# CAROTENOIDS AFFECT THE STRUCTURE AND FUNCTIONS OF THE CYANOBACTERIAL PHOTOSYNTHETIC COMPLEXES

**Summary of the Ph.D. Thesis**

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# 1. INTRODUCTION

Photosynthesis is a process to convert light energy into chemical energy by providing the basic energy source for life on Earth and the oxygenic atmosphere. The photosynthetic electron transport occurs in the thylakoid membrane which accommodates the photosystem I (PSI), photosystem II (PSII) as reaction centers, light-harvesting antennae, cytochrome $b_6f$ (Cyt $b_6f$), and ATP synthase as the major photosynthetic protein complexes (Eberhard et al 2008, Hohmann-Marriott & Blankenship 2011). Cyanobacteria are ecologically important prokaryotes that perform oxygenic photosynthesis. Since the photosynthetic machinery of cyanobacteria show a strong homology with that of eukaryotes, it can be used as a suitable model organism to study the principles of photosynthesis and its regulation that is often difficult to approach in higher plants and algae. Cyanobacteria are also easily amenable to genetic engineering approaches (used in this thesis) and have the potential for industrial biofuel applications as well.

In cyanobacteria, red algae and glaucophytes, the main light-harvesting antenna is the phycobilisome (PBS). The PBS is a giant, multi-subunit pigment-protein complex, which is often the most abundant protein complex of the cell (Stadnichuk et al 2015). Most of the PBSs possess six to eight peripheral rods attached to the central allophycocyanin (APC) core complex by the support of linker proteins. As an antenna for PSI and PSII (Chang et al 2015), the PBSs absorb light in the wavelength range of 500-650 nm, which is less efficiently absorbed by the chlorophyll *a* (Chl *a*) molecules. Beside the light harvesting function, the PBSs also function as a nutrient reservoir. Under macronutrient limited conditions, the PBSs can supply amino acid residues for the cell by programmed PBS degradation. The direct damage of the rod and core linker polypeptides is accounted for the strong light conditions.

PSI is one of the major pigment-protein complexes of the oxygenic photosynthetic organisms and required for ferredoxin-reduction. The evolutionarily conserved PSI structure possesses a high sequence homology from prokaryotic cyanobacteria to higher plants. On the other hand, PSI complexes are present as monomers in plants; whereas those are preferentially organized into oligomers in cyanobacteria (Kruip et al 1994, Watanabe et al 2011). Only partial structure of PSI monomer of *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) has been obtained (Mazor et al 2014), so further studies are needed to reveal the exact structure of trimeric PSI. PSII catalyzes a unique reaction in nature – the light-induced mechanism of water oxidation (Barber 2016). It is the major source for the production of harmful reactive oxygen species (ROS), especially singlet oxygen under high-light stress (Krieger-Liszkay et al 2008).

Carotenoids are the most widely spread pigments in nature that are classified into two subgroups, the oxygen-containing xanthophylls and the carotenes (oxygen free). Carotenoids contain a long conjugated chain of double bonds which determines their light absorption (400-500 nm) properties (Britton 1995). In photosynthetic organisms, carotenoids are the most important elements of the non-enzymatic antioxidant system and also capable to quench triplet excited states of Chls (Domonkos et al 2013). Besides the protective roles, recently carotenoids are also considered to have a major role in the photosynthetic complexes of cyanobacteria (Sozer et al 2010, Toth et al 2015), as well as in plants (Croce et al 2002, Dall'Osto et al 2013). Due to the physicochemical properties, most of the β-carotene is bound to the photosystems (Jordan et al 2001, Guskov et al 2009) and to some less abundant proteins like high-light inducible proteins (HLIPs) (Komenda & Sobotka 2016). In *Synechocystis*, the most abundant xanthophylls are zeaxanthin, echinenone, and myxoxanthophyll. In spite of the high amount of xanthophylls in the thylakoid membrane, their exact localization and structural roles in the photosynthetic complexes have not been

unfolded yet. Several *Synechocystis* mutants have been generated by the inactivation of various genes, which are involved in the carotenoid biosynthesis pathways. Using these mutants, sequential elimination of different carotenoid forms could be obtained, which allows us studying their specific roles in the photosynthetic complexes.

