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A new kinetic model for biochemical oscillations: Graph-theoretical analysis

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ABSTRACT

A graphical analysis demonstrates the ability of slow substrate activation and certain types of cooperativity between the two enzyme active sites to generate sustained oscillations. The analysis allows us to estimate kinetic parameter values for which oscillations exist. The scheme analyzed can explain the cyclical changes in functioning of various motor enzymes. Moreover, this scheme does not generate bistability for any parameter values. The graphical analysis presented is simple and visually clarifies the regulatory role of the details in the kinetic schemes.

1. Introduction

Biochemical networks consist of many enzyme-catalyzed reactions. Some of them are capable of critical kinetic behaviour such as bistability or oscillations [1,2]. This behaviour is widely studied [3–5,28–32].

Graph-theoretic methods simplify modeling of critical phenomena by ruling out certain classes of enzyme mechanisms unable to generate the critical types of kinetics [6–15,30–32]. Moreover, this paper shows that a graph-theoretic approach allows us to estimate the parameter values for which the desired critical regime can be obtained.

Many kinetic models of enzymological reactions use empirical rate equations similar to the classic Michaelis–Menten equation. However, many real enzymological reactions involve irreversible or slow steps [16–19,28]. Therefore, the Michaelis–Menten equation is often unacceptable for modeling.

A graph-theoretic analysis of this paper escapes the problems of the Michaelian approximation by considering mass-action kinetics. Our analysis is based on the methods by Clarke [6] and Ivanova [7,8], applied to the biochemical systems by Goldstein et al. [12–15,20,21,23].

We demonstrate here the detailed graphical analysis on a biochemical example. We hope this simple graphical analysis will be useful for many other practical applications.

An illustrative example considered is a new oscillation mechanism, which paradoxically involves substrate activation. Usually, not the substrate-activated but the substrate-inhibited enzymes are considered in the models of biochemical oscillations [2]. Meanwhile, substrate activation can be of importance for regulation of the biochemical processes [22,33,34].

The graphical analysis of this paper reveals some important properties of kinetic schemes, because their properties depend on their topological structure. We discuss the schemes which can and which can not induce concentration oscillations and which can induce oscillations but can not induce bistability.

2. Results

2.1. A new oscillation model

We analyze the following enzymological system:

$$\begin{aligned} &E_0 + S \underset{2}{\overset{1}{\rightleftharpoons}} E_1, \\ &E_1 + S \xrightarrow{3} E_2 \xrightarrow{4} E_1 + P, \\ &E_2 + S \xrightarrow{5} E_3 \xrightarrow{6} E_2 + P, \\ &\xrightarrow{7} S \end{aligned} \tag{1}$$

Various $E_i (i = 0,1,2,3)$ specify enzyme species, S and P — substrate and product, correspondingly.

In the enzymological system (1) the reversible substrate S binding in the reactions 1 and 2 produces the enzyme activation such that species E_1 and E_2 become catalytically active in respect to this substrate S. Two practically irreversible catalytic processes (reactions 3, 4 and 5, 6) are supposed to be characterized by different kinetic parameters. For example, this difference can be a result of cooperativity for two enzyme subunits. Irreversibility is not of principal importance and is used here for simplicity. Reaction 7 relates to the substrate influx.

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A graph-theoretic analysis of oscillatory kinetic schemes has been performed in our former papers [12–15,20,21] and in the papers by other authors [5–11,30–32]. However, no one of the former models uses substrate activation. A paper by Bayramov [35] modifies our former model [14], which does not consider substrate activation.

Oscillatory models, using multiple substrate interactions in the irreversible reactions, have been studied in application to the peroxidase–oxidase system [28,29]. These models are represented by the kinetic schemes of different topological structure and explain concentration oscillations quite differently [28].

Regulatory ATP binding is shown to activate the specific ATP-hydrolyzing sites in dynein [22,33]. Our paper is not devoted to modeling of dynein's function, however, a new possibility to regulate dynein activity can be of interest for specialists in this field. The oscillatory dynein activity has been discussed elsewhere [24].

