GRAPH –THEORETIC ANALYSIS METHOD OF NON-LINEAR KINETICS OF BIOCHEMICAL REACTIONS

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Using the double barrel graph theory, the algorithmic method for determination of characteristic polynomial coefficients of system nonlinear kinetics equation of biochemical reactions is offered. Several characteristic polynomial coefficients are expressed by stoichiometric coefficients of reagents in the biochemical reaction system and their analytical expressions are obtained.

Key words: nonlinear enzyme kinetic, double barrel graph theory.

INTRODUCTION

At last time, theoretical methods for solution of kinetic problems based on linear graph theory, are widely used in biokinetic studies [1-8]. Linear graphs are used at analysis of the steady- and pre-steady-state (transient phase) of enzyme reactions with a single enzyme form participating in each elementary reaction. In linear mechanisms, the enzyme forms do not interact with each other at any stage. Moreover, each stage of the enzyme reaction in the transient phase according to assumption is a pseudo-first-order reaction because a change of the substrate concentration is negligible during the formation of intermediate enzyme forms.

In living cells, concentrations of many enzymes are often of the same magnitude, as concentrations of the metabolites, i.e. compounds participating in the elementary stages at similar concentrations are equivalent components of elementary reactions in many cases. Such systems are nonlinear. Development of more simple and convenient methods for research of stability of stationary states in nonlinear kinetic systems has not lost the importance. An attraction of the theory of double barrel graphs [9] (bigraphs) allows essentially to simplify the research scheme of stationary state stability.

BASIC DEFINITIONS AND THEORETICAL TREATMENTS

Two types of tops are used in representation of double barrel graph theory, where substances (reagents) are tops of the type A, and reactions are tops of the type B. Tops of different types are connected with oriented branches. The branch going from the substance, participating in the reaction, and other branch going from the reaction to the substance, which is produced as a result of this reaction, form a "positive" pathway. Graphically it is represented as $A_i \rightarrow B_k \rightarrow A_j$, where A is the top - substance, B_k is the top - reaction. Two branches going from two substances, participating in the same reaction, make a "negative" pathway, $A_i \rightarrow B_k \leftarrow A_j$. The closed sequence of ways forms the "cycle". Parity of a cycle is determined by the parity of a number of negative pathways in it. Unity of non-crossed on tops A cycles and branches, going from the substance to the reaction, is called as a "subgraph". The number of tops A in "subgraph" is called to as his "order". The m – order "fragment" is m reactions chosen from the scheme, considered in relation to chosen m substances. Other substances not participating in chosen reactions, do not enter in the "fragment" and are considered as constants. "Fragment" of the scheme refers to "critical" if the sum of coefficients of all his "subgraphs", containing odd number of "even" cycles, is more than the sum of coefficients of all other subgraphs. Subgraphs with odd number of even cycles give the negative contribution to the coefficients of a characteristic polynomial and are the reason of instability.

Stability of the stationary state is investigated by linearization of nonlinear kinetic equations near stationary points. Eigenvalues governing the behavior of the system near these steady states are solutions of the characteristic equation $|\lambda I - J| = 0$, where λ is an eigenvalue, I is the unit matrix, J is the Jacobian. Stability is provided, if the Jacobian matrix for system of kinetic equations with elements:

$$J_{ij} = \sum_{k=1}^{m} \gamma_{ij} \partial \upsilon_k / \partial u_j$$

has eigenvalues with negative real parts. Here, m is the number of reaction stages, $\gamma_{ik} = \beta_{ik} - \alpha_{ik}$, β_{ik} and α_{ik} are the stoichiometric coefficients (numbers of molecules of the compound U_i generated and going into the k-th reaction, respectively), u_i is the concentration of the i-th compound, υ_k is the rate of the k-th reaction. If even though one of coefficients α_i of the characteristic polynomial Jacobian matrix of the reaction system

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

(where m corresponds to the rank of the γ_{ik} matrix of the reaction system) changes its sign from plus to minus, when the reagent concentrations are changed, then the steady state of the system becomes unstable. According to Ivanova [10], if the lower coefficient (α_m) is negative and there are not steady-state points on 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 within the polyhedron (multiple steady states).

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

Thus, the analysis of existence of critical regimes is related with coefficients of the characteristic polynomial (1). The stage of determination of characteristic polynomial

coefficients is the most toilful stage in the analysis of nonlinear systems. In this work, we offer an algebraic algorithmic method for determination of these coefficients by using of bigraphs.

