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Publication date
2004

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Citation for published version (APA):

Yeremenko, N. (2004). *Functional flexibility of photosystem I in cyanobacteria*. [Thesis, fully internal, Universiteit van Amsterdam]. Universiteit van Amsterdam - IBED.

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Chapter 6

Photosystem I trimers from *Synechocystis* sp. PCC 6803 lacking the PsaF and PsaJ subunits bind an IsiA ring of seventeen units

Photosystem I trimers from *Synechocystis* sp. PCC 6803 lacking the PsaF and PsaJ subunits bind an IsiA ring of seventeen units

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Keywords: photosystem I, IsiA, electron microscopy

Summary

We report a structural characterization by electron microscopy and image analysis of a supramolecular complex consisting of photosystem I (PSI) and the chlorophyll-binding protein IsiA from a mutant of the cyanobacterium *Synechocystis* sp. PCC 6803 lacking the PsaF and PsaJ subunits. The circular complex consists of a central PSI trimer surrounded by a ring of seventeen IsiA units, one less than in the wild-type supercomplex. We conclude that PsaF and PsaJ are not obligatory for the binding of the IsiA ring, and that the size of the PSI complex determines the number of IsiA units in the ring. The resulting number of seventeen copies implies that each PSI monomer has a different association to the IsiA ring.

Photosystem I (PSI) is a large multi-subunit pigment-protein complex embedded in the thylakoid membranes of green plants and cyanobacteria. Cyanobacterial PSI is arranged as monomers or trimers within the membrane and the structure of the trimeric complex from *Synechococcus elongatus* has been solved at 2.5 Å resolution by X-ray diffraction (Jordan *et al.*, 2001). It shows the positions of 12 protein subunits (PsaA-F, Psa-M and PsaX) and 127 cofactors.

Under conditions of iron deficiency, a common condition in many aquatic ecosystems, there is an overall reduction of the PSI level relative to that of Photosystem II (PSII). Iron limitation induces high levels of expression of IsiA, a chlorophyll *a*-binding protein with an amino acid sequence similar to that of the CP43 subunit of PSII (Burnap *et al.*, 1993). For the cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942, it has recently been shown that the IsiA protein associates to PSI, forming PSI-IsiA supercomplexes. The supercomplex is composed of trimeric PSI surrounded by an almost circular ring of 18 IsiA molecules (Bibby *et al.*, 2001; Boekema *et al.*, 2001; Nield *et al.*, 2003). Each IsiA subunit binds about 16-17 chlorophyll (Chl) *a* molecules (Andrizhiyevskaya *et al.*, 2002) and serves as a rapid and efficient antenna for PSI in the supercomplex (Andrizhiyevskaya *et al.*, 2002; Melkozernov *et al.*, 2003). Spectroscopic characterization of PSI-IsiA supercomplexes from cyanobacteria grown under iron starvation further suggest that the IsiA ring increases the absorption cross-section of PSI by about 70-100% (Andrizhiyevskaya *et al.*, 2002; Melkozernov *et al.*, 2003). The formation of supercomplexes is not only restricted to cyanobacteria under iron limitation. In a low-light adapted strain of the prochlorophyte *Prochlorococcus marinus* a supercomplex has been found between PSI and 18 Pcb proteins (Bibby *et al.*, 2001) and recently a supercomplex of 2 x 5 Pcb proteins flanking a dimeric PSII reaction center was reported for *Prochloron didemni* (Bibby *et al.*, 2003).

Chl *a* molecules bound to the low-molecular weight proteins of PSI have been suggested to play a role in the energy transfer from the outer IsiA ring towards the reaction center (Nield *et al.*, 2003). Three of these chlorophylls are bound to PsaJ, a subunit containing one transmembrane α -helix (Fromme *et al.*, 2001). PsaF is in close contact to PsaJ, also contains one transmembrane helix and forms hydrophobic interactions with several β -carotene molecules of the PSI core complex. A possible function for PsaF in cyanobacteria could be the stabilization of the ferredoxin binding (Jeanjean *et al.*, 2003) and the formation of an interaction with the external antenna system of phycobilisomes (Fromme *et al.*, 2001). We have used a *psaFJ* mutant of *Synechocystis* sp. PCC 6803 lacking the genes for the PsaF and PsaJ subunits (Zuther *et al.*, 1998) to investigate the role of these small PSI subunits in the PSI-IsiA supercomplex formation.