## 2. **OBJECTIVES**

The present study was aimed at further investigating the influence of carotenoid composition on the structure and organization of the major photosynthetic complexes. In the proposed thesis, cyanobacterium *Synechocystis* was used as a model organism. The main advantages of this species are its entirely sequenced genome and easy transformability. Carotenoids are known to play a major role in photoprotection, regulation of the membrane properties, but less information is available about their structural roles in the photosynthetic complexes. The photosynthetic complexes should be organized and structurally interacted in the thylakoid membrane in order to perform the efficient photosynthetic energy transfer. Based on the previous findings in our laboratory, the following aims were investigated in the proposed thesis:

1. To find out which specific carotenoid species involved and understand the mechanism that eventually leads to the presence of unconnected PBS units, when the carotenoid composition is altered.

2. To study, how xanthophylls influence the oligomerization of PSI in *Synechocystis*. More specifically, which xanthophyll species contribute at what extent to the organization of the PSI complex.

## 3. **METHODS AND TECHNIQUES**

- Construction of *Synechocystis sp.* PCC 6803 mutants
- Absorption spectroscopy
- Steady state and Time-resolved fluorescence spectroscopy
- Oxygen polarography
- Circular-dichroism spectroscopy
- Isolation of thylakoid membranes and cytosolic fractions
- Sucrose density gradient fractionation of photosynthetic complexes
- Protein analysis: SDS, BN, and CN-PAGE
- Chromatographic techniques: HPLC and FPLC

## 4. SUMMARY OF FINDINGS

The dissertation is focused on the role of carotenoids in the assembly, structure, and function of cyanobacterial photosynthetic complexes, namely the phycobilisome antenna and photosystem I trimer.

***Role of carotenoids in the phycobilisome structure***

- The previous research in our laboratory revealed that the lack (*crtB*) or the limited availability (*crtH*) of carotenoids leads to the presence of unconnected PBS rod units and PBSs with shorter rods.
- Here, we have confirmed that a small fraction of β-carotene, which is not connected to the photosystems, is important for the properly assembled PBS. We have proved with a set of carotenoid deficient mutants, including a newly generated myxoxanthophyll deficient mutant (*cruF, in this thesis*), that none of the xanthophylls could significantly influence the structure of PBS.
- We have observed the reduced amount of rod linker proteins in the complete carotenoid deficient mutant *(crtB).* Thus, a higher rate of degradation or inhibition of linker protein synthesis could be accounted for the alteration of PBS structure.
- One of the main functions of carotenoids is the protection against ROS induced damage. Accordingly, the cells with lower carotenoid content (*crtH*) exhibited an increased production of a harmful ROS, so-called $^1O_2$. These cells were also more susceptible to high light treatment but did not show the accumulation of disconnected PC rods of PBS.

- In the *crtH* mutant, the remaining myxoxanthophyll could ensure the ROS protection of the PBS linker proteins. This hypothesis was conclusively rejected by the use of newly created double mutant *(crtH/cruF, in this thesis)*, which also lacks myxoxanthophyll.
- Thus, in spite of the reduced antioxidant protection of the cell, ROS induced direct damage did not seem to affect the phycobiliproteins or rod linkers of a cell possessing limited β-carotene.
- As a next approach, the enzymatic PBS degradation was induced by nitrogen starvation condition in the *crtH* and *crtB* mutants. Surprisingly, a delayed degradation of the disconnected PBS units was observed under nitrogen shortage.
- Our results suggest that the increased amount of unconnected PC units upon limited β-carotene availability is most probably due to an insufficient degradation rate of phycobiliproteins and/or an increased disassembly of PBS. It is rather surprising because the presence of carotenoids has not been identified in the structure of PBS.

***Effect of xanthophylls in the PSI trimeric complex***

- Earlier studies in our research group showed that absence of the majority of xanthophylls resulted in a higher propensity of the PSI trimers to disassemble into monomers. Here, we confirmed the presence of two kinds of xanthophylls, zeaxanthin, and echinenone in the isolated PSI trimeric complex.
- The spectroscopic data showed that both carotenoids have specific and not interchangeable roles in the structural organization of PSI *in vivo* and *in vitro* as well. It predicts the specific sites for the xanthophylls at the PSI complex *in vivo.*
- Our biochemical studies showed that the increased propensity of PSI trimers to disassemble into monomers in the absence of xanthophylls upon treatment by chelating agent EDTA (ethylenediaminetetraacetic acid). Hence, we propose that upon xanthophyll

deficiency, the structural differences in the monomer-monomer interface of the PSI trimer probably led to an easier removal of the structurally important $Ca^{2+}$.

