Investigating the Early Stages of Photosystem II Assembly in *Synechocystis* sp. PCC 6803

**ISOLATION OF CP47 AND CP43 COMPLEXES**

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Biochemical characterization of intermediates involved in the assembly of the oxygen-evolving Photosystem II (PSII) complex is hampered by their low abundance in the membrane. Using the cyanobacterium *Synechocystis* sp. PCC 6803, we describe here the isolation of the CP47 and CP43 subunits, which, during biogenesis, attach to a reaction center assembly complex containing D1, D2, and cytochrome b556, with CP47 binding first. Our experimental approach involved a combination of His tagging, the use of a D1 deletion mutant that blocks PSII assembly at an early stage, and, in the case of CP47, the additional inactivation of the FtsH2 protease involved in degrading unassembled PSII proteins. Absorption spectroscopy and pigment analyses revealed that both CP47-His and CP43-His bind chlorophyll *a* and *β*-carotene. A comparison of the low temperature absorption and fluorescence spectra in the Qy region for CP47-His and CP43-His with those for CP47 and CP43 isolated by fragmentation of spinach PSII core complexes confirmed that the spectroscopic properties are similar but not identical. The measured fluorescence quantum yield was generally lower for the proteins isolated from *Synechocystis* sp. PCC 6803, and a 1–3-nm blue shift and a 2-nm red shift of the 77 K emission maximum could be observed for CP47-His and CP43-His, respectively. Immunoblotting and mass spectrometry revealed the co-purification of PsbH, PsbL, and PsbT with CP47-His and of PsbK and Psb30/Ycf12 with CP43-His. Overall, our data support the view that CP47 and CP43 form preassembled pigment-protein complexes *in vivo* before their incorporation into the PSII complex.

Photosystem II (PSII) is the light-driven water:plastoquinone oxidoreductase of oxygenic photosynthesis, responsible for producing most of the oxygen in the atmosphere (1). It is located in the thylakoid membrane of chloroplasts and cyanobacteria and is a multisubunit lipoprotein complex composed of both intrinsic and extrinsic proteins. Crystal structures of dimeric PSII protein complexes isolated from the thermophilic cyanobacteria *Thermosynechococcus elongatus* (2–5) and *Thermosynechococcus vulcanus* (6, 7) have revealed the organization of the 20 subunits within each monomeric complex and the positions of the various cofactors. These include 35 chlorophyll (*Chl*) a molecules, two pheophytin *a* molecules, 12 carotenoids, two heme molecules, one non-heme iron, two calcium ions, two chloride ions, three plastoquinones, 25 lipids, and the Mn4Ca cluster, which catalyzes water oxidation (4).

We are interested in understanding how PSII is assembled from its individual components. Current models suggest a stepwise assembly in both cyanobacteria and chloroplasts involving distinct intermediates (8–10). However, as with other membrane protein complexes, detailed analysis of PSII assembly complexes is hindered by their low abundance in the membrane, and until now, early assembly intermediates of PSII have not been isolated and biochemically characterized.

Here, we describe the isolation of the CP47 and CP43 subunits from the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803). These PSII subunits each contain six transmembrane α-helices and, in the cyanobacterial PSII holoenzyme, bind *Chl* *a* (16 molecules in CP47 and 13 in CP43) and *β*-carotene (4). They lie on either side of the heterodimeric D1/D2 reaction center complex involved in light-induced transmembrane charge separation, and one of their roles is to act as an inner light-harvesting antenna system (11). CP43 is also involved in ligating the Mn4Ca cluster (3). CP47 and CP43 are tightly bound components of the larger PSII core complex, and relatively harsh treatments are required to remove CP47 and CP43 *in vitro* (12, 13).

It is known that the apoproteins of both CP47 (encoded by the *psbB* gene) and CP43 (encoded by *psbC*) are synthesized and inserted into the thylakoid membrane before their incorporation into the PSII complex of *Synechocystis* 6803 (8). However, it remains unclear whether they are able to bind pigment molecules in this “unassembled” state (10). To address this...
issue, we describe here a novel approach for the isolation and characterization of CP47 and CP43 complexes from _Synechocystis_ 6803. Our results show that both CP47 and CP43 are likely to attach to neighboring low molecular mass (LMM) subunits before assembling into larger PSII complexes.

