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A Theoretical Graph Method for Search and Analysis of Critical Phenomena in Biochemical Systems. II. Kinetic Models of Biochemical Oscillators Including Two and Three Substances

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Abstract—Four kinetic models of hypothetical complex reactions containing minimal two-substance or three-substance oscillators were constructed on the basis of the graphical rules suggested in the preceding work. The kinetic models are thought to be a part of one of four general biochemical systems: 1) system of mutual protein phosphorylation/dephosphorylation; 2) autophosphorylation of multisubunit protein; 3) association/dissociation of proteins or protein-containing structures during protein–protein or protein–ligand interaction; and 4) two-substrate enzymatic reaction with substrate inhibition by one substrate. Graphical rules of oscillator association with surrounding medium were considered. The graphical criteria of the oscillation generator elimination and criteria of oscillation damping were obtained. Both damped and undamped oscillations of reaction components were obtained by numerical integration of the mathematical models of these reactions. The areas of changes of model parameters and variables, within which the oscillations exist, were found.

Key words: graph, oscillations, mathematical simulation

A theoretical graph method for search and analysis of critical phenomena (trigger behavior, multistationary character, and concentration oscillations) in complex biochemical systems was suggested in the first part of this series [1]. It was shown that there was a countable set of kinetic schemes combining two, three, four, etc. substances determining the experimentally observed oscillations in chemical or biochemical systems. Within the framework of this approach, any complex biochemical system can be divided into a proper oscillator (generator) and surrounding medium. The oscillator in this case is a negative graph of order $\boldsymbol{k}$ (connected set of $\boldsymbol{k}$ substances and $\boldsymbol{k}$ reactions responsible for generation of oscillations).

The goal of this work was to consider some graphical rules describing oscillator connections with surrounding medium. An additional goal of this work was to consider four mathematical models of the reaction system containing generators of concentration oscillations. Each model was brought into correspondence with a certain generalized biochemical system, a part of the system being represented by an oscillator including two or three substances and described in the preceding work [1].

METHODS OF INVESTIGATION

Numerical integration of mathematical models and determination of corresponding kinetic parameters was performed by solving a set of ordinary linear differential equations using the Dbsolve computer program developed by I. I. Goryanin (Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia). The equations were compiled based on the law of mass action in accordance with corresponding kinetic schemes.

RESULTS AND DISCUSSION

Certain negative graphs of order $\boldsymbol{k}$ (oscillators) considered in the first part of this series [1] constitute a fraction of the graphs of systems in which oscillations of reaction components were measured experimentally or observed by mathematical simulation. Let us consider two well-known mathematical models of chemical oscillators described by a set of two simultaneous differential equations. These are models of Lotka–Volterra [2, 3] and Prigogine–Lefever (Brussellator) [4].

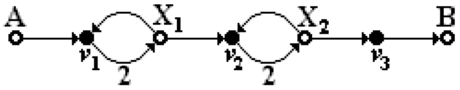
The Lotka–Volterra model is a system of three reactions (**v_1**, **v_2**, and **v_3**) involving two substances (X_1 and X_2)

$$\begin{aligned} &A + X_1 \xrightarrow{v_1} 2 \cdot X_1; \\ &X_1 + X_2 \xrightarrow{v_2} 2 \cdot X_2; \\ &X_2 \xrightarrow{v_3} B, \end{aligned} \tag{1}$$

which are described by a set of simultaneous second order differential equations

$$\begin{aligned} \dot{x}_1 &= k_1 \cdot A \cdot x_1 - k_2 \cdot x_1 \cdot x_2; \\ \dot{x}_2 &= k_2 \cdot x_1 \cdot x_2 - k_3 \cdot B \cdot x_2. \end{aligned} \tag{2}$$

Reaction system (1) includes two autocatalytic stages: autocatalytic supply of substance X_1 at a rate **v_1** from an infinitely large reservoir A and autocatalytic interaction of substance X_2 with substance X_1 (reaction rate, **v_2**). Product X_2 of reaction **v_2** is removed from the system with rate **v_3** to an infinitely large reservoir B. Mechanism (1) includes only direct reactions **v_1**, **v_2**, and **v_3** and assumes infinitely large chemical affinity. Mechanism (1) and set of two simultaneous differential equations (2) correspond to graph **G_1**.



G_1

Graph **G_1** contains two graphical elements (+)-loop, which correspond to autocatalytic stages **v_1** and **v_2** of mechanism (1). In accordance with the rules suggested in the preceding work [1], graph **G_1** gives rise to expressions for calculation of the value and sign of the coefficients **a_1** and **a_2** of the characteristic second order polynomial. Let the right parts of the set of two simultaneous differential equations (2) be equal to zero. Then, the stationary concentrations of substances X_1 and X_2 are:

$$\bar{x}_1 = A \cdot \frac{k_1}{k_4},$$

$$\bar{x}_2 = \frac{B}{A} \cdot \frac{k_2 \cdot k_4}{k_1 \cdot k_3}.$$

Substitution of the found parameters of the stationary point to corresponding equations for calculation of coefficients a_k (**k** = 1, 2, ..., **m**) [1] gives:

$$a_1 = -\frac{v_1}{x_1} + \frac{v_2}{x_1} - \frac{v_2}{x_2} + \frac{v_3}{x_2} = 0 \ , \tag{3}$$

$$a_2 = \frac{v_1 v_2}{x_1 x_2} + \frac{v_2 v_3}{x_1 x_2} - \frac{v_1 v_3}{x_1 x_2} = k_1 \cdot k_3 \cdot A \cdot B \ . \tag{4}$$

It is well known that values of coefficients **a_1** = 0 and **a_2** > 0 over the whole area of changes of system parameters and variables in case of models of so-called two-dimensional oscillators (Lotka, Brussellator, van-der-Pol, Poincare, etc.) correspond to purely imaginary values of roots $\lambda_{1,2}$ of the characteristic polynomial. This gives rise to oscillations of variables X_1, X_2 and a stationary point of the type center in two-dimensional phase space. It is obvious that coefficient **a_1** is equal to zero only if Eq. (3) contains both positive and negative terms. Positive values correspond to elements half-pathway and (–)-loop, whereas negative values correspond only to elements (+)-loop (autocatalysis, $\beta_{ir} > \alpha_{ir}$). Thus, the criterion of existence of a stationary point of the type center in two-dimensional systems is the presence of at least one graphical element (+)-loop in the complete graph of the system.

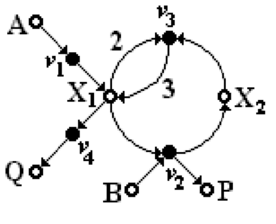
Brussellator is the system of reactions:

$$\begin{aligned} &A \xrightarrow{k_1} X_1, \\ &B + X_1 \xrightarrow{k_2} X_2 + P, \\ &2 \cdot X_1 + X_2 \xrightarrow{k_3} 3 \cdot X_1, \\ &X_1 \xrightarrow{k_4} Q. \end{aligned} \tag{5}$$

System (5) is described by the following set of the second order simultaneous differential equations:

$$\begin{aligned} \dot{x}_1 &= k_1 \cdot A - k_2 \cdot B \cdot x_1 - k_4 \cdot x_1 + k_3 \cdot x_1^2 \cdot x_2 \ , \\ \dot{x}_2 &= k_2 \cdot B \cdot x_1 - k_3 \cdot x_1^2 \cdot x_2 \end{aligned} \tag{6}$$

and graph **G_2**.



