 7 is constructed for the case of the noncompetitive inhibition of enzyme $E_A$ by reagents $S_1$ and $S_2$. In this case the ratio function in models (8) and (21) has the form

$$q=(1+\sigma_1)(1+\sigma_2)/r, \tag{51}$$

while the coefficient of quality with other conditions equal is markedly higher: $K_Q$ = 0.85. The attainment of such quality by the mechanism of direct allosteric regulation [16] would require a fantastic number of activating centers $n_a \sim 10^3$.

Curve 3 in Fig. 7 presents a case of the absence of inhibition of $E_A$ by substrate $S_1$ (indirect activation of E(A, B) by substrate $S_1$ is absent). In this case the ratio function*

$$q=(1+\sigma_1)/r \tag{52}$$

and the coefficient of quality compared with the base-line model is sharply reduced at $K_Q$ = 0.08. The scheme of indirect activation of E(A, B) by product S described by Martiel and Goldbeter [13] has the same low quality ($K_Q$ < 0.10). In this scheme a quadratic product of activation of $E_B$ is used instead of the inhibition of $E_A$, and the indirect activation by substrate $S_1$ is ignored.

Thus, in the absence of indirect substrate activation, mechanism (1) does not realize the potentials offered by a cycle of enzyme covalent modification, i.e., its quality is much lower than in the mechanism of reaction with direct product activation of an allosteric tetramer ($K_Q$ = 0.25).

It is interesting to note that the possible ancient evolutionary precursor of mechanism (1) – a reaction with the isosteric product activation of an oligomeric enzyme [16] with protomers numbering n = 4 – has a very low quality $K_Q$ = 0.08.

## DISCUSSION

Thus, analysis of reaction (1) with an indirect product activation of the enzyme E(A, B) by both reagents permits an unambiguous answer to the question raised in the introduction concerning the possible advantages of mechanism (1) compared with the homologous mechanism of direct allosteric regulation of the oligomer E(R, T) [16].

The main advantage amounts to a sharp increase in the quality of the autogenerator $K_Q$ after replacement of the mechanism of direct allosteric regulation of the oligomeric enzyme E(R, T) [16] by a mechanism of indirect regulation mediated by the cycle of covalent modification $A \rightleftarrows B$ of enzyme E(A, B). The increase in quality from $K_Q$ = 0.23 ... 0.3 to $K_Q$ = 0.77 ... 0.85 occuring after this substitution is equivalent to an increase by two orders of magnitude in the number of allosteric activating centers $n_a$ in the oligomer E(R, T), replacing E(A, B) with a retention of quality. At the same time a high quality is attained in mechanism (1) only during the combined inhibitory competitive ($K_Q$ = 0.77) or noncompetitive ($K_Q$ = 0.85) action of both reagents on the inactivating enzyme $E_A$. And although the inhibitory action of $S_1$ on $E_A$ is not necessary for the generation of autooscillation and trigger regimens, its exception ($K_{i1} \to \infty$) leads to the loss of the main advantage of mechanism (1): The quality is lower ($K_Q$ = 0.08) than in the homologous mechanism of reaction catalyzed by the tetramer E(R, T) ($K_Q$ = 0.23).

Another advantage of mechanism (1), based on covalent modification, is the fact that enzymes providing the simplest hyperbolic kinetics can be used as E(A, B), $E_A$, and $E_B$ for the

*In case (52) $K_S$ should everywhere replace $K_{i1}$ in the expression for dimensionless parameters (4). Therefore, in particular, $\varkappa_s = 1$.

physical realization of this mechanism. In other words, these enzymes do not necessarily have to be oligomers consisting of a large number of subunits or oligomers at all. This property of mechanism (1) may be extremely important when it is used as part of a biotechnological device based on the enzyme immobilization, as enzymes lacking a complex labile quaternary structure should be much more stable than subunit enzymes.

Mechanism (1), of course, has its faults. First, this mechanism is more complex – it involves at least three enzymes (E(A, B), $E_A$ and $E_B$) instead of the one E(R, T) in the allosteric homologue [16]. Second, the energy dissipated in the $A \rightleftharpoons B$ cycle must be expended for the regulation of E(A, B). By Stadtman's estimation [1], a significant fraction of the ATP flux generated by the energy metabolism of Escherichia coli is expended for the covalent modification of glutamine synthetase. And, finally, in mechanims (1) hierarchical relationships between enzyme concentrations (assumption 3) must be observed to ensure a high quality. And although a hierarchy of enzyme concentrations is in fact observed in the cascade controlling the glycogen cycle [17], the realization of this hierarchy requires special and, possibly, complex mechanisms regulating the synthesis and breakdown of enzymes.

In conclusion it should be noted that mechanism (1) belongs to a broad class of polyenzymatic systems whose regulation is based on the recirculation of matter in closed catalytic cycles. Coenzyme pairs such as ATP/ADP, $NAD^+$/NADH, $NADP^+$/NADPH, acetyl-CoA/CoA, etc., that are mathematicallly equivalent to enzymes, can act in place of enzymes as A/B forms in polyenzymatic systems. An analogue of Eq. (7) can be written for each such pair (assuming a low rate of flux through the $A \rightleftharpoons B$ cycle compared with the rates of $A \rightleftharpoons B$ exchange). In this case, as shown in their time by special investigations [18, 19], stoichiometric $A \rightleftharpoons B$ cycles incorporated in a system of stoichiometric connections of polyenzymatic systems may be responsible for a multitude of diverse nonlinear phenomena: the stepwise or hysteretic dependence of of the reaction rate upon substrate or coenzyme concentration, relaxational oscillations and trigger transitions, and the stabilization of coenzyme concentrations. And just as the balance between A and B defined by Eq. (7) gives rise in reaction (1) to a stepwise dependence of the relative concentration $A(\alpha)$ upon the relative enzyme activity $E_A/E_B(q)$, an analogous stepwise dependence arises in polyenzymatic systems: for example, the relative concentration of ATP ($\alpha_3$) or its equivalent, the energy charge $q = (ATP + 0.5\,ADP)/(AMP - ADP + ATP)$ is dependent in a stepwise manner upon the relative activity of ATPase [18]. In both mechanism (1) and in polyenzymatic systems the insertion of the cycle $A \rightleftharpoons B$ into a positive feedback loop gives rise to hysteresis, autooscillations, and triger phenomena [18, 19].

The authors are grateful to É. É. Shnol' and his associates for assistance in the computational work, as well as E. L. Shevelev for useful discussions and R. R. Chulkova for assistance with the computer.

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