The Fourth International Conference on Computational Systems Biology (ISB2010)
Suzhou, China, September 9–11, 2010

Property of Multiple Equilibria for SSI Metabolic Module*

Hong-Bo Lei[1,†] Ji-Feng Zhang[1,‡]

[1]Key Laboratory of Systems and Control, Academy of Mathematics and Systems Science, Chinese Academy of Sciences, Beijing 100190, P. R. China

Abstract Single substrate and single product with inhibition (SSI) metabolic module is one of the four types of basic building blocks of metabolic networks, and its multiple equilibria property has important influence on that of the whole metabolic networks. In this paper, we characterize the rates of the metabolic reactions by Hill kinetics, construct a special vector space, and give a unified model for SSI modules by using a set of nonlinear ordinary equations with multi-variables. A sufficient and necessary condition is given to describe the injectivity of a class of nonlinear systems, and then, used to study the multiple equilibria property of SSI modules. For the SSI modules in which each reaction has at most one inhibitor, a sufficient condition is derived to ensure the absence of multiple equilibria, i.e. the Jacobian matrix of its rate function is nonsingular everywhere.

Keywords Metabolic Network, SSI Module, Multiple Equilibria.

1 Introduction

Revealing the property of multiple equilibria for a metabolic network is a fundamental and important topic in systems biology. However, in the traditional theoretical analysis, necessary information on model parameters is always required. Due to the limitation of measurement tools, measurement errors and biological variability, most of the model parameters are either uncertain or unavailable. This not only makes it difficult to analyze the model, but also limits the applications of the theoretical results based on a model with fixed parameter values. In contrast to detailed model parameters, the topological structure of a metabolic network is relatively easier to be obtained and is invariant for many cases. Hence, a structure-oriented analysis should be much more useful on understanding qualitative dynamics of metabolic networks.

There are some pioneering works in structure-oriented study on multiple equilibria of networks [2–5, 7], which have recently been surveyed in [6]. A metabolic network in a living cell is a large-scale molecular network and contains a great number of metabolites and reactions, and thus, is generally difficult to be theoretically analyzed as a whole, especially when there is no parameters but only structure information available.

*This work was supported by the National Natural Science Foundation of China (under grant 60821091).
†leihb@amss.ac.cn
‡jif@iss.ac.cn

To overcome such a difficulty, we proposed a structure-oriented modularization framework in [6]: using the modularization idea commonly used in the area of control theory, viewing a metabolic network as an assembly of basic building blocks (called metabolic modules) with specific structures, and investigating the multiple equilibria property of the original network by studying the characteristics of these basic modules and their interactions. Such an idea not only reduces the difficulty in investigating a complex metabolic network, but also makes full use of the structure information, thereby overcomes the limitation of the methods based on models with fixed parameter values.

In particular, in [6] we showed that a metabolic network can be decomposed into four types of basic modules according to the topological structure, and proved that one type of those modules, i.e. the single substrate and single product with no inhibition (SSN) modules, cannot admit multiple equilibria. Here we will focus on another important type of those basic modules, i.e. the single substrate and single product with inhibition (SSI) modules, and investigate their multiple equilibria property.

Comparing with SSN modules, an SSI module contains metabolic reactions which are inhibited by other metabolites. Hence, the topological structure of an SSI module is much more complex from theoretical viewpoint. The metabolites interconnect with each other via reactions without inhibitions in SSN modules, while via reactions with inhibitions in SSI modules. Inhibitions make the metabolites (state variables) couple with each other in SSI modules, which are actually a kind of negative feedback. Moreover, the reaction mechanisms are much more complicated in SSI modules than those in SSN modules. For instance, when the other conditions (such as temperature, pH, the concentration and activity of the enzymes) are unchanged, the reaction rates depend mainly on the substrate concentrations in SSN modules but are simultaneously affected by the substrates, the inhibitions and their interactions in SSI modules.

