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Biochimica et Biophysica Acta 1767 (2007) 528–534
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbabio

Structural response of Photosystem 2 to iron deficiency: Characterization of a new Photosystem 2–IdiA complex from the cyanobacterium *Thermosynechococcus elongatus* BP-1

Julia E.-M. Lax [a], Ana A. Arteni [b], Egbert J. Boekema [b], Elfriede K. Pistorius [c], Klaus-Peter Michel [c], Matthias Rögner [a,*]

[a] *Plant Biochemistry, Faculty of Biology, Ruhr-University Bochum, 44780 Bochum, Germany*
[b] *Department of Biophysical Chemistry, GBB, University of Groningen, 9747 AG Groningen, The Netherlands*
[c] *Molecular Cell Physiology, Faculty of Biology, Bielefeld University, 33501 Bielefeld, Germany*

Received 26 October 2006; received in revised form 12 December 2006; accepted 4 January 2007
Available online 13 January 2007
Dedicated to Prof. Jim Barber on the occasion of his 65th birthday

Abstract

Iron deficiency triggers various processes in cyanobacterial cells of which the synthesis of an additional antenna system (IsiA) around photosystem (PS) 1 is well documented [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, E.J. Boekema, A. Hifney, A.E. Yakushevska, M. Piotrowski, W. Keegstra, S. Berry, K. P. Michel, E.K. Pistorius, J. Kruip, A giant chlorophyll–protein complex induced by iron deficiency in cyanobacteria, Nature 412 (2001) 745–748]. Here we show that PS2 also undergoes prominent structural changes upon iron deficiency: Prerequisite is the isolation and purification of a PS2–IdiA complex which is exclusively synthesized under these conditions. Immunoblotting in combination with size exclusion chromatography shows that IdiA is only bound to dimeric PS2. Using single particle analysis of negatively stained specimens, IdiA can be localized in averaged electron micrographs on top of the CP43 subunit facing the cytoplasmic side in a model derived from the known 3D structure of PS2 [B. Loll, J. Kern, W. Saenger, A. Zouni, J. Biesiadka, Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II, Nature 438 (2005) 1040–4]. The presence of IdiA as integral part of PS2 is the first example of a new PS2 protein being expressed under stress conditions, which is missing in highly purified PS2 complexes isolated from iron-sufficient cells.

Keywords: Photosystem 2; Iron starvation; IdiA; Photosystem 2 structure; *Thermosynechococcus elongatus* BP-1

1. Introduction

Iron deficiency frequently occurs in the natural environment and is known to lead to multiple adaptational processes in cyanobacteria [4,5]. As several components of both photosynthetic and respiratory electron transport chains are iron-containing proteins, both chains are especially affected under conditions of iron limitation. This in turn can lead to an increased production of reactive oxygen species (ROS), mainly caused by photosystem (PS) 2 and PS1 [6–8]. Since several ROS-detoxifying enzymes such as catalases, peroxidases, and some superoxide dismutases contain iron as cofactor, a complex interrelationship between iron starvation and oxidative stress exists [4,9–11]. For various nutrient-limiting conditions multiple modifying reactions of the cyanobacterial transport chains have been described. These adaptational responses fall into the category of acquisition, compensation, and retrenchment [5].

Recently, two proteins, which have been shown to be induced under iron starvation in several cyanobacteria, have gained substantial interest: IsiA (*i*ron-*s*tress-*i*nduced protein *A*) and IdiA

Abbreviations: β-DM, dodecyl-β-D-maltoside; EM, Electron microscopy; IEC, ion exchange chromatography; HIC, hydrophobic interaction chromatography; MALDI-ToF, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PAGE, Polyacrylamide gel electrophoresis; PS, Photosystem; SEC, size exclusion chromatography

* Corresponding author.
E-mail address: matthias.roegner@rub.de (M. Rögner).