**EXPERIMENTAL PROCEDURES**

**Cyanobacterial Strains and Growth Conditions**—The glucose-tolerant strain of _Synechocystis_ 6803 (14) and the _pbaA_ triple deletion strain, TD41 (15), were used in this work. For clarity, TD41 will be referred to here as ΔD1. Strains were grown in liquid BG-11 mineral medium and maintained on solid BG-11 plates containing 1.5% (w/v) agar, both containing 5 mM TES-KOH (pH 8.2) at a light intensity of 40 or 5 microeinsteins m⁻² s⁻¹ of white fluorescent light, respectively, and at 29 °C. The medium was supplemented with 5 mM glucose and, where applicable, chloramphenicol (30 μg ml⁻¹), erythromycin (10 μg ml⁻¹), gentamycin (2 μg ml⁻¹), kanamycin (50 μg ml⁻¹), or spectinomycin (50 μg ml⁻¹).

**Construction of Mutants**—To generate the His-tagged CP47 strain (ΔD1/CP47-His/ΔDftsH2), the gentamycin resistance cassette of plasmid pCP47His-tagGmR (16) was removed by BamH1 digestion. After blunting the ends, an erythromycin resistance cassette was introduced to generate pCP47His-tagEryR. Ultimately, this plasmid was transformed into the ΔD1 mutant strain (15) with the plasmid used to produce the Syn0228GENT strain described previously (17). A control strain expressing His-tagged CP47 in a WT background (strain FtsH2), the gentamycin resistance cassette of plasmid pCP47His-tagGmR (16) was removed by BamH1 digestion. After blunting the ends, an erythromycin resistance cassette was introduced to generate pCP47His-tagEryR. Ultimately, this plasmid was transformed into the ΔD1/ΔDftsH2 strain, which had been generated by transforming the ΔD1 mutant strain (15) with the plasmid used to produce the Syn0228GENT strain described previously (17). A control strain expressing His-tagged CP47 in a WT background (strain PSII-His) was generated by transforming the WT with pCP47His-tagEryR. To generate the His-tagged _Synechocystis_ 6803 mutant strain (ΔD1/CP47-His), a His₄₆-coding sequence was introduced at the 3′-end of the _psbC_ gene (sll0851) by overlap extension PCR using the following primers: CP43 +1000-Fw, 5′-ATATTATCCCTCCCTTCTGTAAGG GTGC-3′; CP43 +1000-Rev, 5′-CTGCGCATTAAAGAATTG GCTAAGGACGCGTC-3′; CP43HT-Fw, 5′-CATCATCAT CATCATCATTAGATGAGACTTTACTTGTGAAC-3′; and CP43HT-Rev, 5′-TGTGAAGTCAGCATGAAACCC-3′. The overlap extension PCR product was cloned into the pGEMEasy vector (Promega), and an erythromycin resistance cassette was introduced at an XmnI site 170 bp downstream of the stop codon of the gene. The genotypes of the mutants were verified by PCR analysis using gene-specific primers.

**Isolation of CP47 and CP43**—The Chl a content of samples was determined by extraction into methanol and absorption measurements at 666 and 750 nm (20). Protein samples were analyzed by Blue native (BN)-PAGE and SDS-PAGE as described previously (18, 19).

**Protein Analysis**—The Chl a content of samples was determined by extraction into methanol and absorption measurements at 666 and 750 nm (20). Protein samples were analyzed by Blue native (BN)-PAGE and SDS-PAGE as described previously (21). Unless stated otherwise, BN-8–12% (w/v) polyacrylamide and 18% (w/v) SDS-polyacrylamide gels containing 6 M urea were used. The resulting gels were either Coomassie Blue- or silver-stained (22) or electroblotted onto PVDF membrane using the iBlot system (Invitrogen) according to the manufac-
Isolation of CP47 and CP43

turer’s instructions. Immunoblot analyses were performed using specific primary antibodies and a horseradish peroxidase-conjugated secondary antibody (GE Healthcare). Signals were visualized using a SuperSignal West Pico chemiluminescence kit (Pierce). The primary antibodies used in this study were as follows: anti-CP43 (directed against *Chlamydomonas reinhardtii* PsbC, serum P6, kindly provided by B. Diner), anti-CP47 (directed against barley PsbB (residues 380–394)), anti-D1 (directed against the C-terminal peptide) (23), anti-D2 (directed against the C-terminal peptide) (24), anti-His tag (Invitrogen), and anti-PsbH (from *Synechocystis* 6803) (25).