Owing to these inherent characteristics, both the modeling procedure and theoretical analysis for SSI modules are much more difficult than those for SSN modules. Specifically, first, the intricate topological structure makes the modeling procedure for SSI modules much complicated. It is relatively easy to describe the rate of a metabolic reaction based on Hill kinetics if its inhibitors are known. But in a general SSI module, each reaction may be inhibited by other metabolites, and each metabolite may act as an inhibitor for other reactions. Hence, it is difficult to construct a unified model for SSI modules. Second, the strong coupling in SSI modules makes the model analysis difficult. The metabolites mutually restrain each other via inhibitions in SSI modules, which may result in a loop or other complex structure. Therefore, we have to consider all the metabolites simultaneously, which makes the dimension reduction of the system useless. Third, the complicated mechanisms of the reactions in SSI modules make the reaction rate equations more complex. In fact, the reaction rate is an increasing function of one variable in SSN modules, and is a polynomial that is increasing in any of its variables in the work [2–4] of Craciun et al.. But, in SSI modules, the reaction rate involves multiple variables, and is increasing in the substrate concentrations and decreasing in the inhibitor concentrations, which is also the essential difference between this work and that of Craciun et al..

The above characteristics of SSI modules makes the analytical skills developed for the SSN module cases no longer applicable. To overcome these difficulties, we first construct a special vector space, and represent the unified model of SSI modules via a system of nonlinear ordinary equations in a vector form. And then, we investigate the multiple

equilibria property of SSI modules through analyzing a sufficient and necessary condition of the injectivity of a particular nonlinear system. For the SSI modules in which each reaction has at most one inhibitor, we derive a sufficient condition for the absence of multiple equilibria, i.e. the Jacobian matrix of the rate function is nonsingular everywhere.

This paper is organized as follows. Section 2 describes what the SSI metabolic module is and how to model it based on Hill kinetics. Section 3 gives a main result of this paper, i.e. a sufficient condition for the absence of multiple equilibria of a common type of SSI modules. Section 4 gives several general remarks and future topics to conclude this paper.

2 Modeling SSI metabolic modules

In this section, we will give the definition of SSI metabolic module first, and then the general modeling approach based on Hill kinetics.

2.1 SSI metabolic module

Definition 2.1.
([6]) A metabolic reaction is called a single substrate and single product (SS) reaction, if it contains only one substrate and one product; otherwise, called a multiple substrates or multiple products (MM) reaction. An SS (or MM) reaction is called an SS (or MM) reaction with inhibition, SSI (or MMI) for short, if there exist some inhibitors of the reaction; otherwise, called an SS (or MM) reaction with no inhibition, SSN (or MMN) for short.

Remark 2.1.
A reaction will be viewed as two reactions if it is reversible. For example, take $A \overset{E}{\rightleftharpoons} B$ *as the forward reaction* $A \overset{E}{\longrightarrow} B$ *and the reverse reaction* $B \overset{E}{\longrightarrow} A$.

Definition 2.2.
For a group of SS metabolic reactions (including SSN and SSI reactions), take each metabolite as a node. If two nodes are contained in a same reaction, link them with a directed edge (arrow) from the substrate to the product, and call such an edge reaction edge. If a metabolite can inhibit some reaction, link the corresponding node and reaction edge with a line that contains a bar at the end near the reaction edge. Then we get a graph, called reaction graph of the group of SS reactions.

Now, we give an example to show how to get a reaction graph. Suppose that there are two SS reactions: $A \to B$, $C \to D$, and the metabolite D is an inhibitor of the first reaction. The corresponding reaction graph is shown in Figure 1.

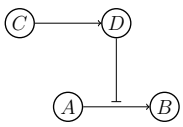

Figure 1: A reaction graph.

Definition 2.3.
In the reaction graph of a group of SS metabolic reactions, a node is called an input node,

if the direction of each reaction edge that connects with it is from it to some other node; a node is called an output node, if the direction of each reaction edge that connects with it is towards it. The other nodes are called state nodes. A state node that directly connects with an input (or output) node is called a head (or an end) node.

