Structure Determination and Improved Model of Plant Photosystem I

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Photosystem I functions as a sunlight energy converter, catalyzing one of the initial steps in driving oxygenic photosynthesis in cyanobacteria, algae, and higher plants. Functionally, Photosystem I captures sunlight and transfers the excitation energy through an intricate and precisely organized antenna system, consisting of a pigment network, to the center of the molecule, where it is used in the transmembrane electron transfer reaction. Our current understanding of the sophisticated mechanisms underlying these processes has profited greatly from elucidation of the crystal structures of the Photosystem I complex. In this report, we describe the developments that ultimately led to enhanced structural information of plant Photosystem I. In addition, we report an improved crystallographic model at 3.3-Å resolution, which allows analysis of the structure in more detail. An improved electron density map yielded identification and tracing of subunit Psak. The location of an additional ten β-carotenes as well as five chlorophylls and several loop regions, which were previously uninterpretable, are now modeled. This represents the most complete plant Photosystem I structure obtained thus far, revealing the locations of and interactions among 17 protein subunits and 193 non-covalently bound photoco-factors. Using the new crystal structure, we examine the network of contacts among the protein subunits from the structural perspective, which provide the basis for elucidating the functional organization of the complex.

During oxygenic photosynthesis, solar energy is converted into chemical energy for all higher forms of life on Earth. This process is driven by a photosynthetic apparatus within the thylakoid membranes of cyanobacteria, algae, and plants. The photosynthetic reactions are performed by two photosystems: Photosystems I (PSI) and II (PSII).

The abbreviations used are: PSI and -II, Photosystems I and II; LHCI and -II, light-harvesting complexes I and II; LHCII and -II, light-harvesting complexes I and II; β-DM, n-dodecyl-β-o-maltoside; β-DTM, n-dodecyl-β-o-thiomialtose; Chl, chlorophyll; Tricine, N-(2-hydroxyethyl)-glycine; PEG, polyethylene glycol; MES, 4-morpholineethanesulfonic acid; BisTris, 2-[bis(2-hydroxyethyl)-amino]-2-(hydroxyethyl)propane-1,3-diol; BCR, β-carotene.
proteins that are not present in the cyanobacteria counterpart, namely PsaG and PsaH, and four proteins of the light-harvesting complex (Lhca1 through Lhca4). However, the low resolution structural data allowed identification of only the main structural elements, and important features were unavailable, such as the identity and conformation of the amino acid side chains. By intense analysis of plant biochemistry and systematic investigation of membrane protein crystallization procedures, we improved purification methods and optimized the crystallization conditions for plant PSI, which enabled the recent determination of the x-ray crystal structure at 3.4-Å resolution, by merging data from 10 different crystals (16). However, the peripheral parts of the complex, including the LHCl, were poorly resolved, therefore prompting us to collect more complete data. The improved crystal structure reported here was determined from three independent and relatively complete data sets exhibiting monoclinic P21 symmetry with the β angle of ~91.2°. A picture at near atomic detail of 12 of 18 protein subunits is presented. The positions of 3159 amino acids were assigned, as were those of 173 chlorophylls (145 revealing the orientation of the Qx and Qy transition dipolar moments), 15 carotenoids, 2 phyloquinones, and 3 Fe₄S₄ clusters, providing the most detailed picture of a plant PSI current available.

In this report, we evaluate the critical steps involved in obtaining better structural information. We emphasize the straightforward biochemical procedure, which could be extrapolated and utilized in improving other plant membrane complex purification and crystallization protocols, as well as crystallographic aspects. From the improved crystal structure, we also examine the network of contacts among the PSI subunits and discuss the unique structural elements of plant PSI in respect to functional design.