Reverse-phase HPLC Pigment Analysis—Pigments were extracted into 80% (v/v) acetone/water at 4 °C under dim light conditions, and the sample was centrifuged at maximum speed for 1 min in a microcentrifuge to pellet precipitated proteinaceous material before injection. The injection volume was 20 μl, and the pigments were resolved using a Gemini 5-μm C6-phenyl 110-Å column (Phenomenex). The following program was run at a flow rate of 1 ml min⁻¹: 0–4 min at 100% system B, 4–20 min linear gradient from 100% system B to 40% system B and 60% system C, 20–24 min at 40% system B and 60% system C, 24–35 min at 100% system B, with system A being 65% (v/v) acetonitrile and 35% (v/v) water, system B being 90% (v/v) acetonitrile and 10% (v/v) water, and system C being 100% ethyl acetate. The run was monitored at 440 nm using a Jasco MD-2015 Plus diode array detector. Chl a and β-carotene quantification was performed after calibration with pigment standards (purchased from Sigma) of known concentrations.

Spectroscopic Analyses—CP43-His and CP47-His samples were diluted in buffer containing 20 mM BisTris·HCl (pH 6.5), 20 mM NaCl, 0.09% (w/v) β-DM, and 75% (v/v) glycerol. The samples for the absorption experiments were diluted to an absorbance of ~0.5 cm⁻¹ at the Q₅ maxima, whereas for the fluorescence experiments, an absorbance if ≤0.1 cm⁻¹ was used. The low temperature measurements were performed in an Utreks helium flow cryostat (5 K) or in an Oxford liquid nitrogen bath cryostat (77 K). Absorption spectra at 5 K were recorded in a home-built setup equipped with a 150-watt tungsten-halogen lamp, a monochromator, and a photodiode detector. The spectra were recorded using lock-in detection with 1-nm spectral resolution. Absorption spectra at 77 K were recorded in a PerkinElmer Lambda 40 UV-visible spectrophotometer with 1-nm spectral resolution. Fluorescence spectra at 77 K were recorded in a Jobin Yvon Fluorolog-3-11 fluorometer. The excitation wavelength was 488 nm (10-nm full-width half-maximum), and the spectral resolution was 1 nm. The fluorescence quantum yield was estimated by integrating the total fluorescence and comparing with the known value of the fluorescence quantum yield for higher plant CP43 at room temperature (19).

Mass Spectrometric Analysis—Low molecular mass proteins were identified using the methods described previously (26). For offline electrospray ionization MS, 20 μl of purified CP47-His and CP43-His complexes, respectively, were precipitated overnight in 80% (v/v) acetone/water at ~20 °C. After centrifugation at 13,000 × g for 10 min, the supernatant was discarded, and the pellet was air-dried. The dry pellet was dissolved in solution containing 70% (v/v) acetone, 19% (v/v) water, 10% (v/v) 2-propanol, and 1% (v/v) formic acid. Dissolved proteins were directly applied to a nanospray emitter. Mass spectra were obtained using a Waters Q-Tof Premier spectrometer equipped with a nanoelectrospray ionization source. For scanning LMM proteins, mass spectra at a mass range of 800–2500 m/z were acquired. Data were recorded at a capillary voltage of 0.8 kV and a cone voltage of 37 V. After MS data collection at a rate of 1 s/scan, scans were averaged. After acquisition of fragment ion spectra at a collision energy between 26 and 40 eV, data were analyzed using MassLynx/Biolynx 4.1 software. Sequence tags obtained from the fragment spectra were used for similarity search (EMBL-EBI Data Bank). The samples were analyzed in several repetitions.