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doi:10.1016/j.bbabio.2007.01.003

(*i*ron *d*eficiency *i*nduced protein *A*). IsiA is the light-harvesting chlorophyll (Chl) *a*-binding protein CP43' [12,13]. In *Synechocystis* sp. PCC 6803 [1,14,15] and *Synechococcus elongatus* PCC 7942 [2] a ring of 18 IsiA proteins surrounds a trimeric PS1 complex. Within this supercomplex, the IsiA ring increases the PS1 antenna size by at least 70% [16] and the PS1 absorption cross-section by approximately 2-fold [17], serving as an efficient antenna. Recently, it has been shown that IsiA can also interact with PS1 monomers, surrounding them with single or double rings with multiple copies [18]. In addition to its function as antenna and as Chl *a* storage [19], IsiA has been suggested to protect PS1 and PS2 against excessive light through non-radiative energy dissipation [20–22]. Besides being expressed under iron starvation and oxidative stress [4,9,11,23], IsiA has also been shown to be up-regulated under sodium chloride stress [24] and in the stationary growth phase [25].

IdiA, the other stress responsive protein, has been shown to be highly expressed under iron starvation in *Synechococcus elongatus* PCC 6301/PCC 7942, *Synechocystis* sp. PCC 6803, and *Thermosynechococcus elongatus* BP-1 (subsequently called *T. elongatus* BP-1) [4,26–29]. In *Synechococcus elongatus* PCC 7942 IdiA is a protein of 40 kDa with a pI of 10.5. It has no transmembrane helices [28]. In *Synechococcus elongatus* PCC 6301/7942 parts of the IdiA protein pool become processed, resulting in a protein of 35 kDa. IdiA has similarity to a family of bacterial periplasm-located iron-binding proteins called SfuA, FbpA or HitA [30–33], and its expression is regulated by the positively acting transcription factor IdiB, which is also up-regulated under iron limitation [34]. While genes encoding IdiA-similar proteins have been found in all so far sequenced cyanobacterial genomes, no counterpart seems to exist in green algae and higher plants [4]. In *Synechococcus elongatus* PCC 6301/7942, IdiA has been shown to be mainly associated with the thylakoid membrane [27]. Thylakoid membrane preparations from cells, which were grown under mild iron deficiency and which express IdiA in highly elevated amounts, have an about 75% higher oxygen evolving activity (calculated on Chl basis) than preparations from cells, which were grown under iron-sufficient conditions [35]. A protective effect of IdiA on PS2 is supported by the observation that a *Synechococcus elongatus* PCC 7942 IdiA-free mutant showed a higher sensitivity of the photosynthetic water oxidation towards oxidative stress than wild type (WT), especially when caused by iron limitation [28]. A more detailed characterization of the IdiA-deletion mutant indicated that IdiA possibly interacts with PS2 [36] as the reduced PS2 activity, relative to the WT activity, was associated with an accelerated degradation of the D1 protein [28,36]. This result and the observation that iron-starved IdiA-expressing *Synechococcus* cells show a higher resistance towards the herbicide bentazone [37], suggests that IdiA protects PS2 at the acceptor side against damage in a direct or indirect way. However, since IdiA is lost during the PS2 preparation from the mesophilic *Synechococcus elongatus* PCC 7942, it has so far been impossible to prove a direct association of IdiA with PS2. In *Synechocystis* sp. PCC 6803 two IdiA-similar proteins are present, Slr1295 and Slr0513, both being suggested to be part of the cellular iron acquisition system in this cyanobacterium [38–40]. Toelle et al. [29] have shown that Slr0513 is indeed located in the periplasm and in part in the spheroplast fraction. However, Slr1295 was only detected in the spheroplast fraction and shown to co-purify with PS2 complexes, suggesting different functions of the two IdiA-similar proteins in *Synechocystis* sp. PCC 6803.

Bioinformatic analysis of the genomic sequence of *T. elongatus* BP-1 revealed that this cyanobacterium also contains an IdiA-similar protein (Tll0513, subsequently called IdiA) with a predicted molecular mass of 39.281 kDa and a pI of 10.1 having no transmembrane helices. Fortunately, an antiserum raised against IdiA from the mesophilic *Synechococcus elongatus* PCC 6301 also cross-reacted with the protein from the *T. elongatus* BP-1. Like in the mesophilic *Synechococcus*, also in *T. elongatus* BP-1, IdiA is localized at the thylakoid membranes of iron-starved cells [27]. Since PS2 complexes from *T. elongatus* BP-1 are known to be very stable and have been well-characterized both structurally and functionally [41], this organism is an excellent starting material for the isolation of PS2 complexes from cells grown under mild iron starvation to investigate whether IdiA is indeed associated with highly purified PS2 and to characterize these complexes.

