

Rapid report

Photosystem I trimers from *Synechocystis* PCC 6803 lacking the PsaF and PsaJ subunits bind an IsiA ring of 17 unitsRoman Kouřil^a, Nataliya Yeremenko^b, Sandrine D'Haene^c, Alevtyna E. Yakushevskaya^a, Wilko Keegstra^a, Hans C.P. Matthijs^b, Jan P. Dekker^c, Egbert J. Boekema^{a,*}^a Department of Biophysical Chemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands^b Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, Faculty of Science, University of Amsterdam, Nieuwe Achtergracht 127, 1018 WS Amsterdam, The Netherlands^c Faculty of Sciences, Department of Physics and Astronomy, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands

Received 25 July 2003; accepted 11 August 2003

Abstract

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* PCC 6803 lacking the PsaF and PsaJ subunits. The circular complex consists of a central PSI trimer surrounded by a ring of 17 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 17 copies implies that each PSI monomer has a different association to the IsiA ring.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Photosystem I; IsiA; Electron microscopy

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 [1]. It shows the positions of 12 protein subunits (PsaA–F, PsaI–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 [2]. For the cyanobacteria *Synechocystis* PCC 6803 and *Synechococcus* 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 [3–5]. Each IsiA subunit binds about 16–17 chlorophyll (Chl) *a* molecules [6] and serves as a rapid and efficient antenna for PSI in the supercomplex [6,7]. 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% [6,7]. 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 [8] and recently a supercomplex of 2 × 5 Pcb proteins flanking a dimeric PSII reaction center was reported for *Prochloron didemni* [9].

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 [5]. Three of these chlorophylls are bound to PsaJ, a subunit containing one transmembrane α -helix [10]. PsaF is in close contact with PsaJ, which also

* Corresponding author. Tel.: +31-50-3634220/3634225; fax: +31-50-3634800.

E-mail address: boekema@chem.rug.nl (E.J. Boekema).

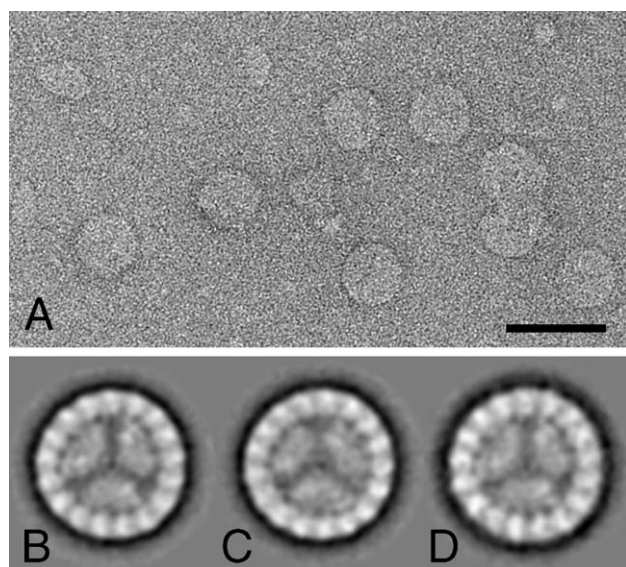


Fig. 1. (A) Part of an electron micrograph of purified PSI–IsiA complexes from *Synechocystis* PCC 6803 *psaFJ*-null mutant. (B–D) Classification of 2200 single particle top-view projections. Average images represent sums of 832 (B), 548 (C) and 576 (D) projections. The space bar in (A) equals 50 nm.

contains one transmembrane helix and forms hydrophobic interactions with several α -carotene molecules of the PSI core complex. A possible function for Psf in cyanobacteria could be the stabilization of the ferredoxin binding [11] and the formation of an interaction with the external antenna system of phycobilisomes [10]. We have used a *psaFJ*-null mutant of *Synechocystis* PCC 6803 lacking the genes for the Psf and Psj subunits [12] to investigate the role of these small PSI subunits in the PSI–IsiA supercomplex formation.

