Biophysical Chemistry 172 (2013) 53–60

Contents lists available at SciVerse ScienceDirect

Biophysical Chemistry

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

Glycolytic oscillations in a model of a lactic acid bacterium metabolism

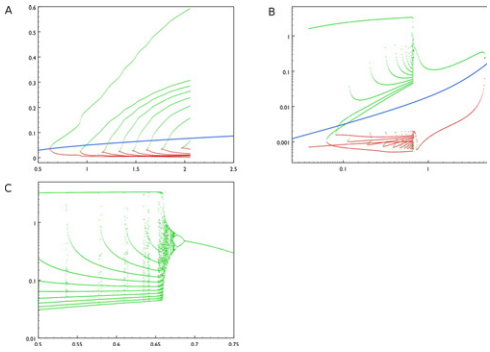
Jennifer Levering *, Ursula Kummer, Konrad Becker, Sven Sahle

Department of Modeling of Biological Processes, COS Heidelberg/BioQuant, University Heidelberg, 69120 Heidelberg, Germany

HIGHLIGHTS

- Glycolytic oscillations in a kinetic model for *Streptococcus pyogenes* occur within physiologically feasible parameter ranges.
- The stoichiometry of the system as well as its allosterically regulated enzymes can give rise to these oscillations.
- We employed established and new optimization methods for finding oscillatory regimes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 October 2012
Received in revised form 12 November 2012
Accepted 12 November 2012
Available online 12 December 2012

Keywords:
Glycolytic oscillations
Kinetic modelling
Computational simulation
Optimization

ABSTRACT

Glycolytic oscillations in yeast have been extensively studied. It is still unclear, if these oscillations are caused by the allosteric enzyme phosphofructokinase or the stoichiometry of glycolysis which contains an autocatalysis with respect to ATP. Bacterial glycolysis shows a different stoichiometry, however, also containing a stoichiometric autocatalysis. For *Escherichia coli*, the regulation of the enzyme phosphofructokinase is also assumed to be a major reason for oscillations to occur. We investigated glycolytic oscillations in a quantitative kinetic model for *Streptococcus pyogenes* set-up on the basis of experimental data. We found oscillations within physiologically feasible parameter ranges. We investigated the origin of these oscillations and conclude that, again, both the stoichiometry of the system, as well as its allosterically regulated enzymes can give rise to these oscillations. For the analysis we employed established and new optimization methods for finding oscillatory regimes and present these in the context of this study.

1. Introduction

Glycolytic oscillations have been extensively studied in yeast experimentally and computationally. They have been reported in intact yeast cells [1] as well as in cell free extracts [2]. Until now, many studies have been carried out to pin down the source for the generation of glycolytic oscillations.

In general, there are two hypotheses for the origin of oscillations. The first one is that oscillations in glycolysis result from the allosteric effects on the enzyme phosphofructokinase (PFK), e.g. through inhibition by adenosine triphosphate (ATP) or other compounds produced during glycolysis [3–9]. Sel'kov developed a simple kinetic model of the PFK reaction including substrate inhibition and product activation and investigated the role of PFK in glycolytic oscillations [4]. According to this study, all glycolytic reactions except for the PFK are not required for the appearance of oscillations. This finding is confirmed by experimental studies testing the ability of various

* Corresponding author. Tel.: +49 62215451275; fax: +49 62215451483.
E-mail address: jennifer.levering@bioquant.uni-heidelberg.de (J. Levering).

0301-4622/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bpc.2012.11.002

glycolytic substrates to initiate glycolysis and oscillations in yeast extracts [5]. It was found that fructose-6-phosphate (F6P) was the last metabolite within the glycolytic sequence that is able to induce oscillations. Goldbeter and Lefever studied an open monosubstrate enzyme reaction describing the allosteric enzyme PFK activated by its product adenosine diphosphate (ADP) [6]. They showed that this simple model can lead to instabilities and, interestingly, the computationally observed sustained oscillations agree with experimental findings. Recently, du Preez et al. [10] re-calibrated an existing steady state kinetic model of yeast glycolysis constructed by Teusink et al. [11]. Using a small subset of experimental data the existing kinetic model was adapted to describe limit-cycle oscillations and intercellular synchronization. Interestingly, the greatest changes were required for the PFK reaction again underlining the importance of the PFK for generating the oscillations.

On the other hand, some studies indicate that glycolytic oscillations could also be the result of an autocatalysis caused by the stoichiometry of the system, namely the production of four molecules of ATP in the course of glycolysis whereas two ATP are required during the first steps of glycolysis [12–16]. Again, this hypothesis was underlined by computational analyses. Sel'kov constructed a simple kinetic model of glycolysis in which the conversion of a substrate into a product takes place in three steps [12]. In the absence of allosteric regulations the model is capable of generating oscillations. Aon et al. demonstrated that a strongly simplified glycolytic model without taking into account the allosteric regulation of PFK is able to exhibit sinusoidal-, square- and spike-like oscillations [15]. Cortassa et al. developed a simple four step kinetic model of glycolysis in order to demonstrate that the presence of the autocatalytic loop through ATP is sufficient for the occurrence of glycolytic oscillations [13,14]. Chandra et al. developed a simple two-state model of glycolysis which included allosteric regulation of PFK and pyruvate kinase (PYK) and was able to qualitatively reproduce the experimental behaviour [16]. Once more, the authors identified the autocatalytic loop as sufficient for the occurrence of oscillations.

