Role of phosphatidylglycerol in cyanobacterial cell fission

Scientific background

Phospholipids are indispensable structural parts of membrane matrix and they actively participate in the membrane and cellular processes. They have also significant role in energy storage and act as signaling molecules. Moreover, lipids could influence the characteristics and functions of membrane proteins. Phospholipids have important role in stress responses, such as cold, light and osmotic stresses.

Membranes of nonphotosynthetic bacteria and eukaryotic cells consist of various forms of phospholipids. Thylakoid membranes of cyanobacteria, green algae and higher plants have a rather specific lipid composition. Phosphtidylglycerol (PG) is the only phospholipid constituent of photosynthetic membranes and it has essential functional roles.

In several photosynthetic complexes the presence of PG molecules was detected by X-ray crystallography. PG has been shown in the core complex of photosystem I (PSI) and photosystem II (PSII) in thermophilic cyanobacterium, *Thermosynechococcus elongatus* as well as in the light harvesting antenna complex (LHCII) of PSII reaction center (RC) in higher plants. In PSII RCs PG was localized in the vicinity of Q_B, the secondary quinone acceptor. In PSI PG molecules were localized around the phylloquinone molecules, which participate in the energy transfer in the core protein complex of PSI reaction center. The role of PG in photosynthetic organisms has been studied using either biochemical or molecular genetic approaches. PG molecules are important for both the formation and functioning of photosynthetic apparatus. We have to take into account that the PG is essential and if its content of the cells decreases under certain percentage it would be lethal. Therefore it is difficult to conclude the direct role of the PG in many processes.

Cyanobacteria, the ancestor of chloroplasts are ideal model organisms for studying the photosynthetic and other cellular processes. The availability of the complete genome sequence of several cyanobacterial strains opened the way for studying the structural and functional roles of the PG *in vivo*. Generation of PG-deficient mutant strains is possible without perturbing the synthesis of other lipids, since phosphatidic acid (PA) is a benching point on the biosynthetic pathway toward PG synthesis. The biosynthesis of PG from PA to PG shown on Figure 1.

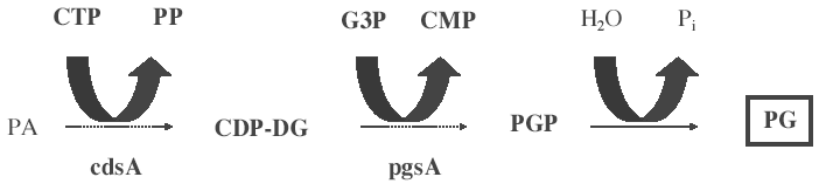


Figure 1: PG biosynthesis

PA is converted to CDP-diacylglycerol (CDP-DG) by cytidin-diacylglycerol synthase and then to phosphatidylglycerol-phosphate (PGP) by PGP synthase. The last step of PG biosynthesis is the dephosphorylation of PGP by PGP phosphatase. PG biosynthesis can be blocked at either the PA to CDP-DG transition or at the CDP-DG to PGP transition.

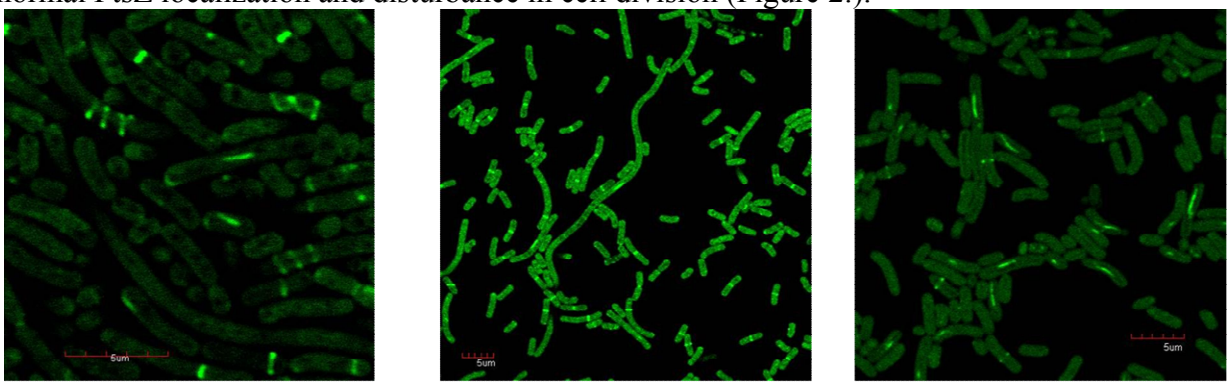
PG is essential for the cells however cyanobacteria are able to take up PG molecules from the culturing media. The uptake of the synthetic lipid molecules from culture medium can preserve cellular functions and study the effect of PG depletion from the cells. A decrease of intracellular

PG content occurs gradually by each cell division. In earlier studies our group has already demonstrated the role of PG in structure and function of photosynthetic complexes. Interestingly, morphological studies by electron microscopy revealed that the cell morphology is seriously affected by PG-depletion. PG depletion inhibits cell division although the synthesis of proteins and lipids, except PG, still continues. These observations emphasized a specific role of PG in cell division membrane-related, therefore we decided to elucidate whether the PG is involved in the cyanobacterial cell division process.