EXPERIMENTAL PROCEDURES

Purification of Plant PSI—Isolation of thylakoid membranes from 12-day-old pea was performed based on the previously described method (17); however, the procedure was shortened to obtain crystallization trials as soon as possible. All procedures were carried performed in dim light at 4–6 °C. For PSI isolation, we adopted the strategy of selective extraction of the complex from partially solubilized membranes. PSI complexes were isolated from thylakoids that were pretreated with the detergent n-dodecyl-β-D-maltoside (β-DM) (Glycon, Inc.) at 1.55 mg of β-DM/mg of chl for solubilization of PSII, ATP-synthase, and b6f complexes. Approximately 20 ml of thylakoid membranes containing 3.0 mg of chl/ml were then solubilized by β-DM to give a final concentration of 6.0 mg of detergent/mg of chl. Unsolubilized material was removed by ultracentrifugation at 110,000 × g for 15 min. The supernatant was applied to a DEAE-cellulose column (DE-52, Whatman, Inc., 1.5 × 18 cm) that was pre-equilibrated with 20 mm Tricine-Tris (pH 7.4) containing 0.2% β-DM. The column was washed with 25 ml of the same buffer, and PSI was eluted with a 0–200 mM NaCl linear gradient (130 ml in each chamber) in 20 mm Tricine-Tris (pH 7.4) containing 0.2% β-DM. First, dark green fractions containing mainly PSI and LHClII complexes were properly separated from the remainders of other photosynthetic complexes. Fractions containing PSI were precipitated by the addition of 50% (w/v) PEG 6000 (Hampton, Inc.) to give a final concentration of 8%, followed by centrifugation at 10,000 × g for 5 min. The pellet was dissolved in ~6 ml of solution containing 20 mm Tricine-Tris (pH 7.4), 0.05% n-dodecyl-β-D-thiomaltoside (β-DTM, Glycon, Inc.). The PSI was applied onto a 10–30% sucrose gradient containing the same buffer and centrifuged using the SW-40 rotor (Beckman, Inc.) at 37,000 rpm for 15 h. Supplemental Fig. S1 presents the fraction of DEAE-cellulose column after sucrose gradient centrifugation. The wide green band containing PSI was collected and loaded onto a DEAE-cellulose column (0.5 × 4 cm) pre-equilibrated with 20 mm Tricine-Tris (pH 7.4). After washing with 3 ml of buffer, PSI was eluted using the same buffer containing 200 mM ammonium acetate and 0.05% β-DTM. An essential prerequisite for protein crystallization is a pure, homogenous solution in which all molecules are as identical as possible. To remove impaired and partly denatured PSI complexes, which might be caused by the DEAE-cellulose column, another purification step was added: a second sucrose gradient. The collected dark green fraction was applied onto a 10–30% sucrose gradient and centrifuged in an SW-60 rotor (Beckman) at 57,000 rpm for 4 h. The purified PSI appeared as a dark band in the middle of the tube, and to avoid protein heterogeneity, only the middle of the band was used for crystallization. The material was precipitated with 10% PEG 6000 and 50 mM ammonium acetate and subsequent centrifugation at 10,000 × g for 4 min. The resultant pellet was dissolved in ~0.5 ml of a solution containing 2 mm Tricine-Tris (pH 7), 0.015% β-DTM and adjusted to a chlorophyll concentration of 3.0 mg/ml. The identification of protein material from selected stages of purification and analysis of PSI purity by SDS-PAGE is shown in supplemental Fig. S2.

Crystallization—Crystallization trials were set up using the sitting drop variant of the vapor-diffusion technique at 277 K. Crystallizing PSI directly from the sucrose gradient, thus keeping a considerable sucrose concentration, increased the crystal growth reproducibility and quality. We also observed that faster purification improved crystallization. All crystallizations were set up manually on 24-well plates. Aliquots (4 µl) of purified proteins were mixed with equal volumes of reservoir solution (22.5 mM MES-BisTris, 0.5% PEG 400, 10 mM succinic acid, 3.5–6.5% PEG 6000, 0.015% β-DTM, pH 6.0–6.3) and equilibrated against 0.5 ml of reservoir solution. Crystals appeared in an elongated rectangular shape of dark green color and varying thickness. Initial crystals emerged after 24 h and reached maximum size within 7 days, a faster crystal formation compared with our previous studies, which lead to 4.4-Å solution (15). The speedy purification and the formation of crystals in a relatively short period seem to be essential for retaining the complex integrity, thereby yielding crystals of higher quality with enhanced resolution.

Crystals were transferred in four steps of increasing PEG 6000 concentrations, up to a final concentration of 40%. After an incubation of up to 7 days, crystals were frozen in liquid nitrogen or directly at 100 K by the nitrogen stream at the synchrotron beam line.

