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Enzymatic System Realizing Two-factor Model

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Enzymatic system which can realize the two-factor model was investigated by computer simulation. In two-factor model, the output of the system is controlled only by the difference in magnitude of excitatory and inhibitory factors, independently of their absolute magnitudes.

The present study revealed that the two-factor model could be realized by either a multi-feedback-system or a conjugate system.

INTRODUCTION

It has been believed that a dynamic behavior of physiological or biochemical system in various hierarchical levels is fundamentally regulated by a combinatory action of an excitatory (activatory) and an inhibitory factor. For an enzymatic system, in general, the enzymatic activity, hence the output of the system, is thought to be continuously regulated by the action of an activator or an inhibitor; i.e., the enzymatic activity changes continuously in propotion to the concentration of the activator or the inhibitor.

On the other hand, it has been well known that some physiological systems respond to the net magnitude of competing excitatory and inhibitory factors. For instance, neuron is firing only when the excitatory input exceeds the inhibitory input, as represented by $F(x, y) = \{1; x \geqq y, 0; x < y\}$, where $F(x, y)$ indicates the output of the system, and x and y are the magnitudes or amounts of the excitatory and inhibitory inputs, respectively.

Rosen (1967, 1968, 1970) has introduced an idea similar to above into biochemical systems and called it two-factor model. In his basic model, an enzymatic system produces opposing two-factors, an activator (x) and an inhibitor (y), and the output is controlled by the function $F(x, y)$. Although such the discrete input may offer a new type of energy or material production, the significance of two-factor model will be emphasized if the output carries an information as to control the other biochemical systems.

Thus, the participation of two-factor model in the regulation of enzymatic reaction system can produce specific output that may not be produced by continuous systems. However in his approach, Rosen has simply and a *priori*

* Notations in Roman indicate the chemical species and those in italic the molar concentration of corresponding species.

assumed the function $F(x, \mathbf{y})$ as for the analysis of general physiological system.

The practical significance of two-factor model in enzymatic reaction systems strongly depend upon the circumstance whether the enzymatic system which realizes the function $F(x, y)$ can be found under reasonable assumptions or not. The present paper deals mainly with the study on the $F(x, y)$-realizing enzymatic system by means of the computer simulation.

COMPUTATION

Feedback and conjugate reactions are thought to be fundamental systems for the regulation of complicated enzymatic reaction. In the present study, simulations were conducted under the assumption that enzymatic reaction system which can realize a threshold function $F(x, y)$ is essentially composed of either a feedback-system or a conjugate reaction system.

Feedback-system

A Dynamic analysis

A typical example of analysed schemes is shown in Fig. 1. Two factors, the activator (x) and the inhibitor (y) are assumed to act on feedback loop and X_1 is taken as the output to be regulated by the fuction $F(x, \mathbf{y})$. The activator (x) enhances the feedback input (for example X_2 in the first loop) and the inhibitor (y) reduces the feedback input. Thus, the feedback input X_2 in the first feedback loop is modified to $(x/y)X_2$ by the two factors. This

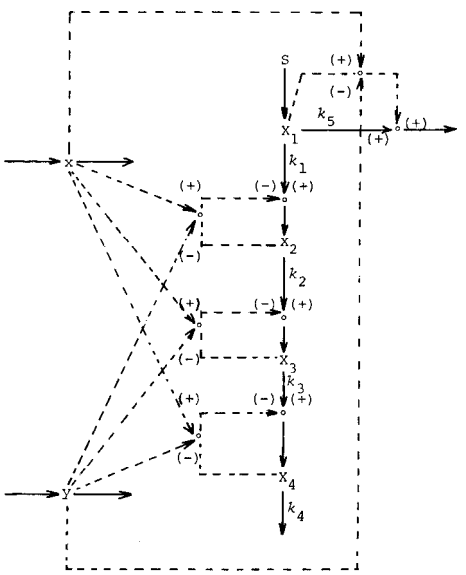


Fig. 1. Three-feedback model. Dotted line indicates the transmission of information only.

modified feedback input changes rate constant k_1 at $X_1 \rightarrow X_2$ step to $k_1/(X_2x/y) = k_1y/xX_2$. Thus, two factors act on the feedback with their ratio, $f=y/x$. In mathematical sence, it is easily presumed that, for realizing the function $F(x,y)$, the mathematical model must contain a term of $(f)^n$ with large n-value, i.e., f must act simultaneously on several feedback loops. For simplify the computation, the simulations were performed on the schemes with n-value less than 4.