RESULTS

Isolation of CP47-His and CP43-His from *Synechocystis* 6803—The low abundance of PSII assembly intermediates in WT *Synechocystis* 6803 is a major barrier to their isolation. We have adopted a 3-fold approach to aid purification of unassembled CP47 and CP43 complexes. First, we incorporated His₆-tags at the C terminus of each protein to allow purification by immobilized metal affinity chromatography. Previous work has already shown that the presence of His-tags at these positions does not prevent assembly of active PSII (27, 28). Second, we blocked assembly of the PSII holoenzyme using a parental strain, ΔD1, which is unable to make the D1 reaction center subunit (8, 15), and finally, in the case of CP47, we also inactivated the membrane-bound FtsH2 protease (Slr0228), which leads to elevated levels of unassembled PSII subunits, including the CP47 apoproteptide, in the ΔD1 strain (17). Construction and validation of the ΔD1/CP47-His and ΔD1/CP47-His/ΔFtsH2 strains are described under “Experimental Procedures.”

The CP47-His and CP47-His complexes were successfully purified from detergent-solubilized thylakoid membranes by immobilized metal affinity chromatography (supplementary Fig. 1). Small amounts of residual high molecular mass contaminants were removed by a subsequent size exclusion chromatography step (Fig. 1, A and B). Both CP47-His and CP43-His were largely monodisperse as assessed by BN-PAGE (Fig. 1C).

Protein Composition—SDS-PAGE combined with immunoblot analyses confirmed the purity of each preparation and the presence of His-tagged CP47 and CP43 (Fig. 1D). In the case of CP47-His, we were also able to detect the presence of PsbH, a neighboring low molecular mass subunit in the PSII holoenzyme (Fig. 1D). Co-purification of PsbH and CP47-His was further demonstrated by two-dimensional BN-PAGE (supplemental Fig. 2). Together, these data confirm earlier conclusions that CP47 and PsbH are capable of forming a complex at an early stage in PSII assembly (25). Interestingly, despite the absence of D1, the D2 reaction center subunit, which interacts with CP47 in the holoenzyme, was also detected in the CP47-His complex, albeit at substoichiometric levels (Fig. 1D). Analysis by mass spectrometry confirmed the presence of PsbH and also identified PsbL and PsbT (Table 1). In the case of CP43-His, low levels of a CP43 degradation product were detected by immunoblotting (designated by an asterisk in Fig. 1D) (data not
shown), and the PsbK and Psb30/Ycf12 subunits were identified by mass spectrometry (Table 1).

Pigment Composition—A notable feature of both the CP47-His and CP43-His complexes was the presence of bound pigment. Reverse-phase HPLC pigment analyses confirmed the presence of both Chl $a$ and $\beta$-carotene in CP47-His and CP43-His complexes (supplemental Fig. 3), with Chl/carotenoid ratios of $\sim$7.7 for CP47-His and 5.0 for CP43-His. The wavelengths of maximum absorption in the red region ($\lambda_{max}$) measured at room temperature were 674 nm for CP47-His and 671 nm for CP43-His (Fig. 2A), close to the values reported for CP47 and CP43 isolated by fragmentation of larger complexes (13). The relative amplitudes of the Soret and carotenoid absorption bands in the room temperature absorption spectra for CP47-His and CP43-His were also quite similar to their spinach counterparts (Fig. 3), consistent with similar Chl/carotenoid ratios in each type of complex.

