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Supercomplexes of IsiA and Photosystem I in a mutant lacking subunit PsaL

Roman Kouřil[a], Nataliya Yeremenko[b], Sandrine D'Haene[c], Gert T. Oostergetel[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 of Biodiversity and Ecosystem Dynamics, Universiteit van Amsterdam, Nieuwe Achtergracht 127, 1018 WS Amsterdam, The Netherlands*

[c]*Division of Physics and Astronomy, Faculty of Sciences, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands*

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Abstract

The cyanobacterium *Synechocystis* PCC 6803 grown under short-term iron-deficient conditions assembles a supercomplex consisting of a trimeric Photosystem I (PSI) complex encircled by a ring of 18 IsiA complexes. Furthermore, it has been shown that single or double rings of IsiA with up to 35 copies in total can surround monomeric PSI. Here we present an analysis by electron microscopy and image analysis of the various PSI–IsiA supercomplexes from a *Synechocystis* PCC 6803 mutant lacking the PsaL subunit after short- and long-term iron-deficient growth. In the absence of PsaL, the tendency to form complexes with IsiA is still strong, but the average number of complete rings is lower than in the wild type. The majority of IsiA copies binds into partial double rings at the side of PsaF/J subunits rather than in complete single or double rings, which also cover the PsaL side of the PSI monomer. This indicates that PsaL facilitates the formation of IsiA rings around PSI monomers but is not an obligatory structural component in the formation of PSI–IsiA complexes.

Keywords: Photosystem I; IsiA; PsaL; Electron microscopy

1. Introduction

Photosystem I (PSI) in cyanobacteria is organized as a trimer or as a monomer. The structure of the trimer from the thermophilic cyanobacterium *Thermosynechococcus elongatus* has been resolved to 2.5 Å by X-ray crystallography [1]. It shows the position of the PsaL subunit in the center of the structure. The presence of this subunit is essential for the trimeric association [2], which is unique for cyanobacteria. In green plants, the closely associated PsaH subunit, not present in cyanobacteria, prohibits trimer formation [3].

Under conditions of iron deficiency, the iron-stress-inducible *isiAB* operon is induced [4]. This leads to the synthesis of, among others, the chlorophyll-binding protein IsiA (or CP43′). The IsiA present in the thylakoid membranes of iron-stressed cyanobacteria is likely to be present in several pools. It was shown by electron microscopy that after short-time stress, the PSI trimer can be encircled by 18 copies of IsiA [5,6]. Spectroscopic evidence has indicated that IsiA provides an additional light-harvesting function [7], and, thus, its function is explained by the supply of more light to the gradually shrinking pool of active PSI centers. Free IsiA is also present, in smaller or larger complete and incomplete ring-like structures [8]. This IsiA moiety forms a rather mobile fraction within the thylakoid membrane [9]. IsiA has also been suggested to associate to Photosystem II (PSII), where it may act as a quencher of

* Corresponding author. Tel.: +31 50 3634225; fax: +31 50 3634800.
E-mail address: boekema@chem.rug.nl (E.J. Boekema).

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excess excitation [10,11]. IsiA may also be regarded as a storage for chlorophyll that cannot be bound anymore to PSI units, as excitation of free chlorophyll would trigger dangerous oxygen ion formation. A similar function has been attributed to HLIPS chlorophyll proteins [12].

We recently showed that a mutant of *Synechocystis* PCC 6803 without the PsaF and PsaJ subunits forms an IsiA ring of 17 units around trimeric PSI [13]. The smaller number of bound IsiA proteins was explained by the smaller circumference of the PSI trimer of the mutant (the PsaF and PsaJ subunits are located at the outer rim of the PSI trimer [1]) and it was concluded that the size of the PSI complex determines the number of units in the IsiA ring [13]. After prolonged iron-deficiency, the number of bound IsiA complex can be much higher [8]. A monomeric PSI complex can be surrounded by 12–14 IsiA copies in an inner ring and another 19–21 copies in a second ring.

In order to investigate the role of the PsaL subunit in the formation of PSI–IsiA supercomplexes at various times after the start of culture in an iron-free medium, we analyzed a PsaL$^-$ deletion mutant of *Synechocystis* PCC 6803 [2], and investigated detergent-solubilized supercomplexes by electron microscopy and image analysis of a large set of single particle projections.

