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Response of Enzymatic Feedback System to Oscillatory Input

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The response of the enzymatic feedback system to an oscillatory input was studied in order to characterize the model system with emphasizing specially the qualitative difference in response of the system from that of a linear feedback system. The frequency response-curve of the enzymatic feedback system exhibits single or double peaks and the positions of these peaks in the frequency axis shift as the feedback constant increases. The distorted wave form on the reactant concentration is observable only in the case that the input had a low frequency or low angular velocity. The stationary value of the end product changed scarcely against the ω value of the input except for the input with a certain value of angular velocity which causes a peak in the frequency response-curve. The frequency of the input is held almost invariably through the oscillations of the reactants in the system.

INTRODUCTION

It has been reported that the appearance of oscillatory behavior in consecutive enzymatic reaction-systems may not be particular but general ones, and this behavior could be fundamentally caused by feedback systems (Okamoto ***et al.,*** 1976). This suggests a solid possibility that a feedback control system may play a major role in the appearance of oscillation of an intermediate in an enzymatic reaction system ***in vivo.***

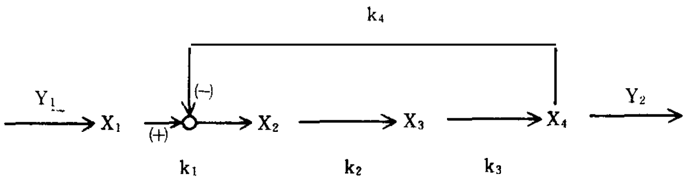
It is easily presumable that in the real enzymatic reaction systems ***in vivo,*** the input of the feedback system has not only a stationary but also an oscillating mode. The oscillating input may be introduced, when a reactant produced by the other feedback sub-systems locating in adjacent position flows in the system subject to the analysis. In such a case, it is expected that the output of the system would have a oscillatory mode different from that of the input. For example, differences would appear in a wave form, an amplitude and a frequency of the wave.

On the other hand, it has been well known that the frequency response test is one of the valuable methods for the characterization of general feedback systems. This method is concerning with the question how the amplitude ratio of the output to the input changes with the increase in an angular velocity or frequency of the sinusoidal input. Furthermore, the natural frequency of the system, which is defined as a frequency of oscillation produced by stationary input, can be calculated from this method.

The present paper deals with the frequency response of the enzymatic feedback system, using the previously reported model (Okamoto et al., 1976), and with the qualitative difference in the frequency response between the nonlinear and the linear feedback systems.

COMPUTATION

As described in the previous paper, the following reaction scheme was assumed to be a typical biochemical feedback system:



Scheme 1.

where X_i represents the reactant or the intermediate; k_1, k_2 and k_3 are the rate constants of corresponding steps. k_4 is considered to be a feedback constant. The detailed operation mode at the summing point in the feedback loop was described in the previous paper. Y_1 and Y_2 are an input and an output of the system, respectively. The value of output (Y_2) is assumed to be constant during the reaction, while the input (Y_1) is assumed to be given by the following equation :

$$Y_1 = A + B\,sin(\omega t) \tag{1}$$

where B and ω denote an amplitude and an angular velocity (defined as frequency), respectively. The rate equation of Scheme 1 in the form of the simultaneous differential equation may be written as follows:

$$\left.\begin{aligned}
\frac{dX_1}{dt} &= Y_1 - (k_1/k_4X_4)X_1 \\
\frac{dX_2}{dt} &= (k_1/k_4X_4)X_1 - k_2X_2 \\
\frac{dX_3}{dt} &= k_2X_2 - k_3X_3 \\
\frac{dX_4}{dt} &= k_3X_3 - Y_2 \\
\frac{dY_1}{dt} &= Bcos(\omega t)
\end{aligned}\right\} \tag{2}$$

The numerical solution of the simultaneous differential equation (2) was obtained either by means of MRKGM subprogram, that is slight modification of the Runge-Kutta-Gill method (Okamoto *et al.*, 1975), or by the ADSL (Analog to Digital Simulation Language) application program.

All the computations were performed by a FACOM digital computer (Model 230-75) in the Computer Center of Kyushu University.

RESULTS

Frequency response

Figure 1 shows a typical damped oscillatory pattern of the concentration of X_4 vs time under the conditions that the input (Y_1) was held at a constant value (stationary state). After about 18 min from the initiation of the reaction, the concentration of X_4 reached to a certain constant level.

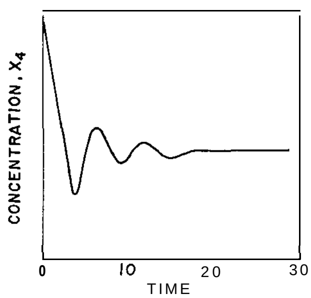


Fig. 1. Typical damped oscillation. Scheme 1 was numerically analyzed assuming that Y_1 is the constant value.

