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The PsbQ protein defines cyanobacterial Photosystem II complexes with highest activity and stability

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Light-induced conversion of water to molecular oxygen by Photosystem II (PSII) is one of the most important enzymatic reactions in the biosphere. PSII is a multisubunit membrane protein complex with numerous associated cofactors, but it continually undergoes assembly and disassembly due to frequent light-mediated damage as a result of its normal function. Thus, at any instant, there is heterogeneity in the subunit compositions of PSII complexes within the cell. In particular, cyanobacterial PSII complexes have five associated extrinsic proteins, PsbO, PsbP, PsbQ, PsbU, and PsbV. However, little is known about the interactions of the more recently identified PsbQ protein with other components in cyanobacterial PSII. Here we show that PSII complexes can be isolated from the cyanobacterium *Synechocystis* sp. PCC 6803 on the basis of the presence of a polyhistidine-tagged PsbQ protein. Purification of PSII complexes using a tagged extrinsic protein has not been previously described, and this work conclusively demonstrates that PsbQ is present in combination with the PsbO, PsbU, and PsbV proteins in cyanobacterial PSII. Moreover, PsbQ-associated PSII complexes have higher activity and stability relative to those isolated using histidine-tagged CP47, an integral membrane protein. Therefore, we conclude that the presence of PsbQ defines the fully assembled and optimally active form of the enzyme.

membrane protein complex | oxygen evolution | photosynthesis | *synechocystis*

The oxygen in the earth's atmosphere is largely produced by oxygenic photosynthetic organisms, which use light energy to convert water into molecular oxygen and to provide reducing equivalents for carbon fixation. The enzyme that catalyzes the water-oxidation reaction is Photosystem II (PSII), a protein complex found in the thylakoid membranes of cyanobacteria and chloroplasts. PSII is composed of numerous components, including >20 protein subunits, both membrane intrinsic and extrinsic, as well as redox active cofactors such as chlorophyll (Chl), pheophytin, quinone, manganese, calcium, chloride, iron, and heme groups. All of these components are intricately arranged within the PSII complex for optimal activity.

Recent structural analyses have provided new insights into the arrangement of the PSII subunits within the complex (1–4). However, PSII is a dynamic protein complex that is constantly irreversibly damaged as a consequence of its normal functioning. The majority of the damage is localized on one protein, D1, which must be proteolytically removed from the complex and replaced with a newly synthesized copy (reviewed in refs. 5 and 6). During this damage-and-repair cycle, the components of the PSII complex must dissociate during the degradation phase and then reassociate during the biosynthesis phase of the cycle. Despite the presence of a repair machinery, the PSII complexes within the thylakoid membranes are a mixture of populations in different states of assembly and disassembly. Although the crystallographic models represent a fraction of the PSII complexes within the thylakoid membrane, they do not provide the complete picture of different forms of PSII.

One area of intense debate on cyanobacterial PSII structure involves the extrinsic proteins associated with the water oxida-

tion machinery. Five extrinsic proteins, PsbO, PsbP, PsbQ, PsbU, and PsbV, have been shown to copurify with PSII complexes isolated from *Synechocystis* sp. PCC 6803 (*Synechocystis* 6803) using a histidine-tagged version of the large integral membrane protein CP47 (7, 8). However, only the PsbO, PsbU, and PsbV proteins have been observed in the structural studies (1–3). The more recently identified PsbP and PsbQ proteins have been shown to play a role in the regulation and stabilization of the water oxidation complex in PSII (8–12), but it is not clear how they associate with PSII complexes with respect to the other extrinsic proteins. Although one PsbQ protein is proposed to be associated with each PSII complex, the relative abundance of PsbP in PSII is a matter of debate (8, 11, 12). Also, the PsbP and PsbQ proteins are likely to have lipid modifications at their N termini that anchor them to the membrane more tightly than the other extrinsic proteins (8, 9). Therefore, many questions remain about how and when the PsbP and PsbQ proteins are incorporated into cyanobacterial PSII complexes.

