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Glycolytic oscillations in a model of a lactic acid bacterium metabolism

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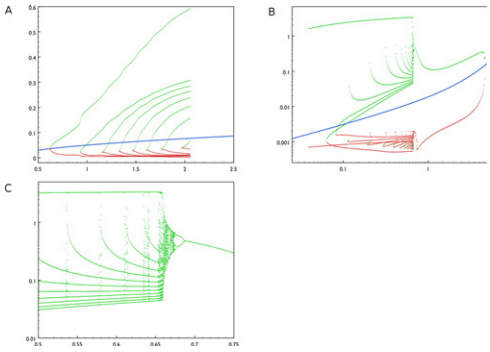
HIGHLIGHTS

- 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



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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

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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

size 100) as implemented in COPASI [29]. The different approaches for the optimization task are described in the Results section 2.

Scaled parameter sensitivities on the oscillation frequency were calculated in COPASI and subsequently analysed using MATLAB (The MathWorks, Inc.). Absolute scaled sensitivities higher than 100 were ignored. Frequency control was visualized using a heat map showing the frequency control for each parameter in all 50 oscillating models.

3. Results

3.1. Kinetic model of glycolysis in S. pyogenes

Lactic acid bacteria rely on substrate-level phosphorylation for their energy synthesis and ferment sugars primarily to lactate via the glycolytic pathway followed by pyruvate degradation. The main part of the external sugar is taken up via the high-affinity phosphoenol-pyruvate-dependent PTS and is directly converted into G6P. Besides the PTS lactic acid bacteria possess a low-affinity glucose permease (GlcP) allowing the diffusion of glucose across the membrane. The conversion of intracellular glucose to pyruvate proceeds via the Embden–Meyerhof–Parnas pathway. The main part of pyruvate is converted into lactate. Besides this, pyruvate can be converted into acetyl-CoA and formate. Acetyl-CoA is metabolised to acetate via acetyl phosphate yielding one additional molecule ATP. Furthermore, acetyl-CoA is converted into acetaldehyde which is further metabolised to ethanol. A schematic representation is depicted in Fig. 1. For a more detailed description of the kinetic model see [26].

In contrast to yeast and other bacteria like *E. coli*, *S. pyogenes* lacks the allosteric regulation of the enzyme PFK but possesses an additional stoichiometric feedback loop introduced into glycolysis by the presence of the PTS. However, there are also allosterically regulated reactions like the PTS (inhibited by ATP and FBP and activated by inorganic phosphate (Pi)), the glucokinase (inhibited by G6P and ATP), the sugar phosphatase II (activated by HPr-ser-P), the GAPDH (inhibited by NADH), the pyruvate kinase (activated by G6P and inhibited by Pi), the lactate dehydrogenase (activated by FBP and Pi