2. Materials and methods

2.1. Cell growth and thylakoid membrane preparation

T. elongatus BP-1 was grown in 4 L penicillin flasks at 45 °C in BG-11 medium bubbled with CO_2-enriched air (5%) and illuminated with white light of 80–100 μmol photons m^{-2} s^{-1} as in [41]. For growth under iron-deficient conditions cells grown several times in regular BG11 medium, were harvested, washed once with iron-deficient BG11 medium, and were inoculated into fresh BG11 medium from which iron was omitted. The inoculum corresponded to an $OD_{750\ nm}$ of 0.3. After 4 days of iron-deficient growth in a 4 L penicillin flask with a constant illumination of ~100 μmol photons m^{-2} s^{-1}, cells with an $OD_{750\ nm}$ of ~4.0 were harvested for preparation of PS II complexes. After washing and resuspending in about 100 ml of buffer B (20 mM MES, pH 6.5, 10 mM $CaCl_2$, 10 mM $MgCl_2$, 0.5 M mannitol), lysozyme (Sigma, 50,000 units/mg protein, 3 × crystallized) was added to a final concentration of 0.2% (w/v), and cells were incubated in the dark at 37 °C for 90 min on a shaker. Cells were disrupted in presence of DNase (Sigma, 400–800 units/mg protein, lyophilized powder protein ≥ 85%) by a French Press treatment (20,000 psi), centrifuged (Sorvall GSA rotor, 18,000 rpm, 20 min, 4 °C), and washed with buffer B.

2.2. Purification of PS2 complexes

PS2 complexes were solubilized from thylakoid membranes with dodecyl-β-D-maltoside (β-DM) (1.2% (w/v)) and sodium cholate (0.5% (w/v)) and separated by centrifugation. The supernatant was loaded onto a sucrose gradient consisting of 14% sucrose (27 ml in buffer B with 0.03% (w/v) β-DM) on top of an 80% sucrose (w/v) pillow in the same buffer. After centrifugation (Beckman, SW28 rotor, 18 h, 4 °C) the PS2 complexes (visible as a green band in the upper layer) were collected with a syringe and further purified by hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEC) according to [41]. Homogeneity of the PS2 preparation was analyzed by analytical size exclusion HPLC [41].

2.3. Polyacrylamide gel electrophoresis (PAGE) and immunoblotting

Polypeptide composition of the PS2 core complexes was analyzed by SDS PAGE using 12% polyacrylamide gels containing 6 M urea according to Schaegger and Jagow [42]. Immunoblot analysis was done after SDS PAGE

(12% polyacrylamide gel according to [39] without urea) and blotting of the proteins onto a PVDF membrane using the capillary blot method.

Thereafter, the membrane was blocked with TBS-T buffer (10 mM Tris–HCl, pH 8, 150 mM NaCl, 0.05% (v/v) TWEEN 20) containing 5% (w/v) skimmed milk powder for 1 h, followed by washing for 2×5 min with TBS-T buffer. The following antisera were used as first antibody (incubation time 2 h): anti-IdiA (directed against IdiA of *Synechococcus elongatus* PCC 6301 [26] and anti-PsbO (directed against PsbO of oat [43]). Each antiserum was diluted in TBS-T buffer. The membranes were washed again 2×5 min with TBS-T buffer and the secondary antibody (anti-rabbit alkaline phosphatase conjugate, Sigma) was applied for 1 h. After washing 2×5 min with TBS-T buffer, the membrane was equilibrated with alkaline phosphatase buffer (100 mM Tris–HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$). For staining, 10 µl nitrotetrazolium blue (50 mg/ml NBT in 70% (v/v) *N*,*N*-dimethylformamide) and 10 µl 5-bromo-4-chloro-3-indolyl phosphate (50 mg/ml BCIP in *N*,*N*-dimethylformamide) in 15 ml of alkaline phosphatase-buffer were added.

2.4. Mass spectrometry

For matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF) analysis isolated and purified PS2 complexes were spotted in a dried droplet application to the sample plate, using sinapic acid and ferulic acid as matrices. After calibration with a standard (Sequazyme Kit, Applied Biosystems), protein masses between 1000 and 20,000 (*m*/*z*) were determined in a linear mode with a MALDI-ToF mass spectrometer (Voyager DE Pro, Applied Biosystems).

