

Sufficient Conditions for Coordination of a Nonlinear Biochemical System under External Forcing

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One of the fundamental properties of biochemical networks in cells is the preservation of viability under conditions of stress and environmental perturbation. This requires that the system maintains coordination in the sense that a stable (time-dependent) state can form under such conditions. Although this property emerges from the characteristics of the constitutive enzymes, the general conditions that must be satisfied by a network to maintain a viable state are not known. The essential features of enzymatic reactions are saturation kinetics and a nonlinear dependency of reaction rate on metabolite concentration. For a simple branched system we derive sufficient conditions on these features that, if satisfied, imply that the resulting metabolic processes remain coordinated when the system is exposed to external perturbations of any type, amplitude, or frequency. Upon removal of the perturbation, the unperturbed state will be recovered. When the system does not satisfy the sufficient conditions, it is shown that coordination depends on the nature of the perturbing signals. Furthermore, once coordination is lost, it cannot be retrieved by removal of the perturbing force and therefore the system cannot settle to any finite state. These results are supported by numerical analysis using three types of external signals: namely, rectangular, sinusoidal, and noisy signals.

1. Introduction

If the components of a biochemical system are coordinated such that the runaway accumulation of metabolites is prevented, then the system can produce a stable (time-dependent) state and therefore be capable of sustaining metabolism. In contrast to chemical systems, this is by no means guaranteed by the nonlinear, saturating nature of enzyme-catalyzed biochemical reactions¹ and so only a restricted class of systems may lead to coordinated states. All biochemical systems are subject to varying environmental conditions, and these produce fluctuations in metabolite flux. These fluctuations can profoundly change the state of nonlinear biochemical systems^{2,3} and result in the breakdown of coordination.^{1,4} In order for metabolic processes to persist when exposed to different stress conditions and external fluctuations, the latter should be avoided. However, the relationship between the system and the environment parameters that maintain coordination are not known, and the following two important issues need to be addressed. The first is to obtain sufficient conditions under which biochemical systems maintain coordination, independently of the forms of external perturbing signals. The second is to understand how these conditions are maintained when the systems are modulated.

The essential components of biochemical systems are enzyme-catalyzed reactions. In general, enzyme-catalyzed reactions have two main characteristics: enzyme saturation and a nonlinear dependence of reaction rates on species concentrations. At saturation of an enzyme, the reaction rate catalyzed by the enzyme reaches a maximum, and further increase of substrate concentration cannot increase the reaction rate. These features are captured in Michaelis–Menten type of reaction kinetics, and they are universal for enzymatic reactions due to the

catalyzing mechanism of enzymes. The kinetics of enzymatic reactions is highly nonlinear in character, due mainly to the regulations of enzymes, and as a consequence are capable of generating complex temporal and spatial structures.^{5,6} Recently, we have shown^{1,4,7} that for biochemical systems with bifurcations the interactions of the above two characteristics may have consequences for system coordination. This work studies a general system comprising a branched network with an input and three enzyme-catalyzed reactions and will analytically determine the sufficient conditions for system coordination under any external forcing. We will show that, if the sufficient conditions are satisfied, the resulting metabolic processes remain coordinated when the system is exposed to external perturbations of any type, amplitude, or frequency. Upon removal of the perturbation, the unperturbed state will be recovered. When the system does not satisfy the sufficient conditions, it is shown that coordination depends on the nature of the perturbing signals. Furthermore, once coordination is lost, it cannot be retrieved by removal of the perturbing force and therefore the system cannot settle to any finite state. These results are supported by numerical analysis using three types of external signals: namely, rectangular, sinusoidal, and noisy signals.

2. Model System

The following reaction scheme is employed to study the sufficient conditions for coordination of nonlinear biochemical systems under external forcing.

The system, Figure 1, features the conversion of S into P and a branched sink, respectively. The positive feedback of P to E_1 is the source producing oscillations in the system. It is assumed that E_1 obeys the concerted transition model of Monod et al.⁸ and E_2 and E_3 follow the simple Michaelis–Menten kinetics. Therefore, the governing equations can be written in

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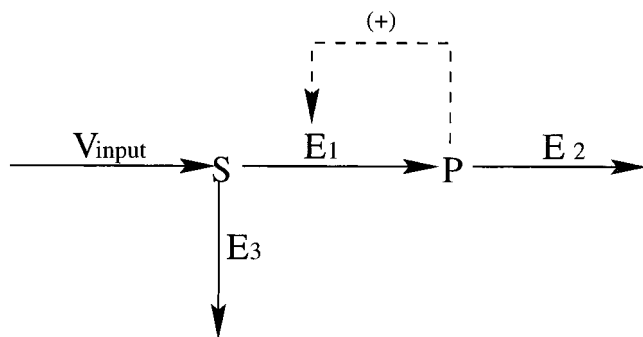


Figure 1. Reaction scheme. The system comprises two species, S and P, and three enzymes, E₁, E₂, and E₃. S is converted into P with a positive feedback and into a branched sink.