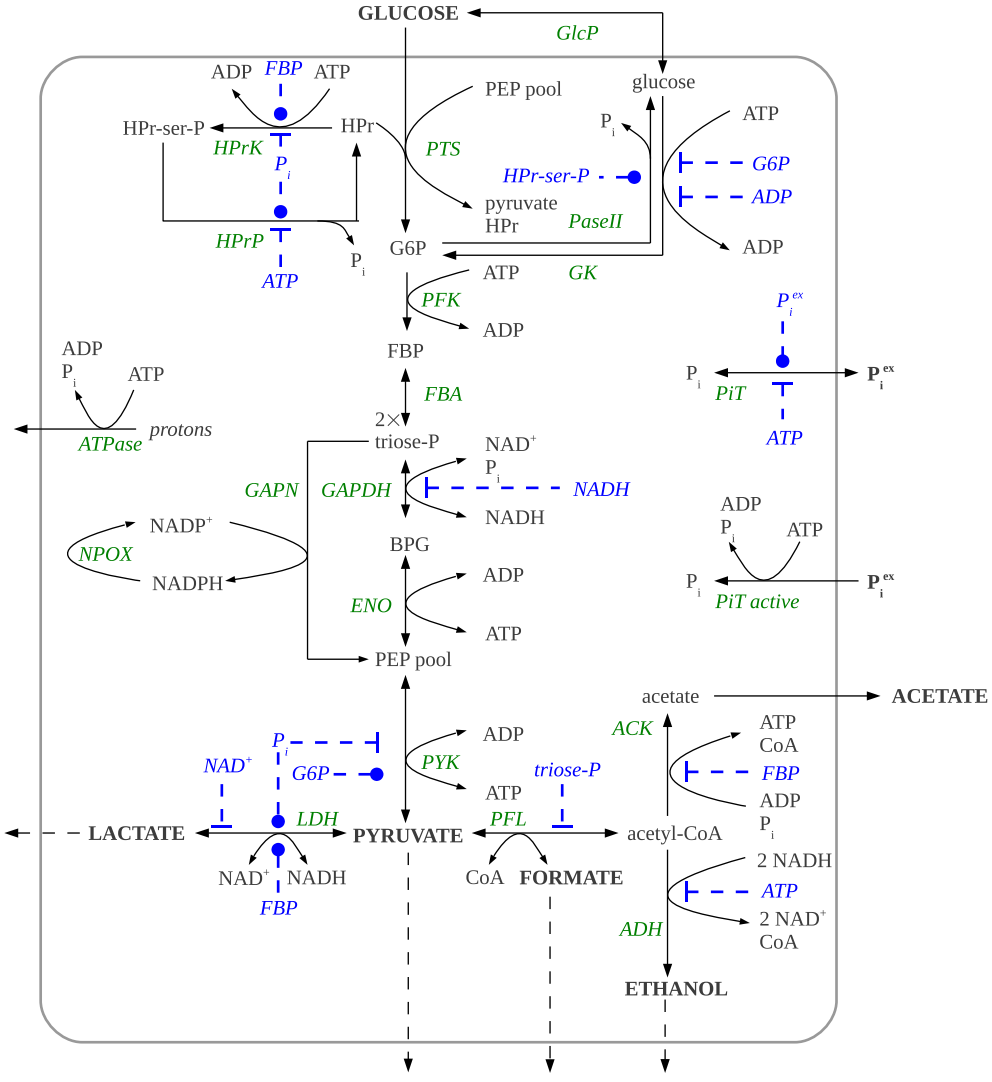

Fig. 1. Overview of molecular interactions of *S. pyogenes* glycolysis. Allosteric regulation (blue) is divided in inhibition (barred arrows) or activation (open circle-ends). Enzymes are listed in green, end products and extracellular metabolites are bold. In the model the permanent supply of CDM-LAB medium is represented by constant glucose, phosphate and acetate concentrations and outflow reactions of all end products, i.e. pyruvate, lactate, ethanol and formate. Abbreviations: 1,3-bisphosphoglycerate, BPG; coenzyme A, CoA; enolase, ENO; glucokinase, GK; lactate dehydrogenase, LDH.

and inhibited by NAD^+), the pyruvate formate lyase (inhibited by the triose phosphates), the acetate kinase (inhibited by FBP) and finally the alcohol dehydrogenase (inhibited by ATP) (see Fig. 1).

As mentioned above, while fitting a detailed model of glycolysis in *S. pyogenes* to experimental data, we observed that several of the solutions indeed showed transient oscillatory behaviour. Fig. 2 exemplarily shows the concentration changes of G6P and FBP in one fitted model. Both metabolites oscillate with equal frequency but different amplitudes and phase angles relative to each other. We would like to stress that the experimental data did not show indications of oscillations, but represent crude time-courses in response to glucose pulses [26]. Nevertheless, the fact that the fitted models often show oscillations while being in agreement with the experimental data could indicate that oscillatory regimes are close to realistic parameter sets.

Since the PFK in our model is not regulated, it cannot be the cause of the oscillations. In order to investigate the components important for these oscillations, the model has been transformed into a model of continuous cultured cells allowing a steady state. Therefore, the concentrations of extracellular metabolites like glucose, acetate and phosphate are fixed and the outflow reactions for ethanol, pyruvate, lactate and formate are introduced. Furthermore, the metabolite "extracellular pyruvate" is removed from the model and "intracellular acetate" as well as a membrane transport for acetate is included. The essential reactions of the model are shown in Fig. 1; the kinetic equations and their parameters, the differential equations and the information about the initial conditions are listed in the Supplementary material.

Once more, it is of importance to note that the requirement of PEP for sugar uptake and, thus, for initiating glycolysis introduces another loop into the system since PEP is produced in the lower part of glycolysis. The regulation of sugar uptake via the PTS system by a FBP activated ATP-dependent protein kinase introduces another loop. These feedback loops together with the one introduced by the autocatalytic loop of ATP render the model into a very complex system.

