Biochemistry (Moscow), Vol. 67, No. 4, 2002, pp. 473-484. Translated from Biokhimiya, Vol. 67, No. 4, 2002, pp. 567-579.
Original Russian Text Copyright © 2002 by Ermakov, Goldstein.

Simplest Kinetic Schemes for Biochemical Oscillators

G. L. Ermakov* and B. N. Goldstein

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences,
Pushchino, Moscow Region, 142290 Russia; fax: (7-0967) 79-0553; E-mail: ermakov@venus.iteb.serpukhov.su

Received March 19, 2001
Revision received May 17, 2001

Abstract—The topological structure of the simplest critical fragments in biochemical systems has been characterized. The conditions are considered where the critical fragments induce oscillations of the concentrations of the system participants. To illustrate, three biochemical systems (transport of ions through a membrane, protein phosphorylation, and two-substrate reaction) are discussed. The kinetic schemes of these systems contain one of the discovered critical fragments. Relaxation oscillations of the concentrations of the system participants were demonstrated using the numerical integration method.

Key words: graph, critical fragment, mathematical modeling

It is common knowledge that many of the biochemical systems are capable of functioning in an oscillatory regime. However, the role of such oscillations is not clearly understood. Unexplored types of oscillators may occur in the living cell. Therefore, new theoretical methods of analysis of metabolic pathways are necessary to search the fragments, which have the ability to generate oscillations of the reagent concentrations, and to assess the prerequisites to the initiation and sustenance of oscillations.

Mathematical modeling of biochemical oscillators uses often notions (regulation, positive or negative feedback) adopted from the technical sciences. The notion of feedback or direct linkage implies the participation of inhibitor or activator in regulation of a certain enzyme. However, oscillations may be caused by other mechanisms. When modeling enzymatic reactions, the empiric expressions for the dependence of the steady-state velocity of the enzymatic reaction on the concentration of ligand (activators or inhibitors) are often used. Besides, it should be noted that the application of the standard formulae and analogies usually do not allow the correct mechanism of initiation and sustenance of oscillations to be established.

There is a method of analysis of the kinetic schemes of the biochemical processes that is free of such disadvantages. This is the method based on graph theory. Graph theory has found wide utility in analysis of the kinetics of complex reactions and the revealing of the relation between the structure of the kinetic scheme and the kinetic behavior. The method under discussion allows the topological elements, which are constituents of oscillators, to be found in the kinetic schemes composed in accordance with the law of mass action. All the simplest biochemical oscillators may be systematized using a theoretical approach based on graph theory. Goldstein and Volkenstein [1], Clarke [2], and Ivanova [3] studied the time-dependent behavior of systems of reactions with the aid of graphs. The method elaborated by Ivanova is especially useful for analysis of oscillations. Goldstein and Ivanova [4] applied this method to analysis of different biochemical systems.

The goal of the present work was to describe the simplest kinetic schemes (the critical fragments) that might be responsible for the critical phenomena in a network of biochemical reactions and to analyze the conditions where these critical fragments initiate oscillations of the concentrations of the components of the system.

METHODS OF INVESTIGATION

The topological classification of the simplest biochemical oscillators proposed in the present work is based on graphic constructions. Therefore, first we should present the main notions of graph theory in a more illustrative form than was done before.

1. Kinetic graphs. The rate of the change in the concentration of a substance X_i is equal to the algebraic sum of the rates of the consumption (l stages) and formation (p stages) of X_i in all r ($r = 1, 2, ..., R$, where $R = l + p$) elementary reactions:

---

* To whom correspondence should be addressed.

$$v^{(X_i)} \equiv \frac{dx_i}{dt} \equiv \dot{x}_i = \sum_{r=1}^{R} (\beta_{\mathrm{ir}} - \alpha_{\mathrm{ir}}) \cdot v_{\mathrm{r}}$$

$$(i = 1, 2, \ldots, m). \qquad (1)$$

Here x_i is the concentration of the substance X_i, α_{ir} and β_{jr} are the stoichiometric coefficients for the substrates (a stage of the consumption of the substance X_i) and products (a stage of the formation of the substance X_j). The first index (i or j) refers to the number of the substance and the second index (r) corresponds to the number of the elementary stage where X_i is consumed or X_j is formed.

For a complex chemical (biochemical) reaction proceeding with participation of substances X_i ($i = 1, 2, \ldots, n$) there is a system of ordinary differential equations of the m-th order ($m < n$) (m independent variables and ($n - m$) laws of conservation representing linear integrals of the system of differential equations). In accordance with the law of mass action the rate of the elementary stage r for the reactions following the bimolecular mechanism may be written as follows:

$$v_r = k_r \cdot x_i^{\alpha_{ir}} \cdot x_j^{\alpha_{jr}}, \qquad (2)$$

where k_r is the rate constant for the stage.

Each complex reaction described by the system of differential equations (1) may be presented as a bigraph. The reactants (X_i) correspond to apices of one type (circles **O**), and the rates (v_r) of the elementary stages of the reaction are displayed as apices of other type (squares ■). The graph of arbitrary complexity contains elements of three types: half-pathway (○——▸■), positive pathway (○——▸■——▸○), and negative pathway (○——▸■◂——○). To explain Eq. (1) and corresponding basic graphic elements, Table 1 presents an open reversible reaction proceeding with participation of three substances. Thus, a complex biochemical reaction can be represented as a system of differential equations (1) and a graph equivalent to this system.

2. Analysis of stability of the system in the neighborhood of the steady-state point. The time-dependent behavior of a chemical (biochemical) system after a small disturbance in the neighborhood of the steady-state is being studied using linearized kinetic (differential) equations. After corresponding transformations we obtain a linearized system of differential equations of the m-th order:

$$\Delta\dot{x}_i = \sum_{j=1}^{m} \left.\frac{\partial \dot{x}_i}{\partial x_j}\right|_{\bar{x}_1 \ldots \bar{x}_n} \cdot \Delta x_j = \sum_{j=1}^{m} b_{ij} \cdot \Delta x_j \ (i = 1, 2, \ldots, m). \qquad (3)$$

The variables in the system of linear differential equations (3) are deviations Δx_i from the steady-state values of the concentrations of substances $\bar{x}_i$, and the constant coefficients are quantities of the type

$$b_{ij} = \left.\frac{\partial \dot{x}_i}{\partial x_j}\right|_{\bar{x}_1 \ldots \bar{x}_n} \qquad (i, j = 1, 2, \ldots, m). \qquad (4)$$

composing square matrix $B = \|b_{ij}\|$ (Jacobian) with m rows and m columns.

Substitution of $\dot{x}_i$ from Eq. (2) into Eq. (4) gives the expression for the matrix elements b_{ij} of the Jacobian:

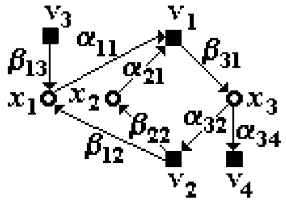
$$b_{ij} = \sum_{r=1}^{R} (\beta_{ir} - \alpha_{ir}) \cdot \frac{\partial v_r}{\partial x_j} = \sum_{r=1}^{R} (\beta_{ir} - \alpha_{ir}) \cdot \alpha_{jr} \cdot k_r x_i^{\alpha_{ir}} \cdot$$

$$x_j^{\alpha_{jr}-1} = \sum_{r=1}^{R} (\alpha_{jr} \cdot \beta_{ir} - \alpha_{jr} \cdot \alpha_{ir}) \cdot \frac{v_r}{x_j}. \qquad (5)$$

Summation in Eq. (5) is performed over all the elementary stages r proceeding with the participation of the substance X_i.