G_2

Assuming that the right parts of Eq. (6) are equal to zero, the value of the single stationary point can be found:

$$\bar{x}_1 = A \cdot \frac{k_1}{k_4},$$

$$\overline{x}_2 = \frac{B}{A}\cdot\frac{k_2\cdot k_4}{k_1\cdot k_3}.$$

Graph $\boldsymbol{G_2}$ can be used to set up equations for calculation of coefficients $\boldsymbol{a_1}$, $\boldsymbol{a_2}$. Substitution of the stationary point values into this equation gives:

$$a_1 = \frac{v_2}{x_1} + \frac{v_4}{x_1} + \frac{v_3}{x_2} - 2\cdot\frac{v_3}{x_1} = k_4 + A^2\cdot\frac{k_1^2\cdot k_3}{k_4^2} - B\cdot k_2, \quad (7)$$

$$a_2 = \frac{v_3 v_4}{x_1 x_2} = A\cdot\frac{k_1^2\cdot k_3}{k_4} > 0\,. \quad (8)$$

Positive value of leading coefficient $\boldsymbol{a_2}$ at any values of system parameters provides the uniqueness of the stationary point. The area of changes in system parameters, within which the following condition is met $\boldsymbol{a_1} > 0$ ($\boldsymbol{a_1} < 0$), in two-dimensional phase space corresponds to an asymptotically stable (unstable) stationary point of the type node/focus. Undamped oscillations are generated in this system only if the system parameters correspond to a single stationary point that is unstable and encircled by a stable limiting cycle. This condition is met in case of alternating-sign values of coefficient $\boldsymbol{a_1}$, which, like in the case considered above, are determined by the presence of a graphical element (+)-loop in the complete graph of the system.

The models of Prigogine–Lefever and Lotka–Volterra considered above, as well as all two-dimensional oscillators, whose mechanisms can be represented as a graph, contain graphical element loop. The presence of this graphical element is a sufficient condition for existence of oscillation of components of these systems. Moreover, other models of chemical oscillators of higher order described in the literature (oregonator [5], Bray reaction of catalytic degradation of hydrogen peroxide [6], peroxidase reaction (EC 1.11.1.7) [7], etc.) contain graphical element loop as a destabilization component.

Because negative graph of order $\boldsymbol{k}$ (oscillator) is a part of the complete graph of a complex reaction involving X_i ($i = 1, 2, ..., \boldsymbol{n}$) substances described by $\boldsymbol{n - m}$ balance equations ($\boldsymbol{n > m > k}$), there should be at least one output half-pathway emerging from at least one substance node belonging to this negative graph. Output half-pathways may either belong to the pathways connecting negative graph with the other parts of the complete graph of the system or correspond to the reactions of removal of given substance from the system. Let these half-pathways be called **connections** of negative graph of order $\boldsymbol{k}$ with **surrounding medium**. The surrounding medium in this case is assumed to be represented either by the rest of the system or/and another metabolic system connected to the initial system through one of its metabolites.

Under stationary conditions, the right parts of the simultaneous differential equations of the mathematical models of biochemical systems are equal to zero. This fact is graphically illustrated by the following obvious rule: **for any node X_i ($i = 1, 2, ..., n$) under stationary conditions the algebraic sum of all emerging and incident half-pathways multiplied by corresponding weight (stoichiometric coefficient) is equal to zero (Rule 10)**. This statement allows the graphical rule of connections between negative graph of order $\boldsymbol{k}$ (oscillator) with its surrounding to be formulated. In the general case, all or some substances belonging to the negative graph may have several incident and/or emerging pathways and/or half-pathways. However, it will be shown below in this work that it is sufficient to consider the **emerging half-pathways** alone. At a certain number and topology of **connections** and topology of pathways belonging to a negative graph, the connections of the negative graph with surrounding medium are able to eliminate the negative value of the graph upon approaching the stationary values of the system variables.

Let us consider four kinetic models of phenomenological reactions, including some the second order and the third order negative graphs (oscillators) described in the first part of this work [1]. The kinetic model of a system of five reactions involving four substances combined by one balance equation is shown in Fig. 1. The mechanism illustrated by Fig. 1a contains the second order negative graph ${}^2\boldsymbol{G_2}$ (Fig. 1d), which has been described in the first part of this work [1]. Because in the general case graph ${}^2\boldsymbol{G_2}$ is a part of a complex system, it may contain the half-pathways emerging from or incident to one or the two substances of the graph. In addition, according to the law of conservation of the number of atoms of reagents and products in each elementary act of reactions $\boldsymbol{v_1}$ and $\boldsymbol{v_2}$, the minimum number of system components including graph ${}^2\boldsymbol{G_2}$ is equal to four (Fig. 1c).

The complete graph (Fig. 1c) of the list of reactions (Fig. 1a) is described by the set of the third order simultaneous differential equations written on the basis of the law of mass action. The graph shown in Fig. 1c can be used to set up the analytical expression for the coefficients $\boldsymbol{a_1}$, $\boldsymbol{a_2}$, and $\boldsymbol{a_3}$ of the third order characteristic polynomial, because each coefficient $\boldsymbol{a_i}$ (i = 1, 2, 3) is equal to the algebraic sum of all subgraphs of order i contained in the complete graph of the reaction list:

$$a_1 = \frac{v_4}{x_1} + \frac{v_3}{x_2} + \frac{v_2}{x_3} + \frac{v_1}{x_4} > 0\,, \quad (9)$$

$$a_2 = \frac{v_4\cdot v_3}{x_1\cdot x_2} + \frac{v_4\cdot v_2}{x_1\cdot x_3} + \frac{v_3\cdot v_2}{x_2\cdot x_3} - \frac{v_1\cdot v_2}{x_1\cdot x_2}, \quad (10)$$

