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Simple Critical Schemes in Non-autocatalytic Systems of Biochemical Reactions

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Abstract—Kinetic behavior of various reaction systems consisting of three compounds was analyzed in order to find and study the origin of multiple-steady-state and auto-oscillatory behavior in biochemical systems. More than 3000 such reactions were considered. Using the bigraph method, six simple critical reaction schemes were found. It is shown that the presence of these fragments in the composition of more complex biochemical systems may be the origin of the multiple steady-states and auto-oscillations.

Key words: multiple steady-states, auto-oscillations, graph theory

Multiple steady-states and auto-oscillation modes in biochemical systems play an important role in biological processes. Now many scientists consider that specific stoichiometry of intracellular biochemical reactions is the origin of these modes. Studies of stoichiometry of enzymatic catalysis allows partial understanding of molecular mechanisms of such important biological phenomena as bistability and auto-oscillations. The existence of oscillation mode and/or multiple steady-states in biochemical system will be further called critical kinetic phenomena.

Autocatalytic reaction mechanism is one of destabilizing factors of the steady-states in biochemical systems [1]. Many modes explaining various types of critical behavior in biochemical systems are based on this mechanism. It should be noted that equation terms like kx, kxy, and kx^2 ($k > 0$) are usually called autocatalytic. The presence of autocatalytic mechanisms is considered to be necessary and sufficient for initiation of critical modes. However, in some cases it is very difficult to interpret the elementary auto-oscillation steps in chain reactions in molecular-biophysical sense [2]. That is why it is interesting to reveal simple critical schemes consisting of non-autocatalytic reactions and being a destabilizing factor in more complex systems. The goal of this work was to reveal the sequences of reactions which can cause unstable steady-state and/or multiple steady-states of a biochemical system as a whole.

CRITICAL SCHEMES IN NON-AUTOCATALYTIC SYSTEMS OF REACTIONS

For analysis of kinetic schemes, we used a method based on bigraph theory [3, 4]. This method relates the structure of a kinetic scheme to the critical phenomena (multiple steady-states, auto-oscillations) arising in it. The reaction rates of kinetic schemes are defined by the mass-action expression; dynamic behavior is defined by differential kinetic equations. Possible critical phenomena can be revealed by analysis of the characteristic polynomial of the linearized system of kinetic equations. Coefficients of this polynomial are evaluated by the method based on analysis of the reaction scheme [4, 5], to which bigraphs with top-compounds (A-top) and top-reactions (B-top) correspond. Tops of different types are linked by arrows. An arrow from interacting compound to reaction and another one from reaction to its product form a "positive pathway". Graphically it is presented as $A_i \rightarrow B \rightarrow A_j$, where A-tops are compounds and B-top is a reaction. Two arrows from two compounds participating in the same reaction form a "negative pathway" $A_i \rightarrow B \leftarrow A_j$. A closed sequence of pathways forms a "cycle". An even cycle is formed by an even number of negative pathways in it. A subgraph is a combination of cycles not crossing in A-tops and arrows from compound to reac-

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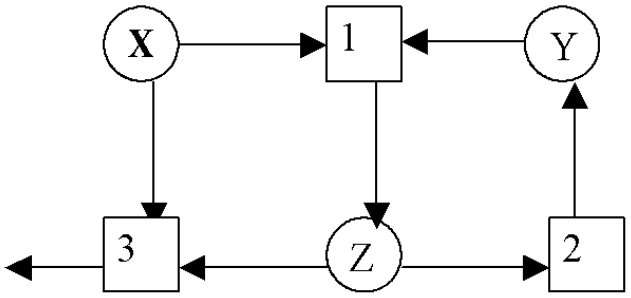

Fig. 1. Bigraph scheme of a fragment of the first type. Letters in circles are the names of interacting compounds, and numbers in squares are the numbers of reactions. Arrows from interacting compounds to reactions show participation of a certain compound in a certain reaction, and arrows from reaction to compounds show production of a certain compound in a certain reaction.