If the system is stabile, deviation Δx_i is damped out with time. When the system is instable, deviation Δx_i increases with time. The system (3) may be solved for:

Table 1. Relationship between the structure of graph and the analytical dependences

Stoichiometric equation of the reaction	Bigraph of the reaction	Rate of change in concentration (x_i) of substance X_i	Kinetic equation for rate (v_r) of elementary stages of the reaction
$\xrightarrow{v_3} \alpha_{11} \cdot X_1 + \alpha_{21} \cdot X_2 \underset{v_2}{\overset{v_1}{\rightleftharpoons}} \beta_{31} \cdot X_3 \xrightarrow{v_4}$		$\dot{x}_1 = \beta_{12} \cdot v_2 + \beta_{13} \cdot v_3 - \alpha_{11} \cdot v_1$ $\dot{x}_2 = \beta_{22} \cdot v_2 - \alpha_{21} \cdot v_1$ $\dot{x}_3 = \beta_{31} \cdot v_1 - \alpha_{32} \cdot v_2 - \alpha_{34} \cdot v_4$	$v_1 = k_1 \cdot x_1^{\alpha_{11}} \cdot x_2^{\alpha_{21}}$ $v_2 = k_2 \cdot x_3^{\alpha_{32}}$ $v_3 = k_3$ $v_4 = k_4 \cdot x_3^{\alpha_{34}}$

$$\Delta x_i = C_i \cdot e^{\lambda t} \qquad (i = 1, 2, ..., m) . \qquad (6)$$

Substitution of the expression for variable (6) in the system of differential equations (3) and corresponding transformations give the system of linear uniform equations:

$$\begin{aligned} &C_1 \cdot (b_{11} - \lambda) + C_2 \cdot b_{12} + ... + C_m \cdot b_{1m} = 0 ; \\ &C_1 \cdot b_{21} + C_2 \cdot (b_{22} - \lambda) + ... + C_m \cdot b_{2m} = 0 ; \\ &. \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \\ &C_1 \cdot b_{m1} + C_2 \cdot b_{m2} + ... + C_m \cdot (b_{mm} - \lambda) = 0 . \end{aligned} \qquad (7)$$

Here unknown quantities are C_i ($i = 1, 2, ..., m$) and the coefficients b_{ij} are determined according to Eq. (5). The system (7) is solvable if determinant (8) is equal to zero:

$$\begin{vmatrix} (b_{11} - \lambda) & b_{12} & . \ . \ . & b_{1m} \\ b_{21} & (b_{22} - \lambda) & . \ . \ . & b_{2m} \\ . & . & . \ . \ . & . \\ b_{m1} & b_{m2} & . \ . \ . & (b_{mm} - \lambda) \end{vmatrix} =$$

$$= \det(B - \lambda \cdot E) = 0, \qquad (8)$$

where B is square matrix (Jacobian) of coefficients b_{ij} ($i, j = 1, 2, ..., m$), E is a unit matrix. Determinant (8) is referred to as *characteristic polynomial* (9) of matrix B:

$$\det(B - \lambda \cdot E) = (-1)^m \cdot \lambda^m + (-1)^{m-1} \cdot \lambda^{m-1} \cdot A_1 +$$

$$+ ... (-1)^{m-k} \cdot \lambda^{m-k} \cdot A_k + ... + A_m = 0 . \qquad (9)$$

There is a subsequent alternation of signs in Eq. (9) at any value of m: $\lambda^m - \lambda^{m-1} A_1 + \lambda^{m-2} A_2 - \lambda^{m-3} A_3 + ... \pm A_m = 0$. If m is even, the plus sign stands before A_m; if m is odd, the minus sign stands before A_m. The characteristic polynomial can be written in the following form:

$$p(\lambda) = \lambda^m + \lambda^{m-1} \cdot a_1 + ... + \lambda^{m-k} \cdot a_k +$$

$$+ ... + a_m = 0 . \qquad (10)$$

According to definition, coefficients a_k are expressed as follows:

$$\begin{aligned} &a_1 = (-1)^1 \sum_{i=1}^{m} b_{ii} ; \\ &. \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \\ &a_k = (-1)^k \sum_{i=1}^{l} M_i ; \\ &. \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \\ &a_m = (-1)^m \cdot \det B . \end{aligned} \qquad (11)$$

From Eqs. (11) it follows that coefficient a_1 with minus sign is equal to the sum of all diagonal elements of matrix B. Coefficients $a_2, a_3, ..., a_{m-1}$ with the corresponding signs are equal to the sums of all diagonal minors of the second order ($M_i = M^{i_1, i_2}_{j_1, j_2}$), third order ($M_i = M^{i_1, i_2, i_3}_{j_1, j_2, j_3}$), and so on. Coefficient a_m is equal to the determinant of matrix B with corresponding sign.

According to definition, the diagonal minor of the k-th order ($M^{i_1, i_2, ..., i_k}_{j_1, j_2, ..., j_k}$) of matrix B is the determinant consisting of elements of matrix B that are located at the intersection of rows $i_1, i_2, ..., i_k$ and columns $j_1, j_2, ..., j_k$, when $i_1 = j_1$, $i_2 = j_2$, ..., $i_k = j_k$. The number of l diagonal minors in the sum (11) for coefficient a_k is the number of combinations from m in k.

Each element b_{ij} is determined according to Eq. (5). Therefore coefficients a_k are the algebraic sums of the following form:

$$a_k = \Sigma \, N \cdot \frac{v_{r_1} ... v_{r_k}}{x_{i_1} ... x_{i_k}} . \qquad (12)$$

The number of multipliers in numerator and denominator of each summand of sum (12) is exactly equal to k. Coefficient N is a combination of stoichiometric coefficients α_{ir} and β_{jr}; summation is being performed over all terms of developed determinants.

When solving characteristic equation (10), we obtain the values of roots $\lambda_1, \lambda_2, ..., \lambda_m$. The general solution of the system (3) has the following form:

$$\Delta x_i = D_{i_1} \cdot e^{\lambda_1 t} + ... + D_{i_m} \cdot e^{\lambda_m t} \ (i = 1, 2, ..., m) . \qquad (13)$$

If all λ_i are negative quantities, the steady-state of the system under study is stable, since deviation Δx_i ($i = 1, 2, ..., m$) from the steady-state concentrations $\bar{x}_i$ ($i = 1, 2, ..., m$) are damped out with time according to the law (13). If only one of the coefficients λ_i is a positive quantity, the steady-state is instable. *Thus, to obtain sufficient prerequisites for instability, we do not need to search for roots λ_i of characteristic polynomial (10); suffice it to analyze the signs of coefficients a_k since it is precisely the signs of coefficients that determine the signs of roots of characteristic polynomial λ_i.*

3. Conditions of appearance of the critical phenomena (bistability and oscillations). If the alteration in some parameters is accompanied by the change in sign of one of the coefficients a_k in Eq. (10) from plus to minus, an originally stable state becomes instable. Ivanova [5] showed that the condition $a_m > 0$ at all values of the concentrations (or concentration parameters) implied the existence of a single steady-state point. This point (state) may be stable or instable. If one of the junior coefficients of Eq. (10) $a_{m-1}, a_{m-2}, ..., a_1$ becomes negative, i.e., provided that $a_m > 0$ and $a_{m-1} < 0$ or $a_{m-2} < 0$, ..., $a_1 < 0$, the single steady-state point inside the region of parameters may be instable. These conditions are the sufficient prerequisite to the existence of a stable limiting cycle near the instable steady-state point, i.e., the prerequisite to initiation of undamped oscillations.