To further investigate cyanobacterial PsbQ, we took a direct approach to isolate and characterize the population of PSII complexes containing the PsbQ protein. Using a strain of *Synechocystis* 6803 that expresses a histidine-tagged version of the PsbQ protein, we show it is possible to purify active PSII complexes. Analysis of the PsbQ-associated PSII complexes revealed that PsbQ is present in addition to the PsbO, PsbU, and PsbV proteins and confers enhanced activity and stability to the complex. We propose that the PsbQ-associated PSII complexes represent the fully functional form of cyanobacterial PSII, whereas the PSII complexes isolated using a histidine-tagged CP47 protein are a more heterogeneous mixture of different populations of PSII.

Results

The QHis strain, which expresses a polyhistidine-tagged version of the cyanobacterial *psbQ* gene, was generated in *Synechocystis* 6803 to facilitate isolation of PSII complexes containing the PsbQ protein. The construct was designed such that the native promoter of the *psbQ* gene and the next downstream gene (*ureD*) remained intact. A sequence coding for octa-histidine was added to the 3' end of the *psbQ* gene to produce a C-terminal histidine-tagged expressed PsbQ protein. A gentamycin resistance marker was also introduced between the *psbQ*-histidine tag fusion and *ureD* genes, for selection purposes (Fig. 1A). Because *Synechocystis* 6803 contains multiple copies of its genome per cell, complete segregation of the mutant construct at the *psbQ* locus was verified by PCR analysis (Fig. 1B). The QHis strain

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Abbreviations: *Synechocystis* 6803, *Synechocystis* sp. PCC 6803; Chl, chlorophyll; PSII, Photosystem II.

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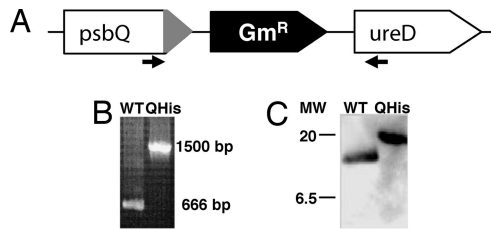


Fig. 1. Construction of the QHis strain and segregation analysis. (A) *psbQ* locus of the QHis strain. The *psbQ* gene, which contains additional sequence at its 3' end encoding an octa-histidine tag (gray), is followed by a downstream gentamycin resistance marker and the next downstream gene, *ureD*. The arrows show the location of the primers used for segregation analysis. (B) PCR segregation analysis of WT and QHis cells. The sizes of the products in base pairs (bp) are shown on the right. (C) Immunodetection of the PsbQ protein in WT and QHis cells. The sizes and positions of the molecular mass standards are shown on the left.

results in a larger PCR product due to the insertion of the gentamycin resistance marker (834 bp). Immunodetection showed that QHis cells express a PsbQ protein with a larger molecular weight (Fig. 1C), because of the presence of an octa-histidine tag.

The N terminus of the plant PsbQ protein contains a number of features critical for its association with PSII (13–15). Because few of these elements are conserved in cyanobacteria (13), it is difficult to predict how PsbQ associates with cyanobacterial PSII. However, cyanobacterial PsbQ has hydrophobic properties conferred by a lipid anchor at its N terminus (9). Structural work has shown that the C terminus is far from the face of PsbQ that is predicted to interact with PSII in plants (13, 14). Therefore, we reasoned that the presence of a C-terminal histidine tag should not affect the association and function of PsbQ within the PSII complex.

Photosynthetic activity, as measured by the rates of photoautotrophic growth and oxygen evolution, in QHis cells was compared with that of WT and $\Delta psbQ$ cells to determine the effect of the C-terminal histidine tag on PsbQ function. No differences in photoautotrophic growth or oxygen evolution activity were observed between WT and the QHis strain under nutrient replete conditions (Fig. 2). Because the $\Delta psbQ$ mutant displays photosynthetic defects on depletion of Ca^{2+} or Cl^{-} from the medium (8, 10), further analysis of oxygen-evolution activity in the QHis strain was conducted in Ca^{2+} -deficient medium (Table 1). Although the $\Delta psbQ$ mutant showed reduced activity relative to WT, the QHis strain evolved oxygen at rates comparable to WT. Also included for comparison is the HT3 strain, which contains a histidine-tagged version of the CP47 membrane protein and is frequently used for isolation of PSII complexes from *Synechocystis* 6803 cells. Based on these results, the C-terminal histidine tag did not appear to alter the function of PsbQ in the QHis mutant.