2. Materials and methods

2.1. Cell culture and isolation of PSI–IsiA complexes

A *Synechocystis* PCC 6803 mutant lacking PsaL was designed according to Ref. [2]. It was grown at 30 °C in BG11 medium at a light intensity of 50 μmol photons m^{-2} s^{-1} in ambient air. Iron deficiency was achieved by omitting all iron sources from the BG11 medium. Cells were harvested 6 days after inoculation in iron-free medium for short-term iron stress and 15 days for long-term iron stress. Subcellular preparations and isolation of PSI–IsiA complexes by FPLC were made according to Ref. [8].

2.2. Electron microscopy

EM was performed as described in Ref. [14]. Briefly, EM specimens were prepared on glow-discharged carbon-coated grids, using 2% uranyl acetate as a negative stain. EM was performed on a Philips FEG20 electron microscope. Semi-automated data acquisition was used to record a total of 4675 2048×2048 pixel images at 66,850× magnification with a Gatan 4000 SP 4K slow-scan CCD camera. The step size (after the binning) was 30 μm, corresponding to a pixel size of 4.5 Å at the specimen level and projections were selected for single particle averaging [15] with Groningen Image Processing software. Projections were aligned by multireference alignment and aligned images were subjected to multivariate statistical analysis (MSA). After MSA, particles were classified and summed and class sums were used in a next cycle of multireference alignment, MSA and classification. Final sums within homogeneous classes were obtained by reference-free alignment procedures [16].

3. Results

Purified supercomplexes from a PsaL mutant from *Synechocystis* PCC 6803 were investigated after short and long iron stress conditions. Multiple types of PSI–IsiA complexes were found by EM and image analysis. In the case of short iron stress, the variation in shape of the complexes is rather small. Classification indicated the presence of an incomplete double ring of IsiA associated to PSI monomers. The inner ring consists of either 6 (Fig. 1a,b) or 7 copies (Fig. 1c–e), whereas the outer ring is formed by 7–10 copies of IsiA. These same five types of complexes were also found under long-term iron stress, where the number of complexes with 7 IsiA copies in the inner ring was relatively higher. A larger complex with incomplete rings of 8 and 12 copies was also found (Fig. 1f). The individual IsiA copies are well resolved in the smallest particles, with 6 inner IsiA copies (Fig. 1a,b), whereas in the larger ones individual copies are somewhat fuzzy (Fig. 1f). This is explained by a higher flexibility in binding positions in the larger particles. Very small numbers of PSI-free IsiA-only supercomplexes were found (Fig. 1n).

In addition, monomers with either one or two complete IsiA rings were observed at long-term iron stress conditions (Fig. 1g–l). The total number of such complexes was about half of the ones with incomplete rings. In comparison to the complexes with incomplete rings, the individual copies of IsiA are poorly resolved, despite the fact that some of these supercomplexes have a larger size. A similar case of fuzzy details was previously observed for wild-type PSI monomers with double IsiA rings, where it was demonstrated that slight flexibilities between the rings lead to the loss in resolution [8]. Judging from the features and size, the complexes of Fig. 1g,h appear to have rings of 13 and 14 IsiA copies. The double rings in Fig. 1i have 12 plus 19 copies. The complexes of Fig. 1j,k both have 13 plus 20 copies but differ in overall shape. The largest double ring is formed by 14 plus 21 copies (Fig. 1l). Very similar complexes with the same numbers of IsiA copies in single or double rings have been observed for PSI monomers from wild-type *Synechocystis* [8].

No complexes consisting of PSI trimers were found in the PsaL mutant. Such complexes, like the one with a single ring of 18 IsiA copies (Fig. 1o), are abundant in wild-type *Synechocystis* and *Synechococcus* [5,6]. This is not surprising since the mutant lacks the PsaL subunit, which is essential for trimer formation [2]. Nevertheless, it appears that a unique dimeric complex can be formed. From its size and shape, it is likely that the complex in the middle is a dimeric PSI, surrounded by two rings of IsiA (Fig. 1m). The number of IsiA copies is 15 for the inner and 22–23 for the

