ROLES OF SUBSTRATE INHIBITION AND ENZYME ISOMERIZATION IN KINETICS OF BIOCHEMICAL OSCILLATIONS

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The chemical kinetic model is investigated to determine the condition under which a substrate inhibition and enzyme isomerization can lead to biochemical oscillation. A kinetic model for the two-center enzyme which under certain conditions allows the existence of oscillatory behavior is suggested. The main kinetic requirements for the existence of oscillatory regimes in biochemical reaction systems are distinguished.

Key words: enzyme oscillations

INTRODUCTION

Oscillatory behavior has been observed in many enzyme reaction systems [1]. Many investigators of biochemical oscillations believe that its chemical kinetic source has been generally attributed to an autocatalytic reaction mechanism. Even there is an opinion of necessity of autocatalysis for the oscillatory phenomena in enzyme reaction systems [2]. On the other hand, in number of studies it is suggested that substrate inhibition kinetics can also be a source of oscillatory behavior in chemical systems, particularly enzyme reactions. Spangler and Snell [3,4] studied a two-enzyme model system in which the product of one enzyme-catalyzed reaction acts as inhibitor for the other enzyme. This two-enzyme model was shown to exhibit bistability and sustained oscillations. Sel'kov [5] investigated a single enzyme model involving both substrate inhibition and product activation and observed oscillatory phenomena. The oscillations observed were attributed to the substrate inhibition kinetics, but no proof of this assertion was given. Seelig [6] investigated a model involving a single enzyme with substrate inhibition kinetics only (no product activation) and observed oscillations. However, this model involves two substrates, only one of which is an inhibitor. It is not clear whether the existence of multiple substrates is the necessary condition for oscillatory behavior in a system governed by substrate inhibition. Goldstein and Ivanova [7] considered a model of enzyme reaction system with both substrate inhibition and the enzyme isomerization and observed oscillatory phenomena. This model involves the double substrate inhibition, i.e. the substrate inhibits two conformational changed (isomerized) enzyme forms. Shen and Larter [8] investigated a model involving substrate inhibition and autocatalysis. Their investigation shows that though oscillatory behavior is observed in this system, it is caused by either autocatalytic properties of the mechanism or substrate inhibition coupled with product inhibition. They concluded that only substrate inhibition is insufficient for oscillatory phenomena in this mechanism. Several examples exit in the literature [9,10,11], but they comprise only evidence of sufficient conditions for oscillatory behavior, but which are not necessary. As it is obvious from the mentioned above, the autocatalysis mechanism is not necessary condition for the oscillatory phenomena in biochemical reaction networks, as well. Then the principle question arises about necessity of additional conditions for oscillations in chemical reaction systems, particularly enzyme reactions. In this paper we show that (1) neither autocatalysis, nor any other concrete reaction mechanism or its combinations (such as, substrate inhibition, product inhibition etc.) is not necessary condition for the oscillations; (2) for the oscillation phenomena it is necessary the existence of so called "critical reaction fragments".

THE METHOD OF ANALYSIS

Our analysis of non-linear kinetics of chemical reaction networks was carried out on the base of double-barrel graph theory [12,13,14]. The method connects structure of the kinetic schemes with the critical phenomena arising in it (multiplicity of stationary states, self-oscillations). It is known, that the kinetic behavior of system is determined by a characteristic polynomial of linearized systems of the kinetic equations:

$$P = \lambda^n + a_1\lambda^{n-1} + a_2\lambda^{n-2} + \ldots + a_m \qquad (1)$$

If in a stationary state even one of the coefficients in (1) has a negative sign this state becomes unstable and in the system there can be multiple steady states or self-oscillations. Ivanova [12] proved, that if the lower non-zero coefficient (a_m) was negative and there were no steady-state points at the border of the polyhedron of invariance determined by the material balance equations in the phase space, then there should be several steady-state points inside the polyhedron (multiple steady-states).

If $a_m>0$ at any concentration, then there is a single steady-state point (if the boundary conditions are fulfilled). In this case, if another coefficient $a_{m-k}<0$, then a single steady-state point can be unstable. A stable limited cycle i.e. self-oscillations occurs in the vicinity of this single unstable steady-state point.

