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Regulatory design in a simple system integrating membrane potential generation and metabolic *ATP* consumption. Robustness and the role of energy dissipating processes

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ABSTRACT

Bacterial physiological responses integrate energy-coupling processes at the membrane level with metabolic energy demand. The regulatory design behind these responses remains largely unexplored. *Propionigenium modestum* is an adequate organism to study these responses because it presents the simplest scheme known integrating membrane potential generation and metabolic ATP consumption. A hypothetical sodium leak is added to the scheme as the sole regulatory site. Allosteric regulation is assumed to be absent. Information of the rate equations is not available. However, relevant features of the patterns of responses may be obtained using Metabolic Control Analysis (MCA) and Metabolic Control Design (MCD). With these tools, we show that membrane potential disturbances can be compensated by adjusting the leak flux, without significant perturbations of ATP consumption. Perturbations of membrane potential by ATP demand are inevitable and also require compensatory changes in the leak. Numerical simulations were performed with a kinetic model exhibiting the responses for small changes obtained with MCA and MCD. A modest leak (10% of input) was assumed for the reference state. We found that disturbances in membrane potential and ATP consumption, produced by environmental perturbations of the cation concentration, may be reverted to the reference state adjusting the leak. Leak changes can also compensate for undesirable effects on membrane potential produced by changes in nutrient availability or ATP demand, in a wide range of values. The system is highly robust to parameter fluctuations. The regulatory role of energy dissipating processes and the trade-off between energetic efficiency and regulatory capacity are discussed.

1. Introduction

Microbial physiology integrates surface processes occurring at the cytoplasmic membrane level with volume processes taking place in the interior of the organism. The transport of molecules relies on the existence of electrochemical potentials, the membrane potential being the main driving force for many of them [1]. Maintaining the membrane potential in the physiological range of values requires energy consumption and is a central target of regulatory mechanisms. On the other hand, metabolism consumes energy to synthesize metabolites required for cellular maintenance and growth. It is also highly regulated, to achieve a coordinated production of cellular components. Membrane and metabolic processes share many cellular components, but little attention has been paid to how the action of these processes is integrated. Can these two types of processes be independently regulated? If so, in what way?

Propionigenium modestum is an adequate system to study this type of questions because it presents the simplest reaction scheme known integrating membrane potential generation and metabolic energy consumption. The bacterium is strictly anaerobic, obtaining its total energy needs from succinate fermentation [2]. The nutrient is used to produce an electrochemical gradient of Na^+ which is the energy source to synthesize the *ATP* needed in metabolic processes. The steps involved in this process have been well established by Dimroth and colleagues [3–11]. It starts with the transfer of the *CoA* portion from propionyl-*CoA* to succinate, producing succinyl-*CoA* and propionate, which leaves the cell. Succinyl-*CoA* is rearranged to (R)-methylmalonyl-*CoA*, which is then converted to (S)-methylmalonyl-*CoA*. A Na^+ pump decarboxylase couples the decarboxylation of (S)-methylmalonyl-*CoA* to the transport of Na^+ ions to the external milieu across the cytoplasmic membrane. The product of this last reaction, propionyl-*CoA*, is employed to convert a new succinate molecule into succinyl-*CoA*. The overall process transforms one mole of succinate into one mole of propionate and one mole of CO_2. The electrochemical Na^+ gradient thus generated is coupled to *ATP* synthesis by a Na^+-translocating *ATP* synthase. This reaction is the only source of *ATP*, driving all the *ATP*-consuming metabolic pathways. Energy metabolism in *P. modestum* is entirely based on Na^+ as coupling ion and does not involve substrate level or electron transport

Abbreviations: MCA, Metabolic control analysis; MCD, Metabolic control design

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phosphorylation. This type of energy metabolism, in which the electrochemical Na^+ gradient couples decarboxylation with *ATP* synthesis, was termed decarboxylation phosphorylation.

The energy coupling device described must maintain the membrane potential and the *ATP* consumption at physiological values in order to ensure normal metabolic functioning. To achieve this goal, we assume that a certain part of the energy accumulated in the gradient is dissipated through a Na^+leak, which is uncoupled to *ATP* production. Brand and colleagues have extensively studied the physiological significance of mitochondrial proton leak in animal cells and tissues, finding that several important regulatory functions rely on this type of energy dissipating process [12–16]. In the present work we show, for the reaction scheme of *P. modestum* [3,8], that the Na^+ leak is required to perform essential adaptive regulatory responses. For instance, when changes in the nutrient concentration, external Na^+ concentration or metabolic *ATP* demand take place, the membrane potential can be kept at physiological values in a wide range of conditions by adjusting the leak only. In addition, the regulatory responses based on adjusting the leak do not significantly affect the *ATP* consumption, which is set to satisfy the metabolic demand for *ATP*.