For this aim let's rewrite elements of the Jacobian matrix in the form:

$$J_{ij}=\sum_{k=1}^{m}(\beta_{ik}-\alpha_{ik})\partial\upsilon_k/\partial u_j=\sum_{k=1}^{m}\{(\beta_{ik}\partial\upsilon_k/\partial u_j-\alpha_{ik}\partial\upsilon_k/\partial u_j)_{i\neq j}+(\gamma_{ik}\partial\upsilon_k/\partial u_j)_{i=j}\}=$$

$$=\sum_{k=1}^{m}(P_{ji}^k+N_{ji}^k+H_i^k)$$

where,

$$P_{ji}^k=\beta_{ik}\frac{\partial\upsilon_k}{\partial u_j},\quad N_{ji}^k=-\alpha_{ik}\frac{\partial\upsilon_k}{\partial u_j},\quad H_i^k=\gamma_{ik}\frac{\partial\upsilon_k}{\partial u_i}. \tag{2}$$

From the point of view of the double barrel graph theory, these quantities have a real topological sense: P_{ji}^k is the positive pathway from A_j-top to A_i-top through B_k-top, i.e. the pathway corresponds to the generation of compound i in the reaction k; N_{ji}^k is the negative pathway from A_j- top to A_i-top through B_k-top, i.e. the pathway corresponds to interaction of compounds j and i in the reaction k; H_i^k is the half-pathway (or segment) of compound i involved in the reaction k. Here it is necessary to note that in non-autocatalytic reactions $\beta_{ij}=0$ at $\alpha_{ij}\neq 0$ and therefore in this case we obtain the value for half-pathway as in [11]:

$$H_i^k=-\alpha_{ik}\frac{\partial\upsilon_k}{\partial u_i}$$

This expression is true only for non-autocatalytic reactions.

If the reaction proceeds according to the mass action law, i.e., if

$$\upsilon_k=K_k u_1^{\alpha_{1k}}\ldots u_n^{\alpha_{nk}}$$

where K_k is the rate constant of k-th reaction stage, then expressions for pathways and half-pathways are simplified:

$$P_{ij}^k=\alpha_{ik}\beta_{jk}\frac{\upsilon_k}{u_i};\ N_{ij}^k=-\alpha_{ik}\alpha_{jk}\frac{\upsilon_k}{u_i};\ H_i^k=\alpha_{ik}\gamma_{ik}\frac{\upsilon_k}{u_i} \tag{3}$$

(υ_k is the rate of the stage k, u_i is the concentration of the i– th compound).

Let's write the sum of elements of the i-th column of the Jacobian matrix. This sum will be called by the nodal polynomial for the i-th A-top of a bigraph. The nodal polynomial is equal to the sum of all pathways (positive and negative) and half-pathways originating from the given i - th A-top:

$$M_i=\sum_{j=1}^{n}\sum_{k=1}^{m}\left(P_{ij}^k+N_{ij}^k+H_i^k\right) \tag{4}$$

For the determination of coefficients α_i of the characteristic polynomial (1), using the conception of the nodal polynomial, we offer following rules of determination:

- α_1 is the sum of all half-pathways originating from all nodes with the negative sign, i.e., α_1 is determined as the sum of contributions of all possible first-order "subgraphs":

$$\alpha_1=-\sum_{i=1}^{n}\sum_{k=1}^{m}H_i^k \tag{5}$$

- α_2 is the sum of all twin products of all nodal polynomials, i.e., α_2 is determined as the sum of contributions of all possible two-order "subgraphs":

$$\alpha_2=\sum_{i=1}^{n}\sum_{j=1}^{n}M_iM_j\quad(i\neq j) \tag{6}$$

- α_3 is the sum of all triple products of all nodal polynomials, i.e., α_3 is determined as the sum of contributions of all possible three-order "subgraphs":

$$\alpha_3=\sum_{i=1}^{n}\sum_{j=1}^{n}\sum_{k=1}^{n}M_iM_jM_k\,(i\neq j,\ i\neq k,\ j\neq k),\ etc. \tag{7}$$

Some of members obtaining in these products are equal to zero. These members are determined by following rules of "member reduction":

- a product where the index of one B–top presents more than once is equal to zero;
- products where pathways do not form cycles (determined by lower indices of the pathways) also are equal to zero (cyclic products are included into the sum with the opposite sign);

- members corresponding to the product of the pathway and the half-pathway are equal to zero.