$$a_3 = \frac{v_4\cdot v_3\cdot v_2}{x_1\cdot x_2\cdot x_3} > 0\,. \quad (11)$$

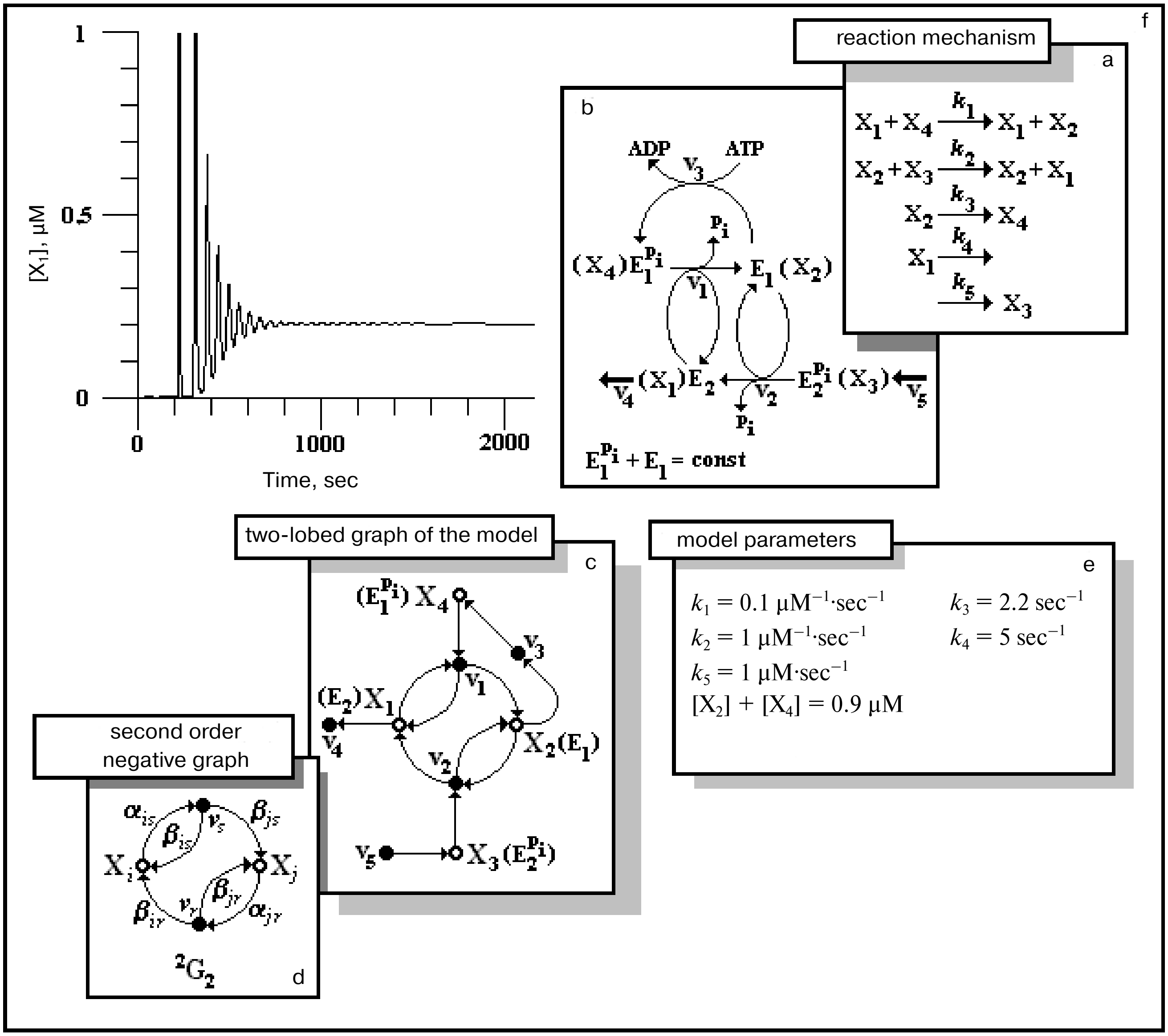


Fig. 1. Mathematical model of a complex reaction whose complete graph contains the second order negative graph (oscillator) inducing damped oscillations of system components: a) succession of reactions of interconversion of substances X_1, X_2, X_3, and X_4, where k_1-k_5 are the rate constants of the second and first order; b) scheme of hypothetical mechanism of mutual phosphorylation–dephosphorylation of two proteins (E_1, E_2). Protein forms E_2, E_1, E_2^{pi}, and E_1^{pi} correspond to substances X_1, X_2, X_3, and X_4, respectively, where E_i and E_i^{pi} are dephosphorylated and phosphorylated forms of the protein, respectively; c) complete two-lobed graph of reactions. Symbols (○) and (●) correspond to substance node (X_i) and reaction rate (v_r) node of elementary reaction, respectively; d) second order negative graph of the complete graph of the system, the presence of which is one of the conditions for the existence of oscillations of system components. α_{ir} and β_{jr} are the stoichiometric coefficients; e) model parameters; f) damped oscillations of concentration of component X_1 obtained from numerical solution of the fourth order set of simultaneous ordinary differential equations derived from the law of mass action. Differential equations were solved using the Dbsolve computer program developed by I. I. Goryanin (Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia).

The presence of a negative component in Eq. (10) and positive value of leading coefficient $\boldsymbol{a}_3$ constitute the sufficient condition of the existence of oscillation of the components of the system. The following expressions are valid under stationary conditions: $v_4 = v_2$ and $v_3 = v_1$ (Fig. 1c). In this case, the positive subgraph in Eq. (10), which is composed of the product of the emerging half-pathways v_4 and v_3 $(+v_4 \cdot v_3/x_1 \cdot x_2)$, under stationary conditions is equal by modulus (but has opposite sign) to the subgraph $-v_1 \cdot v_2/x_1 \cdot x_2$. Thus, as the stationary level is attained, the value of coefficient $\boldsymbol{a}_2$ becomes strictly positive. It can be shown similarly that for all types of the second order negative graphs of the topological types $^2\boldsymbol{G}_1$, $^2\boldsymbol{G}_2$, and $^2\boldsymbol{G}_3$ considered in the first part of this work [1], the following statement (Rule 11) is valid **if there is at least one half-pathway emerging from any substance node belonging to this graph. Because the second order negative graph composed of positive pathways and containing graphical element loop or having at least one stoichiometric coefficient β_{ir} unequal to one is a part of the system of order $n > 4$, it contributes negatively to coefficient a_2 only under prestationary conditions (Rule 11).**

Numerical integration of the mathematical model of the reactions considered above (Fig. 1f) revealed that the chosen values of the system parameters corresponded to damped oscillations (Fig. 1e). Some complex biochemical systems may incorporate the reaction sequence shown in Fig. 1a as a subsystem. For example, reactions including substances X_2, X_4 and X_1, X_3 can correspond to an open system of two interacting proteins $\mathbf{E}_1$ (E_1^{pi}) and $\mathbf{E}_2$ (E_2^{pi}), in which the protein activity is modified as a result of interaction (mutual protein phosphorylation/dephosphorylation) (Fig. 1b). The results of numerical integration of the mathematical models showed that under physiological concentrations of these substances and appropriate values of parameters (Fig. 1e), the system underwent damped oscillations, which lasted for 10 min.