This means that oscillations can arise if in the graph of common reaction network there is a critical fragment of lower order. This allows searching for the reason of oscillations in critical fragments of the lowered order. Such fragments in various reaction mechanisms can arise. They may be both autocatalytic and non-autocatalytic mechanisms, although, the number of such reaction mechanism is not large [15]. Therefore this shows, that neither autocatalysis, nor any other concrete reaction mechanism or its combinations is not the necessary condition for the oscillation phenomena.

The existence of lowered order of critical subgraph (reaction fragment) is the necessary condition, but un-sufficient for the arising of oscillatory behavior. Our structural-kinetic analysis allows us to support some additional requirements, which can lead to sustained oscillations. (1) From our careful study of the oscillatory kinetic models we have shown that the presence of the flow terms for the substrate (and/or other non-balanced compounds) is crucial for oscillatory behavior. (2) The rates of reaction stages, which consist of the critical fragment, could be sufficiently larger than the other reaction rates. In this case, if the sum of contributions of high-ordered subgrpahs remains positively, then oscillatory behavior is possible.

THE ROLE OF THE ENZYME ISOMERIZATION IN BIOCHEMICAL OSCILLATIONS

It is well-known, that enzymes in a solution exist in several (usually in two) isomerized forms [16]. Transitions between the conformers have essential significance for the oscillation behavior. Conformers of the enzyme, as a rule, have various affinities to substrate. In solutions these enzyme conformers interact with one substrate and thus create competition between these two forms of enzyme. It leads to the occurrence in the graph of two negative ways sequence, which enters into an even cycle and together with catalytic cycle forms the critical fragment. Thus there is an opportunity for existence of an oscillatory mode. To illustrate the role of enzyme isomerization in oscillation phenomena we had chosen the Guinoprotein Glucose Dehydrogenase enzyme (GDH), as an example. Experimental data show the biphasic cooperativity containing two sets of apparent kinetic parameters. The data allow to suggest, that GDH have two subunits in the two states of mutual interactions and the two catalytic cycles of GDH have different rate limiting steps [17]. Summarizing the literary data it is possible to present the basic scheme of reactions, catalyzed by the GDH, as follows:

1. $E + A \rightarrow EA;\ EA + S \rightarrow EAS;\ EAS \rightarrow EBP;$
   $EBP \rightarrow E^*B+P;\ E^*B \rightarrow E^* + B;$
2. $E + S \leftrightarrow ES$
3. $E^* \rightarrow E$
4. $E^* + A \rightarrow E^*A;\ E^*A + S \rightarrow E^*AS;\ E^*AS \rightarrow E^*BP;$
   $E^*BP \rightarrow E^*B+P;\ E^*B \rightarrow E^*+B.$

Here E and E^* are the different isomerized forms of free enzyme, A is the coenzyme, S is the substrate, B is the oxidized coenzyme and P is the product. As in other dehydrogenase reactions, in this scheme the linkage stage of a substrate to enzyme-coenzyme complex and the stage of isomerization of enzyme are rate limiting stages [16,17]. Stopping at slow stages of the reaction catalyzed by GDH, it is possible to present reaction in the following sequence: the substrate (S) contacts with the first catalytic center of enzyme (E). The first catalytic act occurs and enzyme passes to another isomerized form (E*) at which catalytic center of the second subunits becomes accessible for the substrate. It is a new isomerized form of enzyme, which has other affinity to a substrate, and is catalytic active, too. It can form active enzyme - substrate complex (E^*AS) and create a product (P). As well the conformation transition of the second isomerized form of enzyme into the first can be occurred ($E^* \rightarrow E$). Thus, as a result of two catalytic acts in the system there will be a biphase accumulation of a product with different parameters. Besides, this scheme permits the substrate inhibition: one can assume, that it occurs at linkage of the substrate to the second active center in the first conformation of enzyme and forms the inactive enzyme-substrate complex (ES), which is in a good agreement with experimental data [16]. Thus, stopping at slowly-stages, it can be written:

1. $E + A + S \xrightarrow{1} E^* + B + P;$
2. $E^* \xrightarrow{2} E;$
3. $E^* + A + S \xrightarrow{3} E^*AS$
4. $E + S \xrightarrow{4} ES$
5. $E^*AS \xrightarrow{5} E^* + B + P$
6. $ES \xrightarrow{6} E + S$

