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Frequency Conversion Mechanism in Enzymatic Feedback Systems

MASAHIRO OKAMOTO† AND KATSUYA HAYASHI

Department of Agricultural Chemistry, Faculty of Agriculture, Kyushu University 46-02, Fukuoka 812, Japan

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The frequency conversion mechanism in enzymatic feedback systems which can keep the system's natural frequency against oscillating perturbations has been investigated with computer simulations. The results indicate that the feedback system, including time-delay element, has the property that the resulting period of the sustained oscillation is an integral multiple of the system's natural period related to frequency entrainment by relaxation oscillators, and the interaction of this system and oscillating inputs could be represented by the theoretical equation proposed for the synchronization of two interacting oscillating systems.

1. Introduction

Many investigators have experimentally proved that oscillatory chemical reactions exist in the homeostatic regulation systems. Various oscillatory behaviors are observable in living organisms, such as secretion of hormones, heart beat and circadian rhythms, which may prove to be caused by chemical events (Higgins, 1967; Chance *et al.*, 1973). Focused on the oscillations in metabolic pathways, most of the models propose that oscillations arising from enzymatic regulation refer to a negative feedback system by the end product of the chain (Morales & McKay, 1967; Walter, 1969*a*,*b*, 1970).

On the other hand, it has been reported that the oscillation of the concentration of each intermediate in a glycolysis pathway exhibits its "natural" frequency; for instance, the period of the oscillation of NADH absorbance in glycolysis is about 37 sec for intact yeast cell (Higgins, 1967) and 4 min for muscle cell. What mechanism can keep its natural frequency against perturbations introduced to the system? This problem may lead to an understanding of the frequency conversion mechanism; the natural

† Address all correspondence to Dr. M. Okamoto, Department of Microbiology and Immunology, 6730 Medical Science Building II, The University of Michigan, Medical School, Ann Arbor, Michigan 48109, U.S.A.

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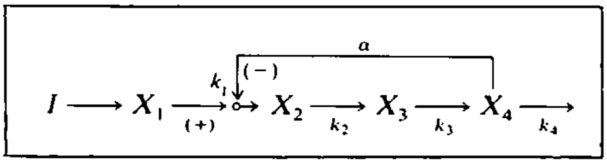
frequency of the system is maintained against oscillatory perturbations of various frequencies. Thus, this mechnism is very important in the metabolic control in order for the system to synchronize the frequencies of interacting systems and to keep the natural frequency constant.

Marek & Stuchl (1975) first observed the synchronization experimentally in two interacting oscillatory systems using the Belousov reaction (Noyes, Field & Körös, 1972); oxidation of malonic acid by bromate in sulphuric acid with ceric/cerous ions as catalyst. In enzymatic reactions, Boiteux, Goldbeter & Hess (1975) revealed the relation of the frequency of phosphofructokinase and that of glucose input in glycolysis. By injecting a periodic rate of glucose to the phosphofructokinase reaction system they investigated the time-courses of NADH absorbance. In their study, they clarified the domains of entrainment of NADH absorbance by the fundamental frequency, 1/2-harmonic and 1/3-harmonic of a sinusoidal source of glucose. However, a model of the frequency conversion mechanism which maintains the natural frequency against oscillatory inputs with various frequencies has not yet been proposed. This paper deals with the exploration of the enzymatic control system which allows a frequency conversion mechanism.

2. Model

Since the feedback system has been recognized to be the universal control mode causing the enzymatic oscillations, we will analyze the feedback system as an oscillator. Consider the following consecutive reactions (Scheme 1, each assumed to include an irreversible step) where k_i is the rate constant of the corresponding step. Assume that I is exogenous substrate supply which is maintained constant and n-molecules of the end product X_4 inhibits co-operatively the reaction step of $X_1 \rightarrow X_2$ (end product inhibition). The α represents the magnitude or gain of the feedback loop and is assumed to be constant.