Cyanobacterial PSII complexes have usually been isolated by using a histidine-tagged version of the membrane protein CP47 and metal-affinity chromatography (7, 16). In the present study, the histidine-tagged PsbQ protein was used to purify PSII complexes from the QHis strain. Solubilized QHis membranes were applied to a Ni^{2+} affinity column, Chl absorbance was monitored at 436 nm throughout the procedure, and PsbQ-associated complexes were eluted by a buffer containing excess histidine (Fig. 3A). The elution peak was confirmed to contain PSII by 77 K fluorescence measurements, which showed PSII-characteristic peaks at 683 and 691 nm (Fig. 3B). Purification of PSII complexes by a virtue of a tagged extrinsic protein has not been previously described, and our results demonstrate that PsbQ is a bona fide subunit of cyanobacterial PSII.

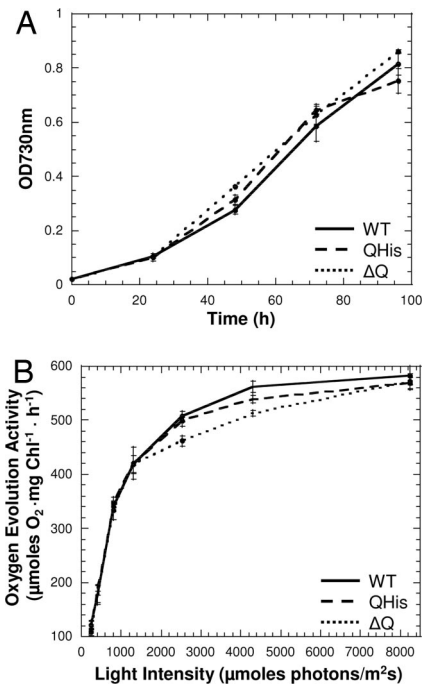


Fig. 2. Photosynthetic activity of the QHis strain. (A) Photoautotrophic growth of WT, QHis, and $\Delta psbQ$ cells in BG11 medium. Error bars represent the standard error of the mean ($n = 3$). (B) Oxygen-evolution activities of WT, QHis, and $\Delta psbQ$ (ΔQ) cells in BG11 medium. Error bars represent the standard error of the mean ($n = 3$).

The PsbQ-associated PSII complexes were then compared with PSII complexes isolated by using a histidine-tagged CP47 protein. The protein profiles of PsbQ- and CP47-tagged PSII complexes were very similar (Fig. 4). As expected for the PsbQ-tagged PSII complexes, the WT PsbQ protein was absent, and the histidine-tagged PsbQ with a higher apparent molecular mass was present. Notably, the Psb27 protein was absent from PsbQ-associated PSII complexes. This 11-kDa extrinsic protein has been implicated as a factor in PSII biogenesis (17–19), and this result suggests that Psb27 cannot bind to PSII complexes that contain the PsbQ protein.

Previously, we have shown that PSII complexes that lack the PsbQ protein showed a partial loss of the PsbV protein (cytochrome c_{550}) (9). Although the PsbQ protein is absent in the current PSII models (1–3), this observation indicated a structural link among these proteins, in which the PsbQ protein stabilizes PsbV within the complex. Therefore, we hypothesized that the population of PsbQ-associated PSII complexes would also retain more PsbV protein relative to the CP47-tagged PSII complexes. To test this hypothesis, the molar ratios of cytochrome c_{550} in PsbQ- and CP47-tagged PSII complexes were compared (Table 2). Indeed, the PsbQ-tagged PSII complexes retained more PsbV than the CP47-tagged PSII complexes. Also shown for comparison are a number of other CP47-tagged PSII complexes, which lack one or more extrinsic proteins. In agreement with the previous study that showed that PsbV is destabilized in the absence of PsbQ (9), the $\Delta psbQ$ /CP47-tagged PSII complexes retained less PsbV than CP47-tagged complexes. Treatment of PSII samples with 1 M $CaCl_2$ results in the release of the extrinsic proteins PsbO, PsbU, and PsbV, and consequently both the $CaCl_2$ -washed CP47-tagged and PsbQ-tagged PSII samples showed a further reduction in cytochrome c_{550} content. No cytochrome c_{550} signal was observed in the $\Delta psbV$ /CP47-tagged complexes isolated from a strain in which *psbV* was genetically deleted.

