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Kuhara, Satoru
Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

Iwamoto, Shinichi
Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

Fujita, Atsushi
Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

Hayashi, Katsuya
Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

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Feedback Control of Enzymatic Reaction-Diffusion System I

Satoru KUHARA, Shinichi IWAMOTO, Atsushi FUJITA and Katsuya HAYASHI

Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University 46-02 Fukuoka 812

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An enzymatic reaction model, in which the enzymes are fixed at respective spatial positions and the moving reactants drived by free diffusion react with the fixed enzymes, was built to simulate a metabolism in cellular conditions. Further, it was assumed in the model that the final product inhibits the first enzyme by the negative feedback. The dynamical behavior of the model was surveyed by computer simulation to evaluate its capability of the constant-value control or homeostatic control.

It was found as a result that the model system exhibited a weak controllability on the homeostasis of the metabolites against the external perturbation added to the system. Some discussions were also made on the mechanism of homeostatic control of metabolites under cellular conditions.

INTRODUCTION

The principal mode responsible to homeostasis of the enzymatic or metabolic pathways under cellular conditions has been believed to be feedback control generated automatically by the enzymatic reaction system itself. According to the biochemical findings, an enzymatic feedback system is classified to a type of parametric control in which an activity of the regulatory enzyme is negatively and directly controlled by an intermediate or the product of the system through the feedback loop, and most regulatory enzymes, often called key enzyme, belong to the class of allosteric enzyme. The regulatory behavior of several allosteric enzymes in closed system (in *vitro* or in test-tube) has been well studied, and from the experimental results, especially from the sigmidoial curve between the activity and substrate concentration, it was speculated that allosteric enzyme would display an excellent capability of automatic and homeostatic control on the metabolites under cellular condition against an external perturbation.

On the other hand, it has been well recognized that the dynamic behavior of certain enzymatic reactions in an open system deviates frequently and profoundly from that observed in a closed system. Thus, it has been needed to follow the controllability of an allosteric enzyme in an open system. Because of the experimental difficulties, first the behavior of the allosteric enzyme in the open system was mainely followed by computer simulation technique using an appropriate model system.

Morales and McKay *et al.* (1967) and Higgins *et al.* (1973) studied the behavior of enzymatic feedback system including an allosteric enzyme as the regulatory element, and found that the activity of allosteric enzymes in the presence of negative effector can be represented by a type of the Hill equation. The computer simulation of such systems revealed that certain feedback systems can originate the oscillation of intermediates in enzymatic reaction system. Sakamoto *et al.* (personal communication) formulated the all transformations among the states of the allosteric enzyme, the processes of effector binding and the reaction of substrate by differential equation. They solved numerically the differential equations and found that the time courses of enzyme-states exhibited the sustained oscillations. Furthermore, they analyzed the feedback system consisted sequentially of allosteric enzyme and Michaelis-Menten enzyme, and revealed that this type of feedback system did not show any controllability on automatic and homeostatic control of the product.

Okamoto *et al.* (1977, 1978) investigated the controllability of various structures of enzymatic feedback system in a homogeneous medium. As a result, it was found that feedback structures which provide a large pool of the input substrate or a branched pathway above the regulatory enzyme were able to reject to some extent the effect of external perturbation, but did not exhibit the full of homeostasis.

The control theory insists that in the optimal regulator the feedback element should be time-dependent and that the deviation of the real state of the system from the desired one should be stored (integrated with time) within the system. In essential, these basic features should also be realized in the enzymatic feedback system, and it is clear that this is not the case for the enzymatic feedback system postulated based upon the biochemeical findings. In this connection, Okamoto *et al.* (1980, 1983) have attempted to suppose the structure of the ideal or faultless enzyme able to realize the perfect homeostasis in homogeneous medium, and clarified that a hysteretic enzyme and a twofactor realizing enzyme are the strong candidates for such the faultless enzyme.

In a living cell, enzymes seem to localize at the respective sites. For the practical discussion on the enzymatic feedback system, therefore, it is necessary to build first the model of the feedback system in an inhomogeneous medium corresponding to the cellular conditions, in which the enzymes are fixed at the respective sites and the reactants move in the space by free or facilitated diffusion.

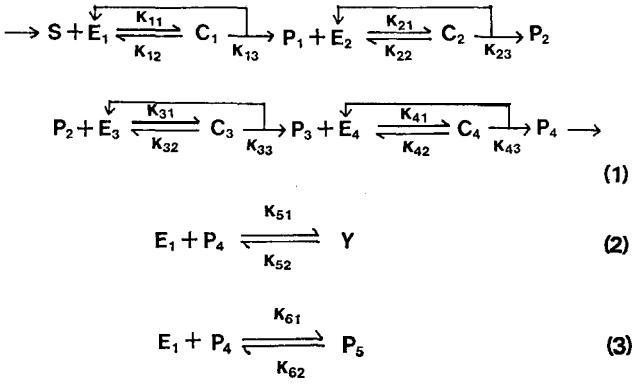
On the other hand, nonlinear rection-diffusion systems have been well analyzed with respect to the travelling wave, the pattern formation, oscillating behavior and so on. For example, stemmed from the analysis of Turing Model (Rosen, 1970), chemical reaction such as Belousov-Zhabotinskii reaction have been comprehensively investigated. From accumulated knowledge on the dynamical behavior of the nonlinear reaction-diffusion system, it is expected that the spatially irregular distribution of metabolites in the cell would be originated from the nature of enzymatic reaction systems, If the system satisfy some constraints to exhibit the above characteristics. Thus it is very

important to elucidate actual aspect of the such constraints for understanding the cellular device responsible to the spatial distribution of metabolites, which certainly carries some physiological roles in the cellular activity. Along this line, in *the* present study, the controllability of the enzymatic feedback system was analyzed first by means of computer simulation.

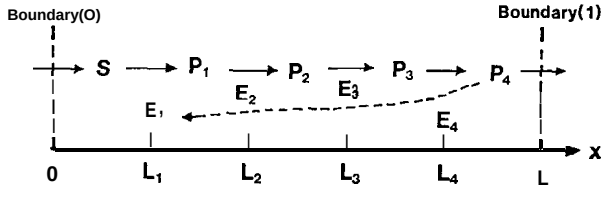
NUMERICAL SOLUTION OF STATE EQUATION

Model scheme

An enzymatic reaction system consisted of four different enzymes E_1-E_4 is assumed to be the model system as shown in Scheme 1. The substrate



Scheme 1



Scheme **2**

S is continuously supplied to the system and products $P_i, i=1$, 2, 3 are the substrate of the next enzymes, respectively. The product P_4 inhibits the activity of the enzyme E_1 either as a competitive inhibitor (Scheme 1-(2)) or as a negative allosteric effector (Scheme 1-(3)). P_5 represents the fraction of product P_4 that bound to E_1. The enzymes E_1-E_4 are assumed to be fixed at L_1-L_4 in one-dimensional space $(0, L)$ as shown in Scheme 2. The enzyme-substrate complexes C_1-C_4, enzyme-inhibitor complex Y and P_5 are

also fixed at the corresponding postions. The substrate and the products $P_1 - P_4$ move freely in the space by diffusion and permeate through the boundaries (0 and L) according to the given boundary conditions.