A hypothetical mechanism of the system of five reactions including four substances and corresponding to the complete graph (Fig. 2b) containing the third order negative graph (Fig. 2c) of type $^3\boldsymbol{G}_2$ [1] is shown in Fig. 2a. The mathematical model of the reaction list shown in Fig. 2a contains four independent variables. In this case, the coefficients of the fourth order characteristic polynomial of the system are:

$$a_1 = \frac{v_1}{x_1} + \frac{v_3}{x_2} + \frac{v_5}{x_2} + \frac{v_2}{x_3} + \frac{v_3}{x_4} > 0 \ , \quad (12)$$

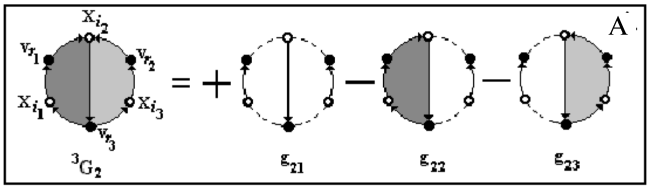
$$a_2 = \frac{v_1 v_5}{x_1 x_2} + \frac{v_1 v_2}{x_1 x_3} + \frac{v_1 v_3}{x_1 x_4} + \frac{v_2 v_3}{x_2 x_3} + \frac{v_2 v_5}{x_2 x_3} + \frac{v_3 v_5}{x_2 x_4} + \frac{v_2 v_3}{x_3 x_4} > 0 \ , \quad (13)$$

$$a_3 = \frac{v_1 v_2 v_5}{x_1 x_2 x_3} + \frac{v_2 v_3 v_5}{x_2 x_3 x_4} - \frac{v_1 v_2 v_3}{x_1 x_2 x_3} \ , \quad (14)$$

$$a_4 = \frac{v_1 v_2 v_3 v_5}{x_1 x_2 x_3 x_4} > 0 \, . \quad (15)$$

The presence of a negative component in coefficient $\boldsymbol{a}_3$ and positive value of coefficient $\boldsymbol{a}_4$ constitute the sufficient condition for the existence of oscillation of components of this system. The results of the numerical integration of the mathematical model describing this mechanism (Fig. 2a) at the given values of model parameters (Fig. 2f) are shown in Fig. 2g. The third order negative graph shown in Fig. 2c is a part of this mechanism. This graph contains a single half-pathway (**connection**) emerging from the substance node X_2. It follows from this analysis that the phase space of the system variables contains the only one stationary point of the type stable focus, which is characterized by damped oscillations of system components.

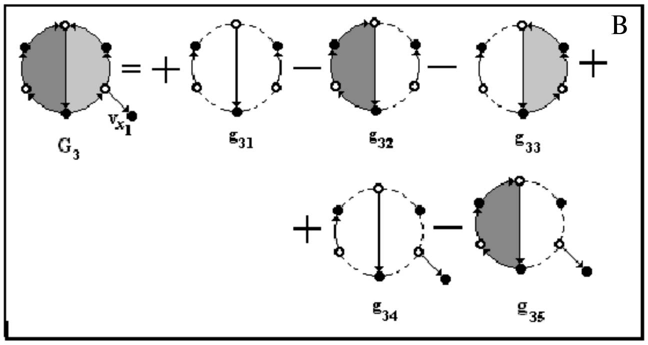
Let us consider all possible variants of topology of the connections of the negative graph $^3\boldsymbol{G}_2$ with its potential surrounding medium. This analysis is intended to demonstrate that any combination containing one or two emerging half-pathways (connections) gives rise to elimination of its negative value under stationary conditions. In graphical terms, the third order negative graph $^3\boldsymbol{G}_2$ (scheme **A**) is the algebraic sum of three of its subgraph components $^3\boldsymbol{G}_2 = \boldsymbol{g}_{21} - \boldsymbol{g}_{22} - \boldsymbol{g}_{23}$:



The value and sign of graph $^3\boldsymbol{G}_2$ is determined by the values and signs of its components (the third order subgraphs).

$$^3G_2 = + \frac{v_{r_1} v_{r_2} v_{r_3}}{x_{i_1} x_{i_2} x_{i_3}} - \frac{v_{r_1} v_{r_2} v_{r_3}}{x_{i_1} x_{i_2} x_{i_3}} - \frac{v_{r_1} v_{r_2} v_{r_3}}{x_{i_1} x_{i_2} x_{i_3}} = - \frac{v_{r_1} v_{r_2} v_{r_3}}{x_{i_1} x_{i_2} x_{i_3}} \quad (16)$$

Let at least one half-pathway X_{i_3} emerge from the node v_{x_i} of the graph $^3\boldsymbol{G}_2$. Then, new graph $\boldsymbol{G}_3$ is equal to the sum of five third order subgraphs taken with corresponding sign (scheme B).



Because subgraphs $\boldsymbol{g}_{34}$ and $\boldsymbol{g}_{35}$ have equal values but opposite signs, they cancel one another. Thus, the value and sign of graph $\boldsymbol{G}_3$ coincide with the value and sign of the third order negative graph $^3\boldsymbol{G}_2$ of scheme **A**.

It can be shown that for all variants of topology of negative graph of order $\boldsymbol{k}$, **the half-pathway emerging from**