the following form

$$\frac{d[S]}{dt} = V_{\text{input}} - V_{E_1} - V_{E_3} \quad (1)$$

$$\frac{d[P]}{dt} = V_{E_1} - V_{E_2} \quad (2)$$

$$V_{E_1} = \frac{V_{E_1}^{\text{max}} [S] \left(1.0 + \frac{[S]}{k_S}\right) \left(1.0 + \frac{[P]}{k_P}\right)^2}{L + \left(1.0 + \frac{[S]}{k_S}\right)^2 \left(1.0 + \frac{[P]}{k_P}\right)^2} \quad (3)$$

$$V_{E_2} = \frac{V_{E_2}^{\text{max}} [P]}{k_{m2} + [P]} \quad (4)$$

$$V_{E_3} = \frac{V_{E_3}^{\text{max}} [S]}{k_{m3} + [S]} \quad (5)$$

Here k_S and k_P are the dissociation constants of S and P for E₁, respectively. k_{m2} and k_{m3} are the Michaelis constants of E₂ and E₃. L is the allosteric constant of E₁. $V_{E_1}^{\text{max}}$, $V_{E_2}^{\text{max}}$, and $V_{E_3}^{\text{max}}$ are the maximum activities of E₁, E₂, and E₃, respectively.

Under changing environments, the effects of different types of external forcing on the system are assumed to be realized by the following equation:

$$V_{\text{input}} = V_0(1 + \theta) \quad (6)$$

where θ is the forcing term, which is bounded between -1 and 1 to guarantee $V_{\text{input}} \geq 0$. θ can take any form as long as it satisfies $-1 \leq \theta \leq 1$. V_0 is a constant. The forcing of external signals can be superimposed on other parameters and concentrations in a similar way.

3. Results

(a) Sufficient Conditions of System Coordination under External Forcing. System coordination requires that the total (average) flux into each species pool must be equal to its total (average) output flux.^{1,4,7} For the governing mass-balance eqs 1 and 2, these coordination conditions are described by

$$\langle V_{\text{input}} \rangle = \langle V_{E_1} \rangle + \langle V_{E_3} \rangle \quad (7)$$

$$\langle V_{E_1} \rangle = \langle V_{E_2} \rangle \quad (8)$$

where the symbol $\langle \rangle$ denotes the time average. If V_{E_1} , V_{E_2} , and

V_{E_3} are not restricted, eqs 7 and 8 can always be satisfied. However, for enzyme-catalyzed reactions, V_{E_1} , V_{E_2} , and V_{E_3} are bounded by the upper limits $V_{E_1}^{\text{max}}$, $V_{E_2}^{\text{max}}$, $V_{E_3}^{\text{max}}$, respectively, the validity of eqs 7 and 8 may depend on the relations between the time-dependent concentrations, $[S]$ and $[P]$,¹ and the emergence of dynamical structures may have consequences in system coordination.^{1,4} In particular, since external forcing may change the dynamical state of the system, it may affect system coordination. Figure 2 illustrates how coordination is maintained by the system in a stable steady-state subjected to a periodic forcing in V_{input} .

If the perturbed system is to remain coordinated, the runaway accumulation of both $[S]$ and $[P]$ must be prevented. The condition that ensures the value of $[S]$ is bounded by the dynamics can be readily obtained. Mathematically, provided $V_{E_1}^{\text{max}} + V_{E_3}^{\text{max}} > \langle V_{\text{input}} \rangle$, $[S]$ cannot increase indefinitely. This condition can be deduced directly from eqs 1, 3, and 5, since it guarantees that for any $[P]$, $\int d[S] < 0$ when $[S] \rightarrow \infty$ and where the limits of the integration are such that the value of $\langle V_{\text{input}} \rangle$ converges. Therefore, irrespective of the product concentration, $[P]$, this guarantees the substrate concentration, $[S]$, remains finite. In Figure 2b, this guarantees that areas 1 and 3 are compensated by areas 2 and 4, respectively. The condition that the value of $[P]$ is bounded by the dynamics is more complicated. In Figure 2c, V_{E_1} is persistently bigger than V_{E_2} for areas 1 and 3. Therefore, during these stages, $[P]$ persistently accumulates. This in turn stimulates further increases in V_{E_1} through positive feedback (eq 3). Since V_{E_2} approximately remains unchanged for areas 1 and 3, the runaway accumulation of $[P]$ and concomitant loss of coordination can only be prevented if the value of $[S]$ declines sufficiently rapidly to compensate for the $[P]$ -stimulated increase in V_{E_1} . Once $[S]$ is thus reduced, the value of V_{E_3} will also be reduced at a rate that depends on the value of $[S]$, $V_{E_3}^{\text{max}}$, and k_{m3} . Conservation of average flux in eq 7 ensures that this will result in an increase of V_{E_1} that will favor the accumulation of $[P]$ again. Therefore in order to maintain coordination, there must be an interplay between V_{E_1} , V_{E_2} , and V_{E_3} such that areas 1 and 3 are equal to areas 2 and 4 in Figure 2c, respectively. However, if $[P]$ increases to a sufficiently large value in area 1 or 3, such a compensation may not be possible. Once this happens, V_{E_1} remains persistently larger than V_{E_2} , and $[P]$ increases indefinitely. Therefore, the destruction of coordination condition eq 8 is characterized by $[P] \rightarrow \infty$.¹ When $[P] \rightarrow \infty$, $V_{E_2} \rightarrow V_{E_2}^{\text{max}}$, and $V_{E_1} \rightarrow V_{E_1}^{\text{max}} [S] / (k_S + [S]) = (V_{E_1})_{\text{limit}}$. Mathematically, as long as $V_{E_2}^{\text{max}} > \langle (V_{E_1})_{\text{limit}} \rangle$, eqs 2, 3, and 4 guarantee $\int d[P] < 0$ when $[P] \rightarrow \infty$ and where the limits of the integration are such that the value of $\langle (V_{E_1})_{\text{limit}} \rangle$ converges. Under these conditions, the value of $[P]$ remains bounded for any value of $[S]$.