State equation

The state equations of the enzymatic feedback system in inhomogeneous medium may be derived from Schemes 1 and 2 as

$$\frac{\partial [S]}{\partial T} = D_s \frac{\partial^2 [S]}{\partial X^2} - k_{11}[S][E_1] + k_{12}[C_1]$$

$$\frac{\partial [P_1]}{\partial T} = D_1 \frac{\partial^2 [P_1]}{\partial X^2} - k_{21}[P_1][E_2] + k_{22}[C_2] + R_1$$

$$\frac{\partial [P_2]}{\partial T} = D_2 \frac{\partial^2 [P_2]}{\partial X^2} - k_{31}[P_2][E_3] + k_{32}[C_3] + k_{23}[C_2]$$

$$\frac{\partial [P_3]}{\partial T} = D_3 \frac{\partial^2 [P_3]}{\partial X^2} - k_{41}[P_3][E_4] + k_{42}[C_4] + k_{33}[C_3]$$

$$\frac{\partial [P_4]}{\partial T} = D_4 \frac{\partial^2 [P_4]}{\partial X^2} + k_{43}[C_4] + R_2 \qquad (1)$$

$$\frac{\partial [E_1]}{\partial T} = -k_{11}[S][E_1] + k_{12}[C_1] + R_3$$

$$\frac{\partial [E_2]}{\partial T} = -k_{21}[P_1][E_2] + (k_{22} + k_{23})[C_2]$$

$$\frac{\partial [E_3]}{\partial T} = -k_{31}[P]2\,[E_3] + (k_{332} + k_{33})[C_3]$$

$$\frac{\partial [E_4]}{\partial T} = -k_{41}[P_3][E_4] + (k_{42} + k_{43})[C_4] \qquad (2)$$

$$\frac{\partial [C_1]}{\partial T} = k_{11}[S][E_1] - k_{12}[C_1] - R_1$$

$$\frac{\partial [C_2]}{\partial T} = k_{21}[P_1][E_2] - (k_{22} + k_{23})[C_2]$$

$$\frac{\partial [C_3]}{\partial T} = k_{31}[P_2][E_3] - (k_{32} + k_{33})[C_3]$$

$$\frac{\partial [C_4]}{\partial T} = k_{41}[P_3][E_4] - (k_{42} + k_{43})\,[C,] \qquad (2')$$

For the system without feedback inhibition (abbreviated as no.),

$$R_1 = k_{13}[C_1]$$
$$R_2 = R_4 = 0$$
$$R_3 = k_{13}[C_1] \qquad (3)$$

for the competitive inhibition (abbreviated as compet.),

$$\frac{\partial[Y]}{\partial T}=k_{51}[P_4][E_1]-k_{52}[Y]$$

$$R_1=k_{13}[C_1]$$

$$R_2=-k_{51}[P_4][E_1]+k_{52}[Y]$$

$$R_3=k_{13}[C_1]-k_{51}[P_4][E_1]+k_{52}[Y]$$

$$R_4=[Y] \tag{4}$$

and for allosteric effector (abbreviated as allost),

$$\frac{\partial[P_5]}{\partial T}=k_{61}[P_4][E_1]-k_{62}[P_5]$$

$$R_1=k_{13}[C_1]/(1+\alpha[P_5])$$

$$R_2=-k_{61}[P_4][E_1]+k_{62}[P_5]$$

$$R_3=k_{13}[C_1]/(1+\alpha[P_5])$$

$$R_4=0 \tag{5}$$

The conservation equations are

$$[E_1]+[C_1]+R_4=E_{10}\delta_{X,L1}$$

$$[E_n]+[C_n]=E_{n0}\delta_{X,Ln}$$

$$n=2,3,4 \tag{6}$$

where brackets represent the molar concentration of chemical species, D_s and D_n, $n=1,\ldots,4$, are diffusion coefficient of substrate and products $P_n, n=1,\ldots,5$, T the reaction time, X the distance from boundary (0), k_{ij} the rate constant of reaction steps indicated in Scheme 1, δ is the Kroneker's delta and E_{n0} $n=1,\ldots,4$, represent the total concentration of respective enzymes.

The initial conditions are assumed to be

$$[S(0,0)1=S_0, \quad [S(0,X)]=0, \quad 0<X\leqq L$$

$$[P_n(X,0)]=0, \quad n=1,2,\cdots,5, \quad 0\leqq X\leqq L$$

$$[C_n(X,0)]=0, \quad n=1,2,\cdots,4, \quad 0\leqq X\leqq L$$

$$[E_n(L_n,0)]=E_{n0}, \quad n=1,2,\cdots,4$$

$$[E_n(X,0)]=0, \quad n=1,2,\cdots,4, \quad X\neq L_n$$

$$[Y(X,0)]=0, \quad 0\leqq X\leqq L \tag{7}$$

and the boundary conditions are assumed to be

$$[S(0,T)]=S_0$$

$$\frac{\partial[P_n(0,T)]}{\partial X}=a[P_n(0,T)], \quad n=1,\cdots,5$$

$$\frac{\partial[S(L,T)]}{\partial X}=a[S(L,T)]$$

$$\frac{\partial[P_n(L,T)]}{\partial X}=a[P_n(L,T)] \quad n=1,\cdots,5$$

$$a>0, \quad \text{constant} \tag{8}$$

These boundary conditions imply that the all reactants pass through the boundaries of both sides in proportion to their own concentrations, except that the concentration of substrate at boundary (0) is held at constant value.