Data Collection and Structure Determination—X-ray diffraction data were collected at the European Synchrotron Radi-
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Optimization of Crystal Quality—Initially, the crystal quality varied markedly within a crystallization batch, and hundreds of crystals should have been screened to identify single crystals that diffracted substantially better than others. Therefore, the ability of different additives to improve crystal quality was examined. Importantly, we found that crystals grew much better and exhibited improved diffraction properties in the presence of 0.015% β-DTM. This might be due to the effect of detergent on binding monomers in a crystal lattice, in agreement with a few membrane protein structures, which show specific interactions between protein and detergent head groups (23). The source and batch of the detergent also played a substantial role in successful crystallization. A similar effect was described in the crystallization of the Na+/H+ antiporter (24, 25). However, higher levels of detergent tended to inhibit crystal formation, presumably due to “empty” micelles, which prevented contacts between protein detergent micelles.

Despite this improvement in the reproducibility and quality of crystals, plant PSI crystals suffered from anisotropic x-ray scattering. In protein crystallography, anisotropy of x-ray diffraction occurs when the strength of lattice contacts of a crystal is lower in one cell direction than another. Thus, changing the detergent used for crystallization and adding different detergents as additives to crystallization conditions was attempted, however it did not lead to significant progress. Because the phenomenon of an anisotropic kind of diffraction was attributed to the spatial form of crystals, which were long but thin (supplemental Fig. S3a), we attempted to obtain new crystal morphology by screening different crystallization conditions. A new crystal form was found by substitution of 8 mM ammonium citrate with 10 mM succinic acid and reduction of the pH of crystallization buffer from 6.7 to 6.0. Comparison of the two crystal forms (pH 6.7 versus pH 6.0) presented in supplemental Fig. S3 shows that new crystals appeared to be in a quadrangle shape and much smaller in size, yet they were thicker, proposing a more homogeneous lattice formation. Comparison of the anisotropy analysis (26) and unit cell parameters between the two crystal forms shown in supplemental Fig. S4 demonstrates that, for crystals of the new form, the anisotropy was notably reduced.

Further improvement of the diffraction quality was achieved due to post-crystallization dehydration soaking. We performed the dehydration procedure by transferring PSI crystals through a series of solutions of increasing concentrations of precipitant (PEG 6000) and incubated the crystals for at least 24 h at each dehydration solution. Crystals subjected to this controlled dehydration showed dramatic improvement of diffraction properties. A strong correlation was found between the length of the dehydration period, solvent content, decrease in unit cell dimensions, and the diffraction quality. The majority of dehydrated crystals diffracted beyond 4 Å at a synchrotron source, and some exhibited diffraction of ~3-Å resolution. The dehydration also triggered a new crystal order, and decreased the cell volume by ~30%, compared with the previous low resolution PSI structure (15). Essentially, this allowed more accurate data to be collected, which eventually led to the 3.4-Å (16) and the currently improved 3.3-Å resolution structure determination.

Data Collection—There were few caveats hidden in the data collection process for obtaining sufficient and reliable structural information. First, relatively weak diffracting crystals did not allow adequate x-ray scattering measurements to be obtained using a house source generator. Thus, data collection as well as initial screening were performed at synchrotrons with available highly intense beam sources (European Synchrotron Radiation Facility ID23-2 and Swiss Light Source PXI). Second, well diffracting crystals were found to have relatively small sizes, which required only highly focused beam lines, such as European Synchrotron Radiation Facility ID23-2. Third, plant PSI crystals are very sensitive to radiation damage even at cryo temperatures. Because of radiation sensitivity, the diffraction limit dropped significantly during data collection, which for that reason was restricted to only ~20 good quality frames per crystal portion. This, in conjunction with relatively small crystal sizes, did not permit complete data collection from a single crystal. Hence, our previous crystal structure was based on the merged data from 10 different crystals (16). Fourth, the dehydration procedure yielded considerably variable crystal geometries. In some cases, the β angle of unit cell dimensions of data collected from crystals of the same batch varied from 91° to 98°, changing the overall volume between 2.4 [m^-3] and 3 [m^-3]. Because crystallographic restrictions do not allow data with different geometrical parameters to be combined, this often meant that only limited sections of specific crystals were able to produce usable structural information. This obstacle significantly challenged structure determination. Together, the inherent variability of plant PSI crystals, their fragility, and radiation damage sensitivity meant that it was essential to collect and analyze more than 200 different data sets (400 Gb of data) to construct sufficient and relatively complete crystallographic data from different preparations with isomorphic parameters and reasonable statistics, which eventually led us to the previous structure determination at 3.4-Å resolution (16).

