Native-like Photosystem II Superstructure at 2.44 Å Resolution through Detergent Extraction from the Protein Crystal

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SUMMARY

Photosystem II (PSII) catalyzes a key step in photosynthesis, the oxidation of water to oxygen. Excellent structural models exist for the dimeric PSII core complex of cyanobacteria, but higher order physiological assemblies readily dissociate when solubilized from the native thylakoid membrane with detergent. Here, we describe the crystallization of PSII from Thermosynechococcus elongatus with a postcrystallization treatment involving extraction of the detergent C12E8. This resulted in a transition from Type II to Type I-like membrane protein crystals and improved diffraction to 2.44 Å resolution. The obtained PSII packing in precise rows, interconnected by specific pairs of galactolipids and a loop in the PsbO subunit specific to cyanobacteria, is superimposable with previous electron microscopy images of the thylakoid membrane. The study provides a detailed model of such a superstructure and its organization of light-harvesting pigments with possible implications for the understanding of their efficient use of solar energy.

INTRODUCTION

Membrane proteins are essential components of basic biological processes, but revealing their structure is difficult. Problems arise from their large hydrophobic surface area, which is necessary to stabilize the protein in the native lipid bilayer but renders it insoluble in water. A detergent is needed to extract the protein from the membrane, whereupon a detergent belt around the hydrophobic surface is formed (Garavito and Ferguson-Miller, 2001). This belt is not incorporated into type I crystals, where layers of membrane proteins are stacked with protein contacts between transmembrane regions (Russo Krauss et al., 2013; Michel, 1983). In contrast, the space between protein units in type II crystals is usually large enough to accommodate the original detergent belt at the expense of stable protein contacts, which likely contributes to the difficulty in obtaining high-quality crystals for diffraction experiments. Among the many detergents available, n-dodecyl-β-D-maltoside (βDM) provides the largest number of successful crystallization trials (Parker and Newstead, 2012).

Photosystem II (PSII) is the water-plastoquinone oxidoreductase of oxygenic photosynthesis. This protein complex usually occurs as a homodimer, and, as crystallized, each monomer consists of 17 membrane-intrinsic and three membrane-extrinsic protein subunits and harbors nearly 100 cofactors (Guskov et al., 2010). PSII uses solar energy to split water into molecular oxygen, protons, and electrons at the catalytic center—the manganese cluster (Mn4CaO5) (Müh and Zouni, 2011; Kawakami et al., 2011). The electrons are delivered to plastocyanin in a two-step proton-coupled electron transfer process, and the formed plastoquinol delivers electrons via the cytochrome b6f complex to photosystem I (Müh et al., 2012). X-ray structures of the dimeric PSII core complex (dPSIIcc) from thermophilic cyanobacteria have been reported with resolutions up to 1.9 Å (Guskov et al., 2009, 2010; Umena et al., 2011) (Figure 1). In addition, structures of a monomeric form (mPSIIcc) (Broser et al., 2010), herbicide-inhibited dPSIIcc (Broser et al., 2011), and dPSIIcc with Ca2+ substituted by Sr2+ (Koua et al., 2013) were reported. In all cases, βDM was used as detergent, while the recent structures of Thermosynechococcus vulcanus PSIIcc feature an additional detergent, n-heptyl-β-thioglycoside (HTG) (Umena et al., 2011; Koua et al., 2013).

The dPSIIcc contains a high amount of integral lipids, which are important for stability and functional integrity (Guskov et al., 2010; Kern and Guskov, 2011). Detergent molecules are present in the structure at specific positions, suggesting that they may have replaced lipids. The high structural similarity of βDM to certain lipids (e.g., digalactosyldiacylglycerol; DGDG) was believed to facilitate this exchange (Guskov et al., 2009; Kern and Guskov, 2011). This idea prompted us to replace βDM with a detergent that is structurally dissimilar to galactolipids. We show here that high-quality crystals of active dPSIIcc from T. elongatus can indeed be obtained with the detergent octaethylenglycolmonododecylether (C12E8). Although the lipid/detergent exchange cannot be quantified completely, the
T. elongatus

In our protocol, dPSIIcc from crystals, show high oxygen evolution activity similar to the (pink), cytochrome monomer for clarity. Further indicated are the membrane-spanning low-subunits PsbC (CP43) and PsbB (CP47) (light green) are omitted in the left monomer for clarity. Further indicated are the membrane-spanning low-molecular-weight subunit PsbY, as well as the extrinsic subunits PsbO (pink), cytochrome C550 (PsbV, blue) and PsbU (gray). See also Figure S1 and Table S1.

Figure 1. Architecture of T. elongatus dPSIIcc

Each monomer of dPSIIcc consists of 17 transmembrane and 3 membrane-extrinsic subunits. View along the membrane plane based on the new 2.44 Å resolution structure. Subunits PsbA (D1, yellow) and PsbD (D2, orange) contain inter alia the Mn₄CaO₅ cluster (magenta), RC Chl a (P₀, and P₇₆₀, green), nonheme iron (Fe, orange), and plastoquinones (Q₀ and Q₆, pink). The antenna subunits PsbC (CP43) and PsbB (CP47) (light green) are omitted in the left monomer for clarity.

