Enzyme Isomerization and Concentration Oscillations in Five-Component Biochemical Systems

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Abstract—The kinetic behavior of mnemonic enzymes is analyzed. Based on sufficient conditions for initiation of oscillations, the possibility of concentration oscillations in an open five-component enzymatic system is shown.

Key words: nonlinear kinetics, mnemonic enzyme, oscillation

Oscillatory phenomena have been observed in some enzymatic systems [1]. Autocatalytic steps are usually considered to be a chemical source of oscillations in these systems [2]. However, there are some models in which autocatalysis is not the origin of auto-oscillations. Thus, it was shown that substrate inhibition can also be an origin of oscillations in enzymatic systems [3-5]. Enzyme isomerization in the course of reaction can also result in oscillatory behavior [6, 7]. In this case, various enzyme forms (isomers) compete for the same substrate.

As shown in [7], an oscillatory mode can arise from enzyme inactivation during the enzymatic reaction, but these oscillations will decay.

It is well known that autocatalytic steps are difficult to consider in the chemical sense. From this viewpoint, enzymatic systems not containing autocatalytic steps are of particular interest.

How simple can an oscillatory non-autocatalytic enzymatic system be? In this work, we shall try to answer this question. It is shown that in enzymatic reactions including isomerization, immobilization, or substrate inhibition of the enzyme, non-decaying concentration oscillations are possible.

## ENZYME ISOMERIZATION DURING THE REACTION

Enzymes in solutions are known to exist in several mutually transforming isomeric forms. Enzymes subject to conformational changes are called hysteresis enzymes [8, 9]. In so-called mnemonic enzymes, conformational changes are induced by the enzyme binding to the substrate or product [10, 11]. Almost all regulatory enzymes are hysteresis or mnemonic enzymes [9-11].
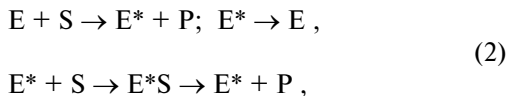
Usually conformers possess different affinity to the substrates and other ligands; that is why ligand binding results in a shift in the relationship between conformers and thus to the regulation of the enzyme activity.

In many enzymatic reactions a substrate is supposed to play a dual role: a substrate in a common sense and also an inhibitor in excess quantities; since two different conformations of the enzyme have different affinity to the substrate, a fine substrate auto-regulation of the enzyme activity can be expected. It should be noted that in these mechanisms only one isomeric form of the enzyme is supposed to be catalytically active, i.e., the transformed conformer does not catalyze the reaction.

We suggest that another type of fine regulation of the rate of enzymatic reaction is possible. The following sequence of reactions catalyzed by a mnemonic enzyme can be presented:

$$E + S \leftrightarrow ES \rightarrow E^* + P; \ \ E^* \rightarrow E \ ,$$

$$E^* + S \leftrightarrow E^*S \rightarrow E^* + P. \tag{1}$$
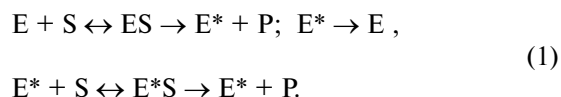
The stage of conformational transformations is known to be the limiting stage of enzymatic reaction with conformational changes of the enzyme [1, 9]. Supposing that the reaction rate is mainly defined by the rates of "slow" stages, the reaction system (1) can be presented in a simpler form:

$$E + S \rightarrow E^* + P; \ E^* \rightarrow E ,$$

$$E^* + S \rightarrow E^*S \rightarrow E^* + P ,$$
                                                                    (2)

where E, E*, and E*S are various forms of the enzyme. Reaction (1) can be considered as a modification of a scheme of mnemonic enzyme. After a catalytic act, conformation of mnemonic enzyme is changed and it "remembers" interaction with the substrate, slowly recovering its initial state.

The abovementioned modification is that the substrate is supposed to interact with the enzyme with the changed conformation and participate in the second catalytic act. Thus, two conformers of the enzyme compete for the same substrate and this results in formation of "critical" (auto-oscillation and/or multiple steady states) phenomena in the system (this question is considered below). Consequently, reaction scheme (2) can be a crucial nucleus in a reaction system of higher order.

