Biophysical Chemistry 206 (2015) 40–57

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

A new model for the aerobic metabolism of yeast allows the detailed analysis of the metabolic regulation during glucose pulse

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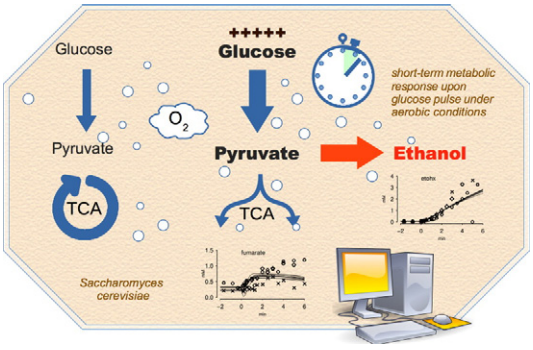
HIGHLIGHTS

- A new detailed kinetic model of aerobic energy metabolism of yeast is presented.
- Analysis of dynamics of metabolites after a glucose pulse
- Kinetics of PDC, ADH and PDH bypass pave the way for the onset of the Crabtree effect.
- TCA cycle can operate in two branches after a glucose pulse.
- Flux through the TCA cycle is regulated in two blocks.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 April 2015
Received in revised form 23 June 2015
Accepted 25 June 2015
Available online 4 July 2015

Keywords:
Aerobic glycolysis
Glucose repression
Short-term Crabtree effect
Respiro-fermentative growth
Metabolic regulation

ABSTRACT

The onset of aerobic fermentation (the so-called Crabtree effect) in yeast has long been of interest. However, the underlying mechanisms at the metabolic level are not yet fully understood. We developed a detailed kinetic model of the aerobic central metabolism of *Saccharomyces cerevisiae* comprising glycolysis, TCA cycle and major transport reactions across the mitochondrial membrane to investigate this phenomenon. It is the first one of this extent in the literature. The model is able to reproduce experimental steady state fluxes and time-course behavior after a glucose pulse. Due to the lack of parameter identifiability in the model, we analyze a model ensemble consisting of a set of differently parameterized models for robust findings. The model predicts that the cooperativity of pyruvate decarboxylase with respect to pyruvate and the capacity difference between alcohol dehydrogenase and the pyruvate dehydrogenase bypass play a major role for the onset of the Crabtree effect.

1. Introduction

Cells and organisms being able to best adapt to their environment are most likely to survive and to reproduce themselves. A well-known example for such an adaptation is the fact that Baker's yeast (*Saccharomyces cerevisiae*) rapidly converts sugar to ethanol when exposed to excess glucose even under aerobic conditions. This complex trait – known as the Crabtree effect – is thought to have been developed by yeast cells as an evolutionary beneficial adaptation to successfully compete with other microbes even though using primarily fermentation for ATP production is energetically less efficient [1].

In some circumstances this behavior is nevertheless undesired. For example, the biomass yield of yeast cells – a workhorse for many important biotechnological applications – is often decreased by the production of ethanol when cultivated on sugars under fully aerobic conditions. This generally cannot be avoided as transient exposure to glucose excess may

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http://dx.doi.org/10.1016/j.bpc.2015.06.010
0301-4622/

occur in industrial fed-batch reactors due to imperfect mixing and feeding with highly concentrated sugar solutions [2]. Interestingly, cancer cells also exhibit metabolic adaptations similar to the Crabtree effect (but referred to as the Warburg effect) which are undesired as they promote tumor growth and invasiveness [3].

Depending on the time scale one distinguishes short-term (which is in the focus of this study) and long-term Crabtree effects. Short-term aerobic fermentation (within a few minutes) is driven solely by metabolic regulation evidenced by data showing unchanged enzyme capacities during this time span. In contrast, long-term aerobic fermentation (>45 min) is considered to occur via regulation of the expression of glucose-sensitive genes [4].

It is assumed that glucose overflow metabolism is the primary mechanism behind the Crabtree effect (e.g. [1]). Thus, glucose excess leads to high glycolytic rates causing an overflow of the branch point metabolite pyruvate under the assumption that respiratory metabolism is already saturated [5]. However, the latter is still under debate [6,7]. The surplus of available pyruvate is then directed via pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) towards ethanol. Pronk et al. suggested that this is also driven by the kinetic properties of PDC, especially its cooperativity [5]. In addition, van Urk et al. highlight the role of differences in expression levels of PDC, ADH and pyruvate dehydrogenase (PDH) bypass enzymes [6].

S. cerevisiae belongs to a minority of respiro-fermenting yeasts which also repress respiration as an additional mechanism to further increase ethanol production. Whether this phenomenon seen in long-term adaptation may also play a role in the short-term Crabtree effect and, thus, contributes to the overflow of ethanol also at shorter time scales is under debate [1,8].

Despite these attempts to mechanistically explain the short-term events in Crabtree-positive yeasts a complete quantitative understanding of how yeast cells, and potentially also cancer cells, do rapidly and reversibly regulate their energy metabolism is still lacking. It has been shown that experimental observations together with quantitative computational modeling are valuable tools to solve such complex issues [9].