3.2. Optimization techniques for finding oscillatory regimes

With the model at hand, we wanted to search for oscillatory regimes within a physiologically plausible parameter space in dependence on different components for the model. The model has more than 130 kinetic parameters which makes the parameter space high dimensional and the characterization of the oscillatory regime difficult. Therefore, we employed numerical global optimization techniques. The approach is as follows: First a (heuristic) indicator function is defined that can be calculated from a simulation of the model and indicates the presence of oscillatory behaviour. Then global optimization of this function in parameter space is performed. The performance of this approach naturally depends on the choice of the indicator function. We investigated three fundamentally different types of indicator functions, each of which could successfully find oscillatory behaviour.

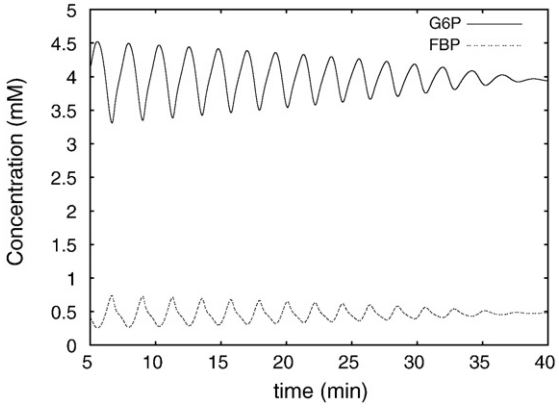

Fig. 2. Typical oscillation pattern observed after fitting the glycolytic model of *S. pyogenes* to glucose-pulse data [26]. Exemplary, the metabolites G6P and FBP are displayed. Both metabolites show damped oscillations with equal frequency but different amplitudes and phase angles relative to each other.

3.2.1. Method A: A function based on the eigenvalues of the Jacobian of the system in a steady state

Typically the origin of an oscillation is a Hopf bifurcation. A necessary condition for a Hopf bifurcation is that the real part of a complex conjugate pair of eigenvalues changes its sign from negative to positive. Therefore a function based on these eigenvalues can indicate the presence of oscillations. This approach has been described and implemented in software by Chickarmane et al. [30]. The specific optimization target used by Chickarmane et al. is based on minimizing the product of the real parts of all eigenvalues, augmented with terms that favour the presence of complex eigenvalues. This approach is able to identify a Hopf bifurcation point, but it does not take into account that for the emergence of a stable oscillation from a steady state the Hopf bifurcation must necessarily involve the largest two eigenvalues of the Jacobian. du Preez et al. have recently applied a slightly different approach, where the ratio of the absolute values of the imaginary and the real part of a pair of complex conjugate eigenvalues is maximized [10]. This requires that before optimization the system is already in a state that has complex eigenvalues which is not generally the case when starting with random initial values for the optimization. Since our study requires that oscillatory parameter sets are routinely found from random initial values, we employ an optimization target that delivers the two largest eigenvalues to become complex and if this has been achieved aims for positive real parts. The function, as implemented in the software COPASI, is defined as follows. If the largest eigenvalues λ_1 and λ_2 are already complex, the optimization target is

$$f = \lambda_1^R \cdot \begin{cases} \dfrac{\left|\lambda_1^I\right|}{0.01 + \left|\lambda_1^I\right|} & \text{for } \lambda_1^R > 0 \\ 2 - \dfrac{\left|\lambda_1^I\right|}{0.01 + \left|\lambda_1^I\right|} & \text{for } \lambda_1^R \leq 0 \end{cases}$$

where superscripts *R* and *I* indicate the real and imaginary parts, respectively. This expression favours large real parts and non-zero imaginary parts of the complex conjugate pair of eigenvalues. If the eigenvalues are real, the target function is

$$f = 2 \cdot \lambda_2^R - \begin{cases} 2 \cdot \lambda_1^R & \text{for } \lambda_1^R > 0 \\ 0 & \text{for } \lambda_1^R \leq 0 \end{cases}$$

This expression favours minimizing the difference between the real parts of the largest two eigenvalues, based on the fact that a vanishing difference between these real parts is a prerequisite for the occurrence of a complex conjugate pair. The function is constructed in a way that it is continuous for all λ_1 and λ_2.

