Final Report (PD OTKA 108551)

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The main aim of this post-doctoral research grant was to investigate the cooperative effect of lipids, carotenoids and proteins in photosynthetic apparatus at various stress conditions, such as low- and high-temperature stresses, and high-light induced photoinhibition. The model organism used in this study was *Synechocystis* sp. PCC6803 cyanobacterial strain and various mutants derived from. With help of mutants I could study the roles of polyunsaturated lipids (AD mutant containing just saturated and monounsaturated lipids), among carotenoids the specific roles of xanthophylls (in RO mutant just carotenes are present, xanthophylls are missing). ROAD mutant was on the main focus of this study, because this mutant makes possible investigation in the same time the combined effect of lipids and xanthophylls on the structure and function of photosynthetic apparatus. Among the photosynthetic pigment-protein complexes I focused on PSI oligomers. PsaL mutant contains just PSI monomers, but PSI trimers are completely missing.

New cyanobacterial mutants like RO/PsaL (contain just xanthophylls and PSI monomers) and ROAD/PsaL (is a xanthophyll-less mutant, does not have polyunsaturated lipids, as well as PSI trimers) were successfully generated during this study. I followed not just PSI but also other pigment-protein complexes like PSII and the light harvesting complexes of cyanobacteria, the so called phycobilisomes, which are part of the photosynthetic apparatus, too.

During the three years period of grant I followed the work plan, and the obtained results were presented at various conferences, and published in international peer reviewed journals (Zakar et al. 2016; Zakar et al. 2017a).

Six months prolongation of my post-doctoral grant was very useful, I could perform extra experiments required by the reviewers for the submitted article, which has been published (Vajravel et al. 2017). The prolonged period made also possible to submit two other papers (Zakar et al. 2017b; Petrova et al. 2017) and to present one part of the grant results in a new manuscript, hope to be submitted very soon (Klodawska et al. 2017).

In this report I am going to summarize my results in three main points:

I) Lipid-carotenoid-protein interaction on membrane level (lipid remodeling, carotenoid reorganization)

II) Structural studies (additive effect of lipids and carotenoids on PSI oligomerization, phycobilisome structural stability)

III) Functional investigations (synergetic effect of polyunsaturated lipids, xanthophylls in temperature and light stress responses)

I. I performed detailed analysis of lipid content in wild-type and various *Synechocystis* mutant strains using a mass spectrometry based lipidomic approach. Surprisingly the lipid content of xanthophyll-less and polyunsaturated –lipid less mutant was substantially remodeled. Non bilayer to bilayer forming lipid ratios decreased significantly in the mutants. Bilayer forming lipids like digalactosyldiacylglycerol (DGDG), the sulfolipid sulfoquinovosyldiacylglycerol (SQDG), and the phospholipid phosphatidylglycerol (PG) can compensate the loss in membrane carotenoid and polyunsaturated lipid content. Bilayer lipids are important in stabilizing the unprotected and unbalanced membranes. The observed changes were not only on the lipid class levels, but also on lipid species, too. Changes in the saturation level of specific lipid classes like PG and SQDG proved to be very important in adaptation to temperature stresses, like moderate low and moderate high temperatures. Remodeling makes the thylakoid membranes extremely flexible and adaptive to stress conditions. The obtained remodeling results reveal that lipids and carotenoids can act cooperatively in this process (Zakar et al. 2017a). The removal of polyunsaturated lipids also resulted in the reorganization of the xanthophyll content, increasing the xanthophyll to β-carotene ratio. This demonstrates that lipids and carotenoids act cooperatively in maintaining and protecting membrane structures (Zakar et al. 2017a).

I analyzed the effects of PsaL protein deletion (which resulted in monomerization of PSI complexes) on the lipid composition of thylakoid membranes. In PSI trimer-less mutant I could detect dramatic change in the non-bilayer to bilayer lipid ratios, and on the fatty acid content of lipid molecules. The obtained results show that PSI monomerization both induces changes in lipid composition and affects dynamics of membranes (FTIR measurements), thus lead to the conclusion that the presence of PS I trimer is indispensable to maintain the native properties of thylakoid membranes in cyanobacteria in changing environmental conditions. To our knowledge this is a first report on how lipid components of a natural membrane responds to a change in oligomerization state of a protein component (unpublished results, manuscript close to submission (Klodawska et al. 2017).

II. I combined a variety of methods (clear native gel electrophoresis, FPLC and circular dichroism spectroscopy) to analyze the cooperative effect of lipids and carotenoids on the structure of photosynthetic protein-complexes. Saturated and monounsaturated lipids as well as xanthophylls may stabilize PSI trimers. These results suggest additive cooperation between the lipids and carotenoids, in which xanthophylls have a prevailing impact (Zakar et al. 2017a). However, the γ-linolenic acid deficient Spirulina I22 mutant showed similar trimer monomer ratio as it was detectable in xanthopyll-less mutant's (RO, ROAD and RO/PsaL) case. The lack of this polyunsaturated lipid resulted in PSI monomerization (Zakar et al. 2017b).