For peptide mass fingerprint (PMF) of proteins separated by SDS PAGE including 6 M urea, the respective gel bands were digested with trypsin solution (12.5 ng/µl trypsin in 25 mM NH_4HCO_3, Promega, 18,000 units/mg protein). α-Hydroxycinammic acid was used as matrix material.

2.5. Electron microscopy (EM) and image analysis

EM was performed according to [44]. EM specimens were prepared on glow-discharged carbon-coated grids using different negative stains, and EM was performed on a Philips FEG120 electron microscope. Semi-automated data acquisition was used to record a total of 300 2048×2048 pixel images at 77,200× magnification with a Gatan 4000 SP 4K slow-scan CCD camera. Step size (after the binning) was 30 µm corresponding to a pixel size of 3.8 Å at the specimen level. Projections were selected for single particle averaging with the Groningen Image Processing software and aligned by a multi-reference alignment followed by multivariate statistical analysis (MSA). Thereafter, particles were classified and summed, and the class sums were used in a next cycle of multi-reference alignment, MSA, and classification. Final sums within homogeneous classes were obtained by reference-free alignment procedures.

3. Results

3.1. Isolation of PS2 complexes from cells grown under iron-deficient conditions

T. elongatus BP-1 cells were grown in BG11 medium with or without iron for 4 days. After harvest and cell breakage, PS2 complexes were solubilized with β-DM and enriched by sucrose gradient centrifugation, followed by further purification using hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEC) (for details see Materials and methods). Fig. 1A shows the IEC elution profile of PS2 complexes isolated from cells grown under iron-deficient conditions. There is no principal difference to the elution profile of cells grown under iron-sufficient conditions [41], i.e. the PS2 complexes consist of three fractions: (i) PS2 monomers, (ii) PS2 dimers with high activity, and (iii) PS2 dimers with low activity. The reason for the low activity of the second dimeric PS2 complexes, which is a minor fraction, is presently unknown. The major characterizations of this report focus on the monomeric and the first dimeric PS2 peak. Size exclusion chromatography (SEC) confirmed that the first PS2 peak from iron-starved cells indeed represents monomers (retention time 19.5 min) and the second peak (retention time 20.5 min) represents PS2 dimers (not shown). Fig. 1B shows

