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Okuyama, Kaoru
Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

Okamoto, Masahiro
Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

Aso, Yoichi
Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

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

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Studies on the Threshold Mechanism of Enzymatic Reaction

Kaoru Okuyama, Masahiro Okamoto, Yoichi Aso and Katsuya Hayashi

Laboratory of Sericultural Chemistry, Faculty of Agriculture, Kyushu University

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A reaction system containing bimolecular subsystems was studied as a model system which realizes chemically a threshold behavior. An analog computer was used for preliminary determination of parameter set that is attainable to steady-state, and digital computation was performed to obtain a numerical solution of the rate equation. A certain relationship among input concentration, Michaelis constant, and the substrate concentration (output) with respect to the threshold-type response of the system has been clarified. When oscillatory input was adopted to the system, the output concentration vibrated with the same angular velocity as input and it became clear that the basic behavior of the system was not altered by such an oscillatory input.

INTRODUCTION

It has been well observed that a living organism shows threshold behaviors at various levels, such as enzymatic reaction, drug effect, neurophysiology or eco-system (Pavlidis, 1973; Chance *et al.*, 1973). For a simple enzymatic reaction, an allosteric mechanism has been proposed for the explanation of a sigmoidal relationship between the activity and the substrate concentration. A similar analysis has been adopted to the characteristic equilibrium phenomena on the interaction between hemoglobin and oxygen molecule. For the drug effect, the threshold in dose-response relation has been considered to be arisen from the consumption of the drug by the combination with some kind of inhibitor or by metabolic action of an enzyme system in the body.

A chemical reaction system is known to be lacking in inertia term. The discontinuity of the output (response) for the continuous change in the input concentration has, therefore, been hardly explained by the terms of chemical reaction system, if the special consumption mechanisms as described above were not taken into consideration. On the contrary, at the present time, the discontinuity of response, for instance induction period, with respect to the reaction time under the constant input has been well explained both experimentally and theoretically.

On the other hand, the progress in mathematical biology in recent years has been accompanied increasingly by the implication that the threshold function, which is frequently assumed in handling mathematically the subject, would be realized by purely chemical systems, because the mathematical model

including the threshold function can evenly be adoptable to all biological events (Rosen, 1970). Contrary to such a theoretical prediction, sophisticated threshold-realizing chemical reaction system are not confirmed yet. When the details of threshold-realizing system become clear, the firm guarantee to the assumed threshold function will be given and the analysis of the regulation mode in biological reactions *in vivo* will be made more easily and precisely.

Walter *et al.* (1967) have studied model systems involving a series of enzymatic reactions and reported that some systems showed a sigmoidal relationship between the activity and the substrate concentration. Ličko (1972) studied a model system containing bimolecular subsystems and found that unsaturated threshold-type response resulted from such a reaction system.

Their works should be appreciated since the chemical threshold had been first theoretically predicted to be arisen from the purely chemical system, without considering any specific molecular mechanism such as allosteric effect. However, it is likely that there are some uncertain points, for instance, Ličko has analyzed the rate equation by steady-state approximation and, nevertheless, has not specified the allowable region for the values of parameters.

In the present paper, as a preliminary step to establishing the general models for threshold-generating system, the authors analyzed the Ličko's model by numerical computation and simulated its behavior, especially the responses to the oscillating input.

COMPUTATION

Ličko's model system shown in Fig. 1 was analyzed. In the model system, X and Y represent the intermediates and are taken as the input and the output of the system, respectively.

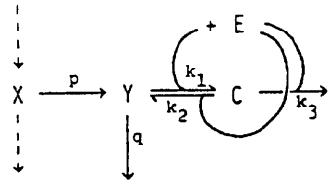

Fig. 1. Model reaction system for computation.

E and C represent enzyme and substrate, and p, q, k_1, k_2 and k_3 are rate constants. $X(t)$, $Y(t)$, $E(t)$ and $C(t)$ represent the concentrations of X, Y, E and C at the reaction time t. X component may be supposed to be an intermediate of a certain main reaction system (dotted line) and the precursor of Y component which is assumed to be the output and the substrate of enzyme in the subsystem.