Further analysis of different xanthophyll-mutants pointed to the fact that among xanthophylls especially echinenone and zeaxantin are important for the fine-tuning of the PSI trimer structure. These xanthophylls could be part of the complex or be embedded in the membrane in the vicinity of PSI (Vajravel et al. 2017). The exact localization of xanthophylls in the photosynthetic complexes is yet to be determined.

A non-invasive technique like circular dichroism (CD) made possible also to study the influence of lipids and carotenoids on the structural integrity of light harvesting complexes of Cyanobacteria, the so called phycobilisomes. The obtained results show that among carotenoids xanthophylls play an important role in stabilizing the structure of phycobilisomes (Zakar et al. 2016). Differential scanning calorimetry (DSC) proved to be a powerful tool to investigate the structural integrity of these phycobilisomes in whole cells (Petrova et al. 2017). The obtained data demonstrate that the calorimetric profiles of cyanobacteria cells are dominated by a sharp symmetric transition at about 63 °C that originates from phycobilisomes denaturation. It is shown that the thermal stability of phycobilisomes can be studied in their native cell environment and that their transition temperature can be used as a marker for the phycobilisomes structural integrity within the cells. This opens up the possibility to assess the influence of a variety of factors (like xanthophyll- or polyunsaturated lipid deficiency, temperature and light stress) that might affect the stability and structural organization of phycobilisomes in intact cells and hence widen our knowledge on the role of the structural organization of phycobilisomes in optimizing the photosynthetic processes of cyanobacteria.

During the structural studies I faced with the fact that when we isolate the protein complexes from their native environment (for clear native gel, FPLC experiments) this might affect the fine structure or oligomeric status of protein complexes. Therefore it is good to develop and apply also other non-invasive, close to *in vivo* methods (like CD and DSC) for characterizing the structure of protein-complexes (Zakar et al. 2017b; Petrova et al. 2017).

III. Variable chlorophyll *a* fluorescence (OJIP), low-temperature fluorescence (77K) and oxygen-evolution measurements made possible to follow carotenoid and lipid deficiency-induced functional changes in the photosynthetic apparatus. In these functional studies I combined the temperature stress with the high light, too. High light is very dangerous for photosystem. It targets mainly PSII pigment-protein complexes, and causes so called photoinhibition. I followed also the so called recovery from photoinhibition process. The sensitivity of photosynthesis to high light is enhanced by xanthophyll deficiency. The lack of polyunsaturated lipids has much less effect on light sensitivity. Interestingly, combined xanthophyll and polyunsaturated lipid deficiency has a synergic effect in sensitizing the photosynthetic activities to high light. Functional studies suggest that, such cooperation between the lipids and xanthophylls are more pronounced at moderate low and moderate high temperatures. Both xanthophylls and polyunsaturated lipids affect mainly the recovery processes from photoinhibition. In contrast to earlier observations, moderate low and moderate high temperatures did not have major effects on the recoveries of only xanthophyll, or only polyunsaturated lipid mutants. However, cells with a combined deficiency in lipids and carotenoids were found extremely sensitive to light at suboptimal temperatures. This highlights the cooperation between lipids and carotenoids in alleviating stress effects (Zakar et al. 2017a).

In summary, results obtained during this post-doctoral OTKA grant study revealed that both unsaturated lipids and xanthophylls are required for ensuring the structural and physiological basis of efficient stress protection.

List of cited peer reviewed articles, submitted publications with peer review process and article manuscript:

Klodawska K, **Laczko-Dobos H**, Kis M, Strzalka K, Szalontai B, Gombos Z, Malec P (2017) PS I trimer is required to maintain lipid composition and dynamic properties of thylakoid membranes in cyanobacterium Synechocystis PCC6803. Manuscript close to submission

Petrova N, Todinova S, **Laczko-Dobos H**, Zakar T, Vajravel S, Taneva S, Gombos Z, Krumova S (2017) Structural integrity of phycobilisomes in intact Synechocystis sp. PCC 6803 wild type and mutant cells evaluated by means of differential scanning calorimetry. Submitted to Photosynth Res

Vajravel S, Kis M, Klodawska K, **Laczko-Dobos H**, Malec P, Kovacs L, Gombos Z, Toth TN (2017) Zeaxanthin and echinenone modify the structure of photosystem I trimer in Synechocystis sp. PCC 6803. Biochim Biophys Acta 1858 (7):510-518. doi:10.1016/j.bbabio.2017.05.001

Zakar T, Herman E, Vajravel S, Kovacs L, Knoppova J, Komenda J, Domonkos I, Kis M, Gombos Z, **Laczko-Dobos H** (2017a) Lipid and carotenoid cooperation-driven adaptation to light and temperature stress in Synechocystis sp. PCC6803. Biochim Biophys Acta 1858 (5):337-350. doi:10.1016/j.bbabio.2017.02.002

Zakar T, Kovacs L, Vajravel S, Herman E, **Laczko-Dobos H**, Gombos Z (2017b) Determination of PS I oligomerization in various cyanobacterial strains and mutants by non-invasive methods. Submitted to Photosynthetica

Zakar T, **Laczko-Dobos H**, Toth TN, Gombos Z (2016) Carotenoids Assist in Cyanobacterial Photosystem II Assembly and Function. Front Plant Sci 7:295. doi:10.3389/fpls.2016.00295