Crystal Structure of Monomeric Photosystem II from *Thermosynechococcus elongatus* at 3.6-Å Resolution*§

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The membrane-embedded photosystem II core complex (PSIIcc) uses light energy to oxidize water in photosynthesis. Information about the spatial structure of PSIIcc obtained from x-ray crystallography was so far derived from homodimeric PSIIcc of thermophilic cyanobacteria. Here, we report the first crystallization and structural analysis of the monomeric form of PSIIcc with high oxygen evolution capacity, isolated from *Thermosynechococcus elongatus*. The crystals belong to the space group C222₁, contain one monomer per asymmetric unit, and diffract to a resolution of 3.6 Å. The x-ray diffraction pattern of the PSIIcc-monomer extracts exhibit less anisotropy (dependence of resolution on crystal orientation) compared with crystals of dimeric PSIIcc, and the packing of the molecules within the unit cell is different. In the monomer, 19 protein subunits, 35 chlorophylls, two pheophytins, the non-heme iron, the primary electron source in oxygenic photosynthesis (1–3). Several x-ray crystal structures of homodimeric PSIIcc from thermophilic cyanobacteria have been published with resolutions ranging from 3.8 to 2.9 Å (4–9). The monomers in the dimer are related by a non-crystallographic C2 rotation axis. Each monomer contains 17 membrane-intrinsic and three membrane-extrinsic protein subunits, the latter being located at the luminal side of the membrane. The redox-active cofactors are harbored by the heterodimeric protein matrix formed by subunits D1 (PsbA) and D2 (PsbD) and are arranged in two pseudo-C2 symmetric branches.

This entity, referred to as reaction center (RC), consists of four chlorophyll a (Chla) molecules, two pheophytins a (PheoD1, PheoD2), two plastoquinones (PQ) QA and QB, and the water oxidizing complex (WOC). Whereas the tightly bound QA acts as electron transmitter, the mobile QB is the substrate of the quinone reductase part of PSIIcc. An additional plastoquinone molecule (PQb) of unresolved function has been located next to the QB binding site in the recent crystal structure of dimeric PSIIcc at 2.9-Å resolution. In the RC, light-induced charge separation takes place leading to the oxidation of P₄₇₃ to the cation radical P₄D₁⁺. The electron is transferred via PheoD₁ and QA to QB, P₄D₁⁺ has an exceptionally strong oxidizing power (1.25 V (10)) and is able to abstract electrons via Yz from the WOC, a heteronuclear Mn₆Ca cluster located at the luminal side of PSII. After the accumulation of four redox equivalents in the so-called S-states (S₂, S₃, ..., S₅) of the WOC (11), water is oxidized to molecular oxygen.

The crystals of dimeric PSIIcc belong to the orthorhombic space group P2₁2₁2₁ and diffract to a maximal resolution of 2.9 Å. The monomers in the dimer are related by a non-crystallographic C2 rotation axis. Each monomer contains 17 membrane-intrinsic and three membrane-extrinsic protein subunits, the latter being located at the luminal side of the membrane. The redox-active cofactors are harbored by the heterodimeric protein matrix formed by subunits D1 (PsbA) and D2 (PsbD) and are arranged in two pseudo-C2 symmetric branches.

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The x-ray diffraction pattern is highly anisotropic (the attained resolution depends on the orientation of the crystal in the x-ray beam), which limits the useful resolution range (12, 13). This feature seems to originate from the packing of the dimeric complexes in the unit cell, but no other crystal form has been found for cyanobacterial PSIcc so far.

