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Mathematical Modeling of Bacterial Metabolism

A. N. Golomysova[a], M. Gomelsky[b], and P. S. Ivanov[a]

[a] *Department of Biophysics, Faculty of Physics, Moscow State University, Moscow, 119991 Russia*
[b] *Department of Molecular Biology, University of Wyoming, E. University Ave., Dept. 3944, Laramie, WY, 82071 USA*
e-mail: agolomy@yandex.ru

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Abstract—A variant of mathematical model construction for the anaerobic metabolism of a purple nonsulfur bacterium based on a flux balance analysis is presented in this paper. The model includes all the central metabolic pathways of the bacterial cell, as well as pathways for biosynthesis of amino acids and bacteriochlorophyll. It is shown that the results of modeling of bacterial photoheterotrophic growth on acetate agree with experimental values for the known metabolite pathways, which shows that the constructed model adequately describes actual cellular processes.

Key words: metabolism modeling, linear programming, flux balance analysis, stoichiometric model

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INTRODUCTION

The reconstruction and modeling of complex biological systems, such as cellular metabolism, is one of the main goals of systems biology. This modeling includes not only the formation of a biochemical reaction network, but also a prediction of metabolite flux intensity (reaction rate) through each network branch and the system's behavior under various medium conditions [1].

In recent years the approach known as Flux Balance Analysis (FBA) or metabolic flux analysis (MFA), which is also called stoichiometric modeling below, has been successfully applied to the characterization of the flux distribution inside cells [2–4]. Linear optimization of steady-state metabolite flux distribution is carried out in this method under a predetermined reaction stoichiometry. The assumption of a steady-state implies that the yield rate of any metabolite is considered to be equal to its consumption rate, and, as a result, the problem is reduced to a system of linear equations. As the quantity of reactions in a metabolic network is usually more than the quantity of metabolites, the system of equations appears to be underdetermined and to have an infinite number of solutions. When applying FBA, one solution, which yields the maximum of a certain linear combination of fluxes called the objective function, is selected out of the domain of possible solutions using linear programming. Selection of the objective function is crucial, as it simulates the goal of cellular activity. For example, researchers are often interested in the solution that corresponds to the maximum cellular growth rate (or maximum biomass production), because the fastest-growing organisms survive natural selection [5]. An alternative method is to seek the solution that provides the maximum yield of energetic equivalents (Adenosine Triphosphate or ATP), which are necessary not only for growth, but for all types of cellular activities [6].

When it's impossible to construct detailed kinetic models to study complex metabolic systems, due to lack of sufficient data, flux models are often used, owing to their simplicity. These models both help to explain the known cellular behavior [3, 4] and investigate the possibilities of affecting cellular metabolism [7, 8].

The flux models of several bacterial cells have been constructed and software methods for their construction on the basis of annotated genomes have been developed [8].

In this work, we present flux model of main metabolic pathways for purple nonsulfur bacterium of the *Rhodobacter* genus [9]. The photoheterotrophic growth of the bacterium was investigated for the case of an oxygen deficit. This type of growth is of a special interest, as it's characterized by a large bacteriochlorophyll yield [10] and bacterial ability to accumulate polyhydroxybutyrate [11] and produce molecular hydrogen. This bacterium is considered as a potential producer of hydrogen fuel, which makes the modeling of its metabolism especially topical [12].

1. STOICHIOMETRIC MODEL

Stoichiometric or flux models usually include two main parts. The first part is the list of biochemical reactions for all the metabolic pathways represented in the model. The activity of the corresponding enzyme in a particular organism is checked for each reaction. The second part of a model is a mathematical algorithm, which transforms the input set of stoichiometric coefficients and boundary conditions into the output values of reaction rates.