However, consolidation of data from different crystals has obvious limitations, and the cost associated with this strategy of data collection increases in the terms of the quality of structural information. In our case, it is especially critical because plant PSI is a super-complex that consists of two loosely bound complexes (the core complex and LHCl) that may adopt somewhat different relative positions. In addition, taking into account that several subunits are loosely associated with the main body of plant PSI, protein subunit composition may also differ among crystals. Thus, to obtain homogeneity of crystallographic data and improve the quality of the merged structural information, we focused our efforts on finding individual crystals that would allow complete data collection from the same crystal. Very
recently, few such crystals were identified and despite the fact that some inconsistency was observed for data collected from different parts of these crystals, we were able to complete an entire data set at 3.3-Å resolution from a single crystal. This data set belongs to the space group P21, with unit-cell parameters of approximately \( a = 121, b = 189, c = 129, \beta = 91.2^\circ \) (supplemental Table S1). These parameters are distinct from the previously published unit-cell dimensions that resulted from a different shrinking protocol (16). Compared with our previous work, we found a significant improvement of crystallographic data in both the completeness of the data set and the merging statistics (supplemental Table S1). The processing of current x-ray diffraction data were associated with a distinct improvement in the electron density (see supplemental Fig. S5 for examples), which allowed the identification of many additional structural elements discussed below.

Structure Determination Using Theoretical Model—In protein crystallography, the solution of x-ray diffraction data is dependent on two parameters: wave amplitude (intensity) and wave relative phase. The latter is crucial for constructing electron density maps; however, it is systematically lost in measurements because light detectors are only able to measure the intensity. Without phases, it is impossible to apply Fourier transform to structural factors to get the electron density distribution within the unit cell of a crystal. Experimental phases are the most difficult task in structure determination and are usually calculated by quantitative attachment of heavy atoms, which provide anomalous scattering. However, in our case, despite extensive efforts to obtain experimental phases, insertion of heavy atoms caused severe damage to the diffraction properties of crystals, probably due to the delicate nature of the protein complex and very dense crystal packing. Hence, phases were determined using molecular replacement.

Three different models were used as initial search models: a cyanobacterial PSI (PDB code 1BJ0) (13), the 4.4-Å resolution structure of plant PSI (PDB code 1QZV) (15), and a theoretical atomic model of plant PSI (PDB code 1YO9) derived from the synthesis of the two above-mentioned structures (27). Interestingly, structure determination using the theoretical model yielded better refinement statistics for the previous crystal structure determination (16) as well as for the improved model reported in the current report. To eliminate model bias, a composite omit map was calculated in the final stages of refinement. The phase determination from the model was accurate enough to generate an electron density map that revealed additional features that were missing from the theoretical model. This electron density map eventually resulted in a PSI structure composed of 18 protein subunits, 3159 assigned amino acids, as well as 191 photochemical cofactors (Fig. 1).

Taking into account the recent progress of computational methodologies (28, 29), which is based on rapid expansion of the structural membrane protein data base (30), it is evident that the consistently developing theoretical models are becoming closer to valid protein structures (31). Thus, we suggest that the theoretical models based on advanced computational modeling techniques may provide a useful and reliable source for the initial phase determination of x-ray diffraction data of large protein complexes for which experimental phasing provides a challenging barrier.

Overall Structure—Plant PSI consists of two separate functional units: the PSI core, and the LHCI peripheral antenna. The plant core complex is similar to that of cyanobacteria, whereas LHCI is unique to plants and green algae. The core complex consists of 12 protein subunits, and the largest 2, PsaA and PsaB, form a symmetry-related dimer, which binds the majority of pigments. The symmetry arises from gene duplications of the ancient gene, and forms a fundamental building block of PSI transmembrane electron transfer (32, 33). LHCI functions to increase the effectiveness of the photosynthetic process in plants by engaging light harvesting in a broader spectral region and delivering the photon excitation energy to the core complex. In addition, LHCI plays an important role in photoprotection (34). Overall, the quality of electron density maps has considerably improved, permitting not only the refinement of previously identified structural elements but also the assignment of many details that were not found in the previous structure of plant PSI. Differences in the improved quality of the electron density from previous structural data are discussed in supplemental Fig. S5.