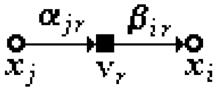
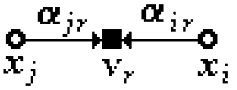
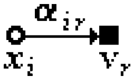
Necessary and sufficient prerequisites to initiation of undamped oscillations may be stated only with regard to the possible steady-state points on the boundary of the region (this is checked rather easily). The region of existence of oscillations is determined by the positive values of concentrations and limited by the conditions of conservation of the sum of definite concentrations. Since the coefficients of the characteristic polynomial are determined with the aid of graph theory, the sufficient prerequisites to initiation of oscillations are stated in terms of graph theory, i.e., through the consideration of the structure of the kinetic scheme of the reaction.

4. Fragments of graph responsible for appearance of critical phenomena. In whole graph of the system of the reactions under study (*m* apices-substances and *r* apices-reactions) we can select a fragment of *k* apices-reactions and *k* apices-substances connected by arrows. The appearance of coefficient a_k of the characteristic polynomial (10) is due to fragments of the *k*-th order of the whole graph of the reactions. The algebraic sum of all the fragments of the *k*-th order determines the sign and value of the coefficient a_k. A fragment is called critical if it gives a negative contribution to the coefficient a_k. The absolute value of a contribution made by the critical fragment is determined by the ratio of its rates v_r and concentrations x_i in the steady-state and can be calculated from Eq. (5).

For each of the diagonal minors of the *k*-th order of Jacobian *B*, which compose coefficient a_k, there is a certain part of the graph. The numbers of the substances involved in this part correspond to the numbers of rows and columns of the corresponding minor. When developing corresponding minors in accordance with the rules of developing determinants, we obtain the algebraic sums of the products of matrix elements of the Jacobian of the type (12). Each product in expression (12) corresponds to a definite subgraph of a fragment of the *k*-th order. For each matrix element b_{ij} determined by Eq. (5) there is a definite element of graph of the reaction: half-pathway, positive or negative pathway. Each of the apices-substances in the subgraph is the origin of one and only one positive (negative) pathway or half-pathway.

Table 2 summarizes graphic elements: positive pathway, negative pathway, and half-pathway that comprise any complex schemes (graphs) of the reactions. Here corresponding matrix elements b_{ij} of the Jacobian calculated from Eq. (5) are also given. Table 3 contains all possible cycles and other combinations of elements corresponding to the product of matrix elements $b_{ij}b_{ji}$. For element b_{ii} of

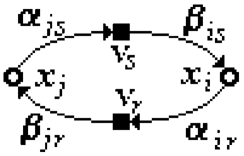
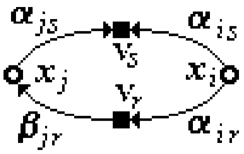
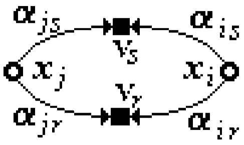
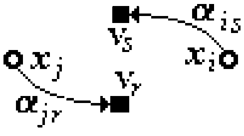
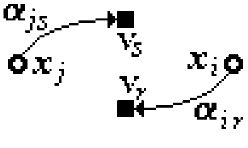
Table 2. Value and sign of matrix elements of the Jacobian $\| b_{ij} \|$ corresponding to the basic elements of a graph of the reactions (positive pathway, negative pathway, and half-pathway)

Graphic presentation of matrix element of Jacobian b_{ij}	Rate of the change in concentration (x_i) of substance X_i	Kinetic equation for rate of reaction following the law of mass action	Sign and value of matrix element of Jacobian $\| b_{ij} \|$, $b_{ij} = \frac{\partial \dot{x}_1}{\partial x_1}$
Positive pathway  	$\dot{x}_i = \beta_{ir} \cdot v_r$	$v_r = k_r \cdot x_j^{\alpha_{jr}}$	$b_{ij} = \alpha_{jr} \cdot \beta_{ir} \cdot \frac{v_r}{x_j}$
Negative pathway  	$\dot{x}_i = -\alpha_{ir} \cdot v_r$	$v_r = k_r \cdot x_j^{\alpha_{jr}} \cdot x_i^{\alpha_{ir}}$	$b_{ij} = -\alpha_{ir} \cdot \alpha_{jr} \cdot \frac{v_r}{x_j}$
Half-pathway  	$\dot{x}_i = -\alpha_{ir} \cdot v_r$	$v_r = k_r \cdot x_i^{\alpha_{ir}}$	$b_{ii} = -\alpha_{ir} \cdot \alpha_{ir} \cdot \frac{v_r}{x_i}$

the Jacobian there are all half-pathways coming out of the substance X_i. For element b_{ij} there are pathways (positive or negative) connecting X_j and X_i, positive direction being the direction from substance X_j to substance X_i. With the above-mentioned relationships between the graphic elements and elements of matrix B we can construct the Jacobian without resort to calculation of derivatives by formula (5) and simplify its development using only the graph of the reactions.

Thus, the search of critical phenomena (oscillations, multistationarity) in the system under study reduces to search of the fragments of a graph determining the negative sign of coefficients of the characteristic polynomial (10). The theoretical approach based on graph theory

Table 3. Graphic presentation and the values of all possible matrix elements of Jacobian $\|b_{ij}\|$ forming the coefficient $a_2 = (-1)^2 \cdot \sum_{k=1}^{l} (b^k_{ii} \cdot b^k_{jj} - b^k_{ij} \cdot b^k_{ji})$ of the characteristic polynomial

Graphic presentation of products of matrix element $b_{ij}b_{ji}$ and $b_{ii}b_{jj}$ of Jacobian $\|b_{ij}\|$	Value and sign of the products of matrix elements $b_{ij}b_{ji}$ and $b_{ii}b_{jj}$ of Jacobian $\|b_{ij}\|$	Contribution of products of matrix elements of Jacobian to the coefficient a_2 at $\|\alpha_{ir}\| = \|\beta_{jr}\| = 1$
Even cycle $b_{ij}b_{ji}$  	$+\alpha_{js}\beta_{is}\alpha_{ir}\beta_{jr}\frac{v_s v_r}{x_j x_i}$	$-\frac{v_s v_r}{x_j x_i}$
Odd cycle $b_{ij}b_{ji}$  	$-\alpha_{js}\alpha_{is}\alpha_{ir}\beta_{jr}\frac{v_s v_r}{x_j x_i}$	$+\frac{v_s v_r}{x_j x_i}$
Even cycle $b_{ij}b_{ji}$  	$+\alpha_{js}\alpha_{is}\alpha_{ir}\alpha_{jr}\frac{v_s v_r}{x_j x_i}$	$-\frac{v_s v_r}{x_j x_i}$
Two half-pathways $b_{ii}b_{jj}$  	$+\alpha_{is}\alpha_{is}\alpha_{jr}\alpha_{jr}\frac{v_s v_r}{x_i x_j}$	$+\frac{v_s v_r}{x_j x_i}$
Two half-pathways $b_{ii}b_{jj}$  	$+\alpha_{js}\alpha_{js}\alpha_{ir}\alpha_{ir}\frac{v_s v_r}{x_j x_i}$	$+\frac{v_s v_r}{x_j x_i}$

allows us, without resort to calculations, to find topology of the critical fragment responsible for instability of the system under certain conditions. Using this method we can "design" or "insert" the critical element in the system of the reactions in order to obtain the desired kinetic regime.