The new dimensionless variables and parameters are defined as follow:

$$S=\frac{k_{11}}{k_{12}}[S]$$

$$P_n=\frac{k_{n+11}}{k_{n+12}}[P_n], \quad n=1,2,3$$

$$P_4=\begin{cases}\dfrac{k_{11}}{k_{12}}[P_4] & \text{for no.}\\ \dfrac{k_{51}}{k_{52}}[P_4] & \text{for compt.}\\ \dfrac{k_{61}}{k_{62}}[P_4] & \text{for allost.}\end{cases} \tag{9}$$

$$E_n=[E_n]/E_{n0}, \quad n=1,\cdots,4$$

$$P_5=[P_5]/E_{10}$$

$$Y=[Y]/E_{10} \tag{10}$$

$$x=X/L, \quad t=T/\theta, \ \theta=L^2/D_s$$

$$\eta_n=D_n/D_s, \ n=1,\cdots, \ 4 \tag{11}$$

$$\sigma_n=k_{n1}E_{10}\theta, \quad n=1,\cdots,4$$

$$\sigma_5=\begin{cases}0 & \text{for no.}\\ k_{51}E_{10} & \text{for compt.}\\ k_{61}E_{10} & \text{for allost.}\end{cases} \tag{12}$$

$$\varepsilon_1=\begin{cases}\dfrac{k_{13}k_{21}}{k_{22}}E_{10}\theta & \text{for no. and compt.}\\ \dfrac{k_{13}k_{21}}{k_{22}[1+E_{10}P_5]}e & \text{for allost.}\end{cases}$$

$$\varepsilon_2=\frac{k_{31}k_{23}}{k_{32}}E_{20}\theta$$

$$\varepsilon_3=\frac{k_{41}k_{33}}{k_{42}}E_{40}\theta$$

$$\varepsilon_4 = \begin{cases} \dfrac{k_{11}k_{43}}{k_{12}} E_{40} & \theta \text{ for no.} \\ \dfrac{k_{51}k_{43}}{k_{52}} E_{40} & \theta \text{ for compt.} \\ \dfrac{k_{61}k_{43}}{k_{62}} E_{40} & \theta \text{ for allost.} \end{cases} \tag{13}$$

The dimensionless state equations may be written as, using the above definitions,

$$\left.\begin{aligned} \zeta_{n2} &= k_{n2}\theta \\ \zeta_{n3} &= k_{n3}\theta \end{aligned}\right\}, \quad n=1,2,\ldots.6 \tag{14}$$

$$\frac{\partial S}{\partial t} - \frac{\partial^2 S}{\partial x^2} + \sigma_1\{SE_1 + E_1 + R_1 - 1\} = 0 \tag{15}$$

$$\frac{\partial P_1}{\partial t} - \eta_1\frac{\partial^2 P_1}{\partial x^2} + \sigma_2\{P_1 E_2 + E_2 - 1\} + \varepsilon_1\{E_1 + R_1 - 1\} = 0 \tag{16}$$

$$\frac{\partial P_2}{\partial t} - \eta_2\frac{\partial^2 P_2}{\partial x^2} + \sigma_3\{P_2 E_3 + E_3 - 1\} + \varepsilon_2\{E_2 - 1\} = 0 \tag{17}$$

$$\frac{\partial P_3}{\partial t} - \eta_3\frac{\partial^2 P_3}{\partial x^2} + \sigma_4\{P_3 E_4 + E_4 - 1\} + \varepsilon_3\{E_3 - 1\} = 0 \tag{18}$$

$$\frac{\partial P_4}{\partial t} - \eta_4\frac{\partial^2 P_4}{\partial x^2} + R_2 + \varepsilon_4\{E_4 - 1\} = 0 \tag{19}$$

$$\frac{\partial E_1}{\partial t} + \zeta_{12}\{SE_1 + E_1 + R_1 - 1\} + R_3 = 0 \tag{20}$$

$$\frac{\partial E_2}{\partial t} + \zeta_{22}\{P_1 E_2 + E_2 - 1\} + \zeta_{23}\{E_2 - 1\} = 0 \tag{21}$$

$$\frac{\partial E_3}{\partial t} + \zeta_{32}\{P_2 E_3 + E_3 - 1\} + \zeta_{33}\{E_3 - 1\} = 0 \tag{22}$$

$$\frac{\partial E_4}{\partial t} + \zeta_{42}\{P_3 E_4 + E_4 - 1\} + \varepsilon_{43}\{E_4 - 1\} = 0 \tag{23}$$

$$\frac{\partial Y}{\partial t} + \zeta_{52}\{Y - P_4 E_1\} = 0 \tag{24}$$

$$\frac{\partial P_5}{\partial t} + \zeta_{62}\{P_5 - P_4 E_1\} = 0 \tag{25}$$

$$R_1 = \begin{cases} 0 & \text{for no. and allost.} \\ Y & \text{for compt.} \end{cases}$$

$$R_2=\begin{cases}0 & \text{for no.}\\ \tau_5(P_4E_1-Y) & \text{for compt.}\\ \sigma_5(P_4E_1-P_5) & \text{for allost.}\end{cases}$$

$$R_3=\begin{cases}\zeta_{13}(E_1-1) & \text{for no.}\\ \zeta_{13}(Y+E_1-1)+\zeta_{52}(P_4E_1-Y) & \text{for compt.}\\ \zeta_{13}(E_1-1)/(1+\alpha E_{10}P_5) & \text{for allost.}\end{cases} \tag{26}$$

Difference equation and numerical solution

The time interval (0, t) and the space region (0, 1) are divided into subintervals with a small width $\Delta t=k$ and subregions with a small length $\Delta x=h$, respectively, and the lattice on time- and space-axis is constructed. The notations i and j indicate the number of the points on the both axes counted from $x=0$ and $t=0$, and pair (i, j) represents the lattice point. The difference equations are derived from dimensionless state equation using the Crank-Nicolson method (1947). The difference equations for the system involving the allosteric inhibition may be writtern as follow:
From equation (15),

$$-\frac{\lambda}{2}S(i-1,j+1)+\left\{1+\lambda+\frac{k}{2}\sigma_1E_1(i,\ \text{j+l}))\text{S(i,}\ j+1)\right.$$

$$-\frac{\lambda}{2}S(i+1,j+1)$$

$$=\frac{\lambda}{2}\{S(i-1,j)+S(i+1,j)\}+(1-\lambda)S(i,j)$$

$$-\frac{k}{2}\sigma_1\{S(i,j)E_1(i,j)+2E_1(i,j)\ \text{-2)}$$

$$I=k/h^2,\quad \Delta t=k,\ \Delta x=h. \tag{27}$$

is obtained. From equations (16)-(18),

$$-\frac{\lambda}{2}\eta_nP_n(i-1,j+1)+\left\{1+\lambda+\frac{k}{2}\sigma_{n+1}E_{n+1}(i,j)\right\}P_n(i,j+1)$$

$$-\frac{\lambda}{2}\eta_nP_n(i+1,j+1)$$

$$=\frac{\lambda}{2}\eta_n\{P_n(i-1,j)+P_n(i+1,j)\}+(1-\lambda\eta_n)P_n(i,j)$$

$$-\frac{k}{2}\sigma_{n+1}\{P_n(i,j)E_{n+1}(i,j)-2E_{n+1}(i,j)-2\}$$

$$-k\varepsilon_n\{E_n(i,j)-1\}$$

$$n=1,2,3 \tag{28}$$

from equation (19),

$$-\frac{\lambda}{2}\gamma_4 P_4(i-1,j+1)+\left\{1+\lambda\gamma_4+\frac{k}{2}\sigma_5 E_1(i,j)\right\}P_4(i,j)$$