The *Synechocystis* PCC 6803 *psaFJ*-null mutant was grown in BG11 medium as in Ref. [12]. Iron-free growth was in BG11 medium without added source of iron. Culture inoculation was by 20–30-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 h. Cells were broken and thylakoid membranes were prepared as in Ref. [13]. PSI–IsiA particles were solubilized with *n*-dodecyl- β -D-maltoside (β -DM) as detergent as in Ref. [6] and isolated by FPLC as described in Ref. [14]. 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 [4]. Electron microscopy was performed on a

Philips CM20FEG electron microscope at $66,850\times$ magnification. Images of 2000×2000 pixels were recorded with a Gatan 4K slow-scan CCD camera with a step size of $15\text{ }\mu\text{m}$ 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

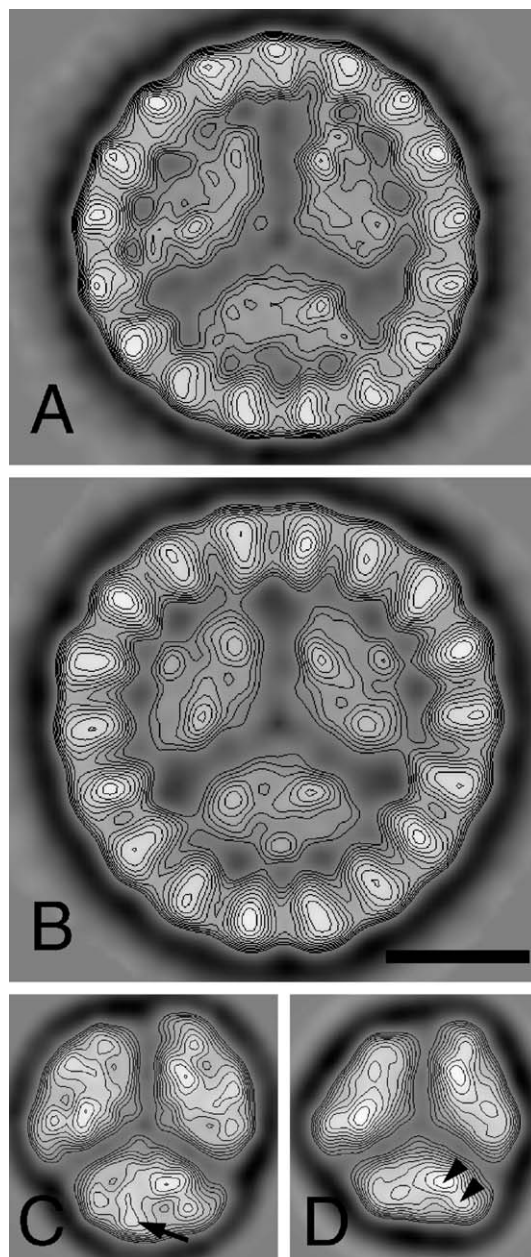


Fig. 2. Comparison of PSI projections. (A) Contoured version of the PSI–IsiA complex from the *psaFJ*-null mutant of *Synechocystis* PCC 6803; (B) native PSI–IsiA complex from *Synechococcus* PCC 7942 (from Ref. [4]); (C) native PSI trimer from *Synechocystis* PCC 6803 (from Ref. [17]); (D) PSI trimer from a AFK6 strain of *Synechocystis* PCC 6803 lacking Psf and Psj (from Ref. [17]). On the images of B, C and D 3-fold rotational symmetry was imposed. A black arrow indicates the position of the Psf/J density in the native trimer; arrowheads indicate the two most prominent densities in the trimer lacking Psf/J. The space bar for (A) and (B) is 10 nm.

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 [15,16], followed by multivariate statistical analysis [15] and classification [16].

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 in Fig. 1B represents non-tilted molecules because it shows a strong 3-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* PCC 6803 [4] and 32.4 nm from the native PSI–IsiA complex of the related cyanobacterium *Synechococcus* PCC 7942 [3] (we previously reported 34.5 nm for the diameter [4], 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 Psf/J mass in a very similar way as was found previously in an electron microscopy study of single PSI trimers [17] (Fig. 2A,D). Comparison to the wild-type supercomplex further indicates that the absence of the peripheral Psf 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 Psf and Psj. These results demonstrate that IsiA can associate with 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 an 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 Psf and Psj subunits are not of prime importance for the structural integrity of PSI–IsiA supercomplexes. The relative func-

tionality of the supercomplex with 17 IsiA copies awaits detailed study.

Acknowledgements

We thank Dr. Martin Hagemann (Universität Rostock, Germany) for making available the *psaFJ-null* 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 Dutch Scientific foundation NWO/ALW.