Use of C₁₂E₈ offers an unexpected route to highly diffracting crystals. A prominent method among postcrystallization treatments to improve crystal quality is controlled dehydration (Russo Krauss et al., 2013; Heras and Martin, 2005). This method was used by Umena et al. (2011) to improve dPSIIcc crystals from T. vulcanus, using the detergent combination βDM/HTG, and ultimately yielded a resolution of 1.9 Å. We were not able to reproduce this procedure with dPSIIcc from T. elongatus in βDM. However, with C₁₂E₈, a dehydration procedure resulted in a significant improvement of the crystals. In this article, we present the structure of dPSIIcc from T. elongatus at 2.44 Å resolution, featuring a complete subunit composition including the subunit PsbY in both monomers (Figure 1) and an arrangement of dimers akin to structures observed in the native thylakoid membrane (Mörschel and Schatz, 1987; Folea et al., 2008).

On the basis of our structural data, we conclude that a crystal transformation occurs that involves not only dehydration but also an in-crystal reorganization of membrane protein packing from type II to type I-like due to detergent extraction. As such, this might be of interest not only for PSI2 research but also for the structural biology of membrane proteins in general.

RESULTS

Purification of C₁₂E₈-dPSIIcc

In our protocol, dPSIIcc from T. elongatus is purified using C₁₂E₈ instead of βDM. The corresponding preparations are referred to in the following text as C₁₂E₈-dPSIIcc and βDM-dPSIIcc, respectively. The purified C₁₂E₈-dPSIIcc, as well as redissolved crystals, show high oxygen evolution activity similar to the activity obtained for βDM-PSIIcc (Kern et al., 2005). C₁₂E₈-dPSIIcc contains all 20 protein subunits, including the low-molecular-weight subunit PsbY identified by MALDI-TOF-mass spectrometry (MALDI-TOF-MS) and the crystal structure (Figure 1). Whereas our previous purifications with βDM (Kern et al., 2005) yielded monomeric and dPSIIcc in almost equal amounts (ratio, 0.7 ± 0.3), the preparation with C₁₂E₈ yields almost exclusively dPSIIcc.

Crystallization and High-Pressure Freezing

C₁₂E₈-dPSIIcc crystals grew to 10–400 μm in the longest dimension and belonged to the same orthorhombic space group P₂₁2₁2₁, with unit cell constants similar to those of the dPSIIcc crystals obtained with βDM (Loll et al., 2005) and βDM/HTG (Umena et al., 2011). Analysis of C₁₂E₈-dPSIIcc crystals before postcrystallization treatment at room temperature (RT), or at 100 K after high-pressure freezing (HPF) in the absence of cryoprotectant, yielded diffraction to 6 Å resolution and showed that the untreated crystals are similar to βDM-dPSIIcc (Loll et al., 2005) with regard to dimer architecture and crystal packing. In particular, loose packing along the a axis, as well as the absence of direct protein-protein contacts, suggests the presence of flexible—and, thus, unobserved—detergent belts along the membrane interface (Figure 2). Such a packing is characteristic for type II crystals (Michel, 1983).

Transformation from Type II to Type I-like Crystals

In order to provide cryoprotection and to improve crystal quality by dehydration, we washed the crystals in a series of transformation buffers containing increasing concentrations of polyethylene glycol (PEG) 5000 monomethyl ether (MME) (in steps of 5% every 5 min up to 40–50%), but no C₁₂E₈. This treatment routinely led to a dramatic improvement in resolution from 6 Å to 2.44–3.5 Å (Figure S2A available online; Table 1). While the P₂₁2₁2₁ symmetry was maintained, unit cell dimensions decreased from a = 141.0 ± 3.1 Å, b = 232.0 ± 0.9 Å, c = 309.9 ± 5.6 Å to...
a = 116.4 ± 0.4 Å, b = 218.4 ± 0.9 Å, c = 300.7 ± 2.6 Å, corresponding to a 25% reduction in unit cell volume. Noticeably, variation in the crystallographic a axis was reduced significantly to below 1 Å. In the light of the apparent drastic change in unit cell dimensions, two paths to the observed tightly packed crystal form appear plausible: (1) transformation of individual crystals and (2) selection of crystals from two subpopulations, one with the large initial unit cell, which only shows interpretable diffraction images at low concentrations of PEG, and a second crystal type with a small unit cell diffracting well only at a high PEG concentration. To decide between these possibilities, we subjected individual crystals to the same treatment in a controlled way (Figure S2B). Crystals were pretested at RT and minimal X-ray dose, returned to individual buffer drops, treated, and tested again in the X-ray beam. For 18 crystals, initial unit cell parameters were determined, and all showed the large cell. All three crystals, which subsequently survived the combined stress of remounting and transformation, showed the small unit cell. We conclude that predominantly one type of crystal exists, and this undergoes a transformation involving a 25% reduction in unit cell volume.