SCHEME 1

It has been speculated that the feedback control would be realized by the co-operative behavior of the allosteric enzyme as a feedback control element. Morales & McKay (1967) and Walter (1970) introduced the following simplified equation for the operation at summing point in feedback control system,

$$Z = k_1/(1+\alpha(X_4(t))^n). \tag{1}$$

It is generally expected that the inhibitor molecule (X_4 in Scheme 1) must be moved to the position of the regulatory enzyme (summing point) by forces such as diffusion or active transport. Thus, we considered this time-consuming process causing time-delay in the feedback system. In this study, we assumed the following mechanism for the operation at summing point in Scheme 1 with reference to equation (1):

$$Z' = k_1/(1+\alpha(X_4(t-\tau))^n) \tag{2}$$

where τ and n denote the time-delay and the number of allosteric effectors per allosteric enzyme molecule (Morales & McKay, 1967) or Hill coefficient (Higgins *et al.*, 1973), respectively. The mathematical model of Scheme 1, therefore, may be written as follows:

$$\begin{aligned} \dot{X}_1 &= I - Z'X_1 \\ \dot{X}_2 &= Z'X_1 - k_2X_2 \\ \dot{X}_3 &= k_2X_2 - k_3X_3 \\ \dot{X}_4 &= k_3X_3 - k_4X_4. \end{aligned} \tag{3}$$

During $0 < t < \tau$, the concentration of $X_4(t-\tau)$ was assumed to be 0.

3. Frequency Conversion Mechanism

In general, the frequency conversion mechanism can be represented by Fig. 1. In Fig. 1(a), T_0 is the natural period ($1/f$ where f is the natural frequency) or autonomous period (Boiteux, Goldbeter & Hess, 1975). Figure

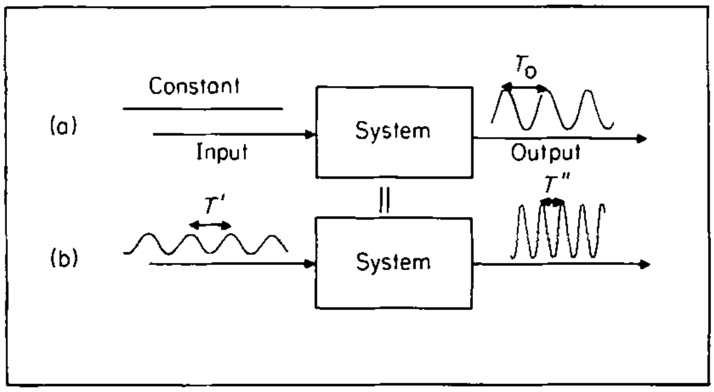


FIG. 1. Frequency conversion mechanism. (a) T_0, natural period of the system. (b) Frequency conversion mechanism when $T' \neq T''$.

1(b) shows the interaction of the system (oscillatory system) with periodic source of substrate. If $T'' \neq T'$, it can be thought that this system has the frequency conversion mechanism; however, in this study, we explored the system which can basically convert the input period T' to the system's natural period T_0. Therefore, in the following section, we will deal with the frequency conversion mechanism which is represented by $T'' = T_0$ (or $= nT_0$) for the variation of T'.

4. Synchronization Effect

Assume the oscillating mode of input (I) in Scheme 1 as

$$I(t) = 10{\cdot}5 + 10{\cdot}0 \sin (2\pi/T)t \tag{4}$$

where T indicates the period of oscillating input.

First, the possibility of frequency conversion for Scheme 1 with no time-delay ($\tau = 0$ in equation (2)) was examined. The results are shown in Fig. 2, a system essentially with no oscillatory property. When the sinusoidal