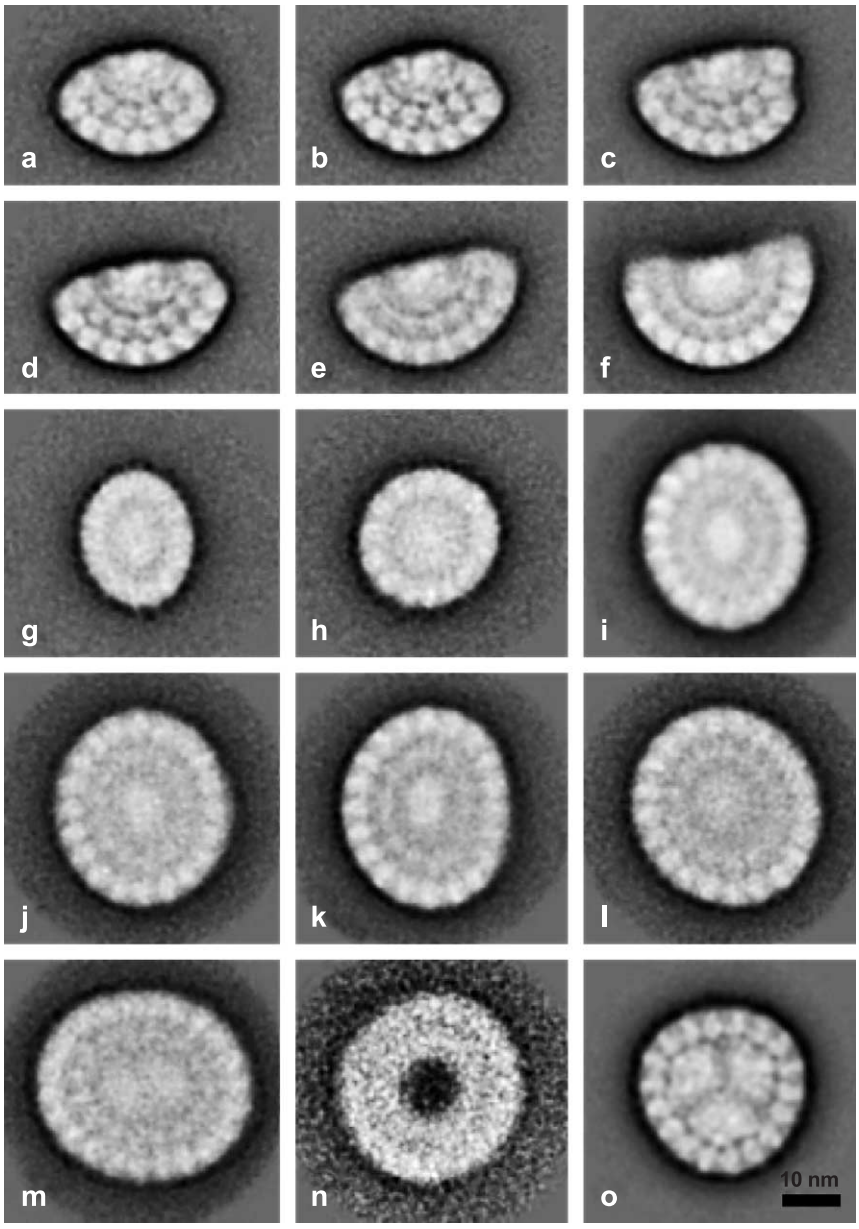

Fig. 1. Processed top views of PSI–IsiA and IsiA supercomplexes obtained by electron microscopy. (a–e) Results of statistical analysis and classification of particles from PsaL mutant *Synechocystis* PCC 6803 under short iron stress. From a total of 11,794 projections, 90% could be assigned to five specific complexes (a–e) with relative frequencies of 26%, 28%, 12%, 17%, and 17%, respectively. (f–n) Results of classification of particles under long iron stress in which nine additional types of complexes were found. From a total of 22,450 projections, 73% could be assigned to complexes presented in a–e plus f–n. The relative frequencies of the complexes a–n were 5% (a), 11% (b), 3% (c), 6% (d), 39% (e), 5% (f) 5% (g), 4% (h) 12% (i), 6% (j plus k), 2% (l), 2% (m) and 0.2% (n). (o) The common PSI–IsiA complex from wild-type *Synechocystis* PCC 6803 under short iron stress is shown for comparison [8].