The *Synechocystis* sp. PCC 6803 *psaFJ* mutant was grown in BG11 medium as in (Zuther *et al.*, 1998). Iron-free growth was in BG11 medium without added source of iron. Culture inoculation was by 20 to 30 fold dilution of cells that were pregrown in normal medium, and cells were washed in iron-free medium prior to further culturing. For the present study cells harvested 48 h after inoculation were used. Iron-limitation was not obligatory to induce the formation of PSI-IsiA supercomplexes, but highest yield in supercomplexes was after an iron-limitation of 48-64 hours. Cells were broken and thylakoid membranes were prepared as in (Scholts *et al.*, 1996). PSI-IsiA particles were solubilized with *n*-dodecyl- β -D-maltoside (β -DM) as detergent as in (Andrizhiyevskaya *et al.*, 2002) and isolated by FPLC as described in (Boekema *et al.*, 1999). An FPLC fraction that eluted at a retention time comparable to the supercomplex of wild type *Synechocystis* from iron deficient medium was used for electron microscopy analysis. Samples of purified single particles were negatively stained in the presence of 0.03% β -DM using the droplet method with 2 % uranyl acetate on glow discharged carbon-coated copper grids (Boekema *et al.*, 2001). Electron microscopy was performed on a Philips CM20FEG electron microscope at 66,850 x magnification. Images of 2000 x 2000 pixels were recorded with a Gatan 4K slow-scan CCD camera with a step size of 15 μ m and a binning factor of 2, corresponding to a pixel size of 0.449 nm at the specimen level. Single particle projections were extracted from the digital images and analyzed with Groningen image processing ("GRIP") software on a PC cluster. A total of 2200 single particle projections were extracted from 680 images by selecting all discernible particles. The analysis of these images was started with multi-reference alignment (Harauz *et al.*, 1998; Van Heel *et al.*, 1992), followed by multivariate statistical analysis (Harauz *et al.*, 1998) and classification (Van Heel *et al.*, 1992).

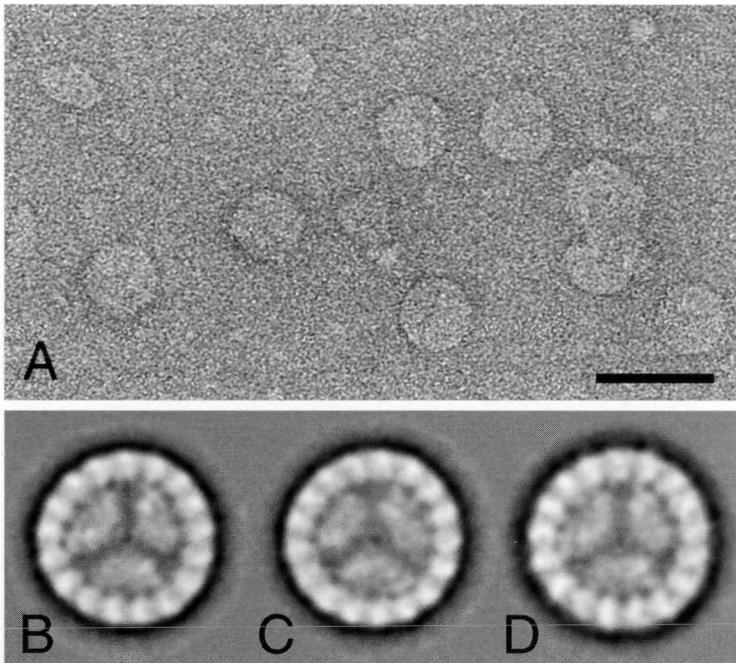


Figure 1. (A) Part of an electron micrograph of purified PSI-IsiA complexes from *Synechocystis* sp. PCC 6803 *psaFJ* mutant. (B-D) Classification of 2200 single particle top-view projections. Average images represent sums of 832 (B), 548 (C) and 576 (D) projections. The space bar in (A) equals 50 nm.

The electron microscopy images showed large numbers of circular shaped top-view projections and some degradation products (Fig. 1A). Repeated cycles of multi-reference alignment, multivariate statistical analysis and classification of the projections were done, and similar projections were clustered. The three predominant views are presented in Fig. 1B-D. All three images show a trimeric PSI molecule surrounded by 17 copies of IsiA. The image of Fig. 1B represents non-tilted molecules because it shows a strong three-fold rotation symmetry for the PSI trimer. Moreover, the 17 copies of IsiA all look rather similar. In the other two classes (Fig. 1C, D) one of the three PSI monomers appears smaller, indicating a slight tilt of molecules on the carbon support film. The apparent tilt is also visible from the appearance of the IsiA molecules, which look less homogeneous than in Fig. 1B. The diameter of the mutant complex plus detergent shell is 29.5 nm, which is clearly smaller than 33 nm for the native PSI-IsiA complex from *Synechocystis* sp. PCC 6803 (Boekema *et al.*, 2001) and 32.4 nm from the native PSI-IsiA complex of the related cyanobacterium *Synechococcus* PCC 7942 (Bibby *et al.*, 2001). (We previously reported 34.5 nm for the