The common restriction of all these eigenvalue based methods is that the starting point for the optimization needs to be a model with a steady state. For some models it cannot be expected that a steady state exists, or is easily found, for a random set of parameters. For the model studied here, however, this turned out to be not a problem. Another limitation is that the analysis based on eigenvalues of the Jacobian can only provide necessary but not sufficient criteria for the existence of a Hopf bifurcation. Thus the method occasionally will find a parameter set where the model exhibits an oscillatory instability but no stable oscillation.

3.2.2. Method B: A function based on the rate of change of a state variable

The second approach is based on a simulation of the time course of the model and thus does not require the existence of a steady state at the start of the optimization. Heuristically, if a model shows stable

oscillatory behaviour, the *amount of change* of a concentration over the course of a simulation will be large, and more specifically, it will grow unrestricted if the duration of the simulation is increased. The indicator function is defined as $\left(\int dt\left|\frac{d}{dt}[\mathrm{FBP}]\right|\right)/[\mathrm{FBP}]_{\max}$, where [FBP] is the concentration of fructose-1,6-bisphosphate over a simulation and $[\mathrm{FBP}]_{\max}$ is its maximal value. The important property of this function is that it has a large value when the concentration of FBP changes a lot compared to its maximal value over the time of the simulation. In our experience this is a reliable indicator for the presence of oscillations. This approach has been applied by Wegner et al. [31]. The drawback of this method is that while we know that large values of the function generally indicate oscillations, it is difficult to judge when the value is *large enough*, so a reliable criterium for stopping the optimization is missing.

3.2.3. Method C: A function based on count of maxima

A very straight forward method to optimize for the presence of oscillations is to consider the number of maxima of the time course of a concentration. While being very simple, this worked surprisingly well. The advantage is that it is easily possible to aim at a certain frequency of oscillations by optimizing for a certain number of maxima during a defined simulation duration. In this study we minimized the function $(\mathrm{maxima}-10)^2$ over a time course of 1000 s. The maxima were detected and counted in the software COPASI by using discrete events triggered by the change in sign (from positive to negative) for the concentration change. A limitation of this approach is that the indicator function (as it is discrete) has no gradient that could be utilized by the optimization methods. This restricts the choice of suitable optimization methods and could generally lead to worse performance of optimization.

For the model studied here all three methods performed well. However, Method C was more efficient in detecting oscillations in a shorter time period as compared to Methods A and B. Efficiency was determined on the basis of average success in finding oscillatory models when ten optimizations were run for 30 min for 10 rounds (in each round we found on average 1.9, 3.3 and 4.5 oscillations by using Methods A, B and C, respectively). Since it is also easy to specify a target frequency for the oscillation, we decided to focus on Method C. Initially, we used broad parameter ranges (one order below and above experimentally measured values; otherwise 0.01–100 mM for binding constants, 0.1–1000 mM/s for Vmax values and 0.001–1000 mM/s for apparent transport rates) to search for oscillatory regions and found plenty of oscillatory solutions meaning that the initial finding of some oscillatory trajectories is not a coincidence and that indeed there are oscillations with realistic parameter sets. Obviously, it is not surprising to find oscillations in a system with feedbacks, however, it is not always clear, if oscillations in a specific system occur with parameters within physiological bounds.

3.3. Searching for the oscillophore

Similar to the discussion on yeast glycolytic oscillations described above, it is obviously interesting to study which parts of the system are contributing the most to the destabilisation of the steady state and the onset of the oscillations. In this specific case, it can be either the stoichiometric autocatalysis or the allosteric regulation of enzymes other than the PFK. In order to investigate this, we changed the model such that one model version contained no allosteric regulations of any enzyme at all, otherwise leaving all equations untouched. In other words all inhibitory or activatory terms in the kinetic equations were deleted. Searching for oscillatory regimes in the open parameter space once again resulted in different sets of parameters which displayed oscillations showing that the present stoichiometry is definitely sufficient to cause nonlinear behaviour with physiological parameters. Additionally, with this model, we fitted the experimental data again to ensure that at least qualitatively, experimental trends can be followed without the allosteric interaction. This worked, however and obviously, the allosteric interactions are needed to perform a more quantitative fit.