Finally we have the sufficient conditions that guarantee the system remains coordinated under any external forcing as

$$V_{E_1}^{\text{max}} + V_{E_3}^{\text{max}} > \langle V_{\text{input}} \rangle \quad (9)$$

$$V_{E_2}^{\text{max}} > \langle (V_{E_1})_{\text{limit}} \rangle = \frac{\int_{\alpha}^{\beta} \frac{V_{E_1}^{\text{max}} [S]}{k_S + [S]} dt}{\beta - \alpha} \quad (10)$$

In eq 10, $[S]$ is determined by the following differential equation:

$$\frac{d[S]}{dt} = V_{\text{input}} - \frac{V_{E_1}^{\text{max}} [S]}{k_S + [S]} - \frac{V_{E_3}^{\text{max}} [S]}{k_{m3} + [S]} \quad (11)$$

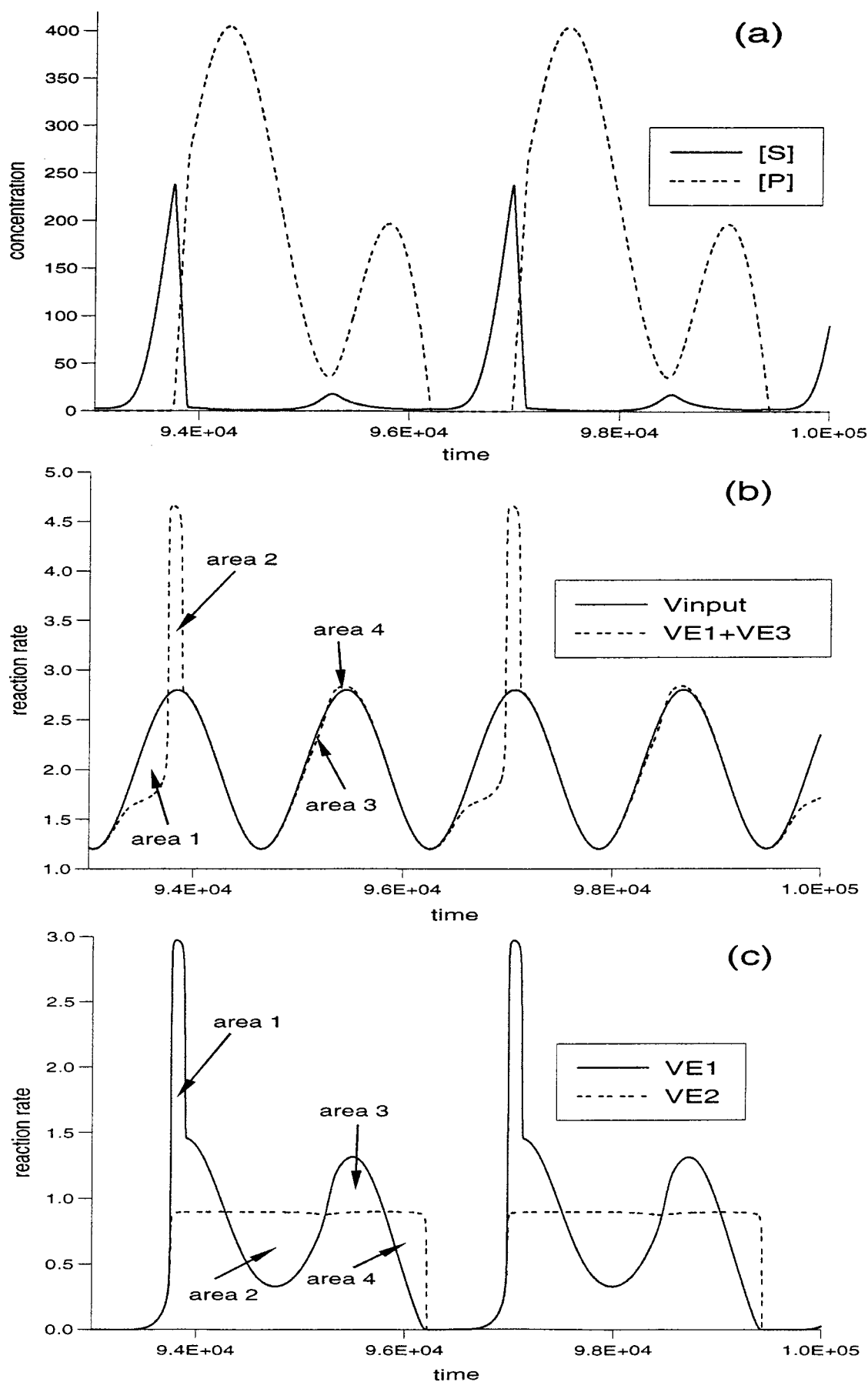


Figure 2. Explanation of system coordination for a stable steady-state subject to a sinusoidal signal ($V_{input} = V_0(1 + \epsilon \sin(2\pi t/T))$ with $T = 1611$ s and $\epsilon = 0.40$. $V_{E_3}^{max} = 0.9$ and other parameters are included in Table 1). (a) Dependence of $[S]$ and $[P]$ on time; (b) the maintenance of coordination condition eq 7; and (c) the maintenance of coordination condition eq 8. Initial condition: $[S] = 1.0$, $[P] = 1.0$.

for any time-dependent V_{input} , and where α and β are the lower and upper limits of the integration which depend on temporal behavior of $[S]$. If $[S]$ changes periodically, the integration can be made over an exact period. If $[S]$ is aperiodic or noisy, the system should be integrated over a sufficiently long time, to allow $\langle(V_{E_1})_{\text{limit}}\rangle$ to converge. In order to illustrate the dependence of system coordination on kinetic parameters, a special case, namely, $k_S = k_{m3}$, is analyzed in detail. For $k_S = k_{m3}$, the relation $\langle(V_{E_1})_{\text{limit}}\rangle/V_{E_3} = V_{E_1}^{\text{max}}/V_{E_3}^{\text{max}}$ is always valid for any type of external forcing. For this case, eq 10 is simplified into

$$V_{E_2}^{\text{max}} > \frac{V_0(1 + \langle\theta\rangle)V_{E_1}^{\text{max}}}{V_{E_1}^{\text{max}} + V_{E_3}^{\text{max}}} \quad (12)$$

It is clear that the sufficient condition for system coordination requires that the input rate and the maximum activities of all enzymes involved in the network be related. In addition, since V_{E_1} , V_{E_2} , and V_{E_3} have upper limits $V_{E_1}^{\text{max}}$, $V_{E_2}^{\text{max}}$, and $V_{E_3}^{\text{max}}$, respectively, the necessary condition for the system to have any convergent state is