For the analysis we normalize all the concentration variables in the system (1):

$$x_0 = [E_0], x_1 = [E_1], x_2 = [E_2], x_3 = [E_3], x_4 = [S] \tag{2}$$

The concentrations of the enzyme species are interconnected by the concentration conservation:

$$x_0 + x_1 + x_2 + x_3 = 1 \tag{3}$$

Therefore, only 4 of 5 concentration variables are independent variables.

2.2. Graph-theoretical analysis

We represent the reaction system (1) by the following bipartite Graph 1:

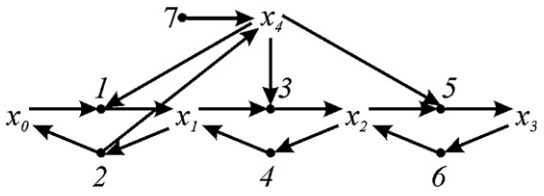
The elementary reactions are shown here by points and indicated by numbers, 1 to 7. Product is omitted in Graph 1 because the product-forming reactions are assumed to be irreversible.

Analysis of system stability and connected phenomena of oscillations and bistability requires the solution of linearized kinetic equations. This solution gives the characteristic polynomial. Its coefficients can be analyzed without finding eigenvalues [6].

Theorems by Ivanova [7,8] prove the following. If no equilibrium points exist on the border of the phase space (space of the concentration variables), then all phase trajectories go to inside of the phase space. If also the characteristic polynomial coefficient of the highest order (a_4 in our case) is positive at any concentration values, then a single equilibrium point exists in the phase space. If also the next coefficient (a_3 in our case) is negative in the equilibrium point (steady state), then this steady state is unstable. This is a sufficient condition for the sustained concentration oscillation in the system (the limit cycle around the single unstable equilibrium point).

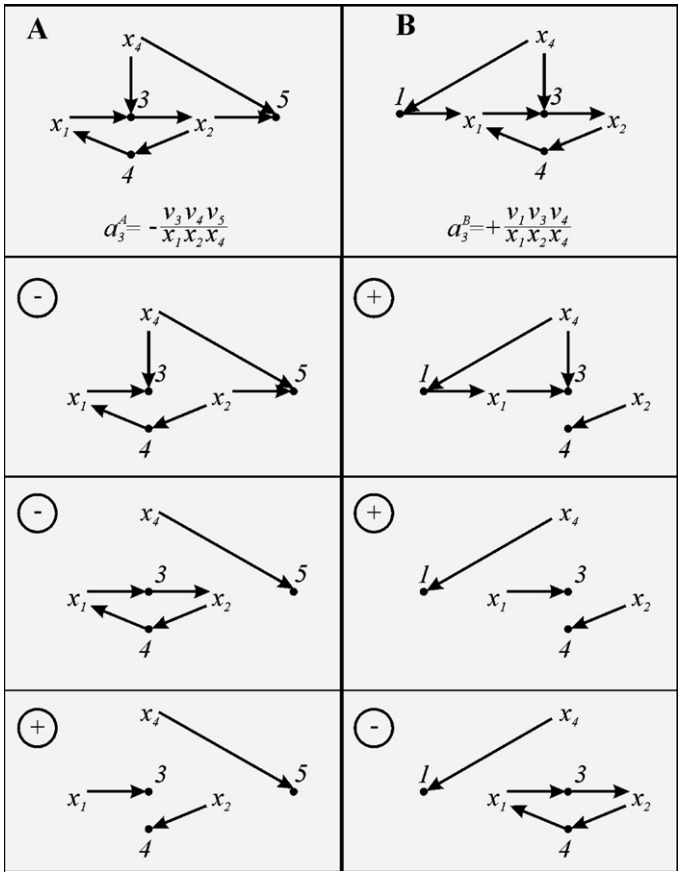
It is seen that the analysis of the system, represented by Graph 1, requires the sign determination for the coefficients (a_4 and a_3) of the characteristic polynomial.

