ELSEVIER

Biophysical Chemistry 120 (2006) 207–214

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Dissipation and maintenance of stable states in an enzymatic system: Analysis and simulation

Junli Liu *

Computational Biology Programme, Scottish Crop Research Institute, Dundee, DD2 5DA, UK

Received 23 November 2005; received in revised form 29 November 2005; accepted 29 November 2005
Available online 27 December 2005

Abstract

The constraint-based analysis has emerged as a useful tool for analysis of biochemical networks. An essential assumption for constraint-based analysis is the formation of a stable steady state. This work investigates dissipation and maintenance of stable states in a simple reversible enzymatic reaction with substrate inhibition. Under mass-action kinetics, the conditions under which the reaction maintains a stable steady state are analytically derived and numerically confirmed. It is shown that, in order to maintain a steady state in the regulated reaction, maximal enzyme activity must be much higher than input rate. Moreover, it is revealed that requirements for large enzyme activity are due to substrate inhibition. It is suggested that high activities of enzymes may play a vital role in protecting a stable state from its catastrophic collapse, giving an additional explanation to an intriguing problem—why the activities of some enzymes greatly exceed the flux capacity of a pathway. In addition, dissipation of the enzymatic reaction is analysed. It is shown that the collapse of stable states is always associated with a point at which dissipation is the highest. Therefore, in order to maintain a stable state, dissipation of the reaction must be less than a critical value. Moreover, although external forcing may not change net mass flow, it may lead to collapse of stable states. Furthermore, when stable states collapse at a critical forcing amplitude and period, dissipation also reaches a highest value. It is concluded that collapse of stable steady state in the enzyme system with substrate inhibition always corresponds to critical points at which dissipation is highest, regardless if the reaction is forced or not. Therefore, for the substrate inhibited reaction, maintenance of stable states is intrinsically related to level of dissipation.

Keywords: Constraint-based analysis; Stable steady states; Dissipation; Excess of enzyme activities; Forced reactions; Kinetic constraints

1. Introduction

In a living system, a metabolic network comprises many complicatedly connected reactions, exchanges material and energy with its surrounding, and dissipates energy. Therefore, a metabolic network is an integrative and open system, its study needs to define a boundary at which materials are transported into or out of the network [1]. By analysing a network and its boundary fluxes as a whole, constraint-based approach has emerged as a useful tool for analysis of the integrated functions of the network [2–10]. Broadly speaking, constraint-based approach analyses the possible flux distributions under the constraints of stoichiometry, thermodynamics and kinetics, and links them with possible phenotypic outcomes. Those constraints restrict different aspects of the network. Specifically, stoichiometric constraints restrict the molar relation of reactants; thermodynamic constraints confine the direction of reactions [1,11–15]; and kinetic constraints guarantee the formation of stable states that are necessary for implementing biological functions [16,17]. In a network, each enzymatic reaction is an essential component for constructing the network, and it also has a boundary at which substrate is supplied and product is removed. In general, an enzyme-catalysed reaction is embedded in a pathway, taking product molecules of the preceding reaction step and supplying substrate for the subsequent step [18]. Enzyme molecules bind with substrate molecules to form an intermediate complex. Subsequently, the intermediate complex releases product molecules and the enzyme becomes available for catalysis again. Finally, the freed enzyme molecules bind substrate molecules again, and the process repeats. Enzyme catalysis can be inhibited or activated by compounds which are themselves reaction products, and the consequent network of

* Tel.: +44 1382 568500; fax: +44 1382 562426.
E-mail address: jliu@scri.sari.ac.uk.

0301-4622/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2005.11.011

feedback and feedforward reactions are the basis of biological functioning in cells.

If an enzyme-catalysed reaction cannot establish a stable state when it is with substrate input and product removal, the network comprising the same reaction cannot reach a stable state. In this sense, a single enzyme-catalysed reaction is an important prototype for understanding the formation of stable states in a network. This work examines three aspects about maintenance of stable states in an enzyme reaction system with substrate inhibition.

Firstly, based on mass-action kinetics, the conditions under which the reaction maintains steady states are analytically derived and numerically confirmed. It is shown that, in order to maintain a steady state in the regulated reaction, maximal enzyme activity must be much higher than input rate. Moreover, it is revealed that requirements for high enzyme activity are due to substrate inhibition. It is suggested that, when enzymes are regulated, high activities of enzymes may play a vital role in protecting a stable state from its catastrophic collapse, giving an additional explanation to an intriguing problem—why the activities of some enzymes greatly exceed the flux capacity of a pathway [19,20].

Secondly, in terms of the Second Law of Thermodynamics, any reaction with non-zero flux dissipates energy. An enzymatic reaction comprises a number of elementary reactions. The total dissipation of those elementary reactions represents the thermodynamic dissipation of the enzyme reaction. Furthermore, dissipation is closely associated with the concept of thermodynamic constraints [1,11–15]. Is the maintenance of a stable state related to other properties of the system? For example, is the collapse of a stable state related to dissipation? We will examine the relation between dissipation and maintenance of stable states.

Thirdly, when an enzyme system settles onto a stable state, how does external forcing affect the state? What is the relation between dissipation and the changes induced by external forcing? We examine the effects of forcing period and amplitude on the maintenance of stable states, and further investigate the relation between dissipation and maintenance of stable states.