In addition to dimeric PSIcc, a monomeric form with high oxygen evolution capacity has been prepared (14–16). It is therefore attractive to crystallize the PSIcc monomer in attempting to achieve a more suitable crystal packing for x-ray structure analysis. In earlier work, the monomer was found to aggregate in solution (15). Therefore, it was considered to be inappropriate for crystallization. We succeeded in developing a novel method for purifying an intact monomeric PSIcc that crystallizes in a different form. The new crystals enabled us to obtain the first x-ray structural model of the PSIcc monomer at 3.6-Å resolution. Based on these data, we address several open questions concerning the role of protein subunits and lipids for the oligomeric state of PSIcc and the assembly/disassembly of PSIcc during the repair cycle of photodamaged subunit D1 (17–19). We also discuss the possibility of improving the structural analysis of the WOC by orientation-dependent spectroscopy.

EXPERIMENTAL PROCEDURES

Protein Purification—The initial purification steps of PSIcc followed the preparation protocol published in (14) with slight modifications. After two consecutive chromatography steps, the fraction containing the PSIcc monomer was concentrated to ~5 mM Chla and loaded onto a third column (diameter of 15 mm, length of 410 mm, Toyopearl DEAE 650S, Tosoh Bio-science) pre-equilibrated with 20 mM MES-NaOH (pH 5.0), 20 mM CaCl2, 0.5 M betaine monohydrate, 0.02% (w/v) n-dodecyl-β-D-maltoside (DDM). After washing at a constant salt concentration for 6 cv with a flow rate of 3 ml/min, monomeric PSIcc was eluted in a linear salt gradient (MgSO4, 0–50 mM, 8 cv). The fraction was concentrated in Amicon stirring cells using a Millipore Biomax 100 membrane (Millipore, MA). For further concentration to volumes <1 ml and to change the buffer conditions after ion exchange chromatography, the sample was washed three times with 10 mM MES-NaOH (pH 6.0), 5 mM CaCl2, 5 mM MgCl2, 0.02% (w/v) DDM using Sartorius Ultra Free 100 concentrators in a centrifuge at 3000 × g at 4 °C. Samples were concentrated to 3 mM Chla and either directly used for crystallization or stored in liquid nitrogen.

Crystallization—A broad crystallization screen was set up using a robot and the sitting drop vapor diffusion method and yielded conditions that were further optimized. Crystals were grown using the microbatch method by mixing the protein solution (3 mM Chla corresponding to ~25 mg/ml of protein) with the same volume of precipitant solution. Between 4 and 10 µl of the finally obtained solution (containing 1.5 mM Chla, ~19% (w/v) PEG 400, 0.1 M PIPES, pH 7.0, 0.2 M CaCl2, 0.01% (w/v) DDM) was placed either in a 96-well plate (IMP@CT, Greiner-Bio-one, Germany) or in the middle of a Teflon tube (inner diameter 1 mm; YCM Europe) and closed with sealing tape or sealing plaster. The crystals grew in 3 to 6 days at 18 °C in the dark and were directly flash-cooled in a nitrogen gas stream at 100 K after soaking with 28% (w/v) PEG 400 to provide cryoprotection.

Crystallographic Data Collection and Analysis—The data set was collected at the European Synchrotron Radiation Facility (ESRF, beam line ID 29), integrated and scaled with XDS (20). The structure of the PSIcc monomer was resolved by the molecular replacement method with the Phaser program (21) using one monomeric part of the 2.9-Å resolution structure of homodimeric PSIcc (8) (PDB entry 3BZ1) as search model. Model rebuilding and refinement were done using COOT (22) and the CNS 1.2 package (23), respectively. The structure was refined with the rigid-body procedure for the protein part of the complex and the annealing procedure for the cofactors. The final model of PSIcc monomer shows R/Rfree factors of 0.297/0.308, with root mean square deviations from ideal geometry of 0.013 Å for bond lengths and 2.1° for bond angles.

Analytical Ion Exchange and Gel Permeation Chromatography—For analytical anion exchange chromatography, a small column (5 mm diameter, 200 mm length, Toyopearl DEAE 650 S) was used connected to an Äkta FPLC system (ÄKTA purifier, Amersham Biosciences) with simultaneous detection at 205, 280, and 680 nm. Gel permeation chromatography experiments were conducted as described in Ref. 15, with simultaneous detection at 222, 280, and 680 nm.