The papers by Ivanova [7,8] show that the coefficients of the characteristic polynomial are determined as the sums of the values of the sub-graphs in the graph.



Graph 1. Graphical representation of the enzymological system (1).

Table 1
Two of the third order fragments of Graph 1.



Left column shows a negative Fragment A and its three sub-graphs characterized by different signs. Fragment B in the right column is shown to be positive as obtained by summation of the two positive and the single negative sub-graphs.

The value of a sub-graph equals the product of the values of the cycles constructing this sub-graph. A directed cycle can involve reaction paths (→•→) and interaction paths (→•←). A cycle, involving the even number of interaction paths and any number of reaction paths, has the negative value. A cycle, involving the odd number of interaction paths, has the positive value. The absolute value of a cycle equals its multiplied reaction rates divided by its multiplied species concentrations [7,8]. The simplest cycle degenerates into the so-called positive half-path (→•) [7,8].

The comparison of various graph fragments gives us the estimation of the kinetic parameters required for the oscillatory phenomenon.

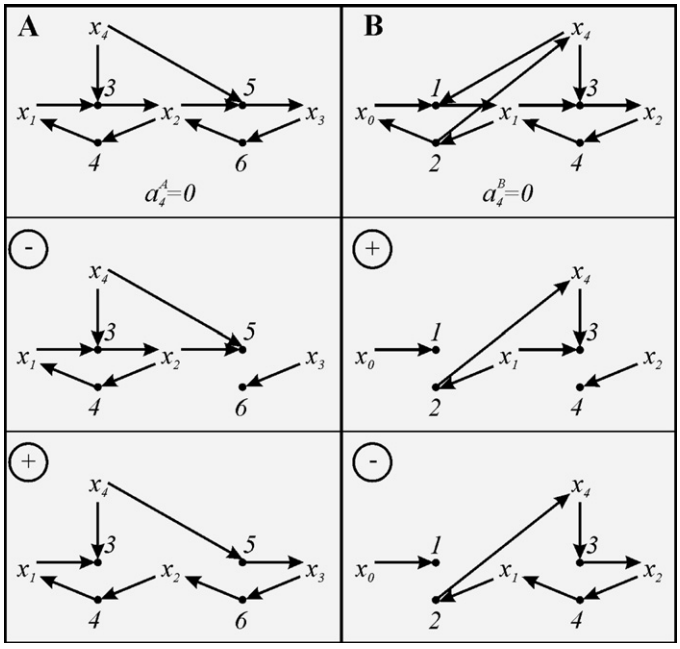
We show that some graph fragments in Graph 1 have values zero producing no effect on the kinetic behaviour.

Moreover, we show that a fragment, producing the negative (destabilizing) a_3 coefficient, becomes eliminated in the a_4 coefficient. Thereby, we show that Graph 1 can induce oscillations but never can induce bistability.

We analyzed the fragments of Graph 1, involving 3 species and 3 reactions, and other fragments, involving 4 species and 4 reactions (see Tables 1 and 2). These fragments induce terms in the coefficients (a_3 and a_4) of the characteristic polynomial. We denote as v_r the steady-state rate of the r-th reaction, and as x_j the steady-state concentration of the j-th species.

Table 1 shows two graph fragments (A and B) of Graph 1. Both fragments are the fragments of the third order (involve 3 species and 3 elementary reactions). In two columns of Table 1 the sub-graphs of the corresponding fragments are shown.

Table 2
Two of the fourth order fragments of Graph 1.



Both fragments do not induce any terms in a_4.

All sub-graphs of a fragment have the same absolute value, because they all involve the same species and the same reactions. However, the different sub-graphs can be with different signs dependent of the sub-graph structure. Their signs are shown in the corresponding corners in Table 1.

The upper sub-graph in the column for Fragment A is a negative (even) cycle with two interaction paths ($x_1 \rightarrow 3 \leftarrow x_4$, $x_4 \rightarrow 5 \leftarrow x_2$). Their direction is determined by the direction of the reaction path ($x_2 \rightarrow 4 \rightarrow x_1$). The product of the two interaction paths and the single reaction path gives rise to the negative cycle with the $-(v_3v_4v_5)/(x_1x_2x_4)$ value.