The rate equation for this system may be written as:

$$\frac{dY(t)}{dt} = pX_0 - k_1E(t)\,Y(t) + k_2C(t) - qY(t) \qquad (1)$$

$$\left.\begin{aligned}\frac{dE(t)}{dt} &= -k_1E(t)\,Y(t)+(k_2+k_3)C(t)\\ \frac{dC(t)}{dt} &= k_1E(t)\,Y(t)-(k_2+k_3)C(t)\end{aligned}\right\} \qquad (1)$$

where $X(t)$ is assumed to have time-invariant value X_0. Since $C(t)\leqq E(0)\leqq X_0$, $E(t)\leqq E(0)$ and $Y(t)\leqq X_0$ under the reaction conditions, the equation (1) can be replaced by the equation (2),

$$\left.\begin{aligned}\frac{dY'(t)}{dt} &= p-k_1E(0)E'(t)\,Y'(t)+k_2C'(t)-qY'(t)\\ \frac{dE'(t)}{dt} &= -k_1X_0E'(t)\,Y'(t)+(k_2+k_3)\frac{X_0}{E(0)}C'(t)\\ \frac{dC'(t)}{dt} &= k_1E(0)E'(t)\,Y'(t)-(k_2+k_3)C'(t)\end{aligned}\right\} \qquad (2)$$

where

$$Y'(t) = Y(t)/X_0,\ E'(t) = E(t)/E(0),\ C'(t) = C(t)/X_0.$$

The initial concentrations were assumed to be $Y(0) = C(0) = 0$. The threshold value θ was defined by

$$\theta = \frac{k_3E(0)}{p} \qquad (3)$$

according to Ličko and the apparent Michaelis constant K_m was defined by

$$K_m = \frac{k_2+k_3}{k_1}$$

The first computations were made to find out the parameter set which results in the steady-state output of $Y(t)$ under constraints of $\theta = 10$ and $K_m = 0.1$. For this purpose, an analog computer was used to solve the equation (2). On the basis of obtained parameter sets, next the behaviors of the model system were predicted by changing the values of θ, K_m and X_0, respectively. In these computations, the equation (1) was solved directly by a digital computer by means of MRKGM subprogram (Okamoto *et al.*, 1975) that is a slight modification of Runge-Kutta-Gill method.

It will be highly possible that the concentration of an intermediate in an enzymatic reaction system *in vivo* oscillates owing to the existence of a feedback control system. Thus, it is assumed to be probable that the input $X(t)$ of the model system has an oscillating mode. As a most simple mode, $X(t)$ was assumed to be given by following equation:

$$X(t) = X_0+B\ \sin\ (\omega t) \qquad (4)$$

where B and ω denote an amplitude (concentration) and an angular velocity, respectively. $X_0 = 10$, $B = 0.5$ and $\omega = 0.5$ rad/sec were used in the computation. With this oscillating input, the time-course of $Y(t)$, $E(t)$ and $C(t)$ were computed.

All computations were performed by a Hitachi analog computer Model ALS-

220 in the Biotron Institute and FACOM 230-75 digital computer in the Computer Center of Kyushu University.

RESULTS

Determination of parameter set

The parameter set containing parameters p, q, k_1, k_2, k_3 and $E(0)$ which gives the steady-state with respect to the output $Y(t)$ was determined by analog computer under the constraints of $\theta = 10$ and $K_m = 0.1$. Table 1 shows typical examples of parameter sets, a and b, obtained by the computation.

Table 1. Parameter set determined by analog computer.

Set \ Parameter	p (sec^{-1})	q (sec^{-1})	k_1 ($M^{-1}sec^{-1}$)	k_2 (sec^{-1})	k_3 (sec^{-1})	$E(0)$ (M)
a	0.01	0.1	40.0	3.5	0.5	0.2
b	0.1	1.0	120.0	7.0	5.0	0.2

Since, on the analog computation, the parameters are fed through the potentiometers, too small values for parameters could not be used on computation. Furthermore, a large difference in parameter values in the same differential equation introduces a large computation error. The parameter sets shown in Table 1 were thus obtained in consideration of such a limitation on analog computation. These results, therefore, do not mean that there are no parameter sets other than those shown in the table.

Behaviors of model system

Around the parameter sets shown in Table 1, there may be many possible sets including various combinations of parameter values. In Table 2, several

Table 2. Parameter set used for digital computation.