1.1 Formation of the List of Reactions

Metabolic analysis begins with consideration of the central part, which includes glycolysis, the pentosophosphate pathway, the tricarboxylic acid cycle, and the Calvin cycle [13, 14]. The biochemical reactions for these parts, as well as the genes encoding the corresponding enzymes, have been thoroughly investigated. The list of reactions for the main metabolism was obtained for the species *Rhodobacter sphaeroides* from the KEGG (Kyoto Encyclopedia of Genes and Genomes) database [15]. Although acetate is one of the growth substrates for this bacterium, the lack of the glyoxylate pathway enzyme, which is necessary for acetate fixation, is one of the metabolic peculiarities of bacteria of the *Rhodobacter* genus. An ethylmalonyl bypass pathway has been discovered in *Rhodobacter sphaeroides* recently, which hasn't been entered into the KEGG database yet. The corresponding reactions were added to the list in accordance with [16].

As cellular growth implies the synthesis of the main building blocks of the cell, the synthesis pathways of all amino acids and substance efflux for synthesis of the lipids were added to the system. Lists of reactions for these pathways were obtained from the KEGG database as well.

Moreover, we included photosynthesis stages, which are of a special interest in phototrophic bacterial growth in the list of reactions. As purple bacteria have cyclic photosynthesis, while the considered processes are steady-state, we reduced photosynthesis to two reactions, namely photon absorption and proton passage through the membrane. A difference in proton concentrations at the membrane is created due to the mentioned fluxes, which is spent on the synthesis of the energetic equivalents ATP and NADH (Nicotinamide adenine dinucleotide) according to the model in [17]. If no limitations are imposed on the photosynthesis rate, the cell will produce an unlimited amount of energy. However, it's difficult to calculate the numerical values of the upper rate boundary for such a complex process. Therefore, it's assumed that the amounts of bacteriochlorophyll and carotenoids, which are necessary for light absorption, are the limiting factors in the model. Carotenoid biosynthesis reactions were taken from the KEGG database, and MetaCyc (Multiorganism Metabolic Pathway and Enzyme Database) was used for bacteriochlorophyll [18].

As purple bacteria have the ability to produce hydrogen [12] and accumulate polyhydroxybutyrate [11], the corresponding biochemical reactions were added to the model as well.

The majority of the metabolites that are involved in only two reactions were excluded from the model for the sake of simplification, and the corresponding reactions were replaced by one. The resulting list includes 257 reactions and 216 metabolites.

1.2 Flux Balance Analysis (FBA)

The list of reactions was transformed into a matrix of stoichiometric coefficients $\mathbf{S}$ with dimension $m \times n$, where m is the quantity of reactions and n is the number of metabolites in the model. In other words, the rows of the matrix $\mathbf{S}$ correspond to metabolites, and the columns to reactions. The active mass law for the system of reactions may be written as follows:

$$\frac{d\mathbf{x}}{dt} = \mathbf{S} \cdot \mathbf{v}, \quad (1)$$

where $\mathbf{x}$ is the vector of metabolite concentration and $\mathbf{v}$ is the vector of reaction (flux) rates.

The metabolite concentrations are constant in the steady-state approximation, and (1) is as follows:

$$\mathbf{S} \cdot \mathbf{v} = 0. \quad (2)$$

This is the basic limitation for the system used in FBA. In addition, limitations are imposed on reaction rates:

$$\alpha_i \le v_i \le \beta_i, \quad i = 1, \ldots, m, \quad (3)$$

where the constants α and β are deduced from the known limitations on the activity of enzymes catalyzing the corresponding reactions.

Closed cycles and alternative pathways may usually be observed in the system of biochemical reactions; therefore, the quantity of reactions appears to be more than the number of metabolites. This means that the quantity of linear equations in (2) is less than the quantity of variables, and the solutions of (2) form a polyhedron in the multidimensional space of fluxes. The indicated variety of solutions describes the possibilities of cellular metabolism.

To simulate the particular metabolic regime, one solution, which corresponds to the extreme value of the objective function, is selected from the domain of solutions. The objective function is constructed as a linear combination of fluxes:

$$v_{\text{of}} = \mathbf{c} \cdot \mathbf{v}, \quad (4)$$

where $\mathbf{c}$ is the vector of coefficients of the objective function.