Results

Several proteins involved in bacterial cell division were identified mainly from *Escherichia coli*. and the encoding genes of these proteins have been also identified in cyanobacteria. Their role and importance in cell division are studied mainly in *Synechocystis* sp. PCC6803 and S*ynechococcus* sp. PCC7942 strains. As the earliest step of cell fission the FtsZ together with other specific proteins form a ring structure (the Z ring) at the division site, which is ubiquitous in bacteria, archea and also found in plastids as well as in mitochondria.. Location of Z ring is governed by MinC, MinD and MinE proteins, encoded on the *minB* operon. MinC together with the membrane associated MinD regulates cell division by suppressing formation of the Z ring. MinE prevents the activity of the MinCD heterodimer at the cell center where the Z ring can be formed

We have created several mutant constructs for investigating the role of PG and proteins involved in Z-ring formation. Standard molecular biological methods were used for the mutagenesis of *Synechococcus* sp. PCC 7942. FtsZ protein, was fused with Green Fluorescence Protein (FtsZ-GFP) for its *in vivo* cellular localization. The FtsZ-GFP construct was inserted into the genome of *Synecochoccus* PCC7942 at the NSII target site under the control of the *ftsZ* gene promoter. The NSII target site is generally used as a gene insertion site; this insertion does not have any influence on the growth. We were able to detect the GFP signal in the dividing cells indicating our fusion construct was functional. We inactivated Min proteins that are involved in the regulation of the Z-ring localization, in the FtsZ-GFP mutant to study their role in cell division. Using laser scanning confocal microscope in all Min mutants we could observe abnormal FtsZ localization and disturbance in cell division (Figure 2.).



***ΔminC*-FtsZ-GFP** ***ΔminE*-FtsZ-GFP** ***ΔminDE*-FtsZ-GFP**

Figure 2.

However, the FtsZ-GFP mutant cells also showed elongated phenotype due to the overexpression of the FtsZ. To elucidate the overexpression of the FtsZ we constructed a mutant in that *ftsZ* gene was inactivated by deletion and antibiotic resistance-cartridge-insertion, in addition FtsZ-GFP was expressed (Δ*ftsZ*-FtsZ-GFP). The mutant cells showed normal division with localization of the GFP signal at the midcell region and the size of the cells were rather uniform. For studying the role of PG in cell division we inactivated the *cdsA* gene (Δ*cdsA*-Δ*ftsZ*-FtsZ-GFP) and studied the effect of PG depletion on division ring localization.

Since we studied the effect of PG depletion on cell division therefore we checked the lipid content of the Δ*cdsA*-Δ*ftsZ*-FtsZ-GFP cells at various stages of PG depletion using thin layer chromatography and gas chromatographic measurements. Relative percentage of PG content decreased to at least to half of the PG supplemented cells during PG depletion before cells died.

Using laser scanning confocal microscope we followed the GFP signal localization to monitor the effect of PG depletion on division ring formation and morphological changes. PG supplied cells showed normal cell division, but we saw disordered FtsZ ring in the PG-depleted cells. With the gradual PG depletion cell elongation was more pronounced (Figure 3.). We could recover the cell division processes with the re-addition of PG to the culture, which indicates the direct role of this anionic lipid in cell division of cyanobacteria. Following a longer-term depletion we could not recover the cells even by adding the PG and the cultures dyed in a few days demonstrating PG is essential for the cells.