Absorption at 5 K and Comparison with Spinach—To assess the optical properties of the pigments bound to CP47-His and CP43-His in more detail, we measured low temperature
Absorption spectra at 5 K and compared them with those of the widely studied CP47 and CP43 complexes isolated by detachment from larger spinach PSII core complexes (Fig. 2B) (29–32). In the Chl Soret region, the spectral shape is almost identical for both inner antenna complexes, with two bands at 416 and 436 nm identical in value to their spinach counterparts. The carotenoid absorption bands for CP47-His are at 469 and 502 nm (467 and 502 nm for spinach CP47), and those for CP43-His are at 467 and 499 nm (462 and 497 nm for spinach CP43). Fig. 2B (inset) shows an enlargement of the Chl Q<sub>y</sub> absorption region. For CP47-His, the absorption spectrum showed two bands at 668 and 676 nm and a shoulder around 683 nm. The absorption red tail extends to 700 nm, suggesting the presence of a spectral component in the red edge of the spectrum (also observed for spinach at 690 nm). Overall, when comparing CP47-His from *Synechocystis* 6803 with spinach CP47, the spectral shapes show some differences in positions and relative amplitude, such as stronger and weaker absorptions for the cyanobacterial inner antenna protein around 667 nm and in the red edge, respectively. For CP43-His, the absorption spectra show a broad maximum at 668.5 nm, two shoulders at 673 and 679 nm, and a narrow band at 684.5 nm. The absorption spectra of the spinach CP43 inner antenna (19) display a very similar spectral shape, the main difference between the two organisms being the 2.5-nm red shift of the narrow band at 684.5 nm in *Synechocystis* 6803 with respect to the 682 nm narrow band in spinach.

**Absorption and Fluorescence at 77 K and Comparison with Spinach**—Fig. 4 shows the absorption and fluorescence spectra at 77 K normalized to a value of 1 at the absorption and fluorescence maxima for CP47 (Fig. 4A) and CP43 (Fig. 4B) from *Synechocystis* 6803 and spinach to facilitate the comparison of their respective spectral shapes. The second derivatives of the absorption (multiplied by −4) are displayed to identify the spectral components present in the spectra. For CP47, the second derivatives of the absorption spectra show differences at wavelengths shorter than 675 nm, with maxima at 658.5 and 660 nm and 670.5 nm for spinach. At wavelengths longer than 675 nm, the peak positions at 676.5 and 682.5 nm are the same. However, the red edge is less pronounced in cyanobacterial CP47-His, which suggests that, although the 682.5 nm band is at the same position in both organisms, this band is narrower in *Synechocystis* 6803. This could be an indication of a more “defined” protein environment or a lower degree of electron-phonon coupling (coupling of the electronic transitions to protein lattice vibrations, which induce broadening of the absorption) in CP47-His. The fluorescence spectrum also shows a narrower and blue-shifted spectral distribution in *Synechocystis* 6803 with respect to spinach, which indicates that the 682.5 nm pigment(s) are emitting states with large contributions to the overall fluorescence spectrum and that the red chlorophyll (peaking around 690 nm in spinach) gives a smaller contribution. However, a “red” chlorophyll is probably also present in *Synechocystis* 6803, in view of the shoulder at around 690 nm in the 77 K emission spectrum. For CP43, the second derivatives confirm that the narrow red band is shifted from 682 nm in spinach to 684.5 nm in *Synechocystis* 6803 and that the other absorption bands peak at the same wavelengths in both organisms. The 2-nm red shift is also observed in the fluorescence spectra (which peak at 682 nm in spinach and 684 nm in *Synechocystis* 6803), indicating that the pigment(s) giving rise to the narrow absorption bands are states
have addressed a very basic question: at what stage can chlorophyll and carotenoid molecules be inserted into the inner antenna proteins CP47 and CP43 of PSII? To address this, we have generated His-tagged strains of *Synechocystis* 6803 to allow rapid isolation of unassembled CP47-His and CP43-His protein complexes from the membranes of a ΔD1 deletion strain. In the case of CP47, we also exploited the observation that the levels of unassembled CP47 increase from 10 to 20% of WT levels to 100% of WT levels when the FtsH2 thylakoid protease is inactivated (17). Thylakoid FtsH proteases appear to play an important role in removing unassembled protein subunits (17). Consequently, the use of FtsH mutants might be a useful strategy to increase levels of other unstable complexes or assembly complexes in the thylakoid membrane in both cyanobacteria and chloroplasts.