2. Dissipation and maintenance of steady states: a reversible enzymatic reaction with substrate inhibition as an example

The simplest enzyme kinetic model involving substrate inhibition is the Michaelis–Menten formalism modified by adding a binding process between substrate-enzyme complex and free substrate [21,22]. When we consider that this reaction is embedded in a pathway, substrate is supplied by a preceding reaction, and product is removed by a subsequent reaction. Therefore, the reaction system is described as

$$\begin{aligned}
&\xrightarrow{V_{\text{in}}} S\\
E + S &\underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{ES}\\
\text{ES} &\underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E + P\\
\text{ES} + S &\underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} \text{ES}_2\\
P &\xrightarrow{k_P}
\end{aligned}$$

The governing mass-balance equation of this reaction system is

$$\begin{aligned}
\frac{\mathrm{d}[S]}{\mathrm{d}t} &= V_{\text{in}} - k_1[E][S] + k_{-1}[\text{ES}] - k_3[\text{ES}][S] + k_{-3}[\text{ES}_2]\\
\frac{\mathrm{d}[E]}{\mathrm{d}t} &= -k_1[E][S] + k_{-1}[\text{ES}] + k_2[\text{ES}] - k_{-2}[E][P]\\
\frac{\mathrm{d}[\text{ES}_2]}{\mathrm{d}t} &= k_3[\text{ES}][S] - k_{-3}[\text{ES}_2]\\
\frac{\mathrm{d}[P]}{\mathrm{d}t} &= k_2[\text{ES}] - k_{-2}[E][P] - k_P[P]
\end{aligned} \tag{1}$$

Here total enzyme concentration, E_0, is conserved, i.e. $[E]_0 = [E] + [\text{ES}] + [\text{ES}_2]$. Therefore, $[\text{ES}] = [E]_0 - [E] - [\text{ES}_2]$. V_{in} is the input rate of substrate, S. Initially, it is assumed that the external pool from which S is supplied is buffered, and therefore V_{in} is a constant. Product P is transported out of the system with a first-order reaction, whose rate constant is k_P.

The steady state of Eq. (1) can be obtained by setting its right hand to be zero. At a steady state, after applying the conventional notation of Michaelis–Menten formalism for kinetic parameters, we have

$$\begin{aligned}
&V_{\text{in}} - \frac{V_{\max}([S] - K_1K_2[P])}{K_m + \left(1 + \dfrac{[S]}{K_3}\right)([S] + K_4[P])} = 0\\
&\frac{V_{\max}([S] - K_1K_2[P])}{K_m + \left(1 + \dfrac{[S]}{K_3}\right)([S] + K_4[P])} - k_P[P] = 0
\end{aligned} \tag{2}$$

where $K_1 = \frac{k_{-1}}{k_1}, K_2 = \frac{k_{-2}}{k_2}, K_3 = \frac{k_{-3}}{k_3}, K_4 = \frac{k_{-2}}{k_1}, K_m = \frac{k_{-1}+k_2}{k_1}$, and $V_{\max} = k_2E_0$.

Therefore, at a steady state,

$$\begin{aligned}
[P] &= \frac{V_{\text{in}}}{k_P}\\
[S] &= \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}
\end{aligned} \tag{3}$$

with

$$\begin{aligned}
a &= \frac{V_{\text{in}}}{K_3}\\
b &= \left(1 + \frac{K_4[P]}{K_3}\right)V_{\text{in}} - V_{\max}\\
c &= V_{\text{in}}(K_m + K_4[P]) + K_1K_2V_{\max}[P]
\end{aligned} \tag{4}$$

In addition,

$$\begin{aligned}
k_2[E] &= \frac{k_mV_{\max}}{K_m + ([S] + K_4[P])\left(1 + \dfrac{[S]}{K_3}\right)}\\
k_2[\text{ES}_2] &= \frac{K_3V_{\max}([S] + K_4[P])[S]}{K_m + ([S] + K_4[P])\left(1 + \dfrac{[S]}{K_3}\right)}\\
[\text{ES}] &= E_0 - [E] - [\text{ES}_2]
\end{aligned} \tag{5}$$

Analysis of Eqs. (2)–(4) reveals that a steady state for which all concentrations are non-negative and finite only exists in a certain range of values of parameters, implying that kinetic

constraints [16,17] restrict the formation of a steady state. Specifically, if [S] at a steady state is non-negative and finite, concentrations of all other species are non-negative and finite. Therefore, we examine the conditions for maintaining non-negative and finite] [S].

In terms of Eqs. (3) and (4), since a and c are always non-negative, there are two possibilities. If b is positive, there is no positive solution for [S], since $-b\pm\sqrt{b^2-4ac}<0$. If $b<0$, non-negative and finite solutions for [S] may exist only if $b^2-4ac>0$. Therefore, Eqs. (6) and (7) are the steady-state conditions for [S]

$$b<0 \tag{6}$$

$$b^2-4ac>0 \tag{7}$$

Eq. (6) leads to

$$V_{\max}>\left(1+\frac{K_4[P]}{K_3}\right)V_{\text{in}}. \tag{8}$$

Eq. (7) gives rise to

$$V_{\max}>\frac{-\beta+\sqrt{\beta^2-4\gamma}}{2}, \tag{9}$$

with

$$\begin{aligned}\beta &= -2\left(1+\frac{K_4[P]}{K_3}\right)V_{\text{in}}-\frac{4K_1K_2[P]}{K_3}V_{\text{in}}\\ \gamma &= \left(1+\frac{K_4[P]}{K_3}\right)^2V_{\text{in}}^2-\frac{4}{K_3}(K_m+K_4[P])V_{\text{in}}^2\end{aligned}. \tag{10}$$