Definition 2.4 (SSI module)**.**
For a metabolic network, denote $\tilde{\mathscr{M}}$ the set of all the metabolites, and $\tilde{\mathscr{R}}$ the set of all the reactions. The triple $(\mathscr{S},\mathscr{R},\mathscr{I})$ is called an SSI module within the metabolic network, if the following conditions are satisfied:

(i) $\mathscr{S} \subset \tilde{\mathscr{M}}$ is nonempty.
(ii) $\mathscr{R} \subset \tilde{\mathscr{R}}$ is nonempty and constituted of all the reactions which are relevant to the metabolites in $\mathscr{S}$. Here, "a reaction is relevant to a metabolite S" means that S is the reactant, product or inhibitor of this reaction.
(iii) The reactions in $\mathscr{R}$ are all SS (including SSN and SSI) reactions.
(iv) $\mathscr{I} \subset \mathscr{S} \times \mathscr{R}$ is nonempty, and its element $(D, A \to B)$ means the metabolite D is an inhibitor of the reaction $A \to B$.

Here, $\mathscr{S}$, $\mathscr{R}$ and $\mathscr{I}$ are called the state node set, the reaction set and the inhibition set of the SSI module $(\mathscr{S},\mathscr{R},\mathscr{I})$, respectively.

2.2 Modeling SSI modules

Two broad classes of enzyme inhibitions, i.e. irreversible and reversible, are generally recognized [1]. In an irreversible inhibition, the inhibitor combines with or destroys a functional group on the enzyme that is essential for its activity, and we do not consider it here. In contrast, in a reversible inhibition, the inhibitor dissociates very rapidly from its target enzyme because it becomes very loosely bound with the enzyme. Three types of reversible inhibitions are observed: competitive, uncompetitive and noncompetitive. Next we will introduce those reversible inhibitions [1].

A competitive inhibitor can combine reversibly with the active site of the enzyme and compete with the substrate. In the following reactions, the metabolite I is acting as a competitive inhibitor of the reaction $S \to P$,

$$\begin{aligned} S + E &\rightleftharpoons SE \to P + E \\ I + E &\rightleftharpoons IE, \end{aligned}$$

where S, E, P and I are substrate, enzyme, product and inhibitor, respectively. Based on the Michaelis-Menten kinetics with the conservation condition on E, the rate of the reaction $S \to P$ can be described as

$$v = \frac{V_{max} C_S}{K_M(1 + C_I/K_C) + C_S}, \tag{1}$$

where C_S and C_I represent the concentrations of the substrate S and the inhibitor I, respectively; V_{max} means the maximum rate of the reaction, K_M is the Michaelis-Menten constant, and K_C is the competitive inhibition constant with respect to I.

An uncompetitive inhibitor cannot combine with a free enzyme, but only with an enzyme-substrate complex, and precludes the complex from converting into product. In

the following reactions, the metabolite I is acting as a uncompetitive inhibitor of the reaction $S \to P$,

$$\begin{aligned} S+E &\rightleftharpoons SE \to P+E \\ I+SE &\rightleftharpoons SEI. \end{aligned}$$

In this case, the rate of the reaction $S \to P$ can be described as

$$v = \frac{V_{max}C_S}{K_M + C_S(1+C_I/K_U)}, \tag{2}$$

where K_U is the uncompetitive inhibition constant with respect to I.

An noncompetitive inhibitor can combine with both free enzyme and enzyme-substrate complexes. In the following reactions, the metabolite I is acting as a noncompetitive inhibitor of the reaction $S \to P$,

$$\begin{aligned} S+E &\rightleftharpoons SE \to P+E \\ I+E &\rightleftharpoons EI \\ I+SE &\rightleftharpoons SEI. \end{aligned}$$

In this case, the rate of the reaction $S \to P$ can be described as

$$v = \frac{V_{max}C_S}{K_M(1+C_I/K_C) + C_S(1+C_I/K_U)}. \tag{3}$$

Although the above three types of reversible inhibitions were observed in experiments, from the theoretical viewpoint, (3) is a general (or an approximate) expression of (1) and (2) with appropriate parameter values. Hence, we will take (3) to describe the rate of reaction $S \to P$ when I is known to be an inhibitor. Generally, for the reaction $\alpha \to \beta$, if $I_1, \cdots, I_q$ are inhibitors, then we can use

$$v_{\alpha\to\beta} = \frac{V_{max}^{\alpha\to\beta}(C_\alpha)^{n_{\alpha\to\beta}}}{K^{\alpha\to\beta}\prod_{i=1}^{q}(1+C_{I_i}/K_{CI_i}^{\alpha\to\beta}) + (C_\alpha)^{n_{\alpha\to\beta}}\prod_{i=1}^{q}(1+C_{I_i}/K_{UI_i}^{\alpha\to\beta})} \tag{4}$$

to describe the reaction rate; and if there is no inhibitor, then we take

$$v_{\alpha\to\beta} = \frac{V_{max}^{\alpha\to\beta}(C_\alpha)^{n_{\alpha\to\beta}}}{K^{\alpha\to\beta} + (C_\alpha)^{n_{\alpha\to\beta}}}, \tag{5}$$

where $n_{\alpha\to\beta}$ is the Hill coefficient.

