The physiological role of phosphatidyl-glycerol in an obligate photoautotrophic cyanobacterium, *Synechococcus* sp. PCC7942

Abstract of Ph.D thesis

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Introduction

The cyanobacteria played dominant role in shaping Earths face from the distant past since approximately 3 billion years ago. Due to their ability of oxygenic photosynthesis they possess still significant role in biological oxygen, and biomass production of the biosphere. The phylogenetic distribution of cyanobacterial strains shows extreme diversity in which the filamentous strains display monophyletic and the unicellular strains show polyphyletic properties based on genetic, and morphological studies. The functional and structural relationship between chloroplasts and cyanobacteria is evident. As a result of this similarity the cyanobacteria are excellent model organisms in the study of photosynthetic processes; amongst them the unicellular, obligatory photoautothrophic *Synechococcus* sp. PCC7942 and *Synechocystis* sp. PCC6803 strains are particularly important.

The light reactions of photosynthesis take place on the so called thylakoid membranes of cyanobacteria and chloroplasts using membrane embedded protein complexes. From the viewpoint of photochemical charge separation the PSI and PSII complexes are particularly important. The biological processes of photosynthesis are highly affected by the lipid composition of thylakoid membranes, therefore the interactions between lipids and proteins in this membrane have functional importance. In cyanobacteria the thylakoid membranes have very conserved lipid composition, which is rather different from that of bacterial membranes and very similar to the lipid composition of higher plant chloroplast thylakoids. The majority of thylakoid lipids are neutral glicoglicerolipids from which the neutral MGDG and DGDG could be found in the largest amount. As a structural element of the membranes both lipid classes play important roles in oxygenic photosynthesis, due to determining the physical properties of thylakoids. As the minorities of thylakoid lipids, the anionic SQDG and PG have also important roles in protein-lipid interactions as it was shown in previous studies.

Synechocystis sp. PCC6803 and *Synechococcus* sp. PCC7942 cyanobacterial strains are the most popular model organisms in researching the role of membrane lipids in photosynthesis. The genome sequences of these two strains are available and there are lots of experiences accumulated during the genetic manipulation of them. In the fatty acid composition of membrane lipids there is a remarkable difference between the two strains.

The *Synechocystis* sp. PCC6803 contains polyunsaturated fatty acids and thus it is more similar to higher plant systems. However *Synechococcus* sp. PCC7942 strain incorporates only monounsaturated fatty acids into its lipids. The role of SQDG could be different in the two strains, because SQDG in *Synechocystis* sp. PCC6803 is essential compared to *Synechococcus* sp. PCC7942 where it is dispensable membrane lipid component. The SQDG mutant *Synechococcus* sp. PCC7942 shows physiological disturbances only under phosphate limiting conditions.

During the past few years many information have arisen from the studies of *Synechocystis* sp. PCC6803 Δ*pgsA*, Δ*cdsA* and PAL/Δ*cdsA* mutants. These mutants are auxothrophic mutants that are unable to synthesize PG *de novo*, but they could take it up from the growth medium. Removing the PG from the growth medium causes typical phenotypic changes that could be followed during the starvation period. These data suggest that in early phase of PG starvation mainly PSII functions are disturbed and defects on PSI can only be detected after longer term (14 days) PG depletion. From these results it is clear that PG has dominant effect on the oligomerisation state of thylakoid membrane protein complexes.

Goals

In order to clarify the possible strain specific role of PG in *Synechococcus* sp. PCC7942 the aims of the theses were the following:

1. The construction of an obligatory photoautotrophic, *Synechococcus* sp. PCC7942/Δ*cdsA* mutant strain, which has remarkably different fatty-acid composition from that of *Synechocystis* sp. PCC6803 model strain.

2. Identify the effect of PG starvation on the lipid composition, and fatty-acid composition of *Synechococcus* sp. PCC7942/Δ*cdsA* strain

3. Comparison of the physiological effect of PG depletion between *Synechocystis* sp. PCC6803/Δ*pgsA*, PAL/Δ*cdsA* and *Synechococcus* sp. PCC7942/Δ*cdsA* mutant strains. Identification of the role of PG on the oligomerisation state of photosynthetic complexes in the thylakoid membrane.

4. Describing the basic photosynthetic activities in *Synechococcus* sp. PCC7942/Δ*cdsA* mutant in the presence and in the absence of exogenously added PG to the medium.