In contrast to untreated crystals, after PEG treatment, C12E8-dPSIIcc formed continuous rows in a way that strictly excludes the detergent belts around at least part of the dimer, similar to type I membrane protein crystals (Michel, 1983). A second, anti-parallel row of dimers exists by virtue of the initial crystal packing; and on crystal transformation, both layers appear twisted by an angle of approximately 45°. Thus, dimers in each row rotate about two axes to form continuous rows. Further crystal contacts along the crystallographic b and c axes are common with untreated C12E8-dPSIIcc or βDM-dPSIIcc crystals and are mediated by the extrinsic PsbV/PsbU and PsbO/PsbZ subunits.

Crystal Packing Reminiscent of Biological Membranes

The particular arrangement of dimers in transformed C12E8-dPSIIcc crystals is similar to the packing of dPSIIcc in cyanobacterial thylakoid membranes observed earlier with electron microscopy (EM). According to these studies, dPSIIcc forms linear rows (Mörschel and Schatz, 1987) or similar two-dimensional (2D) crystalline arrays (Folea et al., 2008) with a center-to-center spacing of 10–12 nm. We have overlaid an electron micrograph of such rows (E.J. Boekema, personal communication) with the exact arrangement of C12E8-dPSIIcc in the dehydrated crystals (Figure 3). The perfect match suggests that C12E8-dPSIIcc exists in a native-like molecular packing in these crystals. Protein contacts between dimers are formed by a handshake of PsbO subunits on the lumenal side. Additional contacts are mediated by two monogalactosyldiacylglycerol (MGDG) lipids (785 and 789) per monomer (Figure 4) that were not seen in PSIIcc crystal structures (Umena et al., 2011; Guskov et al., 2009). These join the membrane interfaces of internal antennae subunits PsbC (CP43) and PsbB (CP47) between chlorophyll a (Chl a) 639 and 612, respectively.

Table 1. Data Collection and Refinement Statistics

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Rmsds, root-mean-square deviations.

*Values in parentheses are for highest resolution shell.

Figure 3. Overlay of Packing in Dehydrated C12E8-dPSIIcc Crystals and Electron Micrographs of Cyanobacterial Thylakoid Membranes

Left: EM image from partly solubilized membranes from Synechocystis sp. PCC 6803 recorded by Folea et al. (2008) showing vertical rows of dimers within membrane fragments viewed from the luminal side. Right: overlay of the EM image with the crystal packing from the new 2.44 Å resolution structure, with dimers colored alternating blue/green. PsbO subunits (dark, reaching across the intersection of monomer and dimer grooves) and bridging MGDG lipids (red, dimer-dimer groove) are highlighted. The EM image was scaled from 12.2 nm to 11.6 nm dimer repeat size.
Figure 4. Arrangement of dPSIIcc Dimers in Dehydrated C12E8-dPSIIcc Crystals

Three exemplary dimers with 116.4 Å spacing are shown as a slice through the membrane normal with overlaid functional elements. Transmembrane helices of the core antennae CP47 (purple) and CP43 (blue) are shown along with Chl a (green). The RC is encircled, and the exchangeable plastoquinone Qb (pink) points outward from the dimer axis. Monomer-monomer (PsbM, diamond) and dimer-dimer (square) interaction sites are highlighted. Dimer contacts mediated by CP43 and CP47 (top insert) include the newly identified MDGD 785 and MGDG 789 lipids, as well as Chl a 612 (shown as sticks along with interacting amino acid residues). Potential hydrogen bonds are shown as dashed lines. Electron density maps are shown for MGDGs: 1s refined 2mFo-DFc in blue and 2.5s mFo-DFc, refined in absence of the lipid, in green. Interdimer contacts mediated by PsbO (bottom left insert) are mediated by the cyano loop (De Las Rivas and Barber, 2004) previously identified as exclusive to cyanobacteria. PsbO sequences (National Center for Biotechnology Information accession numbers) from T. elongatus (NP_681234.1), Synechocystis sp. PCC 6803 (NP_441796.1), Synechococcus sp. PCC 7002 (YP_001733535.1), Fischerella sp. PCC 9339 (WP_017310215.1), C. reinhardtii (XP_001694699.1), A. thaliana (AAEM5169.1), Pea (Pisum sativum, UniProt: P14226), and Spinach (Spinacia oleracea, UniProt: P12359) were aligned with ClustalW (Larkin et al., 2007).
Cofactors in C12E8-dPSIIcc
The structure of C12E8-dPSIIcc from a transformed crystal was solved at 2.44 Å resolution by molecular replacement using the structure of T. vulcanus dPSIIcc (Umena et al., 2011) as a search model and contains all subunits from the latter with the addition of PsbY, previously only observed with low occupancy (Guskov et al., 2009; Kawakami et al., 2007) or in a single monomer (Koua et al., 2013). All 35 Chl a, two pheophytin a, 11 β-carotene, two plastoquinone, one nonheme iron, one bicarbonate, and two heme cofactors could be identified (representative electron density maps are shown in Figure S1). No electron density is observed at the plastoquinone binding site Qb identified previously (Guskov et al., 2009). Two chloride ions near the Mn4CaO5 cluster (Figure S2a) occupy the positions Cl1 (Cl1A in Broser et al., 2011) and Cl2 (as identified by Umena et al., 2011). No electron density is observed at the Clb1 position (Broser et al., 2011) and at Cl3 near PsbU (Umena et al., 2011). Three additional Ca2+ ion positions previously found in the presence of 10 mM CaCl2 (Umena et al., 2011) are unoccupied, which is likely due to the absence of calcium in our transformation buffer. However, Ca1 bound to PsbO in the Umena et al. (2011) study coincides with a contact between successive dimers in crystals of C12E8-dPSIIcc (Figure S3) and may play a role in the association of dPSIIcc superstructures (see Discussion). A total of 304 water molecules could be placed in C12E8-dPSIIcc and are located predominantly in membrane-extrinsic regions. Heavy atoms within the Mn4CaO5 cluster occupy the same positions as in the 1.9 Å resolution structure (Figure S2a). Protein side chains coordinating the cluster could be positioned, but individual oxygen atom positions within the cluster could not be discerned. Therefore, the Mn4CaO5 cluster was modeled as rigid body, conserving the geometry of the highest resolution (1.9 Å) model (Umena et al., 2011) available. Of the coordinated water molecules previously identified (Umena et al., 2011), only W1 and W2 coordinated to Mn4 in the water oxidizing complex could be independently placed.