The mathematical model (rate equation) for the scheme in Fig. 1 may be written as,

$$\begin{aligned}
\dot{X}_1 &= S-(k_1 f/X_2)X_1-(k_5X_1/f)X_1 \\
\dot{X}_2 &= (k_1 f/X_2)X_1-(k_2 f/X_3)X_2 \\
\dot{X}_3 &= (k_2 f/X_3)X_2-(k_3 f/X_4)X_3 \\
\dot{X}_4 &= (k_3 f/X_4)X_3-k_4X_4
\end{aligned} \tag{1}$$

where S is the constant input, and dot indicates the operator d/dt and $X_i = X_i(t)$. f is taken as parameter in simulation. The sign (+) and (−) in Fig. 1 indicate positive and negative effects, respectively, and dotted lines show the flow of information but not flow of material.

The simultaneous nonlinear differential equation (1) was numerically solved by the modified Runge-Kutta-Gill method (MRKGM) using a FACOM 230–75 (Okamoto *et al.* 1975).

B Steady-state approximation.

When the system had attained to the steady-state, the following algebraic equations are obtained from Eq. (1),

$$\begin{aligned}
&S-(k_1 f/X_2)X_1-(k_5X_1/f)X_1 = 0 \\
&(k_1 f/X_2)X_1-(k_2 f/X_3)X_2 = 0 \\
&(k_2 f/X_3)X_2-(k_3 f/X_4)X_3=0 \\
&(k_3 f/X_4)X_3-k_4X_4=0
\end{aligned} \tag{2}$$

The solutions on X_i are,

$$\begin{aligned}
X_1 &= (1/f)^3(S-k_5(1/f)X_1^2)^4/k_1k_2k_3k_4 \\
X_2 &= (1/f)^2(S-k_5(1/f)X_1^2)^3/k_2k_3k_4 \\
X_3 &= (1/f)^2(S-k_5(1/f)X_1^2)^2/k_3k_4 \\
X_4 &= (S-k_5(1/f)X_1^2)/k_4
\end{aligned} \tag{3}$$

With assuming that $S=k_1=k_2=k_3=k_4=1$,

$$X_1-(1/f)^3(1-k_5(1/f)X_1^2)^4=0 \tag{4}$$

is obtained. For the system with n-feedback loops, following general equation is obtained on X_1,

$$X_1-(1/f)^n(1-k_5(1/f)X_1^2)^{n+1}=0 \tag{5}$$

This equation was numerically solved by JARATD subprogram of FACOM SSL and the relation between the output X_1 and l/f value was visualized for each n-value.

Conjugate reaction system

Typical enzymatic conjugate reaction scheme subjected to the simulation is shown in Fig. 2. System shown at the bottom has switching function for active (E,) and inactive (E_i) enzymes. Top is a conjugate reaction system in which the active enzyme participates as a catalyzer in production of outputs. The outputs of whole system are X_1 and X,.

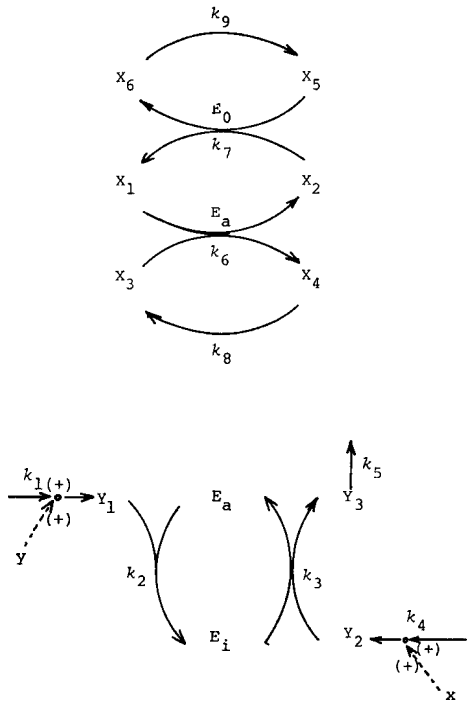


Fig. 2. Conjugate reaction model. Dotted line indicates the transmission of information only. E_a acts on step of $X_1 \rightarrow X_2$ in the upper scheme.

The excitatory factor x and inhibitory factor y produce practical activator Y_2 and inhibitor Y_1, respectively, from external pools. It is assumed that x and y are sort of catalyzer and are not consumed by the reactions, remaining at their constant concentrations. E_0 is an enzyme catalyzing $X_2 \rightarrow X_1$ reaction and assumed to have a constant total concentration.