Dynamic Light Scattering—Dynamic light scattering was performed using a DynaPro Titan instrument with a tunable laser diode at 833 nm wavelength (Wyatt Technology Corporation, Santa Barbara, CA). The protein was dissolved in 100 mM PIPES (pH 7.0), 10 mM CaCl2, 0.5 M betaine monohydrate, 0.03% (w/v) DDM and filtered through a Millex sterile filter (0.22 µm pore size) into a 100-µl cuvette. To obtain the diffusion coefficient Dp, autocorrelation functions of 50 measurements were averaged and analyzed using the instrumental software (Dynamics 6.9.2.9, Wyatt Technology Corporation, Santa Barbara, CA).

Spectroscopic Quantitation of Carotenes—Signals of redissolved crystals of monomeric and dimeric PSIcc were extracted in 80% (v/v) aqueous acetone and spectra were recorded in the wavelength region from 800 to 400 nm, normalized to 664 nm, and the absorbance difference was calculated. By using the molar extinction coefficient for Chla (76,800 M⁻¹ cm⁻¹ at 664 nm (24)) and β-carotene (144,000 M⁻¹ cm⁻¹ at 454 nm (25)) a difference of 1.3 ± 0.2 β-carotene/36 Chla between monomeric and dimeric PSIcc was calculated (assuming that the absorption of pheophytins at 664 nm is reduced by ~50% compared with Chla).

Mass Spectrometry and SDS-PAGE—MALDI-TOF MS analysis was conducted using an Ultraflex II Spectrometer (Bruker Daltonics, Germany) in the linear mode using sinapinic acid as matrix. SDS-PAGE was performed with a Phast System (Amersham Biosciences) using precast HD-SDS gels. Gels were run and silver stained following the protocol of the manufacturer.

Oxygen Evolution Activity Assay—Oxygen evolution of PSII samples was measured at room temperature using a home built Clark-type electrode (26). The excitation was performed either with saturating continuous white light from a tungsten lamp passed through a heat filter or with repetitive 1-Hz flashes.
from a xenon flash lamp. The sample was diluted to 20–50 μM Chl a in a buffer containing 20 mM MES-NaOH (pH 5.0–6.0), 20 mM CaCl₂, and 10 mM MgCl₂. Artificial electron acceptors added were either 2 mM 2,6-dichloro-p-benzoquinone for continuous excitation or 2 mM K₃[Fe(CN)₆] and 0.4 mM phenyl-p-benzoquinone for single flash excitations. The electrode was calibrated using air-saturated and nitrogen-saturated water at atmospheric pressure.

RESULTS

Preparation and Characterization of PSIIcc Monomer—Following the published preparation protocol (14), which comprises two ion exchange chromatography steps, we obtained essentially equal amounts of PSIIcc monomer and dimer (the monomer: dimer ratio of 40 preparations was 1.05 ± 0.45). The fraction of monomeric PSIIcc shows high oxygen evolution activity, but contains significant amounts of other proteins (as revealed by SDS-PAGE, supplemental Fig. S1, lane 1) not found in the fraction of dimeric PSIIcc. This contamination is due to phycobilisome proteins, a small amount of monomeric PSI, and ATP synthase. The latter occurs in nearly the same amount as the PSIIcc monomer (for characterization of ATP synthase from Thermosynechococcus elongatus, see supplemental data). The detection of ATP synthase by absorption at 280 nm is difficult due to the absence of bound pigments and its low tryptophan content (~0.2% assuming a complex formed by α₃β₃γδεabb’c₁₀ (27)). However, ATP synthase could be monitored at a wavelength of 205 nm (Fig. 1), where mainly the protein backbone absorbs (28).