$$\langle V_{\text{input}} \rangle = V_0(1 + \langle\theta\rangle) < V_{E_1}^{\text{max}} + V_{E_3}^{\text{max}} \quad (13)$$

for $i = 1, 2$.

It is clear that the condition eq 9 is both sufficient and necessary for coordination of the system. If external signals are superimposed on kinetic parameters and/or species concentrations, eq 10 can be simplified into different forms. For example, in addition to the superimposition of external signals on input rate, if external signals are also superimposed on $V_{E_1}^{\text{max}}$ in the form of $V_{E_1}^{\text{max}} = (V_{E_1}^{\text{max}})_0(1 + \theta_1)$, eq 10 becomes

$$V_{E_2}^{\text{max}} > \frac{V_0(1 + \langle\theta\rangle)(V_{E_1}^{\text{max}})_0(1 + \langle\theta_1\rangle)}{(V_{E_1}^{\text{max}})_0(1 + \langle\theta_1\rangle) + V_{E_3}^{\text{max}}} \quad (14)$$

However, sufficient condition for system coordination under the forcing of any external signals can always be determined by eqs 9 and 10.

For many types of external forcing such as sinusoidal or uniformly distributed noise, their average values are zero. For this case, eq 12 or 14 is further simplified into a relationship which only includes kinetic parameters, by setting $\theta = 0$ and $\theta_1 = 0$. Figure 3 summarizes the dependence of system coordination conditions on kinetic parameters for any type of external signals with zero average values. When eq 13 is satisfied, in terms of Figure 3 and eq 12, to maintain system coordination under changing environments, increasing either V_0 or $V_{E_1}^{\text{max}}$ needs to increase $V_{E_2}^{\text{max}}$, and increasing $V_{E_3}^{\text{max}}$ may reduce $V_{E_2}^{\text{max}}$. When $V_{E_1}^{\text{max}}$ increases to infinity, $V_{E_2}^{\text{max}}$ should increase to V_0 . When $V_{E_3}^{\text{max}}$ increases to infinity, $V_{E_2}^{\text{max}}$ may reduce to zero.

For external signals with nonzero average value, the sufficient conditions may also depend on the average of external signals, eqs 12 or 14. When sufficient conditions are satisfied, the system is always coordinated when it is subjected to any type of external forcing. When the sufficient conditions are not satisfied, the coordination of the system depends on the values of kinetic parameters and the amplitudes and periods of the external signals. This will be demonstrated in Section (b).

