Phosphoglycerate Mutases Function as Reverse Regulated Isoenzymes in *Synechococcus elongatus* PCC 7942

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Introduction

Phosphoglycerate-mutase (PGM) is an ubiquitous glycolytic enzyme, which in eukaryotic cells can be found in different compartments. In prokaryotic cells, several PGMs are annotated/localized in one compartment. The identification and functional characterization of PGMs in prokaryotes is therefore important for better understanding of metabolic regulation. Here we introduce a method, based on a multi-level kinetic model of the primary carbon metabolism in cyanobacterium *Synechococcus elongatus* PCC 7942, that allows the identification of a specific function for a particular PGM. The strategy employs multiple parameter estimation runs in high CO2, combined with simulations testing a broad range of kinetic parameters against the changes in transcript levels of annotated PGMs. Simulations are evaluated for a match in metabolic level in low CO2, to reveal trends that can be linked to the function of a particular PGM. A one-isoenzyme scenario shows that PGM2 is a major regulator of glycolysis, while PGM1 and PGM4 make the system robust against environmental changes. Strikingly, combining two PGMs with reverse transcriptional regulation allows both features. A conclusion arising from our analysis is that a two-enzyme PGM system is required to regulate the flux between glycolysis and the Calvin-Benson cycle, while an additional PGM increases the robustness of the system.

Abstract

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understand the regulation of PGMs and thereby to validate their annotation.

We here propose the use of kinetic modeling for such analysis. Kinetic modeling is a standard method for predicting the behavior of biological systems [9]. However, reactions catalyzed by isoenzymes are commonly described by single enzymatic kinetics, which would not explain hidden regulatory mechanisms. In order to address this challenge, we have designed a multi-level kinetic model of carbon core metabolism of *Synechococcus*. The model not only helps in the validation of the annotation but also in the

![Figure 1. Scheme of the primary carbon metabolism, encoded as a kinetic model of *Synechococcus elongatus* PCC 7942.](image)

The model includes the Calvin-Benson cycle, sucrose and glycogen synthesis, photosynthetic pathways, glycolysis and sink reactions, representing the adjacent pathways. Green color represents the reaction catalyzed by phosphoglycerate mutase (PGM) and indicates its cardinal position in the crossroads of metabolic pathways (need for complex model). Note: The reactions are described in the model by reversible and irreversible Michaelis-Menten kinetics; reversibility of particular reaction is indicated by two little arrows.

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![Figure 2. Comparison of fold changes in concentration for 3PGA and in expression levels of the four annotated PGM isoenzymes in cells of *Synechococcus elongatus* PCC 7942 after shifting from high to low CO2 level.](image)

Note: synpc7942_0485 probably represents a gene encoding a phosphoserine phosphatase but we cannot exclude if it functions as PGM.

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explanation of regulatory mechanism for PGM isoforms in one compartment.

Materials and Methods
The model was developed and simulations were executed using the SimBiology toolbox of MATLAB (Mathworks Inc.). The routine employed for parameter estimation was a hybrid genetic algorithm (ga_hybrid, Mathworks Inc.). The model is available in the supplement (Model S1).

Systems Biology Workflow
The kinetic model, a successor of the corrected Zhu model [10,11] of the Calvin-Benson cycle, was redesigned for cyanobacteria, extended (photorespiratory pathways, glycolysis) and validated on available metabolic data from Synechococcus el. PCC 7942 [6]. The constraints of the model are: 1) The ATP · (ADP + ATP)−1 ratio was maintained in the physiological range 0.74–0.76 [12], both in high and low CO2 steady states and 2) the biomass production in high CO2 was calculated from growth rate data. On average, Synechococcus shows a 3.4-fold higher growth at 5% CO2 (defined as high CO2, [6]) compared to ambient air CO2 (defined as low CO2, [6]).

The parameter estimation for up to four annotated isoenzymes of PGM was run in high CO2 steady state and the result (kinetic parameters) stored if the difference between the simulated and experimental metabolic data was lower than 15%. We also assumed that, in the case of isoenzymes in non-compartmented prokaryotic cell, a change in gene expression in the steady state is equal to changes in protein activity, i.e., minimal or no post-translation modifications. For the unidentified part of carbon metabolism - reaction catalyzed by PGM - four transcriptomic profiles (four PGM isoforms) were tested for combinations of one to four isoforms and the quality of match with metabolic data in low CO2 was stored for each profile.

