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Switching mechanism for branched biochemical fluxes: Graph-theoretical analysis

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Abstract

A graph-theoretical method is applied to characterize the structure of a simplest switching mechanism of common biochemical importance. This mechanism is based on competition of two coupled substrate-binding pathways for a single substrate. No other regulatory interactions are shown to be needed for the switching phenomenon to be observed. It is shown that switch in branch effluxes is observed as bistability or reciprocal oscillations, depending on the value of steady influx. Frequency of reciprocal efflux oscillations in branches is regulated by steady influx. Therefore, the switching mechanism can function as the coding mechanism in the manner of "influx steady level–efflux frequency". The calculated kinetic equations for the switching mechanism demonstrate very steep transitions in the branch fluxes without using high non-linearity of these equations.

Keywords: Graph-theoretical method; Switching mechanism; Oscillations; Signal coding

1. Introduction

Switching phenomena in branched biochemical reaction networks are understood as disruption for one of the branched fluxes in answer to a biochemical signal. These phenomena follow a stability loss, and usually manifest like bistability transitions [1].

This paper shows that sustained oscillations in branched fluxes proceed in counter-phases and produce a switching phenomenon too. Moreover, the same kinetic mechanism can function as oscillatory as well as discrete switching mechanism with only a single kinetic parameter changed.

Obviously, the switching phenomena depend on the topological structure of the biochemical network. Therefore, topological (graph-theoretical) methods should be useful to study these phenomena.

Indeed, many oscillatory mechanisms are identified and classified with graph-theoretical methods [2–4]. Typically, these phenomena are explained by positive feedback (product activation or autocatalysis).

Negative feedback regulation has received less attention. Only recently, a mechanism, based on negative regulation, was proposed for calcium oscillations [5].

This paper considers a most simple kinetic mechanism of common importance based on negative interactions. Negative interactions originate here from competition of two coupled reactions for a single substrate. Such kind of competition is very reasonable in branched biochemical fluxes, therefore, it should be widely spread in biochemical networks. No additional regulatory interactions are shown here to be required for switching phenomena to be observed.

Moreover, a switching mechanism, considered in this paper, demonstrates its ability to encode the steady value of the influx into the frequency of reciprocal oscillations in two branched effluxes.

2. Theory

2.1. Graphical method

I follow here a graphical method of our previous paper [9] based on the approach by Clarke [6] and Ivanova [7,8]. Graphical analysis of non-linear biochemical systems uses linearization

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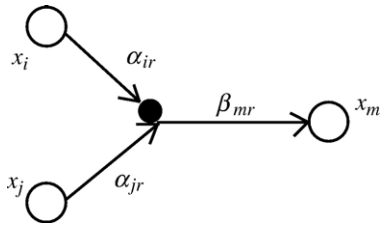


Scheme 1.

of kinetic equations. Therefore, a graphical analysis of linear enzyme systems (method by King and Altman [10] and its modifications [11–13]) is a part of a common method.

Graphical analysis of sustained oscillations and bistability was not illustrated in [9] by simply understandable examples. Therefore, I demonstrate here in detail the validity of this analysis on a simple kinetic mechanism before solving the corresponding differential equations. This analysis allows me to reveal a new switching mechanism and estimate the parameter values for switching phenomena to be observed.

Non-linear kinetic schemes have the following reactions: Scheme 1 represents the utilization of the substances, x_i, x_j, in a single rth reaction:

$$\alpha_{ir}x_i + \alpha_{jr}x_j \rightarrow \beta_{mr}x_m, \tag{1}$$

where α_{ir}, α_{jr}, β_{mr} are stoichiometries, x_m is synthesized in reaction (1). Reactions are shown by closed circles, and substances by open circles.

Kinetic equations for a reaction network are then written as follows:

$$\frac{dx_i}{dt} = \sum_r (-\alpha_{ir} + \beta_{ir})v_r \tag{2}$$

where summation is over all r reactions. Substances x_i participate in these reactions as substrates and products.