(b) Numerical Analysis. When the system is subjected to different types of external forcing, it has different responses. In what follows, by employing numerical analysis, we investigate the coordination of the system when it is subjected to

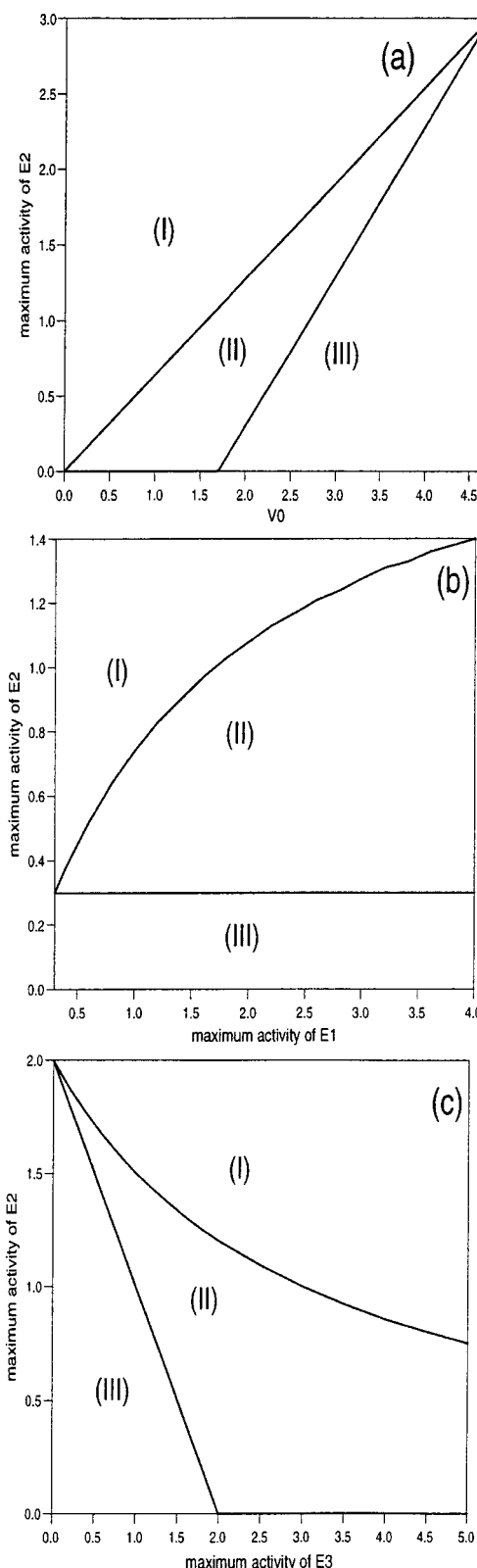


Figure 3. Dependence of system coordination on kinetic parameters (other parameters are included in Table 1.) (a) V_0 vs $V_{E_2}^{\text{max}}$, (b) $V_{E_1}^{\text{max}}$ vs $V_{E_2}^{\text{max}}$, and (c) $V_{E_3}^{\text{max}}$ vs $V_{E_2}^{\text{max}}$. In (a), (b), and (c), region (I): system coordination is always maintained for any type, amplitude, or frequency of external signals; region (II): the maintenance of system coordination depends on the type, period, and amplitude of external signals (see Figures 4 and 5); and region (III): system coordination is always destroyed for any external signals. All regions for $V_0 > 4.7$ in Figure 3a and $V_{E_1}^{\text{max}} < 0.3$ in Figure 3b belong to region (III).

TABLE 1: Parameters Corresponding to the Reaction Scheme in Figure 1

parameter	value
V_0	2.0
$V_{E_1}^{\max}$	3.0
k_S	1.0
k_{m2}	1.0
L	1.0×10^6
k_P	1.0
$V_{E_3}^{\max}$	1.7
k_{m3}	1.0

three types of external signals – namely, rectangular, sinusoidal, and noisy signals.

A rectangular signal is a pulsing signal with pulsing length, T , and pulsing amplitude, ϵ . This signal can be described by eq 6, with ϵ taking two values ϵ and $-\epsilon$ ($\epsilon \leq 1$) in turn. The pulsing length, T , is the same for both $\theta = \epsilon$ and $\theta = -\epsilon$. Thus, $\langle \theta \rangle = 0$, and therefore $\langle V_{\text{input}} \rangle = V_0$.

A sinusoidal signal can be described by setting $\theta = \epsilon \sin(2\pi t/T)$ in eq 6. Here ϵ (≤ 1) is forcing amplitude, and T is forcing period. It is clear that, for sinusoidal signals, $\langle \theta \rangle = 0$, and therefore $\langle V_{\text{input}} \rangle = V_0$. Similarly, quasi-periodic signals can also be generated.

A noisy signal can be realized by setting $\theta = \epsilon \xi(T)$ in eq 6. Here, ϵ (≤ 1) is referred to noise amplitude; $\xi(T)$ is equally distributed random numbers between -1 and 1 ; and T is the duration of the pulse of a single noise event. In this work, noise pulse is also set to occur every T s, which is always equal to the duration time. An equally distributed $\xi(T)$ will guarantee $V_{\text{input}} \leq 0$ in eq 6 for $\epsilon \leq 1$. For this type of noise signal, $\langle \theta \rangle = 0$ and $\langle V_{\text{input}} \rangle = V_0$ is also held. For the sake of simplicity, in what follows, T and ϵ for three types of signals are referred to as period and amplitude of the signals. Their physical significances for three different types of signals are described as above.