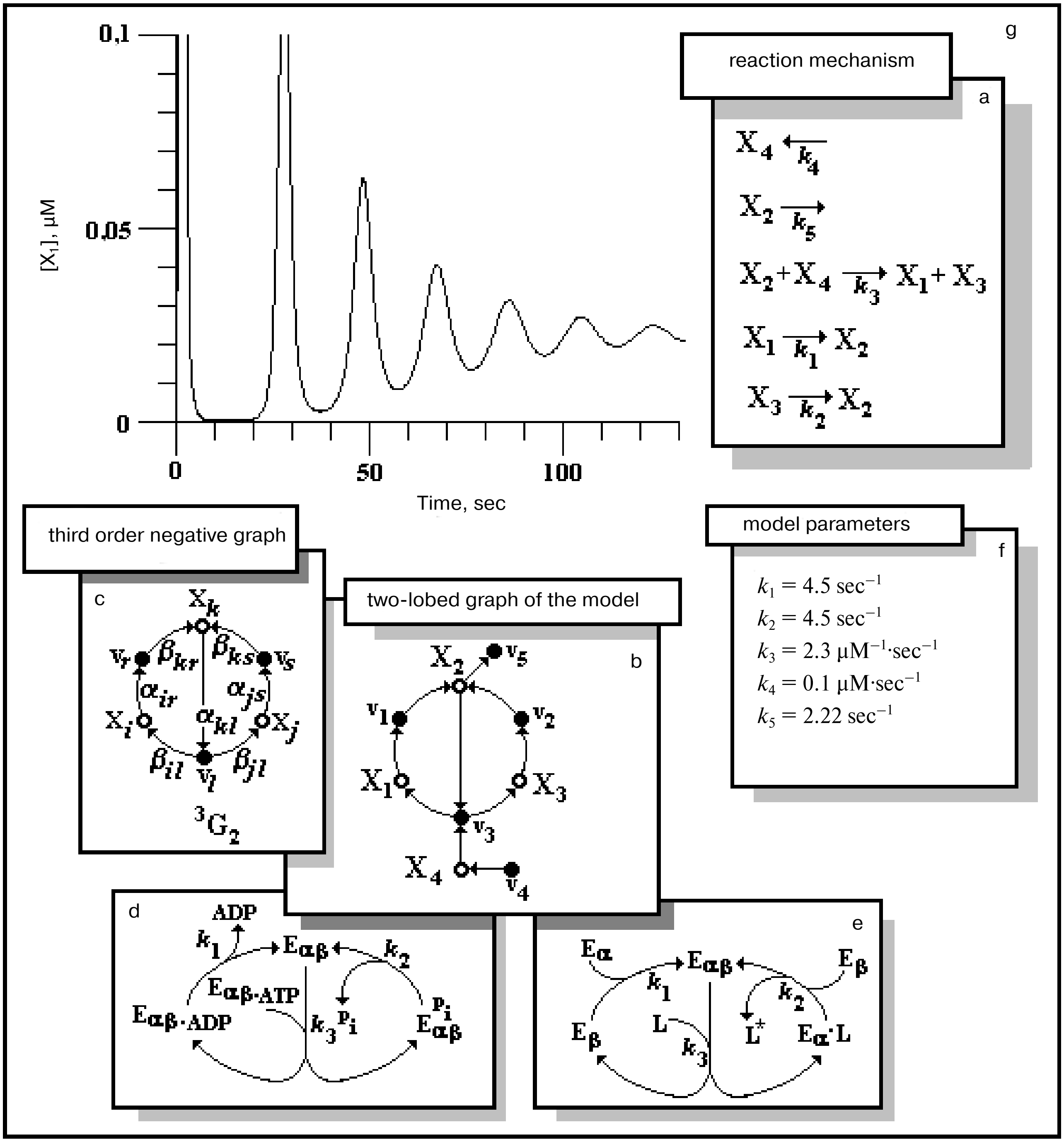
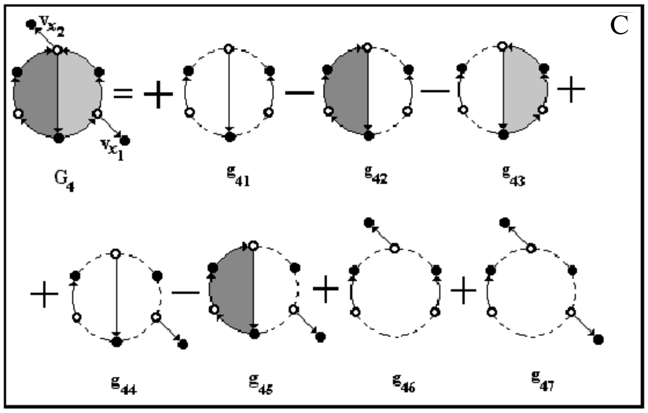


Fig. 2. Mathematical model of a complex reaction whose complete graph contains the third order negative graph (oscillator) inducing damped oscillations of system components: a) succession of reactions of interconversion of substances X_1, X_2, X_3, and X_4, where k_1-k_5 are the rate constants of the second and first order; b) complete two-lobed graph of reactions. Symbols (○) and (●) correspond to substance node (X_i) and reaction rate (v_r) node of elementary reaction, respectively; c) third order negative graph of the complete graph of the system, the presence of which is one of the conditions for the existence of oscillations of system components. α_{ir} and β_{jr} are the stoichiometric coefficients; d) scheme of hypothetical mechanism of autophosphorylation of a multisubunit protein ($E_{\alpha\beta}$); e) scheme of hypothetical mechanism of interaction between ligand (L) and multisubunit protein ($E_{\alpha\beta}$), which may undergo reversible dissociation–association involving protein subunits (E_α and E_β); f) model parameters; g) damped oscillations of concentration of component X_1 obtained as a result of numerical solution of the fourth order set of simultaneous ordinary differential equations derived from the law of mass action. Differential equations were solved using the Dbsolve computer program developed by I. I. Goryanin (Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia).

the node, which does not belong to any even-numbered cycle, does not eliminate the negative graph containing the even-numbered cycle (Rule 12). Therefore, in graph $\boldsymbol{G_3}$, the half-pathway emerging from the substance nodes X_{i_1} or X_{i_3} does not cause its elimination. However, it follows from the topology of graph $\boldsymbol{G_3}$ that under stationary conditions $v_{r_3} = v_{r_1} + v_{r_2}$, $v_{r_1} = v_{r_3}$, and $v_{r_2} = v_{r_3} - v_{x_1}$. Therefore, to provide equilibrium at each substance node, it is necessary to introduce either a sink (emerging half-pathway v_{x_i}) from nodes X_{i_1} or X_{i_2}, or from the two nodes simultaneously. Let us consider the first configuration of the connections, i.e., graph $\boldsymbol{G_4}$ and its third order subgraph components (scheme **C**):



Taking into account connections v_{x_1} and v_{x_2}, the value of graph $\boldsymbol{G_4}$ in this case can be calculated as:

$$G_4 = -\frac{v_{r_1}v_{r_2}v_{r_3}}{x_{i_1}x_{i_2}x_{i_3}} + \frac{v_{r_1}v_{r_2}v_{x_2}}{x_{i_1}x_{i_2}x_{i_3}} + \frac{v_{r_1}v_{x_1}v_{x_2}}{x_{i_1}x_{i_2}x_{i_3}} \,. \qquad (17)$$

It follows from the condition of existence of stationary state or the rule of node equilibrium in graph $\boldsymbol{G_4}$ that $v_{x_1} = v_{r_3} - v_{r_2}$ and $v_{x_2} = v_{r_1} + v_{r_2} - v_{r_3}$, i.e., $v_{r_1} = v_{r_3}$ and $v_{x_2} = v_{r_2}$. Substitution of these expressions into Eq. (17) and further simple transformations demonstrate that the value of graph $\boldsymbol{G_4}$ is equal to zero.

It can be shown similarly that any combination of half-pathways v_{x_i} emerging from the substance nodes of the graph of topology $^3\boldsymbol{G_2}$, upon approaching a stationary state of the system universally causes elimination of its negative value. The introduction of reversible stages, i.e., pathways/half-pathways incident to any substance node, gives rise to the same result. The final conclusion can be drawn that the presence of the third order negative graph $^3\boldsymbol{G_2}$ in the complete graph of a complex biochemical system at any types of the topology of connections between the graph $^3\boldsymbol{G_2}$ and surrounding medium is the **sufficient** condition for generation of only **damped** oscillations of reaction components.

General schemes of two phenomenological biochemical systems containing negative graph $^3\boldsymbol{G_2}$ as a part are shown in Figs. 2d and 2e. The motifs of protein autophosphorylation and reversible association–dissociation of protein during its interaction with ligands schematically shown in Figs. 2d and 2e, respectively, are very common for cell metabolism. It follows from the results described above that such systems contain **a potential generator of damped oscillations**.