To improve the purity of the PSIIcc monomer preparation, we applied an additional chromatography step using a weak anion exchange matrix. As under the formerly used chromatography conditions at pH 6.0 no further separation between the PSIIcc monomer and ATP synthase could be achieved (Fig. 1), the pH of the applied buffer was varied. Whereas at pH 6.8 the separation of the compounds remains incomplete, a clear separation was obtained by decreasing the pH value to 5.0. This condition was consequently used to obtain pure PSIIcc monomer that enabled us to grow single crystals suitable for x-ray diffraction experiments.

We found no indication that exposition of monomeric or dimeric PSIIcc to pH 5.0 has a deleterious effect on the oxygen evolution capacity, in agreement with the reversible inhibition of PSIIcc at acidic pH described earlier (29). Although the oxygen evolution activity of dimeric and monomeric PSIIcc was decreased when measured at pH 5.0, it was fully restored when the buffer was changed to pH 6.0 after long term incubation (4 or 24 h) at pH 5.0. The final monomeric and dimeric PSIIcc samples, as utilized for crystallization, show similar oxygen evolution activity rates ranging from 2400 to 3800 μmol O₂/(mg Chl a h)⁻¹. Furthermore, measurements of the oxygen evolving activity per single flash using the PSIIcc monomers prepared as described above revealed the same oxygen evolution capacity of (¼ mol O₂/(39–68 mol Chl a × flash)) as known from dimeric PSIIcc used in crystallographic studies so far ((¼ mol O₂/(37–73 mol Chl a × flash)) taken from Ref. 14). Neglecting double hits and misses (30), the theoretical limit for fully active centers is ¼ mol O₂/(36 mol Chl a × flash) taking into account the smaller absorption of 2 phycocyanin a compared with 35 Chl a per center.

A combination of MALDI-TOF-MS (supplemental Fig. S2) and SDS-PAGE (supplemental Fig. S1, lane 5) revealed that the PSIIcc monomer contains the same protein subunits as the PSIIcc dimer and is of sufficient purity for protein crystallization. Mass spectrometry also confirmed that the small membrane intrinsic protein subunits of the PSIIcc monomer are N-terminally processed (supplemental Table S1) in the same way as described for the dimeric form (8). Gel permeation chromatography showed a single peak corresponding to a molecular mass of 457 ± 20 kDa for monomeric PSIIcc similar to the value reported earlier (15).

To further analyze the aggregation behavior of monomeric PSIIcc, samples were investigated by using dynamic light scattering at different protein concentrations that are in a suitable range for protein crystallization. The use of a laser at 833 nm wavelength avoids disturbance of the measurements due to light absorption by protein-bound chlorophylls and therefore allows for measurements at high protein concentrations. We obtained a monodisperse particle distribution (9.1 ± 1.3% polydispersity) with a hydrodynamic radius of 5.9 ± 0.1 nm that remained invariant at protein concentrations between 0.8 and 6.0 mg/ml (supplemental Fig. S3). In contrast, the crude fraction of monomeric PSIIcc isolated without the third chromatography step (as in Ref. 14) forms aggregates with higher molecular mass similar to those reported in Ref. 15.

Crystallographic Analysis of PSIIcc Monomer—The green colored, diamond-shaped and plate-like crystals of the PSIIcc

FIGURE 1. Preparation of monomeric PSIIcc. Chromatograms of analytical FPLC runs (Toyopearl-DEAE650S) at different pH values, using a linear salt gradient for elution. The detector wavelengths were 280 (black line) and 205 nm (blue line). For clarity, the intensities corresponding to the two wavelengths were normalized to the peak of the PSIIcc monomer.