$$-\frac{\lambda}{2}\gamma_4 P_4(i+1,j+1)$$

$$=\frac{\lambda}{2}\gamma_4\{P_4(i-1,\ j)+P_4(i+1,j)\}+(1-\lambda\gamma_4)P_4(i,j)$$

$$-\frac{k}{2}\sigma_5\{P_4(i,j)E_1(i,j)-2P_5(i,j)\}$$

$$-k\varepsilon_4\{E_4(i,j)-1\} \tag{29}$$

from equation (20),

$$\left\{\frac{1}{k}+\frac{\zeta_{12}}{2}(S(i,j+1)+1)+\frac{\varepsilon_5}{2}\right\}E_1(i,\text{j-t-1})$$

$$=\left\{\frac{1}{k}+\frac{\zeta_{12}}{2}(S(i,j)+1)-\frac{\varepsilon_5}{2}\right\}E_1(i,j)+\zeta_{12}$$

$$+\zeta_{13}/\left[1+\frac{\alpha E_{10}}{2}\{P_5(i,j+1)+P_5(i,j)\}\right] \tag{30}$$

from equations (21)-(23),

$$\left\{\frac{1}{k}+\frac{1}{2}(\zeta_{n2}P_{n-1}(i,j+1)+\zeta_{n3})\right\}E_n(i,j+1)$$

$$=\left\{\frac{1}{k}+\frac{1}{2}(\zeta_{n2}P_{n-1}(i,j+1)+\zeta_{n3})\right\}E_n(i,j)+\zeta_{n2}+\zeta_{n3}$$

$$n=2,3,4 \tag{31}$$

and from equation (25),

$$\left(\frac{1}{k}+\frac{\zeta_{62}}{2}\right)P_5(i,j+1)$$

$$=\left(\frac{1}{k}-\frac{\zeta_{62}}{2}\right)P_5(i,j)+\frac{1}{2}\zeta_{62}\{P_4(i,j+1)E_1(i,j)+P_4(i,j)E_1(i,j)\}$$

(32)

are derived. The difference equations for the system without feedback or with beedback involving competitive inhibition have fundamentally the same form as above and are not shown here.

Boundary conditions at $x=1$ may be written as,

$$\left(\frac{\partial C}{\partial x}\right)_{x=1} = \frac{1}{h}\left(\nabla_1 + \frac{1}{2}\nabla_2 + \frac{1}{3}\nabla_3 + \frac{1}{4}\nabla_4\right) C(N+1, i) \tag{33}$$

where ∇_i is backward difference operator, C represents the concentration of reactants and i and $x=1$ is denotedd by N. When $C(N, j)$ are abbreviated by C(N), $\nabla_i C(N+1)$ are written as

$$\begin{aligned}
&\nabla_1 C(N+1) = C(N+1) - C(N)\\
&\nabla_2 C(N+1) = C(N+1) - 2C(N) - C(N-1)\\
&\nabla_3 C(N+1) = C(N+1) - 3C(N) + 3C(N-1) - C(N-2)\\
&\nabla_4 C(N+1) = C(N+1) - 4C(N) + 6C(N-1) - 4C(N-2) + C(N-3)
\end{aligned} \tag{34}$$

With equation (34), equation (33) can be transformed to

$$\left(\frac{\partial C}{\partial x}\right)_{x=1} = \frac{1}{h}\left(\frac{35}{12}C(N+1) - 4C(N) + 3C(N-1) - \frac{4}{3}C(N-2) + \frac{1}{4}C(N-3)\right) \tag{35}$$

The difference equations of the state equation, equations (27)-(32), are formulated up to $i=N+1$ to obtain $C(N+1, j)$. Thus, $C(N+1)$ in equation (35) can be obtained by numerical solution of difference equation of the state equation. The boundary conditions $\partial C/\partial x|_{x=0}$ is formulated by a similar form to equation (35). All difference equations (27)-(35) were numerically solved by the Gauss elimination method with $h=\Delta x=0.01$ cm and $k=\Delta t=0.01$ sec.

RESULTS

Time course in homogeneous medium

The reaction time course was calculated with assuming that the enzymatic reaction system in Scheme 1 is operating in a homogeneous medium. It was further assumed that the substate was supplied to the system to maintain the constant concentration and that the final product was removed from the system at the rate proportional to its concentration (k_{33} P,). The result is shown in Fig. 1. The products P_1-P_3 attained rapidly to the same stationary concentration. The accumulation of P_5 was due to the small rate of its removal from the system.

Enzymatic reaction system without feedback

An instance of spatial distributions of the reactants in the system which is laking in the feedback loop is shown in Fig. 2. The value of patameters adopted in the calculation are shown in the legend of the figures. The time courses of the reactants at $x=0.9$ are shown in Fig. 3. The products P_1-P_4 attained to stationary state within $t=0.2$ while the substrate took much longer time to reach the stationary value. The fact that P_4 was higher than P_3 may be due to that the examining point, $x=0.9$, is near the

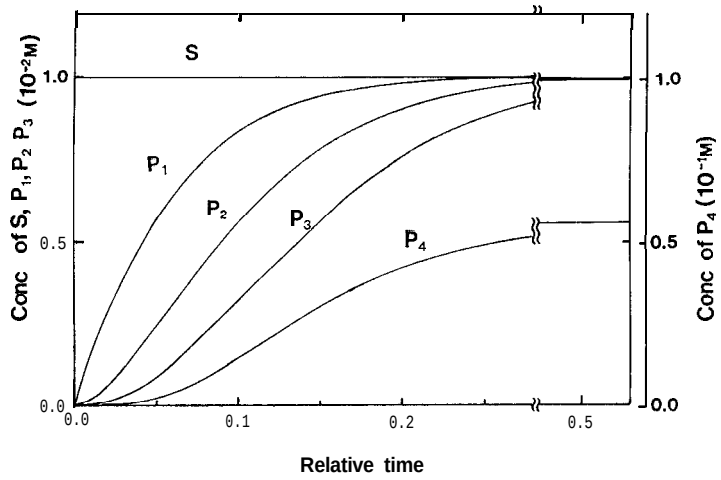


Fig. 1. Time course of enzymatic reaction in Scheme 1 in homogeneous medium. Values of parameters are $k_{11}=k_{21}=k_{31}=k_{14}=1000\,M^{-1}sec^{-1}$, $k_{12}=k_{22}=k_{32}=k_{42}=50\,sec^{-1}$, $k_{13}=k_{23}=k_{33}=k_{43}=30\,sec^{-1}$ and $k_{53}=30\,sec^{-1}$. P_4 was removed by $k_{53}P_4$. Substrate concentration was 0.1 M and all enzymes were 1.0 M.