EXPRESSION OF POLYNOMIAL COEFFICIENTS VIA STOICHIOMETRIC COEFFICIENTS OF REAGENTS

The above-stated rules allow us to express coefficients α_i of the characteristic polynomial (1) through stoichiometric coefficients of reagents. Really, taking into account (3) and (4) in (5), we obtaine for α_1:

$$\alpha_1 = \sum_{i=1}^{n}\sum_{k=1}^{m} \alpha_{ik}\gamma_{ik}\frac{\overline{\upsilon}_k}{\overline{u}_i} \qquad (8)$$

Other coefficients are expressed through stoichiometric coefficients with point of view of reduction rules. In the present work, results of calculations for characteristic polynomial coefficients α_2 ,α_3 and α_4 are presented:

$$\alpha_2 = \sum_{i=1}^{n}\sum_{j>i}^{n} \alpha_{ik_1}\alpha_{jk_2}(\gamma_{ik_1}\gamma_{jk_2} - \gamma_{ik_2}\gamma_{jk_1})\frac{\overline{U}_{k_1}}{\overline{u}_i}\frac{\overline{U}_{k_2}}{\overline{u}_j} \quad k_1\neq k_2,\ k_1=1,\ 2\ \ldots\ m,\ k_2 = 1,\ 2\ \ldots\ m \qquad (9)$$

$$\alpha_3 = \sum_{i=1}^{n}\sum_{j>i}^{n}\left\{\sum_{l>j}^{n} b_{31}b_{32} - \sum_{l\neq i,j}^{n} b_{31}(b_{33} + b_{34})\right\}\frac{\overline{\upsilon}_{k_1}}{\overline{u}_i}\frac{\overline{\upsilon}_{k_2}}{\overline{u}_j}\frac{\overline{\upsilon}_{k_3}}{\overline{u}_l} \qquad (10)$$

where

$$b_{31} = \alpha_{ik_1}\alpha_{jk_2}\alpha_{lk_3},$$
$$b_{32} = \gamma_{ik_1}\gamma_{jk_2}\gamma_{lk_3}$$
$$b_{33} = \gamma_{ik_2}\gamma_{jk_1}\gamma_{lk_3},$$
$$b_{34} = \gamma_{ik_3}\gamma_{jk_1}\gamma_{lk_2},\quad k_1\neq k_2\neq k_3,\ k_1=1,2\ldots m,\ k_2=1,2\ldots m,\ ,\ k_3=1,2\ldots m.$$

For the coefficient α_4 we obtaine:

$$\alpha_4=\sum_{i=1}^{n}\sum_{j>i}^{n}\left\{\sum_{l>j}^{n}\sum_{h>l}^{n} b_{41}b_{42}-\sum_{l>i}^{n}\left(\sum_{h>l}^{n} b_{41}b_{33}\gamma_{hk_4}+\sum_{h\neq i,j,l}^{n} b_{41}b_{34}\gamma_{hk_4}+\sum_{h>i}^{n} b_{41}(b_{43}-b_{44})\right)\right\}\frac{\overline{U}_{k_1}\overline{U}_{k_2}\overline{U}_{k_3}\overline{U}_{k_4}}{\overline{u}_i\overline{u}_j\overline{u}_l\overline{u}_h}$$

here,

$$b_{41} = b_{31}\alpha_{hk_4}$$
$$b_{42} = b_{31}\alpha_{hk_4},$$
$$b_{43} = \gamma_{ik_4}\gamma_{jk_1}\gamma_{lk_2}\gamma_{hk_3},$$
$$b_{44} = \gamma_{ik_2}\gamma_{jk_1}\gamma_{lk_4}\gamma_{hk_3},$$
$$k_1\neq k_2\neq k_3\neq k_4\ ,\ k_1=1,\ 2\ldots\ m,$$
$$k_2=1,2\ldots m\ ,\ k_3=1,\ 2\ldots\ m,\ \ k_4=1,\ 2\ldots\ m.$$

In this formulas $\overline{\upsilon}_k$, and $\overline{U}_i$ are the stationary rate of k_i-th reaction and the stationary concentration of i-th reaction component, respectively.