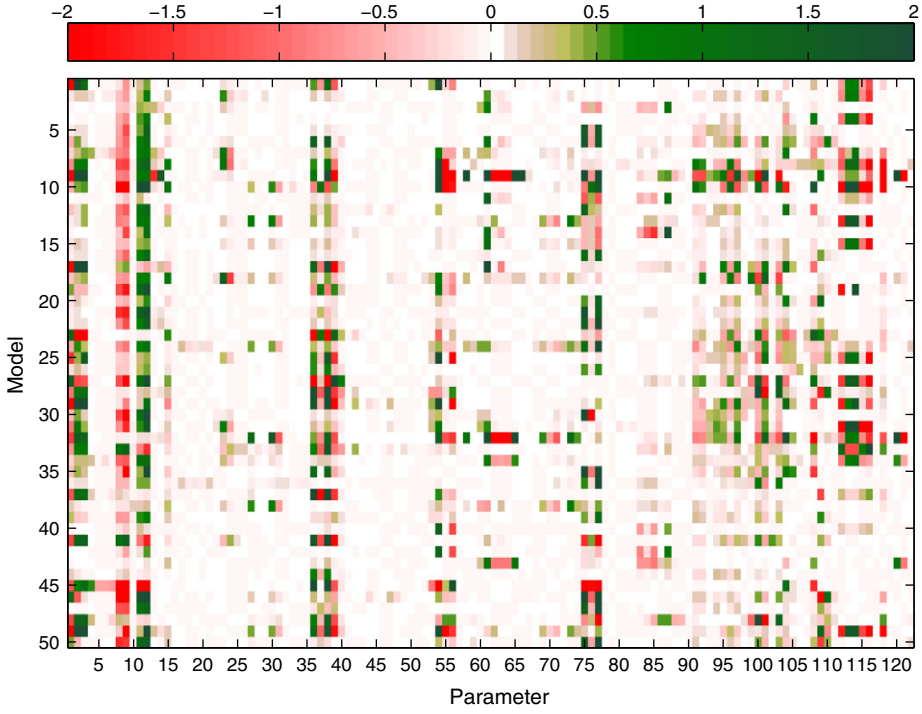

Fig. 3. Scaled sensitivities of the frequency to the model parameters for all 50 oscillating glycolytic models of *S. pyogenes*. The order of the parameters is given in the Supplementary material.

In another attempt, we fixed the concentrations of ATP, ADP, pyruvate and PEP in the model rendering it insensitive towards the stoichiometry of these species. Once again, we searched for oscillatory solutions in the broad, but physiological meaningful parameter space. Once more, we found oscillatory solutions indicating that the allosteric feedbacks in the system are also sufficient by themselves to cause oscillations.

In order to narrow down our search, we subsequently decreased the ranges for our parameters based on experimental data for the related *Lactococcus lactis*. In this case, parameters of *L. lactis* — as listed in the supplement — were allowed to vary by 0.1–10 times the experimentally determined parameter taken from [32–36]. The above analysis with both model versions was repeated and once more, both of the mechanisms were sufficient to generate oscillations. Therefore, we conclude that both mechanisms are sufficient and able to cause oscillatory behaviour with similar parameter sets indicating that only, if all experimental parameters are known in detail, one could in principal determine which mechanism is the most important. However, one should keep in mind that biological parameters are not fixed, e.g. Vmax values will change over orders of magnitude during different environmental conditions rendering such an analysis only possible for one specific state of the cell.

3.4. Frequency control analysis

In addition to analysing which parts of the system are crucial for the destabilisation of the steady state, we analysed the sensitivity of the frequency to small changes in the model parameters. For yeast, the control of the frequency was extensively studied. Based on a core model simulating experimental results with adequate accuracy Bier et al. demonstrated that frequency control is distributed over all processes and regulatory interactions in the system [37]. This finding and, in particular, that the control does not reside solely in the PFK reaction was confirmed by Teusink et al. who studied the control of glycolytic oscillations in three existing models [38]. Using a Fourier transformation Reijenga et al. described the oscillating concentrations as a function of frequencies [39]. By means of control analysis they found out that the frequency control was distributed among all enzymes. Madsen et al. [17] investigated the mechanism of glycolytic oscillations in intact yeast cells and yeast extracts and, again, affirmed that the frequency control is distributed over the network reactions. Here, PFK, hexokinase, glycogen formation, ATP consumption, GAPDH, alcohol dehydrogenase (ADH), glycerol formation and flow of the continuous-flow stirred tank reactor affect the frequency. In the case of yeast extracts, the frequency is mainly controlled by glucose inflow and ATP consumption.