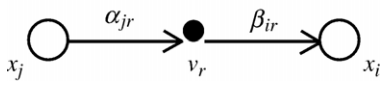
The following kinetic equations are written for a small displacement $\Delta x_i = x_i - \overline{x}_i$ of concentrations x_i (i=1,2,..., n) from their steady-state values $\overline{x}_i$:

$$\begin{aligned}\frac{d\Delta x_i}{dt} &= \sum_{r,j}(-\alpha_{ir} + \beta_{ir})\frac{\partial v_r}{\partial x_j}\Delta x_j \\ &= \sum_j b_{ij}\Delta x_j, (i,j = 1,2,...,m; r = 1,2,...,R)\end{aligned} \tag{3}$$

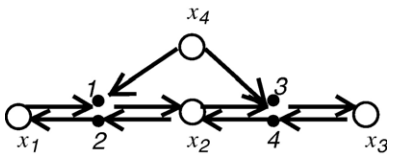
where coefficients b_{ij} are the following matrix elements obtained with mass action law for reaction rates v_r:

$$b_{ij} = \sum_r (-\alpha_{ir} + \beta_{ir})\alpha_{jr}\frac{\overline{v}_r}{\overline{x}_j}, (r = 1,2,...,R) \tag{4}$$

Here R is the number of reactions, m is the number of independent concentrations.



Scheme 2.



Scheme 3.

Linear systems involve only non-branched steps:

Scheme 2 (in contrast with Scheme 1) does not involve more than one branch entering the same v_r. In accordance with Eq. (4), reaction $x_j \rightarrow x_i$ (Scheme 2) corresponds to the matrix element,

$$b_{ij} = (\alpha_{jr}\beta_{ir})(\overline{v}_r/\overline{x}_j).$$

The solution of kinetic Eq. (3) leads [6] to the characteristic polynomial:

$$p(\lambda) = \lambda^m + a_1\lambda^{m-1} + a_2\lambda^{m-2} + ... + a_m = 0, \tag{5}$$

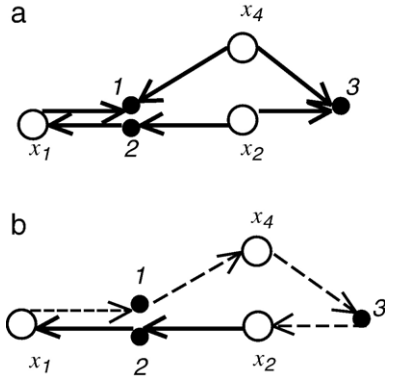
where coefficients a_i are related to elements b_{ij} by:

$$\begin{aligned}a_1 &= (-1)^1\sum_i b_{ii}, a_2 = (-1)^2\left(\sum_{i,j} b_{ii}b_{jj} - \sum_{i,j} b_{ij}b_{ji}\right), \\ a_3 &= (-1)^3\left(\sum_{i,j,k} b_{ii}b_{jj}b_{kk} - \sum_{i,j,k} b_{ii}b_{jk}b_{kj} + \sum_{i,j,k} b_{ij}b_{jk}b_{ki}\right), ...\end{aligned} \tag{6}$$

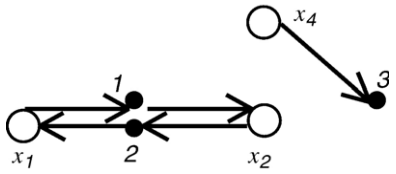
One can see from Eq. (6) that coefficients a_i are constructed by cyclic element products, such as $b_{ij}b_{ji}$, $b_{ij}b_{jk}b_{ki}$, etc. and by elements b_{ii}, which are not cycles, if simplest feedback loops are absent in the system.

All cyclic terms are eliminated from coefficients a_i for linear enzyme systems, therefore, all coefficients a_i for linear systems are positive trees (or sub-trees) of reaction branches [13].

Non-linear systems, involving branched reactions as in Scheme 1, can induce destabilizing negative a_i coefficients due to feedback cycles of reactions.