A simple mechanism of an open successive reaction including four substances is shown in Fig. 3a. It was shown in [8, 9] that the successive mechanism was the sufficient condition for the existence of single and stable stationary point in the phase space of the system variables. Conversely, the presence of stages of interaction between intermediate substances in the list of reactions is the sufficient condition for existence of multiple stationary state [9]. It can be shown using the theoretical graph method suggested in this work that the presence of at least one stoichiometric coefficient $\beta_{ir} > 1$ in the reaction list of the successive mechanism gives rise to single stationary point of the type of asymptotically stable focus, i.e., to generation of damped oscillations of system components.

The complete graph shown in Fig. 3b represents the reaction list shown in Fig. 3a. The complete graph shown in Fig. 3b contains the third order negative graph (Fig. 3c) of type $^3\boldsymbol{G_{12}}$ [1]. Analysis of such systems revealed that the presence of any **even-numbered cycle** containing **any** number of substances in the complete graph of the system in the presence of at least one stoichiometric coefficient larger than one ($\beta_{ir} > 1$) was the sufficient condition for the existence of a stable stationary point of the type of asymptotically stable focus. This means that the biochemical system containing an even-numbered cycle under the conditions specified above and certain values of system parameters contains a potential generator of **damped** oscillations.

The damped oscillations shown in Fig. 3f are generated by one of the components of the mechanism shown in Fig. 3a. These curves were calculated by numerical integration of a set of the fourth order simultaneous differential equations. The coefficients of the fourth order characteristic polynomial are equal to:

$$a_1 = \frac{v_1}{x_1} + \frac{v_2}{x_2} + \frac{v_4}{x_2} + \frac{v_3}{x_3} + \frac{v_2}{x_4} + \frac{v_7}{x_4} > 0 \,, \qquad (18)$$

$$a_2 = \frac{v_1v_2}{x_1x_2} + \frac{v_1v_4}{x_1x_2} + \frac{v_1v_3}{x_1x_3} + \frac{v_1v_2}{x_1x_4} + \frac{v_1v_7}{x_1x_4} + \frac{v_2v_3}{x_2x_3} + \frac{v_3v_4}{x_2x_3} + \frac{v_2v_7}{x_2x_4} + \frac{v_2v_4}{x_2x_4} + \frac{v_4v_7}{x_2x_4} + \frac{v_2v_3}{x_3x_4} + \frac{v_3v_7}{x_3x_4} > 0 \,, \qquad (19)$$

$$a_3 = (1-\beta_{32})\cdot\frac{v_1v_2v_3}{x_1x_2x_3} + \frac{v_1v_2v_4}{x_1x_2x_3} + \frac{v_2v_3v_4}{x_2x_3x_4} + \frac{v_1v_2v_3}{x_1x_3x_4} + \frac{v_1v_2v_3}{x_1x_2x_4} \,, \qquad (20)$$

$$a_4 = \frac{v_1v_2v_3v_4}{x_1x_2x_4x_4} + \frac{v_1v_3v_4v_7}{x_1x_2x_4x_4} + (1-\beta_{32})\cdot\frac{v_1v_2v_3v_7}{x_1x_2x_4x_4} \,. \qquad (21)$$

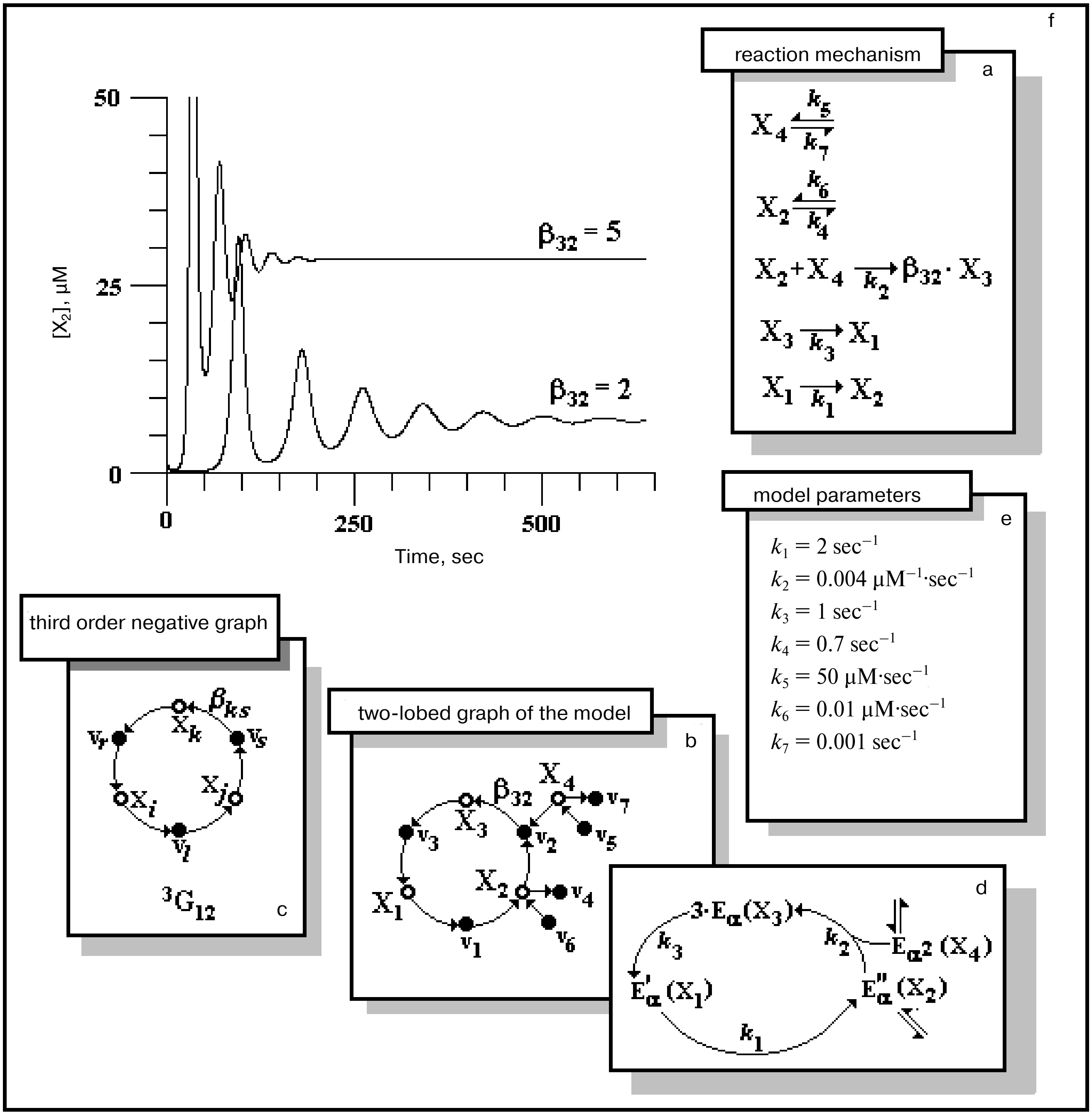
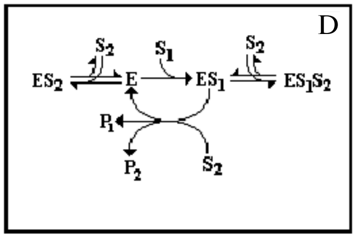


