Stability of metabolic pathways with irreversible reactions

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Abstract—**By making minimal assumptions on the kinetics of chemical reactions we study the stability of steady states in metabolic pathways in relation to the topology of the metabolic network. Here we report our results on metabolic pathways with irreversible reactions. We show that the steady states of linear pathways are always locally asymptotically stable. This is not necessarily true for branched and cyclic pathways, but stability in such networks is assured under mild conditions.**

I. Introduction

Understanding the dynamics of metabolic networks is a challenging task. Most theory has focused on the analysis of models for specific metabolic pathways. It is, however, not clear whether, and to what extent, the results obtained extend to metabolic pathways in general. Many of the metabolic pathways studied today comprise thousands of metabolites and reactions. Moreover, for most reactions, the laws governing the kinetics are not known or only incompletely specified. Therefore, a structural modelling approach based on qualitative assumptions on network topology and reaction kinetics is required.

Previous studies along these lines [1-5] arrived at general conclusions regarding the stability of steady states in chemical reaction networks with mass-action kinetics. However, often the assumption of mass-action kinetics is typically not satisfied in real metabolic networks. Recently progress has been achieved for networks with general nonlinear rate laws, provided that all stoichiometric coefficients are equal to 0 or ±1. Flach and Schnell [6] proved quite generally the local stability of steady states in metabolic networks with reversible single-substrate-single product kinetics. Their proof applies to pathways with linear and branched topology, and can be expected extend to pathways with cyclic topology. Reznik and Segré derived complementary results, first numerically [7] and later analytically [8], for cyclic pathways with irreversible single-substrate-single-product reactions. By means of a different proof, we showed quite generally [9] the local stability of steady states in case of networks involving only single-substrate-single product reactions, irrespective of network topology. This result was later extended to networks including multiple-substrate-multiple-product reactions [10]. We concluded that the steady state of metabolic networks with nonlinear kinetics is locally stable whenever all stoichiometric coefficients are 0 or ±1.

Although in most metabolic reactions only one molecule of each metabolite participates [11], this is not always the case. For example, several molecules of cofactors like ATP, ADP, NAD or NADP are involved in a single metabolic reaction. Therefore we will drop here the standard assumption that the stoichiometric assumptions are limited to 0 and ±1. Instead, we will assume that all reactions are irreversible. Although in principle all chemical reactions are reversible due to the laws of thermodynamics, many reactions in metabolic pathways can be considered irreversible in practice [12].

II. Assumptions

A metabolic pathway is a set of chemical species, also called metabolites, together with metabolic reactions (catalysed by enzymes) in which these metabolites participate [13]. The dynamics of a metabolic pathway consisting of m metabolites and n chemical reactions is described by a system of ordinary differential equations [11]:

$$\dot{x} = Sv(x) \tag{1}$$

Here x is the m-dimensional vector of metabolite concentrations, S is the $m \times n$ stoichiometric matrix and $v(x)$ is an n-dimensional vector of reaction rates, which are functions of the metabolite concentrations. In the stoichiometric matrix rows correspond to different metabolites and columns correspond to reactions. The entry S_{ij} represents the number of molecules of metabolite i used in reaction j. If i is a substrate of a reaction j then $S_{ij} < 0$, if i is a product then $S_{ij} > 0$. If i does not participate in a reaction j then $S_{ij} = 0$.

We assume that all metabolic reactions are *'monotonic'* in the sense that reaction rates are faster at higher substrate concentrations and not affected by product concentrations due to irreversibly of reactions. Reaction

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O. Ivanov, Groningen Centre for Systems Biology, Nijenborgh 7, 9747 AG, Groningen, The Netherlands (corresponding author, phone: +31624775700; email: o.ivanov@rug.nl).

A. J. van der Schaft, Johan Bernoulli Institute for Mathematics and Computer Science, University of Groningen, Nijenborgh 9, 9747 AG, Groningen, The Netherlands.

F. J. Weissing, Groningen Centre for Systems Biology and Centre for Ecological and Evolutionary Studies, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.

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rates are also not affected by metabolites which are neither substrates nor products of these reactions. Hence, we assume:

$$\frac{\partial v_j}{\partial x_i}\begin{cases} > 0, & \text{if } i \text{ is a substrate in reaction } j \\ = 0, & \text{otherwise} \end{cases} \tag{2}$$

Michaelis-Menten kinetics and Hill kinetics all satisfy these assumptions. The *gradient matrix* $G(x) \equiv \partial v(x) / \partial x$ consists of *n* rows of derivatives of reactions rates with respect to the *m* metabolites. Since metabolite *i* is a substrate in reaction *j* if $S_{ij} < 0$ and a product if $S_{ij} > 0$, it is obvious from (2) that the matrix *G* has the same sign pattern as $-S^{\mathrm{T}}$.