Similarly, other sub-graphs are constructed. They are characterized by the signs shown in Table 1.

Summation of the three sub-graphs for Fragment A gives the negative term a_3^A in the coefficient a_3 as shown in Table 1. The similar procedure for Fragment B gives the positive term a_3^B in the coefficient a_3 as shown in Table 1.

One can see that two Fragments, A and B, which seem to be of similar structure, have different signs. The negative fragment A, when being great enough in its absolute value, can induce the negative a_3 coefficient. Therefore, this fragment can induce instability in the system. Fragment A involves two negative cycles, but Fragment B involves only one negative cycle. The cycle with the two interaction paths in Fragment A can be interpreted as the "double-negative feedback" which is known to induce instability [25]. Therefore, Fragment A is of special interest as one of the simplest destabilizing fragments in the kinetic scheme.

Comparison of Fragments A and B shows that their absolute values differ only in their reaction rates, v_5 and v_1. This fact gives one of the estimations:

$$v_5 > v_1, \tag{4}$$

which is needed to obtain the negative value for summed terms in the coefficient a_3.

In the estimations of the parameters we take into account the following equalities of the steady-state reactions rates:

$$v_1 = v_2; v_3 = v_4; v_5 = v_6; v_7 = v_3 + v_5 \tag{5}$$

All estimations relate to the independent reaction rates only.

There is a positive fragment in Graph 1 topologically identical to Fragment B:

$$\binom{3\ 5\ 6}{x_2 x_3 x_4} \tag{6}$$

Here 3, 5, and 6 relate to elementary reactions, and x_2, x_3, and x_4 to species in the fragment. The comparison with Fragment A gives the following inequality:

$$x_4 > k_6 / k_3. \tag{7}$$

There are two more positive fragments in Graph 1:

$$\binom{1\ 3\ 5}{x_1 x_2 x_4}, \quad \binom{2\ 3\ 4}{x_1 x_2 x_4}. \tag{8}$$

They differ in their reactions only. Substitution of reactions 1 and 2 in these fragments by their reverse reactions, 2 and 1, does not change their values. The comparison of the negative Fragment A value with the values of all positive fragments gives the following estimation:

$$v_3 = v_4 > v_5 = v_6; v_3 = v_4 \gg v_1 = v_2 \tag{9}$$

From Eqs. (7) and (9) we obtain the following inequality:

$$k_5k_6 < k_3k_4 \tag{10}$$

This inequality determines the cooperativity for the two enzyme active sites.

Consider now the fragments of the 4-th order inducing the terms in the a_4 coefficient. Table 2 shows two fragments having the value zero. Fragment A here is characterized by the two sub-graphs. One of them is the negative combination of the 3-rd order Fragment A of Table 1 with the positive half-path ($x_3 \rightarrow \bullet 6$). Another sub-graph is the positive odd cycle. Their summation gives the value zero ($a_4^A = 0$). Fragment B in Table 2 topologically differs from Fragment A in Table 2 only by the reversibility of the reaction ($x_0 + x_4 \rightleftharpoons x_1$). Therefore, Fragment B includes Fragment A and the two sub-graphs shown in Table 2. The sum of the sub-graphs in Fragment B equals zero ($a_4^B = 0$). Therefore, both Fragments A and B of Table 2 induce no terms into the a_4 coefficient. Table 2 demonstrates how the negative Fragment A in Table 1 is eliminated in the fragments of the higher order. This phenomenon is rather unusual for oscillatory kinetic models. This fact means that Graph 1 never can induce bistability for any parameter values. Our analysis shows that a positive fragment of the fourth order exists in Graph 1. Therefore, the a_4 coefficient is always positive.