Lipids in C12E8-dPSIIcc
Endogenous lipids previously reported at 2.9 Å resolution (Guskov et al., 2009) and confirmed at 1.9 Å (Umena et al., 2011) were also observed in the C12E8-dPSIIcc structure (Table S1). Four phosphatidylglycerols (PGs) and one MGDG identified at 1.9 Å resolution could be confirmed at 2.44 Å resolution, while four MGDGs and one DGDG were initially identified in their place at 2.9 Å. This is probably due to a wrong assignment at the lower resolution. Fatty acid tails of lipids in the QB cavity were only partially resolved, indicating flexibility. Only two pairs of lipids could clearly be identified at the monomer-monomer interface. The lipid 667 assigned to a sulfoquinovosyldiacylglycerol (SQDG) in T. vulcanus at 1.9 Å (Umena et al., 2011) was modeled as MGDG due to the absence of the sulfate group in the electron density map. We do, however, acknowledge the strong evidence for SQDG presented in the Umena et al. (2011) study. This represents the only difference in composition of observed lipids within the dimer envelope. Identification of further lipids at the monomer-monomer interface was not possible. Instead, elongated electron density, which could originate from detergent, PEG 5000 MME, or partially resolved lipids, was modeled as individual alkyl chains and labeled as unknown ligand (UNL). The same positions have previously been modeled as either highly mobile lipids (Guskov et al., 2009), detergent molecules (Umena et al., 2011) or UNL, indicating that lipid composition at the interface is poorly defined in all current high-resolution structures. Of the lipids previously modeled on the surface of dPSIIcc, just a single one (SQDG 768), wedged in a groove between PsbF and PsbX, was retained. In contrast, two MGDG lipids were retained on the surface of subunits CP47 and CP43, which were not previously identified in jDM-dPSIIcc (Guskov et al., 2009) or replaced by HTG (Umena et al., 2011). These lipids mediate contacts within rows of C12E8-dPSIIcc.

Unlike jDM, the detergent C12E8 does not have an identifiable carbohydrate head group and could not be interpreted in the crystal structure. Of 13 detergent molecules surrounding the dimer, as suggested by the 1.9 Å resolution (Umena et al., 2011), 5 were modeled in the present study as unknown (alkyl chains) and four as MGDG 785 and 789. Residual density was observed at three additional positions. Altogether, the endogenous lipids intrinsic to PSIIcc, including those in the Qb cavity, were retained in C12E8-PSIIcc, while it is likely that those at the periphery, including the monomer-monomer interface, were replaced. Exceptions are two pairs of lipids bound in a specific manner at both the monomer-monomer and dimer-dimer interfaces.

PEG Affects the CMC of C12E8
The transformation of crystals from type II to type I was possible only with C12E8 but not with jDM. To rationalize this observation, we determined the effect of PEG on the critical micelle concentration (CMC) of the detergents. The CMC can be considered as the equilibrium constant for micelle formation, $m \frac{D}{D_m} = \text{CMC}_0$, where $D$ is a detergent monomer and $D_m$ is a micelle with aggregation number $m$. The quantity $\ln(\text{CMC}/\text{CMC}_0)$, where $\text{CMC}_0$ is the CMC in the absence of PEG, can be interpreted as the change in the Gibbs free energy of micelle formation due to PEG. Its increase with PEG concentration indicates a stabilization of detergent monomers in the solution. According to Figure S2C, this effect is larger for C12E8 than that for jDM. Such a stabilization of monomers should not only shift the equilibrium of micelle formation to the left (i.e., increase the CMC) but should also shift the following equilibrium to the right: (type II crystal) $\rightleftharpoons$ (type I crystal) $+ D$. Here, $x$ is an unknown quantity of detergent monomers, $D$, in the mother liquor. Thus, we propose that, by stabilizing the detergent in the solution, PEG contributes to the detergent extraction from the crystals. This effect is more pronounced for C12E8 than for jDM, which is probably one reason why the transformation takes place only with C12E8.