Table 1. Photosynthetic activity of cells in the presence and absence of calcium

Light intensity, μmol of photons per m^2 per s	BG11				BG11 $-\text{Ca}^{2+}$			
	WT	ΔpsbQ	QHis	HT3	WT	ΔpsbQ	QHis	HT3
8,250	604 \pm 14	541 \pm 12	558 \pm 12	638 \pm 3	401 \pm 7	263 \pm 9	388 \pm 7	345 \pm 9
2,525	537 \pm 7	473 \pm 6	500 \pm 2	585 \pm 2	369 \pm 7	190 \pm 7	324 \pm 17	338 \pm 9
800	380 \pm 13	336 \pm 10	364 \pm 9	399 \pm 8	255 \pm 3	152 \pm 3	224 \pm 9	242 \pm 7

Oxygen evolution rates are given in units of μmol of $\text{O}_2 \cdot \text{mg}^{-1}$ of $\text{Chl} \cdot \text{h}^{-1} \pm$ the standard error of the mean ($n = 3$).

The extrinsic proteins of PSII enhance the stability of the four manganese atoms that comprise the catalytic core of the oxygen-evolving center of PSII (20). Because of the enhanced stabilization of the PSII extrinsic proteins in the PsbQ-associated complexes, the abundance of Mn associated with these PSII complexes was also determined (Table 2). In this analysis, the PsbQ-tagged PSII complexes had increased Mn content relative to the CP47-tagged PSII complexes, whereas the $\Delta\text{psbQ}/\text{CP47}$ and $\Delta\text{psbV}/\text{CP47}$ -tagged PSII samples showed decreases in the amount of associated Mn. As with the cytochrome measurements, when the extrinsic proteins are removed by 1 M CaCl_2 treatment, the manganese atoms are also released in both the CP47- and PsbQ-tagged PSII samples. This result, along with the above data, highlights the role of PsbQ in the stability of the oxygen-evolving center on the luminal side of PSII.

The above results indicate that a small percentage of CP47-tagged PSII complexes are not fully assembled, thereby resulting in lower amounts of PsbV and Mn on a per-Chl basis. On the

other hand, the PsbQ-tagged PSII complexes represent a population enriched for fully assembled complexes. Notably, the yield of PsbQ-tagged PSII complexes from our purification procedure is $\approx 25\text{--}30\%$ that of CP47-tagged PSII on a per-Chl basis. Thus, the PsbQ-associated PSII complexes represent a smaller subpopulation of CP47-containing PSII complexes.

Differences in the protein and cofactor contents between the CP47- and PsbQ-tagged PSII complexes suggested possible changes in activity or stability between these two types of PSII. Indeed, the PsbQ-tagged PSII complexes had higher rates of oxygen-evolution activity compared with CP47-tagged PSII (Fig. 5). This result is also consistent with the PSII population hypothesis, in that PsbQ-associated PSII complexes have a higher activity than the average activity of the populations of PSII present in the CP47-tagged PSII sample. No difference in PSII activity was observed at the cellular level for the QHis strain (Fig. 2), which gives further evidence that the isolated PsbQ-tagged PSII is only a fraction of the total PSII in the cells. Thus, QHis cells also contain the small percentage of partially assembled PSII, but isolation of the PsbQ-associated complexes selects those complexes that are fully assembled.

Treatment with the small reductant hydroxylamine (NH_2OH) is an effective tool for assessing the stability of the oxygen-evolving center in PSII complexes (9, 21). If the oxygen-evolving center is