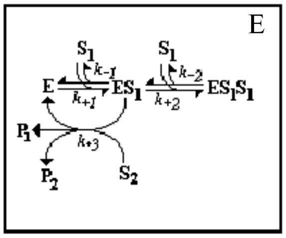
Fig. 3. Mathematical model of a complex reaction whose complete graph contains the third order negative graph (oscillator) inducing damped oscillations of system components: a) succession of reactions of interconversion of substances X_1, X_2, X_3, and X_4, where k_1-k_7 are the rate constants of the second and first order; β_{32} is the productive stoichiometric coefficient; b) complete two-lobed graph of reactions. Symbols (o) and (●) correspond to substance node (X_i) and reaction rate (v_r) node of elementary reaction, respectively; c) third order negative graph of the complete graph of the system, the presence of which is one of the conditions for the existence of oscillations of system components. β_{ks} is the stoichiometric coefficient; d) scheme of hypothetical mechanism of interaction between protein homodimer ($E_{\alpha}2$) with its protomer (E_α), where E'_α and E''_α are different conformations of protein E_α; e) model parameters; f) damped oscillations of concentration of component X_2 obtained by numerical solution of the fourth order set of simultaneous ordinary differential equations derived from the law of mass action. Differential equations were solved using the Dbsolve computer program developed by I. I. Goryanin (Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia).

It follows from Eqs. (18)-(21) that positive sign of coefficient $\boldsymbol{a}_4$ and negative sign of coefficient $\boldsymbol{a}_3$ constitute the sufficient condition for the existence of damped oscillations. The values of system parameters, at which the sufficient conditions $\boldsymbol{a}_3 < 0$ and $\boldsymbol{a}_4 > 0$ are observed, are shown in Fig. 3e. The theoretical graph analysis of the mechanism shown in Fig. 3a revealed the following specific features of the mechanism. 1) Positive values of coefficients $\boldsymbol{a}_4$ is provided by the presence of half-pathway $\boldsymbol{v}_4$ at substance node X_2. 2) The introduction of reversible influx of substance at any other position (X_1 or X_3) causes cancellation of corresponding subgraphs and makes the coefficient $\boldsymbol{a}_4$ vanish. 3) The introduction of back reactions ($\boldsymbol{v}_{-1}$, $\boldsymbol{v}_{-2}$, and $\boldsymbol{v}_{-3}$) in addition to direct reactions ($\boldsymbol{v}_1$, $\boldsymbol{v}_2$, and $\boldsymbol{v}_3$) causes only the appearance of additional positive summands in coefficient $\boldsymbol{a}_3$ but does not change qualitatively the dynamic behavior of the system.

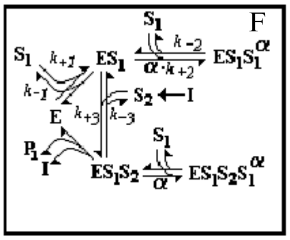
The general mechanism of the reversible association–dissociation of a certain multisubunit protein (E) is schematically shown in Fig. 3d. The necessary conditions of the existence of damped oscillations in such systems are: a) system openness by one of the reaction components; b) the presence of a cycle of conversion of a certain component. In the hypothetical example considered above, a certain protein homodimer E_{α^2} during its interaction with a protomer E''_{α} dissociates into protomer components E_{α}. As a result of conformational rearrangement of protein molecule, the protomers may exist in several forms: $E_{\alpha} \rightarrow E'_{\alpha} \rightarrow E''_{\alpha}$ (Fig. 3d). For the sake of simplicity, back stages of biochemical reactions are not shown in Fig. 3d, because introduction of reversible stages does not cause qualitative changes in the phase portrait of the model but only changes the values of the system parameters at which damped oscillations (see above).

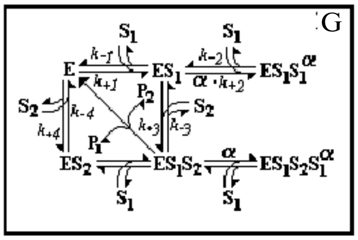
The kinetic model of the phenomenological two-substrate enzymatic reaction with inhibition by one substrate (scheme **D**) is shown in Fig. 4. This model is a modification of the model of a possible enzymatic mechanism of substrate inhibition suggested in [10] (scheme **E**).



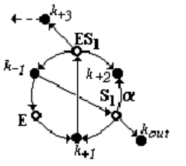


The mechanism shown in Fig. 4a and scheme **D** implies formation of two inhibitory enzyme–substrate complexes $\mathbf{ES_2}$ and $\mathbf{ES_1S_2}$. The formation of the complex $\mathbf{ES_2}$ implies that in addition to the productive enzyme–substrate complex $\mathbf{ES_1}$ (reaction v_3, Fig. 4b), there is noncatalytic binding of substrate $\mathbf{S_2}$ to free enzyme form (reaction v_2, Fig. 4b). This mechanism (scheme **D** and Fig. 4a) includes an oscillator (the third order negative graph ${}^3\boldsymbol{G}_{20}$ [1]) (Fig. 4c). The third order graph ${}^3\boldsymbol{G}_{20}$ is a part of many kinetic schemes of chemical and enzymatic reactions. For example, the description of two enzymatic reactions with substrate inhibition includes various modifications of ordered (schemes **E**, **F**) or disordered (scheme **G**) mechanisms of binding of substrates $\mathbf{S_1}$ and $\mathbf{S_2}$ to enzyme.

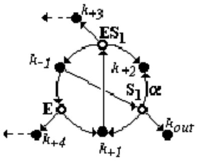




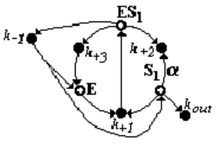
The graphical analysis of these data revealed that the complete graphs of the four schemes considered above (**D**, **E**, **F**, and **G**) contained only one type of negative graph (namely, graph ${}^3\boldsymbol{G}_{20}$). The schemes of three mechanisms considered below in more detail (**E**, **F**, and **G**) show only the part of the complete graph that contains the third order negative graph and all half-pathways emerging from the substance nodes belonging to the graph (graphs $\boldsymbol{G}_5$, $\boldsymbol{G}_6$, and $\boldsymbol{G}_7$, respectively).



G_5

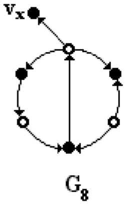


G_6

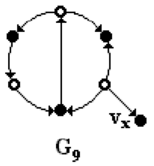


G_7

It can be shown that of all possible combinations of emerging half-pathways (connections) belonging to three substances of the third order graph ${}^3\boldsymbol{G}_{20}$, only two variants do not cause elimination of its negative value (graphs $\boldsymbol{G}_8$ and $\boldsymbol{G}_9$).