DISCUSSION

dPSIIcc Purified in an Alternative Detergent
The current work explores the possibility of obtaining high-quality crystals of active, water-splitting dPSIIcc in an alternative detergent. The original motivation to use C12E8 instead of jDM was to avoid replacement of endogenous lipids in the protein complex and, thus, to enhance the stability and integrity of dPSIIcc. Compared to the 1.9 Å resolution structure of dPSIIcc, the same integral lipids were found within each
monomer, while fewer lipid molecules were found at the mono-
mer-monomer interface and at the dimer-dimer surface. None-
theless, C12E8 enhances the stability and integrity of dPSIIcc,
as suggested by the following results: PSIIcc is as active in
C12E8 as it is in jDM. In C12E8, almost exclusively, dPSIIcc
is obtained after membrane solubilization and protein purifica-
tion, whereas a significant amount of mPSIIcc is obtained
when using jDM. Furthermore, the low-molecular-weight sub-
unit PsbY is fully occupied in both parts of the PSII dimer,
whereas it was at least partially lost in all earlier preparations
based on jDM.

The lipids observed previously, but not in the current structure,
are located in regions of high mobility as suggested by crystallo-
graphic B-factors (Guskov et al., 2009; Umena et al., 2011).
Along with water molecules and ligands modeled as unknown,
their observability is expected to be highly dependent on crystal-
lographic resolution. Complete assignment of lipids at the mono-
mer-monomer interface remains elusive. With the exception of
two pairs of lipid molecules, lipids at this interface appear either
flexible or readily exchanged by detergent, but a distinction
between these two possibilities cannot be made. Most impor-
tant, two previously unobserved lipids on the protein surface,
MGDG 785 and 789, were retained during extensive purification
in C12E8. They are found at the interface between PSIIcc dimers
in the new crystal structure, suggesting that they are crucial in
stabilizing dimer-dimer contacts, which might be of physio-
logical relevance (discussed later). Retention of MGDG lipids at
the interface between dimers appears to be crucial for stability
of the transformed crystal and provides a model for dimer-dimer
interaction in vivo. Preliminary analysis of electron density from
three other crystals with resolutions between 2.5 and 2.9 Å
suggests that MGDG 785 and 789 were routinely copurified
with C12E8-dPSIIcc.

Unexpected Transformation from Type II to Type I-like
Crystals
A twist in the use of C12E8 was that type I crystals of dPSIIcc were
obtained. Even more surprising was the route to these crystals:
First, type II crystals are formed that had the same space group
(P212121) as the earlier crystals of jDM-dPSIIcc. In a second
step, originally designed to dehydrate the crystals, these are
transformed into type I crystals with the retention of space group
but reduced unit cell constants. Note that a transformation from
type II to type I requires a significant reduction in the detergent
content of the crystal.

The Effect of Detergent on Crystal Dehydration
Protein crystals contain a high amount of solvent, and manipula-
tion of solvent content by dehydration has been established as a
promising method for improving crystal resolution (Heras and
Martin, 2005). This method was applied earlier to membrane pro-
teins, but so far, the focus has been on the extraction of water
from the crystal. For example, the resolution of plant photo-
system I was recently improved from 4.4 to 3.3 Å through a com-
bination of detergent choice and dehydration in 40% PEG 6000
after crystallization, resulting in a 30% reduction in unit cell size
(Amunts et al., 2010). No evidence for detergent extraction was
reported, and it was observed that the dehydration procedure
yielded a variability in crystal geometry. A drastic change in res-
olution was achieved with the 1.9 Å resolution structure of
dPSIIcc (Umena et al., 2011). A complex dehydration protocol
was reported, implying the use of a mixture of jDM and HTG.
This resulted in a reduction of unit cell size by 10% and the
creation of two additional crystal contact patches between
membrane-extrinsic regions. Previous dehydration experiments
with dPSIIcc using jDM alone did not result in any significant
improvement of crystal quality. In particular, the crystallographic
a axis, along which packing is predominantly determined by the
detergent belt, never decreased below 126 Å in jDM-dPSIIcc
crystals, compared to 122 Å in jDM/HTG (Umena et al., 2011)
and 116 Å in C12E8 crystals. This finding suggests that manipu-
lation of the detergent belt might be a key step in the successful
transformation of membrane protein crystals. However, with the
use of C12E8, we found a path to improved crystals by allowing
not only water but also detergent to be extracted. In our case,
the crystal volume was reduced by 25% and the resolution
improved from around 6 Å resolution to at least 2.5 Å. In contrast
to mere dehydration, the transformation from type II to type I
allowed for the creation of new crystal contacts between mem-
brale-intrinsic regions of the protein.