Consider a possibility of the arising of oscillation behavior in the suggested scheme of reaction. Five variable concentrations participate in the system. We shall denote their dimensionless quantities as:

$$c_1=S/S_0,\ c_2=E^*/E_0,\ c_3=E/E_0,\ c_4=ES/E_0,\ c_5=E^*S/E_0,$$

there, S_0 is the stationary concentration of the substrate, E_0 is the total concentration of enzyme (the sum of all enzyme and enzyme complexes). These variables are connected with a balance ratio:

$$c_2 + c_3 + c_4 + c_5 = 1$$

Hence, only four variables are independent and therefore, the characteristic polynomial of system will be of the 4-th order:

$$P = \lambda^4 + a_1\lambda^3 + a_2\lambda^2 + a_3\lambda + a_4 \qquad (2)$$

The sign of smallest nonzero coefficient a_4 of the characteristic polynomial for the given system is determined by the critical fragment of the 4-th order. If $a_4 > 0$ for any c_i in invariant area, i.e. in area where

$$c_i > 0,\ c_2 + c_3 + c_4 + c_5 = 1,\ c_1 \le c_{max},$$

then the stationary point is unique. Thus it is taken into account, that on a border of invariant polyhedron there are no stationary points. If, besides the inequality $a_3 < 0$ in a unique point, then this unique stationary point is unstable and is carried out around this point there is a steady limited cycle.

Stationary rates of reactions are connected by means of the following equality:

$$\upsilon_1=\upsilon_2,\ \upsilon_3=\upsilon_5,\ \upsilon_4=\upsilon_6,\ \upsilon_0=\upsilon_1+\upsilon_3.$$

Thus, from 12 stationary rates and concentrations seven are independent. Using a method of calculation of the characteristic polynomial coefficients [13,14] we obtain the expressions for the coefficients of characteristic polynomial

a_1, a_2, a_3 and a_4. The coefficients a_1 and a_2 are positive for any positive values of independent variables. An analysis of expression for a_3 shows, that in the field of self-oscillations the quantities $\upsilon_4=\upsilon_6$ should be small enough. Therefore neglecting the members containing υ_4 and υ_6 in the expression for a_3 one obtains more simple expression for a_3 as:

$$a_3 = \frac{\upsilon_2\upsilon_5}{c_1c_2c_3c_5}\{\upsilon_2(c_3 - c_5) + \upsilon_5(c_2 + 2c_3)\}$$

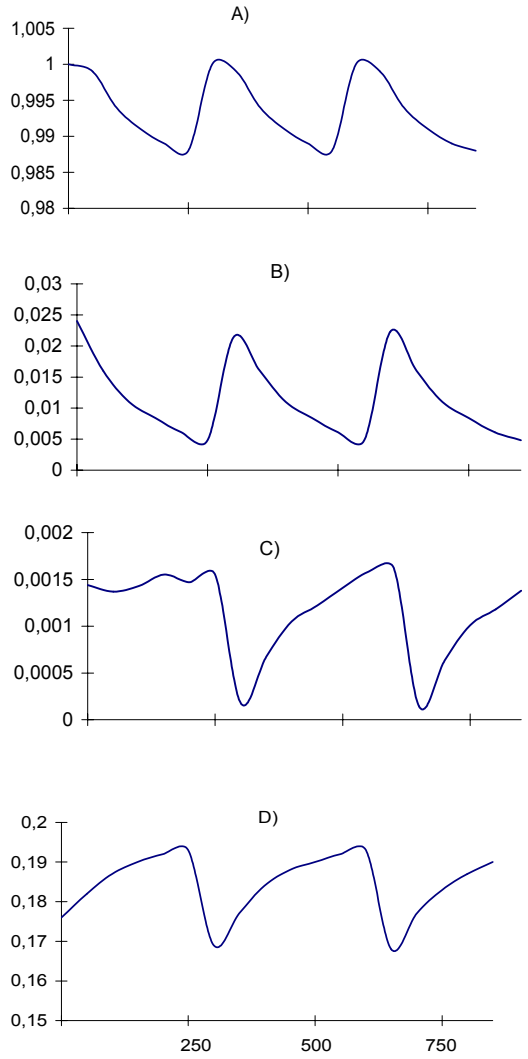

Fig.1. A time dependence of relative concentrations of the reaction scheme, considered above: A) substrate concentration (c_1); B) Free enzyme concentration (c_2); C) concentration of isomerized enzyme (c_3); D) concentration of E^*AS (c_5). Curves are obtained for the following values of relative concentrations and rate constants: $c_1=1$, $c_2=0.024$, $c_3=0.002$, $c_5=0.15$, $k_1=200$, $k_2=200$, $k_3=40$, $k_4=0.001$, $k_5=300$, $k_6=0.4$.