- We found that a more rigorous purification protocol was able to eliminate the spectral difference between wild-type and xanthophyll deficient PSI trimers. This finding led to the proposition that the xanthophylls are either closely associated or present in the peripheral part of the PSI complex and can also interact with the surrounding lipid matrix.
- We propose that xanthophylls are performing mostly fine regulation of the PSI structure. It is rather surprising because xanthophylls were not considered to have a vital structural role in the photosynthetic complexes of cyanobacteria.
- It is interesting to note that the structural role of xanthophylls in PSI that was discovered in this thesis work has further supported by detecting both zeaxanthin and echinenone molecules in the recently published high-resolution, partial structure of *Synechocystis* PSI.
- We also observed a low number of zeaxanthin and echinenone molecules in the PSI complexes of *Thermosynechococcus elongatus*. Based on the previous work in our laboratory, the stability of the PSII complex is also influenced by the absence of xanthophylls in *Synechocystis*. Thus, it seems that the structural function of xanthophylls for photosystems could be a more general phenomenon, not only specific for *Synechocystis* species or PSI.

## 5. LIST OF PUBLICATIONS (MTMT: 10058037)

***Publications included in the thesis***

1. Vajravel S, Kis M, Kłodawska K, Laczko-Dobos H, Malec P, Kovács L, Gombos Z, Toth T.N (2017) Zeaxanthin and echinenone modify the structure of photosystem I trimer in *Synechocystis sp.* PCC 6803. *Biochim. Biophys. Acta* 1858(5): 510-518. (IF: 4.93).
2. Vajravel S, Kovács L, Kis M, Rehman A. U, Vass I, Gombos Z, Toth T.N (2016) β-Carotene influences the phycobilisome antenna of cyanobacterium *Synechocystis sp.* PCC 6803. *Photosynthesis Res* 130(1): 403-415. (IF: 3.86).

***Other publications***

1. Zakar T, Herman E, Vajravel S, Kovacs L, Knoppová J, Komenda J, Domonkos I, Kis M, Gombos Z, Laczko-Dobos H (2017) Lipid and carotenoid cooperation-driven adaptation to light and temperature stress in *Synechocystis sp.* PCC6803. *Biochim. Biophys. Acta* 1858(5): 337-350. (IF: 4.93).
2. Petrova N, Todinova S, Laczko-Dobos H, Zakar T, Vajravel S, Taneva S, Gombos Z, Krumova S (2018) Structural integrity of *Synechocystis sp.* PCC 6803 phycobilisomes evaluated by means of differential scanning calorimetry. *Photosynthesis Res* (in-press). (IF: 3.86).
3. Zakar T, Kovacs L, Vajravel S, Herman E, Kis M, Laczko-Dobos H, Gombos Z (2018) Determination of PS I oligomerisation in various cyanobacterial strains and mutants by non-invasive methods. *Photosynthetica* (in-press). (IF: 1.4).

## 4. SCIENTIFIC EXPOSURE

***Oral presentations***

1. Department of Plant Physiology and Biochemistry seminar series, Jagiellonian University, Krakow, Poland (2016).
2. Institute of Plant Biology seminar series, Biological Research Center, Szeged, Hungary (2016).

***Poster Presentations***

1. 42nd FEBS Congress on "From Molecules to Cells and Back", Jerusalem, Israel (2017).
2. 8th International Conference on "Photosynthesis and Hydrogen Energy Research for Sustainability", Hyderabad, India (2017).
3. Straub-Napok, Biological Research Center, Szeged, Hungary (2016).
4. International Meeting on "Photosynthesis Research for Sustainability", Crete, Greece (2015).
5. European Plant Science Retreat, Paris, France (2015).
6. 7th Symposium on Microalgae and Seaweed Products in Plant/Soil Systems "Contribution to Sustainable Agriculture", Masonmagarovar, Hungary (2015).
7. Straub-Napok, Biological Research Center, Szeged, Hungary (2015).

***Individual Research Training***

1. Laboratory of Photosynthesis, Institute of Microbiology, Center Algatech, Trebon, Czech Republic (2016 & 2017).
2. Department of Plant Physiology and Biochemistry, Jagiellonian University, Krakow, Poland (2016).

3. Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria (2016).