G_8



G_9

Of two substrate–enzyme mechanisms considered above (schemes **E**, **F**, and **G**), only the mechanism illustrated by scheme **E** (this mechanism was described in more detail in [10]) provides all necessary conditions for generation and existence of oscillations of the system components. The main cause of the oscillator (graph ${}^3\boldsymbol{G}_{20}$) elimination in the mechanisms of schemes **F** and **G** consists in the fact that a branch of the third order negative graph incorporated in these schemes is represented by the reaction of

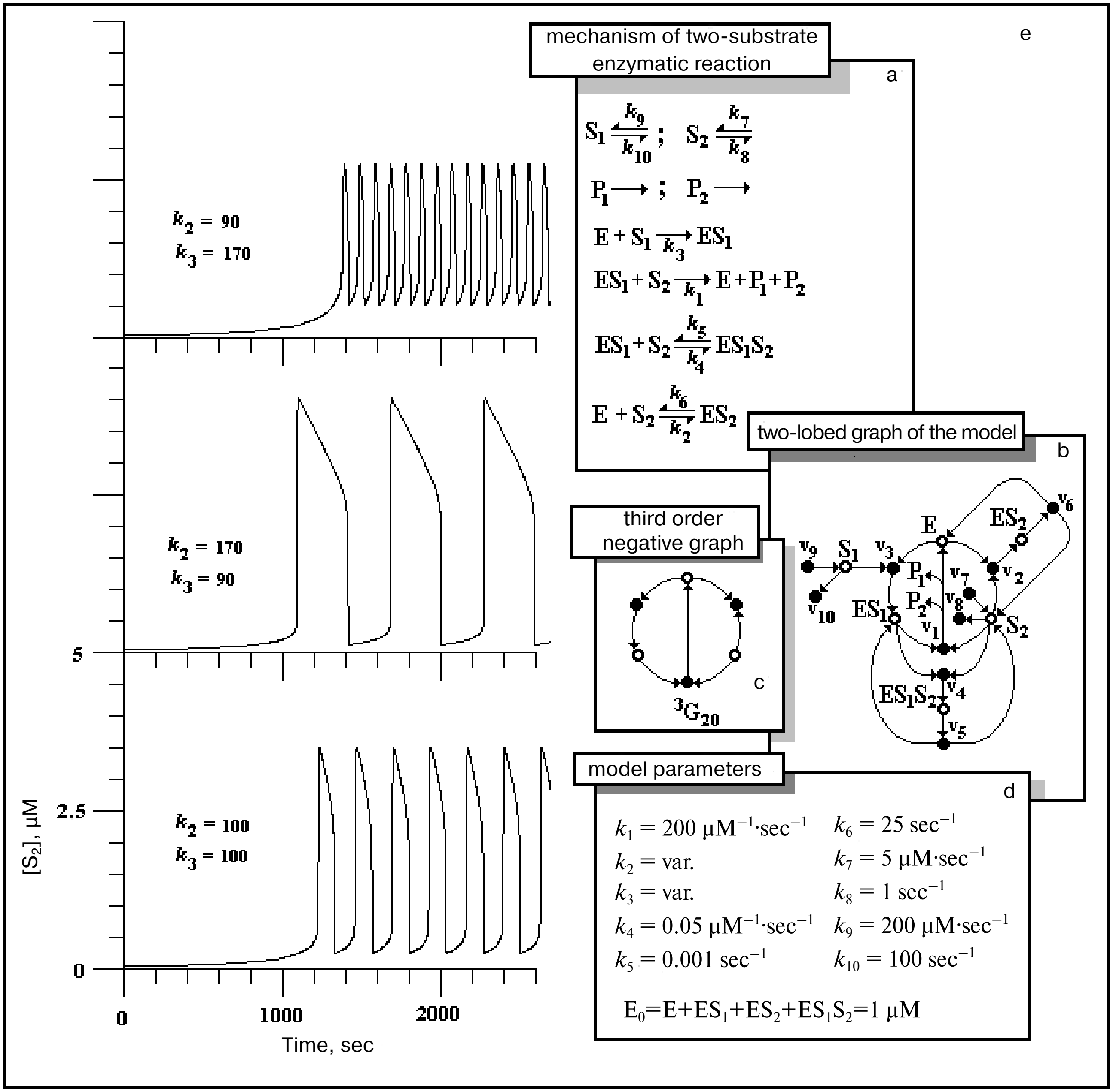


Fig. 4. Mathematical model of a phenomenological open two-substrate enzymatic reaction with substrate inhibition, the mechanism of which contains an oscillator capable of inducing (under certain values of system parameters) undamped oscillations of system components: a) reaction mechanism, where S_1, S_2 and P_1, P_2 are substrates and products of reaction, respectively; E and ES_1 are the free form of the enzyme and productive substrate–enzyme complex, respectively; ES_2 and ES_1S_2 are the inhibitory enzyme–substrate complexes; k_1-k_{10} are the rate constants of the first and the second order; b) complete two-lobed graph of reactions. Symbols (○) and (●) correspond to substance node (X_i) and reaction rate (v_r) node of elementary reaction, respectively; c) third order negative graph (oscillator) of the complete graph of the system, the presence of which is one of the conditions for the existence of oscillations of system components; d) model parameters; e) undamped oscillations of the substrate S_2 concentration obtained by numerical solution of the set of the fifth order simultaneous ordinary differential equations derived from the law of mass action. Differential equations were solved using the Dbsolve computer program developed by I. I. Goryanin (Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Russia).

the $\boldsymbol{k_{-1}}$ reversible stage of binding of substrate $\mathbf{S_1}$ to enzyme (graphs $\boldsymbol{G_5}$ and $\boldsymbol{G_6}$). Such a topology gives rise to the appearance of an additional odd-numbered cycle in graph $^3\boldsymbol{G_{20}}$, the negative value of the graph $^3\boldsymbol{G_{20}}$ being eliminated by the additional odd-numbered cycle.

The results of numerical integration of the mathematical model of this mechanism (scheme **D**) are shown in Fig. 4e. The values of the model parameters, at which **undamped oscillations** of system components were generated, were found (Fig. 4d). The model analysis performed in this work revealed that there was a sufficiently broad range of variation of model parameters, within which the oscillations in the system were maintained. Variable ratio of two model parameters (rate of formation of productive enzyme–substrate complex $\mathbf{ES_1}$ (rate constant $\boldsymbol{k_3}$) and formation of inhibitory complex $\mathbf{ES_1S_2}$ (rate constant $\boldsymbol{k_2}$)) was considered as an example of a possible regulator of the oscillation frequency.

Thus, the results of this and preceding [1] works demonstrated that the well-known mathematical tests of the existence or absence of critical phenomena (trigger behavior, multistationary character, and concentration oscillations) in biochemical/chemical systems had obvious graphical representation and tests in the complete graph of complex reaction. The graphical rules suggested in this work allow the subsystem/oscillator (aggregate of substances and reactions) responsible for generation of concentration oscillations of system components to be detected in the schemes of the complex reactions. The validity of the approach suggested in this work was supported by the results of numerical integration of mathematical models of both hypothetical complex reactions and experimentally observed oscillators in chemical systems.

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