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—The green colored, diamond-shaped and plate-like crystals of the PSIIcc
monomer grew to maximum dimensions of ~1.0 × 0.6 × 0.2 mm (Fig. 2). The diffraction pattern showed a diffuse background scattering as observed for crystals of PSIIcc dimer (12), but was nearly isotropic (supplemental Fig. S4A). We were able to collect and process a dataset to 3.6-Å resolution (Table 1). The crystals belong to the orthorhombic space group C2221 with unit cell constants a = 119.89 Å, b = 224.69 Å, c = 337.28 Å and one PSIIcc monomer in the crystal asymmetric unit (solvent content, 61.6%). The structure was determined by molecular replacement using one monomer of the 2.9-Å resolution model of 25-Å thickness in agreement with simple geometric models (31, 32). The only exception is the region close to the C terminus of PsbM, where the detergent belts of the two adjacent monomers are probably partly fused or squeezed. Interestingly, in the crystals of monomeric PSIIcc a crystallographic C2 axis is located between the PsbM subunits of two neighboring monomers leading to an arrangement, in which their dimerization surfaces are facing each other (Fig. 3C). In the following, the term dimerization surface exclusively refers to monomeric PSIIcc and corresponds to the monomer-monomer interface in dimeric PSIIcc.

**Protein Subunits and Cofactors**—The quality of the data allows the unambiguous assignment of the main chain folding of 19 polypeptide subunits, and no major changes compared with the dimeric PSIIcc structure could be detected. No electron density was found for the peripheral subunit PsbY, although its presence would not lead to sterical conflicts within the crystal. Nevertheless, PsbY could be detected in the major-ﬁeld density (supplemental Fig. S2 and Table S1). The extended lumenal loop of PsbO provides two hydrogen bonds with the neighboring monomer (Fig. 3A, circle A), thereby interacting with the N-terminal loop of PsbF (PsbO-Lys112–PsbF-Pro10) and the N-terminal short α-helix of PsbE (PsbO-Ser115–PsbE-Asp12). Another hydrogen bond is found between PsbO-Thr51 and the N terminus of PsbA (PsbA-Asn12, Fig. 3A, circle B). The contacts along the b-axis are not resolved so far (Fig. 3B), but there are two regions, where protein subunits of different monomers approach each other by 5–6 Å. These comprise the C termini of PsbM (PsbM-Gln33, Fig. 3C) and the N terminus of PsbH (PsbH-Arg4) facing the loop of PsbO that connects transmembrane α-helices c and d. Because the monomers are oriented with their membrane normal (corresponding to the non-crystallographic C2 axis of the PSIIcc dimer) perpendicular (~87°) to the crystallographic b-axis, their membrane planes are nearly parallel to this axis (Figs. 2 and 3B). Therefore, the detergent belt may prohibit close interactions between neighboring complexes in this direction. There is enough space between the PSIIcc monomers to accommodate a detergent belt in the form of a monolayer ring of 25-Å thickness in agreement with simple geometric models (31, 32).

**Crystal Packing of PSIIcc Monomer**—Although the unit cell contains eight monomeric PSIIcc complexes, its volume is only slightly (~3%) larger than the unit cell of crystallized dimeric PSIIcc with four dimers. This indicates an almost equally dense molecular packing in both crystal forms. Fig. 3A shows the packing of PSIIcc monomers in the unit cell viewed along the crystallographic b-axis. Three types of crystal contacts can be distinguished (Fig. 3A, circles A, B, and ellipse C). The major-
the electron density (supplemental Fig. S5A). This fact enables us to confirm the arrangement of all chlorins in the RC and of all 29 Chl\(a\) molecules bound to the core antenna proteins CP43 (13 Chl\(a\)) and CP47 (16 Chl\(a\)). In cases where flexible phytyl chains are not stabilized by contacts with protein and/or other cofactors, their assignment is rather difficult. Therefore, the similarity with the structure of the PSIIcc dimer was used as a guide. The reliability of the assignment of each cofactor was probed with the calculation of OMIT maps, which serve to reduce possible model bias problems introduced after molecular replacement.

For most of the carotenoids, the electron density is not continuous. Their localization is mainly based on the electron density of the ionone rings and the analogy to the \(\beta\)-carotene positions in the PSIIcc dimer. This resulted in the assignment of 11 \(\beta\)-carotenoids. Absorption spectroscopy of redissolved crystals confirmed this number (see “Experimental Procedures”). The \(\beta\)-carotene missing in the structure of the PSIIcc monomer is located at the monomer-monomer interface in dimeric PSIIcc and therefore may detach due to its exposed localization in the monomer. The best defined \(\beta\)-carotene in the PSIIcc monomer is Car\(_{559}\) close to cytochrome \(b_{559}\).