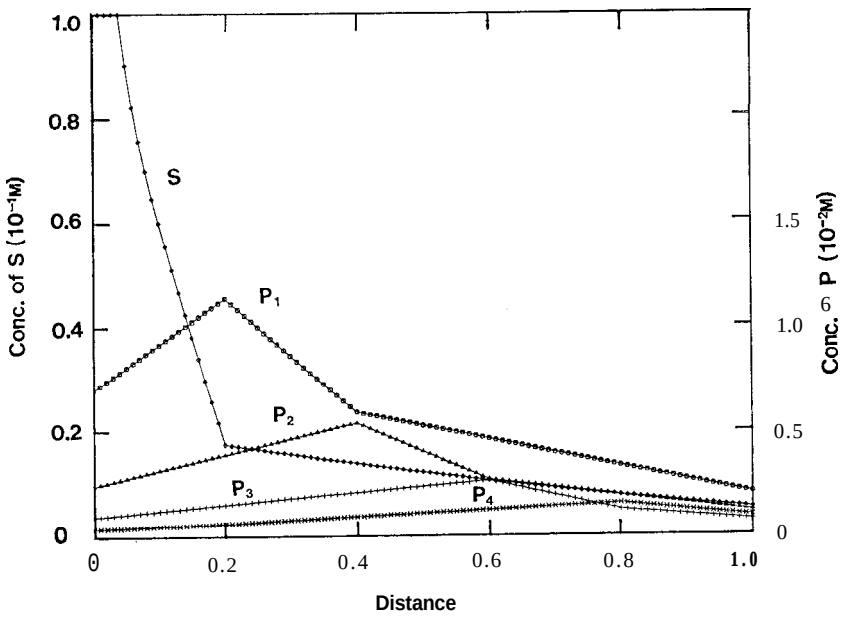


Fig. 2. Spatial distribution of the products in enzymatic reaction system without feedback in inhomogeneous medium. Parameters adopted for calculation were the same as those in Fig. 1 and $L_1=0.2$, $L_2=0.4$, $L_3=0.6$ and $L_4=0.8$ cm, $D_s=10^{-5}$, $D_1=D_2=D_3=D_4=10^{-4}\,cm^2\,sec^{-1}$.

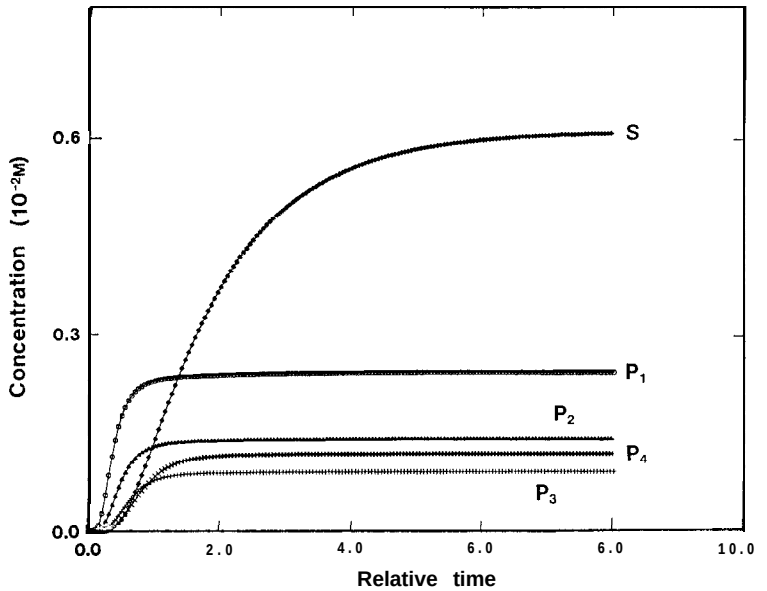


Fig. *3.* Time course of reactants at $x=0.9$ in the enzymatic system without feedback. Conditions were the same as those in Fig. 2.

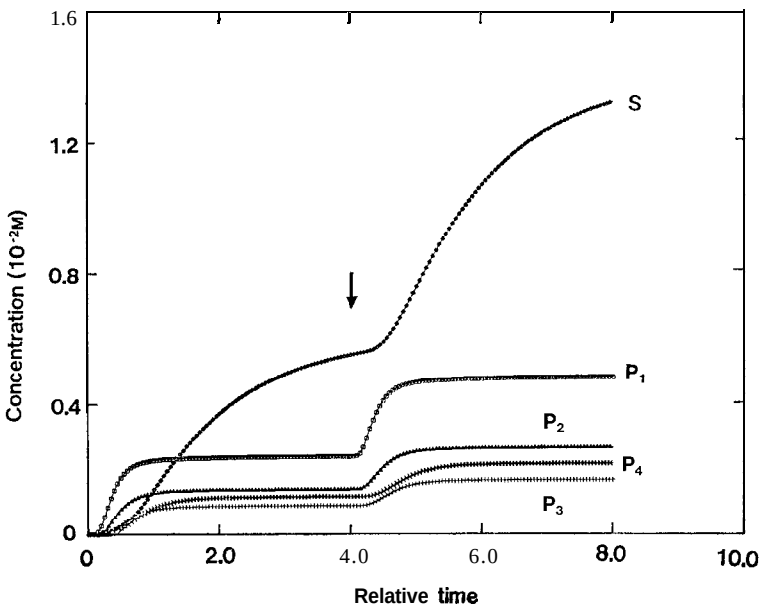


Fig. 4. Time course of the reactants at $x=0.9$ in the system without feedback. S was changed at time indicated by arrow from 0.1 to 0.2 M.

location of $E_4(x=0.8)$. After all products had attained to stationary state, the concentration of the substrate at $x=0$ was increased twofold in the step function and the calculation of itme course was proceeded. This situation may correspond to that the system was perturbed by an external forces causing a large supply of the substrate through bundary (0). The calculated result is shown in Fig. 4. The concentrations of the products at new stationary state doubled exactry as expected, showing no capability of autocontrol against the external perturbation.