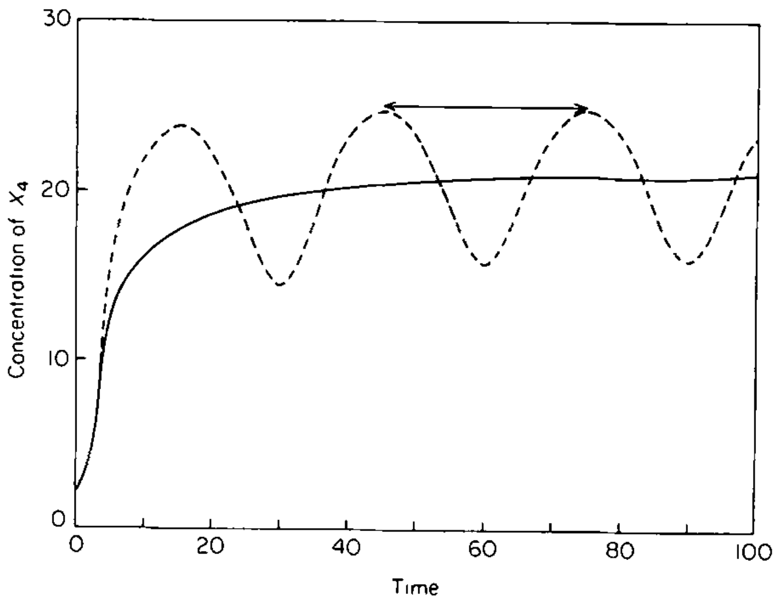


FIG. 2. Time-course of $X_4(t)$ in Scheme 1 without time-delay ($\tau = 0$). ——, $I = 10{\cdot}5$; – – –, $I = 10{\cdot}5 + 10{\cdot}0 \sin (2\pi/30{\cdot}0)t$. The period of $X_4(t)$ is 30·0. Rate parameters: $\alpha = 0{\cdot}0005$, $n = 3$ (equation (2)), $k_1 = k_2 = k_3 = 1{\cdot}0$, $k_4 = 0{\cdot}5$. Initial concentrations: $X_1 = X_2 = X_3 = 1{\cdot}0$, $X_4 = 2{\cdot}0$.

input ($T = 30{\cdot}0$ in equation (4)) was introduced to the system, $X_4(t)$ showed a sustained oscillation with the period of 30·0 which is the same as the period of the oscillating input. Thus, the system does not have the possibility of converting the frequency of the input.

Next, fixing the time-delay (τ) at 4·0, the time-courses of $X_4(t)$ were calculated under conditions of the constant input (I) ($I = 0{\cdot}5, 10{\cdot}5, 20{\cdot}5$).

As shown in Fig. 3, $X_4(t)$ exhibited sustained oscillation with a period of 13·0 for $I = 10{\cdot}5$ and 14·0 for $I = 20{\cdot}5$. We did not observe sustained oscillation of $X_4(t)$ for $I = 0{\cdot}5$. Thus, Scheme 1 with $\tau = 4{\cdot}0$ has natural period (T_0) between 13·0 and 14·0 according to the conditions specified above. The oscillatory patterns of $X_4(t)$ were investigated under the assumption that the oscillatory input (equation (4)) was introduced. As described already, if this system provides the mechanism of frequency conversion,

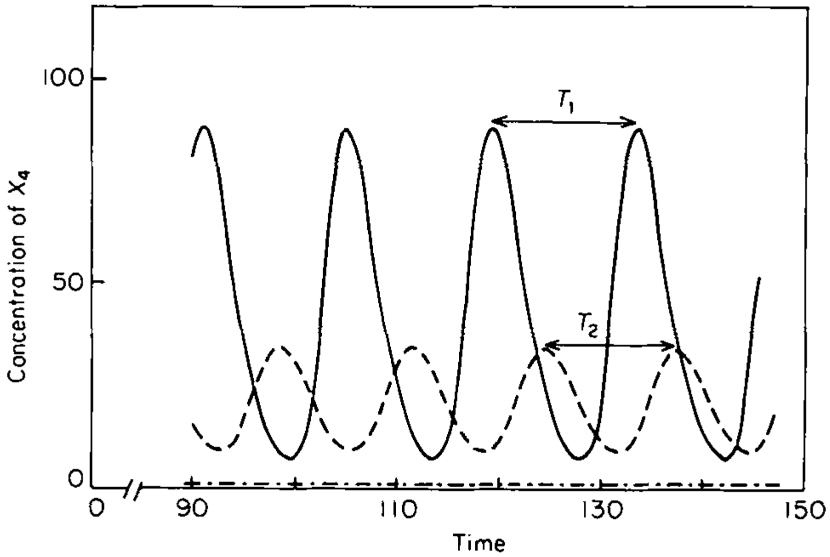


FIG. 3. Time-course of $X_4(t)$ in Scheme 1 with $\tau = 4{\cdot}0$. ——, $I = 20{\cdot}5$; – – –, $I = 10{\cdot}5$; — · — · —, $I = 0{\cdot}5$. The periods, T_1 and T_2, are 14·0 and 13·0, respectively. The rate parameters and the initial concentrations are the same as in Fig. 2.

