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Kinetic metabolic modelling for the control of plant cells cytoplasmic phosphate

Mathieu Cloutier, Jingkui Chen, Frithjof Tatge, Virginie McMurray-Beaulieu, Michel Perrier, Mario Jolicoeur *

Canada Research Chair in Applied Metabolic Engineering, Bio-P² Research Unit, Department of Chemical Engineering, École Polytechnique de Montreal, P.O. Box 6079, Station Centre-ville, Montreal, Quebec, Canada H3C 3A7

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ABSTRACT

A previously developed kinetic metabolic model for plant metabolism was used in a context of identification and control of intracellular phosphate (Pi) dynamics. Experimental data from batch flask cultures of *Eschscholtiza californica* cells was used to calibrate the model parameters for the slow dynamics (growth, nutrition, anabolic pathways, etc.). Perturbation experiments were performed using a perfusion small-scale bioreactor monitored by *in vivo* ^{31}P NMR. Parameter identification for Pi metabolism was done by measuring the cells dynamic response to different inputs for extracellular Pi (two pulse-response experiments and a step-response experiment). The calibrated model can describe Pi translocation between the cellular pools (vacuole and cytoplasm). The effect of intracellular Pi management on ATP/ADP and phosphomonoesters concentrations is also described by the model. The calibrated model is then used to develop a control strategy on the cytoplasmic Pi pool. From the identification of the systems dynamics, a proportional-integral controller was designed and tuned. The closed-loop control was implemented in the small-scale NMR bioreactor and experimental results were in accordance with model predictions. Thus, the calibrated model is able to predict cellular behaviour for phosphate metabolism and it was demonstrated that it is possible to control the intracellular level of cytoplasmic Pi in plant cells.

1. Introduction

Plant cell metabolism possesses unique properties that can hinder the technological and commercial success of a bioprocess. One of these features is the capacity to accumulate high levels of nutrients. As an example, five- to 10-fold variations in intracellular inorganic phosphate (Pi) concentration are observed over the time course of plant cells cultures (van Gulik et al., 1993; Lamboursain and Jolicoeur, 2005). This can affect the cells secondary metabolites production potential (Lamboursain and Jolicoeur, 2005) and growth kinetics (Cloutier et al., 2007b) [...].

Understanding plant cell metabolic regulation is a challenging issue. As regards phosphate, there is regulation for Pi management under Pi limitation (Plaxton, 1998), which allows plant cells to optimize their use of Pi. Abel et al. (2000) observed that Pi-limited tomato cells exhibited a significant increase in phosphodiesterase secretion, which allows degrading extracellular nucleotides debris. Wu et al. (2003) observed that 29% of the genes in *Arabidopsis thalina* were up- or down-regulated in Pi-limiting conditions. Many of the down-regulated genes in these conditions were related to functional groups such as photosynthesis and nitrogen assimilation. Intracellular Pi also plays a central role in the regulation of enzymes activity through reversible phosphorylation processes. Many enzymes in plant glycolysis are regulated by this process, among which are pyruvate kinase, phosphoenolpyruvate carboxykinase and sucrose phosphate synthase (Huber et al., 1994). Intracellular Pi may also affect energetic shuttles concentrations and equilibrium (ATP/ADP) since it is directly involved in the conversion of ADP to ATP.

Therefore, plant cell metabolism and its regulation by Pi pools is a complex issue and has to be addressed properly, especially as its intracellular dynamics are important. Control of plant primary metabolism by metabolites concentrations (as opposed to the traditional view of gene and enzyme regulated metabolic pathways) is a subject that is gaining attention recently. Plant metabolomics for primary metabolism was reviewed by Fiehn (2006) and studies on plant metabolomics show that metabolites level can be the controlling element in plant metabolism. Borland et al. (1999) reported that the intracellular malate concentration can control the crassulacean acid metabolism in plants under certain conditions and Carrari et al. (2003) also reported that the regulation of TCA cycle by metabolic intermediates is significant in plants.

These reports on intracellular dynamics of plant metabolism partly explain why little success is achieved when applying the

* Corresponding author. Tel.: +1 514 340 4711x4525; fax: +1 514 340 4159.
E-mail address: mario.jolicoeur@polymtl.ca (M. Jolicoeur).

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classical tools of metabolic engineering to improve plant-based bioprocesses. Metabolic flux analysis (MFA) and metabolic control analysis (MCA) can be used to improve the catalytic properties of cells through a better understanding of their metabolic features. Even though these approaches are now applied to plant cells (Rontein et al., 2002; Hughes et al., 2004; Sriram et al., 2004; Ratcliffe and Shachar-Hill, 2006) many challenges are still encountered because of the limited quantitative data on the specific dynamics and regulation of plant cell metabolism. Moreover, the common approaches (MFA, MCA, isotopomer balancing, etc.) to study metabolic systems are mostly based on steady-state analysis, which is obviously not representative of a majority of plant cells bioprocesses.

Since plant cells intracellular nutrients levels affect pant metabolism and complicate the analysis of the system, the modulation or control of these nutrients levels will be a fundamental advance in our capacity to understand and improve the metabolic properties of plant cells. In this study, the predictive capacity of a kinetic metabolic model will be used as a basis to develop a closed-loop control strategy for plant cells cytoplasmic Pi concentration in a small-scale bioreactor monitored by ^{31}P NMR. This is, to the best of the authors' knowledge, the first time that closed-loop control is achieved on intracellular concentration [...]. This study will thus provide powerful analytical and experimental tools for future works on the regulation and control of metabolic systems.