2. Methods

2.1. Differential equations representation

The relevant information of the energy metabolism of *P. modestum* (Section 1) is incorporated in a differential equations model, representing the dynamic and steady-state description of the system (Section 3.1). The starting system, having five differential equations, is reduced to a system of two equations. The original rate equations are transformed into reduced rates, which depend on membrane potential and *ATP* only. This reduced system is the one used to perform a steady-state MCA and MCD.

2.2. Metabolic Control Analysis

The theory of Metabolic Control Analysis (MCA) expresses the systemic responses of metabolic variables, quantified by the control coefficients, in terms of the sensitivity properties of the component reactions, represented by the elasticity coefficients [17–23]. These expressions may be used to calculate the control coefficients from the experimentally determined elasticity coefficients. If the elasticity coefficients have not been measured, the expressions may also be used to obtain some general features of the patterns of responses. For instance, the fact that not all intermediates in the reaction scheme directly affect all the rates results in that many elasticity coefficients are zero. In addition, the signs of the elasticity coefficients (normally positive for substrates and activators, and negative for products and inhibitors) condition the patterns of responses that can be obtained. In this way, the signs of control coefficients, their lower or upper bounds and inequalities among them may be obtained with very limited information. In Section 3.2, we use MCA to analyze this type of properties for the reaction scheme of *P. modestum*.

2.3. Metabolic Control Design

Metabolic Control Design (MCD) solves the inverse problem to MCA, i.e. it calculates the sensitivity properties of the component reactions that are needed to obtain a predetermined pattern of systemic responses [24–25]. The control coefficients are not all independent. MCD starts by stating all the constraints involved. These are structural, kinetic or those imposed by design. The theory of MCD expresses the elasticity coefficients as functions of the independent control coefficients. Combining these expressions with those of MCA, all the control coefficients may be expressed in terms of the independent ones. These equations are useful to determine which types of responses may be expected to simultaneously occur, and which patterns of responses are not possible.

Application of MCA and MCD to systems with conservation constraints is normally solved incorporating a link matrix, which is used to correct the values of the elasticity coefficients [19, 24]. An alternative way to solve this problem is, first, to reduce the system and, afterwards, to apply to the reduced system the theory of MCA and MCD developed for systems without conservation constraints. This second way is the one used below. It has the great advantage of leading to much simpler expressions relating component and system coefficients. The additional difficulty is that the signs of the elasticity coefficients of the reduced system must be deduced from those of the non-reduced one, a task that is nevertheless reasonably simple for the system under study.

It is important to remember that traditional MCA and MCD were developed for infinitesimal changes. Control and elasticity coefficients are systemic properties and their values change when the parameters of the system are changed. Therefore, the results obtained strictly hold for the reference state. Several contributions have extended MCA and MCD to large changes [26–30]. However, the type of structure studied here may not be analyzed with the formalisms for large changes developed to date.

2.4. Numerical simulations

Numerical simulations were used to study the steady-state behavior of the system when subject to large parameter changes. The parameter values of the rate equations were assigned so that the system responds to small changes as calculated using MCA and MCD. For this aim, the following steps were carried out. First, adequate rate laws were assigned to each of the processes in the reaction scheme. The reduced rates were algebraically differentiated and scaled to obtain the expressions of the elasticity coefficients. These were used to calculate the control coefficients, in terms of the parameters and the reference values of the variables. The elasticity coefficients are independent of the corresponding protein concentrations, because the rate equations used are proportional to those concentrations. The protein concentrations were assigned so that the fluxes take pre-established values. The kinetic constants were drawn from plausible ranges of values, keeping those that render suitable values of the control coefficients. A set of parameter values, fulfilling all the conditions deduced with MCA and MCD, was used to perform the numerical simulations. The system of differential equations was integrated until the steady state of all the variables was reached. The steady-state values of the reference state and the states after parameter perturbations were determined. Calculations were performed using the program Mathematica (Wolfram Research Inc).

3. Results and discussion

3.1. Dynamic and steady-state description of the variables

In the reaction scheme of Fig. 1, we represent the processes of Na^+ gradient generation (v_G), *ATP* synthesis from the electrochemical gradient (v_S) and *ATP* consumption by metabolic processes (v_M), described in Section 1. Our study will focus on the interplay between *ATP*-generating membrane processes and *ATP*-consuming metabolic processes. Therefore, the steps transforming succinate into propionate and CO_2, and the network of reactions consuming *ATP* are each aggregated into single steps. A passive transport of anions (v_A) was incorporated to fulfill electroneutrality. The reaction scheme also includes a step corresponding to the Na^+ leak (v_L). This rate represents the dissipation of the Na^+ gradient uncoupled to *ATP* synthesis. The Na^+ leak in *P. modestum* has not been experimentally measured but, as we shall see, it turns out to be an essential piece in the regulatory design of the system.

The two transporters and the leak are electrogenic, i.e. they carry net electric currents contributing to the electrical potential across the membrane. Thus, the rates of the transporters and of the Na^+ leak depend on membrane potential, V_m, as represented in the scheme. The rates depend on the concentrations of their corresponding substrates and products,