In the current 3.3-Å crystal structure, the entire plant PSI super-complex contains over 10% more atoms than previously described and reveals a previously unidentified small protein subunit. The elusiveness of these elements in the previous structure may be attributed to the generally poorer data, or because it was not presented in some or the majority of the 10 crystals from which the data were derived. The overall structure is shown in Fig. 1, and the newly identified elements are indicated. The current improved plant PSI model at 3.3-Å resolution includes 18 protein subunits, 173 chlorophylls, 15 \( \beta \)-carotenoids, 3 \( Fe_3S_4 \) clusters, and 2 phyloquinones. 10 of 12 plant chlorophylls, and 3 Fe4S4 clusters, and 2 phyloquinones. 10 of 12 plant
PSI core complex subunits (PsaA, PsaB, PsaC, PsaD, PsaE, PsaF, PsaI, PsaJ, PsaK, and PsaL) are closely related to the PSI of cyanobacteria and show remarkable uniformity in the composition and homology of individual protein sequences. Other plant subunits of the core complex (PsaG and PsaH) perform unique structural functions related to the harboring of LHCI and LHCII, respectively, and are discussed elsewhere (35, 36).

Additionally, an electron density was observed at the PsaK side of the core complex (supplemental Fig. S6). Because none of the additional subunits known to be assembled into PSI (PsaO (37) and PsaP (38)) could be unambiguously traced in the density, we tend to ascribe it to an unidentified polypeptide, namely PsaR, and thus modeled it as polyalanine. This protein contains a single transmembrane helix that has no direct contacts with the PsaA-PsaB heterodimer and, according to the structure, is unable to bind PSI in the absence of PsaK. PsaR is located in between the PsaK and Lhca3 subunits, suggesting that it may be involved in LHCI stabilization. A relatively weak electron density of this protein subunit suggests loose association with the core of PSI, which might be the reason for its absence in the previous crystallographic model derived from 10 different crystals (16). It is tempting to suggest that the presence of this alleged subunit resulted in a larger unit-cell volume and the β = 91° crystal form.

Chlorophylls—In the core complex, the positions and orientations of most chlorophyll molecules were the same and many were refined. However, in addition to the previously identified 168 chlorophylls, 5 more chlorophyll molecules were found in the structure of plant PSI. One of these newly found chlorophylls is Chl1303, which is located in the gap region between the core complex and LHCI (Fig. 2a). Gap chlorophylls are critically important, because they mediate excitation energy transfer from Lhca2 to the core complex. The presence of gap chlorophylls is rather unique to plant PSI, because experiments performed with PSII super-complexes showed that dissociation into individual pigment proteins does not produce a significant loss of pigments (39). In addition, gap chlorophylls cannot be reconstituted with a protein moiety by in vitro methods due to their unique location. Thus, the crystal structure of plant PSI provides important insights into this exclusive phenomenon, which would be unobservable by the other available methods. In our structure, the gap chlorophyll Chl1303 is found between subunit PsaB and two neighboring light-harvesting proteins Lhca1 and Lhca4 (Fig. 2a). Chl1303 is situated ~16 Å from Chl4010 of Lhca4, and as close as 14 Å to Chl1003 of Lhca1. This range is favorable for fast excitation energy transfer. From the opposite side, the two gap chlorophylls 1302 and 1305 are located in close proximity (11 Å and 13 Å, respectively). Thus, the structure suggests that the Chl1303 position is sufficient for excitation energy transfer from the Lhca1–4 dimer to the core through chlorophylls 1302 and 1305. Importantly, the detection of Chl1303 at this position now provides a structural logic for the location of Chl1302, because, in the previously described model (16), Chl1302 was not linked to any other chlorophyll coordinated by one of the LHCI units, and we were unable to explain its functional significance.

The data at 3.3-Å resolution also allowed the refinement of the position of another gap chlorophyll, Chl1308, and permitted assignment of Chl2005 coordinated by Lhca2 (Fig. 2b). Although Chl2005 is located in a similar position as in LHCI (40), in our previous work, the electron density contained no structural information with respect to this chlorophyll. Chl1308 considerably contrasts with its previously identified position; however, the improved electron density at this region allowed not only unambiguous identification of its location, but also of the circular orientation of the porphyrin ring. This chlorophyll is located between Chl2005 (11-Å distance) and additional gap Chl1309 (10-Å distance), mediating the excitation energy transfer from Lhca2 to the core complex. Overall, as the resolution improves, more important details begin to emerge.