θ(M) \ Parameter	K_m (M)	p (sec^{-1})	q (sec^{-1})	k_1 (M^{-1} sec^{-1})	k_2 (sec^{-1})	k_3 (sec^{-1})	$E(0)$ (M)
1.0	0.001	0.01	0.1	60.0	0.01	0.05	0.20
	0.010	0.01	0.1	40.0	0.35	0.05	0.20
	0.100	0.10	1.0	40.0	3.50	0.50	0.20
	1.000	0.10	1.0	12.0	11.50	0.50	0.20
	2.000	0.10	1.0	12.0	14.00	10.00	0.01
10.0	0.01	0.01	0.1	60.0	0.1	0.5	0.2
	0.10	0.10	1.0	120.0	7.0	5.0	0.2
	0.50	0.10	1.0	12.0	1.0	5.0	0.2
	1.00	0.10	1.0	12.0	7.0	5.0	0.2
20.0	0.01	0.01	0.1	60.0	0.1	0.5	0.4
	0.10	0.10	1.0	120.0	7.0	5.0	0.4
	1.00	0.10	1.0	12.0	7.0	5.0	0.4
	1.00	0.10	1.0	12.0	2.0	10.0	0.2

sets selected from such possible sets are shown. In addition, Table 2 also includes the sets determined by the same method under the constraint of $\theta = 1$ or 20.

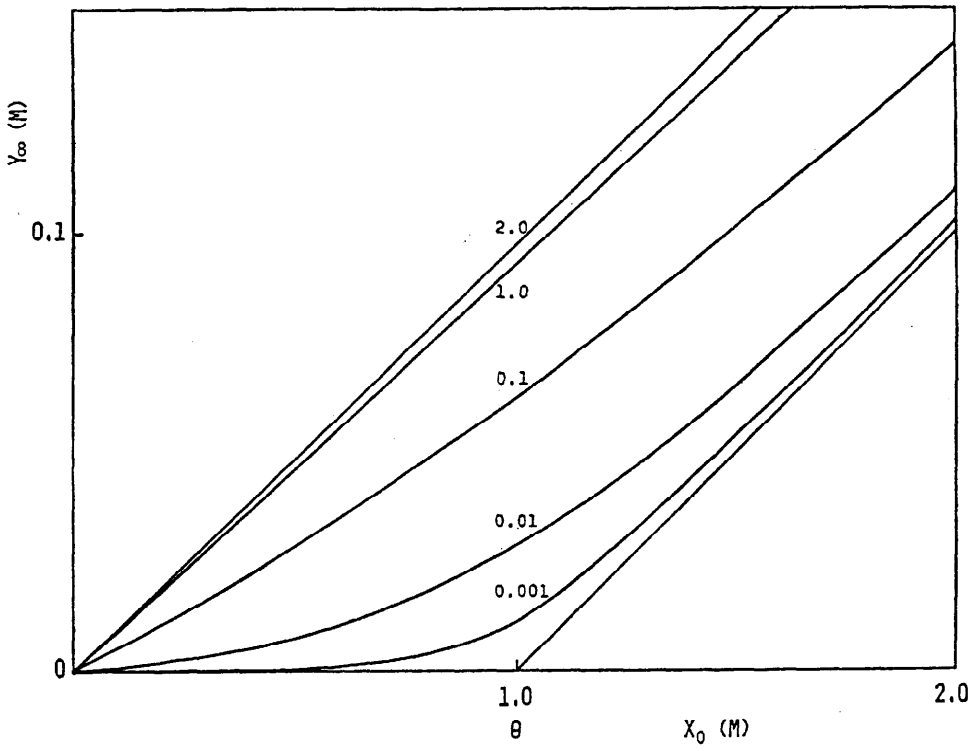

Fig. 2. Computed results on equation (1) with parameter sets listed in first section of Table 2. The value on line represents K_m value.

For the inspection of behaviors of the model system, the equation (1) was numerically solved by digital computer using these parameter sets. Figure 2 shows the computed relation between input X_0 and output $Y(t)$ at steady-state (Y_∞) under the fixed value $\theta = 1$. The value on each line indicates the K_m value. At K_m value of 0.001, the curve shows nearly threshold character. From the effect of K_m value on the shape of calculated curve, it is obvious that much smaller values of K_m may give a typical threshold relation between the input and the output. The line through $X_0 = 1.0$ is the theoretical one and it will be realized when one used the extremely small value of K_m.

The computed results with $\theta = 10$ are shown in Fig. 3. The same relation as in Fig. 2 was observed. Figure 4 shows the results computed with $\theta = 20$ and $E(0) = 0.4$. The curve in the figure with $K_m = 0.1$ was not shifted at all with changing $E(0)$ value from 0.4 to 0.2, although $E(t)$ and $C(t)$ values at steady-state became half with the change in the $E(0)$ value. Thus, the effect of $E(0)$ value on the output Y_∞ seems to be considerably small.