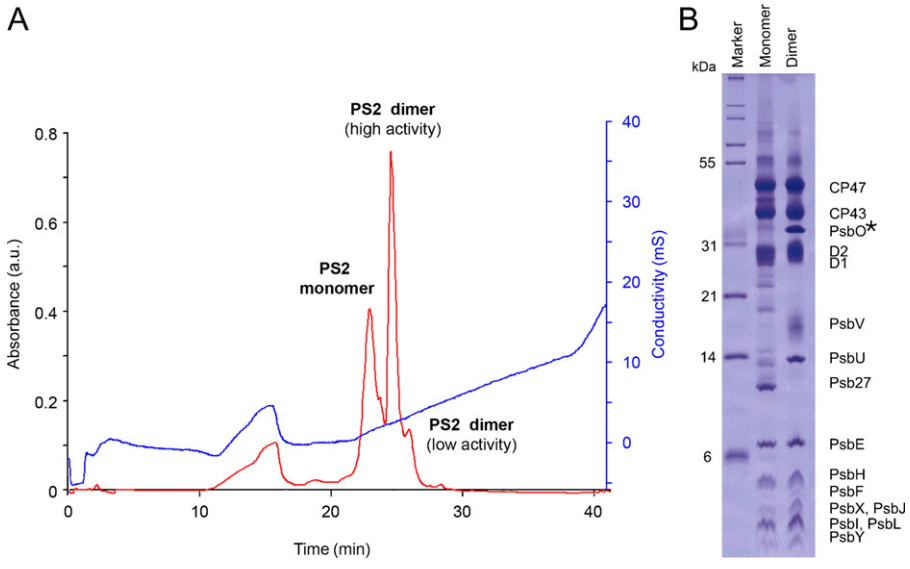

Fig. 1. (A) Elution profile of PS2 (isolated from iron-deficient cells) from the IEC column. Elution was performed with a magnesium sulphate gradient of 0.01 M to 0.4 M; the corresponding conductivity in mS is shown as blue line. Absorbance was recorded at 280 nm (red line). For details see text. (B) SDS urea PAGE of monomeric and dimeric (high activity) PS2 fraction of iron-deficient cells from the IEC column. SDS PAGE was performed with a 12% polyacrylamide gel containing 6 M urea according to [42]. Each lane contains 40 µg proteins. Staining was done with Coomassie Brilliant Blue (for details see Materials and methods). The proteins were identified in analogy to known protein patterns of other PS2 preparations from *T. elongatus* BP-1 and Maldi-ToF-MS analysis. The PsbO protein band has been labelled with an asterisk to indicate that in PS2 dimers the PsbO band also contains the IdiA protein.

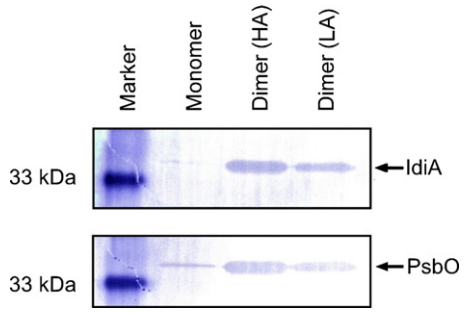

Fig. 2. Immunoblot analyses of PS2 complexes isolated from *T. elongatus* BP-1 cells grown under iron deficiency. The three PS2 peak fractions from the IEC column (PS2 monomers, PS2 dimers with high oxygen evolving activity (HA) and PS2 dimers with low oxygen evolving activity (LA)) were separated by 12% SDS PAGE according to [42], with each lane containing 20 μg proteins. Immunological detection was with the anti-IdiA antiserum (dilution 1:500) or with the anti-PsbO antiserum (dilution 1:1000) and a secondary antibody coupled to alkaline phosphatase.

the SDS urea PAGE pattern after staining with Coomassie Brilliant Blue for the PS2 monomers (peak 1 of Fig. 1A and lane 2 of Fig. 1B) and PS2 dimers (peak 2 of Fig. 1A and lane 3 of Fig. 1B) isolated from iron-starved cells. The protein pattern of both samples basically corresponds to those of PS2 complexes isolated from cells grown under iron-sufficient conditions [41]. The dimeric PS2 fraction showed all major PS2 subunits: (i) the D1 and D2 reaction center peptides, (ii) the core antenna proteins CP47 and CP43, (iii) the α-subunit of the Cyt b_{559} protein, and (IV) the extrinsic peptides PsbO, PsbU, and PsbV.

In contrast, the monomeric PS2 fraction did not contain the three extrinsic proteins (or only minor amounts of them). Instead, this fraction contains the lipoprotein Psb27, which is suggested to be involved in PS2 assembly [45]. In comparison to the highly active dimeric PS2 complexes, monomeric PS2 complexes contain more contaminations with unidentified proteins. Below CP47 another protein band is seen, which may represent a CP47 degradation product. Several bands in the range of 14 to 21 kDa possibly represent phycobilisome subunits. No significant differences in SDS urea PAGE were observed with respect to the small PS2 subunits (<10 kDa) of monomeric and dimeric PS2 complexes, which was also confirmed by MALDI-ToF MS. This implies that PS2 subunits of cells grown under iron-sufficient conditions are also present in PS2 complexes of cells grown under iron-deficient conditions (data not shown).

3.2. Immunological detection of IdiA

Since the SDS urea PAGE pattern of PS2 complexes isolated from iron-starved cells did not provide evidence for the presence of an additional protein in the 35 kDa region after staining with Coomassie Brilliant Blue, the PS2 complexes were probed after SDS Page and immunoblotting with an antiserum raised against the isolated IdiA protein from the mesophilic *Synechococcus elongatus* PCC 6301 [26]. In addition, an antiserum raised against the PsbO protein, a major PS2 subunit (apparent mass 35 kDa, calculated mass 29.6 kDa), known to lack transmembrane helices, was also used. All three PS2 fractions eluated from the IEC column (Fig. 1A) were analyzed. As shown in Fig. 2, IdiA was detected in PS2 dimers with high and PS2 dimers with low activity (see Fig. 1A), while monomeric PS2 complexes contained hardly any IdiA protein. All three PS2 fractions contained the extrinsic PsbO protein. The highest amount was present in PS2 dimers with a high oxygen-evolving activity. Thus, the immunoblot experiments clearly show the presence of IdiA in highly purified PS2 dimers. This result was verified by peptide mass fingerprint analysis after trypsin digestion (Table 1).