Materials and Methods

Organisms and growth conditions

Synechococcus sp. PCC 7942/Δ*cdsA* cells were selected and grown on BG11 agar plates supplemented with 500 μM sodium acetate. *Synechococcus* sp. PCC 7942 WT and mutant cells were cultured in unbuffered BG11 liquid medium supplemented with 500 μM sodium acetate. The cultures were irradiated at 30 °C with 30 to 35 μmol photons $m^{-2}s^{-1}$ of continuous white light. Aeration was performed without additional CO_2, using a gyratory shaker at 100 rpm. 50 mL liquid cultures were maintained in 150 mL Erlenmeyer flasks in the presence of 20 μM dioleoyl-PG (18:1/18:1PG; P-9664; Sigma, St. Louis), 50 μg mL^{-1} kanamycin or 8 μg mL^{-1} chloramphenicol. PG depletion was carried out by washing the cells twice with PG-free medium, and culturing them thereafter in PG-free medium.

Synechocystis sp. PCC 6803/Δ*pgsA* mutant cells were grown photoautotrophically in BG11 medium supplemented with 5 mM HEPES-NaOH (pH 7.5), and 20 μg mL^{-1} kanamycin. Growth conditions, PG supplementation and PG depletion were similar to those used for *Synechococcus* sp. PCC 7942 cells.

Mutant generation, transformation of cells

Standard molecular biological methods were used during the targeted insertional mutagenesis of *Synechococcus* sp. PCC 7942. *Escherichia coli* DH5α and XL1-Blue cells (grown in Luria broth at 37 °C) were transformed using standard methods. The *cdsA* gene was identified by homology search using the published sequence of the *Synechocystis* sp. PCC 6803 *cdsA* gene (http://bacteria.kazusa.or.jp/cyanobase/). RCU1 5'-CTCGAGCAACGCTTGCTTAT-3' and RCD1: 5'-AATTCGCATTGCCGCTGAGG-3' primer pair was used to amplify the *cdsA* locus of *Synechococcus* sp. PCC 7942/Δ*cdsA*. The amplified 1596 bp *cdsA* PCR fragments were cloned into pMPM-A2 recipient vector and verified by automatic sequencing. The 209 bp *Mun*I/*Hinc*II fragment of the *cdsA*

gene was replaced by a kanamycin resistance cassette or a chloramphenicol resistance cassette from pZE21 and pZA3 vectors, respectively. The resulting plasmids carrying chloramphenicol resistance, pRC5C1, and kanamycin resistance, pRC5K1 were used for targeted insertional mutagenesis of the *cdsA* gene. Transformation of WT *Synechococcus* sp. PCC 7942 cells was done according to an optimized method. The segregation state of the transformants was checked by PCR amplification of the *cdsA* locus from the mutant chromosome.

Lipid analysis

Lipids were extracted from intact cells according to standard lipid analytic methods (Bligh and Dyer 1959). Lipid classes were separated by thin layer chromatography and fatty acids were analyzed on Supelco SP2330 capillary columns in a Hewlett Packard HP6890 gas chromatography equipment as described earlier

Spectroscopic measurements of cell density, proteins and pigments

Aliquots of cyanobacterial cells were collected and OD was measured at 750 nm using a Shimadzu UV-3000 Spectrophotometer (Columbia, MD). Cells were pelleted and extracted with acetone:methanol (7:2) The concentration of Chl was calculated from the absorbance at 663 nm of the extracted samples using an extinction coefficient of 82 mM^{-1} cm^{-1}. The protein content of extracted and pelleted cells was measured by the Lowry method..

Absorption spectra were taken using protein normalized cultures in 3ml quartz cuvettes using a Shimadzu UV-1601 spectrophotometer. The cultures were adjusted to 200µg/ml protein content. The baseline was corrected using two blank cuvettes filled with BG11 medium turned with the opaque side throughout the light path. The initial OD at 750nm was adjusted to zero and spectra were taken using normal scanning speed against a blank sample. The resulted spectra were further normalized to each-other using the absorption maximum of the phycobiliproteins (625nm).

Measurements of photosynthetic oxygen-evolving activity

Photosynthetic oxygen evolution in intact cells was measured with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, U.K.). Whole electron transport chain was measured from H_2O to CO_2 and PSII oxygen-evolving activity from H_2O to exogenously added artificial quinone molecules (500 μM p-BQ). Light from an incandescent lamp at a saturating light intensity of 500 μmol photons $m^{-2}s^{-1}$, equipped with a red optical filter was used in all oxygen evolution measurements. The Chl concentration of the samples was adjusted to 5 μg mL^{-1}.

Flash-induced fluorescence relaxation kinetics

The kinetics of the decay of flash-induced variable Chl fluorescence were measured in the 150 μs to 100 s time range by a double modulation fluorometer (PSI Instruments, Brno).