SUFFICIENT CONDITIONS FOR INITIATION OF NON-DECAYING OSCILLATIONS IN FOUR-COMPONENT REACTION SYSTEMS

Within the first approximation, the kinetic behavior of a four-component chemical system is determined by the type of the roots of the characteristic equation of the linearized system of differential equations:

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

where λ is an eigenvalue of the Jacobian of the system and a_i ($i = 1-4$) are the real coefficients which are determined by the scheme of interaction of the components of the reaction system.

Solution of Eq. (3) by Ferrari's method results in the third-order equation, which is a restatement of Eq. (3):

$$y^3 - a_2y^2 + (a_1a_3 - 4a_4)y - a_1^2a_4 + 4a_2a_4 - a_3^2 = 0.$$
                                                                    (4)

Discriminants of Eqs. (3) and (4) are the same:

$$D = -108Q ,$$
                                                                    (5)

where

$$Q = \left(\frac{P}{3}\right)^3 + \left(\frac{q}{2}\right)^2 ,$$

$$P = -\frac{a_2^2}{3} + (a_1a_3 - 4a_4) ,$$

$$q = -\frac{2a_2^3}{27} + \frac{a_2}{3}(a_1a_3 - 4a_4) + (4a_2a_4 - a_1^2a_4 - a_3^2).$$
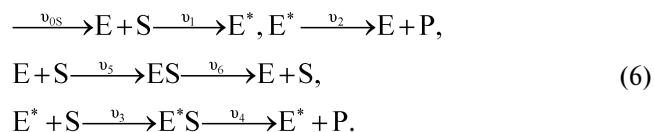
It is obvious that if the discriminant (D) of Eq. (3) is negative, the roots of this equation are complex-conjugated; this corresponds to the oscillatory behavior of a dynamic system. If the real parts of the roots of polynomial (3) are negative, oscillations will decay, but if the real part of at least one pair of the roots of Eq. (3) is positive, arising oscillations will not decay. In this case, if concentrations of all reagents are limited at $t \rightarrow \infty$ (if concentration of at least one reagent increases indefinitely, the considered model has an essential drawback and of course, should be re-considered), non-decaying auto-oscillations arise in the system. Signs of the real parts of the roots of characteristic equation (3) are determined by the Rauss−Gurvits criterion. From the aforesaid it can be assumed that if at least one of the coefficients a_i is negative, Eq. (3) has roots with a positive real part. So, a sufficient condition for existence of non-decaying oscillations in a four-component dynamic systems is simultaneous fulfillment of the following two conditions: negativity of the discriminant of Eq. (4) and negativity of one of the coefficients a_1, a_2, a_3 in Eq. (3).

POSSIBILITY FOR INITIATION OF CONCENTRATION OSCILLATIONS IN A FIVE-COMPONENT OPEN NON-AUTOCATALYTIC SYSTEM

Analysis of existing models of the systems of chemical reactions with oscillatory behavior shows that special interactions between the reagents are the physicochemical origin of initiation of oscillation modes. It can be definitely stated that interaction of reagents at the autocatalytic reaction stages is one of these special interactions. Competition of several interrelating reagents for a certain component of the system of reactions is another scheme causing oscillatory behavior of the reaction system [12]. Competitive interaction of enzymes (or isomers of the same enzyme) is a widespread phenomenon in biochemical reaction systems.

In the abovementioned hypothetical scheme (2), there arises a competition between the catalytically active conformers of the mnemonic enzyme, and thus the oscillation mode can be initiated.