Eqs. (9) and (10) lead to

$$\begin{aligned}V_{\max}>&\left(1+\frac{K_4[P]}{K_3}\right)V_{\text{in}}+\frac{2K_1K_2[P]}{K_3}V_{\text{in}}\\ &+2V_{\text{in}}\sqrt{\frac{K_1K_2[P]}{K_3}\left(1+\frac{K_4[P]}{K_3}+\frac{K_1K_2[P]}{K_3}\right)+\frac{(K_m+K_4[P])}{K_3}}.\end{aligned} \tag{11}$$

Since the first term in Eq. (11) is the same as the right hand of Eq. (8) and the last two terms are positive in Eq. (11), it is clear that, as long as Eq. (11) is valid, Eq. (8) is also valid. Therefore, Eq. (11) is the condition under which the system establishes a non-negative and finite steady state. Once a steady state exists, it has been shown that the state does not lose its local stability [22].

Eq. (11) can be employed to comprehensively analyse the effects of kinetic parameters on the formation of steady state. It can be seen that increasing V_{in} requires the increase in $V_{\max}$. Similarly, increasing any of K_1, K_2, K_3, and K_4 also requires the increase in $V_{\max}$. However, increasing K_3 corresponds to the decrease in $V_{\max}$.

Numerical analysis confirms that Eq. (11) is the condition for maintaining a steady state in the system when values of the kinetic parameters change. For example, if all rates constants are unity (i.e., $K_1=K_2=K_3=K_4=1$, $K_m=2$.), Eq. (11) becomes Eq. (12).

$$V_{\max}>V_{\text{in}}\left\{(1+3V_{\text{in}})+2\sqrt{2(V_{\text{in}}^2+V_{\text{in}}+1)}\right\}. \tag{12}$$

Fig. 1 shows an example of how Eq. (12) affects the evolution of the reaction system. In terms of Eq. (12), when $V_{\text{in}}=2.0$, $V_{\max}>28.97$. When $V_{\max}=29.0>28.97$, the system develops to a steady state for substrate S and product P (Fig. 1a). Simultaneously, E, ES and ES_2 reaches a steady state as well (data not shown). However, when $V_{\max}=28.90<28.97$, the system cannot establish a steady state. Substrate S increases infinitely and product P infinitely approaches zero (Fig. 1b). Moreover, E and ES approaches zero, and ES_2 approaches E_0 (data not shown). Consequently, the catalysing cycle in the system breaks down and the system cannot implement biological functions. In a similar manner, based on Eq. (11), effects of all kinetic parameters on formation of steady states can be numerically analysed. For example, if $K_3=0.5$ ($K_1=K_2=K_4=1$, $K_m=2$), Eq. (11) gives $V_{\max}>52.533$ for $V_{\text{in}}=2.0$, numerical calculation confirms that if $V_{\max}=52.54>52.533$, the system develops to a steady state. If $V_{\max}=52.52<52.533$, no steady states exist.

Eqs. (11) and (12) show that maintenance of a steady state requires a highly nonlinear relationship between the input flux and the maximal enzyme activity. If the reaction is not

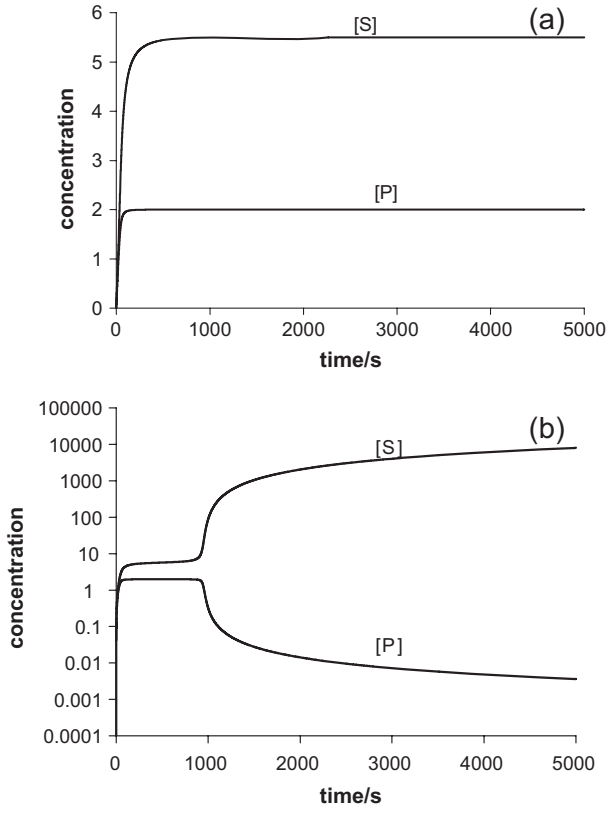

Fig. 1. Effects of Eq. (12) on the formation of steady states. $V_{\text{in}}=2.0$. (a) $V_{\max}=29.0$, a steady state is established. (b) $V_{\max}=28.90$, no steady states exist. In (b), the y-axis is in logarithmic scale.

inhibited by the substrate S, as long as $V_{max} > V_{in}$, the system establishes a steady state. Therefore, the nonlinearity of Eqs. (11) and (12) is the consequence of substrate inhibition.