2. Dynamic modelling for control of plant cell metabolism

The model used in this study is based on previous works (Leduc et al., 2006; Cloutier et al., 2007a) on hairy roots metabolism. Here the model will be applied to shake flask cultures of *Eschscholtiza californica*. The model is also used to analyse Pi compartmentation, regulation and metabolism of the same *E. californica* cell line cultivated in a perfusion small-scale bioreactor monitored by ^{31}P NMR. The ^{31}P NMR experiments yield valuable insight on phosphate and energy metabolism by rapid and quantitative measurement of Pi pools (vacuolar and cytoplasmic) and phosphated metabolites (phosphomonoesters, ATP and ADP) during perturbation experiments. Pi is often the limiting nutrient in plant cultures and has fast uptake and allocation dynamics, as observed by Cloutier et al. (2007a) in a simulation of *Catharanthus roseus* central primary metabolism. Thus it is believed that the assessment of these dynamics with the appropriate experimental tools is fundamental for the development of an efficient modelling strategy. The use of data on the energetic state of the cells (quantitative ATP and ADP measurements) will allow a precise evaluation of the influence of energy metabolism on the glycolytic fluxes. As reported by Urbanczyk-Wochniak et al. (2003) and Alonso et al. (2005), the energetic management in plant cells might be a significant factor in the control of the glycolysis. The use of data sets from different experiments (shake flask and perfusion small-scale bioreactor) with different timescales (days versus hours) allowed testing the capacity of a dynamic model to describe plant cell behaviour in different experimental conditions.

Finally, the most common argument in favour of dynamic modelling is the capacity to predict experimental results and to improve bioprocess strategy (culture conditions, medium composition and feeding, etc.). In this work, the dynamic model was used to develop a control loop for the cytoplasmic Pi (CPi) concentration. The implementation of this experimental strategy was simplified by the use of the model as a tool to simulate the system composed of the cells cultivated in the small-scale bioreactor and the control loop.

3. Model description

The model for *E. californica* suspension (Fig. 1) is based on the model presented for hairy roots in Cloutier et al. (2007a). This fully dynamic model includes 46 reactions and 41 metabolic species. A complete description of the model, stoichiometric equations, fluxes regulation and kinetic parameters is presented in Appendix A. The hypotheses for model development were discussed in Leduc et al. (2006) and Cloutier et al. (2007a) with extensive description of pathways and regulations mechanisms. The approach of identifying parameters with experimental time profiles for metabolites and nutrients was also discussed in previous works. The presentation of the model in Appendix A is thus limited to the structural changes (i.e. changes other than parameters values) that were made to describe the culture conditions and the observed plant cell metabolic behaviour in the specific context of the present work.

4. Development of a controller for cytoplasmic phosphate

Since *in vivo* NMR allows for a fast and on-line measurement of the Pi pools in the cells, it is possible to implement a control strategy on Pi metabolism using the extracellular Pi (EPi) concentration as the manipulated variable. As was observed by Mauch et al. (1997) the intracellular dynamics of a metabolic system can be affected by an adequate perturbation in extracellular concentrations. The manipulated variable used in this study (EPi) is easily modulated since the NMR small-scale bioreactor is operated in perfusion mode. CPi was chosen as the controlled variable because it is the first storage pool for Pi and is involved in the regulation of many reactions of the primary metabolism of plant cells, as mentioned previously.

The controller designed in this study is a proportional-integral (PI) controller. This design was chosen from the direct synthesis method (Ogunnaike and Ray, 1994). In that regards, a first order with time delay model was identified for the process and the desired closed-loop response was set as a first order. With these specifications, the design by direct synthesis will yield a PI controller. This design also has the advantage that it does not require the measurement of the derivative on the CPi signal, which could be problematic because of the relatively low precision ($\approx$15%, as discussed in Rebeille et al., 1985) and time resolution (30 min) of NMR measurements in the conditions used for this study. The controller was implemented in discrete form with a control action every hour. The experimental conditions dictate this relatively low time resolution for the control actions, as measurements are taken every 30 min and delays are present in the system (medium circulation) and in the processing of NMR signals. Thus, actuating the controller every hour ensure that a response will be measured in the system before making any further decision on EPi feeding. The discrete form of the controller is written as follows:

$$EPi_n = EPi_{n-1} + K_c\left[(e_n - e_{n-1}) + \frac{\Delta t}{\tau_i}e_n\right] \quad (1)$$

where EPi_n is the actuated EPi concentration, EPi_{n-1} is the EPi concentration at iteration '$n-1$', K_c is the proportional gain of the controller, τ_i is the integral time constant, Δt is the time between two control actions (1 h in this case) and e_n and e_{n-1} are the errors (setpoint minus measurement) on CPi at iteration 'n' and '$n-1$', respectively.

More details on the parameters tuning for this controller are provided in Results and discussion section. In that regards, model simulations including the control loop will be used for further tuning of controller parameters. Thus, the calibrated