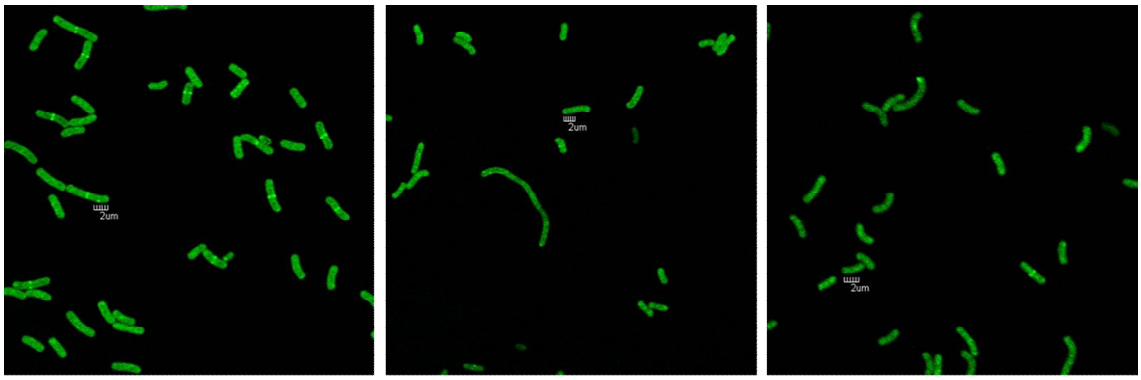


Figure 3. CLSM pictures of Δ*cdsA*-Δ*ftsZ*-FtsZ-GFP mutant cells

We studied the physiological properties of mutant cells, mainly focusing on photosynthetic parameters, and compared those to the characteristics of wild-type cells. Before cell division the cyanobacterial cells should reach an appropriated metabolic state and cell size. Cyanobacteria being photosynthetic bacteria their photosynthetic parameters could be representative for their general state.

We participated in the development of a new technique for monitoring photosynthesis in individual cells. Our results were published in the Biophysical Journal (Krumova SB et al. 2010).

We also used differential scanning calorimetry for the identification of photosynthetic complexes based on their calorimetric transitions (Laczko-Dobos H et al. 2011).

The changes in photosynthetic electron transport rates during PG deprivation were followed by measuring the redox changes in PSI reaction centres (P700) of bacterial cells by P700 and chlorophyll fluorescence measuring system. Our results demonstrated the negative effect of the

PG depletion on PSII activity and indicated that the rate of respiration is hampered in the lack of PG. We performed more detailed investigations to reveal the role of PG in the function of photosynthetic reaction centres and measurements of fluorescence yield indicated that PG depletion could affect the quinone binding site of PSII (Itoh S et al. 2012).

2D gel electrophoresis analysis revealed that the PG depletion affected the protein pattern of photosynthetic complexes, i.e. increase of PSII monomer on the expense of PSII dimer and the monomerization of PSI trimer could be observed (Figure 4.).

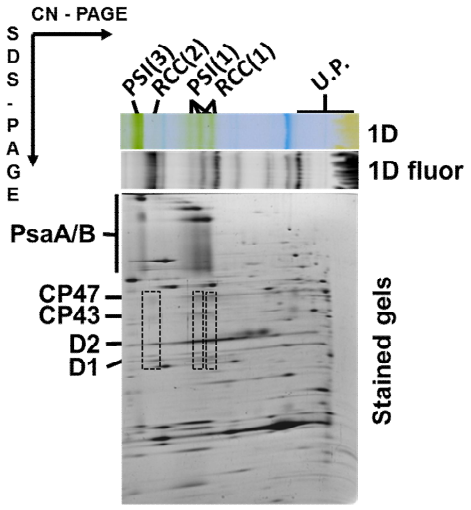


Figure 4. 2D gel electrophoresis of 12 days PG-depleted Δ*cdsA*-Δ*ftsZ*-FtsZ-GFP mutant cells

Cell-division processes are in close interaction with the membrane therefore membrane characteristics and components could have an impact on it. We studied the effect of the alteration in the membrane structural organization on the membrane characteristics and photosynthetic parameters. We summarized our results in several scientific papers published in international journals (Dankov KG et al. 2011, Klodawska K et al. 2012, Dobrikova AG et al. 2013).

During our investigations we got general overview on the role of membrane components and photosynthetic complexes so we summarized the collected information in review articles (Sozer O et al. 2011, Domonkos I et al. 2013).

To get a complete view about the role of PG in the cell division and in the photosynthetic processes we performed comparative proteomic analysis on PG-supplemented and PG-depleted cyanobacterial cells by mass spectrometry. Our analysis revealed 80 PG-regulated proteins involved in various cellular processes including photosynthesis, respiration, metabolism, transport, transcription, and translation (Talamantes T. et al 2014).

On the whole, our results revealed several role of PG in different cyanobacterial cellular processes among others including division ring localization and photosynthetic processes. However, further investigation needed for determination of the precise role and to unravel the function of the different participant in the various cellular processes.

Our publications related to the project and cited in the report:

Dankov KG et al. 2011 Plant Physiol Biochem 49: 629-35.
Dobrikova AG et al. 2013 Physiol Plant 147: 248-60.
Domonkos I et al. 2013 Prog Lipid Res doi: 10.1016/j.plipres.2013.07.001.
Itoh S et al. 2012 BBA 1817: 287-97.
Klodawska K et al. 2012 Acta Biochim Pol 59: 87-90.
Krumova SB et al. 2010 Biophys J 99: 2006-15.
Laczko-Dobos H et al. 2011 Photosynth Res 107: 237-46.
Sozer O. et al. 2011 Front Biosci (Landmark Ed.) 16: 619-43.
Tatjana T et al. 2014 accepted in Proteomics