Let $(\mathscr{S}, \mathscr{R}, \mathscr{I})$ be an SSI module. Take the state node set $\mathscr{S}$ as a basis and construct a vector space $\mathbb{R}^{\mathscr{S}} = \{\sum_{S\in\mathscr{S}} z_S S : z_S \in \mathbb{R}\}$. As a convention, if α (or β) in the reaction $\alpha \to \beta$ is an input (or output) node, view it as a zero vector in $\mathbb{R}^{\mathscr{S}}$; otherwise, view it as a vector α (or β) in $\mathbb{R}^{\mathscr{S}}$. Let C_S represents the concentration of the metabolite S. Then the vector $C = \sum_{S\in\mathscr{S}} C_S S \in \mathbb{R}^{\mathscr{S}}$ can represent the concentrations of all the metabolites in $\mathscr{S}$. Note that the reaction $\alpha \to \beta$ consumes α and generates β with the rate $v_{\alpha\to\beta}$ simultaneously. Thus, we can obtain a unified model for a general SSI module as.

$$\frac{dC}{dt} = \sum_{S\in\mathscr{S}} \dot{C}_S S = \sum_{\alpha\to\beta\in\mathscr{R}} v_{\alpha\to\beta}(\beta-\alpha) \triangleq R(C,p), \tag{6}$$

where p is the corresponding vector-valued parameter, $v_{\alpha\to\beta}$ is in agreement with (4) if reaction $\alpha\to\beta$ is with inhibitions, and (5), otherwise. $R(C,p)$ is called the rate function of the module. Noticing that all parameters in (4) and (5) are positive, and $n_{\alpha\to\beta}\geq 1$, we denote such a parameter space by P. Since the concentration of each metabolite is positive, we just need to discuss the system (6) in $\mathbb{R}_+^{\mathscr{S}}=\{\sum_{S\in\mathscr{S}} z_S S : z_S\in\mathbb{R}_+=(0,\infty)\}$.

Definition 2.5.
For a fixed parameter p_0, an equilibrium of the model (6) is a state C that satisfies $\dot{C}=0$, i.e. a solution of the algebraic equations $R(C,p_0)=0$. The system (6) or the SSI module is said to have the capability of multiple equilibria, if there exists a parameter p_0 such that $R(C,p_0)=0$ has more than one positive solutions.

3 Model Analysis

Lemma 3.1.
([6]) Let $F(\cdot)$ be a real function defined on $\mathbb{R}^n$, and D be a subset of $\mathbb{R}^n$. For a system described by a set of ordinary differential equations $\frac{dx}{dt}=F(x)$, if F (also called the vector field of the system) is injective in D, then the system cannot admit multiple equilibria in D, i.e. $F(x)=0$ has at most one root in D.

Lemma 3.1 provides a sufficient condition for the absence of multiple equilibria of a general system, but such a condition is difficult to be verified. Hence, we need to convert it into an equivalent one which is relatively easy to be verified. For some simple cases, for example, $f(x):\mathbb{R}\to\mathbb{R}$ is continuously differentiable function of one variable, then its injectivity is equivalent to that its differential is nonzero everywhere. Unfortunately, there is no such an equivalence for a general high dimensional map. As an counterexample, taking $F(x,y)=(\frac{1}{3}(x-1)^3,y)^T$, it is obvious that $F(x,y)$ is injective on $\mathbb{R}^2$, but the determinant of its Jacobian matrix is $det(JF)=(x-1)^2$, which is zero on line $x=1$; and taking $F(x,y)=(\sqrt{2}e^{x/2}\cos(ye^{-x}),\sqrt{2}e^{x/2}\sin(ye^{-x}))^T$ [8], the determinant of its Jacobian matrix is $\det(JF)\equiv 1$, but $F(0,y+2k\pi)=F(0,y)$, which means that F is not injective. Nevertheless, for some particular high dimensional map, its injectivity and the nonsingularity of its Jacobian matrix is equivalent. We will give such a class of maps in the following lemma with proof being given in Appendix A.