5. Numerical integration. To analyze the kinetic models and determine corresponding kinetic parameters, we found the solution of the system of ordinary linear differential equations using the program Dbsolve developed by I. I. Goryanin (Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia). Equations were composed on the basis of the law of active masses and corresponded to definite kinetic schemes.

RESULTS AND DISCUSSION

It is of interest to discuss the structure of the fragment of a graph of third order that would give a negative contribution in coefficient a_3. At definite values of parameters such a fragment may result in the negative value of a_3 (this is a sufficient prerequisite to existence of critical phenomena). In the subsequent discussion we accept that stoichiometric coefficients take the following values: $\alpha_{ir} = 1$ and $\beta_{jr} = 1$ or 0.

The sign of coefficient a_k is determined by three factors: first, by the sign before the sums in Eqs. (11), second, by the sign before each summand of the sum (11), which is defined by the rules of developing determinants, and lastly by the sign of each element b_{ij} according to Eq. (5) and description in Table 2.

Consider the absolute value, sign, and graphic equivalent of coefficients a_1, a_2, and a_3 of the characteristic polynomial (10). The graphic expression of coefficient a_1 is a set of subgraphs where each substance is an origin of only one half-pathway (the arrows in graph of the reaction have the following form: origin of each arrow is the apex-substance and the end of the each arrow is the apex-reaction proceeding with the participation of the given substance). For all biochemical systems coefficient a_1 is positive. According to Eq. (5) each of the elements b_{ii} comprising a_1 is actually equal to

$$b_{ii} = \sum_r \left(-\alpha_{ir} \cdot \alpha_{ir} \cdot \frac{v_r}{x_i}\right),$$

where summation is performed over all stages proceeding with the participation of the substance X_i.

Substitution of the quantity b_{ii} with its sign in expression (11) for a_1 shows that the following inequality is always valid: $a_1 > 0$.

According to definition (11), coefficient a_2 is equal to the sum of all diagonal minors of the second order of the Jacobian of the system of m-th order. The rules for developing determinants give coefficient a_2 as an algebraic sum of paired products $+b_{ii}b_{jj}$ and $-b_{ij}b_{ji}$. The graphic expression of $+b_{ii}b_{jj}$ is a set of all subgraphs where each two substances X_i and X_j are the origin of only one half-pathway. According to definition (5), each element b_{ii} and b_{jj} is negative. Therefore their product is always positive. The graphic expression of the product $b_{ij}b_{ji}$ is a set of subgraphs forming a cycle, which connects the substances X_i and X_j. The closed consequence of even number of negative pathways and/or any number of positive pathways with the same direction is referred to as *even cycle*, otherwise cycle is *odd* [5, 6].

Table 3 summarizes all graphic structures corresponding to coefficient a_2. After the substitution of the values of matrix elements the sum of the negative products $-b_{ij}b_{ji}$ corresponding to even cycles and the positive products $+b_{ii}b_{jj}$ will give zero since the absolute values of $-b_{ij}b_{ji}$ and $+b_{ii}b_{jj}$ are identical. Thus, for most of biochemical systems coefficient a_2 is equal to zero or has positive sign.

According to definition (11), coefficient a_3 is equal to the sum of all diagonal minors of the third order of the Jacobian of the system of m-th order. For each minor $M_{i,j,k}^{i,j,k}$ there are parts of whole graph involving three substances, namely X_i, X_j, and X_k, and all pathways and half-pathways incoming and issuing out of each substance. After developing each minor we obtain the algebraic sum of triple products of elements of the Jacobian. Each product corresponds to a definite subgraph involving substances X_i, X_j, and X_k.

Consider for example minor $M_{1,2,3}^{1,2,3}$ for the part of the whole graph involving substances X_1, X_2, and X_3. After developing the minor we obtain: $M_{1,2,3}^{1,2,3} = b_{11}b_{22}b_{33} + b_{21}b_{32}b_{13} + b_{31}b_{23}b_{32} - b_{11}b_{23}b_{32} - b_{22}b_{13}b_{31} - b_{33}b_{12}b_{21}$. In this case the product $b_{11}b_{22}b_{33}$ combines all half-pathways issuing out of each substance one by one. For products $b_{12}b_{23}b_{32}$ and $b_{13}b_{32}b_{12}$ there are cycles passing through all three substances. In the first case the cycle passes through substances in the direction from X_1 to X_3. In the second case the cycle passes in the opposite direction. For products with minus sign (according to the rules of developing determinants) $-b_{11}b_{23}b_{32}$, $-b_{22}b_{13}b_{31}$, and $-b_{33}b_{12}b_{21}$ there are half-pathway issuing out of the substance X_1 and a cycle passing through the substances X_2 and X_3 for first product and so on. Taking into account the sign in determination of coefficient a_3 in Eq. (11) and signs at developing minors of the third order, we can conclude that only subgraphs possessing a positive value give a negative contribution in the coefficient.

Table 4 demonstrates the basic critical fragments of the third order. To illustrate, table shows the full analysis of one of the critical fragments. Eliminating subgraphs having the same absolute value but different signs (Table 5) and taking into account the sign of coefficient a_3 according to determination (11), we obtain that the value of the given fragment is equal to $-v_1v_2v_3/x_1x_2x_3$. The non-eliminated subgraph corresponding to this value is indicated in Table 5 by bold lines. As a part of the complex scheme (graph) of the reactions, the critical fragment gives one of the summands in the sum (12). Because of the existence of negative summands in the sum (12) coefficient a_3 may have negative value at definite values of v_i and x_i. Thus, the given fragments may initiate oscillations of the concentrations of the reagents in the system.