Experimental Data
Relative transcriptomic and metabolomics data of high CO2 as well as low CO2 Synechococcus cells were taken from [6]. A considering of two environmental conditions was necessary to known and implement the changes in transcriptomic level of PGMs in the model and helpful for constraining the model by doubling the amount of metabolic data. For 3PGA, cellular concentrations were calculated using the data from [13]. These authors reported that cyanobacteria contain 1300 nmol 3PGA/g fresh weight. According to our calibration of cyanobacterial fresh weight and total cellular volume, this amount corresponds to 5.39 mM 3PGA in the total cell volume in low CO2 grown cells. The concentration of 2-phosphoglycerate was recalculated on the basis of known ratio to 3PGA [6].

Figure 3. Quality for the match of simulated and measured data in low CO2 for single PGM scenario in dependence on (i) varying PGM activities fitted in high CO2 and (ii) regulation by transcript amounts. Figures represent the match between simulated and measured data in low CO2 in dependence of estimated kinetic parameters (Vmax and Km values for preferred substrate and product) in high CO2 for a single PGM scenario. The Vmax fitted to steady state in high CO2, was modified by the amount of PGM isoforms taken from the changes in mRNA values (one by one) after shift from high to low CO2. Results are shown for randomly chosen set of twenty parameters estimation runs for PGM and the best fit (Nr. 12). The left figure shows the match for preferred product of PGM, 2-phosphoglycerate (2PGA). The black solid line shows the impact of 1.7-fold down-regulated enzyme, corresponding to PGM4; gray contours indicate the difference in matching the data if PGM is 15.4-fold up-regulated (corresponding to PGM2). In order to illustrate the impact of transcriptomic changes for the other two annotated PGMs, the results for 1.4-fold down-regulated (circle) and 2.7-fold up-regulated (square) isoenzymes are presented in the case of the best fit. The right figure shows the match for preferred substrate of PGM, 3-phosphoglycerate (3PGA); colors/lines have the same meaning as for the figure on the left. Notes: 1) the top boundary of axis y shows results equal or worse than match ratio equals to 3, 2) The match ratio is calculated as X/Y where X(Y) is a higher(lower) number from a pair of simulated and experimental values for a particular data point, 3) Fit from high CO2 was included (saved as a result) if the difference between simulated and measured data was smaller than 15%, 4) each point represents an independent simulation run compared to experimental data - the lines improve the perception for the differences in match ratios and have no other meaning.

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Results and Discussion

When One Enzyme is not Enough

The implementation of isoenzymes within a kinetic model, when localized in one compartment, deals with two extremes. First, for only one enzyme and the model not being constrained by a lack of data, there are still non-identifiability problems due to multiple sets of parameter value combinations that are equally able to fit the data related to a particular state. Second, considering two or more enzymes, the combinational explosion in the parameter space leads to computational requirements that make it virtually impossible to fit the model to sets of experimental data for different states of the system.

Various studies have focused on the response of cyanobacteria to changes in the environment, by considering a range of omics data [6,14,15], which allow constraining the model. On the basis of these results, we were aiming to develop a method that would permit the identification and analysis of isoenzymes in a one-compartment system and within a reasonable amount of time (days to weeks). We here particularly focus on PGMs because of their key position in regulating the flux of carbon through the Calvin-Benson cycle and its export into glycolysis in cyanobacteria. This position of PGMs in the metabolic network makes it impossible to fit the model to sets of experimental data for different states of the system.

Reverse Regulated Versus Co-regulated Isoenzymes

We have shown that the one-isoenzyme scenario cannot keep the expected balance between 3PGA and 2PGA (Fig. 3). We can either get a robust system, which is however out of the physiological range, or a system that is very sensitive to changes in the parameter values. The natural next step in the analysis is to test for two PGMs in one compartment. This approach requires metabolic data from two steady states and transcriptomic data describing the shift between these two states, in our case, from high to low CO2. However, parameter estimation for two isoenzymes, running for two steady states of metabolism, has enormous computational demands. At this point, data collected from the single PGM scenario, based on the constrained model, proved to be very useful.