**FIGURE 2. Positions and newly identified chlorophylls in the gap region between the core complex and LHCI.** The comparison of chlorophyll arrangement between the current (blue) and the previous model (yellow orange) (16) reveals that positions and orientations of several chlorophylls in the gap region were refined, and additional chlorophylls were identified. On the top panel the enlarged region is depicted by the white quadrangle. a, chlorophylls 1003, 1302, 1303, 1305, and 4010 are depicted. Gap chlorophyll 1303 was not identified previously at this position. Center to center distances suggest that Chl1303 mediate excitation energy transfer between Chl1003 (Lhca1) and Chl1302 (gap) and also possibly between Chl4010 (Lhca4) and Chl 1305 (gap). b, chlorophylls 1308, 1309, 2005, and 2012 are depicted. Chl2005 (Lhca2) was not identified previously at this position. The position and orientation of Chl1308 (gap) was refined in the current model. Center to center distances suggest that these chlorophylls form a pathway for excitation energy transfer from Lhca2 to the core complex.
and yield more accurate data and further insights into the structural role of gap chlorophylls in PSI. Importantly, the structure also suggests that gap chlorophylls impose geometrical and functional constraints, which play a part in the construction of unique Lhca binding sites, contributing to the structural integrity of the PSI-LHCl super-complex.

In addition, Chl1125 was identified, which is coordinated by PsaA at a position conserved with the cyanobacterial counterpart (13). Chlorophylls 1145 and 1148 may occupy artificial positions, because they are relatively distant from their neighbors and are situated in the alleged site where LHClI may interact with PSI during state transition (41–43). New chlorophylls coordinated by PsaK were also found.

Carotenoids—In addition to the previously identified 5 carotenoids, 10 new carotenoids are found in the current 3.3-Å resolution structure and modeled as complete β-carotenes (BCRs). The carotenoids are embedded into the lipid phase and coordinated by PsaA (BCR2, BCR3, BCR11, BCR12, BCR14, and BCR18), PsaB (BCR4, BCR5, BCR6, BCR10, BCR17, and BCR21), PsaF (BCR14 and BCR 16), PsaG (BCR6), Psaj (BCR12), Psal (BCR 18, BCR19, BCR20, and BCR21), and PsaL (BCR19). Carotenoids play an important role in photoprotection (44). In addition, because in several carotenoids each pole of β-carotene is coordinated by different subunits or by distant parts of the same subunits, they are vital for the structural integrity of PSI. Most of the β-carotene positions and coordinations are conserved between cyanobacteria and plants (Fig. 3). Previously we found that BCR16 moved considerably from its position in T. elongatus, because cyanobacterial subunit PsaX is not present in plant PSI. In the current crystal structure, a similar situation is observed for BCR21 and the subunit PsaX also missing from plant PSI. Although one pole of BCR21 is as close as 5.5 Å to Chl1201 (PsaB), the other pole is situated 5 Å from Chl1243, instead of in contact with PsaM as in the cyanobacterial structure (13). Interestingly, in the region between Lhca2, Lhca3, and PsaA, there is an additional relatively strong electron density, which we attribute to the unique carotenoid in the gap region between the core complex and LHCI.

Subunit PsaK—Subunit K is poorly resolved in all PSI structures, including the high resolution structure of T. elongatus (13). Although the overall spatial arrangement between the plant PSI transmembrane core and corresponding cyanobacterial part is highly conserved, there is considerable diversity in the location of PsaK subunit (Fig. 4). PsaK, a nuclear-encoded, 9-kDa subunit located on the PsaA side of the core complex, shares the same genetic origin with PsaG, which evolved via gene duplication (45). In the previous crystal structure, the position of PsaK was poorly resolved, and likely part of its alleged assignment was incorrect. The current maps enabled modeling of this subunit. PsaK contains two transmembrane α-helices connected by a positively charged stromal loop, such that both the N and C termini are located in the luminal side of thylakoid membrane. In the plant PSI structure, the position of PsaK is relatively closer to the PsaL pole than its position in the available crystal structure from cyanobacteria (13). In the cyanobacterial PSI trimer, PsaK is positioned in the region facing the adjoining monomer. Thus, PsaK might somehow contribute to trimer stabilization in cyanobacteria. However, PSI in eukaryotes functions as a monomeric unit. Interestingly, PsaK was recently identified in marine virus genomes containing PsaA, PsaB, PsaC, PsaD, PsaE, PsaK, and a unique PsaL-PsaF fusion protein (46). Even though PsaL, PsaI, PsaM, and PsaX are absent in these genomes, the presence of PsaK suggests its importance and antiquity.