Scheme 4. a, b.



Scheme 5.

Feedback activation is a well-known mechanism for destabilization and connected phenomena in biochemical networks [2,3]. Less studied are the kinetic schemes without feedback activation, an example of which is presented in Scheme 3.

Its destabilizing effect is explained by competition of different binding reactions (reactions (1) and (3)) for a single substance (substance x_4 in Scheme 3).

Consider this phenomenon in detail. The destabilizing structure, shown by heavy arrows in Scheme 3, adds three terms to the coefficient a_3 of the characteristic polynomial according to Eq. (4), all of these terms have the same absolute value, $(\bar{v}_1\bar{v}_2\bar{v}_3/\bar{x}_1\bar{x}_2\bar{x}_4)$, and differ only in their signs. Schemes 4–6 give graphical representation of these terms.

Scheme 4(a, b) shows the graphical representation (the cycle) for one of these terms, $a_3^{(1)}=-(b_{41}b_{24}b_{12})$.

According to Eq. (6), this cyclic product of three b_{ij} elements is taken with the sign minus. Matrix elements, b_{41}, b_{24}, b_{12}, correspond here to the following reactions in Scheme 3 (elements are expanded according to Eq. (4)):

$$x_1 \rightarrow x_4, b_{41} = (-\alpha_{11}\alpha_{41})(\bar{v}_1/\bar{x}_4) = -\bar{v}_1/\bar{x}_4 \tag{7}$$

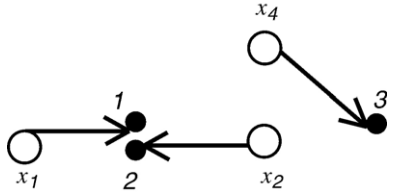
$$x_4 \rightarrow x_2, b_{24} = (-\alpha_{43}\alpha_{23})(\bar{v}_3/\bar{x}_2) = -\bar{v}_3/\bar{x}_2 \tag{8}$$

$$x_2 \rightarrow x_1, b_{12} = (+\alpha_{22}\beta_{12})(\bar{v}_2/\bar{x}_1) = +\bar{v}_2/\bar{x}_1 \tag{9}$$

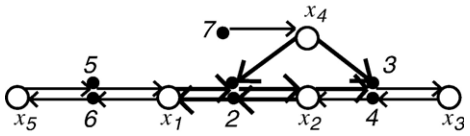
Therefore, the value of the term $a_3^{(1)}$ is

$$a_3^{(1)} = -(b_{41}b_{24}b_{12}) = -(\bar{v}_1\bar{v}_2\bar{v}_3/\bar{x}_1\bar{x}_2\bar{x}_4) \tag{10}$$

Here b_{41} and b_{24} are represented by the interaction reactions, shown in Scheme 4a by meeting arrows $\rightarrow\bullet\leftarrow$. Their direction is determined by reaction $x_2 \rightarrow x_1$, which dictates the direction for all reactions in the cycle, as shown in Scheme 4b by dotted arrows.



Scheme 6.



Scheme 7.

Scheme 5 shows the graphical representation of the second term $a_3^{(2)}$:

$$a_3^{(2)} = +(b_{21}b_{12})(b_{44}) \tag{11}$$

where

$$b_{21} = (+\alpha_{11}\beta_{21})(\bar{v}_1/\bar{x}_2) = +\bar{v}_1/\bar{x}_2 \tag{12}$$

$$b_{44} = (-\alpha_{43}\alpha_{43})(\bar{v}_3/\bar{x}_4) = -\bar{v}_3/\bar{x}_4 \tag{13}$$

and b_{12} is given in Eq. (9).

