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Spatiotemporal dynamics of the Calvin cycle: Multistationarity and symmetry breaking instabilities

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ABSTRACT

The possibility of controlling the Calvin cycle has paramount implications for increasing the production of biomass. Multistationarity, as a dynamical feature of systems, is the first obvious candidate whose control could find biotechnological applications. Here we set out to resolve the debate on the multistationarity of the Calvin cycle. Unlike the existing simulation-based studies, our approach is based on a sound mathematical framework, chemical reaction network theory and algebraic geometry, which results in provable results for the investigated model of the Calvin cycle in which we embed a hierarchy of realistic kinetic laws. Our theoretical findings demonstrate that there is a possibility for multistationarity resulting from two sources, homogeneous and inhomogeneous instabilities, which partially settle the debate on multistability of the Calvin cycle. In addition, our tractable analytical treatment of the bifurcation parameters can be employed in the design of validation experiments.

1. Introduction

The development of techniques for increasing plant biomass holds the promise of engineering plants which can be used for production of biofuels in a sustainable carbon-neutral fashion. Plant biomass is the outcome of complex biochemical reactions reflecting the necessity for balancing conflicting demands for resources to maintain cell vitality and function with those to support growth. Plant growth depends on the uptake and assimilation of inorganic nutrients and the photosynthetic assimilation of carbon dioxide (CO_2) via the Calvin cycle (Stitt and Krapp, 1999). This CO_2-assimilating pathway takes place in the chloroplast of photosynthetic plant cells yielding carbon skeletons necessary for maintenance of the entire plant metabolism. Therefore, understanding the mechanisms of the Calvin cycle can propel the design of techniques for manipulation of its efficiency.

The study of cell metabolism has traditionally focused on determining the factors that influence metabolic rates, at levels of both metabolic pathways and the whole organism (Heinrich and Schuster, 1996). Although there has been a significant progress in the structural analysis of metabolic pathways in order to understand and predict the distribution of cellular fluxes (Palsson, 2000; Schuster et al., 2000; Grimbs et al., 2007), addressing the problem of efficient biomass production requires elucidation of the dynamical properties of plant metabolic models. The question arises as to whether there exists a qualitative dynamical feature of plant-specific metabolic pathways which results in possibilities for increasing the production of biomass.

Multistationarity is a qualitative feature of systems, characterized by the existence of multiple positive steady states, with great potential for application in biotechnology. Biological entities (*i.e.*, genes, proteins), biochemical pathways, and cells operate in one of multiple exclusive states at any given time. For instance, a gene can either be expressed or not expressed, glycolysis and gluconeogenesis represent mutually exclusive metabolic states, and a stem cell may be at an undifferentiated state or committed to differentiating to a particular lineage (Chatterjee et al., 2008). As pointed out in Prigogine and Nicolis (1967), there are at least two sources for multistationarity: (1) instabilities with respect to space-independent (*homogeneous*) perturbations, whereby the system goes from one to another homogeneous steady state, which may or may not be stable, and (2) instabilities with respect to space-dependent (*inhomogeneous*) perturbations, when the diffusion plays a crucial role by increasing the manifold of possible perturbations. From a biotechnological perspective, altering the control of multistationarity in

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biological systems offers means for manipulating the outcome of a particular biochemical process.

Given a stimulus, the control of a biological switch, characterized with two *stable* steady states, is established via perturbation of the stimulus' concentration: When it changes over a threshold value, the entire system undergoes a transition from one to the other stable state, without residing in an in-between state due to the instability of the latter. The stimulus which exhibits such a property is referred to as *bifurcation parameter*. Bifurcation parameters can be endogenous or exogenous to the system. Typical endogenous bifurcation parameters include the kinetic parameters associated with a particular biochemical reaction, while exogenous parameters include conservation relations of some chemical element. We note that the response of individual biochemical reactions to changes in the bifurcation parameter is continuous and graded; however, the combination of these graded responses gives rise to a bistable (switching) behavior.

For experimental validation of bistability, one relies on the threshold property for the applied stimulus: The threshold concentrations of the stimulus for the two possible transitions between the steady states (from the first to the second steady state and vice versa) are different. Therefore, two response curves can be generated by adding/subtracting small increments of the stimulus, resulting in a *hysteresis diagram*. However, such experimental approaches on a population level could have contradicting results; namely, the compounded effect of the individual bistable cellular responses may appear graded for the population itself. The contrast between population and single cell levels has been illustrated experimentally in a number of systems, including *Xenopus levis* oocytes (Bagowski et al., 2001, 2003). We point out that the experimental set up for monitoring the photosynthetic response in plants may be further hindered by the heterogeneous population of cells in a leaf or a rosette, since not all cells demonstrate photosynthetic capacity. However, experimental approaches relying on isolated chloroplasts may prove useful in the study of the existence of multistationarity in photosynthetic processes.