Consider as an example the system of interconversions of four substances involving one of the discovered critical fragments of type (I) presented in Table 4:

Table 4. Some types of the critical fragment of the third order

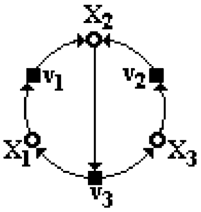
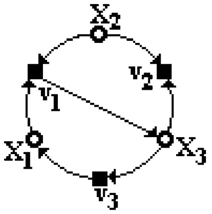
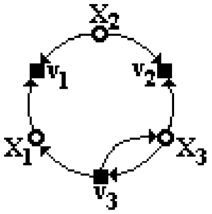
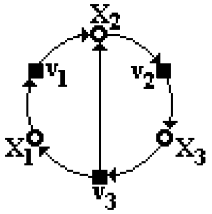
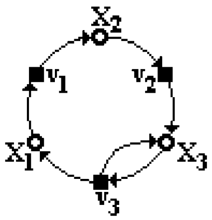
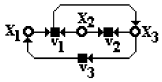
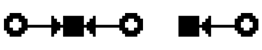
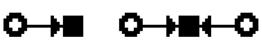
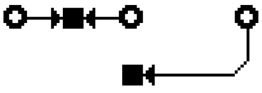
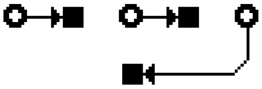
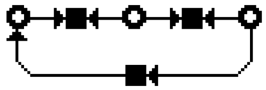
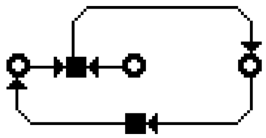
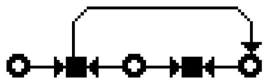
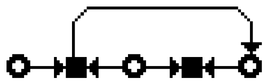
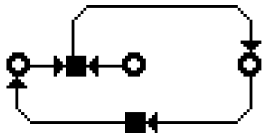
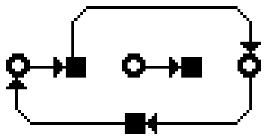
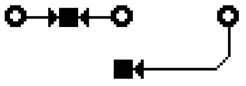
Critical fragment of the third order	Rate of change in concentration (x_i) of substance X_i	Jacobian $\|b_{ij}\|$, $b_{ij} = \dfrac{\partial \dot{x}_i}{\partial x_j}$
I 	$\dot{x}_1 = k_3 \cdot x_2 - k_1 \cdot x_1$ $\dot{x}_2 = k_1 \cdot x_1 + k_2 \cdot x_3 - k_3 \cdot x_2$ $\dot{x}_3 = k_3 \cdot x_2 - k_2 \cdot x_3$	$\begin{vmatrix} -\frac{v_1}{x_1} & \frac{v_3}{x_2} & 0 \\ \frac{v_1}{x_1} & -\frac{v_3}{x_2} & \frac{v_2}{x_3} \\ 0 & \frac{v_3}{x_2} & -\frac{v_2}{x_3} \end{vmatrix}$
II 	$\dot{x}_1 = k_3 \cdot x_3 - k_1 \cdot x_1 \cdot x_2$ $\dot{x}_2 = -k_1 \cdot x_1 \cdot x_2 - k_2 \cdot x_2 \cdot x_3$ $\dot{x}_3 = k_1 \cdot x_1 \cdot x_2 - k_2 \cdot x_2 \cdot x_3 - k_3 \cdot x_3$	$\begin{vmatrix} -\frac{v_1}{x_1} & -\frac{v_1}{x_2} & \frac{v_3}{x_3} \\ -\frac{v_1}{x_1} & -\frac{v_1}{x_2}-\frac{v_2}{x_2} & -\frac{v_2}{x_3} \\ \frac{v_1}{x_1} & \frac{v_1}{x_2}-\frac{v_2}{x_2} & -\frac{v_2}{x_3}-\frac{v_3}{x_3} \end{vmatrix}$
III 	$\dot{x}_1 = k_3 \cdot x_3 - k_1 \cdot x_1 \cdot x_2$ $\dot{x}_2 = -k_1 \cdot x_1 \cdot x_2 - k_2 \cdot x_2 \cdot x_3$ $\dot{x}_3 = -k_2 \cdot x_2 \cdot x_3$	$\begin{vmatrix} -\frac{v_1}{x_1} & -\frac{v_1}{x_2} & \frac{v_3}{x_3} \\ -\frac{v_1}{x_1} & -\frac{v_1}{x_2}-\frac{v_2}{x_2} & -\frac{v_2}{x_3} \\ 0 & -\frac{v_2}{x_2} & -\frac{v_2}{x_3} \end{vmatrix}$
IV 	$\dot{x}_1 = k_3 \cdot x_3 - k_1 \cdot x_1$ $\dot{x}_2 = k_1 \cdot x_1 - k_2 \cdot x_2 + k_3 \cdot x_3$ $\dot{x}_3 = k_2 \cdot x_2 - k_3 \cdot x_3$	$\begin{vmatrix} -\frac{v_1}{x_1} & 0 & \frac{v_3}{x_3} \\ \frac{v_1}{x_1} & -\frac{v_2}{x_2} & \frac{v_3}{x_3} \\ 0 & \frac{v_2}{x_2} & -\frac{v_3}{x_3} \end{vmatrix}$
V 	$\dot{x}_1 = k_3 \cdot x_3 - k_1 \cdot x_1$ $\dot{x}_2 = k_1 \cdot x_1 - k_2 \cdot x_2$ $\dot{x}_3 = k_2 \cdot x_2 - k_3 \cdot x_3 + k_3 \cdot x_3$	$\begin{vmatrix} -\frac{v_1}{x_1} & 0 & \frac{v_3}{x_3} \\ \frac{v_1}{x_1} & -\frac{v_2}{x_2} & 0 \\ 0 & \frac{v_2}{x_2} & -\frac{v_3}{x_3}+\frac{v_3}{x_3} \end{vmatrix}$

Table 5. Value and contribution of the critical fragment (II) 

to the coefficient $a_3 = (-1)^3 \cdot \Sigma\,(M^{1,2,3}_{1,2,3} + \ldots + M^{i_1,i_2,i_3}_{i_1,i_2,i_3})$ of the characteristic polynomial

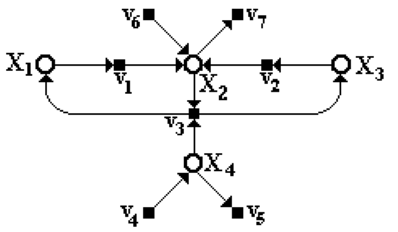
Jacobian $\|b_{ij}\|$ of fragment	Subgraphs of fragment and corresponding values of products $b_{ij}b_{kl}b_{nm}$ of elements of Jacobian		Contribution of fragment to coefficient a_3
$b_{11}\,b_{22}\,b_{33}$ ■□□ □■□ □□■		$-\dfrac{v_1 \cdot v_1 \cdot v_2}{x_1 \cdot x_2 \cdot x_3}$	$-\dfrac{v_1 \cdot v_2 \cdot v_3}{x_1 \cdot x_2 \cdot x_3}$
		$-\dfrac{v_1 \cdot v_2 \cdot v_2}{x_1 \cdot x_2 \cdot x_3}$	
		$-\dfrac{v_1 \cdot v \cdot v_3}{x_1 \cdot x_2 \cdot x_3}$	
		$-\dfrac{v_1 \cdot v_2 \cdot v_3}{x_1 \cdot x_2 \cdot x_3}$	
$b_{21}\,b_{13}\,b_{32}$ □□■ ■□□ □■□		$\dfrac{v_1 \cdot v_2 \cdot v_3}{x_1 \cdot x_2 \cdot x_3}$	
		$-\dfrac{v_1 \cdot v_1 \cdot v_3}{x_1 \cdot x_2 \cdot x_3}$	
$b_{31}\,b_{12}\,b_{23}$ □■□ □□■ ■□□		$\dfrac{v_1 \cdot v_1 \cdot v_2}{x_1 \cdot x_2 \cdot x_3}$	
$-b_{11}\,b_{23}\,b_{32}$ ■□□ □□■ □■□		$\dfrac{v_1 \cdot v_2 \cdot v_2}{x_1 \cdot x_2 \cdot x_3}$	
		$-\dfrac{v_1 \cdot v_1 \cdot v_2}{x_1 \cdot x_2 \cdot x_3}$	
$-b_{22}\,b_{13}\,b_{31}$ □□■ □■□ ■□□		$\dfrac{v_1 \cdot v_1 \cdot v_3}{x_1 \cdot x_2 \cdot x_3}$	
		$\dfrac{v_1 \cdot v_2 \cdot v_3}{x_1 \cdot x_2 \cdot x_3}$	
$-b_{33}\,b_{12}\,b_{21}$ □■□ ■□□ □□■		$\dfrac{v_1 \cdot v_1 \cdot v_2}{x_1 \cdot x_2 \cdot x_3}$	
		$\dfrac{v_1 \cdot v_1 \cdot v_3}{x_1 \cdot x_2 \cdot x_3}$	