The mathematical model of this scheme may be written as,

$$\dot{X}_1 = k_7 E_0 X_2 X_5 - k_6 E_a X_1 X_3$$
$$\dot{X}_2 = k_6 E_a X_1 X_3 - k_7 E_0 X_2 X_5$$
$$\dot{X}_3 = k_8 X_4 - k_6 E_a X_1 X_3$$

$$\begin{aligned}
\dot{X}_4 &= k_6 E_a X_1 X_3 - k_8 X_4 \\
\dot{X}_5 &= k_9 X_6 - k_7 E_0 X_2 X_5 \\
\dot{X}_6 &= k_7 E_0 X_2 X_5 - k_9 X_6 \\
\dot{E}_a &= k_3 E_i Y_2 - k_2 E_a Y_1 \\
\dot{E}_i &= k_2 E_a Y_1 - k_3 E_i Y_2 \\
\dot{Y}_1 &= k_1 y - k_2 E_a Y_1 \\
\dot{Y}_2 &= k_4 x - k_3 E_i Y_2 \\
\dot{Y}_3 &= k_3 E_i Y_2 - k_5 Y_3
\end{aligned} \qquad (6)$$

where X_i and Y_i are concentrations of reactants, k_i is rate constant at a specific step and $X_i = X_i(t), E_a = E_a(t), E_i = E_i(t), Y_i = Y_i(t)$. This simultaneous differential equation was also numerically solved by MRKGM program.

RESULTS AND DISCUSSION

Feedback system (dynamic analysis)

Equation (1) was solved with the initial conditions of $X_1 = X_2 = X_3 = X_4 = 1.0$, and the values of parameters are assumed to be $k_1 = k_2 = k_3 = k_4 = 1.0, k_5 = 0.0025$ for simplicity of numerical solution. The values of S and y were fixed to 1.0 and 20.0, respectively. Value of x was changed to yield various f-values. As a control, the similar computation was performed with assuming that $k_5 = 0$. Without an outlet bypass, the output X_1 increases monotoneously with increase in value of $1/f = x/y$. With supplement of the bypass, X_1 tends to have saturation profile. In the case of one feedback loop (two loops from the bottom in the scheme in Fig. 1 are omitted), no clear saturation was observed as represented by curve 1 in Fig. 3. A sigmoidal curve with a gentle slope was observed for the system with two feedback loops. Three feedback loops gave a typical sigmoidal profile, though its shape is far different from that of the function $F(x, y)$ (schematically represented by —•—).

It is easily presumed from the computational results described above that,

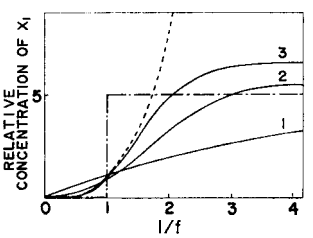


Fig. 3. Steady-state concentration of X_1 as a function of f. (---); without the bypass ($k_5 = 0$), 1; system with one feedback loop on $X_1 \rightarrow X_2$, 2; system with two feedback loops on $X_1 \rightarrow X_2$ and $X_2 \rightarrow X_3$, 3; system with three feedback loops shown in Fig. 1, (—•—); ideal response of X_1 or value of function $F(x, y)$.

if f controls equally a number of feedback loop, the curve of the output X_1 us f will approach to that of $F(x, y)$. In order to see the effect of the number of feedback loop on the curve shape and to simplify the computation, the steady-state approximation was utilized.

Feedback system (steady-state approximation)

Equation (5) was numerically solved for $n=3$, 7 by JARATD subprogram. There are two real roots; one has practical meaning and gave the same curve as that obtained by dynamical analysis (see Fig. 4-A-b), while the other carries no chemical meaning (Fig. 4-A-a). When n-value was taken to be 7, the curve approached more to that of the step-function than the case of $n=3$.

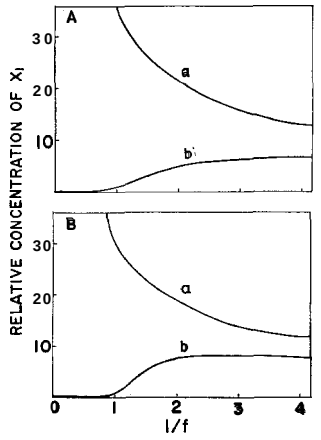


Fig. 4. Steady-state analysis. A; $n=3$, B; $n=7$, a; calculated with one real root which has no chemical meaning, b; calculated with other real root which has chemical meaning.