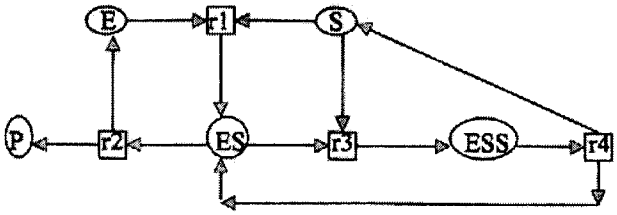
At necessity it is possible to obtain corresponding expressions also for other coefficients. For this purpose it is necessary to express nodal polynomials through stoichiometric coefficients and to reject members which are equal to zero on the appropriate items of the reduction rules. It is necessary to note that the negative members in the obtained expressions of characteristic polynomial coefficients express contributions of destabilizing interactions in the reaction system. Contributions from cyclic fragments in the structure of the reaction graph are such. If the sum of negative members is more than the sum of positive members then the appropriate interaction causes destabilization of a stationary state and it can be the reason of existence of multi steady state and/or self-oscillation regimes in reaction system.

In some relatively simple cases, the obtained expressions of polynomial coefficients allow to predict kinetic behavior of a system without necessary calculations. For example, let us consider the problem of existence of self-oscillation regimes in three – component nonautocatalitic biochemical systems. From the formula (8) it follows, that for all nonauto-catalitic reactions $\alpha_1>0$. The sign of the second coefficient is determined from (10) and it is obvious, that for ordinary biochemical reactions, where at each elementary stage no more than two various components participate, $\alpha_2>0$. This reason leads to the following conclusion: Self-oscillations are impossible in three-component nonautocatalitic, biochemical systems in range of the first-order approximation of stability.

As an example of illustration of the offered approach we shall consider the following system of reactions. It is the scheme of the well-known substrate inhibition reaction.

$$E + S \rightarrow ES \qquad (r1)$$
$$E\ S \rightarrow E + P \qquad (r2)$$
$$ES + S \rightarrow ESS \qquad (r3)$$
$$ESS \rightarrow ES + S \qquad (r4)$$

In this system there are 4 interacting substances (*A*-tops) and 4 reactions (*B*-tops). In the representation of the double barrel graph the considered system is represented as:



In this figure, letters in circles (*A*-tops) mean reagents, and figures in squares (*B*-tops) - the reactions. The arrow going from i-th A-top to j-th B-top means the entry of the i-th reagent in j-th reaction. The arrow going from j-th B-top to i-th *A*-top means formation of i-th reagent at the j-th reaction.

For the research of nonlinear kinetics of the given reaction system we shall write matrixes of stoichiometric coefficients, β_{ij}, α_{ij} and γ_{ij} ($i=1...4$, number of interacting reagents, and $j=1 ... 4$, number of reactions):

$$\begin{array}{c|cccc} & \beta_{ij} & & \alpha_{ij} & & = & \gamma_{ij} \\ S & 0\ 0\ 0\ 1 & & 1\ 0\ 1\ 0 & & & -1\ \ 0\ -1\ 1 \\ E & 0\ 1\ 0\ 0 & - & 1\ 0\ 0\ 0 & = & & -1\ \ 1\ \ 0\ 0 \\ ES & 1\ 0\ 0\ 1 & & 0\ 1\ 1\ 0 & & & \ \ 1\ -1\ -1\ 1 \\ ESS & 0\ 0\ 1\ 0 & & 0\ 0\ 0\ 1 & & & \ \ 0\ \ 0\ \ 1\ -1 \end{array}$$

It is easy to show that the rank of γ_{ij} matrix is equal to 3.

It means, that in the system there is one balance equation, and only 3 from 4 variable of concentrations of reagents are independent. It is obvious, because the total enzyme concentration must conserve:

$$E+ES+ESS=E_o=Const. \qquad (11)$$

Therefore the characteristic polynomial of the system will be in the third order:

$$P = \lambda^3 + \alpha_1\lambda^2 + \alpha_2\lambda + \alpha_3$$

On the other hand, it is easy to show, that on border of a polyhedron of the invariance, determined by the equation (11), there are no stationary points, i.e. there is no a stationary point, where concentrations of some reagents are equal to zero. Therefore if $\alpha_3>0$ then the stationary point of the system is unique. However if $\alpha_3<0$ and other coefficients are positive, then in the system multiple stationary points are presented, i.e. poly-stability takes place. If $\alpha_3>0$ and at least one of other coefficients (α_1 or α_2) are negative, then sustained oscillations arise around this stationary point. Let coordinates of a stationary point will be $(\overline{u_1}, \overline{u_2}, \overline{u_3}, \overline{u_4})$.