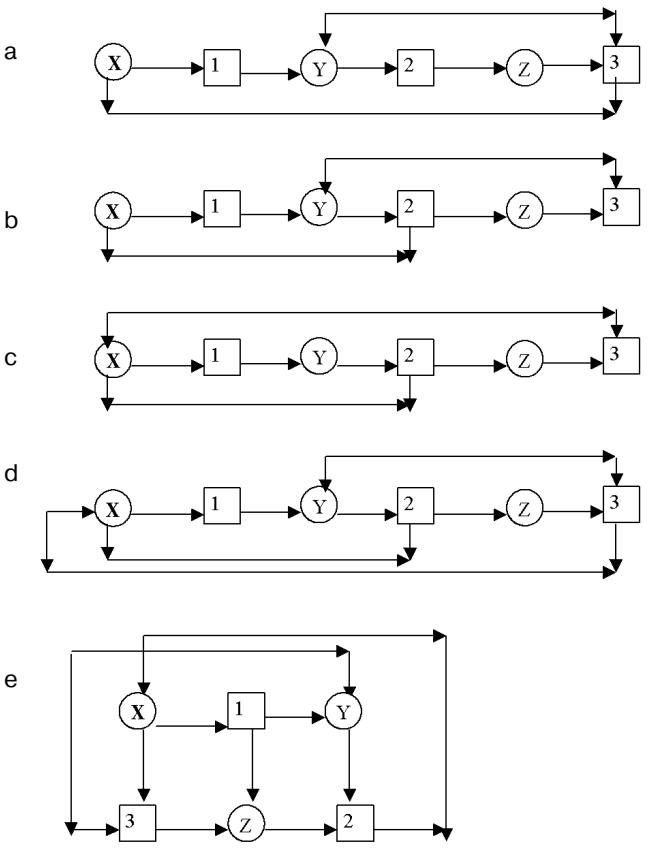

Fig. 2. Bigraph scheme of the fragments of the second type. Designations are the same as for Fig. 1.

tion. The order of a subgraph is equal to the number of A-tops in it. Stoichiometric coefficients of reactions comprising subgraph determine numerical value of its coefficient; the latter is defined as an algebraic sum of the number of positive and negative terms in the contribution of subgraph over m reactions chosen. A "fragment" of the scheme of order m is formed by m reactions chosen from the scheme and considered in relation to m compounds chosen. Other compounds not participating in the chosen reactions are not a part of the fragment. A fragment of the scheme is called "critical" if the sum of coefficients of all its subgraphs with odd number of even cycles is larger than the sum of coefficients of all other subgraphs, that is, gives a negative contribution to the corresponding coefficient of characteristic polynomial. Subgraphs with odd number of even cycles give a negative contribution to coefficients of the characteristic polynomials and are the origin of instability. Possible appearance of critical phenomena is related with the presence of critical fragments in the scheme.

It is well known that kinetic behavior of the system is determined by the characteristic polynomial of linearized system of kinetic equations:

$$P = \lambda^n + a_1 \cdot \lambda^{n-1} + a_2 \cdot \lambda^{n-2} + ... + a_n.$$

If at least one of the coefficients of this polynomial has a negative sign in the steady-state, this state becomes unstable. As shown in [4], the ith coefficient of characteristic polynomial is determined by the sum of contributions from various subgraphs of ith order. This means that if, for example, $a_2 < 0$, the contribution from any two interacting compounds in a_2 is negative and its absolute value is more than the sum of all other positive contributions of double interactions. These interactions of compounds with negative contribution are the origin of instability of the steady-state of kinetic system. In a non-autocatalytic system, a_1 and a_2 coefficients are always positive. This means that the order of the critical subgraph should be more than two; in other words, the number of interacting substances in the critical subgraph is not less than three. Considering this, a search for the elementary critical subgraphs was performed within the limits of the following requirements: 1) number of compounds in the subsystem should be not less than three; 2) number of reactions combining these compounds should be minimal; 3) not more than two compounds can participate in each elementary stage of reaction (the maximal reaction order is bimolecular); 4) none of the elementary stages is autocatalytic; 5) stoichiometric coefficients of interacting compounds do not exceed unity.

Contributions from more than 3000 various schemes were calculated within the limits of these requirements. As a result, six critical fragments were obtained. Bigraph schemes of these critical fragments are presented in Figs. 1 and 2. For biochemical interpretations, these fragments

can be divided into two types by the chemical character of reactions.

The first type of critical fragments. Only one of the found fragments can be related to the first type. It consists of the following stages:

$$X + Y \rightarrow Z,$$

$$Z \rightarrow Y,$$

$$Z + X \rightarrow .$$

This fragment presented in Fig. 1 is nonlinear and has a clear biochemical meaning. Let us consider X as a substrate, Y as a free form of enzyme, and Z as the enzyme–substrate complex; then this fragment corresponds to enzymatic reaction with the substrate inhibition. This means that in non-autocatalytic enzymatic reaction, the substrate inhibition is a destabilizing factor of the steady-state of the system. Under certain conditions, this factor may cause the multiple steady-states and/or auto-oscillations. There appears to be the properties of enzymatic reaction with the substrate inhibition which attracted attention of many scientists studying bistability and auto-oscillations in biochemical systems. Thus, Sel'kov [5], Seeling [6], Thomas [7], Goldstein and Ivanova [8], Lengyel and co-workers [9], Shen and Larter [10] used the mechanisms of the substrate inhibition for modeling bistability and auto-oscillations in biochemical systems. It should be noted that the presence of inhibition stage in this scheme is not sufficient for negativity of its contribution. Really, if the stage of formation of the enzyme–substrate complex (Z) is supposed to be reversible, the negative contribution of this fragment is compensated and the scheme is not critical. Consequently, for this fragment to be critical, the rate of reversible decomposition of the enzyme–substrate complex should be significantly lower than the rate of the catalytic stage.

In some cases this fragment can be the only high-order one and consequently, may be the origin of the multiple steady-states.