$$\begin{aligned}
&X_1 \xrightarrow{v_1} X_2\,;\\
&X_3 \xrightarrow{v_2} X_2\,;\\
&X_2 + X_4 \xrightarrow{v_3} X_1 + X_3\,; \qquad\qquad (14)\\
&\xrightleftharpoons[v_7]{v_6} X_2\,;\\
&\xrightleftharpoons[v_5]{v_4} X_4\,.
\end{aligned}$$

For the system of the chemical equations (14) there is the system of linear differential equations composed on the basis of the law of mass action:

$$\begin{aligned}
&\dot{x}_1 = k_3 \cdot x_2 \cdot x_4 - k_1 \cdot x_1\,;\\
&\dot{x}_2 = k_1 \cdot x_1 + k_3 \cdot x_4 + k_6 - k_3 \cdot x_2 \cdot x_4 - k_7 \cdot x_2\,; \qquad (15)\\
&\dot{x}_3 = k_3 \cdot x_2 \cdot x_4 - k_2 \cdot x_3\,;\\
&\dot{x}_4 = k_4 - k_5 \cdot x_4 - k_3 \cdot x_2 \cdot x_4\,.
\end{aligned}$$

The system under discussion is equivalent to graph:



The Jacobian of the linearized system for this graph has the following form:

$$\left\|b_{ij}\right\| = \begin{vmatrix} -\dfrac{v_1}{x_1} & \dfrac{v_3}{x_2} & 0 & \dfrac{v_3}{x_4} \\ \dfrac{v_1}{x_1} & -\dfrac{v_3}{x_2} - \dfrac{v_7}{x_2} & \dfrac{v_2}{x_3} & -\dfrac{v_3}{x_4} \\ 0 & \dfrac{v_3}{x_2} & -\dfrac{v_2}{x_3} & \dfrac{v_3}{x_4} \\ 0 & -\dfrac{v_3}{x_2} & 0 & -\dfrac{v_3}{x_4} - \dfrac{v_5}{x_4} \end{vmatrix} \qquad (16)$$

The corresponding characteristic polynomial is written as follows:

$$\lambda^4 + a_1 \cdot \lambda^3 + a_2 \cdot \lambda^2 + a_3 \cdot \lambda + a_4 = 0\,. \qquad (17)$$

The sufficient prerequisite to existence of oscillation in system (15) is positive sign of the superior coefficient $a_4 > 0$ and negative sign of any one of the junior coefficients in Eq. (17) ($a_1 < 0$, $a_2 < 0$, or $a_3 < 0$). It was shown previously that for most chemical systems including the system under discussion coefficients a_1 and a_2 are always positive. Thus, the sufficient prerequisite to existence of oscillation in a system of the fourth order has the following form: $a_3 < 0$ and $a_4 > 0$. To illustrate, we shall give the values of all coefficients of Eq. (17).

According to definition (11), the coefficient a_1 is a sum of diagonal elements of Jacobian (16) with minus sign:

$$a_1 = -\sum_{i=1}^{4} b_{ii} = \frac{v_1}{x_1} + \frac{v_3}{x_2} + \frac{v_7}{x_2} + \frac{v_2}{x_3} + \frac{v_3}{x_4} + \frac{v_5}{x_4}\,. \qquad (18)$$

Coefficients a_2 and a_3 are respectively the sum of six and four diagonal minors of matrix (16) with corresponding common sign. If we develop determinants (minors) of the second and third order, take into account all the signs and take all necessary reductions, the following expressions can be obtained:

$$\begin{aligned}
a_2 &= (-1)^2 \cdot (M_{1,2}^{1,2} + M_{1,3}^{1,3} + M_{1,4}^{1,4} + M_{2,3}^{2,3} + M_{2,4}^{2,4} + M_{3,4}^{3,4}) =\\
&= \frac{v_1 \cdot v_7}{x_1 \cdot x_2} + \frac{v_1 \cdot v_2}{x_1 \cdot x_3} + \frac{v_1 \cdot v_3}{x_1 \cdot x_4} + \frac{v_1 \cdot v_5}{x_1 \cdot x_4} + \frac{v_7 \cdot v_2}{x_2 \cdot x_3} +\\
&+ \frac{v_7 \cdot v_3}{x_2 \cdot x_4} + \frac{v_7 \cdot v_5}{x_2 \cdot x_4} + \frac{v_3 \cdot v_5}{x_2 \cdot x_4}\,; \qquad (19)
\end{aligned}$$

$$\begin{aligned}
a_3 &= (-1)^3 \cdot (M_{1,2,3}^{1,2,3} + M_{2,3,4}^{2,3,4} + M_{1,3,4}^{1,3,4} + M_{1,2,4}^{1,2,4}) =\\
&= -\frac{v_1 \cdot v_2 \cdot v_3}{x_1 \cdot x_2 \cdot x_3} + \frac{v_1 \cdot v_2 \cdot v_7}{x_1 \cdot x_2 \cdot x_3} + \frac{v_7 \cdot v_2 \cdot v_3}{x_2 \cdot x_3 \cdot x_4} +\\
&+ \frac{v_7 \cdot v_2 \cdot v_5}{x_2 \cdot x_3 \cdot x_4} + \frac{v_1 \cdot v_2 \cdot v_3}{x_1 \cdot x_3 \cdot x_4} + \frac{v_1 \cdot v_2 \cdot v_5}{x_1 \cdot x_3 \cdot x_4} +\\
&+ \frac{v_1 \cdot v_7 \cdot v_3}{x_1 \cdot x_2 \cdot x_4} + \frac{v_1 \cdot v_7 \cdot v_5}{x_1 \cdot x_2 \cdot x_4}\,. \qquad (20)
\end{aligned}$$

Coefficient a_4 is written as follows:

$$\begin{aligned}
a_4 &= (-1)^4 \cdot \det B = \frac{v_1 \cdot v_2 \cdot v_3 \cdot v_7}{x_1 \cdot x_2 \cdot x_3 \cdot x_4} +\\
&+ \frac{v_1 \cdot v_2 \cdot v_5 \cdot v_7}{x_1 \cdot x_2 \cdot x_3 \cdot x_4} - \frac{v_1 \cdot v_2 \cdot v_3 \cdot v_5}{x_1 \cdot x_2 \cdot x_3 \cdot x_4}\,. \qquad (21)
\end{aligned}$$

Analysis of Eqs. (18) and (19) show that coefficients a_1 and a_2 are positive. Coefficients a_3 and a_4, apart from the positive contributions, have negative contributions caused by the critical fragment of type (I). Consider the values of parameters and variables which provide the sufficient prerequisites to existence of oscillations ($a_3 < 0$ and $a_4 > 0$).