Let us consider scheme (2), accounting for the substrate inhibition of the catalytically active conformer E of the mnemonic enzyme:

$$\xrightarrow{v_{0S}} E + S \xrightarrow{v_1} E^*, E^* \xrightarrow{v_2} E + P,$$

$$E + S \xrightarrow{v_5} ES \xrightarrow{v_6} E + S,$$
                                                                    (6)

$$E^* + S \xrightarrow{v_3} E^*S \xrightarrow{v_4} E^* + P.$$

A system of kinetic equations of the reaction scheme (6) looks as follows:

$$\frac{d[E]}{dt}=\upsilon_2-\upsilon_1-\upsilon_5+\upsilon_6,$$

$$\frac{d[E^*]}{dt}=\upsilon_1+\upsilon_4-\upsilon_2-\upsilon_3,$$

$$\frac{d[E^*S]}{dt}=\upsilon_3-\upsilon_4, \tag{7}$$

$$\frac{d[S]}{dt}=\upsilon_{0s}-\upsilon_1-\upsilon_3-\upsilon_5+\upsilon_6,$$

$$\frac{d[ES]}{dt}=\upsilon_5-\upsilon_6,$$

where

$$\upsilon_1=k_1[E][S],\quad \upsilon_2=k_2[E^*],\quad \upsilon_3=k_3[E^*][S],$$

$$\upsilon_4=k_4[E^*S],\quad \upsilon_5=k_5[E][S],\quad \upsilon_6=k_6[ES].$$

Below are the results of analysis of scheme (6). There is one balance relationship in this system:

$$E + ES + E^* + E^*S = E_0, \tag{8}$$

where E_0 is the total concentration of all enzyme forms in the reaction system. This means that only four of five
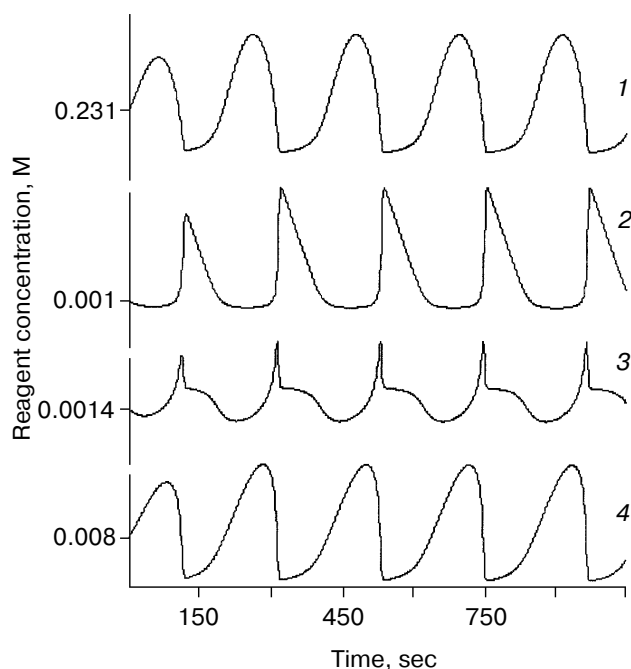
Fig. 1. Concentration oscillations of reagents in reaction system (6). The curves were obtained for the following relative steady-state concentrations: [S] = 0.23 (*1*), [E] = 0.001 (*2*), [E*] = 0.0014 (*3*), [E*S] = 0.008 (*4*) at the rate constants k_1 = 300 $M^{-1}\cdot sec^{-1}$, k_2 = 50 sec^{-1}, k_3 = 50 $M^{-1}\cdot sec^{-1}$, k_4 = 2 sec^{-1}, k_5 = 5 $M^{-1}\cdot sec^{-1}$, k_6 = 0.001 sec^{-1}, and υ_{os} = $8.6\cdot10^{-2}$ $M\cdot sec^{-1}$. For the initial deviation from the steady state [E*] = 0.00014 M^{-1}.

variable concentrations in the system (7) are independent and, consequently, the considered system can be characterized by a fourth-order characteristic polynomial (3). There are seven rates in the considered system. In the steady state the following equalities are established: $v_1 = v_2$, $v_3 = v_4$, $v_5 = v_6$; consequently, three rates are independent, that is, four other rates can be expressed by them. The latter rates are dependent. Any three rates of seven can be chosen as independent: below we choose v_1, v_3, and v_5. It is obvious that the other four rates can be expressed as follows: $v_2 = v_1$, $v_4 = v_3$, $v_6 = v_5$; and $v_{os} = v_1 + v_3$.