Thus, it is logically expected that the curve with a large n-value converges to the step-function. However, this model seems include several defects. For example, the practical molecular mechanism by which the feedback input is modified by factor f can not be surmised from knowledge on enzymology. Furthermore, it is not realistic that the same quality, f, acts in the same mode on several different feedback loops.

Thus, feedback-system which realize the function $F(x, y)$ could be constructed in a mathematical sense. However, practically such a system appears not to be allowable, since it seems to be too much complicated to exist in a real biological entity.

Conjugate reaction system

Equation (6) was numerically solved under the initial conditions of

$$X_1=X_2=0.5, \quad X_3=X_5=10.0,$$
$$X_4=X_6=0, Y_1=Y_2=Y_3=1.0,$$
$$E_a=E_i=0.5, \quad E_0=0.001$$

and at the parameter values of

$$k_1=k_4=k_5=1.0, \quad k_2=k_3=5\times 104$$
$$k_6=k_7=10^3, \quad k_8=k_9=10.0$$

The y-value was fixed to 20.0 and x-value was changed as to yield various f-values.

The computed results are shown in Fig. 5. The concentrations of E_a and E_i changed stepwise at $x/y=1$, and the concentrations of X_2 and X_1 changed in the similar way except for appearance of a little curved corner. The magnitude of curved part seemed to depend upon the total concentration of E_0.

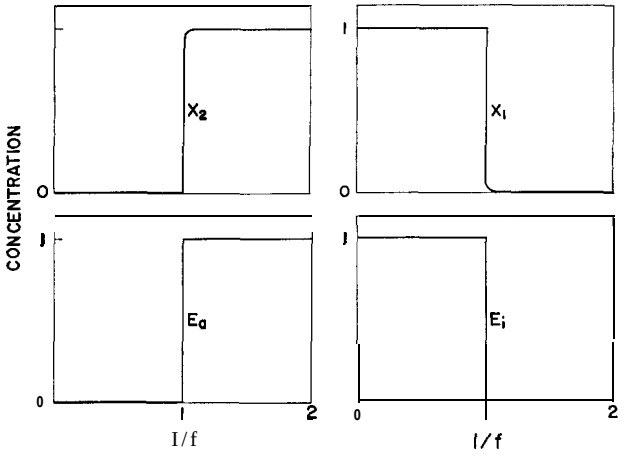
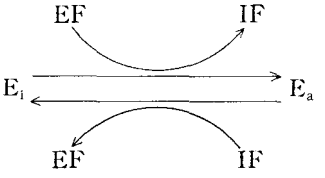


Fig. 5. Steady-state concentrations of E_a, E_i, X_2 and X_1 as a function of f.

When the excitatory factor EF and the inhibitory factor IF are assumed to act directly on an enzyme according to the scheme,



(7)

the equilibrium state is described by,

$$K=\frac{(E_i)(EF)}{(E_a)(IF)}$$
$$(E_a)=\frac{1}{K}\frac{(E_i)(EF)}{(IF)} \tag{8}$$

where parentheses indicate the equilibrium concentration. This equation

means that (E_a) does not form the step-curve under any conditions on the reactants.

The scheme on the actions of the excitatory and the inhibitory factor toward enzyme, which is shown in Fig. 2, has characteristic features relative to the static equilibrium scheme depicted in Eq. (7). The scheme in Fig. 2 transformed the static nature of the action of two factors into the dynamic one through the insertion of the activator, Y_2, and the inhibitor, Y,. When static equilibrium process is inserted to a part of dynamic flow system, its nature is greatly altered. This is the reason why E_a or E, behaves as a step-function, as shown in Fig. 5.

This type of conjugate reaction system may be easily constructed in vivo, since it does not contain any unreasonable element. Therefore, it is highly possible that switching regulation will take place in biochemical system in intact organism, and this may be an important proposal for considering the mechanism of the precise regulation of biochemical system *in vivo*.

In dynamical analysis of physiological phenomena, usually the function $F(x, y)$ is *a priori* assumed to be realized within the interested system, without considering the definite structure of $F(x, y)$-realizing system. The results of the present study may offer one example $F(x,$ y)-realizing system, and contribute to elucidation of details of switching regulation in biological system.

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