In the PSIIcc monomer, electron density is found at the Q\(_A\), Q\(_B\), and Q\(_C\) sites, but can be assigned unambiguously to a PQ only for Q\(_A\) (supplemental Fig. S6A) revealing a position virtually identical to Q\(_A\) in the dimer (8). The electron density at the Q\(_B\) site cannot be assigned to a PQ molecule, but could be modeled as a buffer molecule MES (supplemental Fig. S6B). A patch of electron density is found in the vicinity of Car\(_{559}\) and the heme group of cytochrome \(b_{559}\) which corresponds to the position of the head group of Q\(_C\) identified in the dimer (supplemental Fig. S6C). However, the assignment of this electron density to a specific molecule is difficult.

**Lipids and Detergent Molecules**—The structure of the PSIIcc dimer (8) revealed the presence of 25 integral lipid molecules per monomer (11 monogalactosyldiacylglycerol (MGDG), 7 digalactosyldiacylglycerol (DGDG), 5 sulfoquinovosyldiacylglycerol (SQDG), and 2 phosphatidylglycerol). Using this information about lipid positions, it was possible to assign 22 lipid headgroups in the electron density map of monomeric PSIIcc (supplemental Fig. S5B). This finding is in agreement with the similar lipid composition found for monomeric and dimeric PSIIcc from *T. elongatus* (33).

The positions of the lipid headgroups surrounding the RC are essentially the same as those of their counterparts in the dimer (8), forming three smaller clusters (2–3 lipids) around the RC and one larger lipid cluster consisting of seven lipids (missing one MGDG (LMG218E in PDB code 3BZ1) found in dimeric PSIIcc) at the plastoquinone/plastoquinol exchange cavity. The three lipids found at the periphery of the PSIIcc dimer were also found in the monomer with slightly shifted positions of the luminal headgroups.

(PsbF (cyan) and PsbE (green)), PsbU (pink), PsbV (blue), and PsbO (green), and the remaining small subunits (gray). Lipids (cyan) and detergent molecules (brown) are drawn in space-filling representation. The red circle marks a lipid/detergent cluster located at the dimerization surface.
In dimeric PSIIcc, seven pairs of lipids are located at the monomer-monomer interface due to the non-crystallographic C2 symmetry (Fig. 4B). At the dimerization surface of the PSIIcc monomer, five lipids were found: two lipids pointing to the cytoplasmic side (SQDG 1 and MGDG 2, Fig. 4A, for nomenclature, see supplemental Table S2) and three to the lumenal side (MGDG 3 and DGDG 4 and 5). Whereas the headgroups of the lipids oriented toward the cytoplasm are at the same positions as in the dimer, the headgroups of the remaining lipids are found to be slightly shifted. Furthermore, the electron density suggests that one MGDG found in the dimer (MGDG 5) is replaced by DGDG (DGDG 5) or DDM in the monomer (Fig. 4A). By analyzing the lipid positions in the PSIIcc monomer, we found 10 lipids, including DGDG 4 and DGDG 5 located at the dimerization surface, which follow the pseudo-C2 symmetry of the RC.

Seven detergent molecules (DDM) per monomer could be assigned in the PSIIcc dimer. Three of them are located at the periphery and four at the monomer-monomer interface. We resolved seven DDM molecules in the PSIIcc monomer: three at the periphery and four at the dimerization surface. Two of the three peripheral DDM molecules are at similar positions as in Ref. 8, but the headgroup of the third is shifted by about 7 Å and contributes to crystal contacts. Two of the DDM assigned at the dimerization surface (DDM 6 and 7) are nearly at the same position as described for dimeric PSIIcc. Two further DDM (DDM 8 and 9) were located at new positions in close vicinity to DGDG 4. The headgroup of the latter is rotated and forms polar contacts (Fig. 3C, red circle, supplemental Fig. S5C, and Fig. 4A) with DDM 8. The positions of DGDG 4 and DDM 8 and 9 would interfere with subunit CP47 from the second monomer in the dimer.