Organization of solubilized membrane proteins into crystalline
layers has long been established in 2D crystallography. Both
detergent removal (by dilution, dialysis, or adsorption to a matrix)
and addition of lipids are required (Dolder et al., 1996; Rigaud
et al., 1998). However, detergent removal was reported to be
challenging for low CMC detergents (such as jDM or C12E8).
In our in-crystal transformation, we have demonstrated the
importance of dilution of the detergent by repeated washing
with detergent-free buffer. In contrast, initial controlled dehydra-
tion experiments using the HC1c humidified air stream (San-
chez-Weatherby et al., 2009) could not reliably reproduce crystal
transformation. In these experiments, dehydration only was
controlled by varying humidity, but crystals were mounted with
very little additional solvent, thus preventing dilution of the
detergent.

At present, it is unclear, why the transformation works with
C12E8 but not with jDM. There are two aspects to be consid-
ered: (1) PEG increases the solubility of detergent monomers
in the aqueous phase, as suggested by its effect on the
CMC, which is larger for C12E8 than for jDM (Figure S2C).
Since detergent extraction from the crystal supposedly involves disin-
tegration of detergent belts into monomers that have to enter
the solution, this process is supported by PEG. That C12E8
is more stabilized in aqueous PEG solution than jDM likely
contributes to the different behavior of the two detergents. (2)
The detergent monomers need to have a certain amount of
mobility in the transforming crystal in order to be able to leave
it. In this respect, the flexible and slim C12E8 molecule is likely
superior to jDM, with its bulky and hydrogen-bond-forming
head group.

Comparison with EM Studies of Thylakoid Membranes
Freeze-fracture EM studies of T. elongatus (Mörchel and
Schatz, 1987) have shown extensive alignment of membrane-
embedded particles, indicating that dPSIIcc tends to form long
rows reminiscent of those building up the C12E8-dPSIIcc crys-
tals. Rows, and even arrays, of dPSIIcc were also observed after
mild detergent solubilization of membranes from the mesophilic
cyanobacterium Synechocystis PCC 6803 (Folea et al., 2008). The good match between the electron micrograph and new X-ray structure (Figure 3) suggests that the latter indeed reflects the in vivo situation and, thus, provides a detailed structural model of dPSIIcc in the native membrane. Figure 4 shows a representation containing a row of three dPSIIccs derived from the 2.44 Å resolution structure coordinates. Both monomer-monomer and dimer-dimer contacts contribute to an ensemble comprising multiple CP43 and CP47 antennae and reaction centers (RCs). In this arrangement, plastoquinone exchange between the Q_B pocket and the membrane phase remains possible, as the entrances of the quinone diffusion channels point outward. While monomer-monomer interactions via the PsbM leucine zipper (Guskov et al., 2009) and two loops (Ala 111 to Gly 113 and Glu 54 to Glu 64) in PsbO appear stable during purification, an apparently weaker set of interactions connects dimers. The four endogenous MGDG lipids 785 and 789 connect the core antennae CP47 and CP43 in the vicinity of Chl a 612—the narrowest part of the dimer interface (Figure 4).

Interestingly, another contact is mediated by an additional loop in PsbO, previously termed the cyano loop due to its presence only in the sequence of cyanobacteria but not in green algae and plant PsbO (De Las Rivas and Barber, 2004). The latter form supercomplexes with light-harvesting complexes, prohibiting the formation of rows of dimers (Kouril et al., 2013), which might be related to the absence of the cyano loop sequence. The PsbO-PsbO dimer interaction occurs at a noncrystallographic pseudo-2-fold axis. Superimposition of the new 2.44 Å resolution structure with that at 1.9 Å resolution (Umena et al., 2011), obtained in the presence of 10 mM CaCl₂, suggests the presence of two symmetry-related calcium binding sites (Ca-1) at Thr 138 (Figure S3). Therefore, a role of calcium in the formation or disassembly of dPSIIcc superstructures in vivo is possible but remains hypothetical at present.