Therefore, the value of the term $a_3^{(2)}$ is

$$a_3^{(2)} = -(\bar{v}_1\bar{v}_2\bar{v}_3/\bar{x}_1\bar{x}_2\bar{x}_4) \tag{14}$$

There is a third term originated from the structure shown in Scheme 6:

Its value equals:

$$a_3^{(3)} = -(b_{11}b_{22}b_{44}) \tag{15}$$

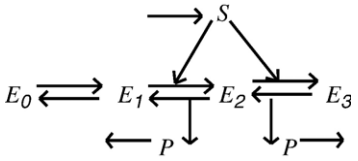
Elements b_{ii} are negative, similar to element b_{44}, shown in Eq. (13).Consequently,

$$a_3^{(3)} = +(\bar{v}_1\bar{v}_2\bar{v}_3/\bar{x}_1\bar{x}_2\bar{x}_4) \tag{16}$$

The sum of these three terms, $(a_3^{(1)}+a_3^{(2)}+a_3^{(3)})$, gives rise to the negative coefficient a_3, if the absolute value of this sum is large enough, as compared with other sub-schemes, involving 3 reactions and 3 substances, in the larger kinetic scheme.

The destabilizing structure, considered above, usually is a part of larger kinetic schemes. If the substances of this structure are connected with other parts by reversible reactions, this connection does not disturb its destabilization property. Such reversible interactions introduce zero steady-state fluxes and do not change the value of the destabilizing structure.

A system can generate sustained oscillations, if the negative characteristic polynomial coefficient is not the highest



Scheme 8.

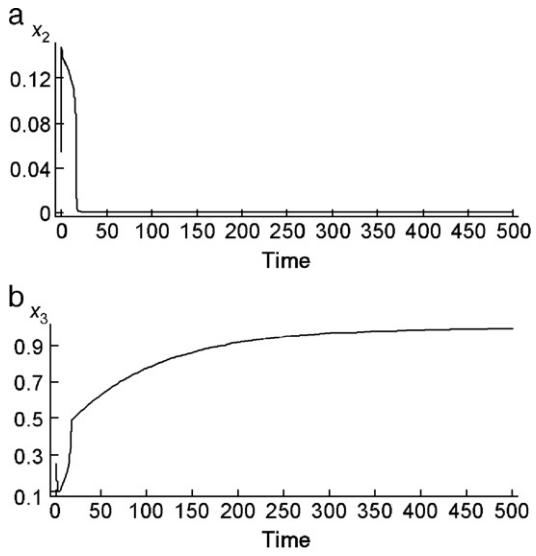


Fig. 1. a. Calculated dependence of the normalized variable x_2 on time (relative units). The dimensionless parameters of Scheme 7 are the following: $k_1=1000$, $k_2=10$, $k_3=500$, $k_4=1.5$, $k_5=0.01$, $k_6=0.1$, $v_7=1.5$. Initial values for variables: $x_1=0.05$, $x_2=0.055$, $x_3=0.25$, $x_4=0.01$. The value x_2 is proportional to the efflux rate, $v_2=k_2x_4$. b. Calculated dependence of the normalized variable x_3 on time. Parameters as in Fig. 1a. The value x_3 is proportional to the rate of another branch efflux, $v_4=k_4x_3$.

coefficient a_m [9]. If $a_m>0$, and some of $a_i<0$ ($i<m$), the system can generate sustained oscillations [9].

If some substances in Scheme 3 are interdependent by one conservation condition, the number of independent substances equals three. One more substance should be added to Scheme 3 for oscillations to be observed (to obtain). Such a modification of Scheme 3 leads us to a simplest oscillatory switching mechanism. Earlier we have shown [9] that the simplest oscillation scheme should involve at least two joint cycles of reactions. Scheme 3 involves two joint cycles, shown in Scheme 4b and in Scheme 5.