From Eq. (21) it follows that $a_4 > 0$, if $v_7 > v_5$ or, after substitution of the corresponding values of the rates of the reactions, $x_2/x_4 > k_5/k_7$. Analogously, substituting the values of the rates of the reactions in Eq. (20) and applying simple transformations, we obtain that $a_3 < 0$, if condition (22) is fulfilled:

$$1 > \frac{k_7}{k_3}\cdot\frac{1}{x_4} + \frac{k_7}{k_1}\cdot\frac{x_2}{x_4} + \frac{k_5}{k_1}\cdot\frac{k_7}{k_3}\cdot\frac{1}{x_4} + \frac{x_2}{x_4} + \frac{k_5}{k_3}\cdot\frac{1}{x_4} + \frac{k_7}{k_2}\cdot\frac{x_2}{x_4} + \frac{k_5}{k_2}\cdot\frac{k_7}{k_3}\cdot\frac{1}{x_4}. \tag{22}$$

Setting the right parts of the system of differential equation (15) to zero, we find the steady-state values of

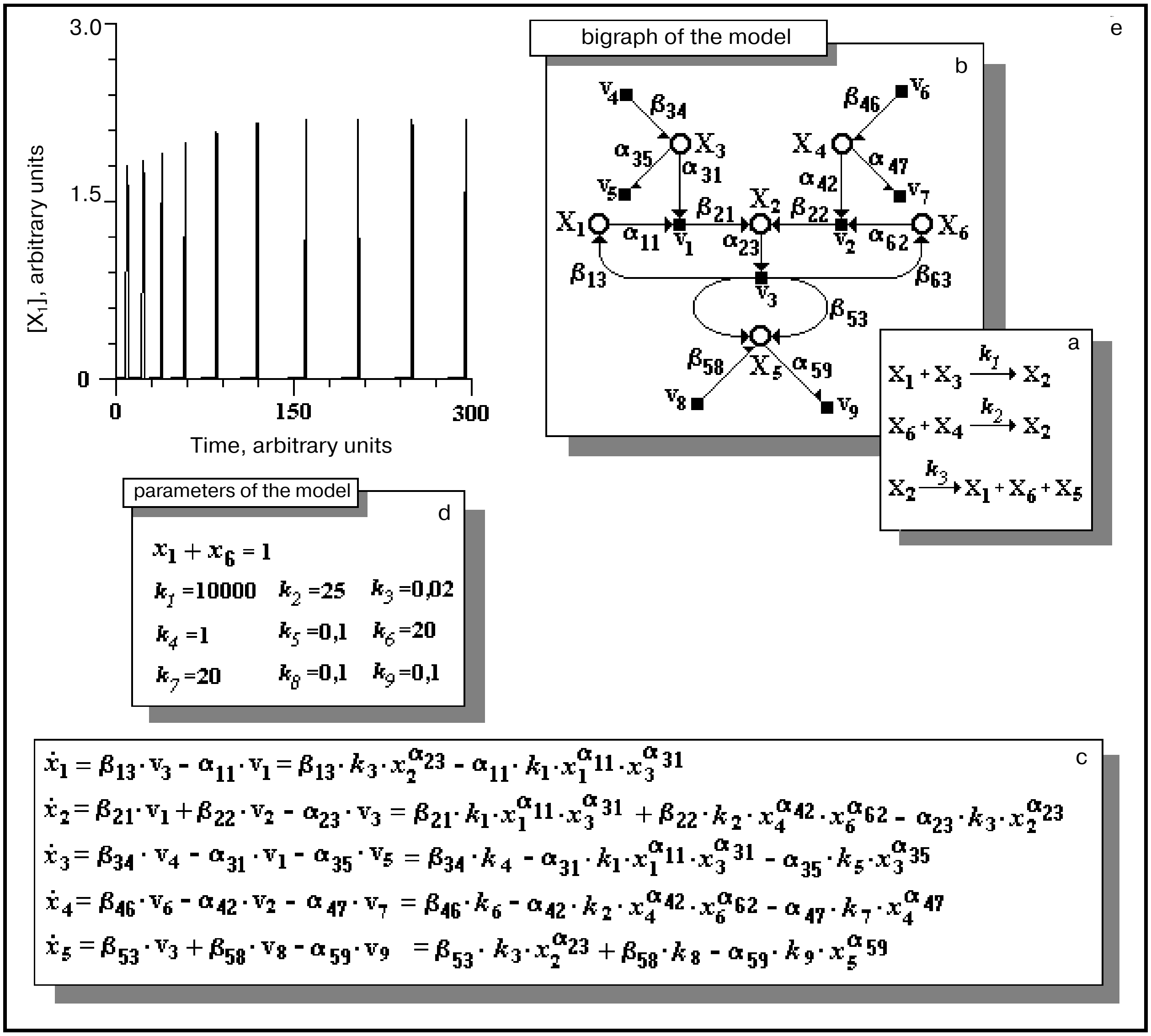


Fig. 1. Mathematical model of the sequence of the reactions involving the critical fragment of the third order responsible for initiation of oscillations of the concentrations of the reagents: a) the sequence of interconversions of the substances X_1, X_2, X_3, X_4, X_5, and X_6 (k_1, k_2, and k_3 the rate constants of the second and first order, respectively); b) bigraph of the reactions (symbols **O** and ■ refer to the substance X_i and the rate of the elementary stage v_r, respectively; α_{ir} and β_{ir} are the substrate and product stoichiometric coefficients, respectively); c) the system of the fifth order of ordinary differential equations composed on the basis of the law of mass action; d) parameters of the model (dimensions of the constants and concentrations of substances are given in arbitrary units); e) undamped relaxation oscillations of the reagent X_1 calculated by numerical solving of the system of differential equations with the aid of program Dbsolve developed by I. I. Goryanin.