Lemma 3.2.
Let $\mathscr{V}$ be an n dimensional vector space on the field of real number $\mathbb{R}$, $\mathscr{S}=\{\varepsilon_1,\cdots,\varepsilon_n\}$ be an orthogonal basis of $\mathscr{V}$, $D\subset\mathscr{V}$ be an open set, and $\mathscr{R}=\{(\alpha_k,\beta_k):k=1,\cdots,m\}$ be a finite subset of $\mathscr{V}\times\mathscr{V}$. For a fixed vector-valued parameter $p\in P$ (P is the parameter space), $F(\cdot,p)$ is a map on D, which has the form

$$F(x,p)=\sum_{k=1}^{m} f_k(x,p)(\beta_k-\alpha_k), \tag{7}$$

where $x=(x_1,\cdots,x_n)$ is the coordinate of the vector $\sum_{i=1}^n x_i\varepsilon_i$ with respect to the basis $\mathscr{S}$, and $f_k(x,p)$ is continuously differentiable with respect to x. Then

(i) if for any $\widetilde{x}\neq\widehat{x}\in D$ and $\widetilde{p}\in P$, there exist $x\in D$, $y(\neq 0)\in\mathscr{V}$ and $p\in P$ such that the following equation holds for all $k=1,\cdots,m$,

$$f_k(\widetilde{x},\widetilde{p})-f_k(\widehat{x},\widetilde{p})=\sum_{i=1}^{n} y_i\frac{\partial f_k}{\partial x_i}(x,p), \tag{8}$$

then the Jacobian matrix of F is nonsingular everywhere on D for all p is a sufficient condition to ensure that F is injective on D for all p;

(ii) if for any $x \in D$, $y(\neq 0) \in \mathscr{V}$ and $p \in P$, there exist $\widetilde{x} \neq \widehat{x} \in D$ and $\widetilde{p} \in P$ such that (8) holds for all $k = 1, \cdots, m$, then the sufficient condition in (i) is also necessary.

Lemma 3.3.
Assume $\mathscr{V}$, $\mathscr{S} = \{\varepsilon_1, \cdots, \varepsilon_n\}$, $D = \{\sum_{i=1}^n z_i\varepsilon_i : z_i > 0\}$ and $\mathscr{R} = \{(\alpha_k, \beta_k) : k = 1, \cdots, m\}$ have the same meanings as in Lemma 3.2. $\{N_1, N_2, N_3, N_4\}$ is a partition of $N = \{1, \cdots, m\}$, i.e. they are disjoint and $\bigcup_{i=1}^4 N_i = N$. Let $\{r_k : k \in N_3 \cup N_4, r_k \in \{1, \cdots, n\}\}$ and $\{q_k : k \in N_2 \cup N_4, q_k \in \{1, \cdots, n\}\}$ be two sequences and $r_k \neq q_k$. Let

$$F(x,p) = \sum_{k=1}^{m} f_k(x,p)(\beta_k - \alpha_k), \tag{9}$$

$$f_k(x,p) = \begin{cases} h_k(p_k) = \dfrac{a_k(u_k)^{n_k}}{b_k + (u_k)^{n_k}}, & k \in N_1, \\ h_k(x_{q_k}, p_k) = \dfrac{a_k(u_k)^{n_k}}{b_k\left(1 + \frac{x_{q_k}}{c_k}\right) + (u_k)^{n_k}\left(1 + \frac{x_{q_k}}{d_k}\right)}, & k \in N_2, \\ h_k(x_{r_k}, p_k) = \dfrac{a_k(x_{r_k})^{n_k}}{b_k + (x_{r_k})^{n_k}}, & k \in N_3, \\ h_k(x_{r_k}, x_{q_k}, p_k) = \dfrac{a_k(x_{r_k})^{n_k}}{b_k\left(1 + \frac{x_{q_k}}{c_k}\right) + (x_{r_k})^{n_k}\left(1 + \frac{x_{q_k}}{d_k}\right)}, & k \in N_4, \end{cases} \tag{10}$$

where p_k is the corresponding vector-valued parameter, its components a_k, b_k, c_k, d_k and u_k are positive real number, $n_k \geq 1$; $p = (p_1, \cdots, p_m) \in P$ (P is the corresponding parameter space). Then the Jacobian matrix of F is nonsingular everywhere on D for all $p \in P$ is equivalent to that F is injective on D for all $p \in P$.