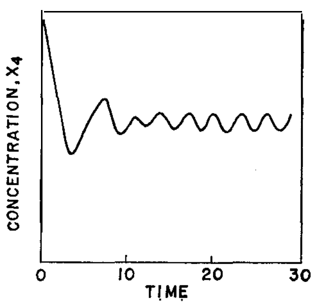


Fig. 2. Effect of a sinusoidal input. Scheme 1 was numerically analyzed assuming that $Y_1 = A + B \sin(\omega t)$.

When the input (Y_1) has a sinusoidal mode represented by equation (1), a typical sustained oscillation of X_4 was observed after 18 min as depicted in Fig. 2. It was easily presumed, therefore, that this sustained oscillation was due to the oscillating input (Y_1) as is evident from the patterns shown in Figs. 1 and 2. The amplitude ratio of the output to the input (B'/B) was obtained for various ω values of the input, where B' denotes the amplitude of the sustained oscillation of X_4.

As described before, the frequency response test is concerning with the change in the ratio (B'/B) with the increase in an angular velocity (ω) of the input under fixing A and B in equation (1) at constant levels. Generally, this test can be visualized by means of Bode diagram, in which the abscissa represents ω on a logarithmic scale and the ordinate shows the gain in dB (decibel) units. The gain, g, is defined by

$$g = 20 \log (B'/B) \qquad (3)$$

Thus, the characteristic of the frequency response of the system can be read from the profile of the Bode diagram.

Frequency response of enzymatic feedback system

The computer simulation of Scheme 1 was performed with varying the ω and k_4 values under fixing the values of other parameters, in order to observe the frequency response. On simulation, the initial concentrations were assumed as:

$X_1(0)$ = 0.821, X,(O) = 0.533, X,(O) = 0.915,
X,(O) = 0.543, Y,(O) = 0.701

The fixed values of the parameters were chosen as follows:

k_1 = **0.704,** k_2 = *0.900,* k_3 = **0.645,**
A = 0.701, B = **0.500**

Figure 3 shows the Bode diagram obtained with changing the k_4 value. The broken line indicates the frequency response of the reaction system without the feedback loop (corresponding to the case of $k_4=0$ in Scheme 1). The

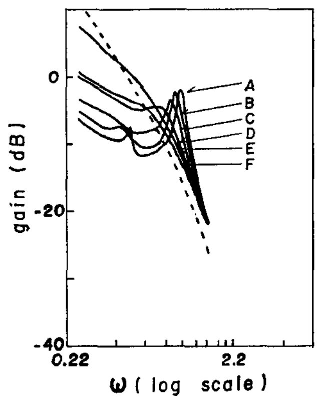


Fig. 3. The Bode diagram for Scheme 1. Curve A, $k_4=7.50$; B, $k_4=6.33$; *C,* $k_4=5.00$ *;* D, $k_4=3.30$ *;* E, $k_4=0.063$; F, $k_4=3.00$. The broken line corresponds to $k_4=0$ (no feedback loop).

characteristic features observable from Fig. 3 can be summarized as follows:

i) The shape of each curve is not simple. Each single curve has several crossing points. ii) The curves with the k_4 value larger than 3.30 exhibit a distinct peak, and the position of the peak shifts toward the right with intensifying the sharpness as the k_4 value increases. The amplitude ratio (B'/B) becomes to increase steeply as the k_4 value exceeds 4.50, as shown in Table 1. iii) The peak appears in the low ω region in Fig. 3 when the k_4 value is over 6.33, and the position of the peak also shifts as in the case of the peak in the right side. iv) In the low ω region, the height of curves can be arranged in the order of the k_4 values from top to bottom, whereas in the right side, the order is nearly inverted.

Table 1. Influence of k_4 value on the amplitude ratio (B'/B) of the peak (see Fig. 3).

k_4	ω value at peak	amplitude ratio
3.30	0.811	0.598
3.50	0.838	0.598
4.50	0.924	0.618
5.00	**0.938**	0.656
6.33	0.993	0.776
7.50	0.997	0.795

Effect of input frequency on wave form of X_4

In order to estimate the effect of the value of angular velocity of the oscillatory input on the wave form of X_4, computations were performed with changing ω value in equation (1) with fixing k_4 value at 6.33. This effect is shown in Fig. 4 with selected ω values; 0, 0.52, 1.00. With $\omega=0$ (A), the pattern of X_4 exhibited the mode of a typical damped oscillation. In the case of $\omega=1.00$ (B), for which the amplitude ratio (B'/B) took a high value (see Fig. 3), the pattern of X_4 showed the same sinusoidal wave as that in the input. With $\omega=0.52$ (C), for which the ratio (B'/B) took a low value (see Fig. 3), the pattern of X_4 showed distorted wave forms. These distorted wave forms appeared only in the case that the input has an extremely small ω value.