We shall proceed now to definition of coefficients α_i. They are determined from the 4x4 dimensional determinant of the Jacobian matrix. Really, we are interested only in signs of these coefficients and arising in these coefficients negative components cause interest with the point of view of a possibility of critical phenomena in the kinetics of given system. According to formulas (8-10) we obtaine:

$$\alpha_1 = \frac{\overline{\upsilon}_1 + \overline{\upsilon}_3}{\overline{u}_1} + \frac{\overline{\upsilon}_1}{\overline{u}_2} + \frac{\overline{\upsilon}_2 + \overline{\upsilon}_3}{\overline{u}_3} + \frac{\overline{\upsilon}_4}{\overline{u}_4} > 0$$

$$\alpha_1 = \frac{\overline{\upsilon}_1\overline{\upsilon}_3}{\overline{u}_1\overline{u}_2} + \frac{\overline{\upsilon}_1\overline{\upsilon}_4}{\overline{u}_1\overline{u}_4} + \frac{\overline{\upsilon}_1\overline{\upsilon}_2}{\overline{u}_2\overline{u}_3} + \frac{\overline{\upsilon}_1\overline{\upsilon}_4}{\overline{u}_2\overline{u}_4} + \frac{\overline{\upsilon}_2\overline{\upsilon}_4}{\overline{u}_3\overline{u}_4} + \frac{1}{\overline{u}_1\overline{u}_3} > \left(2\overline{\upsilon}_1\overline{\upsilon}_3 + \overline{\upsilon}_1\overline{\upsilon}_2 + \overline{\upsilon}_2\overline{\upsilon}_3\right) > 0$$

$$\alpha_3 = \frac{\overline{\upsilon}_1\overline{\upsilon}_2}{\overline{u}_1\overline{u}_3} \cdot \left(\frac{\overline{\upsilon}_4}{\overline{u}_4} - \frac{\overline{\upsilon}_3}{\overline{u}_2}\right)$$

We see that, coefficients α_1 and α_2 are positive at any positive values of concentration of reagents. It means, that sustained oscillations in the considered system are impossible. However, there is an opportunity for existence of the multi-stability. It is possible, if $\overline{u}_2 < \overline{u}_4$, since in the stationary state $\overline{\upsilon}_3 < \overline{\upsilon}_4$ and therefore, $\alpha_3 < 0$. This conclusion can seem unusual, because in many models the multi-stability and self-oscillations coexist and are realized by change of kinetic parameters. This example shows that there are systems admitting multi-stationarity and not admitting self-oscillations. In this example our purpose is to show the certain advantage of the offered method of the analysis of nonlinear kinetics of biochemical reactions. Therefore we do not stop on the analysis of a condition of multi-steady states in the considered system. However we would like to note, that this obtained condition of multi-stability not always hits to an eye at research by other methods.

CONCLUSION

The obtained expressions of polynomial coefficients can seem as complicated at first glance. However, they are, much more simple in comparison with other methods [10,12] and they are easily programmed on electronic means of calculation.

Moreover, the offered method for the determination of characteristic polynomial coefficients has the important advantage: this method allows to distinguish the fragments, which are the critic fragments. In other words, above-stated facts allow to see, that interaction of what reagents in what stages of a reaction are destabilizing factor for the stable

stationary state. It is very important for the understanding of the molecular basis and mechanisms of non-ordinary behavior of biochemical reaction systems.

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BİOKİMYƏVİ REAKSİYALARIN QEYRİ-XƏTTİ KİNETİKASININ QRAF-NƏZƏRİ ANALİZ METODU

İki lüləli qraf nəzəriyyəsinin tətbiqi ilə biokimyəvi reaksiyaların kinetikasının qeyri-xətti tənliklər sisteminin xarakteristik tənliyinin əmsallarının tapılması metodu verilmişdir. Bu əmsallardan bəziləri reagentlərin stexiometrik əmsalları ilə ifadə olunmuş və onların analitik ifadələri alınmışdır.

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ГРАФ-ТЕОРЕТИЧЕСКИЙ МЕТОД АНАЛИЗА НЕЛИНЕЙНОЙ КИНЕТИКИ БИОХИМИЧЕСКИХ РЕАКЦИЙ

С применением теории двудольных графов предложен метод определения коэффициентов характеристического уравнения системы нелинейных кинетических уравнений биохимических реакций. Некоторые из этих коэффициентов выражены стехиометрическими коэффициентами реагентов и получены их аналитические выражения.

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