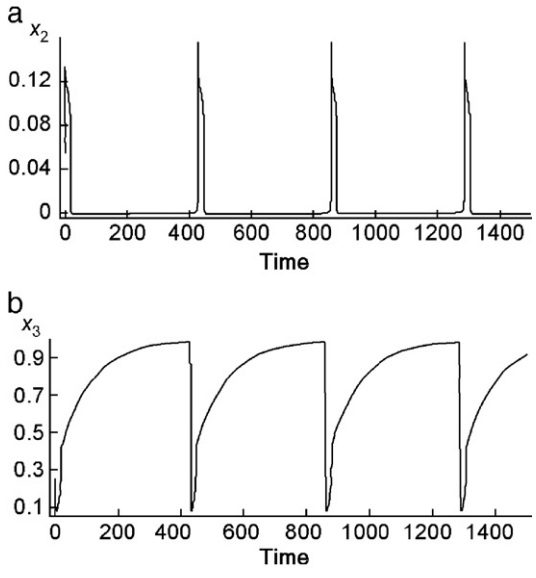


Fig. 2. a. The similar dependence as in Fig. 1a for changed parameter $v_7=1.3$. b. The similar dependence as in Fig. 1b for changed parameter $v_7=1.3$.

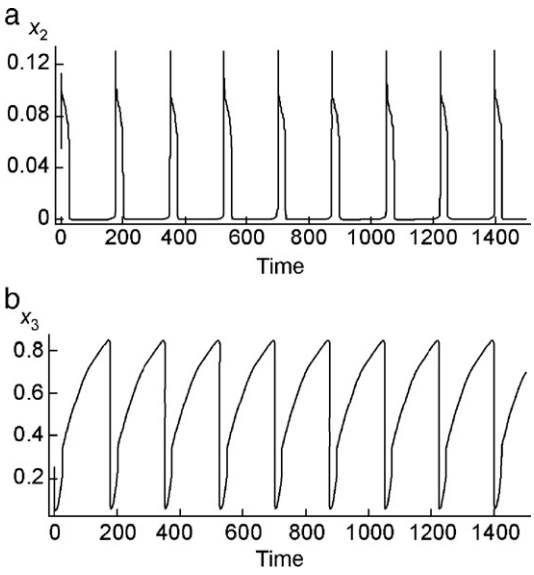


Fig. 3. a. The similar dependence as in Fig. 1a for changed parameter $v_7=1$. b. The similar dependence as in Fig. 1b for changed parameter $v_7=1$.

3. Results

3.1. Switching mechanism

A simplest switching mechanism is obtained by adding reactions (5) and (6) to Scheme 3, as shown in Scheme 7:

Influx to substance x_4, also shown in Scheme 7, is added to compensate x_4 exhaustion.

The following interpretation of Scheme 7 can be represented by Scheme 8:

Here E_0, E_1, E_2, E_3 denote various enzyme forms, and S, P denote substrate and product, correspondingly.

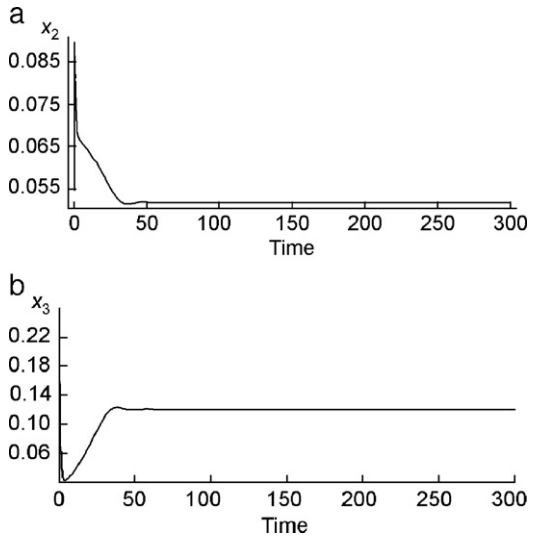


Fig. 4. a. The similar dependence as in Fig. 1a for changed parameter $v_7=0.7$. b. The similar dependence as in Fig. 1b for changed parameter $v_7=0.7$.

Scheme 8 can describe substrate binding at two types of binding sites. A switching phenomenon in this case means the time separation of two product types.

Substrate S in Scheme 8 is one of the variables, being not in excess, in contrast to many mathematical models. Really, in most cellular systems substrates of many enzymes are present in concentrations, comparable with enzyme concentrations.