Madsen et al. examined the general dynamic properties of glycolytic oscillations in yeast experimentally and computationally and analysed the cases of yeast extracts and intact cells separately [17]. They pointed out, that glycolytic oscillations are caused by different mechanisms in extracts and intact cells and concluded that in the case of yeast extracts glycolytic oscillations are driven by the allosteric regulation of the enzyme PFK whereby in intact cells the stoichiometry of the ATP–ADP–adenosine monophosphate system and the allosteric control of PFK are responsible for the appearance of oscillations. Furthermore, the distributed control and the hexose transport kinetics are thought to be involved in the generation of oscillations in glycolysis.

Thus, in summary, there is still an ongoing debate which is the actual central cause of the glycolytic oscillations in yeast. In contrast to yeast, glycolytic oscillations in bacteria have not been very well studied. The stoichiometry of glycolysis in bacteria constitutionally differs from that in yeast. In bacteria, the sugar uptake is carried out by permeases as well as by high-affinity phosphotransferase systems (PTS) which catalyse the import and direct phosphorylation of sugar derivatives like mono- and disaccharides or amino sugars [18]. In this process, phosphoenolpyruvate (PEP) serves as energy source and phosphoryl donor. Consequently, the PTS incorporates a new regulatory loop into glycolysis. Furthermore, there is a different allosteric regulation of PFK in some bacteria and none at all in others.

The occurrence of oscillations in bacterial glycolysis has been computationally investigated by Chuang and Chiou [19] who applied chemical reaction network theory to determine a minimal subnetwork of the model developed by Hatzimanikatis and Bailey [20]. This minimal subnetwork admits an unstable steady state with a positive real eigenvalue which results in undamped oscillations for a small perturbation. However, no explanation for the occurrence of glycolytic oscillations is given.

To our knowledge, so far *Escherichia coli* is the only bacterium for which glycolytic oscillations were observed experimentally [21,22]. Schaefer et al. applied an automated sampling device coupled to a stirred tank reactor to monitor intracellular metabolite concentrations like glucose-6-phosphate (G6P), PEP, glyceraldehyde-3-phopshate (GAP), dihydroxyacetone phosphate (DHAP), 3-phosphoglycerate (3PG) and pyruvate [21]. Trinh et al. observed oscillations in biomass, glucose and metabolite concentrations after the addition of ethanol to *E. coli* grown in a glucose-limited chemostat [22]. Furthermore, glycolytic oscillations in *E. coli* have been computationally studied [23,24]. Both published *E. coli* models include the PTS for sugar uptake and regulation of PFK by PEP and ADP. Ricci presented a mathematical model including PTS, PFK and PYK and investigated the influence of ADP, PEP and F6P on the dynamic regulation of glycolysis during glucose consumption under steady state conditions [23]. The model showed that ADP and ATP exert a major impact on the dynamics of the system. This observation can be explained by the involvement of ADP in the regulation of the flux-controlling enzymes PFK and PYK [23]. Chassagnole et al. developed a kinetic model of the central carbon route in *E. coli* including PTS, glycolysis, pentose phosphate pathway and storage material [24]. The model is capable to describe the oscillations observed in experiments of Schaefer et al. [21]. However, the origin of the oscillations is not discussed.

In some lactic acid bacteria like *Streptococcus pyogenes* the PFK is not an allosteric enzyme and therefore is neither regulated by ATP and fructose-1,6-bisphosphate (FBP) as in yeast nor by APD and PEP as observed in *E. coli*. *S. pyogenes* colonises the skin or throat and causes many human diseases ranging from mild skin infections to serious systemic diseases like rheumatic fever [25]. As being a lactic acid bacterium it relies on substrate-level phosphorylation for its energy synthesis and ferments sugars primarily to lactate via the glycolytic pathway followed by pyruvate degradation.

To our knowledge, the appearance of oscillations in the glycolysis of lactic acid bacteria has not been studied so far. This holds especially true for a species which is lacking the PFK regulation, one of the assumed major reasons for oscillations to occur in other organisms. Previously, we constructed a detailed kinetic model for glycolysis in *S. pyogenes* [26]. This model was fitted to experimental data of glucose pulse experiments. However, the parameters in the model were still unidentifiable which led us to working with ensembles of models all satisfying the constraint of fitting the data and analysing which features are preserved among these models. Several times, we observed oscillatory solutions when fitting the model to experimental data. Therefore, in this study we investigate the potential of this system to show oscillations with physiological parameter values. For this purpose, we used different methods of optimization to scan parameter spaces in search of oscillatory regimes. We used established, as well as new methods to do so and represent these in this manuscript. Moreover, we exemplify different bifurcation scenarios which occurred with different parameter combination. Interestingly, complex oscillations exclusively occurred following a period adding rather than the common period doubling route. Both routes have been observed in biological systems, e.g. period doubling for intracellular calcium oscillations in non-excitable cells by Perc and Marhl [27] and period adding for instance in the peroxidase/oxidase system by Hauser et al. [28].

2. Materials and methods

The mathematical model was formulated using ordinary differential equations, as specified in the Supplementary information. Simulations were performed with the LSODA algorithm as implemented in COPASI [29]. For the set-up and the parametrization of the kinetic model see [26].

In order to find the oscillatory ranges within the parameter space we optimized the parameters using the particle swarm algorithm (swarm