For example, let us consider an opened enzymatic reaction with irreversible substrate inhibition. If the substrate and free enzyme arrive to the system with the inflow rates v_{os} and v_{oe}, respectively, the system of differential equations looks like:

$$dX/dt = v_{os} - k_1XY - k_3XZ - k_4X,$$

$$dY/dt = v_{oe} + k_2Z - k_1XY,$$

$$dZ/dt = k_1XY - k_3XZ - k_2Z.$$

This system can be rewritten as follows:

$$dX/dt = v_{os} - v_1 - v_3 - v_4,$$

$$dY/dt = v_{oe} + v_2 - v_1,$$

$$dZ/dt = v_1 - v_2 - v_3,$$

where $v_1 = k_1XY$, $v_2 = k_2Z$, $v_3 = k_3XZ$, $v_4 = k_4X$.

The characteristic equation of linearized system of kinetic equations can be written as follows:

$$\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 = 0.$$

Calculation of a_1 and a_2 coefficients show that they are positive at all positive values of kinetic parameters and are relatively complex. However, for our purposes it is important only to know the sign of these coefficients, and that is why their analytical expression is not given here. Together with this,

$$a_3 = \frac{\overline{v}_1\overline{v}_3}{\overline{u}_1\overline{u}_2\overline{u}_3}(\overline{v}_4 - \overline{v}_2),$$

where $\overline{u}_i$ and $\overline{v}_i$ are the steady-state concentrations of interacting compounds and the rates of ith reaction, respectively.

As can be seen, if $\overline{v}_4 > \overline{v}_2$ (that is, if the outflow rate of the substrate is less than the rate of its catalytic decomposition), a_3 is positive at all positive values of kinetic parameters, and the multiple steady-states are impossible in the system considered above. If $\overline{v}_4 < \overline{v}_2$, a_3 is negative at all positive values of kinetic parameters, and multiple steady-states exist in the system. In general, the irreversible substrate inhibition can be considered as nonlinear inactivation of enzyme, and such system will be one with multiple steady-states. Thus, multiple steady-states in the system with nonlinear enzyme inactivation may be the origin of trigger behavior of this system. Such behavior of the enzymatic system may be an important regulatory mechanism of cell metabolism.

It should be noted that there are no auto-oscillations in such systems, that is, multiple steady-states are the only possible critical states. This situation looks unusual for the specialists in modeling critical behavior of biochemical systems. In many modes the systems are considered in which the multiple steady-states and auto-oscillation coexist and are realized on changes in kinetic parameters. However, as seen in the given example, there are systems allowing multiple steady-states but not allowing auto-oscillations at any parameter values.

The second type of critical fragments. Critical fragments of the second type (Fig. 2) consist of the following stages:

a) $\begin{cases} X \to Y \\ Y \to Z \\ Z \to X + Y \end{cases}$, b) $\begin{cases} X \to Y \\ Y \to Z + X, \\ Z \to Y \end{cases}$

c) $\begin{cases} X \to Y \\ Y \to Z + X, \\ Z \to X \end{cases}$ d) $\begin{cases} X \to Y \\ Y \to Z + X \\ Z \to X + Y \end{cases}$,

e) $\begin{cases} X \to Y + Z \\ Y \to X + Z \\ Z \to X + Y \end{cases}$.

These schemes are similar in chemical sense. They can be presented as a sequence of the monomolecular reactions with particle multiplication (branched chain reaction). Kinetic equations of autocatalytic reactions can be obtained from kinetic equations of these systems using limiting transfers [11, 12]; that is why these fragments should be called dynamically autocatalytic. Linearity of certain stages is typical of these fragments, and this fact to some extent hinders their direct biochemical interpretation. However, chemical schemes including these critical fragments can be found. For example, a fragment in Fig. 2b is a typical scheme of catalytic reaction with catalyst reduction. Together with this, a possibility of construction of chemical system based on the obtained scheme can be proved more strictly using Korzukhin's theorem [2]: it is always possible to construct a chemical system of kinetic equations whose behavior will to any extent coincide with behavior of a given system of kinetic equations:

$$dX_j/dt = \Psi_j(x_1, x_2, ..., x_n),$$

where Ψ_j $(x_1, x_2, ..., x_n)$ are any polynomials with integer non-negative powers ($i = 1, 2, ..., n$).

It should be also noted that the fragments in Fig. 2 (a-e) may correspond to some multistage biochemical systems or may be obtained as a result of reduction of kinetic equations over the minor parameters. Thus, a fragment of type (b), destabilizing fragment of a single steady-state, was obtained on modeling auto-oscillation of H^+ on erythrocyte membranes [13].

So, analysis of simple kinetic modes using the graph theory methods reveals possible "crisis" in kinetics of the model enzymatic systems based on the structure of their schemes. Such phenomena as hysteresis or auto-oscillations may be considered as induced by "critical fragments" in the kinetic graph. In case of the multiple steady-states, hysteresis arises from the presence of the high-order (that is, containing all independent compounds) critical fragment in the graph. Auto-oscillations can arise if the critical fragment of the lower order is present in the graph. This rule allows search for the origin of auto-oscillation in the lower-order fragments when investigating auto-oscillatory mechanisms. From this viewpoint, the critical fragments found in this study will be useful for understanding molecular mechanisms of critical behavior of biochemical systems.

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