III. RESULTS

To assess the local stability of a steady state x^*, we consider the Jacobian matrix at this steady state:

$$J(x^*) = \left.\frac{\partial (Sv(x))}{\partial x}\right|_{x=x^*} = S \left.\frac{\partial v(x)}{\partial x}\right|_{x=x^*} = S \cdot G(x^*) \tag{3}$$

In view of $G(x^*) \sim (-S)^{\mathrm{T}}$ the Jacobian has a very special structure, which is closely related to that of stoichiometric matrix, i.e. the topology of the metabolic pathway.

A. Stability in metabolic pathways with single-substrate-single-product reactions

A general representation of a linear single-substrate-single-product metabolic pathway is of the form

$$\begin{aligned} &\xrightarrow{v_1} S_{11}x_1 \\ &S_{12}x_1 \xrightarrow{v_2} S_{22}x_2 \\ &\qquad\vdots \\ &S_{m-1\,n-1}x_{m-1} \xrightarrow{v_{n-1}} S_{mn-1}x_m \\ &S_{mn}x_m \xrightarrow{v_n} . \end{aligned} \tag{4}$$

The Jacobian matrix is lower triangular, with negative entries on the diagonal:

$$J = \begin{pmatrix} -S_{12}\dfrac{\partial v_2}{\partial x_1} & & & \\ S_{22}\dfrac{\partial v_2}{\partial x_1} & \ddots & & \\ & \ddots & -S_{m-1\,m-1}\dfrac{\partial v_{n-1}}{\partial x_{m-1}} & \\ & & S_{mn-1}\dfrac{\partial v_{n-1}}{\partial x_{m-1}} & -S_{nm}\dfrac{\partial v_n}{\partial x_m} \end{pmatrix} \tag{5}$$

Hence, all the eigenvalues of *J* are negative, implying that in case of linear single-substrate-single-product pathways steady states are always locally asymptotically stable. In the presence of branching, the Jacobian is still lower triangular with negative diagonal, again implying local stability of steady states.

A general representation of a cyclic single-substrate-single-product metabolic pathway is of the form

$$\begin{aligned} &\xrightarrow{v_1} S_{11}x_1 \\ &S_{12}x_1 \xrightarrow{v_2} S_{22}x_2 \\ &\qquad\vdots \\ &S_{m-1\,n-1}x_{m-1} \xrightarrow{v_{n-1}} S_{mn-1}x_m \\ &S_{mn}x_m \xrightarrow{v_n} S_{1n}x_1. \end{aligned} \tag{6}$$

The Jacobian matrix is lower triangular, with negative entries on the diagonal:

$$J = \begin{pmatrix} -S_{12}\dfrac{\partial v_2}{\partial x_1} & & & S_{1n}\dfrac{\partial v_2}{\partial x_1} \\ S_{22}\dfrac{\partial v_2}{\partial x_1} & \ddots & & \\ & \ddots & -S_{m-1\,m-1}\dfrac{\partial v_{n-1}}{\partial x_{m-1}} & \\ & & S_{mn-1}\dfrac{\partial v_{n-1}}{\partial x_{m-1}} & -S_{nm}\dfrac{\partial v_n}{\partial x_m} \end{pmatrix} \tag{7}$$

The structure of the Jacobian matrix for cyclic metabolic pathways is similar to the one for linear pathways except for an additional positive entry in the upper right corner of the Jacobian. The presence of this additional entry may cause instability. Using the Gershgorin circle theorem [14] it can be shown that stability depends on the entries of the stoichiometric matrix. Specifically, the eigenvalues of *J* have negative real part if:

$$S_{21} \cdot \ldots \cdot S_{mn} > S_{11} \cdot \ldots \cdot S_{m-1n-1} \cdot S_{1n}. \quad (8)$$

Here the left hand side of the inequality (8) represents the product of the stoichiometric coefficients that appear on the diagonal of the Jacobian matrix. These stoichiometric coefficients correspond to the consumption of reaction substrates. The right hand side of the inequality (8) represents the product of the stoichiometric coefficients that appear on the off-diagonal entries of the Jacobian matrix. These stoichiometric coefficients correspond to creation of reaction products. Stability of steady states for metabolic cycles therefore relies on the structure of the network.