Feedback system with competitive inhibition

The calculated spatial distributions of reactants of the feedback system in which E_1 is inhibited competitively by P_4 are shown in Fig. 5, and the time courses at $x=0.9$ are shown in Fig. 6. The fundamental features of the distribution and time course were nearly the same as those of the system without the beedback loop (Figs. 2 and 3). However, the concentration of substate near the stationary state was considerably higher than that of the system without feedback loop while the time courses of the products in both systems were overlapped almost completely. The changes in the concentrations of the products at stationary state caused by twofold increase in the substrate concentration at $x=0.9$ are shown in Fig. 7. Twofold increase in the substrate concentration resulted in about 1.9 times increase in the product

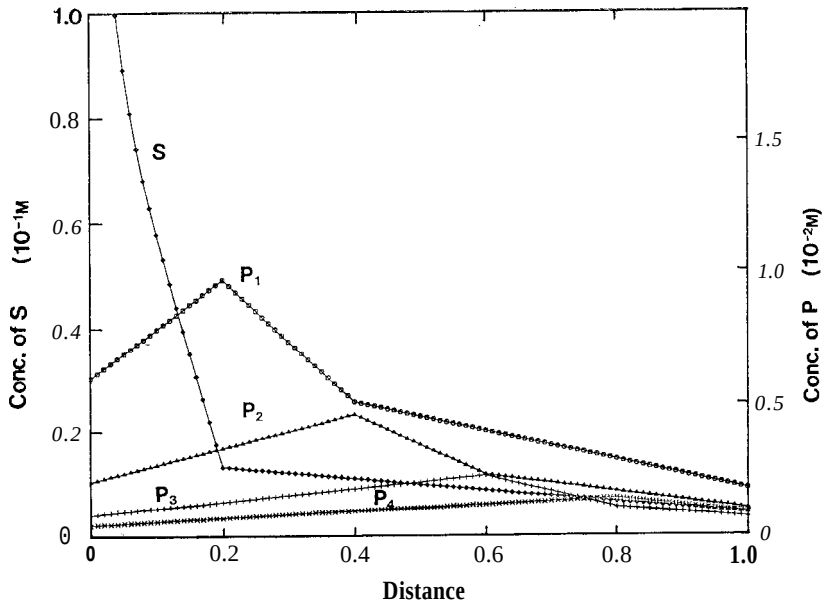


Fig. 5. Spatial distribution of reactants at x -0.4 in the enzymatic feedback system with competitive inhibitor. Conditions were the same as those in Fig. 2, except $k_{51}=2000$ and $k_{52}=10\ sec^{-1}$.

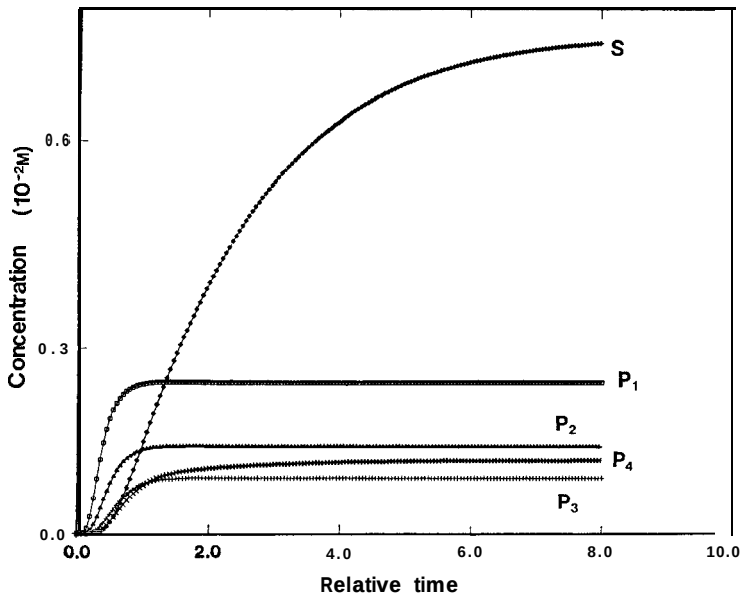


Fig. 6. Time course of reactants at x=0.9 in the feedback system with competitive inhibitor. Reaction conditions were the same as those in Fig. 5.

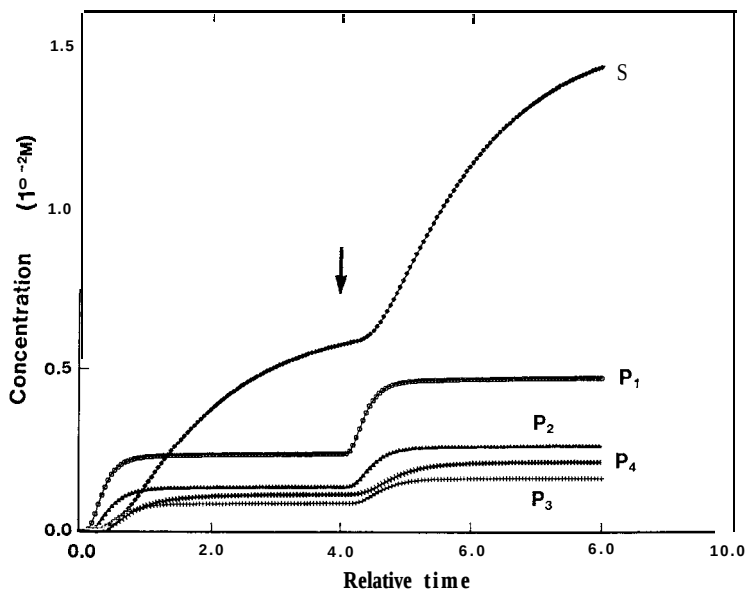


Fig. 7. Time course at x-O.9 in the feedback system with competitive inhibitor. Substrate concentration was increased twofold at time indicated by arrow.

Table 1. Effect of binding constant on the concentration ratio between old and new stationary states.

Upper table is for the case where substrate concentration was increased twofold and lower table is for threefold in crease. Values indicate the ratio of concentration at new stationary state to that at old stationary state.

		Ratio			
k_{51}/k_{52}	S	P_1	P_2	P_3	P_4
100	2.47	1.96	1.86	1.18	1.91
200	2.57	2.00	1.86	1.86	1.85
500	2.79	1.91	1.85	1.82	1.81
1000	3.11	1.86	1.71	1.74	1.81

		Ratio			
k_{51}/k_{52}	S	P_1	P_2	P_3	P_4
100	4.26	2.92	2.79	2.56	2.64
200	4.46	2.89	2.67	2.48	2.54
500	4.92	2.74	2.72	2.48	2.50
1000	5.47	2.59	2.40	2.34	2.40

concentrations at the new stationary state, showing very slight auto-controllability against the external perturbation. The effectiveness of competitive inhibitor P_4 to inactivate enzyme E, may depend on the rate constants of the enzyme-inhibitor complex formation. Table 1 shows the effect of k_{51}/k_{52} (binding constant) on the controllability of the system. The upper figures show the case of twofold increase in the substrate concentration and the lower the case of threefold increase. As can be seen in the table, the increment of the product at a large value of binding costant was about 80 % of the substate increment. Thus, it is clear that the system containing competitive inhibition is able to reject only 20 % of the effect of the external perturbation, and this type of feedback system may not he regarded as the basic mechanism of homeostatic control of metabolism.