the period of $X_4(t)$ must be kept near 13 against oscillatory inputs with the various periods because the maximum value of $I(t)$ is 20·5, the minimum value 0·5 and the average value 10·5. The relations of the period of the oscillatory input, T', and the resulting period of $X_4(t)$, T'', are shown in Table 1 and Fig. 4. It was revealed that the resulting period T'' may be represented by,

$$T'' = nT_0 \tag{5}$$

where T_0 denotes the natural period of Scheme 1 and n is a coefficient. Figure 4 shows the oscillation of $X_4(t)$ in with the period of the oscillatory input $T' = 39{\cdot}0$, which is three-times as large as T_0. When the oscillatory input with the period of nT_0 was introduced to the system, double periodicity, a superimposed period of T' and T_0, was observed.

How is the property that the period of the sustained oscillation is an integral multiple of the system's natural period related to frequency entrainment by relaxation oscillators? Why does the coefficient (n in equation (5))

TABLE 1

Relation between the period of oscillating input (T') and that of $X_4(t)$ (T'') in Scheme 1

T'	T''	T'	T''
0 ($I=0\cdot5$)	0	26·0	13·0×2
0 ($I=10\cdot5$)	13·0	29·0	13·0×11
0 ($I=20\cdot5$)	14·0	33·0	13·0×5
5·0	13·0	36·0	13·0×11
6·5	13·0	39·0	13·0×3
13·0	13·0	42·0	13·0×13
16·0	13·0×6	46·0	13·0×7
20·0	13·0×3	49·0	13·0×11
23·0	13·0×7	52·0	13·0×4

change in accordance with T'-value? Ruelle (1973) has pointed out the mathematical relation of interaction of two oscillating systems; suppose that the periods of two oscillators are $T'$and T_0, respectively. If T'/T_0 is irrational, a periodic solution of the system will not result (no synchronization). However, the resulting period after interaction of two oscillatory systems is close to integral multiples mT' and nT_0, and therefore, n/m is a rational fraction close to T'/T_0. His result may be interpreted as a synchronization effect between two interacting oscillating systems. Taking into acount his result, we checked up the relation between T'/T_0 and n/m based on Table 1 and the results are summarized in Table 2. The T'/T_0 value in each case completely corresponds to its n/m value. These theoretical

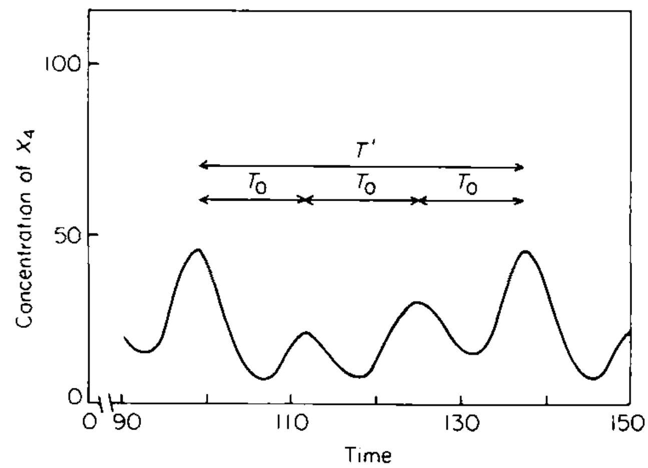


FIG. 4. Time-course of $X_4(t)$ in Scheme 1 with $\tau=4\cdot0$. The period of the oscillating input (T in equation (4)) is 39·0. T' and T_0 are 39·0, 13·0, respectively. Double periodicity was observed.

TABLE 2

Synchronization effect of Scheme 1 and oscillating input

T'	T_0	T'/T_0	T''	m†	n†	n/m
5·0	13·0	0·38	13·0	2·60	1·00	0·38
6·5	13·0	0·50	13·0	2·00	1·00	0·50
13·0	13·0	1·00	13·0	1·00	1·00	1·00
16·0	13·0	1·23	78·0	4·88	6·00	1·23
20·0	13·0	1·54	39·0	1·95	3·00	1·54
23·0	13·0	1·77	91·0	3·96	7·00	1·77
26·0	13·0	2·00	26·0	1·00	2·00	2·00
29·0	13·0	2·23	143·0	4·93	11·0	2·23
33·0	13·0	2·54	65·0	1·97	5·00	2·54
36·0	13·0	2·77	143·0	3·97	11·0	2·77
39·0	13·0	3·00	39·0	1·00	3·00	3·00
42·0	13·0	3·23	169·0	4·02	13·0	3·23
46·0	13·0	3·54	91·0	1·98	7·00	3·54
49·0	13·0	3·77	143·0	2·92	11·0	3·77
52·0	13·0	4·00	52·0	1·00	4·00	4·00