Thus, the following linear programming problem is created in the FBA [7]:

$$\text{maximize of } v_{\text{of}} = \mathbf{c} \cdot \mathbf{v}$$

$$\text{subjected to conditioons} \begin{cases} \sum_{i=1}^{m} S_{ij} v_i = 0, \quad j = 1, \ldots, n, \\ \alpha_i \le v_i \le \beta_i, \quad i = 1, \ldots, m. \end{cases} \qquad (5)$$

The standard simplex method implemented in the VC++ environment was used for numerical solution of (5).

1.3 Selection of the Objective Function

The objective function (4) imitates the goal of cellular activity, therefore, its selection requires special attention. Generally, the biomass production is maximized [3, 5, 7, and 14] in the process of stoichiometric model construction. As a matter of practice it is equivalent to the yields of the metabolites of glycolysis and the tricarboxylic acid cycle in physiological relationships. The rest of the metabolic pathways may be negative in this case; therefore, such a selection of the objective function is not useful for detailed investigations of metabolism. For example, in order to investigate lysine production by the bacterium *Escherichia coli*, the authors of [3] had to introduce an additional objective function related to the metabolism of this amino acid.

Objective functions related to energetic equivalent yield are used more rarely [5, 8]. Although ATP and NADH are necessary not only for growth, but for all types of cellular activity, these molecules are synthesized in sporadic reactions [6]. The data on the directions of these reactions should be introduced into the model explicitly, otherwise, the simulated system will start producing energy in all the theoretically accessible ways, which doesn't agree with the known information about cellular physiology.

Photosynthetic flux is taken as the objective function in the present model, because, on the one hand, it's directly connected with ATP production, and, on the other hand, its rate is limited by the amounts of bacteriochlorophyll and carotenoids [17]. Moreover, the fluxes related to the synthesis of amino acids and lipids were specified in the model using physiological relationships [13, 17].

Limitations (3) weren't introduced to the model for the majority of fluxes, in a similar manner to [7, 8]. A special exception was made for ATP synthesis reactions (their directions were specified) and Calvin cycle reactions, because this particular pathway is a metabolic bottleneck in the case of photoheterotrophic growth [17].

2. EXAMINATION OF THE MODEL

Experimental determination of the rates of reactions in the living cell may be carried out using isotope analysis [20]. However, the required data for the anaerobic phototrophic metabolism of purple bacteria weren't discovered in the available literature. Therefore, examination of the model was carried out by comparison with the known yield rates for particular metabolites, given the known substrate absorption rate.

The authors of [10] showed that the chlorophyll yield rate for the photoheterotrophic growth of *Rhodobacter capsulatus* on acetate is 10 mg per 1 g of biomass growth. When 1 g of acetate was the input of the model the chlorophyll yield was 13 mg. According to [11], the maximum yield rate of polyhydroxybutyrate for the photoheterotrophic growth of *Rhodobacter sphaeroides* on acetate is 0.03 g per 1 g of biomass growth, while the authors of [13] provide the corresponding value of 0.05 g per 1 g of biomass growth. When 1 g of acetate was the input of the model, the polyhydroxybutyrate yield was 16 mg. Thus, the values of the fluxes of carbon-containing compounds obtained in the model agree with experimental data in their order. As the experimental values of fluxes vary approximately tenfold in papers by various authors, the obtained relationship is considered confirmation of the model's performance capability.

CONCLUSIONS

A stoichiometric steady-state approximation model of the anaerobic metabolism of a phototrophic bacterium was constructed in this paper. It includes all the central metabolic pathways of a bacterial cell (glycolysis, the tricarboxylic acid cycle, the pentosophosphate pathway, and the Calvin cycle), as well as other ways of synthesizing amino acids, bacteriochlorophyll, carotenoids, polyhydroxybutyrate, and hydrogen. The simulation of the bacterial photoheterotrophic growth on acetate provided flux values for particular metabolites that are comparable to actual ones. Therefore, the applicability of the model to the description of the metabolism of this bacterium was shown.

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