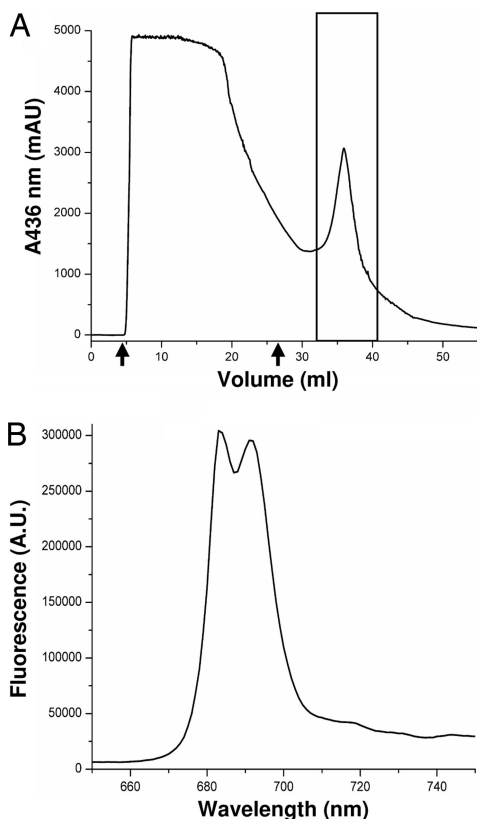


Fig. 3. Isolation of PSII from QHis cells. (A) Chromatogram of PsbQ-tagged PSII isolation. The first arrow designates the start of sample injection, and the second arrow indicates the start of sample elution. The pooled peak fractions are enclosed by a rectangle. (B) Fluorescence emission spectrum at 77 K for PsbQ-tagged PSII. Peaks are located at 683 and 691 nm.

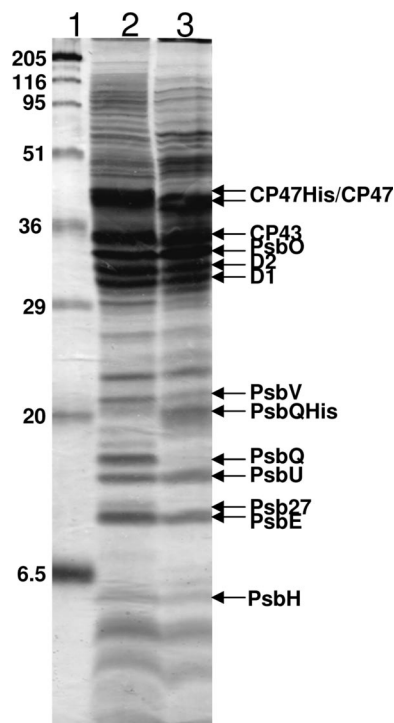


Fig. 4. Polypeptide compositions of isolated PSII. Each lane contained $5 \mu\text{g}$ of Chl-containing sample: (1) molecular mass standards, (2) CP47-tagged PSII, and (3) PsbQ-tagged PSII.

Table 2. Stoichiometry of PSII components

	CP47-tagged	PsbQ-tagged	$\Delta psbQ$ CP47-tagged	CaCl ₂ -washed CP47-tagged	CaCl ₂ -washed PsbQ-tagged	$\Delta psbV$ CP47-tagged
Mn:PSII*	3.63 ± 0.11	4.03 ± 0.08	2.13 ± 0.02	0.50 ± 0.04	0.4 ± 0.05	0.94 ± 0.01
Cyt <i>b</i> ₅₅₉ :PSII [†]	0.6	0.8	0.4	0.1	0.1	N/D

N/D, not detected.

*Molar ratio determined by atomic absorption spectroscopy.

[†]Molar ratio determined from optical difference spectra.

unstable, resulting in a more exposed manganese cluster, such PSII complexes are more susceptible to inactivation by NH₂OH. Thus, NH₂OH inactivation was used as a measure of PSII stability at the cellular and purified PSII complex levels. At the level of whole cells, $\Delta psbQ$ cells were the most susceptible to inactivation by NH₂OH, whereas WT, HT3, and QHis cells were similar in their response to NH₂OH (Table 3). However, at the level of isolated complexes, the CP47-tagged PSII complexes were inactivated to a greater extent by NH₂OH treatment relative to the PsbQ-tagged PSII complexes. As expected, $\Delta psbQ$ /CP47-tagged PSII complexes showed the most inactivation on NH₂OH treatment (Table 4 and ref. 9). Hence, the PsbQ-tagged PSII complexes are more stable than the CP47-tagged complexes. These data confirm the hypothesis that QHis cells contain the same distribution of partially assembled PSII complexes as WT or HT3 cells, but purification of complexes by association with the PsbQ protein enriches for fully assembled PSII complexes.

Discussion

Previous studies have shown that PsbQ copurifies with cyanobacterial PSII, and its absence results in defects in the water-oxidation machinery (7–10). The current study unequivocally proves that PsbQ is a component of cyanobacterial PSII. These findings challenge the current structural models of cyanobacterial PSII, which contain only the PsbO, PsbU, and PsbV proteins (1–3). Recently, a modeling study that compared electron density for plant PSII to the cyanobacterial structure revealed that the plant PsbP and PsbQ proteins appear to have binding sites distinct from that of the cyanobacterial PsbU and PsbV proteins on the luminal face of the complex (22). Although the modeling study demonstrates that the binding sites of each of the proteins do not mutually exclude each other, our data show that PsbQ is present in addition to PsbO, PsbU, and PsbV in cyanobacterial PSII complexes. Therefore, the current structural PSII models do not provide a complete picture of the luminal face of the complex in cyanobacteria, and future studies will need to address this issue.