The single PGM scenario suggests that it is the up-regulated PGM2 which is likely to provide the kinetic regulation for the reaction due to high sensitivity with respect to kinetic parameter values. We therefore tested the dual PGM scenario with two PGMs, denoted as alpha and beta. We employed the parameter values from the best fit provided by the single PGM scenario for alpha PGM (Fig. 4, open square) and varied the parameters for beta PGM, see Fig. 4 (solid triangles); the equilibrium constant is the same for both PGMs. Moreover, for the sake of comparison, we have added the robust case from the single PGM scenario (1.7-fold down-regulated PGM4) and another dual PGM scenario in which both PGMs were co-down-regulated (PGM1 and PGM4).

The comparison with other scenarios clearly shows a large improvement, both in robustness against varying parameter values for the beta PGM and in the ability to describe the experimental data if we proceed from single PGM scenario (Fig. 5, dashed line), over dual co-regulated PGMs (Fig. 5, dotted line) to dual reverse regulated PGMs (Fig. 5, solid line). It is therefore the combination of two PGMs with reverse transcriptomic regulation, PGM2 and PGM4, giving the closest match with experimental data. Moreover, if we have a look at the estimated parameters for the best fit for this scenario (Fig. 4, open square and open triangle), the Michaelis constants for prefered substrate and product for alpha and beta PGMs in Table 1 have nearly interchanged values. This is an expected outcome from a evolutionary point of view for two
enzymes catalyzing the same reaction in one compartment, thus supporting the applied approach. In summary, our in silico experiment led us to the conclusion that reverse gene regulation for two isoenzymes in one compartment, together with the opposite affinity for the substrate and product, provides very good explanation of the measured metabolic data.

The results clearly show high robustness of the system (Fig. 5, solid line) against varying the kinetic parameters (Fig. 4, solid triangles), which is equivalent to noise from fluctuating metabolite concentrations in a single cell. Hence the results support our initial assumption that system of isoenzymes does not require an additional regulatory mechanism, for instance the post-translational modifications. This was implicit in the assumed 1:1 ratio between the transcript level and protein activity for isoenzymes in the non-compartmented cell.

The Case of Two other Annotated PGMs

We have shown that two reverse regulated PGMs are able to explain the experimental data (Fig. 5), i.e., to regulate the interconversion between 3PGA and 2PGA and thus the flux between the Calvin-Benson cycle and glycolysis. This raises the questions whether there is any benefit of having more than two PGMs in one compartment. In order to test the triple PGMs scenario, we have taken the best fits for alpha and beta PGMs (Fig. 4, open square and open triangle) and varied the $k_M$ values for gamma PGM. Our analysis of the triple PGMs scenario shows that there is a negligible improvement in robustness and only

| kinetic parameter | value   | unit       |
|-------------------|---------|------------|
| $K_{eq}$          | 0.056   | dimensionless |
| $V_{max\_alpha}$ | 0.050   | mM         |
| $k_{m\_alpha}$   | 0.042   | mM         |
| $K_{eq\_beta}$   | 1.772   | mM         |
| $k_{m\_beta}$    | 1.257   | mM         |
| $V_{max\_beta}$ | 0.027   | mM         |
| $k_{m\_beta}$    | 0.047   | mM         |

Activity of PGMs is in the model described by reversible Michaelis-Menten kinetics. Note: $V_{max}$ values are normalized to the activity of RuBisCO. The routine employed for parameter estimation was a hybrid genetic algorithm.

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a small improvement in matching experimental data (Fig. 5, blue line). The $k_M$ values for gamma PGM, in the case of the best fit, is in between the $k_M$ values of PGMs alpha and beta (Fig. 4, open circle). There was however no improvement in the biomass production (our estimation of growth speed), which implies that only two PGMs are necessary to control the flux between the Calvin-Benson cycle and glycolysis in the controlled environment. Since two-component response regulators have been proposed for the glycolytic pathway [16], this further supports our conclusion that only two PGMs are essential for the regulation of the system.