For the unforced system with $V_{\text{input}} = 2.0$ and other parameters shown in Table 1, the system has a bifurcation at $V_{E_2}^{\max} = 0.856$, below which oscillations emerge. In terms of the sufficient condition of the system (eqs 9 and 12), the coordination of the system is guaranteed under any type of external signals with zero average if $V_{E_2}^{\max} \geq 1.277$. The necessary condition requires that $V_{E_i}^{\max} > 0.3$ for $i = 1, 2$. For three types of signals (rectangular, sinusoidal, and uniformly-distributed noisy signals), it has been found that when the sufficient conditions for system coordination (eqs 9 and 12) are satisfied ($V_{E_2}^{\max} \geq 1.277$), the external signals with any periods and amplitudes cannot destroy system coordination. For this case, although changes in the type, the period, or the amplitude of external signals may generate different dynamical patterns, eqs 7 and 8 are always guaranteed. Subsequently, for any periods and amplitudes of external signals, both $[S]$ and $[P]$ are always able to settle onto a stable (time-dependent) state after transients die out. When external signals are removed, the system recovers the original (unperturbed) steady state. It is clear that the condition $V_{E_2}^{\max} \geq 1.277$ is sufficient for maintaining system coordination under the forcing of external signals. However, when $V_{E_2}^{\max} < 1.277$, maintenance of system coordination depends on the value of $V_{E_2}^{\max}$, period, and amplitude of the external perturbation as shown in Figures 4 and 5 for applied rectangular and sinusoidal signals, respectively.

Trends for noisy signals have also been examined (data not shown) and are qualitatively similar to those in Figures 4 and 5. For $V_{E_2}^{\max} < 1.277$, the following conclusions can be drawn. The system can process perturbations across a broad range in frequency provided they have small amplitudes, but large

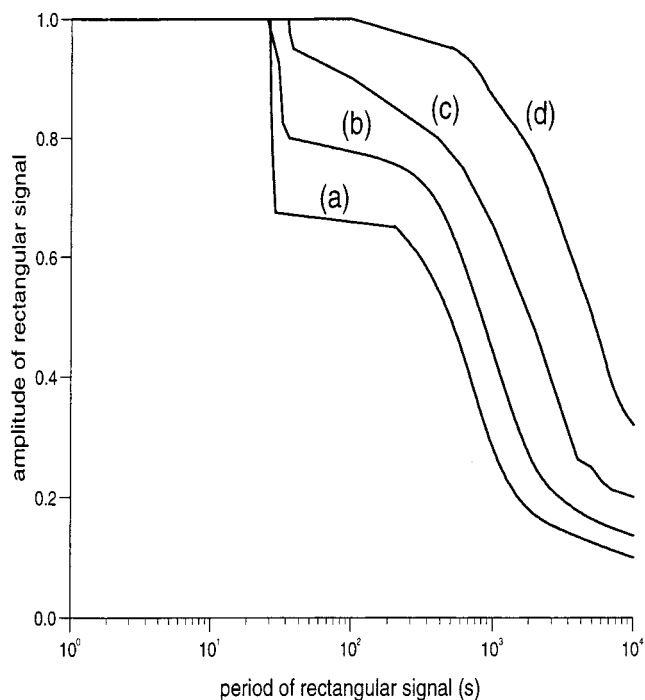


Figure 4. For rectangular signals, dependence of system coordination on $V_{E_2}^{\max}$, period (T), and amplitude of the signals (ϵ). $V_{E_2}^{\max} =$ (a) 0.9, (b) 1.0, (c) 1.1, (d) 1.2. System coordination is maintained in the area below each curve, and it is destroyed in the area above each curve for $\epsilon \leq 1$. For $V_{E_2}^{\max} > 1.277$, system coordination is always maintained. Initial condition: $[S] = 1.0$, $[P] = 1.0$.