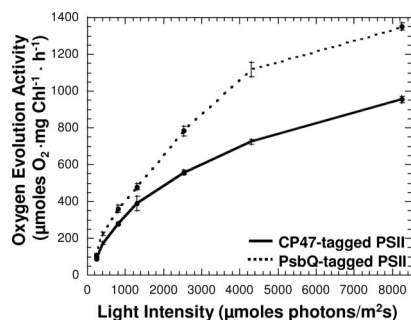


Fig. 5. Oxygen-evolution activity of CP47- and PsbQ-tagged PSII complexes as a function of light intensity. Error bars represent the standard error of the mean ($n = 3$).

The presence of PsbQ has noticeable consequences on the form and functions of the oxygen-evolving center of PSII. In comparison to the CP47-tagged PSII complexes, the PsbQ-tagged PSII retained more PsbV and manganese, resulting in higher activity and stability. These data demonstrate that PsbQ functions to stabilize the luminal components of cyanobacterial PSII. Furthermore, the data also highlight heterogeneity in the CP47-tagged PSII complexes. Although the majority of CP47-tagged complexes are fully functional, a small percentage of these complexes are not, which is consistent with analyses by others (19, 23). The PsbQ-associated PSII complexes represent a population of PSII enriched for fully assembled centers. Thus, the PsbQ-tagged PSII complexes have higher activity and stability than that of the average of the heterogeneous mixture of PSII complexes within the CP47-tagged PSII sample.

PSII is a highly dynamic protein complex, continually undergoing assembly and disassembly, and the details of this cycle are the focus of intense research (5, 6). Fig. 6 illustrates key steps in the PSII life cycle and places the various PSII subpopulations in a temporal context. The D1 protein, initially synthesized as a precursor protein (pD1) with a C-terminal extension, is incorporated into a precomplex containing D2 and cytochrome *b*₅₅₉ (5, 24, 25). The Psb27 protein transiently associates with PSII precomplexes before pD1 processing, manganese cluster assembly, and extrinsic protein association (18, 19). The pD1 protein is processed by the CtpA protease, and the CP47 and CP43 proteins are incorporated into the complex along with a number of other low-molecular-weight components (5, 26). Next, the manganese cluster is assembled, and the extrinsic proteins PsbO, PsbU, PsbV, and PsbQ bind to the luminal side of the complex (27). Finally, two monomers come together to form a PSII dimer. Upon damage of the D1 protein, the luminal side of PSII disassembles, and the damaged D1 protein is proteo-

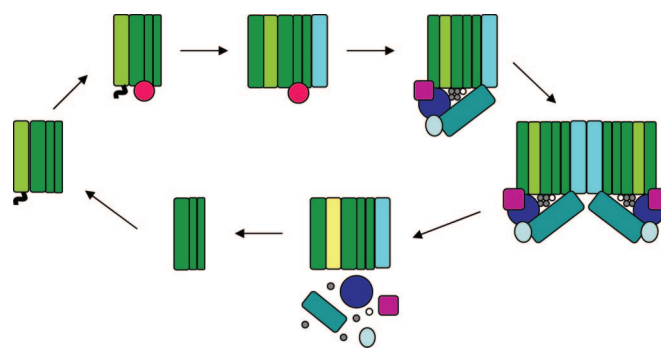


Fig. 6. Model of PSII biogenesis and repair. The PSII subunits are colored as follows: D1 (light green); D2, Cyt *b*₅₅₉, CP43 (dark green); CP47 (cyan); Psb27 (pink); PsbO (teal); PsbV (dark blue); PsbU (light blue); PsbQ (purple); and damaged D1 (yellow). The top half of the cycle represents steps in the synthesis half of the pathway resulting in fully assembled dimers on the far right, and the bottom half of the cycle shows the disassembly of the complex and removal of the damaged D1 protein. Refer to Discussion for further details.