To study the possible existence of non-decaying oscillations, let us check how the abovementioned sufficient conditions for auto-oscillations are met in the four-component system. For this, we shall calculate the coefficients of a characteristic equation (3) of the system of kinetic equations (7). In this work, the coefficients are determined according to the method developed by the author [13, 14]. The coefficients, which can change their signs on change in kinetic parameters, are of interest for us, and a_3 and a_4 of the characteristic polynomial (3) appeared to be such coefficients:

$$a_3=\upsilon_1\upsilon_3\upsilon_5\Big(\frac{1}{c_1c_2c_5}+\frac{1}{c_1c_3c_4}+\frac{3}{c_1c_3c_5}+\frac{1}{c_1c_4c_5}+$$
$$+\frac{1}{c_2c_3c_4}+\frac{1}{c_2c_3c_5}+\frac{1}{c_2c_4c_5}+\frac{1}{c_3c_4c_5}\Big)-$$
$$-\frac{\upsilon_1^2\upsilon_3}{c_1c_2c_3}+\frac{\upsilon_3^2\upsilon_1}{c_1c_2c_4}+\frac{\upsilon_3^2\upsilon_1}{c_1c_3c_4}(\upsilon_1+2\upsilon_3),$$

$$a_4=\frac{\upsilon_1\upsilon_3\upsilon_5}{c_1c_2c_3}\Big(\frac{\upsilon_3}{c_4}-\frac{\upsilon_1}{c_5}\Big)+\frac{\upsilon_3\upsilon_1\upsilon_5}{c_1c_4c_5}\Big(\frac{\upsilon_3}{c_2}+\frac{\upsilon_1+2\upsilon_3}{c_3}\Big).$$
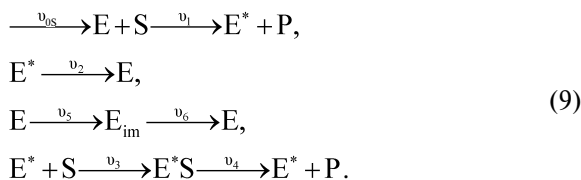
In these formulae c_i (i = 1-5) are steady-state concentrations of the substrate S, conformers of the enzyme E and E*, and complexes ES and E*S, and v_i (i = 0-6) are the steady-state rates of the particular reaction stages, respectively.

As can be seen in the formulae, the coefficients a_3 and a_4 can become negative on change in concentrations of the reagents. If $a_3 < 0$, then according to the Cartesian sign rule, characteristic Eq. (3) will have two positive roots. In this case, one of the two abovementioned sufficient conditions for existence of auto-oscillations is satisfied. At the negative values of discriminant (5), non-decaying oscillations arise. As can be seen in the formulae for a_3 and a_4, for initiation of auto-oscillations in the considered system, it is necessary (but not sufficient!) that $v_1 > v_3$ and $c_4 > c_3$. Besides, the rate constants of inhibition reactions should be negligible compared with the other rate constants. The results of numerical solution of system (7) corresponding to the oscillatory behavior of the system are presented in Fig. 1. The frequency of arising oscillations depends on the rate constants as well as on

the inflow rate of the incoming reagent (substrate). On decrease in the inflow rate the period of oscillations increases.

Thus, competition of two different isomers (E and E*) for the same substrate destabilizes the steady state of the reaction system and promotes initiation of the non-decaying concentration oscillations.

Let us consider another modification of system (2) including immobilization of one of the conformers of the free enzyme:

$$
\begin{aligned}
&\xrightarrow{\upsilon_{0s}} E + S \xrightarrow{\upsilon_1} E^* + P, \\
&E^* \xrightarrow{\upsilon_2} E, \\
&E \xrightarrow{\upsilon_5} E_{im} \xrightarrow{\upsilon_6} E, \\
&E^* + S \xrightarrow{\upsilon_3} E^*S \xrightarrow{\upsilon_4} E^* + P.
\end{aligned}
\tag{9}
$$