FIGURE 3. Carotenoids in plant PSI. Comparison of carotenoid arrangement between plant and cyanobacterial PSI. View is from the stroma with the same orientation as in Fig. 4. Carotenoids of plant PSI are blue; carotenoids of cyanobacterial PSI are red. Cyanobacterial subunit PsaM is shown in as a red schematic. Chlorophylls are shown in transparency for reference. Conservation of the most of carotenoid positions and configurations is demonstrated. On the left, a considerably moved position and different coordination of BCR21 is shown. While in cyanobacteria the luminal pole of BCR21 is coordinated by the unique PsaM subunit (Val-13), in plants additional Chl1241 is found at the respective coordination position of BCR21. Phytol chains were removed for clarity.

FIGURE 4. Position of PsaK in plant PSI. Superposition of plant PSI core complex (blue) on cyanobacteria counterpart (red). a, view from the stroma; b, view along the membrane normal. Conserved transmembrane subunits are shown as ribbon structures. Unique subunits are shown in schematic and surface representation. Note the differences in location of PsaK subunit between plant and cyanobacterial structures. Stromal subunits PsaC, PsaD, and PsaE as well as plant LHCl complex were removed for clarity.
A possible rationalization for conserving this subunit in eukaryotic PSI might be 2-fold: Firstly, PsaK was shown to regulate uncoupling of the LHCI antenna in *Chlamydomonas* cells subjected to iron-deficiency conditions (47, 48), underpinning the importance of this subunit in the regulation of light harvesting under stress. Secondly, PsaK might have a role in state transitions (49). The 16-Å resolution electron microscopy data of single particle analysis of the PSI/H₁₈₅₂₈/LHCI super-complex in *Arabidopsis thaliana* showed a large density at the side of PsaH/L/A/K, which was assigned to the LHCI trimer (41). These results are in agreement with the previously suggested docking site for phosphorylated LHCI (36). In addition, the model of interaction between PSI and LHCI based on x-ray crystal structures (16, 50) fits quite well with the possible interaction between the PsaK loop and the N terminus of LHCI. Following this logic, during the course of evolution, the relative position of PsaK might have been refined by natural selection for optimizing the adaptation of higher eukaryotic organisms to their ecological niche.

**Light Harvesting Complex, LHCI**—Several aspects of the LHCI arrangement in the crystal structure are worth noting. The electron density map in the LHCI region is relatively poor but allowed better assignment of Lhca1–4 subunits. In the current structure, large parts of the loop regions in both the luminal and the stromal sides, which were not previously identified, were assigned (Fig. 1). Consequently, much information was derived concerning contacts not only among these subunits, but also between LHCI and the core complex; this is discussed in more detail below. In addition, the improved structural information allowed better tracing of transmembrane helices of Lhca1 and Lhca3. Importantly, in Lhca3, the conservation of transmembrane helix 3 is now apparent and does not seem to significantly differ from the other three monomers, giving credence to the biochemical analysis of reconstituted Lhca3 (51) and in agreement with sequence analysis (40). The current model includes 15 chlorophylls in Lhca1, which is 1 chlorophyll more than previously reported; 14 chlorophylls in Lhca2, 1 less than previously reported (since 1 Chl was found to be coordinated by the core complex), although the positions of a few chlorophylls in the current structure are different; 17 chlorophylls in Lhca3, 3 more chlorophylls than previously reported; 15 chlorophylls in Lhca4, which is the same as previously reported. This gives rise to a total of 61 chlorophylls associated with LHCI in the current structure of plant PSI. In addition, some non-assigned electron densities are still present in this region, but we could not ascribe it to either additional pigments (39, 52, 54) or any of the Lhca paralogs (55–57). Overall, the current structure provides a much more realistic picture of the spatial organization of the LHCI complex and how it is bound to the core complex. However, we should also point out that the quality of the electron density maps within LHCI is far less well defined than for the core complex. A structure with considerably better resolution, which would be able to identify positions of different types of carotenoids and bound
lipids, will provide additional insights regarding the functional role of LHCI.