From this expression it is seen, that for occurrence of oscillations it is necessary, that $c_3 < c_5$. Therefore we shall consider below the area of concentration value, where this condition is satisfied. The expression for the coefficient a_4 has the form:

$$a_4 = \frac{\upsilon_2\upsilon_4\upsilon_5}{c_1c_2c_3c_4c_5}\{\upsilon_2(c_3 - c_5) + \upsilon_5(c_2 + 2c_3 + c_4)\}$$

For existence of self-oscillations, it is enough, that

$$\upsilon_5(c_2 + 2c_3) < \upsilon_2(c_5 - c_3)$$
$$\upsilon_5(c_2 + 2c_3 + c_4) > \upsilon_2(c_5 - c_3)$$

It is possible to combine these inequalities and as a result a sufficient condition for self-oscillations is obtained in a final form:

$$\frac{\upsilon_2}{\upsilon_5} < \frac{c_4}{c_5 - c_3}$$

If this condition is not fulfilled in the system there will be no self-oscillations, and there will be places of bistability in considered reaction system. Results of numerical calculations, which lead to non-damping self-oscillations, are shown in figure 1.

CONCLUSION

We have continued a study of substrate inhibition scheme originally done by Degn [19] to determine whether oscillatory behavior can be supported by it. Our calculation shows, that oscillatory behavior cannot be sustained only by such a mechanism. The application of double barrel graph theory allows us to support the main requirements, which lead to oscillation behavior: For the existence of oscillations in the system consisting of n reagents, at least m-order critical fragments must be: $m=n-f-1$. Here f is the number of the balance equations (i.e., the number of mass conservation laws). On the other hand we conclude, that the presence of the flow terms for the non-balanced reagents is necessary for the oscillation phenomena. Our third requirement is the existence of considerable difference of reaction rates between critical and non-critical fragments of common reaction networks. Obtained results also allow to predict from a general class of enzymes those that may be good candidates for the generation of oscillatory behavior. We find that soluble two-center dehydrogenases can lead to oscillations. Our model studies of oscillatory conditions for the two-center in the presence of substrate inhibition and the conformation transition may be a convenient basis for the investigation of more complex oscillatory events in biology. For example, in [20] it have been found that glucose stimulation of pancreatic β cells induces oscillations of the membrane potential, cytosolie Ca^{2+}, and insulin secretion. Each of those events depends on glucose metabolism. Both intrinsic oscillations of metabolism and repetitive activation of mitochondrial dehydrogenases by Ca^{2+} have been suggested by authors to decisive for this oscillatory behavior.

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SUBSTRAT İNHİBİRƏSİNİN VƏ FERMENT İZOMERİZASİYASININ BİOKİMYƏVİ RƏQSLƏRİN KİNETİKASINDA ROLU

Biokimyəvi rəqslərin yaranma şərtlərini təyin etmək üçün, substrat inhibirəsi və ferment izomerizasiyası olan fermentativ reaksiyaların kimyəvi modellərinin kinetikası tədqiq edilmişdir. Periodik axımın mövcudluğuna imkan verən, iki-mərkəzli ferment reaksiyası modeli təklif edilmişdir. Biokimyəvi reaksiya sistemlərində periodik rejimlərin mövcudluğu üçün əsas kinetik tələblər ayarlanmışdır.

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РОЛИ СУБСТРАТНОГО ИНГИБИРОВАНИЯ И ИЗОМЕРИЗАЦИИ ФЕРМЕНТА В КИНЕТИКЕ БИОХИМИЧЕСКИХ КОЛЕБАНИЙ

Для определения условия возникновения биохимических колебаний исследована кинетика химической модели ферментативной реакции с изомеризацией фермента и субстратным ингибированием, Предложена кинетическая модель реакции двух-центрового фермента, которая позволяет существование колебательного поведения. Выделены основные кинетические требования для существования колебательных режимов в биохимических реакционных системах.

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