**Extended Alignment of Internal Antenna**

A possible physiological role of the PSII superstructures is related to the efficiency of light harvesting. Figure 4 shows that, in the PSII rows, two CP43 and two CP47 antenna subunits of three PSIIcc dimers each form a slab of connected Chls, in which equilibration of excitation energy transfer should be possible in the picosecond time range. Each slab is close to four RCs and, thus, can deliver excitation energy to, but also receive excitation energy from, each of the four RCs. Each RC, in turn, can exchange excitation energy effectively with two such antenna slabs. The possibility to exchange excitation energy between RC and antenna in both directions is important, because the one-electron processes in the RC (charge separation and recombination) are linked on one hand to the four-electron process of water oxidation in the Mn₄CaO₄ cluster at the luminal side and, on the other hand, to the two-electron process of plastoquinone reduction at the cytoplasmic side. Then, a situation can occur, where, e.g., after three turnovers of the RC, the final electron acceptor (Qₒ) has left the binding pocket, the intermediate electron acceptor Q₁ is reduced, and water oxidation is incomplete. In such a situation, the RC can still accept excitation energy from the antenna system but cannot use it (closed RC). Instead, charge recombination occurs, and the excitation energy is transferred back to the antenna system. In the present PSII superstructure, the excitation energy can be transferred back from the closed RC to one of two antenna slabs, where each slab, in turn, can transmit the energy to three other RCs. Thus, the probability that the excitation energy is recycled is increased 6-fold compared to a single dimer, provided that the other RCs are not closed. Note that, in a single PSIIcc dimer, back excitation energy transfer from the closed RC would have to be to CP47 (and not CP43), then to the second CP47 subunit in the dimer, and from there to the second RC. Furthermore, the probability that this second RC is also closed is higher than the probability that, in the row, all of the six RCs considered earlier are simultaneously closed. Therefore, the arrangement of antennae and RCs in the PSII superstructure enhances the efficiency of solar energy usage. A quantification of this effect is only possible with detailed simulations (Renger and Müh, 2013). Although such simulations are beyond the scope of the present work, the structural data provide the necessary basis for them.

**Conclusions**

We provided a perspective on postcrystallization treatments of membrane protein crystals in general by demonstrating a transformation from type II to type I and a path to high-quality crystals of PSII in a native-like arrangement in particular. Work is in progress to obtain C₁₂E₅-dPSIIcc microcrystals for RT femtosecond X-ray diffraction experiments (Kern et al., 2012, 2013) to unravel the mechanism of photosynthetic water oxidation.

**EXPERIMENTAL PROCEDURES**

**Protein Purification**

Cells of *T. elongatus* were prepared as described elsewhere (Kern et al., 2009). Thylakoid membranes were solubilized with 1.2% (w/w) C₁₂E₈ (Sigma-Aldrich, >98% pure, catalog no. P8925, used without further purification). The protein extract was purified first by two steps of anion exchange chromatography on a Toyopearl DEAE 650 S resin (Tosoh Bioscience) in buffer A: 0.02 M MES-NaOH (pH 6.0), 0.5 M betaine monohydrate, 0.01 M CaCl₂, 0.013% (w/w) C₁₂E₅, using a linear gradient from 0 to 0.1 M MgSO₄. Further purification was performed on a MonoQ resin (GE Life Sciences) in buffer A, using a linear gradient from 0 to 1 M NaCl. Purified C₁₂E₅-dPSIIcc was finally obtained in 0.02 M MES (pH 6.0), 0.01 M CaCl₂ and 0.013% C₁₂E₅, concentrated to 2 mM Chl a (−21 mg/ml dPSIIcc) and stored under liquid nitrogen.

**Size Exclusion Chromatography**

To check the obtained peak fractions for their oligomerization state, we ran PSII samples (0.13 mM Chl a, 300 μl) on a Superose 6 column (diameter, 10 mm; height, 30 cm; GE Healthcare) in a buffer containing 0.02 M MES-NaOH (pH 6.0), 0.5 M betaine monohydrate, 0.01 M CaCl₂, 0.025 M MgSO₄, and 0.013% (w/w) C₁₂E₅ at a flow rate of 0.3 ml/min using an Åkta FPLC System (GE Healthcare). The protein peaks were detected at a wavelength of 280 nm and compared to the retention volumes of standards of βDM-dPSIIcc (750 kDa) and βDM-mPSIIcc (390 kDa) (Zouni et al., 2005).

**Blue Native PAGE**

Blue native PAGE (BN-PAGE) was performed with PSII samples (final concentration, 0.06 mM Chl a) using the protocol of Wittig et al. (2006). Protein standards were treated in the same way as the samples. The gel was destained with 10% acetic acid.

**MALDI-TOF-MS**

MALDI-TOF-MS was performed with an Ultraflex II Spectrometer (Bruker Daltonics) equipped with a 200 Hz solid-state Smart Beam laser. The mass spectrometer was operated in the positive linear mode. PSIcc solution and dissolved PSIcc crystals (1–4 mM Chl a) were prepared on sinapinic acid.
Oxygen Evolution Measurements

Oxygen evolution measurements of PSIIcc solutions were carried out with a Bachofer Clark electrode. Samples were excited with a xenon flash lamp with a flash frequency of 1 Hz. Samples were diluted in buffer: 20 mM MES-NaOH (pH 6.5), 10 mM CaCl2, 10 mM MgCl2 to a final concentration of 20–50 μM Chl a. The artificial electron acceptor K3[Fe(CN)6] was added with a final concentration of 2 mM, and electron donor p-benzoquinone was added with a final concentration of 200 μM.

CMC Measurements

CMC measurements were performed by exploiting the fluorescence enhancement of the dye 8-anilinonaphthalene-1-sulfonate according to Abuin et al. (1997) using a Horiba Jobin Yvon FluoroMax-2 spectrometer.