The theoretical analysis of multistationarity in biological systems is performed on a kinetic model comprising a set of biochemical reactions. Therefore, any conclusions regarding multistationarity of the studied system ultimately depend on the employed model. The general numerical approach relies on conducting stability analysis of a given model through the following steps: (1) a steady state is calculated, (2) perturbation of the system is imposed to establish the stability of the steady state, (3) perturbation of the stimulus' concentration is imposed to check the transition to a new (stable) steady state. The existing studies focus on multistationarity (and multistability) in gene-regulatory and signaling networks (Kaneko and Yomo, 1994; Nakajima and Kaneko, 2008; Koseska et al., 2010; Tyson et al., 2003).

Unlike gene-regulatory and signaling networks, metabolic pathways with capacity for multistationarity can be characterized intuitively as transiting between states which result in different composition and quantity of biomass. Development of detailed kinetic models of metabolic pathways, however, requires information about the rate equations, enzyme-specific kinetic parameters, and substrate/product regulatory mechanism. Nevertheless, recently established mathematical approaches render it possible to infer sound statements about multistationarity of metabolic networks even when kinetic parameters are not known.

With respect to the multistationarity of a set of biochemical reactions, two questions are crucial: (1) Do the biochemical reactions have the capacity for multistationarity irrespective of the kinetic parameters? and (2) Given a (partial) set of kinetic parameters, which element of the biochemical reactions can be considered a bifurcation parameter? To answer the first question, one needs to establish a relation between multistationarity and the underlying structure of the biochemical reactions. Knowing whether a network can operate in more than one steady state only partially addresses the multistationarity analysis, since one still has to determine the regions of the parameter space in which multistationarity occurs. The answer to the second question pinpoints precisely these regions.

Due to the potential for biotechnological applications of multistationarity, the question as to whether the Calvin cycle could operate in multiple steady states is of paramount importance. Despite the large number of models for the Calvin cycle, the analysis of the existence and experimental validation of multiple steady states in this pathway is still fragmentary, usually resulting in contradictory conclusions. Pettersson and Ryde-Pettersson (1988) found two steady states for their model of the Calvin cycle. However, they showed that one of these steady states is unstable and therefore considered to be of no biological relevance, while the remaining stable steady state was in accordance with previous experiments (Flügge et al., 1980; Heldt et al., 1977). Poolman et al. (2000) also demonstrated that their extension of the model of Pettersson and Ryde-Pettersson (1988) exhibits two steady states. Moreover, Poolman et al. (2001) attempted to experimentally verify this result; however, the two observed steady states were found in leafs of different age and therefore have different capacities of utilizing the produced carbohydrates (Olçer et al., 2001). It is still unclear to which extent these results hold within one single chloroplast or leaf. A systematic approach was taken by Zhu et al. (2009), using a sophisticated algorithm to find all roots of a system of polynomials. The application of this approach to a simple model of the Calvin cycle revealed 40 steady states, of which 39 were biological infeasible due to extremely small or even negative metabolite concentrations. Although this analysis was limited to a given set of kinetic parameters, Zhu et al. (2009) concluded that the Calvin cycle can operate in only one steady state.

Here we systematically analyze the capacity for multiple steady states in a model of the Calvin cycle endowed with a hierarchy of kinetic laws based on two mathematical approaches: Chemical Reaction Network Theory (CRNT), together with its extension based on elementary flux modes, and algebraic geometry. The hierarchy of kinetic laws imposed on the set of biochemical reactions describing the Calvin cycle offers the means for determining the necessary and sufficient conditions for the existence of two steady states in this particular model. Moreover, we determine the set of bifurcation parameters which could be helpful in experiment design for validation of our theoretical findings. In addition, we explore the possibility for the existence of symmetry breaking instabilities in a slightly modified model of the Calvin cycle. Our results partially settle the debate about the existence of multistability in a model of the Calvin cycle and contribute an alternative interpretation of the existing experimental data.

The paper is organized as follows: In Section 2 we briefly review the mathematical apparatus needed for studying the relation between the structure of the Calvin cycle and its capacity for multistationarity. The hierarchy of kinetic laws embedded in the Calvin cycle is described in Section 3. The general approach is outlined in Section 4 and then applied in Section 5. We present our findings for the existence of multiple steady states in a model of the Calvin cycle for four types of kinetics: mass action, Michaelis–Menten via mass action, irreversible Michaelis–Menten, and mass action with diffusion kinetics, in Sections 5.1–5.4, respectively. Finally, in Section 6, we conclude with the implications or our findings and the necessity of a carefully tailored experiment for validation.

2. The Structure of a Model for the Calvin Cycle

The Calvin cycle consists of three phases in which there is energy supply in form of ATP and redox elements (NADP/NADPH):