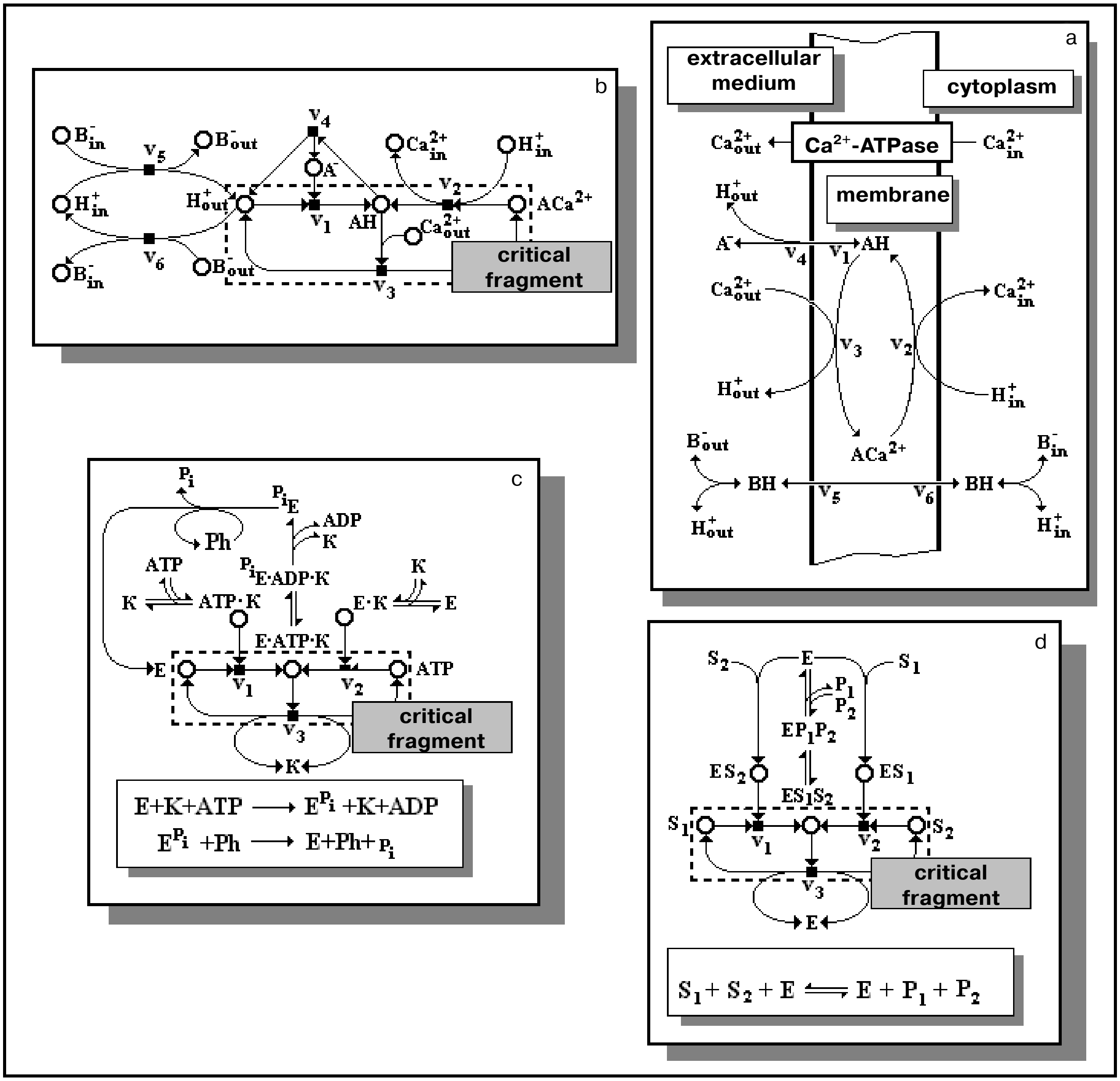


Fig. 2. Several biochemical systems involving the minimum critical fragment of the third order responsible for initiation of oscillations of the concentrations of the reagents: a) scheme of transport of H^+ and Ca^{2+} through the erythrocyte membrane with the participation of ionophore A23187 (A and A^- are the free and ionized forms of ionophore A23187; ACa^{2+} and AH are the forms of ionophore A23187 bound to Ca^{2+} and H^+; B^- and BH are the ionized and protonated forms of carbanions, respectively (pyruvate, lactate, etc.)); b) graph of the reactions for transport of ions through the cell membrane (symbols **O** and ■ refer to the substance X_i and the rate of the elementary stage v_r, respectively); c) phosphorylation of a protein (E, K, and Ph are the protein/enzyme, protein kinase, and phosphatase, respectively); d) simplest mechanism of two-substrate reaction (S, P, and E are substrate, product, and enzyme, respectively). For the sake of simplicity, in schemes (c) and (d) the bigraph is represented only by the part of the scheme that contains the critical fragment.

the rate constants. After substitution of these values in the relationship (22) and corresponding transformations we obtain the following relationship:

$$1 > 1 + \frac{k_6 \cdot x_2}{k_3 \cdot x_4} + \ldots \qquad (23)$$

Since variables and the rate constants are always positive, under steady-state conditions the inequality (23) is not valid at any of the values of variables and parameters. Thus, the system under discussion does not contain a limiting cycle and oscillations are missed. This example shows that if the system contains the critical fragment the above-mentioned inequalities for coefficients may break down. To determine the region of the change in the parameters of the system where oscillations may be initiated, we should carry out an analytical consideration.

Figure 1a shows as an example the kinetic scheme involving the interconversion of six substances. The corresponding graph (Fig. 1b) includes the above-mentioned critical fragment of type (I). For the system of the reactions presented in Fig. 1a with regard to the single relationship of the material balance there is the system of differential equations of the fifth order (Fig. 1c). The numerical solution of the given system showed that undamped relaxation oscillations were initiated (Fig. 1e) in the indicated region of the change in the values of parameters (Fig. 1d).

Figure 2 shows examples of biochemical systems involving the above-mentioned critical fragment (I) of the third order. The scheme presented in Fig. 2 (a and b) was discussed in [7] to explain the oscillations during the transport of H^+ and Ca^{2+} performed by the single transporter through the erythrocyte membrane in opposite directions. Two hypothetical schemes depicted in Fig. 2 (c and d) correspond to the large class of naturally occurring reactions. Among these are the reactions involving phosphorylation of proteins (Fig. 2c) and two-substrate reactions (Fig. 2d).

When analyzing such systems, the main difficulty is connected with the selection of parameters of the system responsible for its instability. It is conceivable that the critical values of parameters fall in a "non-physiological region" and cannot be realized in a real system under *in vivo* conditions. Nevertheless, the approach elaborated in the present work allow us to design systems with desired properties and to model the biotechnological/chemical processes on the basis of the study of the systems working in the corresponding critical regime.

This study was funded by the Russian Foundation for Basic Research (grant 01-04-48346) and INTAS (grant 1504).

REFERENCES

1. Goldstein, B. N., and Volkenstein, M. V. (1968) *Doklady AN SSSR*, **178**, 386-388.
2. Clarke, B. L. (1980) *Adv. Chem. Phys.*, **43**, 1-215.
3. Ivanova, A. N. (1979) *Kinetika i Kataliz*, **20**, 1019-1028.
4. Goldstein, B. N., and Ivanova, A. N. (1987) *FEBS Lett.*, **217**, 212-215.
5. Ivanova, A. N. (1984) *Critical Phenomena in Complex Chemical Systems*: Doctoral dissertation [in Russian], Chernogolovka.
6. Goldstein, B. N. (1989) *Kinetic Graphs in Enzymology* [in Russian], Nauka, Moscow.
7. Goldstein, B. N., Kholmukhamedov, E. L., Ivanova, A. N., and Furman, G. A. (1987) *Mol. Biol. (Moscow)*, **21**, 132-139.