Feedback system with allosteric enzyme

The time course of the reactants at $x=0.9$ in the system involving allosteric enzyme E_1 and negative effector P_4 is shown in Fig. 8. The concentrations of the products at the stationary state were considerably lower than those in the system with competitive inhibition (see Fig. 6). The characteristic features of the time course were the appearance of overshoot on P, and P_2 concentrations. The effects of binding constant, k_{61}/k_{62} in E_1-P_5 complex formation and the constant on the stationary concentration of reactants are shown in Table 2. The binding constant (upper table) changes scarcely the stationary concentration, while the increase in the value of α (lower table) causes a remakable decrease in the concentration of the products and increase in that of the substrate. Fig. 9 shows the effect of increase in the substrate concentration at $x=0.9$ on the concentrations of reactants at the

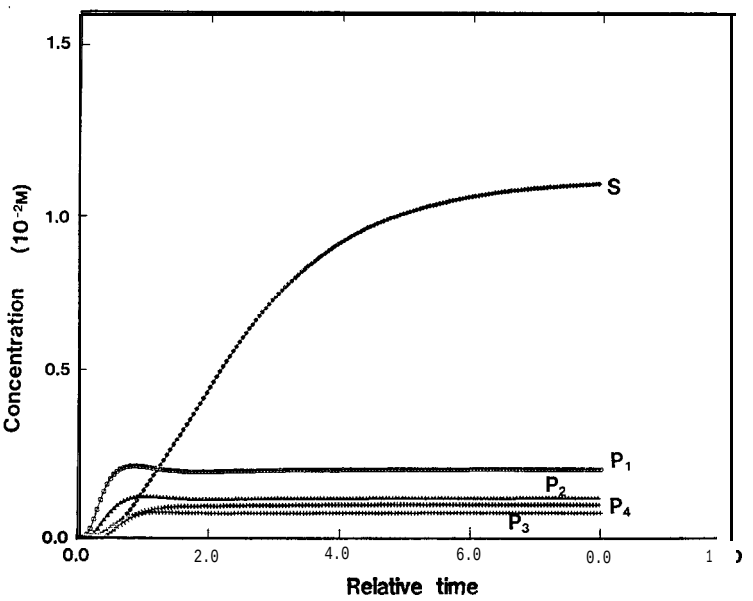


Fig. 8. Time course of reactants at x=0.9 in the feedback system with allosteric effector. Reaction conditions were the same as those in Fig. 6 except $k_{61}=1000, k_{62}=10\ sec^{-1}$ and $\alpha=30$.

Table 2. Effect of binding constant between enzyme and effector and the value of α on stationary concentration of reactants in enzymatic feedback system with allosteric effector.

	Concentration in Steady-State (10^{-2}M)				
k_{61}/k_{62}	S	P_1	P_2	P_3	P_4
100	0.63	0. 24	0.13	0.09	0.11
200	0.67	0.24	0.13	0.09	0.11
500	0.15	0.23	0.13	0.08	0.11
1000	0.85	0.22	0.13	0.08	0. 10
	Concentration in Steady-State (10^{-2}M)				
α	S	P_1	P_2	P_3	P_4
30	0.73	0.20	0.12	0.07	0.10
100	1.03	0.16	0.09	0.06	0. 08
1000	2.22	0.08	0.05	0.03	0. 04
5000	2.65	0.04	0.02	0.01	0: 02

stationary state. The concentrations of products increased in proportion to the substrate concentration. As described above, the value of α affected strongly the concentrations at the stationary state. Table 3 shows the effect of the value of α on the concentration-difference (denoted by ratio) between old and new stationary states. Upper table is for the case where substrate

Table 3. Effect of the value of α on the concentration ratio between old and new stationary states.

	Ratio				
α	S	P_1	P_2	P_3	P_4
30	2.93	1.80	1.67	1.62	1.72
100	2.51	1.63	1.67	1.67	1.65
1000	2.52	1.51	1.51	1.51	1.49
5000	2.40	1.51	1.55	1.50	1.45

	Ratio				
α	S	P_1	P_2	P_3	P_4
30	5.10	2.39	2.48	2.24	2.20
100	4.68	2.13	2.06	2.03	2.01
1000	3.83	1.88	1.87	1.86	1.85
5000	3.56	1.86	1.86	1.86	1.80

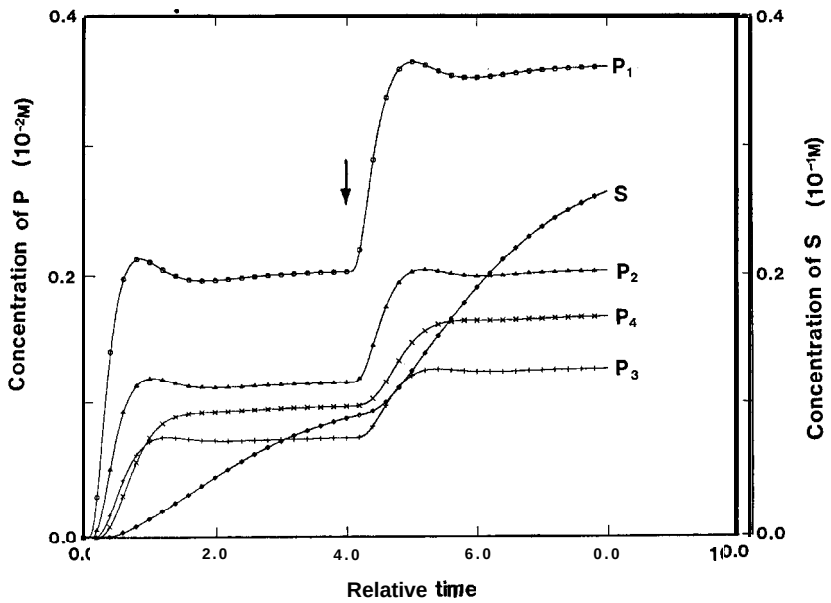


Fig. *9.* Time course in the enzymatic feedback system with allosteric enzyme. Substrate concentration was increased twofold at the time indicated by arrow. Other conditions were the same as those in Fig. 8.

was increased twofold and the lower is for the case of threefold increase. If there is no auto-controllability, the values of ratio on P_1-P_4 would be 2.0 for upper table and 3.0 for the lower, and reversely if there is complete controllability the value would be 1.0 for both cases. The increments of the stationary concentrations of the products was about 45 % of the increment of the substrate concentration.