B. Stability in metabolic pathways with multiple-substrates-multiple-products reactions

For linear pathways with multiple-substrates-multiple-products reactions steady states form manifolds [10]. The Jacobian is a block lower triangular matrix. All the entries on the diagonal blocks are negative. The spectrum of such Jacobian is the union of the eigenvalues of diagonal blocks:

$$B_j = \begin{pmatrix} -S_{ij}\frac{\partial v_j}{\partial x_z} & -S_{ij}\frac{\partial v_j}{\partial x_{z+1}} & \cdots & -S_{ij}\frac{\partial v_j}{\partial x_{z+p}} \\ -S_{i+1j}\frac{\partial v_j}{\partial x_z} & -S_{i+1j}\frac{\partial v_j}{\partial x_{z+1}} & \cdots & -S_{i+1j}\frac{\partial v_j}{\partial x_{z+p}} \\ \vdots & \vdots & \vdots & \vdots \\ -S_{i+kj}\frac{\partial v_j}{\partial x_z} & -S_{i+kj}\frac{\partial v_j}{\partial x_{z+1}} & \cdots & -S_{i+kj}\frac{\partial v_j}{\partial x_{z+p}} \end{pmatrix} \quad (9)$$

The rank of each diagonal block is equal to one. Hence, the only nonzero eigenvalue in each diagonal block B_j is equal to the sum of its diagonal entries, i.e. to the trace, $\lambda_i = Tr(B_i)$. Since the trace of each diagonal block is always negative, the only nonzero eigenvalue λ_i in B_i is negative. Hence, the spectrum of the Jacobian matrix in this case consists of negative and zero eigenvalues. It can be shown [10] that for metabolic pathways with multiple-substrates-multiple-products reactions the steady states form a manifold with a dimension corresponding to the number of zero eigenvalues. Since the rest of the eigenvalues have negative real part, such manifolds of steady states are locally attractive.

The Jacobian matrix for branched pathways with multiple-substrates-multiple-products irreversible reactions has a structure that is similar to the one for linear pathways. In the case there is one metabolite in the branching point the eigenvalues spectrum of the Jacobian also has the same properties. For example, in the branching pathway (10) there is one metabolite x_2 in the branching point:

$$\begin{aligned} &\xrightarrow{v_1} S_{11}x_1 \\ &S_{12}x_1 \xrightarrow{v_2} S_{22}x_2 \\ &\qquad S_{32}x_2 \xrightarrow{v_3} S_{33}x_3 + S_{34}x_4 \xrightarrow{v_5} S_{56}x_6 \\ &\qquad S_{42}x_2 \xrightarrow{v_4} S_{45}x_5 \end{aligned} \quad (10)$$

The Jacobian matrix for the pathway (10) will have the same properties as for the linear pathways with multiple-substrates-multiple-products reactions. In the case there is more than one metabolite in the branching point (11) the Jacobian matrix might have eigenvalues with positive real.

$$\begin{aligned} &\xrightarrow{v_1} S_{11}x_1 \\ &S_{12}x_1 \xrightarrow{v_2} S_{22}x_2 + S_{32}x_3 \\ &\qquad S_{23}x_2 + S_{33}x_3 \xrightarrow{v_3} S_{43}x_4 \\ &\qquad S_{24}x_2 + S_{34}x_3 \xrightarrow{v_4} S_{54}x_5 \end{aligned} \quad (11)$$

This is because the branching leads to the appearance of additional derivatives of reaction rates with respect to substrates in the diagonal blocks B_j of the Jacobian. Such diagonal blocks that corresponds to the branching point do not necessarily have rank deficiency one and their nonzero eigenvalues do not necessarily have negative real part. In the Jacobian for the example (11) the diagonal block corresponding to the branching point is

$$B_2 = \begin{pmatrix} -S_{23}\frac{\partial v_3}{\partial x_2} - S_{24}\frac{\partial v_4}{\partial x_2} & -S_{23}\frac{\partial v_3}{\partial x_3} - S_{24}\frac{\partial v_4}{\partial x_3} \\ -S_{33}\frac{\partial v3}{\partial x_2} - S_{34}\frac{\partial v_4}{\partial x_2} & -S_{33}\frac{\partial v_3}{\partial x_3} - S_{34}\frac{\partial v_4}{\partial x_3} \end{pmatrix}. \quad (12)$$

Generally, the rank of this diagonal block is 2. Structural kinetic modelling method [15] shows that eigenvalues of the Jacobian of the pathway (11) with positive real part are possible.

Structural kinetic modelling also demonstrates that stability is also not guaranteed under all conditions for metabolic cycles with multiple substrates and products. Conditions for stability of steady states in this case are to be found.

IV. CONCLUSIONS

We investigated the local stability of steady states for metabolic pathways with arbitrary stoichiometry; focussing on pathways with irreversible reactions. For linear pathways local stability of steady states is always guaranteed. For branched and cyclic pathways this is not always the case. We provided conditions for local

stability for these types of pathways. We did not discuss the stability properties in metabolic pathways with reversible reactions. Numerical simulations show that even for linear pathways with multiple-substrates-multiple-product-reactions stability is not guaranteed. This contrasts with previous results on stability of steady states in metabolic networks with reversible reactions and stoichiometry 0 or ±1 [6-10]. In such networks steady states are always locally stable. Apparently, stoichiometry plays a crucial role in stability properties of steady states in metabolic networks. A natural example of the influence of stoichiometry on stability of steady states is the well-known phenomenon of glycolytic oscillations [16].

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