outer ring. However, given the poor resolution, it cannot be fully ruled out that it is a dimeric PSII, in which the monomers have about the same dimensions, though they are less round. One argument against the presence of PSII in these structures is the fact that until now only incomplete rings of PSII and a Pcb antenna protein have been observed in *Prochloron dimemni* or *Prochlorococcus* species [17,18]. In addition, a closed antenna ring could hamper the interaction with membrane-inserted plastoquinone, the electron acceptor of PS II, in contrast to the situation at the acceptor site of PS I where ferredoxin acts in the water phase. However, if PSII would be inactive, this argument is not valid, and then the IsiA ring could serve as excitation quencher, as suggested in Refs. [10,11]. Finally, an extensive search within this and other sets of PSI–IsiA particles (over 100,000 picked particles in total) did not reveal any particles reminiscent of the supercomplexes between PSII and Pcb proteins as observed in *P. dimemni* or *Prochlorococcus* species [17,18]. Thus, it seems that there is no evidence for interaction of PSII and IsiA.

4. Discussion

Three different studies of large sets of PSI and IsiA complexes from WT and from PsaF/J and PsaL mutants indicate that IsiA can make a surprising number of structurally different complexes. Although these studies do not show atomic detail, it is evident that the precise association of IsiA to PSI is variable from the fact that trimers can be surrounded by either 18 or 17 IsiA copies, depending on the presence of PsaF/J [13]. In the case of PSI monomers, there is also variability in the association, because a native monomer can be surrounded by either 12, 13 or 14 copies of IsiA [8]. This work indicates that PsaL is not needed at all in the formation of PSI–IsiA complexes with complete rings around wild-type PS I monomers. It also appears that IsiA rings can encircle a dimeric particle, which is probably also PSI (Fig. 1m). The second ring even has 22–23 copies for encircling the inner ring, indicating that the number of IsiA copies can vary by a factor of about 2 in closed rings. Another point of interest is the fact that almost no free IsiA complexes without PSI appeared. It is possible that the monomeric state of PSI, forced by the absence of the PsaL subunits, leads to a higher overall binding of IsiA. The ratio of overall bound IsiA in the mutant and in the wild type was about 1:19.5 and 1:15.5, respectively.

However, the absence of the PsaL subunit seems to reduce the tendency to form complete rings around monomeric PSI, in comparison to the wild type. After long-term iron stress, the majority of PSI particles (~70%) is surrounded by incomplete rings (Fig. 1). In our wild-type study of IsiA structures, incomplete rings were only partially considered [8]. Reexamination of these data sets indicated that less than 10% of the PSI monomers were surrounded by incomplete IsiA rings. This discrepancy by a factor of at least 7 could on the one hand be explained by the loss of domains favoring the binding IsiA on the mutant PSI monomer. On the other hand, the shape of the remaining complex might be a more important factor. The loss of the L subunit leads to a rather elongated PSI monomer and this could weaken the overall interaction between the IsiA ring and PSI. In a very recent similar study of a PsaL mutant of PSI, Aspinwall et al. [19] also showed large numbers of incomplete rings of IsiA around PSI monomers. They did not observe, however, particles with complete rings and surprisingly all IsiA was bound in single incomplete rings only. However, the remarkable differences between the two studies can be explained from the use of different strains. Aspinwall et al. used a double mutant of *Synechocystis* without PS II reaction centers and glucose was added as source of reductant. In the present work, a mutant strain with PSII was used. In this type of strain and with a higher light intensity used, the electron flux to the stromal side of PS I is likely larger than in the PS II less mutant. Conditions for oxidative stress in the present work are more evident than in the work of Aspinwall et al. Oxidative stress is an important de-repressing condition for the *isiAB* operon as demonstrated by Jeanjean et al. [20]. Hence, one may expect a larger induction of IsiA proteins, visible as a higher number of IsiA copies in association with PSI.

Despite the fact that PsaL does not seem to be indispensable for IsiA binding and that a precise orientation of all IsiA molecules towards PSI is probably not necessary for excitation energy transfer, there must be specific domains on PSI for establishing some of the IsiA binding. The question is where these domains could be located. In the complexes of the PsaL mutant, we did not observe any type of incomplete rings binding on the PsaL site of PSI. Moreover, the best-resolved IsiA copies are from particles with six IsiA copies bound at the side of the PsaF/J subunits. Thus, we speculate that domains where IsiA multimers and PSI centers specifically associate are to be present at the PSI periphery close to PsaF/J subunits, but not on these subunits, because in their absence IsiA proteins still can associate.

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