2.3. Numerical analysis

We solve the following kinetic equations corresponding to Graph 1:

$$\begin{aligned}
\frac{dx_1}{dt} &= k_1(1-x_1-x_2-x_3)x_4 - k_2x_1 - k_3x_1x_4 + k_4x_2 \\
\frac{dx_2}{dt} &= k_3x_1x_4 - k_4x_2 - k_5x_2x_4 + k_6x_3 \\
\frac{dx_3}{dt} &= k_5x_2x_4 - k_6x_3 \\
\frac{dx_4}{dt} &= k_7 - k_1(1-x_1-x_2-x_3)x_4 + k_2x_1 - k_3x_1x_4 - k_5x_2x_4
\end{aligned} \tag{11}$$

A solution of these kinetic equations was made with the computer program DBSolve [26]. For the solution the following initial conditions are used:

$$x_0 = 1, x_1 = x_2 = x_3 = 0, x_4 = 0.01 \quad (12)$$

The kinetic parameters are taken in accordance with our estimations ((9) and (10)) such that slowest are the reactions 1 and 2, and the most rapid are the reactions 3 and 5:

$$k_1 = 0.001, k_2 = 0.1, k_3 = 1000, k_4 = 3, k_5 = 500, k_6 = 1.1, k_7 = 0.5 \quad (13)$$

These parameters (in arbitrary units) in their relative values are in agreement with the parameters of the real enzymological system [36,37]. The substrate binding (k_3 and k_5) at the two equivalent active sites we take as rapid as known in the literature [36] for dynein. We suppose the two active sites not to interact during the substrate binding. The product release we suppose to be cooperative. The relation ($k_5/k_6 > k_3/k_4$) is interpreted in the experimental work [36] as the positive cooperativity. We take the relative parameter values because only the relations are conserved for various dynein isoforms [33]. The affinity for ATP in the regulatory ATP-binding sites is always lower than in the primary active sites [37]. This is valid for ATPases of the AAA+mechanoenzyme family [33].

Fig. 1 shows the calculated x_3 oscillation for the parameter values shown in Eqs. (12) and (13).

Fig. 2 shows the changed x_3 oscillation for the changed k_1 value ($k_1 = 0.1$) with other parameters as in Eqs. (12) and (13). One can see that the "spiky" oscillation at $k_1 = 0.001$ becomes more harmonic at ($k_1 = k_2 = 0.1$).

The dependence of the oscillation amplitude and period on k_1 is shown in Fig. 3(a,b).

A similar monotonous dependence is obtained for (k_2). Sustained oscillations are observed for k_2 values less than 25. Increasing k_2 values generate oscillation damping. For increasing k_7 in the domain, $0.1 < k_7 < 1$, and other parameters fixed, both period and amplitude increase, the amplitude increases from 0.08 to 0.75, and the period increases from 48 to 125 in the time units of Figs. 1 and 2.

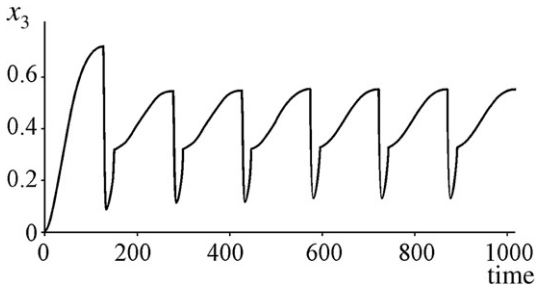


Fig. 1. Oscillatory solution for x_3 obtained from Eq. (11). Initial conditions are as in Eq. (12), and kinetic parameters are as in Eq. (13). All parameters are in arbitrary units.

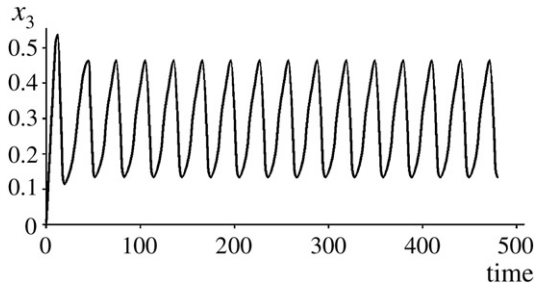


Fig. 2. Oscillatory solution obtained for the same parameters as in Fig. 1 except $k_1 = 0.1$.