The above analysis clearly demonstrates that the maintenance of a steady state in the reaction system is subject to the constraints of Eq. (11). If Eq. (11) is invalid, the catalysing cycle of the reaction breaks down, the reaction cannot perform biological functions. However, is the maintenance of a steady state related to other properties of the system? For example, is the collapse of a steady state related to dissipation? Because energy efficiency is usually considered to be a vital aspect of biological functions [23–26], we further investigate if maintenance of a steady state is related to dissipation of the system.

When a steady state is established in the reaction system, the general form of dissipation of the reaction system can be deduced from the reaction scheme, which is described by Eq. (13).

$$\mathrm{hd} = -V_{in}\mathrm{RT}\left(-\ln\frac{k_1k_2}{k_{-1}k_{-2}} + \ln\frac{[P]}{[S]}\right) \tag{13}$$

where hd is dissipation, $[S]$ and $[P]$ are the steady-state concentration of substrate and product, respectively. R is the gas constant, and T is the absolute temperature. In an isothermal system, RT is a constant. In the following, we use Eq. (14) to describe dissipation.

$$D = \frac{\mathrm{hd}}{\mathrm{RT}} = -V_{in}\left(-\ln\frac{k_1k_2}{k_{-1}k_{-2}} + \ln\frac{[P]}{[S]}\right) \tag{14}$$

where D is dissipation in the unit of RT.

Based on Eqs. (3), (4) and (14), dissipation at a steady-state can be calculated. Although effects of rate constants can be fully examined based on Eq. (14), for the simplicity we set all rate constants to be unity and examine the dependence of dissipation on maximal enzyme activity and input rate. Fig. 2 summarises the results.

For a fixed V_{in}, dissipation increases monotonically with the decrease of V_{max} (Fig. 2a). When V_{max} decreases to such a value that Eq. (12) becomes invalid, the dissipation reaches a highest value, beyond which no stable states exist. For a fixed V_{max}, dissipation increases monotonically with the increase of V_{in}. When V_{in} increases to such a value that Eq. (12) becomes invalid, the dissipation reaches a highest value, beyond which no stable states exist. Effects of rate constants on dissipation have also been examined. It is revealed that, although rate constants may change dissipation quantitatively, they do not change the trend of Fig. 2. Therefore, although collapse of stable states may stem from either the decrease of V_{max} or increase of V_{in}, it always corresponds to a point at which dissipation is the highest. In other words, in order to maintain stable states, the dissipation of the reaction system must not exceed a critical level. Therefore, for the substrate inhibited reaction with Michaelis–Menten formalism, maintenance of stable states intrinsically links with level of dissipation.

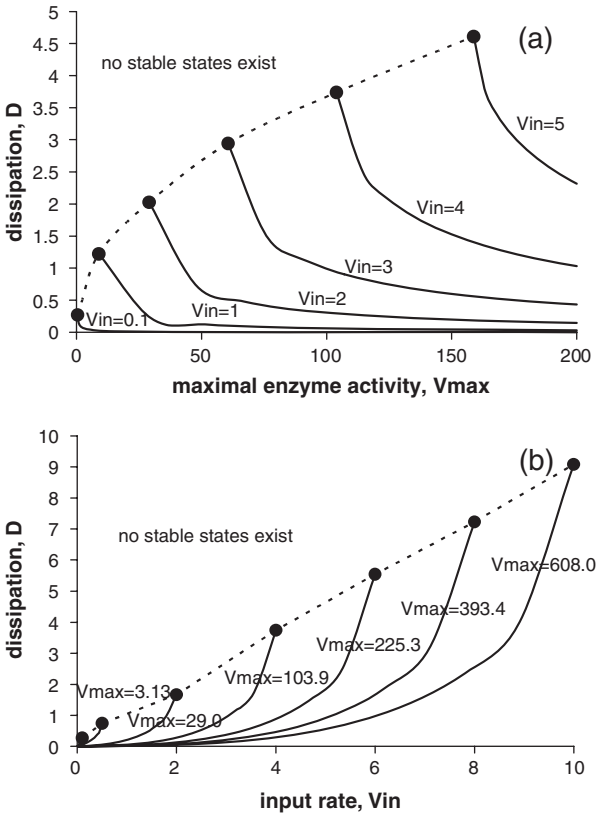

Fig. 2. Dependence of dissipation on maximal enzyme activity and input rate at steady states. (a) and (b) show that, although collapse of stable states may stem from either the decrease of V_{max} or increase of V_{in}, it always corresponds to a point at which dissipation is the highest.

3. Dissipation and maintenance of stable states under forcing

In general, all enzymatic reactions in a living system are subject to a fluctuating input. These fluctuations may stem from varying environmental conditions, or from the interaction between reactions. For example, plant photosynthesis is subject to light intensity fluctuations and the processes of acquiring carbon resources are time-dependent. Moreover, since a particular reaction is always embedded in a pathway, its input is the output of preceding reactions. By taking into account the complex manner in the network of enzymatic reactions in vivo [18], any reaction may be considered to be forced by other reactions.