**Mn₄Ca Cluster**—In the PSIIcc monomer, electron density arising from the metal ions of the Mn₄Ca cluster was found in the same position as reported for the PSIIcc dimer (8). In agreement with EPR measurements on the PSIIcc monomer from *T. elongatus* (16), we assume that the structure of the cluster is essentially the same in monomeric and dimeric PSIIcc. Due to the limited resolution, the assignment of a chloride ion in the vicinity of the Mn₄Ca cluster was not possible.

**DISCUSSION**

In this study, we present the first structure of a monomeric PSIIcc with high oxygen evolution capacity. The results show that in the PSIIcc monomer 19 of the 20 subunits are arranged identically to the corresponding subunits in the dimer. Although PsbY was found to be present in most of the redissolved crystals examined by MALDI-TOF-MS, we obtained no electron density for this subunit in the actual dataset. This finding may be attributed to variant, substoichiometric occupancy
and larger disorder of PsbY within the PSIIEcc monomer. In the PSIIEcc dimer the electron density for this subunit was visible at 3.8-Å resolution (4), but appears to be absent in a dataset at 3.5 Å (6). In agreement with the poorly defined electron density for this subunit even at 2.9-Å resolution in the PSIIEcc dimer (8), we conclude a loose association of PsbY with PSIIEcc.

The almost identical structure of monomeric and dimeric PSIIEcc is also reflected by the location of the tetrapyrrole cofactors and the non-heme iron. In agreement with this finding, the presence of 11 carotenoids at positions expected from dimeric PSIIEcc could be confirmed. As five of the 12 carotenoids in the PSIIEcc dimer are located at the monomer-monomer interface it is remarkable that only one of these five molecules is missing in the monomer. This carotene is found in a bridging position across the monomer-monomer interface.

Concerning plastoquinone cofactors, the assignment of QA is reliable due to the presence of electron density for both the headgroup and the isoprenoid tail. In contrast, it was not possible to assign a PQ in the electron density at the QΔ site. Because QΔ is the substrate of the quinone reductase part of PSIIEcc, it may have left the QΔ site as plastoquinol. Our model suggests that a buffer molecule can occupy the QΔ site in the absence of competing PQ. The penetration of a buffer molecule would be facilitated, if the quinone exchange cavity is more accessible from the aqueous phase. This may be the case, because one of the lipids shielding the QΔ site from the cytoplasm appears to be absent in the PSIIEcc monomer. The electron density found at the QΔ site is not yet assigned to any molecule in our model, but the presence of a PQ cannot be excluded (supplemental Fig. S6C).

The present data do neither allow the detection of fatty acid tails of lipids nor a distinction between the DDGG and the malse headgroup of detergent molecules (DDM). Therefore, the assignment in the PSIIEcc monomer is tentative and based on the 2.9-Å model of dimeric PSIIEcc, which is used as criterion to discriminate these molecules. Consequently, the electron density of sugar headgroups found at positions that significantly differ from those in the model of the PSIIEcc dimer was assigned to DDM molecules.

Despite this limitation, the majority of the lipids and detergent molecules described in the structure of dimeric PSIIEcc are found to be also present in PSIIEcc monomer. Due to the non-crystallographic C2 symmetry in dimeric PSIIEcc, seven pairs of lipids and four pairs of DDM are located at the monomer-monomer interface. In the PSIIEcc monomer, lacking this local symmetry, five lipids and four DDM are found at the dimerization surface. These molecules are located next to the D1 protein (surrounding the RC), to PsbT and PsbM, but not at the surface provided by CP47 (Fig. 4). Apparently, these lipids form a stable complex only with parts of the dimerization surface and probably exert a functional or structural role in the RC or in the repair cycle of D1 rather than being only involved in the dimerization of PSIIEcc.