Crystallography

Initial crystals of C12E5-dPSIIcc were obtained with the ProPlex (Radaev et al., 2008) crystal screen (Molecular Dimensions) using the sitting drop vapor diffusion method. Optimization was performed by microbatch in 96-well IMPACT Plates (Greiner Bio-One GmbH). Two millimolars Chl a equivalent of PSIIi was mixed 1:1 with the optimized crystallization solution: 0.1 M Tris (pH 7.5), 0.1 M (NH4)2SO4 in steps of 5% PEG for 5–10 min (resulting in the transformation from type II to type I) and transferred to liquid N2 at 100 K. Crystals appeared after 1–2 days at RT and grew further in size within a few days.

HPF

PSII crystals were drawn into thin-walled polyimide tubes and were high pressure frozen at 210 MPa and 77 K using a Baltec HPM 010 instrument (Burkhardt et al., 2012, 2013). Polyimide tubes were purchased from GoodFellow GmbH (catalog no. IM307014: inner diameter, 0.155 mm; outer diameter, 0.193 mm; wall thickness, 0.019 mm; catalog no. IM307015: inner diameter, 0.166 mm; outer diameter, 0.196 mm; wall thickness, 0.015 mm). Sample manipulation after HPF was carried out at cryogenic temperatures below 140 K. Polyimide tubes containing the PSII crystals in their frozen growth solution were mounted on hollow steel pins (Dispomed Witt oHG, catalog no. 10092; outer diameter, 0.6 mm) via homemade polyimide clamps.

X-Ray Diffraction Data Collection and Analysis

Diffraction data were measured in three ways: (1) at RT directly in the crystalization solution using a HC1b humidity controller (Sanchez-Weatherby et al., 2009) set to 99% humidity (experiments were performed at beamline 14.3 operated by the Joint Berlin MX-Laboratory and the Helmholtz-Zentrum Berlin [H2B] at the BESSY II electron storage ring in Berlin-Adlershof, Germany; Mueller et al., 2012); (2) at 100 K after HPF; and (3) for measurements including the final data set, crystals were cryoprotected and dehydrated by stepwise transfer to a buffer containing 50% (v/v) PEG 5000 MME, 0.1 M Tris (pH 7.5), 0.1 M (NH4)2SO4 in steps of 5% PEG for 5–10 min (resulting in the transformation from type II to type I) and transferred to liquid N2 at 100 K. Experiment 3 was carried out at BESSY II beamlines 14.1 and 14.2 operated by the Joint Berlin MX-Laboratory and the H2B. Experiments 2 and 3, including collection of the final data set, were performed at beamline P11 at the third-generation synchrotron source PETRA III at DESY. The reported data set was collected from a 250 × 100 × 100 μm3 crystal using a 100-μm-diameter beam with a total dose of 2.8 × 1014 photons (3.6 × 1010 photons per square micrometer) at 1.196 Å X-ray wavelength. Data were integrated to 2.44 Å maximum resolution and scaled with XDS (Kabsch, 2010) (Table 1). The structure was solved by molecular replacement with Phaser (McCoy et al., 2007), using two separate monomers of the 1.9 Å resolution structure (Protein Data Bank [PDB] ID: 3ARC; Umena et al., 2011) as search model. The model was fitted to the electron density using iterative cycles of hand building in COOT (Emsley et al., 2010) and automated refinement in phenix.refine (Afonine et al., 2013). The quality of the protein model was confirmed with MolProbity (Chen et al., 2010). Ninety-six percent of backbone torsion angles were in favored regions of the Ramachandran plot, 0.3% were outliers, and the remaining residues were in additionally allowed regions.

The position and presence of each cofactor was confirmed by generating an omit map after three macrocycles of refinement in phenix.refine in the absence of each cofactor from the model. Geometry restraints for Chl a and pheophytin a were automatically generated by the Grade server v1.001 (Global Phasing). Heme geometry was taken from the atomic resolution structure in PDB entry 3FMU and restraints generated in eLBOW (Moriarty et al., 2009). The heme located in subunit PsbV (cytochrome c551) was modeled as covalently bound to Cys 37 and Cys 40 of the same subunit. The geometry of the Mn4Ca2O5 cluster was taken from the highest resolution structure available (PDB ID: 3ARC) and modeled as rigid body. All other ligand restraints were taken from the CCP4 (Winn et al., 2011) monomer library. The cofactor and residue numbering scheme was matched to PDB entry 3ARC.

ACCESSION NUMBERS

The final model was deposited in the PDB (ID: 4PJ0).

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.str.2014.09.007.

AUTHOR CONTRIBUTIONS

J.H. developed the purification, crystallization, and transformation protocol with the help of M.I. and M.B. (transformation). A.B. performed HPF experiments. M.B., J.H., A.B., and A.M. collected diffraction data. M.B. analyzed diffraction data, built the model, and designed the artwork. F.M. analyzed CMC data. J.H, M.B., J.K., F.M., H.D., and A.Z. designed the experiment, interpreted the results, and wrote the publication.

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Native-like Photosystem II Superstructure