Our results indicate that the isolated His-tagged CP47 and CP43 complexes contain a very similar set of chlorophyll and carotenoid pigments compared with spinach CP47 and CP43 isolated by fragmentation of larger core complexes. Evidence comes from the close similarity in spectral shape and relative amplitudes in the absorption spectra in the visible range from 400 to 750 nm between the *Synechocystis* 6803 complexes and spinach PSII core complexes (Fig. 3) as well as similarities in low temperature absorption and fluorescence spectra (Figs. 2 and 4). Thus, we can conclude that the insertion of pigment into CP47 and CP43 in *Synechocystis* 6803 is able to occur before their incorporation into a larger PSII complex, at least in the PSII assembly mutants studied here. Previous work has shown that the availability of Chl *a* is important for the synthesis and accumulation of full-length CP47 apoprotein (33, 34) and that absence of carotenoid leads to impaired synthesis of CP47 and especially of CP43 (35). Also, accumulation of CP47 and CP43 is severely impaired in site-directed mutants lacking an appropriate amino acid ligand to chlorophyll (36, 37). These data therefore suggest that binding of pigment starts to occur at an early stage in the assembly of CP47 and CP43 in the WT, perhaps even cotranslationally during insertion of the apoproteins into the membrane to help stabilize the complex. A close synchronization between pigment binding and the synthesis and folding of CP47 and CP43 would also help to minimize potential toxic effects of free chlorophyll in the membrane (38). Whether all or only a fraction of the chlorophyll molecules bind to CP47 and CP43 at this early stage of PSII assembly in the WT is unclear. How chlorophyll and carotenoid are presented to the CP47 and CP43 apoproteins is also currently unknown, although there is some suggestion that enzymes involved in chlorophyll biosynthesis might be docked onto the thylakoid membrane (39).

The precise Chl/carotenoid ratio to be expected for CP47-His and CP43-His is still not clear. In the most recent structure, 35 Chl *a* molecules are present per PSII monomer, with 16 molecules bound to CP47 and 13 to CP43 (4). However, the assignment of carotenoids within the PSII structural models has proved to be more difficult. With improved resolution, the number has increased from 7 (3) to 11 (5) and most recently to 12 molecules (4). Analysis by Mühl *et al.* (40) concluded that five carotenoids are associated with CP47 and four with CP43. However, many of these carotenoids are located at the inter-

**FIGURE 4. Comparison of the low temperature absorption and fluorescence spectra in the Qy band region between the CP47 and CP43 inner antenna proteins of *Synechocystis* 6803 and spinach.** Absorption and fluorescence spectra for CP47-His (A) and CP43-His (B) from *Synechocystis* 6803 and for CP47 and CP43 from spinach were recorded at 77 K and normalized to 1 at the absorption and fluorescence maxima. Second derivatives of absorption spectra (2nd Der Abs) are also shown.
faces between subunits, such as between CP43 and PsbK/PsbZ
(41), and at the interface of the two monomers between CP47
and D1-PsbT (40). The Chl/carotenoid ratios determined here
(7.7 for CP47-His and 5.0 for CP43-His) are much greater than
those predicted by Müh et al. (40) (3.2 for CP47 and 3.25 for
CP43) and might reflect absence of carotenoid in the smaller
assembly complexes described here and/or loss of carotenoid
during purification.

Comparison of the low temperature absorption and fluores-
cence spectra of the inner antenna proteins isolated from Syn-
echocystis 6803 and spinach shows that the spectral distribution
of absorption bands is similar but not identical in both organ-
isms. Regarding the functionality of the antenna complexes
with respect to their efficiency of excitation energy transfer to
the reaction center, the fluorescence quantum yields for CP47-
His and CP43-His were calculated and found to be generally
lower than those for their spinach counterparts. The differ-
ences may be of several origins. One explanation might derive
from the fact that the Synechocystis 6803 antenna complexes
are assembly complexes isolated at a stage preceding their
assembly into the core complex (not isolated from the core
complex, as in the case of spinach). This implies that the energy
absorbed by the antenna protein pigments is not “meant” to be
used by the reaction center for photochemistry and that there
might therefore be an intrinsic energy dissipation pathway,
possibly mediated by LMM PSII subunits. Alternatively, the
assembly complexes could be in a protein conformation that is
not favorable for efficient excitation energy transfer (because it
is not needed), which might change to an optimized conforma-
tion once the complexes are integrated into the core complex.
Another possibility is that the differences may arise from vari-
atations in the macrostructure of the inner antenna systems
between the two organisms and sequence differences between
them. In spinach, the core antennas play a role in transferring
excitation energy absorbed by other peripheral antennas: the
minor antennas CP24, CP26, and CP29 and the main light-
harvesting complex II. In Synechocystis 6803, a role as an “inter-
mediary” in excitation energy transfer could be bypassed by the
presence of the dynamic phycobilisomes, which can funnel
excitation energy directly to the reaction center. Therefore,
the requirement of highly efficient excitation energy transfer
would be less of a determinant in Synechocystis 6803 than in spinach.