References

- [1] P. Jordan, P. Fromme, H.T. Witt, O. Kuklas, W. Saenger, N. Krauss, Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution, *Nature* 411 (2001) 909–917.
- [2] R. Burnap, T. Troyan, L.A. Sherman, The highly abundant chlorophyll–protein complex of iron-deficient *Synechococcus* sp. PCC7942 (CP43') is encoded by the IsiA gene, *Plant Physiol.* 103 (1993) 893–902.
- [3] T.S. Bibby, J. Nield, J. Barber, Iron deficiency induces the formation of an antenna ring around trimeric photosystem I in cyanobacteria, *Nature* 412 (2001) 743–745.
- [4] E.J. Boekema, A. Hifney, A.E. Yakushevskaya, M. Piotrowski, W. Keegstra, S. Berry, K.-P. Michel, E.K. Pistorius, J. Kruij, A giant chlorophyll-protein complex induced by iron deficiency in cyanobacteria, *Nature* 412 (2001) 745–748.
- [5] J. Nield, E.P. Morris, T.S. Bibby, J. Barber, Structural analysis of the photosystem I supercomplex of cyanobacteria induced by iron deficiency, *Biochemistry* 42 (2003) 3180–3188.
- [6] E.G. Andrihievskaya, T.M.E. Schwabe, M. Germano, S. D'Haene, J. Kruij, R. van Grondelle, J.P. Dekker, Spectroscopic properties of PSI–IsiA supercomplexes from the cyanobacterium *Synechococcus* PCC 7942, *Biochim. Biophys. Acta* 1556 (2002) 265–272.
- [7] A.N. Melkozernov, T.S. Bibby, S. Lin, J. Barber, R.E. Blankenship, Time-resolved absorption and emission shown that the CP43' antenna ring of iron-stressed *Synechocystis* sp. PCC6803 is efficiently coupled to the photosystem I reaction center core, *Biochemistry* 42 (2003) 3893–3903.
- [8] T.S. Bibby, J. Nield, F. Partensky, J. Barber, Oxyphotobacteria-antenna ring around photosystem I, *Nature* 413 (2001) 590.
- [9] T.S. Bibby, J. Nield, M. Chen, A.W.D. Larkum, J. Barber, Structure of a photosystem II supercomplex isolated from *Prochloron dimemni* retaining its chlorophyll *a/b* light-harvesting system, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 9050–9054.
- [10] P. Fromme, P. Jordan, N. Krauss, Structure of photosystem I, *Biochim. Biophys. Acta* 1507 (2001) 5–31.
- [11] R. Jeanjean, E. Zuther, N. Yermenko, M. Havaux, H.C.P. Matthijs, M. Hagemann, *FEBS Lett.* 594 (2003) 52–56.
- [12] E. Zuther, H. Schubert, M. Hagemann, Mutation of a gene encoding a putative glycoprotease leads to reduced salt tolerance, altered pigmentation, and cyanophycin accumulation in the cyanobacterium *Synechocystis* sp. strain PCC 6803, *J. Bacteriol.* 180 (1998) 1715–1722.
- [13] M.J.C. Scholts, P. Aardewijn, H.S. van Walraven, Membrane vesicles from *Synechocystis* 6803 showing proton and electron transport and high ATP synthase activities, *Photosynth. Res.* 47 (1996) 301–305.
- [14] E.J. Boekema, H. van Roon, F. Calkoen, R. Bassi, J.P. Dekker, Multiple types of association of photosystem II and its light-harvesting antenna in partially solubilized photosystem II membranes, *Biochemistry* 38 (1999) 2233–2239.

- [15] G. Harauz, E.J. Boekema, M. van Heel, Statistical image analysis of electron micrographs of ribosomal subunits, *Methods Enzymol.* 164 (1988) 35–49.
- [16] M. Van Heel, M. Schatz, E. Orlova, Correlation functions revisited, *Ultramicroscopy* 46 (1992) 307–316.
- [17] J. Kruip, P. Chitnis, B. Lagoutte, M. Rögner, E.J. Boekema, Structural organization of the major subunits in cyanobacterial photosystem 1. Localization of subunits PsuC, -D, -E, -F and -J, *J. Biol. Chem.* 272 (1997) 17061–17069.