Recently, two assumed members of PGM family, cofactor dependent PGMs annotated in *Hydrogenobacter thermophilus*, were identified in a wet lab experiment as phosphoserine phosphatases [17]. It is known that phosphoserine phosphatase (PSP) is essential for serine and glycine metabolism [18,19] but no PSP is annotated for *Synechococcus*. The alternative pathways for serine and glycine synthesis in cyanobacteria were proposed [20,21], however, we tested if one or more of the PGMs annotated in *Synechococcus* can be identified as PSP. Cluster analysis of PGM-like proteins shows that each PGM belongs to different protein family (Fig. 6) but, interestingly, PSPs in *Hydrogenobacter thermophilus* are in the same cluster as PGM3 (Fig. 6). Moreover, we have detected conservative regions between that PSPs and PGM3. It is therefore likely that we can identify PGM3 as PSP, however, we cannot entirely exclude that this enzymes functions as PGM as well. Finally, PGM3 is up-regulated in low CO$_2$ (Fig. 3) which lead to speculation on contribution of photorespiration for serine biosynthesis as suggested [20].

As for the PGM1 and PGM4, due to almost the same expression in low CO$_2$ (Fig. 2), we do not have sufficient data to clearly distinguish between these two down-regulated enzymes. We however showed that stronger down-regulation gives higher robustness to the system, as indicated in Fig. 3. There might also be other reasons why there are three, or even four, PGMs in *Synechococcus*. For instance, it is known that the majority of proteins have more than one function [22].

The simultaneous parameter estimation for two PGMs for more steady states is very difficult and nearly impossible for three PGMs. One can therefore assume a rather small likelihood for the right combination of substrate-product affinities for every PGM to occur during evolution. Moreover, our analysis shows that the system in not very sensitive to varying separately the affinity of beta or gamma PGM although the best fit fulfills the expectation regarding the affinities for the product/substrate for two isoenzymes (Tab. 1). Therefore, an occurrence of several PGMs could be explained, from the evolutionary point of view, as the simplest means to achieve the robust response of the regulated system. As we have shown, the number of isoenzymes has a big impact on the system robustness (Fig. 5). Indirect support for such speculation is our analysis for triple PGMs scenario where $k_M$ values, both for beta and gamma PGMs, were varied. The results demonstrate even higher robustness of the system (Fig. 5, green

**Figure 6. Grouping of PGMs and PSPs by using cluster analysis.** ClustalW2 2.1 (http://www.ebi.ac.uk/Tools/) was employed as a tool for protein alignment analysis, codon table for bacteria was selected. PGMs 1–4 (*Synechococcus*) and two PSPs (*Hydrogenobacter thermophilus*) are highlighted. PGM3 is clustered with two PSPs. doi:10.1371/journal.pone.0058281.g006
line) than the case of fixed beta PGM and varied gamma PGM (Fig. 5, blue line). Furthermore, the mean quality of match with experimental data improved by 5.6% in comparison to dual reverse PGMs scenario. Taken together, these results might imply that in the case of essential metabolic crossroads, the occurrence of more than two isoenzymes catalyzing the same reaction, especially in non-controlled environment, work as a buffer keeping the homeostasis of the system.

Despite the fact that our analysis cannot provide a clear cut answer why there are more PGMs, the presented approach contributes to an understanding of enzymatic regulation and provides a ration approach to identify the roles of particular, even non-homologous, isoenzymes.

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Supporting Information

Model S1 The model of primary carbon metabolism in cyanobacterium Synechococcus elongatus PCC 7942 is provided and encoded in SBML L2V4 and set up for triple PGMs scenario, based on the best fits of alpha, beta and gamma PGM (Fig. 4).

Author Contributions

Jointly discussed ideas and concepts: JJ MH DS OW. The modeling was done by: JJ. Contributed to the writing of the final manuscript: OW MH. Provided the experimental data: DS MH. Read and approved the final manuscript: JJ MH DS OW. Conceived and designed the experiments: MH. Performed the experiments: MH DS. Analyzed the data: JJ MH. Contributed reagents/materials/analysis tools: MH OW. Wrote the paper: JJ.