DISCUSSION

The homeostatic control of metabolism in a living cell may be realized through the combination of various mechanisms. However, respective mechanism seems to have essentially the form of feedback system as depicted in Fig. 10. Mechanisms 1, 2 and 3 control the activity of regulatory enzyme and

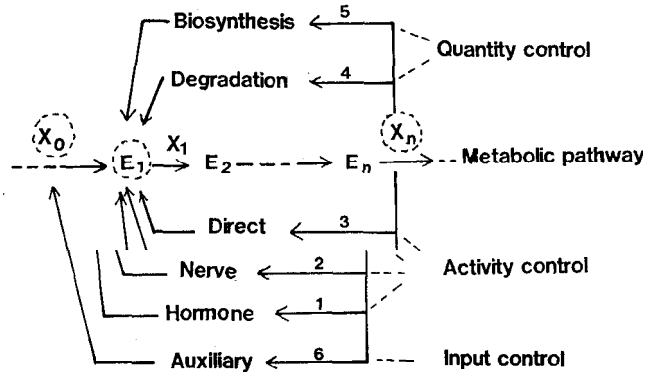


Fig. 10. Various modes of enzymatic feedback systems.

mechanisms 4 and 5 the quantity of the enzyme. Mechanism 6 is thought to belong to the input regulation, while others are parametric regulation. The mechanism 3 is direct feedback system in which X_{n} inhibits enzyme E_1, and it is very likely that basic features of this system are common to all other feedback systems which include complicated feedback element. Thius, the enzymatic reaction system containing direct feedback loop has been investigated in a series of the present study. First, the enzymatic systems with direct feedback in homogeneous medium have been intensively studied on their capabilities of homeostatic control. However, it was concluded that this type of system could not realize the satisfactory control for homeostasis (Okamoto et *al,* 1978).

In a living cell, the reaction conditions of enzymatic system are regarded to be inhomogeneous and it is expected that the enzymatic system with direct feedback in such medium would realize by some possibility the homeostatic control of reactant against the external perturbation. Thus, the present study was carried out to elucidate theoretically the controllability of enzymatic feedback system in an inhomogeneous medium which corresponds to the cellular conditions.

In the present model, the cellular conditions were abstractly represented ; three-dimensional space was reduced to one-dimensional space, and active transport across the membrane, the flow of cytoplasm and the others were neglected. Further it was assumed that the enzymatic reaction is irreversible and the reactants can pass through the boundaries. However, such abstract model may still represent the fundamental nature of the real system.

The activity of allosteric enzymes in the presence of negative effector at stationary state in the closed and homogeneous system has been formulated by Monod *et al. (1965)* and Koshland *et al.* (1966). The precise representation of the rate equation of the allosteric enzyme may be given by simultaneous differential equation without using the allosteric and substrate-binding constants. Since, it was revealed that the rate equation of the allosteric enzymes can be approximated satisfactory by a simple equation of the Hill type (Morales and MacKay (1967)), R_1 in equation (5) was used for the formulation of the activity of allosteric enzyme. The parameter α in R_1 may be related to the value of allosteric constant. The values of various parameters adopted for the numerical calculation were average of general enzymatic reactions. In an enzymatic reaction, it is usual to use a large excess of substrate to the enzyme. However, in the present model of the reaction-diffusion system, the apparent activity of the enzymes becomes to be very low, because a large fraction of substrate passes as the unreacted through the space in which the enzymes are fixed. Consequently, a large excess of enzyme concentration relative to that of the substrate at boundary $x=0$ should be applied for the calculation.

In the present model, the boundary conditions were assumed to be represented by equation (8). These conditions indicate that, except the substrate at $x=0$, all reactants can pass through the boundaries at the rate proportional to their concentrations. This may be not the case for the real enzymatic reactions in living cells. Therefore, the calculation should be continued in succeeding computer simulation with revising the boundary conditions.

The enzymatic reaction system without feedback (Scheme 1) gave rapidly the stationary state in a homogeneous medium. Since the substrate is supplied to the system at a canstant rate and the final product P_4 is removed at the rate proportional to its concentration, the substrate and the products, P_1-P_3 did not accumulated in the system giving the same concentration at the stationary state. In the enzymatic reaction system without feedback inhibition in the inhomogeneous medium, the products, P_1-P_4, attained rapidly to the stationary state with different concentrations, while the substrate took a long time because of no back-flowing of substrate through $x=0$. After the all products had attained to the stationary state, the concentration of substrate at $x=0$ was increased twofold in step-function and the time courses were followed. As can be seen in Fig. 4, the concentrations of the all products were also increased exactly twice. The increase in the substrate concentration may be regarded logically as the addition of the external perturbation to the system through the boundary (0). Consequently, it was concluded that this system has no auto-controllability for the external perturbation.

In the enzymatic feedback system with the competitive inhibition, the time courses (Fig. 6) of all products at $x=0.9$ were nearly the same as those (Fig. 3) in the system without the feedback, when the same substrate concentration was supplied to the system through boundary $x=0$. The twofold in-

crease (perturbation) in the substrate concentration caused an uniform increase in the concentration of the products at the new stationary state, indicating very weak auto-controllability of the system against the perturbation. Since the binding constant k_{51}/k_{52} seems to affect most strongly the auto-controllability of the system, the effect of this constant on the stationary concentration of the products was surveyed. However, as shown in Table 1 it is clear that the binding constant is not sensitive parameter. With sufficiently large binding constant, the increment in the product concentration at the new stationary were 1.8 for twofold increase in the substrate concentration and 2.4 for threefold increase. These values imply that only about 20 % of the effect of external perturbation could be rejected by the feedback and this system cannot be candidate for the autocontrol mechanism on the homeostasis of metabolism.

In the enzymatic feedback system containing allosteric enzyme, the activity of the system was considerably lower than that of the feedback system with competitive inhibition; the stationary concentration of the product was lower and that of the unreacted substrate was higher than those of the competitive inhibition. This means that the inhibitory effect of P_4 as negative allosteric effector on the allosteric enzyme E_1 is stronger than that of P_4 that acts as competitive inhibitor. As can be seen in Fig. 8, the time course of P_1 and P_2 exhibited an overshoot. This will be caused by fact that the appearance of inhibitory effect of P_4 on E_1 took some time-delay owing to the movement of P_4 by free diffusion ; before P_4 reached to E_1, P_1 was formed in a large amount without the inhibition and thereafter the formation of P_1 was surpressed by P_4. In the allosteric inhibition, the binding constant, k_{61}/k_{62} between the allosteric effector and the enzyme E_1 and the constant a may be important factors governing the auto-controllability which relates with the stationary concentration of the products. As shown in Table 2, the binding constant exhibited practically no effect, while the value of α affected sensitively the stationary concentration, suggesting a strong relation with the controllability on homeostasis of the products.

As shown in Fig. 9, the stepwise increase in the substrate concentration caused uniform increase in the product concentrations at the new stationary state. As stated above, the value of a appears to relate strongly to the auto-controllability of the feedback system. It was evident from Table 3 that the feedback system with a large value of a is able to reject about 60 % of the effect of the external perturbation, and that this system is superior to the feedback system with competitive inhibitor with respect to the homeostatic control of the enzymatic products or metabolites. However, it is concluded that the all enzymatic reaction model subjected to the present study has no satisfactory capability of auto-control against the external perturbation. Further, computer simulations are necessary to find out the fundamental structure of feedback system which is available to achieve the homeostatic control of metabolism in living cell.

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