The proof of Lemma 3.3 is given in Appendix B.

Thorem 3.1.
If each reaction in the reaction set $\mathscr{R}$ of an SSI module $(\mathscr{S}, \mathscr{R}, \mathscr{I})$ has at most one inhibitor, then the sufficient condition for the absence of multiple equilibria of the corresponding model (6) is that the Jacobian matrix $\left(\frac{\partial R}{\partial C}(C,p)\right)$ of the rate function $R(C,p)$ is nonsingular for all $p \in P$ and $C \in \mathbb{R}^n_+$.

Proof. Divide the reactions in $\mathscr{R}$ into four classes: reaction that is from an input node to a head node and with no inhibition, reaction that is from an input node to a head node and with inhibition, reaction that is from a state node to other state node or output node and with no inhibition, reaction that is from a state node to other state node or output node and with inhibition, When each reaction in $\mathscr{R}$ has at most one inhibitor, the rate equations of the above four classes of reactions confirm with the function f_k in (10) for k in N_1, N_2, N_3 and N_4, respectively. Thus, the model (6) of this SSI module is a special case of the system (9), which means the results in Lemma 3.3 are still valid for such an SSI module with $\mathscr{V} = \mathbb{R}^{\mathscr{S}}$ and $D = \mathbb{R}^{\mathscr{S}}_+$. □

4 Concluding Remarks

The SSI module is one of the four types of basic building blocks of metabolic networks, whose multiple equilibria property was studied in this paper. Due to the complexity of its topological structure, the strong coupling between each metabolite and the intricacy of the reaction mechanism, it is a difficult task to analyze dynamic properties of SSI modules. In particular, comparing with SSN modules which generally cannot admit multiple equilibria, there exists negative feedback in SSI modules caused by inhibitions, which makes the module structure and the reaction mechanism much more complicated. This paper mainly discussed one common type of SSI modules in which each reaction has at most one inhibitor, which is considered as the first step towards elucidating the design principle of metabolic networks in living organisms. In the near future, we will further discuss the SSI modules in which there are reactions with more than one inhibitor.

Appendix

Due to the limitation of space, the appendix is not published here. If any one is interested in the proof, please email Hong-Bo Lei (leihb@amss.ac.cn).

References

[1] A. Cornish-Bowden. *Fundamentals of enzyme kinetics*. Portland Press, 3rd edition, 2004.

[2] G. Craciun and M. Feinberg. Multiple equilibria in complex chemical reaction networks: I. the injectivity property. *SIAM J. Appl. Math.*, 65(5): 1526–1546, 2005.

[3] G. Craciun and M. Feinberg. Multiple equilibria in complex chemical reaction networks: II. the species-reaction graph. *SIAM J. Appl. Math.*, 66(4): 1321–1338, 2006.

[4] G. Craciun, Y. Tang, and M. Feinberg. Understanding bistability in complex enzyme-driven reaction networks. *Proc. Natl. Acad. Sci., USA*, 103(23): 8697–8702, 2006.

[5] M. Kaufman, C. Soule, and R. Thomas. A new necessary condition on interaction graphs for multistationarity. *J. Theor. Biol.*, 248(4): 675–685, 2007.

[6] H.B. Lei, J.F. Zhang, and L. Chen. Multiple equilibria in metabolic network: SSN module. The 29th Chinese Control Conference, Beijing, China, 29-31, July, 2010.

[7] C. Soulé. Graphic requirements for multistationarity. *ComPlexUs*, 1(3): 123–133, 2003.

[8] M. Chamberland and G. Meisters. A mountain pass to the jacobian conjecture. Canad. Math. Bull., 41(4): 442–451, 1998.