Oscillating input

The time-course of the model system was computed with assuming that the input $X(t)$ is oscillating due to the feedback control system functioning to

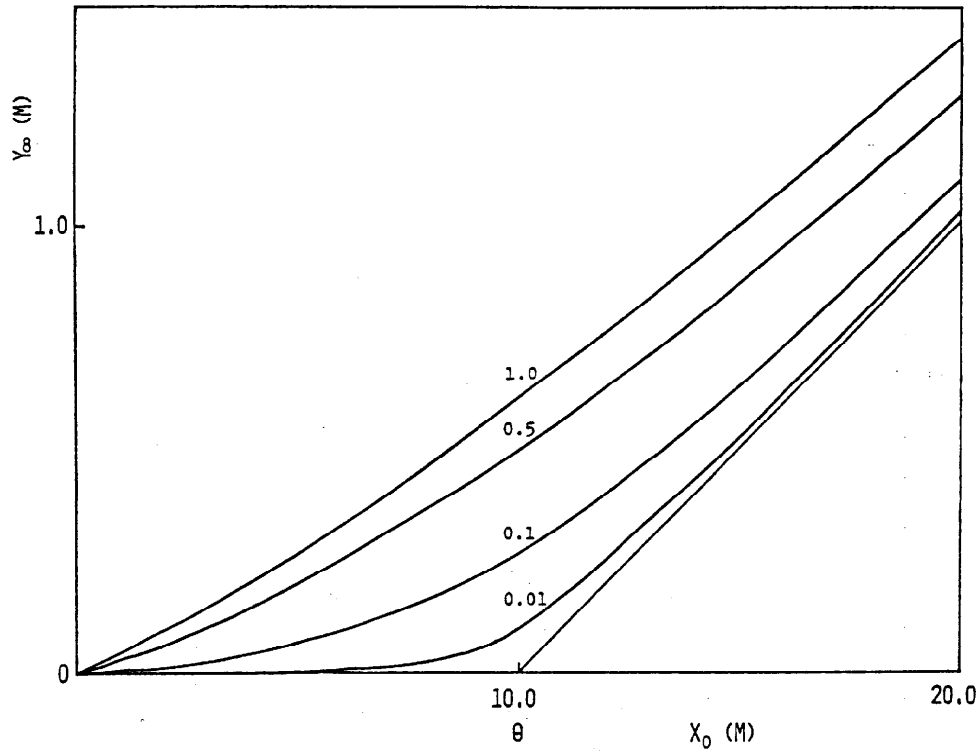

Fig. 3. Computed results with parameter sets listed in second section of Table 2. The value on line represents K_m value.

the formation of $X(t)$ in the main pathway (dotted line in Fig. 1). Figure 5 shows the results with the basic set *a*, magnification of 1 in Table 3. The flat curve shows the time-course of $Y(t)$ with input $X_0 = 10$, whereas the waved curve shows the computed $Y(t)$ with the input $X(t) = 10 + 0.5 \sin(0.5t)$. Figure 6 shows the computed results with the basic set *b*, magnification of 1 in Table 3. Except for the rapid attainment to the steady-state and a large amplitude of the wave, the curve in Fig. 6 has basically the same features as that in Fig. 5. The computed results with various combinations of k_1 and k_2 values at the nearly same K_m value are summarized in Table 3. It is expected from these results that the model system is capable of rectifying or reversely amplifying the oscillation to some extents. Details on the change in the amplitude will be discussed later.

DISCUSSION

It was found on the model system shown in Fig. 1 that the parameter set attainable to the steady-state lay in a limited region, as already suggested by Walter and Morales (1964). This fact infers that the steady-state approximation should be carefully adopted to kinetic analysis of enzymatic reaction, even a simple enzymatic reaction is subjected to the study. Nevertheless, when the output is attainable to the steady-state, the existence of threshold response and the relationship on threshold value (equation 3) derived first by Ličko with steady-