3.3. EM of the PS2–IdiA complexes

Electron microscopy and image processing were performed to investigate whether an additional density could be detected in PS2 dimers and to investigate where IdiA is located in PS2. Preparation of PS2 complexes for electron microscopy using 2% uranyl acetate pH 5.0 (commonly used for staining of the complexes) resulted in a complete loss of IdiA from the PS2 complex. This was verified by applying PS2 complexes to size exclusion chromatography (SEC) at pH 5.0 also resulting in a separation of IdiA from the PS2 complexes (detection by immunoblotting). This separation of IdiA from PS2 complexes is most likely due to the fact that IdiA is a basic protein (calculated pI = 10.1) and therefore, the IdiA–PS2 interactions under the used experimental conditions are weakened. For this reason, we tried various other staining solutions: (i) 2% uranyl oxalate pH 7.0, (ii) 2% phosphotungstate acid pH 7.2, (iii) 2% Nano V (vanadium derivative)

Table 1
Analysis of peptide fragments of IdiA and PsbO

Protein	*m/z* submitted	MH^+ matched	Δppm	Start [aa]	End [aa]	Missed cleavages	Database protein sequence
IdiA	1181.5293	1181.7006	− 145	118	128	0	(R)-VLNSVVPANLR-(E)
	1803.8017	1803.8198	− 10	299	313	0	(K)-AQEMFAMANFEYPVR-(A)
	2046.2481	2046.0885	78	247	264	2	(K)-EQDRAVAAKVGLFFPNQR-(D)
PsbO	1709.2158	1708.8758	199	29	44	0	(K)-QTLTYDDIVGTGLANK-(C)
	2124.4367	2124.0825	167	150	169	0	(K)-NLVASTQPNVTSITTSTDFK-(G)
	2929.2045	2929.8798	− 26	234	260	0	(R)-TGEIAGTFESEQLSDDOMGAHEPHEVK-(I)

In SDS urea PAGE the proteins IdiA and PsbO run in one band, which was excised, trypsin-digested, and analyzed by MALDI-ToF-MS. MS-FIT evaluation of the peptide mass fingerprint data detected three peptides of IdiA and PsbO.

pH 8.0, and (iv) 2% Nano W (organotungstate compound) pH 6.8. Among these, 2% uranyl oxalate pH 7.0 gave the best results.

Figs. 3A and 4C show the negative-stain projection maps of highly active dimeric PS2 complexes at a resolution of 24 Å isolated from *T. elongatus* BP-1 cells grown under iron-deficient or iron-sufficient conditions, respectively. The picture represents a dimeric PS2 particle in which both monomers are very similar as compared with the 2-fold symmetrized projection map (Fig. 3B). The PS2/IdiA map clearly shows some distinct differences to maps from PS2, which has been purified from cells grown under iron-sufficient conditions (Fig. 3C) [41,44]. This is seen in a difference map, in which the strongest additional masses are marked with white triangles and the strongest reduced masses with black triangles (Fig. 3D). The white arrowhead indicates the position of the strongest increased density, which is considered to be occupied by the IdiA subunit. A comparison with the high-resolution X-ray structure indicates that this deficiency is at the position where the PsbO subunit is located (Fig. 4). It could indicate some loss of PsbO during specimen preparation for electron microscopy after purification. However, since the particles are active in oxygen-evolution (not shown), this implies that major parts of dimeric PS2 contain PsbO. The partial absence of PsbO and the presence of IdiA next to this site give the PS2-IdiA particle a clearly different appearance. Other positive differences have smaller amplitudes and do not result in a significant change in the negative-stain profile. Such differences at the periphery of the complex can rather be attributed to different detergent boundary layers than to a different protein composition.