† $T'' = mT' = nT_0$

analyses also mean that Scheme 1 with time-delay is basically the mechanism to synchronize the exogenous oscillatory substrate supply with various periods to its natural period.

5. Discussion

We adopted the model of end product inhibition (Scheme 1) as one of the enzymatic oscillators based on the following two assumptions: first, that there exists a time-delay until the inhibitor molecule (X_4) moves to the regulatory enzyme by some physical forces; and secondly, that the activity of regulatory enzyme changes in accordance with the decreasing sigmoidal function of X_4 describing the effect of inhibition. We assumed, therefore, equation (2) for the operation at summing point in Scheme 1 based on the most widely used form for operating function (equation (1)). The plot of Z' (or Z) vs the concentration of X_4 (inhibitor) used in this study is shown in Fig. 5. We adopted the values of all rate parameters in arbitrary, however, in most of the cases, the concentration of $X_4(t)$ (or $X_4(t-\tau)$) oscillated sustainedly between the values of 5·0 and 45·0. As shown in Fig. 5, apparently, the regulatory enzyme whose activity is Z' is not saturated by inhibitor molecules. This shows that there is no problems on our parameter setting.

The feedback systems have closely related to their oscillatory behaviors because the feedback loop pathway is considered to be a time-retardation realizing device. It has also recognized that enzymatic feedback systems play an important role of constant-value control (homeostatic control, in a wide sense) of metabolisms. In our previous papers (Okamoto, Aso & Hayashi, 1977, 1978; Okamoto *et al.*, 1980), we have clarified that feedback systems causing oscillatory behaviors lack the ability of constant-value control. We expected, therefore, the oscillatory feedback systems play another role in metabolisms.

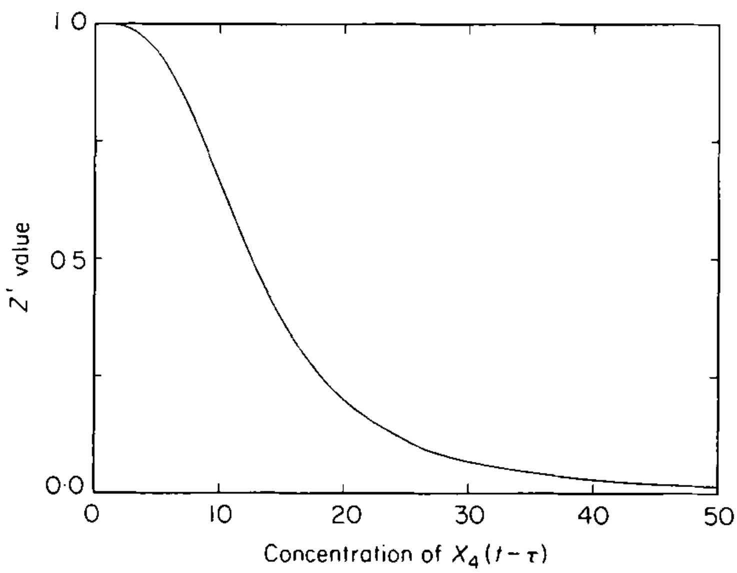


FIG. 5. Sigmoidal activity of Z' value in equation (2). $k_1 = 1{\cdot}0$, $\alpha = 0{\cdot}0005$, $n = 3$.

In the present study, we analyzed the frequency conversion mechanism as one of the possible roles. The results indicate that: Scheme 1 could cause the sustained oscillation due to the time-delay element (τ-value); this oscillatory system had the frequency conversion mechanism; the system keeps the period of end product at the natural period against oscillatory inputs with the various periods; the interaction of this oscillatory system and oscillating input could be represented by the mathematical equation that Ruelle (1973) has proposed on the synchronization of two oscillating systems.

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