Glycolysis is probably one of the most frequently modeled metabolic systems (for a review see [10]). For yeast, full-scale models of glycolysis in intact cells have been developed for anaerobic conditions, e.g. [11–13]. Additionally, a few detailed models of aerobic, glucose-limited glycolysis do exist [4,14–17], three of them with a focus on short-term behavior [4,14,15]. The models reproduce the experimental data rather well. However, mechanistic explanations of the metabolic regulation underlying the short-term aerobic fermentation are very scarce. In all these models, the TCA cycle is either missing or lumped into one or two phenomenological equations. Thus, there is no detailed kinetic model of the TCA cycle for yeast existing in the literature. There are, however, several models of the TCA cycle with a detailed biochemical description for other organisms, e.g. [18–22].

In this study we aim at contributing to the understanding of short-term metabolic adaptation by means of a comprehensive kinetic model of yeast glycolysis, fermentation and TCA cycle. The model helps to explain the experimentally observed dynamic behavior of metabolites and fluxes after a glucose pulse applied to *S. cerevisiae* grown in glucose-limited continuous chemostat cultures at low dilution rates in the presence of oxygen.

We conclude that the cooperativity of PDC with respect to pyruvate as well as the capacity difference between ADH and the PDH bypass are major determinants for the onset of aerobic ethanol formation upon glucose pulse. This holds even for conditions where all other pyruvate utilizing enzymes are not saturated. In addition, TCA cycle fluxes are strongly influenced by the rapid depletion of CoA.

A deeper understanding of the energy metabolism of baker's yeast will be of great importance both for increasing the efficiency at the biotechnological applications of yeast and possibly developing new anti-cancer strategies.

2. Materials and methods

2.1. Computational algorithms and software used

The ODE-model has been implemented in Copasi (Build 89) [23]. Simulations were performed with the LSODA algorithm. Steady-state concentrations were calculated using the Newton method. For parameter estimation we used different global optimization algorithms, mainly Particle Swarm, Genetic Algorithm and Simulated Annealing. All these algorithms as well as Metabolic Control Analysis (MCA) and calculation of sensitivities were used as implemented in the software. The model scheme was created with VANTED v2.1.0 [24] and meets the Systems Biology Graphical Notation [25] (http://www.sbgn.org). Visualization of the data were done with Octave [26] and R (http://www.r-project.org).

2.2. Computation of metabolite concentrations, fluxes and model compartments

Since our studies use experimental data generated by Kresnowati et al. 2006 [27] we compute metabolite concentrations and compartment volumes according to their data. They measured concentrations of metabolites in μmol/g DW which we converted to mM using 2 ml/g DW as cellular volume (personal communication with J. Nielsen). Based on a dry weight (DW) of 15 g of yeast cells in 4 l culture medium we get a total intracellular volume of 0.12 l (Eq. (1)).

$$V_{cell} = \frac{15\mathrm{gDW(yeast)}}{1\mathrm{(culturevolume)}} \cdot \frac{2\mathrm{ml(yeastvolume)}}{\mathrm{gDW(yeast)}} \cdot 4\mathrm{l(culturevolume)} \quad (1)$$

We assumed that in the short time frame after the glucose pulse, the biomass concentration in the chemostat does not change. For calculating the concentrations of the metabolites residing in the mitochondria, we assumed that mitochondria make up about one tenth of the cellular volume and accordingly multiplied the respective metabolite concentrations by the factor of 10.

2.3. Computation of apparent equilibrium constants

The apparent equilibrium constants of the enzymes were taken from the literature wherever available or calculated. The calculation was done as follows: Inorganic phosphate (pi) concentration was kept constant like in other glycolysis models [11,12]. Richard et al. reported an experimentally determined pi concentration of 11 mM [28]. Hynne et al. used in their model a constant concentration of 10 mM [12]. Wu et al. whose experimental conditions apply to our model, reported a value of 50 mM [29]. In the literature, both concentrations are confirmed since a broad concentration range from 10 mM to 75 mM is reported [30]. We used a range between 10 mM to 50 mM for the calculation of the range of the apparent equilibrium constants, to which the model was fitted. Boumans et al. had reported that the physiological ionic strength in yeast lies around 200 mM [31]. The pH in the medium of the chemostat was kept at 5. However, there is varying information in the literature, how the external pH affects the intracellular pH. Valli et al. report that by decreasing the external pH from 7.0 to 2.2, a progressive reduction of the pHi (internal pH) from 7.1 to 5.1 was observed in exponentially grown yeast cells [32]. In contrast, stationary cells, which were able to maintain the pHi constant at around 6.1 when the external pH was in the range of 7.0 to 5.5, experienced a drop in pHi to 5.5 as a consequence of a reduction of the external pH to 5.0. However, Orij et al. report that pHi values in yeast are unaffected by external pH shifts between pH 3.0 and pH 7.5 and that in the chemostat cultures respiring glucose they determined a pHcyt of 6.9 and a pHmit of 7.3 [33]. Therefore we assumed a mitochondrial pH between 6 and 7. Accordingly, the calculation was made using the Standard Transformed Gibbs Energies of Formation of Reactants in kJ mol-1 at 303.15 K (temperature maintained in the cultures), Ionic Strength 0.25 M and for pHs 6 and 7.