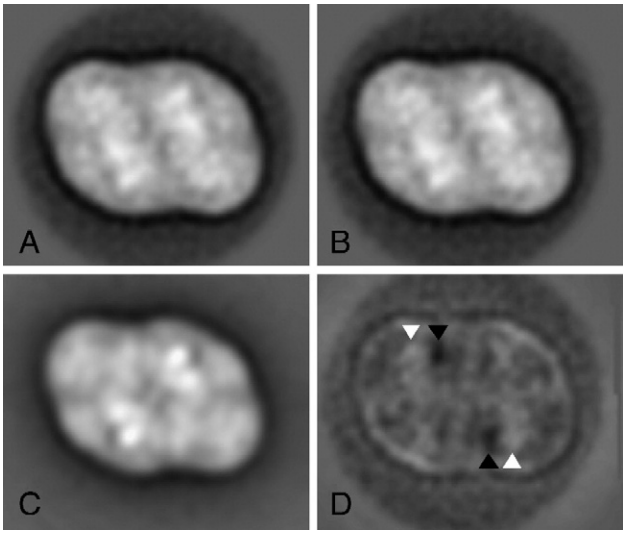

Fig. 3. Single particle analysis of PS2 complexes from *T. elongatus* BP-1 grown under iron-deficient or iron-sufficient conditions. (A) Average projection of the best 4261 dimeric PS2 particles out of the PS2–IdiA particles isolated from cells grown under iron-deficient conditions, analyzed without imposing symmetry. The particles are attached to the EM grid with their lumenal side and thus, are seen from the cytoplasmic side of the thylakoid membrane. (B) Same image as in frame A but with 2-fold symmetry imposed. (C) Image of dimeric PS2 under iron-sufficient conditions (from [44]). (D) Difference image of images A and C. The white arrowhead indicates the position of the strongest additional density difference; the black arrowhead indicates the position of the strongest density deficiency difference.

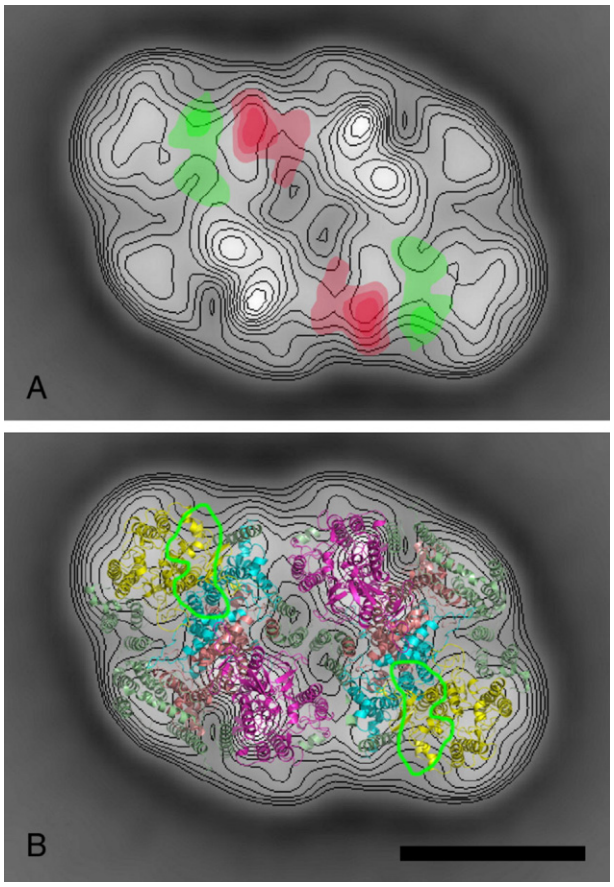

Fig. 4. Comparison of the PS2 electron microscopy projection map with the PS2 high-resolution X-ray structure. (A) The map of C, in contoured version, on which the main additional mass (green overlay) and the main deficient mass (red overlay) are indicated. (B) High-resolution PS2 structure (from [3]); protein data base entry 2AXT.pdb) from the lumenal side with CP43 (yellow), CP47 (purple), D1 (blue) and D2 (pink). Other membrane-bound subunits have been indicated in olive-green; PsbZ and extrinsic subunits have been omitted. IdiA is positioned within the bright green line at the cytoplasmic side. The space bar represents 10 nm.