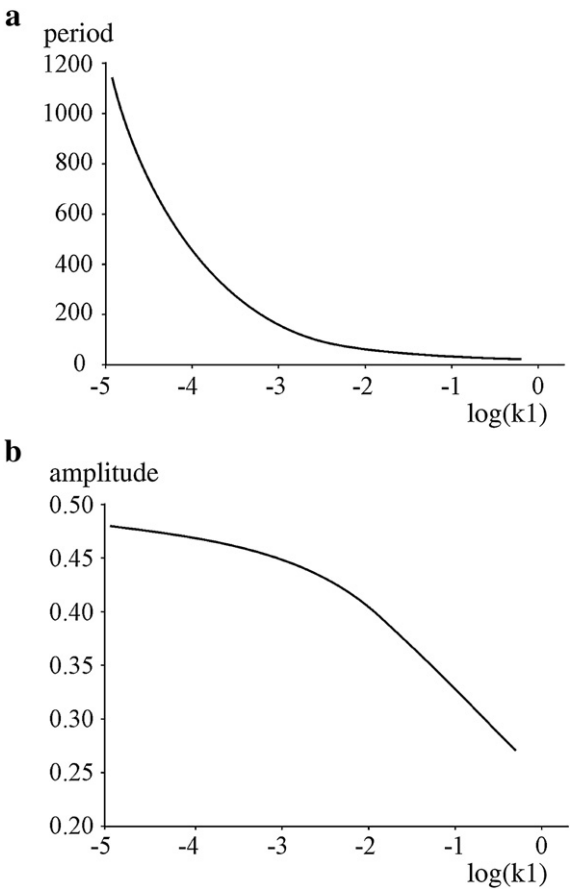


Fig. 3. a. Calculated x_3 oscillation period in its dependence on k_1. Time units are as in Figs. 1 and 2. Other parameters are fixed. b. Calculated x_3 oscillation amplitude in its dependence on k_1. Other parameters are fixed.

The numerical analysis in this section shows that the side slow reactions, 1, 2, and the substrate influx (k_7) are of regulatory importance in Graph 1.

3. Discussion

It has been shown earlier [13,14] that critical phenomena in biochemical networks, such as bistability or sustained oscillations, are induced by the so-called [7,8] "critical fragments" in the kinetic graphs. Some simple critical fragments have been classified earlier [12].

This paper considers one of the critical fragments (Fragment A in Table 1). This critical graph fragment has a simple biochemical interpretation. It represents the combination of the catalytic cycle and the "feedback regulatory loop". The considered regulatory loop can be a result of the cooperativity in two enzyme subunits.

To obtain sustained oscillations, the critical graph fragment should be combined with a side reversible reaction. Reversibility of a side reaction is required not to disturb the steady-state distribution of species in the critical fragment. A side reaction in this paper is the substrate activation never considered earlier in models of concentration oscillations.

Our graphical analysis verifies the former results [14]. We demonstrate how the kinetic parameters can be easily estimated by the graphical procedure to predict the oscillation behaviour.

In the earlier works [19,27], it had been shown that the slow side reaction can periodically restore the diminished activity of the rapid Michaelian enzyme reaction. Such a "restorer" can behave as a generator

of damped oscillations [19,27]. In our Graph 1, the side reaction "restores" the limit cycle (sustained) oscillation.

Substrate activation can be of biochemical importance in preparing enzyme to the catalytic activity. This is the case for many molecular motors [33]. The different role of different substrate-binding sites during dynein functioning is discussed in the recent literature [22,33].

The recent work [34] strongly supports a binding change mechanism for F1-ATPase, which has three substrate-binding sites highly cooperative during substrate binding and hydrolysis. It was shown that ATP binding at the empty site triggers a series of motions leading to ATP hydrolysis and product release at the other two catalytic sites. Our Graph 1 can explain the cyclic functioning of this and other similar enzymes.

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