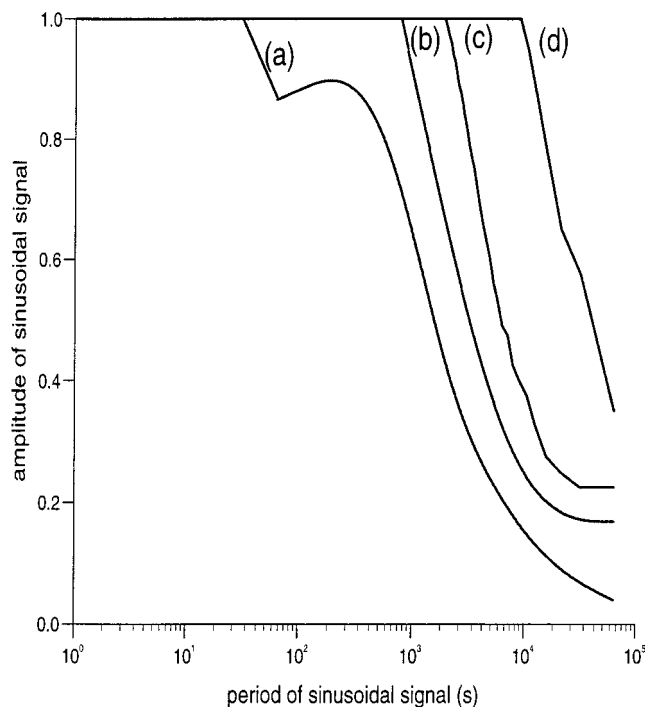


Figure 5. For sinusoidal signals, dependence of system coordination on $V_{E_2}^{\max}$, period (T), and amplitude of the signals (ϵ). $V_{E_2}^{\max} =$ (a) 0.9, (b) 1.0, (c) 1.1, (d) 1.2. System coordination is maintained in the area below each curve, and it is destroyed in the area above each curve for $\epsilon \leq 1$. For $V_{E_2}^{\max} > 1.277$, system coordination is always maintained. Initial condition: $[S] = 1.0$, $[P] = 1.0$.

amplitude perturbations may destroy system coordination. Similarly, the system can withstand large amplitude perturbations provided the frequency is high (period is short), however large amplitude perturbations of long duration (i.e., long period

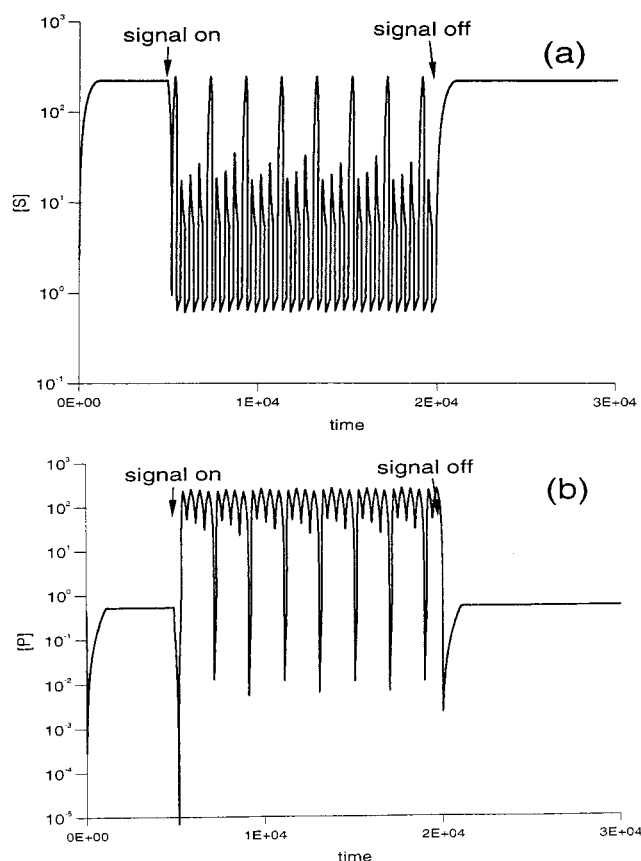


Figure 6. For a rectangular signal with $T = 246$ s and $\epsilon = 0.6$, and at $V_{E_2}^{\max} = 0.9$, system coordination is maintained. (a) and (b) indicate the system returns to the original steady state after the signal is removed. Initial condition: $[S] = 1.0$, $[P] = 1.0$.

or low frequency) will destroy system coordination. Using rectangular signals as an example, Figures 6 and 7 summarize the evolution of the system for a signal with two different periods (246 or 276 s) and the same amplitude ($\epsilon = 0.6$). For a period of perturbation $T = 246$ s, when the rectangular signal is switched on, the system develops to a stable period-4 state (Figure 6a,b). After the signal is switched off, the system returns to the original steady state (Figure 6a,b). Numerical analysis reveals that coordination conditions (eqs 7 and 8) are maintained when the signal is on and after it is removed. For a period of perturbation $T = 276$ s, after the signal is switched on, $[P]$ persistently accumulates and it cannot converge to any finite value (Figure 7b), and $[S]$ tends to develop to the state determined by eq 11 (Figure 7a). Therefore, the system cannot develop to any stable state on the phase plane of $[S]$ and $[P]$. After the signal is removed, $[P]$ continues to accumulate infinitely (Figure 7b) and $[S]$ develops to a steady state with a value of 0.741 (Figure 7a). Numerical calculation indicates coordination condition eq 7 is maintained, but eq 8 is destroyed when the signal is switched on and after it is removed. Therefore, when the system is subjected to such a rectangular signal, it cannot function properly, since $[P]$ will accumulate to infinite values. Importantly, after the signal is removed, system coordination cannot be recovered. Therefore, once external signals cause coordination destruction, the original state is not retrievable, even when external signals are removed, (Figure 7a,b). The other types of signals have similar effects on system coordination.