4. Discussion

We have characterized isolated PS2 complexes from *T. elongatus* BP-1 grown under iron-deficient conditions in comparison with PS2 complexes isolated from cells grown under iron-sufficient conditions [41]. Our results show that IEC and SEC elution profiles as well as polypeptide composition of PS2 complexes isolated from iron-sufficient or iron-deficient cells are basically identical. The only observed major difference was that dimeric PS2 complexes from iron-starved cells contained the IdiA protein. IdiA has been shown to be highly expressed under iron starvation in several cyanobacterial species including *T. elongatus* BP-1 [4]. Previous biochemical experiments indicated that IdiA protects the acceptor side of PS2 from oxidative stress, especially when caused by iron deficiency [36]. This raised the question whether IdiA is an integral component of PS2. Results presented in this paper

clearly show that IdiA is present in highly purified PS2 dimers but is almost absent in PS2 monomers isolated from *T. elongatus* BP-1 cells grown under iron-deficient conditions. Therefore, we concluded that IdiA is an integral part of a modified PS2 complex isolated from iron-deficient cells.

A comparison of our EM data with the structural PS2 model obtained by X-ray diffraction data indicates that IdiA binds at a site, which is in close contact to both, the CP43 and the D1 subunit of PS2 (Fig. 4 green line). Since the structural differences between the two monomers in the non-symmetrized EM map are marginal, it is likely that each monomer within the PS2 dimer binds one IdiA subunit. Unfortunately, as specimens on the EM grid yielded only top-view and no side-view projections, it was not possible to directly decide whether IdiA is located on the cytoplasmic or the lumenal side of the thylakoid membrane. However, an additional aspect of the top view analysis yields strong evidence that IdiA is indeed located on the cytoplasmic side of PS2. Almost all dimeric particles of standard PS2 preparations analyzed by our group bind in top-view position, i.e. with their smooth cytoplasmic side to the carbon support film [41,44]. In contrast, PS2–IdiA complexes predominantly bind with their lumenal side, which is possibly smoothed by some absence of the extrinsic PsbO protein to the support film. Therefore, binding of PS2–IdiA particles with their lumenal side results in maps with a different handedness, i.e. that they have to be mirrored to become compatible with the PS2 maps lacking IdiA.

The presence of IdiA in PS2 dimers isolated from iron-starved *T. elongatus* BP-1 cells is the first example of a new PS2 protein, which becomes expressed under nutrient-deficient conditions and which is missing in highly purified PS2 complexes isolated from iron-sufficient cells. Thus, IdiA is a tightly and specifically associated part of PS2 complexes isolated from iron-starved cells. Although the precise function of IdiA is still unclear, biochemical characterizations [4] indicate that IdiA protects the acceptor side of PS2, which is more exposed under iron limitation due to ongoing phycobilisome degradation. Since IdiA has similarities to iron-binding proteins [40], two possible functions of IdiA could be imagined:

(1) IdiA interacts with the non-heme iron located on the D1/D2 reaction center heterodimer and protects Q_A and Q_B from autoxidation.
(2) IdiA functions as an iron sink under increased D1 degradation during stress conditions when a rapid PS2 repair cycle is initiated [6].

To our knowledge, the only other example for a protein becoming associated with PS2 under oxidative stress conditions is the 22-Ku heat shock protein, which is transported to the lumenal side of PS2 in order to protect the oxygen evolving complex in *Chenopodium album* and *Lycopersicum esculentum* [46,47]. Since no highly purified PS2 complexes have been used in these investigations, it remains to be shown whether this protein is indeed an integral part of these PS2 complexes.

Although the specific mechanism by which IdiA protects PS2 has still to be elucidated, for instance by kinetic studies [48], our results suggest an important function for IdiA in protecting PS2 under stress conditions in cyanobacteria. This is obvious from the specific structural re-arrangement of the dimeric PS2 complex – which is always the active part in contrast to the PS2 monomers – upon binding of IdiA close to the active center of the complex. The functional significance of IdiA in protecting cyanobacterial PS2 is further supported by the fact that all so far sequenced 24 cyanobacterial genomes contain a gene encoding an IdiA-similar protein. The fact that an isolated PS2–IdiA complex of promising stability is now available opens up new possibilities for more detailed structural and functional studies of this complex, which may indicate general principles of stress tolerance for membrane protein complexes.

Acknowledgements

The financial support by the DFG (SFB 480, project C1 (MR), project Mi 635/2-2 (KPM), the European Graduate College 795 (MR)), and the Dutch Science Foundation NWO is gratefully acknowledged. We also thank Regina Oworah-Nkruma and Claudia Koenig for excellent technical assistance.

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