In general, the fluctuations can take different forms. Here, we assume that the external forcing is represented by the following equation:

$$V_{input} = V_0\left(1 + \varepsilon\sin\left(\frac{2\pi t}{T}\right)\right) \tag{15}$$

where ε and T are the forcing amplitude and period, respectively. In order to guarantee $V_{input} \geq 0$, ε is limited to the range of 0 to 1. We note that the average of V_{input} over an

exact period is equal to V_0, implying that the forcing does not change the net input rate of the system. We choose the values of parameters such that the system establishes a steady state when it is not subject to forcing, and study how forcing affects the maintenance of the state and dissipation.

When the system settles onto a steady state without forcing, forcing may drive the system to lose its stable states. Subsequently, the system does not maintain any stable state and it therefore cannot implement biological functions. Fig. 3 shows that two neighbouring forcing amplitudes may lead to completely different evolution of the reaction system. In Fig. 3a, a stable limit cycle sustains, in addition to concentration of substrate and product, all enzyme forms, E, ES and ES_2 periodically change. The reaction system settles onto a stable oscillatory state.

However, in Fig. 3b, the catalysing cycle is destroyed. Consequently, E and ES approaches zero, and ES approaches the total enzyme concentration, E_0. Substrate and product cannot settle onto any stable states.

Fig. 4 summarises the effects of forcing amplitude and period on the maintenance of stable states. When forcing amplitude and period are such that their values are in the range below each curve, the reaction system reaches a stable state. However, when their values are above each curve up to forcing amplitude to be 1, no stable states exist. Specifically, for a fixed input rate (V_0=1.0), as maximal enzyme activity, V_{max}, increases, the system is more possible to establish stable states (Fig. 4a). Moreover, for a fixed V_{max}(V_{max}=26.7), as the input rate decreases, the system is more possible to establish stable states (Fig. 4b). In general, the reaction system can process external signals across a broad range in period provided they have small amplitudes, but large amplitude signals may lead to the collapse of stable states. Similarly, the system can withstand large amplitude signals provided the period is short, however large amplitude signals with long period will lead to the collapse of

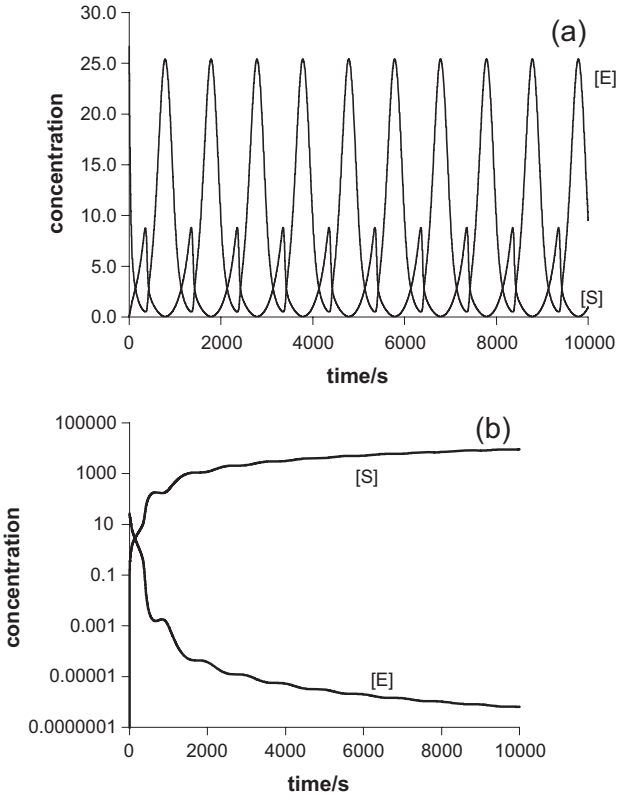

Fig. 3. Effects of two neighbouring forcing amplitudes on evolution of the reaction system., V_{in}=1.0 V_{max}=26.7, T=1000. (a) ε=0.976, the system develops to a stable oscillatory state; (b) ε=0.980, the system cannot develop to any stable states. In (b), the y-axis is in logarithmic scale.

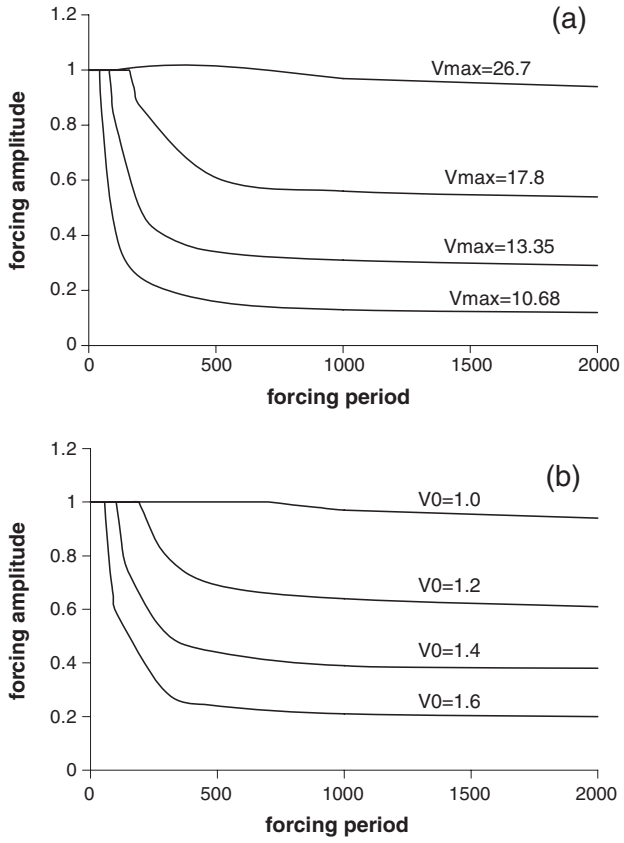

Fig. 4. Effects of forcing amplitude and period on the maintenance of stable states. Below each curve, the reaction system reaches a stable state. (a) V_{in}=1.0. (b) V_{max}=26.7.