**Functional Organization and Inter-protein Contacts in PSI**—The improved structural model at 3.3-Å resolution also paved the way to a better understanding of the functional organization of plant PSI. We examined the structure in the terms of amino acid contacts between protein subunits, shown in Fig. 5. The PsaA/PsaB heterodimer is closely held together and also connected with other subunits of the core through an extensive series of interfacial contacts. This intimately bound protein network explains the difficulty of fractionating the PSI core compared with the PSII core, which can be purified into separate parts.

**LHClI Binding to the Core**—The analysis of the protein-protein interface network of plant PSI, shown in Fig. 5, reveals, for the first time, the bilateral relationships between the core and LHClI complexes, and highlights the roles of several subunits.

LHClI is composed of four subunits, Lhca1-4, that are assembled into two heterodimers arranged like a belt on the “southern hemisphere” of the core. The protein contacts between the core complex and LHClI appear to be relatively weak, which explains the biochemical sensitivity of the PSI-LHClI supercomplex to detergent attack (58–60). It is clear, however, that each of the four light-harvesting proteins fits its specific binding site, because the interface of the core complex formed by subunits PsaG, PsaB, PsaF, PsaJ, PsaA, and PsaK is asymmetric (supplemental Fig. S7). Lhca1 antenna protein is bound to the core through PsaB and PsaG. Previous studies showed that plants in which psaG gene expression was suppressed by antisense technology or eliminated by transposon tagging (61, 62) exhibited compromised stability of the antenna and weaker binding of LHClI to the core. Thus, it was proposed that PsaG somehow stabilizes the peripheral antenna. Our model now provides the structural support for these studies.

Fig. 2 and supplemental Fig. S7 show that interactions between the protein backbone of Lhca4 and Lhca2 and the PSI core occur through small contact surfaces of PsaF and PsaJ, respectively (Lhca2 also interacts with PsaA). Plants lacking PsaF retained an intact antenna complex, however they were severely affected in energy transfer from LHClI (63). ΔpsaF/tobacco plants showed impaired antenna binding and excitation transfer to the PSI core, whereas the functional size of the antenna was not affected (64, 65). Our structure agrees with these studies, suggesting that PsaF and PsaJ were optimized for the interactions with LHClI, although they are probably not crucial for the assembly of the antenna onto the core of PSI. It is noteworthy that the connection of Lhca4 and Lhca2 is also mediated through gap chlorophylls situated in the gap between the two complexes (supplemental Fig. S7). The efficient and specific coupling of Lhca4 and Lhca2 to the core is also confirmed by biochemical analysis (66–68).

Lhca3 appears to interact with PsaA. However, the position of newly traced subunit PsaK suggests that it also binds Lhca3. PsaK was proposed to stabilize LHClI organization, and PSI lacking PsaK, isolated from *Arabidopsis* plants, showed 30–40% less Lhca3, whereas associations with Lhca1 and Lhca4 were unaffected (61, 62). It is likely that additional potential contacts between the two subunits may be also in the future.

Interestingly, recent genetic analysis of PSI from the red algae, *Galdieria sulfuraria*, revealed that it contains the PsaK subunit and one Lhc gene product, which shows significant homology to plant Lhca3 (69).

It is important to note that contacts between the core and LHClI complexes may also be mediated through lipid contribution and phytol moiety of chlorophylls, which are not characterized in the current model. Therefore, a higher resolution structure is needed to address these possibilities.

PsaN is the only subunit located entirely in the thylakoid lumen and can be dissociated from PSI at a high ionic strength (70). In the crystal structure, the position of PsaN is modified in its C terminus, but the section that interacts with Lhca2 and Lhca3 is maintained, suggesting that this subunit may stabilize the heterodimer. However, PsaN might also be involved in orienting the N-terminal domain of PsaF that is crucial for efficient electron transport between the donor (plastocyanin) and PSI (53, 66).

Overall, the 3.3-Å resolution crystal structure provides new information concerning the composition of the subunits of plant PSI and their possible arrangement. The analysis of the intersubunit contact map from the improved structural model presented here may be useful for providing insights into understanding how plant PSI is assembled and turned over after photodamage.

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