Table 3. Accessibility of NH₂OH to PSII donor side in cells

NH ₂ OH, μ M	PSII activity*			
	WT	Δ psbQ	QHis	HT3
0	576 \pm 7 (100 \pm 1.3)	544 \pm 4 (100 \pm 0.7)	572 \pm 11 (100 \pm 1.9)	554 \pm 4 (100 \pm 0.7)
100	468 \pm 7 (81.3 \pm 1.3)	421 \pm 4 (77.5 \pm 0.7)	450 \pm 4 (78.6 \pm 0.6)	450 \pm 4 (81.2 \pm 0.6)
1,000	342 \pm 11 (59.3 \pm 1.9)	280 \pm 14 (51.7 \pm 2.6)	324 \pm 7 (56.6 \pm 1.3)	331 \pm 22 (59.7 \pm 3.8)

*Oxygen evolution rates are given in units of μ mol of O₂ · mg⁻¹ of Chl · h⁻¹ \pm the standard error of the mean ($n = 3$), and the values in parentheses indicate the percent activity \pm the standard error of the mean ($n = 3$) relative to that in the absence of NH₂OH for each strain.

lytically removed from the complex (28). The CP43 and CP47 proteins also dissociate to yield the receptor complex for insertion of the newly synthesized pD1 protein.

During the PSII life cycle, the CP47 protein is assembled into the complex earlier than the PsbQ protein. Accordingly, CP47 is associated with a small percentage of inactive complexes in addition to the fully assembled form, whereas PsbQ is associated with active complexes. In the present study, we show that PsbQ-associated PSII complexes represent an optimally functional form of PSII in cyanobacteria. Therefore, we conclude that the PSII assembly pathway in cyanobacteria culminates in the association of PsbQ, and that the presence of this protein defines complexes with the highest activity in the cell. Further analysis of the different PSII complex subpopulations may provide new insights into the PSII biogenesis pathway.

Materials and Methods

Cyanobacterial Culture Conditions. WT, Δ psbQ, Δ psbQHT3, Δ psbVHT3, HT3, and QHis strains of *Synechocystis* 6803 were grown in BG11 medium (29) at 30°C under 30 μ mol photons m⁻²·s⁻¹. The HT3, Δ psbQHT3, Δ psbVHT3, and QHis strains were grown in BG11 supplemented with 5 mM glucose and antibiotics as follows: 5 μ g/ml kanamycin (HT3)/10 μ g/ml spectinomycin/5 μ g/ml kanamycin (Δ psbQHT3)/1 μ g/ml gentamycin/5 μ g/ml kanamycin (Δ psbVHT3)/1 μ g/ml gentamycin (QHis) at 30°C under 50 μ mol photons m⁻²·s⁻¹ with air bubbling. The HT3 strain was provided by Terry M. Bricker (16). The Δ psbQ and Δ psbQHT3 strains were described previously (8). The Δ psbVHT3 strain was generated by transforming the HT3 strain with the Δ psbV construct (8).

QHis Mutant Construction. The *Synechocystis* 6803 *psbQ* gene was cloned into the pET-41b(+) vector (Novagen, San Diego, CA) in frame with a 3' sequence coding for an octa-histidine tag (8). The *psbQ-His* fragment was amplified and subcloned into the pUC18 vector using the NdeI and XbaI sites. A gentamycin resistance marker was inserted downstream from the *psbQ-His* fusion at the KpnI site. Finally, to ensure targeted double homologous recombination, 500 bp of *Synechocystis* 6803 DNA corresponding to the region 3' of *psbQ* was inserted downstream of the gentamycin resistance marker in the construct using the SacI and EcoRI sites. WT *Synechocystis* 6803 cells were transformed with the plasmid, and positive transformants were selected for growth on gentamycin. Complete segregation of the histidine-tagged version of *psbQ* was confirmed by PCR analysis of the *psbQ* locus.

PSII Purification. PSII from the HT3 and QHis strains were purified according to ref. 7 with modifications for use of an AKTA FPLC system (GE Healthcare, Little Chalfont, Buckinghamshire, U.K.). Solubilized membranes were injected onto a nickel-nitilotriacetic acid agarose column (Qiagen, Valencia, CA) preequilibrated with wash buffer containing 50 mM Mes-NaOH, pH 6.0/5 mM CaCl₂/10

mM MgCl₂/25% glycerol/0.04% β -dodecylmaltoside. After sample loading, the column was washed with three column volumes of wash buffer. PSII was eluted with wash buffer supplemented with 50 mM L-histidine, and 2-ml fractions were collected. Peak fractions corresponding to 10–20 ml after the start of the elution were pooled and precipitated with polyethylene glycol (7). All chromatography steps were performed at 0.3 ml per minute, and absorbance at 436 nm was monitored. PSII complexes were suspended in wash buffer. CaCl₂-washed PSII (1 M) was prepared as described in ref. 7.