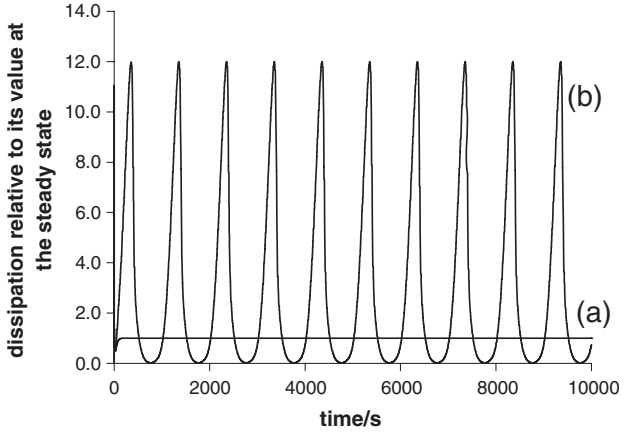

Fig. 5. Temporal dependence of dissipation relative to its value at the steady state. V_{in}=1.0 V_{max}=26.7, T=1000. (a) ε=0.0. (b) ε=0.976.

stable states. We emphasize that the forcing superimposed does not change the (average) input rate. Therefore, under forcing, the (average) mass transported into the reaction system remains the same. However, as demonstrated by Figs. 3 and 4, forcing is able to destroy stable states. Is the collapse of stable states induced by forcing related to dissipation of the system? Therefore, we further investigate effects of forcing on dissipation.

In order to examine how forcing affects dissipation, relative dissipation, R, is defined as

$$R = \frac{\bar{D}}{D_0} \tag{16}$$

where D_0 is dissipation at a steady state when the system is not subject to forcing, and it does not change with time, D is dissipation under forcing, and it changes periodically with time (Fig. 5). $\bar{D}$ is the average of D over an exact period. Therefore, $\bar{D}$ is the dissipation for the oscillatory state.

Fig. 6 shows how forcing affects relative dissipation in the system. In Fig. 6, $V_0=1.0$, $T=1000$, relative dissipation is calculated for five different values of V_{max}. In (a), $V_{max}=8.89$, which is the minimal value calculated from Eq. (12). In this case, if $\varepsilon=0.0$, a steady state is established. However, if ε is any positive value, no stable states exist. In curves (b) to (e), relative dissipation, R is always larger than 1, implying that the system under forcing always dissipates more energy. Importantly, when the system cannot maintain its stable states, the relative dissipation reaches a point, at which dissipation is the highest,. Therefore, under forcing, the system may only accommodate a certain range of dissipation. If dissipation is higher than a critical value, the system cannot maintain any stable states. Further numerical analysis confirms that, when forcing period changes, the collapse of stable states also corresponds a critical point, at which dissipation is the highest (data not shown). Therefore, the collapse of stable states under forcing follows the following scenario: when the system is forced, forcing changes

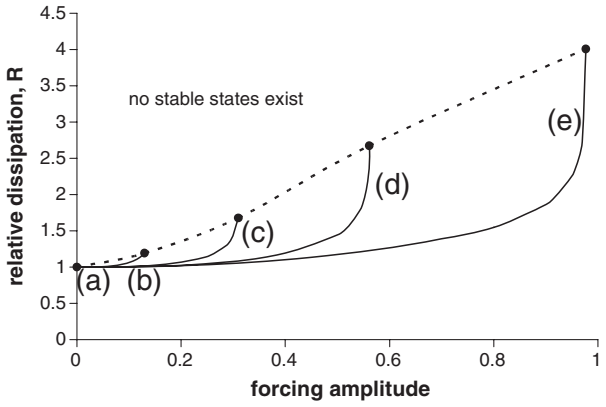

Fig. 6. Dependence of relative dissipation, R, on forcing amplitude, ε, and maximal enzyme activity, V_{max}. $V_0=1.0$, $T=1000$. (a) $V_{max}=8.89$, (b) $V_{max}=10.68$, (c) $V_{max}=13.35$, (d) $V_{max}=17.80$, (e) $V_{max}=26.7$. Each curve is calculated as follows. Firstly, dissipation D_0 is calculated for unforced system (i.e., $\varepsilon=0$). Secondly, for a specific ε, time-dependent dissipation D is calculated. After the transient period dies out, a periodic oscillation is established for D (Fig. 5). Thirdly, average dissipation D is calculated over an exact period. Then relative dissipation R is calculated using Eq. (16).

the system from a steady state to an oscillatory state and the dissipation of the system increases. When forcing amplitude and period are such values that stable states cannot be maintained, dissipation reaches a highest value. Subsequently, the system cannot implement any functions.