Similar to yeast, the oscillation frequency in the model for *S. pyogenes* is affected by several control systems. Fig. 3 shows the results of the sensitivity analysis performed with the ensemble of 50 models which were an outcome of our search for oscillatory regimes as described above. In this heat map we set scaled sensitivities higher than 2 or lower than −2 to 2 and −2, respectively, since these values demonstrate a high control and summarizing the high values enables a closer look at the interesting range of low and intermediate control. We observed that the control is distributed across several parameters. There was no single parameter set with one parameter dominating the frequency control. However, we were able to identify groups of parameters having a higher impact than others in the majority of models which are represented by dark stripes in Fig. 3. As in the case of yeast, the oscillation frequency is controlled by reactions influencing the sugar uptake, i.e. PTS (parameters 11–13), by PFK (parameters 18–19 and 21–22) and by ATPase (parameters 85–87). This is surprising, since the PFK is not allosterically regulated in this model. Furthermore, Vmax of non-

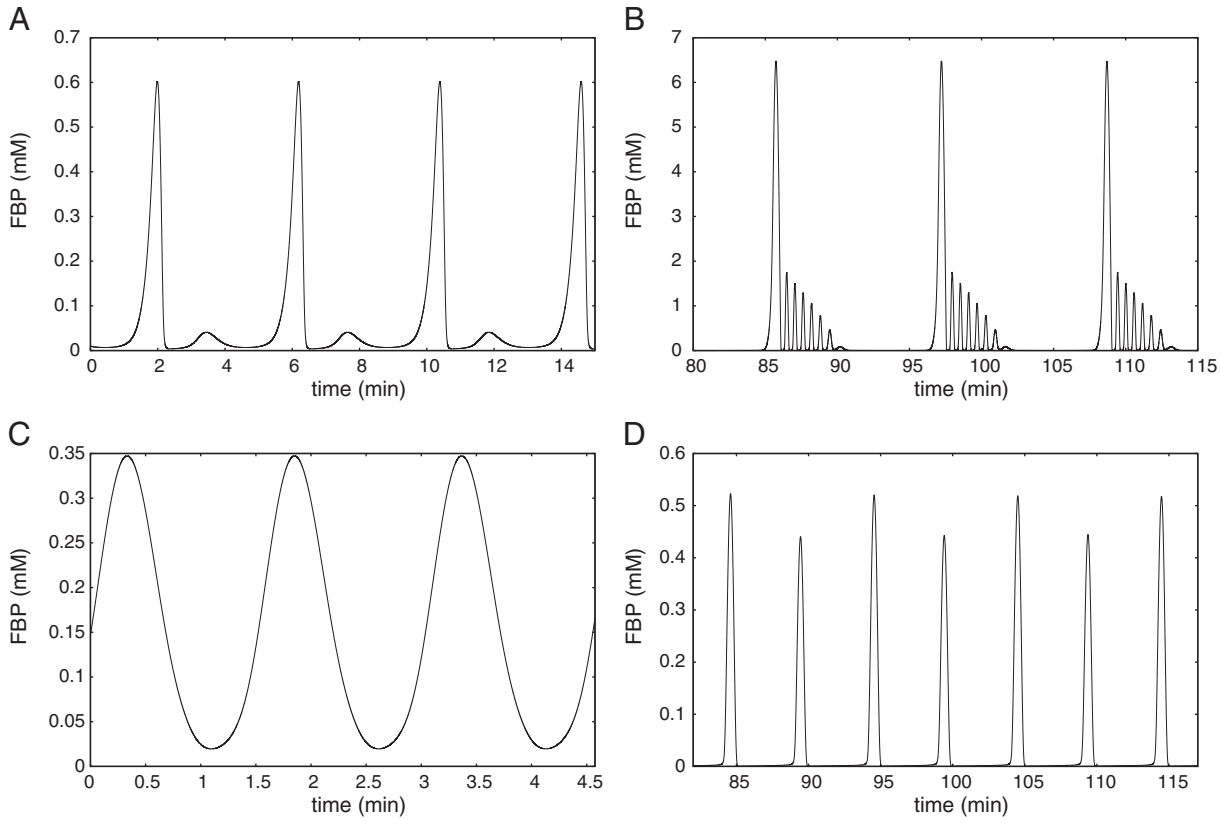

Fig. 4. Complex oscillations observed after optimization and during a constant glucose input rate. (A) and (B) display the FBP concentration for one model describing period adding with different velocity constants for the PTS reaction, (C) and (D)show sinusoidal and period doubling oscillations (representing reverse period doubling as also seen in Fig. 5(C)) for one model with different bifurcation parameters.

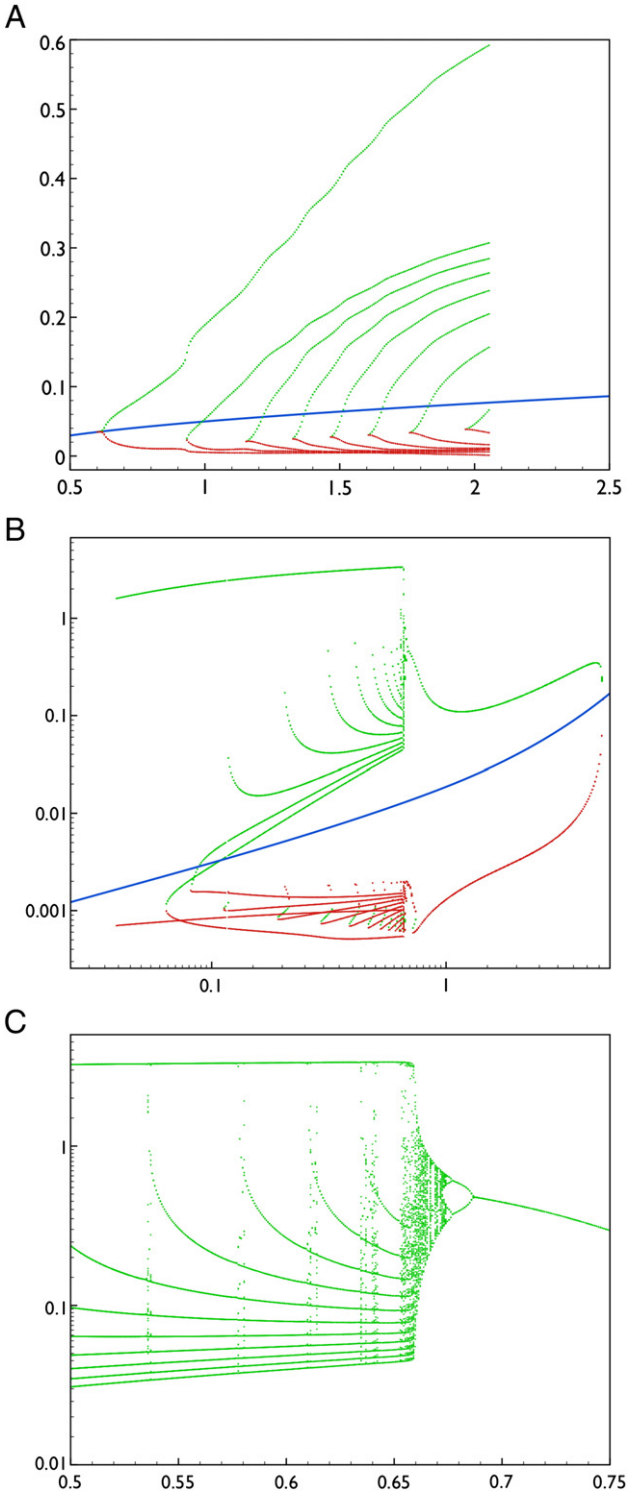

Fig. 5. Bifurcation diagram showing the maximal (green), minimal (red) and steady-state (blue) FBP concentration as a function of the velocity parameter of the PTS reaction. In (A) oscillations emerge via a Hopf bifurcation followed by a period adding sequence for increasing values of the bifurcation parameter. (B) There is a sudden onset of oscillation with an increasing bifurcation parameter followed by a period adding sequence intermittent chaotic regimes. Continuing further beyond the chaotic regimes we find a (reverse) period doubling and a Hopf bifurcation leading to a stable steady state. (C) Is a zoom in section of (B).