Protein analysis

Cells containing 75 μg of Chl were radioactively labeled at 29 °C and at 60 μmol photons m^{-2} s^{-1} with [^{35}S]Met (at >1,000 Ci $mmol^{-1}$, Isotope Institute Ltd, Hungary) for 20 min and then used for the isolation of membranes. Cyanobacterial membranes were prepared by breaking the cells with glass beads (150-200 μm diameter) at 4 °C followed by differential centrifugation.

The isolated membranes were solubilized with dodecyl-β-D-maltoside (dodecylmaltoside/Chl ratio of 40:1, w/w) and analyzed by BN/SDS-PAGE at 4 °C in a 5% to 14% polyacrylamide gel. The whole lane from the gel was excised, incubated for 30 min in 25 mM Tris–HCl buffer (pH 7.5) containing 1% SDS and then layered onto the top of a denaturing gel. The protein composition of the complexes was then assayed by the second dimension SDS-PAGE in a denaturing 12% to 20% linear gradient polyacrylamide gel containing 7 M urea. The protein bands were stained with Coomassie blue.

Results

1. *Synechococcus* sp. PCC 7942/Δ*cdsA* is a mutant defective in PG synthesis and is more susceptible to PG depletion than *Synechocystis* sp. PCC 6803/Δ*pgsA* or PAL/Δ*cdsA*.

2. PG depletion affects the lipid composition of *Synechococcus* sp. PCC 7942/Δ*cdsA*. The decrease in PG content is counterbalanced by an increase in SQDG content in the cells. Added artificial dioleoyl-PG was transformed to essential PG species originally present in the WT cells. However, neither the increased level of SQDG, nor the retailoring of fatty acid composition can compensate for the negative effects of the reduced PG content.

3. The oligomerization of PSII and PSI was severely perturbed by PG depletion in *Synechococcus* sp. PCC 7942/Δ*cdsA* following a short-term PG depletion. The newly synthesized D1 protein was observed in the non- assembled protein region of 2D gels showing the importance of the lipid for the efficient assembly of proteins into complexes.

4. PG depletion resulted in a relatively rapid decrease in PSII oxygen-evolving activity related to the perturbation on the acceptor side of PSII while its donor side was not primarily inactivated. In contrast to *Synechocystis* sp. PCC 6803/Δ*pgsA*, perturbation of the PSII acceptor site in *Synechococcus* sp. PCC 7942/Δ*cdsA* is not caused by the PG-deficiency-induced inhibition of electron transport between Q_A and Q_B and rather seems to be due to a decreased equilibrium constant for sharing the electron between the Q_A-Q_B and Q_AQ_B- states.

List of publications:

Source publication of the thesis:

Bogos B, Ughy B, Domonkos I, Laczko-Dobos H, Komenda J, Abasova L, Cser K, Vass I, Sallai A, Wada H, Gombos Z (2010) Phosphatidylglycerol depletion affects photosystem II activity in *Synechococcus* sp. PCC7942 cells PHOTOSYNTH RES 103:(1) 19-30
IF: 2.681

Publications related to the theses:

Domonkos I, Malec P, Sallai A, Kovacs L, Itoh K, Shen GZ, Ughy B, **Bogos B**, Sakurai I, Kis M, Strzalka K, Wada H, Itoh S, Farkas T, Gombos Z (2004) Phosphatidylglycerol is essential for oligomerization of photosystem I reaction center PLANT PHYSIOL 134: 1471-1478
IF: 5.881

Apostolova EL, Domonkos I, Dobrikova AG, Sallai A, **Bogos B**, Wada H, Gombos Z, Taneva SG (2008) Effect of phosphatidylglycerol depletion on the surface electric properties and the fluorescence emission of thylakoid membranes J PHOTOCH PHOTOBIO B 91:(1) 51-57
IF: 1.838

Laczko-Dobos H, Ughy B, Toth SZ, Kornenda J, Zsiros O, Domonkos I, Parducz A, **Bogos B**, Komura M, Itoh S, Gombos Z (2008) Role of phosphatidylglycerol in the function and assembly of Photosystem II reaction center, studied in a cdsA-inactivated PAL mutant strain of *Synechocystis* sp. PCC6803 that lacks phycobilisomes BBA-BIOENERGETICS 1777:(9) 1184-1194
IF: 4.447

Other publications:

Takacs M, Toth A, **Bogos B**, Varga A, Rakhely G, Kovacs KL (2008) Formate hydrogenlyase in the hyperthermophilic archaeon, *Thermococcus litoralis* BMC MICROBIOL 8: 10.1186/1471-2180-8-88- p.
IF: 2.877