The period of X_4 and that of the input (Y_1) were nearly the same in every case.

Value of X_4 at steady-state

After the concentration of X_4 reached at a stationary level under the conditions that the input has a sinusoidal mode, the stationary value of X_4 is defined as the average of the values at top and bottom in the waved curve. Figure 5 shows the effect of ω and k_4 values on the stationary value of X_4. The broken line indicates the value for the system without the feedback loop. In the case, the stationary value of X_4 decreased almost exponentially with increase in the w value.

As is evident from Fig. 5, the feedback control system has a capability to

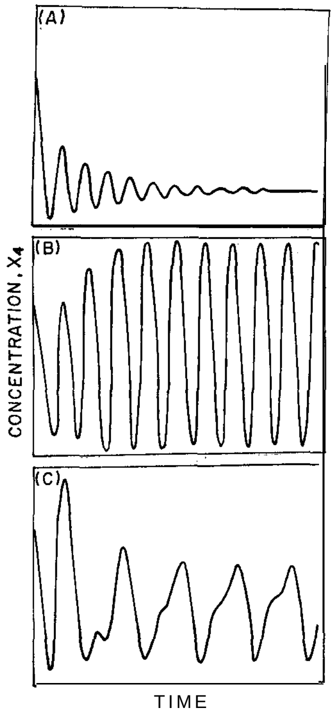


Fig. *4.* Effect of oscillatory input *on* the wave form of X_4. Equation (2) was numerically computed with changing the value of ω. (A), $\omega=0.0$; (B), $\omega=1.00$; (C), $\omega=0.52$.

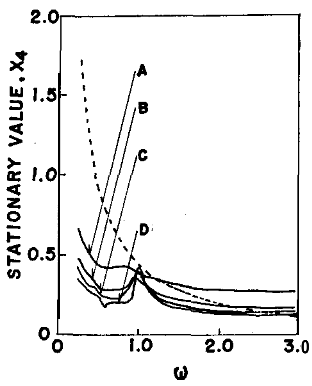


Fig. 5. Influence of ω and k_4 values on the stationary value of X_4. Curve A, $k_4=3.30$; B, $k_4=5.00$; C, $k_4=6.33$; D, $k_4=7.50$. The broken line corresponds to $k_4=0$ (no feedback loop).

keep the stationary value of X_4 unchanged against the various oscillatory inputs, except for the input with certain ω values which causes the peak on the frequency response-curve.

Thus, the results of the simulation on the response of the enzymatic feedback system to the sinusoidal input are summarized as follows:
i) The curve of the frequency response exhibited single or double peaks at the k_4 value larger than 3.30. The positions of the peaks shifted with accompanying the increment of the sharpness as the k_4 value increased. ii) In the case that the input has an extremely small ω value, the pattern of the concentration of X_4 *vs* time showed some distroted forms. In other cases, the X_4 exhibited nearly the same sinusoidal curve as that of the input. iii) The period of X_4 was equal to that of the input in every case. iv) The stationary value of X_4 was kept almost changed against a great change in w value of the input, except the input with a limited ω value.

DISCUSSION

In order to estimate the qualitative difference in the frequency response between nonlinear and linear feedback systems, the following linear feedback system (Diagram 1) is assumed to be a counterpart of the nonlinear system represented by Scheme 1,

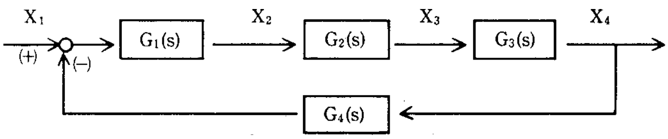


Diagram 1.

where $G_1(s)$ is the transfer function with the complex variable s and can be represented as follows by the Laplace transformation:

$$G_1(s) = k_1/s, \quad G_2(s) = k_2/s, \quad G_3(s) = k_3/s, \quad G_4(s) = k_4, \tag{4}$$

where k_i (i=1, 2, 3) is the rate constant at the step of $X_i \longrightarrow X_{i+1}$, and k_4 is the feedback constant (dimentionless).