A system of kinetic equations describing the reaction scheme (9) is written as follows:

$$
\frac{d[E]}{dt} = \upsilon_2 - \upsilon_1 - \upsilon_5 + \upsilon_6 ,
$$

$$
\frac{d[E^*]}{dt} = \upsilon_1 + \upsilon_4 - \upsilon_2 - \upsilon_3 ,
$$

$$
\frac{d[E^*S]}{dt} = \upsilon_3 - \upsilon_4 ,
\tag{10}
$$

$$
\frac{d[S]}{dt} = \upsilon_{os} - \upsilon_1 - \upsilon_3 ,
$$

$$
\frac{d[E_{im}]}{dt} = \upsilon_5 - \upsilon_6 ,
$$

where

$\upsilon_1 = k_1[E][S]$, $\upsilon_2 = k_2[E^*]$, $\upsilon_3 = k_3[E^*][S]$,

$\upsilon_4 = k_4[E^*S]$, $\upsilon_5 = k_5[E]$, $\upsilon_6 = k_6[E_{im}]$.

Similar to the previous scheme, the balance equation can be written:

$$
E + E_{im} + E^* + E^*S = E_0.
\tag{11}
$$

That is why the characteristic polynomial of the system can be presented by Eq. (3). For the steady-state rates, the following relationships are justified: $v_1 = v_2$, $v_3 = v_4$, $v_5 = v_6$, and $v_{os} = v_1 + v_3$.

To analyze the kinetics of the system, let us calculate the coefficients of Eq. (3) in accordance with scheme (9). It can be easily shown that the first two coefficients of the characteristic polynomial (3) are positive at any values of kinetic parameters and steady-state concentrations of reagents. The coefficients, which can change their signs on change in kinetic parameters, are of interest for us,

and a_3 and a_4 of the characteristic polynomial (3) appear to be such coefficients. The expressions for a_3 and a_4 in the reaction scheme (9) are similar to the expressions for these coefficients for the reaction scheme (7). For initiation of auto-oscillations in system (9), the immobilization rate $v_5 = v_6$ should be negligible compared with the rates of other stages so that the negative term prevailed in this expression. Let us assume that the rate $v_5 = v_6$ is less than the rates of other stages and neglect the terms of this formula containing v_5.

Thus, neglecting the terms containing the steady-state immobilization rates in the expression for a_3, we obtain the simpler formulae for the coefficients a_3 and a_4 in the characteristic polynomial (3):

$$
a_3 \approx -\frac{\upsilon_1^2 \upsilon_3}{c_1 c_2 c_3} + \frac{\upsilon_3^2 \upsilon_1}{c_1 c_2 c_4} + \frac{\upsilon_3^2 \upsilon_1}{c_1 c_3 c_4}(\upsilon_1 + 2\upsilon_3),
$$

$$
a_4 = \frac{\upsilon_1 \upsilon_3 \upsilon_5}{c_1 c_2 c_3}\left(\frac{2\upsilon_3 + \upsilon_1}{c_4} - \frac{\upsilon_1}{c_5}\right) + \frac{\upsilon_3 \upsilon_1 \upsilon_5}{c_1 c_4 c_5}\left(\frac{\upsilon_3}{c_2} + \frac{\upsilon_1 + 2\upsilon_3}{c_3}\right).
$$

This should that auto-oscillations in system (9) can arise at sufficiently low immobilization rate of conformer E. For initiation of oscillations, it is necessary that $a_3 < 0$ and $a_4 > 0$ for any c_i.

These conditions should be met for the following reasons: at $a_3 < 0$, $a_4 < 0$ and $a_3 > 0$, $a_4 < 0$, there exists only one root with a positive real part (according to the Cartesian sign rule). This means that on fulfillment of such conditions, the discriminant of system (5) cannot be negative, that is, Eq. (3) does not have complex-conjugated roots and consequently, oscillatory behavior does not arise.

The results of numerical solution of system (10) are presented in Fig. 2, where changes in independent variables are defined (the fifth component depends on these variables in accordance with the balance Eq. (11)).

It should be noted that multiplicity of steady states in the area of existence of auto-oscillations is impossible in any of the abovementioned schemes, that is, these examples demonstrate that multiplicity of steady states is neither necessary nor sufficient condition for existence of oscillations. This conclusion confirms the idea of [15] and disapproves [16, 17], which state that multiplicity of steady states is a necessary or sufficient condition for oscillations in complex chemical systems.