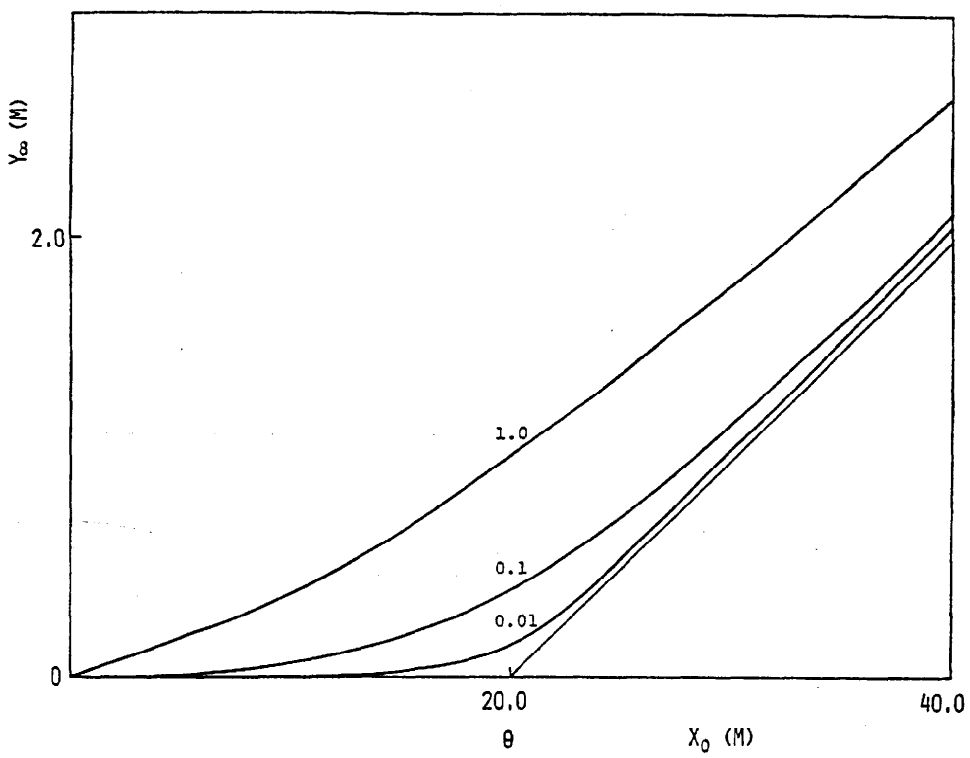

Fig. 4. Computed results with parameter sets listed in third section of Table 2. The value on line represents K_m value.

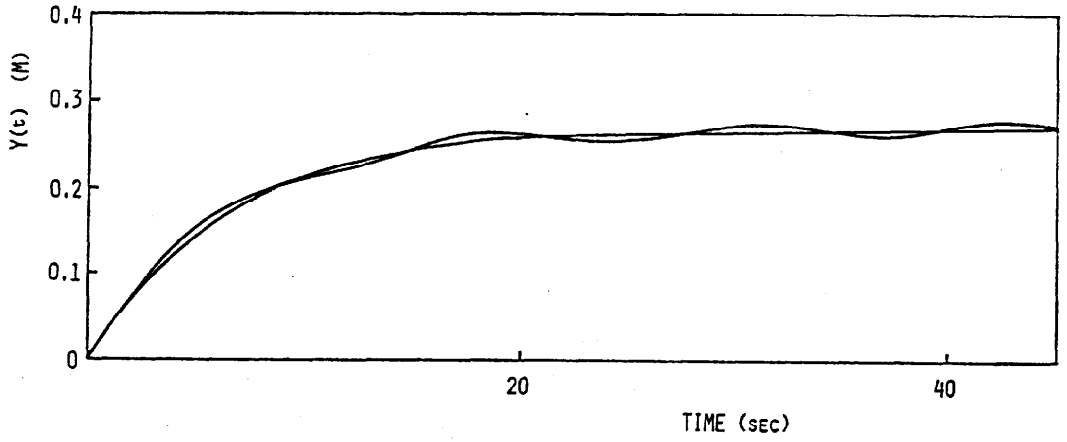

Fig. 5. Computed time-course. Parameter set *a*, magnification 1 in Table 3 was used. Flat curve, constant input; waved curve, oscillating input.

state approximation were verified by the present studies. From the relation between used parameter sets and the computed curves, following equality was deduced under the condition that the p/q takes a fixed value.

$$Y_{\theta_2, nK_m, nX_0} = nY_{\theta_1, K_m, X_0} \tag{5}$$

where $Y_{\theta, K_m X_0}$ is $Y(t)$ value at the steady-state (Y_∞) with arbitrarily defined

Table 3. Parameter set for computation and oscillatory character of output.