In the linear system represented by Diagram 1, it is assumed that there is additivity between X_1 and X_4, though in chemical sense it is believed that such additivity is not realized among the concentrations of different chemical species, unless the concentration is transformed to a suitable thermodynamic quality. Thus, the following operation is adopted at summing point in Diagram 1.

$$Z = X_1 - k_4 X_4 \quad , \tag{5}$$

The over-all transfer function G(s) of the closed-loop in Diagram 1 may be written as,

$$G(s) = \frac{G_1(s)G_2(s)G_3(s)}{1+G_1(s)G_2(s)G_3(s)G_4(s)} \tag{6}$$

By substituting equation (4) to (6), equation (7) is derived.

$$G(s) = \frac{b}{s^3+a} \tag{7}$$

where $a=k_1k_2k_3k_4$, and $b=k_1k_2k_3$. Under sinusoidal steady-state conditions, s is replaced by $i\omega$. Then, the complex transfer function is,

$$G(i\omega) = \frac{b}{(i\omega)^3+a} \tag{8}$$

By deviding into the real and imaginary parts,

$$G(i\omega) = \frac{ab}{\omega^6+a^2} + i\frac{b\omega^3}{\omega^6+a^2} \tag{9}$$

is obtained. The distance of $G(i\omega)$ in the complex s-plane is,

$$|G(i\omega)| = \frac{b}{\sqrt{\omega^6+a^2}} \tag{10}$$

The gain (g) is defined by,

$$g = 20\,log|G(i\omega)| \tag{11}$$

Thus, the gain is represented by,

$$g = 20\,log\left|\frac{k_1k_2k_3}{\sqrt{\omega^6+(k_1k_2k_3k_4)^2}}\right| \tag{12}$$

Figure 6 shows the Bode diagram for Diagram 1 calculated by equation (12), with changing the k_4 value under the conditions of $k_1=0.704, k_2=0.900, k_3=0.645$. The broken line represents the frequency response of the cascade system without the feedback loop. The curves in the frequency response exhibited quite different patterns from those shown in Fig. 3. It is clear from the figure that the curves can be arranged in the order of the k_4 value from top to bottom, and that the gain (g) reached at each constant level in the region of low ω value.

It is well known that the curve in the frequency response of a linear second order system has one peak as shown in Fig. 7. However, the position of the peak does not shift with changing the value of **k.** In contrast to above, in the nonlinear system, it was observed that the position of the peak moved with change of the k_4 value as shown in Fig. 3.

As mentioned above, in a linear feedback system, the angular velocity of the sinusoidal input is held invariant through the reaction steps; the angular velocity on the concentration of each intermediate or the product is the same as that of the input. The real enzymatic feebdack system must be especially nonlinear, because the simple additive mechanism can not be realized at the summing point due to, in general, the difference of the input from the feedback input in their chemical species. As described already, it was observed

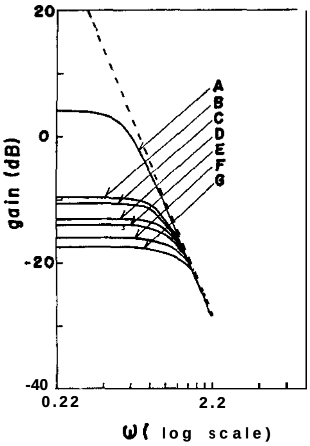


Fig. 6. The Bode diagram for Diagram 1. Equation (12) was computed with changing the value of k_4. Curve A, $k_4=7.50$; B, $k_4=6.33$; C, $k_4=5.00$; D, $k_4=4.50$; E, $k_4=3.30$; F, $k_4=3.00$; G, $k_4=0.633$. The broken line corresponds to $k_4=0$ (no feedback loop).

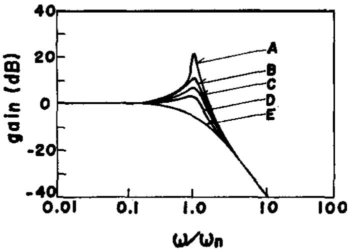


Fig. 1. The typical Bode diagram of a linear second order system. Equation, $G(s) = \omega_n / s^2 + 2k\omega_n s + \omega_n^2$ was calculated. Curve A, $k=0.05$; B, $k=0.10$; C, $k=0.20$; D, $k=0.50$; E, $k=1.0$.

that the angular velocity on an intermediate or the product changed slightly from that of the input, indicating clear similarity of a nonlinear enzymatic system to a linear system. The gain-angular velocity relationship (plotted by the Bode diagram) of an enzymatic system, however, showed a characteristic profile originated from its distinct nonlinearity.

It has been reported that the oscillation on the concentration of some intermediates in glycolysis pathway exhibited the proper frequency according to the species of the source; for instance, the periods on NADH concentration in glycolysis are about 37 sec for intact yeast cell and 4 min for heart muscle extract (Higgins, 1967). Furthermore, it was observed that the frequency of oscillation on the concentration of some intermediates in glycolysis changed considerably from that of the oscillating input (Boiteux et al., 1975). Evidently, a nonlinear enzymatic feedback system is thought to have a complex molecular mechanism by which the frequency of the input can be altered. Furthermore, it is very likely that the external perturbation with oscillating mode may play a critical role for the alteration of frequency mode of enzymatic system. At the present step, however, the molecular mechanism on frequency-alteration has not been clarified. This problem should be elucidated in a near future.

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