We note that these possibilities are not mutually exclusive and
that we cannot conclude from the available data which possi-
bility or possibilities are the right one(s). To shed light on these
issues, it will be necessary to perform further comparative spec-
troscopic studies between assembly complexes and complexes
isolated from the core complex.

An additional novel aspect of our work is the detection of
LMM subunits in the isolated CP43-His and CP47-His com-
plexes. Although our data show that these subunits are capable
of binding to unassembled CP47 and CP43, additional evidence
is required to confirm that such complexes also form in the WT
strain and are not merely “dead-end” complexes that only accu-
mulate in assembly-defective mutants. For instance, we have
shown here that PsbH is capable of forming a complex with
CP47-His (Fig. 1 and supplemental Fig. 2). Low levels of a CP47-
PsbH complex have also been detected in WT thylakoids (25),
and accumulation of full-length unassembled CP47 apoprotein
is impaired in a PsbH-null mutant (25). Together, these
data suggest a stabilizing effect of PsbH on CP47 accumulation
during the early steps of PSII assembly in the WT (25). The
small chlorophyll-a/b-binding-like protein ScpD can also bind
to CP47 in the vicinity of PsbH (42), but ScpD is not expressed
under the illumination conditions used here (data not shown),
which would explain its absence in the CP47-His complex.
Overall, the currently available data suggest that the low level of
unassembled CP47 found in the WT thylakoid membrane
exists predominantly as a CP47-PsbH complex and can include
ScpD under high light conditions. Importantly, we were able to
detect the co-purification of PsbT and PsbL with CP47-His.
These LMM subunits are located next to CP47 in the PSI
holoenzyme and lie at the interface of the two monomers in the
dimeric complex. Whether PsbT and PsbL are present at stoi-
chiometric levels in the CP47-His complex and attach to CP47
early in assembly in the WT is presently unclear.

CP43 has been detected in two distinct complexes (CP43a
and CP43b) in solubilized thylakoid membrane extracts of Syn-
echocystis 6803, so it is likely that CP43 also binds LMM sub-
units at an early stage of PSII assembly in the WT (8). Based on
the PSI crystal structures, three LMM subunits might form a
stable complex with CP43: PsbK, PsbZ, and Psb30/Ycf12 (3–5).
Using mass spectrometry, we have been able to identify PsbK in
the CP43-His complex. A tight association between PsbK and
CP43 has previously been documented in fragmentation stud-
ies of PSII core complexes (39), and the early binding of PsbK to
CP43 during assembly has been inferred from analysis of Chla-
mydomonas PSIII mutants (43). In addition, we have identified
a LMM polypeptide in the His–CP43 complex containing a short
sequence match with Psb30/Ycf12 (Table 1). Hence, we assign
Psb30/Ycf12 as an additional component of the CP43-His com-
plex. Whether PsbZ is also a component of the CP43-His com-
plex remains to be clarified.

In conclusion, we have described the first isolation and char-
acterization of unassembled CP47 and CP43 subunits from the
thylakoid membrane. Our experimental results indicate that
CP47 and CP43 are capable of forming preassembled pigment-
protein complexes containing neighboring LMM subunits
found in the holoenzyme. On the basis of our data here and
earlier results, we propose a modular assembly of PSII in which
D1–PsbI (44), D2–cytochrome b593 (8), CP47–PsbH–PsbL–PsbT,
and CP43–PsbK–Psb30 subcomplexes, the latter possibly includ-
ing PsbZ, are combined together to form first a PSII reaction
center-like complex, then the RC47 complex (a PSI core
complex lacking CP43), and finally a monomeric core complex
(reviewed in Ref. 10). Subsequent formation of oxygen-evolving
PSII would require light-driven assembly of the Mn$_6$Ca cluster
and attachment of the extrinsic proteins.

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