In the dimer, the main protein-protein contact between the monomers is provided by subunit PsbM and its counterpart PsbM’. Our new data revealed that PsbM is at the same position in both, PSIIEcc monomer and dimer. Furthermore, after deletion of PsbM in the mesophilic cyanobacterium Synechocystis PCC 6803, PSIIEcc dimers are still formed (34). This supports our earlier suggestion that direct protein-protein contacts alone are not responsible for dimer formation (8).

The present structural model gives only two hints concerning the question of why the PSIIEcc monomer does not dimerize. (i) One of the two expected binding niches for SQDG at the monomer-monomer interface in the dimer (8) is unoccupied in the monomer (denoted as SQDG 532B in Fig. 4B). This would support a direct role for SQDG rather than phosphatidyldiacylglycerol (35) in dimer formation of PSIIEcc. (ii) Additional detergent molecules were found at positions where they would interfere with dimer formation (labeled 8 and 9 in Fig. 4A). In aqueous solution, the dimerization may be inhibited by the detergent belt.

The D1 protein is characterized by the highest turnover rate among all PSIIEcc polypeptides (19). The repair cycle most probably involves a monomerization of PSIIEcc and N-terminal proteolytic degradation of D1 by FtsH proteases (17). In the structure of the PSIIEcc monomer, the N-terminal part of D1 (including transmembrane α-helix a) is highly exposed to the membrane phase and may be more accessible for FtsH proteases than in the dimer. However, we found no structural indications of a destabilization of D1 in the PSIIEcc monomer and essentially the same oxygen evolution capacity as for the PSIIEcc dimer. Therefore, it is unlikely that the PSIIEcc monomer used for crystallization represents a photodamaged product of dimeric PSIIEcc.

The crystals of the PSIIEcc monomer represent an important step towards the structural elucidation of the Mn₄Ca cluster as they provide a new, highly ordered arrangement of PSIIEcc and N-terminal proteolytic degradation of D1 by FtsH proteases (17). In the structure of the PSIIEcc monomer, the N-terminal part of D1 (including transmembrane α-helix a) is highly exposed to the membrane phase and may be more accessible for FtsH proteases than in the dimer. However, we found no structural indications of a destabilization of D1 in the PSIIEcc monomer and essentially the same oxygen evolution capacity as for the PSIIEcc dimer. Therefore, it is unlikely that the PSIIEcc monomer used for crystallization represents a photodamaged product of dimeric PSIIEcc.

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new crystal form, a better discrimination is possible between absorber-backscatter vectors oriented parallel and perpendicular to the membrane plane. Therefore, it is expected that a full set of polarized extended x-ray absorption fine structure spectra along the crystal axes will significantly expand the available structural information about the Mn$_4$Ca cluster, especially with respect to the manganese-calcium interaction. With these additional constraints, a selection between the currently discussed models may become feasible. These experiments are in progress. The absence of non-crystallographic symmetry in crystals of monomeric PSIcc will also reduce the complexity of the spectra derived from electron paramagnetic resonance studies. The absence of non-crystallographic symmetry in single crystals (40, 41) and thus facilitate the assignments of spectral features.

Besides the still limited information concerning the Mn$_4$Ca cluster, a higher resolved overall structure of PSIcc is indispensable to clarify many questions still open in the present structural models. Therefore, further optimization of the diffraction quality of PSIcc crystals is crucial. To achieve this goal, the new crystal form features the important advantage of significantly higher anisotropy in the diffraction pattern compared with crystals of dimeric PSIcc. Moreover, an improvement of the crystal structure of monomeric PSIcc is also a prerequisite to elucidate the origin of the different oligomerization states of PSIcc, which may help to clarify the mechanism of D1 exchange.

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