4. Concluding remarks

By analysing a reversible enzyme reaction system, this work shows that the maintenance of a stable state requires a highly nonlinear relation between input rate and maximal enzyme activity. This nonlinearity is due to the consequences of substrate inhibition. Moreover, when the reaction system establishes a stable state with a constant input, external forcing may destroy the stable state. Consequently, the system cannot implement any biological functions. Importantly, under certain forcing amplitude and period, maximal enzyme activity is a key factor determining if the system establishes a stable state. Therefore, a reaction system with a certain configuration of enzyme activities may only stably evolve under certain external conditions. Furthermore, this work also shows that, for the specific reaction, collapse of stable steady state is intrinsically related to its dissipation, regardless if the reaction is forced or not.

Evolution of biochemical reactions is subject to constraints. Kinetic constraints [16,17] are conditions under which the reaction establishes stable states. Thermodynamic constraints restrict the direction of the reactions [1,11–15]. Although thermodynamic and kinetic constraints restrict different aspects of the reaction, they can be closely related. Analysis of the reaction system with substrate inhibition shows that system dissipating too much energy may not maintain its stable states. This corresponding relationship may originate from the foundation of kinetic and thermodynamic constraints. Both kinetic and thermodynamic constraints are based fundamentally on elementary reaction steps and mass-action kinetics.

If the reaction system is not inhibited by the substrate, as long as $V_{max}>V_{in}$, the system establishes a steady state. In order to maintain a stable state in the reaction with substrate inhibition, maximal enzyme activity, V_{max}, must be much higher than input rate. For example, in terms of Eq. (12), if $V_{input}=5.0$, then $V_{max}>135.7$; and if $V_{input}=10.0$, then $V_{max}>608.0$. Approximately, maximal enzyme activity is required to be 27- and 61-fold higher than input rate for the two cases, respectively. In other words, in the regulated system, maximal enzyme activity must be drastically increased to maintain a stable state. In a pathway, the activities of some enzymes greatly exceed the flux capacity of the pathway. This intriguing problem attracts much attention [19,20, and references therein]. For example, it has been argued that these high activities are necessary to ensure sufficient net flux in reactions that operate near equilibrium [19]. Recently, by analysing a kinetic model, it is suggested that large activities may reflect a close match of system design to performance requirements [20]. This work shows that, when an enzyme is regulated, high enzyme activities may be essential for forming a stable state. Furthermore, when external fluctuation exists, high activities play a vital role in protecting a stable state from its catastrophic

collapse. Therefore, in addition to the roles previously suggested, it is also possible that high activities of enzymes may be necessary for maintaining a stable evolution in some regulated enzymatic reactions.

Energy conservation and dissipation in biochemical networks are important aspects for understanding biological functions [1,11–15,26]. In particular, in nonlinear autonomous and forced reaction systems, thermodynamic efficiency and dissipation have been systematically studied [23–25]. It has been shown that external forcing may have advantages improving thermodynamic efficiency in oscillatory reactions [27]. This work shows that, under external forcing, if the reaction system dissipates too much energy, the system cannot maintain stable states. Subsequently, no biological functions can be implemented. Therefore, reduction of dissipation by controlling the values of kinetic parameters such as maximal enzyme activity is important for maintaining a biologically functioning state.

Although the rate laws for all reactions follow mass-action kinetics, for an enzymatic reaction the concentration of any form of an enzyme cannot arbitrarily change as it is limited by the total concentration of the enzyme. Therefore, although the saturation and regulation features of enzymatic rate laws cannot be immediately described by mass-action kinetics [28], they are the consequences of the mass-action kinetics in which the concentrations of all forms of an enzyme are limited. Enzymatic kinetics are usually derived from traditional mass-action kinetics together with simplifying assumptions such as the existence of a quasi-steady state [29,30]. At the level of enzymatic reactions, the kinetic rate laws exhibit some special features such as saturation and regulation [28]. Those features are due predominantly to the catalysing functions of enzymes, and they are captured by Michaelis–Menten type kinetics. When a number of enzymatic reactions interplay, based on enzymatic kinetics it has been shown that establishment of a stable state requires specific constraints on kinetic parameters, particularly maximal reaction activities [16,17,31,32]. In order to obtain a stable steady state based on the parameters in literature, many of the parameters need to be adjusted [33,34]. The constraints for formation of stable states in many interplaying enzymes are kinetic constraints for a biological network [17]. Based on mass-action kinetics, this work shows that kinetic constraints in a single enzyme system are the constraints for maintaining the catalysing cycle of the enzyme. Therefore, kinetic constraints exist at the level of both mass-action and enzymatic kinetics.

Acknowledgement

The author would like to acknowledge the Scottish Executive Environmentand Rural Affairs Department (SEERAD) for support.