Basic set \ Magnification		k_1 ($M^{-1}sec^{-1}$)	k_2 (sec^{-1})	K_m (M)	Y_∞ (M)	$Y(t)$ Average amplitude (M)	$Y(t)$ Average period (sec)
a	1	40.0	3.5	0.10	0.27	0.01	ca 13
	10	400.0	35.0	0.09	0.26	0.01	〃
	15	600.0	52.5	0.09	0.26	0.01	〃
	20	800.0	70.0	0.09	0.26	0.01	〃
b	1	120.0	7.0	0.10	0.27	0.03	〃
	10	1200.0	70.0	0.06	0.22	0.03	〃
	20	2400.0	140.0	0.06	0.23	0.03	〃

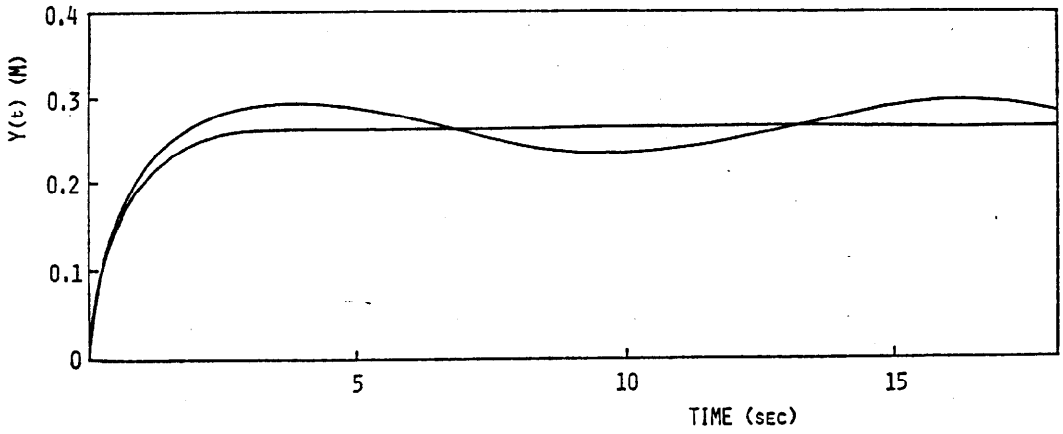

Fig. 6. Computed time-course. Parameter set *b*, magnification 1 in Table 3 was used. Flat curve, constant input; waved curve, oscillating input.

values of θ, K_m and X_0, and n represents the ratio of θ_2/θ_1. This equation may have a meaning that when the p/q value is fixed, the relationship among X_0, Y_∞ and K_m at arbitrarily chosen θ value can be estimated by standardizing the any computed line as a reference. When $\theta = 10$, $K_m = 1$ and $X_0 = 8$ were used, Y_∞ was computed to be 0.477. At $\theta = 1$, the following relation shoud be held:

$$Y_{1,\ 0.1,\ 0.8} = 0.1 \times Y_{10,\ 1,\ 8} = 0.0477$$

At $\theta = 1$, the values of K_m and X_0 which give the value of $Y_\infty = 0.0477$ must be 0.1 and 0.8, respectively. A separate computation with $\theta = 1$, $K_m = 0.1$ and $X_0 = 0.8$ gave the result of $Y_\infty = 0.0477$ in agreement with calculated value.

As described already, the input concentration of the reaction system may have an oscillating character *in vivo*. In such a case, there is no any evidence that the model system still exhibits the threshold response to the oscillating input. Furthermore, it is expected that the output would have different oscillation mode from that of input, because the model system includes a rapid exchange reaction at the formation of C component. The occurrence of beat, reduction or amplification of the wave at output would be arisen owing to the interference of the wave directly came from the input with those passed over the C component once or more. As is evident from Figs. 5 and 6, the steady-state

profile was completely maintained for the oscillating input and the angular velocity of oscillation of output was the same as that of the input. The amplitude of the output altered due to the selection of parameter sets for the computation. In the case with parameter set *a*, average concentration of 10 of the input gave the average concentration of 0.26-0.27 of the output Y_∞ (ratio: ca 0.026), while the amplitude of 0.5 of the input changes to about 0.01 at the output (ratio: 0.02). The average concentration and the amplitude of the input were decreased to nearly the same extent at the output. In the case with parameter set *b*, both ratios were found to be ca 0.022 and 0.06, indicating the amplification of the wave amplitude by the step of the formation of C component in the model system. Thus, it is interesting that the model system has a capability only to amplify the wave amplitude under the examined conditions.

In this computational study of the model system, the parameter sets were determined with assuming that $\theta = 1$, 10 and 20, according to Ličko's report. The value of each parameter included in the sets, therefore, is quite different from that observed on the real enzymatic reaction. The application of the present results to the explanation on threshold-realizing system *in vivo* should be carefully made to avoid the misunderstanding.

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