Enzyme isomerization, $E_0 \leftrightarrow E_1$, is identified for many enzymes and enzyme complexes as inherent conformational transition, leading to enzyme activation.

The second substrate-binding reaction in Scheme 8 can be the inhibitory reaction, as considered in our work [14]. In this case the second product efflux is absent. The switch in this case proceeds between the product release and the dead-end inhibition. A more complex modification of Scheme 8 was considered in our work [15].

The following system of kinetic equations corresponds to Scheme 7:

$$\frac{dx_1}{dt} = -k_1x_1x_4 + k_2x_2 + k_5x_5 - k_6x_1$$

$$\frac{dx_2}{dt} = k_1x_1x_4 - k_2x_2 - k_3x_2x_4 + k_4x_3$$

$$\frac{dx_3}{dt} = k_3x_2x_4 - k_4x_3$$

$$\frac{dx_4}{dt} = v_7 - k_1x_1x_4 - k_3x_2x_4 \qquad (17)$$

The substances along the horizontal line in Scheme 7 are interconnected by the conserved amount of the enzyme:

$$x_1 + x_2 + x_3 + x_3 + x_5 = 1 \qquad (18)$$

The reactions in Scheme 7, shown by heavy lines, should be rapid enough as compared with other reactions. In this case we obtain the negative coefficient of the characteristic polynomial, that is necessary for switching phenomenon to be observed. This fact allows us to estimate the parameter values, kinetic constants k_1,k_2,k_3 for reactions 1, 2, 3 to be greater than constants k_5, k_6 for reactions (5), (6) in Scheme 7. Moreover, variations of the slow outer reaction rate, v_7, in Scheme 7 can modify the oscillatory behavior toward the bistable or stable behavior, as shown in Figs. 1–4. Figs. 1–4 are obtained by solution of Eqs. (17) and (18). Parameters in Eqs. (17) and (18) are shown in figure legends.

Solution of Eqs. (17) and (18) is obtained with the program DBSolve [18]. All variables, x_i, all parameters, k_i, as well as time are here taken dimensionless. Concentrations of enzyme forms are normalized by the total enzyme concentration. Normalized values, x_2 and x_3, in all figures characterize two enzyme activities,$v_2 = k_2x_2$, and $v_4 = k_4x_3$, leading to two branch product effluxes.

Fig. 1a, b show the discrete switch of two effluxes, proportional to x_2 and x_3, at $v_7 = 1.5$. At $v_7 = 1.3$ the switch becomes of oscillatory type (see Fig. 2a,b). Figs. 3a,b and 4a,b show further changes in the switching behavior at $v_7 = 1$ and $v_7 = 0.7$. At $v_7 = 1$ the oscillation frequency is greater than at $v_7 = 1.3$, and at $v_7 = 0.7$ oscillations disappear.

Changes in the oscillation frequency of the effluxes, induced by changes in the value of the steady influx, demonstrate the ability of this mechanism to encode influx into efflux in the manner of "amplitude–frequency".

4. Discussion

A switching mechanism considered is functioning both in discrete and in oscillatory regime. This mechanism is able to separate branch biochemical fluxes practically without futile energy loss. It is seen from Figs. 1–4, that two branch effluxes proceed in counter-phases. Therefore, this switching mechanism directs the steady influx each time completely into one efflux branch.

Similar kinetic mechanisms can be applied to various enzyme system, placed into the branch points of metabolic networks [16].

Moreover, the coding effect to transform the steady influx into the periodic effluxes, demonstrated by Scheme 7, can be important for the cell signaling systems. Various coding mechanisms are discussed in the literature [19]. Scheme 7 can be a new simple mechanism to explain the coding phenomenon.

My analysis shows that substrate participation in two coupled substrate-binding reactions is principal to explain sustained oscillations or bistability transition. If substrate participates only in a single reaction in Scheme 8, this scheme can induce damped oscillations only [17].

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