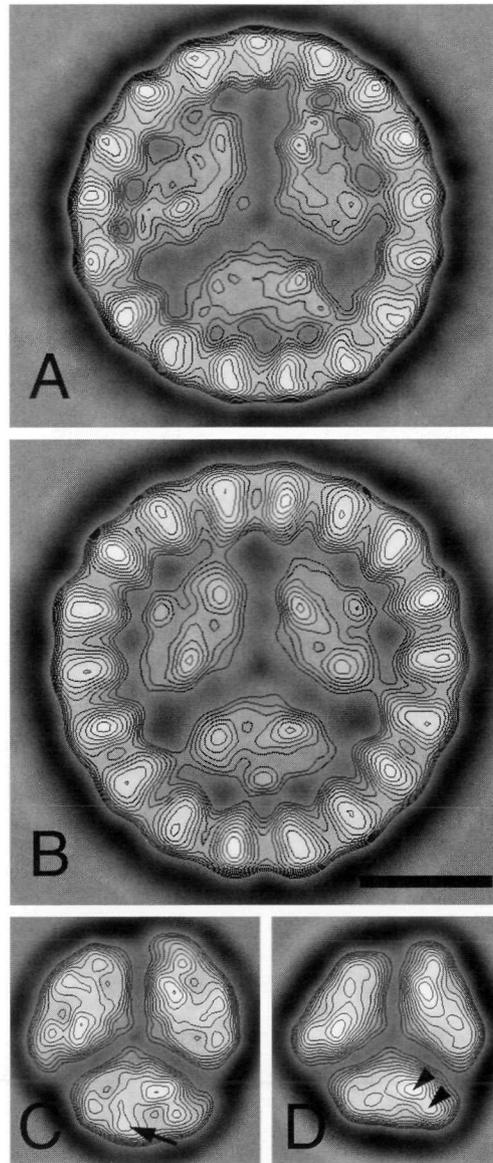


Figure 2. Comparison of PSI projections. (A) Contoured version of the PSI-IsiA complex from the *psaFJ* mutant of *Synechocystis* sp. PCC 6803; (B) native PSI-IsiA complex from *Synechococcus* sp. PCC 7942 (from (Boekema *et al.*, 2001)); (C) native PSII trimer from *Synechocystis* sp. PCC 6803 (from (Kruip *et al.*, 1997)); (D) PSII trimer from a AFK6 strain of *Synechocystis* sp. PCC 6803 lacking PsaF and PsaJ (from (Kruip *et al.*, 1997)). On the images of B, C and D three-fold rotational symmetry was imposed. A black arrow indicates the position of the PsaF/J density in the native trimer; arrowheads indicate the two most prominent densities in the trimer lacking PsaF/J. The space bar for (A) and (B) is 10 nm.

diameter (Boekema *et al.*, 2001), but recalibrating the pixel size with the new CCD camera and with a new densitometer indicated a 6% smaller value).

Comparison of contoured versions of the projections of mutant and wild-type cyanobacterial PSI indicate that the PSI monomer from the mutant supercomplex lacks the combined PsaF/J mass in a very similar way as was found previously in an electron microscopy study of single PSI trimers (Kruip *et al.*, 1997) (Figure 2A, D). Comparison to the wild type supercomplex further indicates that the absence of the peripheral PsaF and -J subunits allows for a 3 nm smaller ring of IsiA molecules to encircle a trimer (Fig. 2A, B). The resulting number of 17 copies, instead of 18 for the wild type, is intriguing because it implies that in the mutant complex each PSI monomer has a different position to the IsiA ring. Nevertheless, a precise organization of PSI and IsiA is possible without PsaF and PsaJ. These results demonstrate that IsiA can associate to PSI in alternative arrangements and that the size of the PSI complex determines the number of IsiA units in the surrounding ring. Obviously, a tight association of IsiA as a 17-unit ring in the mutant is thermodynamically more stable than a more loose association as a 18-unit ring. In the wild type, however, the 17-unit ring will probably not fit the larger circumference of the PSI trimer. The results also demonstrate that the PsaF and PsaJ subunits are not of prime importance for the structural integrity of PSI-IsiA supercomplexes. The relative functionality of the supercomplex with 17 IsiA copies awaits detailed study.

Acknowledgements

We thank Dr. Martin Hagemann (Universität Rostock, Germany) for making available the *psaFJ* mutant for this work and Dr. Gert Oostergetel for his help in EM data recording. This research was supported by grants from the European Commission (RTN2-2001-00092) and the Earth and Life Sciences Foundation (ALW) of the Netherlands Organization for Scientific Research (NWO).

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