Using three types of external signals, numerical analysis confirms that eq 12 is sufficient for maintaining system coordination when the signals are superimposed on the input

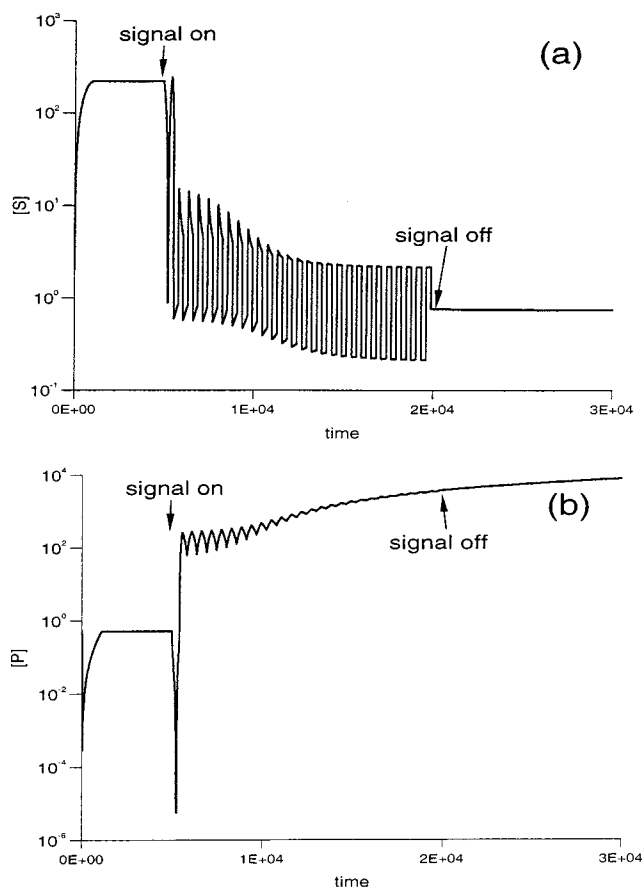


Figure 7. For a rectangular signal with $T = 276$ s and $\epsilon = 0.6$, and at $V_{E_2}^{\max} = 0.9$, system coordination is destroyed. (a) and (b) indicate that, after the signal is removed, $[S]$ converges a limit value (0.741) and $[P]$ continues to accumulate infinitely. Initial condition: $[S] = 1.0$, $[P] = 1.0$.

in the form of eq 6. When eq 12 is not satisfied, the qualitative trend for the effects of the three types of external signals on system coordination is the same. Quantitatively, system coordination depends on the type, the period, and the amplitude of an external signal. It should be emphasized that the changes of average reaction rates are discontinuous from a coordinated condition to an uncoordinated condition. For example, when coordination is maintained, $\langle V_{E_1} \rangle$ is much smaller than $V_{E_2}^{\max}$. Once coordination is lost, $\langle V_{E_1} \rangle$ jumps to a value bigger than $V_{E_2}^{\max}$.

4. Discussion

Using a simple biochemical system with one input and three enzyme-catalyzed reactions, this work reveals that there are sufficient conditions for maintaining system coordination under the forcing of external signals. For a special case, it is explicitly shown that the sufficient conditions require specific relationships of kinetic parameters. When the sufficient conditions are satisfied, the system maintains system coordination under any external forcing. Therefore, when the system is subjected to an external signal, it responds to the signal and its dynamical behavior changes accordingly. When the signal is removed, the system recovers the unperturbed state. However, when the sufficient condition is not satisfied, external signals may or may not destroy system coordination, depending on the type, the amplitude, and the period of the signals. If system coordination is destroyed by external signals, one of species concentrations (here $[P]$) cannot converge to any finite value, and therefore

the system cannot function properly. Once this happens, removal of external signals cannot recover the unperturbed state—destruction of system coordination is a permanent feature once it happens.

As shown in eqs 10 and 11, the sufficient condition for system coordination depends not only on kinetic parameters, but also on the nature of external perturbations. In nature, the evolution of any biochemical system takes place in the context of fluctuating and changing environments. It would be interesting to consider whether this challenge has been met by satisfying the sufficient conditions or by ensuring survival against reasonable extremes. For example, in eq 12, constraints on the kinetic parameters for any specific θ can be determined. System coordination will be guaranteed if eq 12 is satisfied for the largest possible value of θ . The resilience of the system to environmental perturbation will be compromised if the parameters differ from these. Clearly factors in addition to resilience will be important for functioning including the energetic efficiency of the metabolic processes, a factor that is itself parameter dependent.^{9–11}

It is currently possible to modulate enzymatic systems through molecular genetics. One of the main aims of genetic engineering is to increase the intracellular concentrations or secretion of valuable compounds. This work shows that when a biochemical system is modulated, a further question to be addressed is how to guarantee the resilience of the modified systems to intermittent changes in the environment. Any modulations which destroy the validity of sufficient conditions (eqs 9 and 10) would risk the destruction of system coordination.

In large-scale biochemical systems, there are various types of feed-back and feed-forward. The positive regulation of products, as employed in reaction scheme studied here, has been

widely considered to be one of the sources of generating time-dependent behavior.⁶ Other types of regulations such as inhibitions of substrates may also generate time-dependent behavior.¹² It would be interesting to consider how biochemical systems are coordinated when different types of regulations operate in concert. Moreover it would be important to study how restrictive the conditions become as the scale of the biochemical network increases. One possibility is that the conditions become less restrictive as the network complexity increases, but we do not know at present exactly what features of the network size and topology are important in influencing the scaling behavior of the conditions for system coordination.

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