In this work, we tried to answer a question: how simple can a non-autocatalytic system of reactions be? "Simple" means a system with the minimal number of degrees of freedom (the number of independent variables, i.e., concentrations), which have oscillatory mode of reaction.

We state that only two types of the elementary stages—auto-catalysis and competitive interaction of interrelated reagents—can be the source of critical (oscillatory) phenomena. Competitive interaction of the
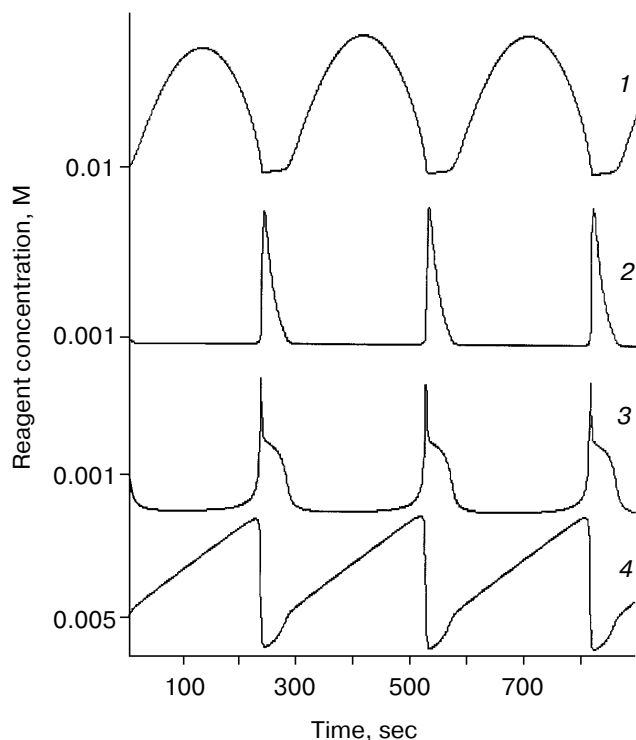
Fig. 2. Concentration oscillations of reagents in the reaction system (9). The curves were obtained for the following relative steady-state concentrations: [S] = 0.01 (*1*), [E] = 0.001 (*2*), [E*] = 0.001 (*3*), [E*S] = 0.005 (*4*) at the rate constants k_1 = 1200 $M^{-1} \cdot sec^{-1}$, k_2 = 12 sec^{-1}, k_3 = 600 $M^{-1} \cdot sec^{-1}$, k_4 = 1.2 sec^{-1}, k_5 = 0.12 $M^{-1} \cdot sec^{-1}$, k_6 = 0.012 sec^{-1}, and v_{os} = 1.8·10^{-2} $M \cdot sec^{-1}$. For the initial deviation from the steady state [E*] = 0.0001 M^{-1}.

enzymes (or isomers of the same enzyme) is widespread in biochemical reaction systems and that is why it deserves particular attention from the viewpoint of nonlinear kinetic phenomena.

The impossibility of auto-oscillations in three-component non-autocatalytic systems, whose elementary stages obey the mass-action expression, was shown by us earlier [12]. In this work, we demonstrate that in the open five-component enzymatic system auto-oscillations are possible.

The schemes of enzymatic reactions considered above are open in relation to the substrate concentration. Flowing systems modeled as open ones describe func-

tioning of biochemical "reactors". Really, biological "reactors" and systems are open; however, the question is what laws do the inflow reagents obey. In this work, we consider that the inflow rate of the substrate is constant, which is a rough approximation (and a drawback, from our viewpoint) of the considered models. Thus, a study of other, more complex laws of inflow of reagents is of special interest.

In this study, we consider mnemonic enzymes, and this choice is deliberate: almost all known mnemonic enzymes are regulatory ones [9, 11]. Critical phenomena in the systems of regulatory enzymes can cause, for example, the oscillatory behavior of cascades of biochemical reactions controlled by the regulatory enzymes and, consequently, can be the microscopic origins of macroscopic physiological periodic processes.

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