phosphorylating GAPDH (GAPN) (parameter 118), PYK (parameters 46–49), pyruvate formate lyase (PFL) (parameters 64–66), $NADP^+$ regenerating reaction (NPOX) (parameters 119–120), active phosphate transport (parameters 122–126) and pyruvate outflow(parameter 128) strongly affect the frequency of glycolytic oscillations in *S. pyogenes*. Interestingly, the fact, that if parameters impact the frequency in a positive or negative way seems to be very conserved irrespective of the exact parameter set. So, even in the absence of knowing all of the parameters in detail, these results are robust and reliable.

3.5. Complex oscillations and bifurcation behaviour

Among the parameter sets fitting the experimental data, as well as among the oscillatory models resulting from our search with the full model, different types of oscillations could be observed, ranging from simple sinusoidal oscillations to complex oscillations including chaotic behaviour. Examples for complex oscillatory time series of models obtained using the optimization approach for finding oscillations and differing in their parameter sets are displayed in Fig. 4. In order to analyse this behaviour further, we investigated the bifurcation behaviour leading to the onset of the complex oscillations. We used the velocity constant of the PTS reaction as bifurcation parameter and plotted the minimal, maximal and steady state FBP concentration as a function of this parameter.

Interestingly, the onset of complex oscillations followed a sequence of period adding bifurcation in all cases, sometimes including a chaotic regime. Some examples for the respective bifurcation diagrams are shown in Fig. 5. In (A) oscillations emerge via a Hopf bifurcation followed by a period adding sequence for increasing values of the bifurcation parameter. The time series from Fig. 4(A) and (B) correspond to different points in this bifurcation scenario. In bifurcation diagram 5(B) (with zoomed in section shown in (C)) there is a sudden onset of oscillation with an increasing bifurcation parameter followed by a period adding sequence intermittent chaotic regimes. Continuing further beyond the chaotic regimes we find (reverse) period doubling (see Fig. 4(D)) and a Hopf bifurcation leading to a stable steady state.

4. Discussion and conclusion

We investigated oscillatory behaviour in a quantitative model for glycolysis of *S. pyogenes*. This was motivated first of all by the fact that we often observed oscillations when fitting the model to experimental data indicating that oscillatory solutions are at least close to the physiological behaviour. Second, the differences in stoichiometry and allosteric regulation of *S. pyogenes* compared to the well studied yeast asked for an investigation of the oscillatory behaviour w.r.t. physiologically relevant parameter ranges. Third, we were interested in developing new methods for systematically scanning large parameter spaces in large biological models for the occurrence of oscillatory behaviour, something which has been done manually until recently.

We observed that oscillations in the model are plausible with physiological parameters, even when narrowing these down to experimental values obtained for *L. lactis* with moderated variations. Moreover, this does not only hold true for the original model which contains two potential causes for oscillations to occur, namely the autocatalysis in the stoichiometry of the system, as well as allosteric feedback regulation of enzymes. It is also true for versions of the model where either cause had been eliminated. This points to the fact that similar to yeast, both mechanisms contributed in physiological parameter ranges to potential oscillations making it close to impossible to really pinpoint the “more important” oscillophore in these mechanisms. Obviously, this would become more feasible, if all the kinetic parameters would really be known in detail, something which is only possible for one specific experimental condition, since especially Vmax values will vary with varying experimental conditions, strains etc.

Searching for oscillations in multi-dimensional parameter spaces has been done mostly manually in the past. Only recently, first automated methods have been established which allow to speedily and effectively scan even large parameter spaces. We added new methods for

this purpose which are based on heuristic criteria, but — at least in this case — outperformed the existing methods. Being able to try out different methods for a specific system will certainly be a good way, since so far, none of the methods seems to work with any arbitrary system. However, once a suitable method is found among the different possibilities, it is fast and efficient to search for oscillations making questions like the one addressed above really feasible to solve, i.e. which features of a complex reaction network are responsible for oscillations to occur given certain parameter boundaries.

Although the model predicts oscillations during a constant glucose input rate this type of behaviour has not yet been experimentally observed. Therefore, the existence of glycolytic oscillations in lactic acid bacteria like *S. pyogenes* cannot be completely precluded.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bpc.2012.11.002.

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