References

[1] H. Qian, D.A. Beard, Thermodynamics of stoichiometric biochemical networks in living systems far from equilibrium, Biophys. Chemist. 114 (2005) 213–220.
[2] J.S. Edwards, R.U. Ibarra, B.O. Palsson, In silico predictions of *Escherichia coli* metabolic capabilities are consistent with experimental data, Nat. Biotechnol. 19 (2001) 125–130.
[3] I. Famili, B.O. Palsson, The convex basis of the left null space of the stoichiometric matrix leads to the definition of metabolically meaningful pools, Biophys. J. 85 (2003) 16–26.
[4] S. Klamt, J. Stelling, Two approaches for metabolic pathway analysis? Trends Biotechnol. 21 (2003) 64–69.
[5] B.O. Palsson, The challenges of in silico biology, Nat. Biotechnol. 18 (2000) 1147–1150.
[6] N.D. Price, J.A. Papin, C.H. Schilling, B.O. Palsson, Genome-scale microbial in silico models: the constraints-based approach, Trends Biotechnol. 21 (2003) 162–169.
[7] C.H. Schilling, B.O. Palsson, Assessment of the metabolic capabilities of *Haemophilus influenzae* Rd through a genome-scale pathway analysis, J. Theor. Biol. 203 (2000) 249–283.
[8] S. Schuster, T. Dandekar, D.A. Fell, Detection of elementary flux modes in biochemical networks: a promising tool for pathway analysis and metabolic engineering, Trends Biotechnol. 17 (1999) 53–60.
[9] S. Schuster, D.A. Fell, T. Dandekar, A general definition of metabolic pathways useful for systematic organization and analysis of complex metabolic networks, Nat. Biotechnol. 18 (2000) 326–332.
[10] J. Stelling, S. Klamt, K. Bettenbrock, S. Schuster, E.D. Gilles, Metabolic network structure determines key aspects of functionality and regulation, Nature 420 (2002) 190–193.
[11] D.A. Beard, E. Babson, E. Curtis, H. Qian, Thermodynamic constraints for biochemical networks, J. Theor. Biol. 228 (2004) 327–333.
[12] D.A. Beard, S. Liang, H. Qian, Energy balance for analysis of complex metabolic networks, Biophys. J. 83 (2002) 79–86.
[13] D.A. Beard, H. Qian, Thermodynamic-based computational profiling of cellular regulatory control in hepatocyte metabolism, Am. J. Physiol., Endocrinol Metabol. Gastrointest. Physiol. 288 (2005) E633–E644.
[14] N.D. Price, I. Famili, D.A. Beard, B.O. Palsson, Extreme pathways and Kirchhoff's second law, Biophys. J. 83 (2002) 2879–2882.
[15] H. Qian, D.A. Beard, S.D. Liang, Stoichiometric network theory for nonequilibrium biochemical systems, Eur. J. Biochem. 270 (2003) 415–421.
[16] J. Liu, Coordination restriction of enzyme-catalysed reaction systems as nonlinear dynamical systems, Proc. R. Soc. Lond., A 455 (1999) 285–298.
[17] J. Liu, Kinetic constraints for formation of steady states in biochemical networks, Biophys. J. 88 (2005) 3212–3223.
[18] L. Stryer, Biochemistry, W.H. Freeman and Company, New York, 1997.
[19] J.F. Staples, R.K. Starez, Honeybee flight muscle phosphoglucose isomerase: matching enzyme capacities to flux requirements at a near-equilibrium reaction, J. Exp. Biol. 200 (1997) 1247–1254.
[20] A. Salvador, M.A. Savageau, Quantitative evolutionary design of glucose 6-phosphate dehydrogenase expression inhuman erythrocytes, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 14463–14468.
[21] H. Degn, Biostability caused by substrate inhibition of peroxidase in an open reaction system, Nature 217 (1968) 1047–1050.
[22] P. Shen, R. Larter, Role of substrate inhibition kinetics in enzymatic chemical oscillations, Biophys. J. 67 (1994) 1414–1428.
[23] J. Ross, M. Schell, Thermodynamic efficiency in nonlinear biochemical reactions, Annu. Rev. Biophys. Biophys. Chem. 16 (1987) 401–422.
[24] M. Al-Ghoul, Dynamics and dissipation in an externally forced system, Phys. Chem. Chem. Phys. 17 (2000) 3773–3783.
[25] J.-F. Hervagault, J.G. Lazar, J. Ross J, Prediction of thermodynamic efficiency in a pumped biochemical reaction, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 9258–9261.
[26] H.V. Westerhoff, K. van Dam, Thermodynamics and Control of Biological Free-energy Transaction, Elsevier, Amsterdam, 1987.
[27] M. Tsuchiya, J. Ross, Advantages of external periodic events to the evolution of biochemical oscillatory reactions, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9691–9695.
[28] R. Heinrich, S. Schuster, The Regulation of Cellular Systems, Chapman and Hall, New York, 1996.
[29] L.A. Segel, M. Selmrod, The quasi-steady-state assumption: a case study in perturbation, SIAM Rev. 31 (1989) 446–477.

[30] I. Stoleriu, D.A. Davidson, J. Liu, Quasi-steady state assumptions for non-isolated enzymatic reactions, J. Math. Biol. 48 (2004) 82–104.
[31] J. Liu, Enhancement and restriction of system coordination by interactions of pathways, J. Biol. Syst. 9 (2001) 169–186.
[32] J. Liu, J.W. Crawford, Sufficient conditions for coordination of a nonlinear biochemical system under external forcing, J. Phys. Chem., B 104 (2000) 2623–2629.
[33] J.M. Rohwer, F.C. Botha, Analysis of sucrose accumulation in the sugar cane culm on the basis of in vitro kinetic data, Biochem. J. 358 (2001) 437–445.
[34] B. Teusink, J. Passarge, C. Reijenga, E. Esgalhado, C.C. van der Weijden, M. Schepper, M.C. Walsh, B.M. Bakker, K. van Dam, H.V. Westerhoff, J. L. Snoep, Can yeast glycolysis be understood in terms of in vitro kinetics of the constituent enzymes? Testing biochemistry, Eur. J. Biochem. 267 (2000) 5313–5329.