Protein Analysis. Electrophoresis and immunodetection were performed by using a 18–24% gradient acrylamide 6 M urea SDS/PAGE system (30). For immunodetection, PsbQ was detected by using specific antiserum (8) after transfer onto a nitrocellulose membrane. Bands were visualized by using enhanced chemiluminescence reagents (WestPico; Pierce, Rockford, IL) on a Fujifilm LAS-1000 Plus imager (Fujifilm, Stamford, CT).

Cofactor Analysis. Chl *a* concentration was determined spectrophotometrically after extraction with methanol (31). The concentrations of manganese were measured on an AA600 atomic absorption spectrophotometer (PerkinElmer Life Sciences, Wellesley, MA). PSII samples were diluted to 5 μ g of Chl per milliliter in deionized water before analysis. The Mn:PSII ratio was calculated based on 41 molecules of Chl/PSII (7). The cytochrome content of PSII samples was measured by reduced minus oxidized (1 mM potassium ferricyanide minus excess sodium dithionite) difference absorption spectra on an Olis DW2000 conversion spectrophotometer (On-Line Instrument Systems, Bogart, GA) at 20 μ g of Chl per milliliter. Cytochrome *c*₅₅₀ content was determined by first modeling the difference absorption spectra as a linear combination of two Gaussian peaks as in ref. 23 and applying an extinction coefficient of 25 mM⁻¹·cm⁻¹ (32) to the cytochrome *c*₅₅₀ peak. The molar ratio of cytochrome *c*₅₅₀ to PSII was calculated based on 41 molecules of Chl/PSII (7).

Oxygen-Evolution Measurements. Steady-state rates of oxygen evolution from cells were measured on a Clark-type electrode in the presence of 1 mM potassium ferricyanide and 0.5 mM 2,6-dichloro-*p*-benzoquinone as electron acceptors at 5–7 μ g of Chl per milliliter in BG11 medium. For analysis under Ca²⁺-limiting conditions, cells were initially grown in BG11 medium and then transferred to BG11–Ca²⁺ medium for 24 h before analysis in Ca²⁺-deficient medium. Oxygen evolution from purified PSII complexes in the presence of electron acceptors was measured at 2 μ g of Chl per milliliter in 50 mM Mes-NaOH (pH 6.0)/20 mM CaCl₂/0.5 M sucrose. For NH₂OH treatment of cells, oxygen evolution was measured after cells were incubated with variable amounts of

Table 4. Accessibility of NH₂OH to PSII donor side in isolated PSII complexes

Sample	PSII activity*	
	0 μ M NH ₂ OH	10 μ M NH ₂ OH
CP47-tagged PSII	834 \pm 12 (100 \pm 1.4)	568 \pm 11 (68.1 \pm 1.3)
Δ psbQ/CP47-tagged PSII	572 \pm 24 (100 \pm 4.1)	301 \pm 10 (52.6 \pm 1.8)
PsbQ-tagged PSII	1,023 \pm 12 (100 \pm 1.2)	789 \pm 18 (77.1 \pm 1.8)

*Oxygen evolution rates are given in units of μ mol O₂ · mg Chl⁻¹ · h⁻¹ \pm the standard error of the mean ($n = 3$), and the values in parentheses indicate the percent activity \pm the standard error of the mean ($n = 3$) relative to that in the absence of NH₂OH for each sample.

NH₂OH in the dark for 2 min at 30°C before the assay at 8,250 μmol photons per m² per second. For NH₂OH treatment of PSII complexes, PSII samples were incubated for 2 min in the dark at 30°C with 0 or 10 μM NH₂OH before the assay at 8,250 μmol photons per m² per second.

Fluorescence Measurements. PSII samples were diluted in wash buffer, and fluorescence emission spectra at 77 K were measured

on a Fluoromax-2 fluorometer with excitation at 420 nm (Jobin Yvon, Longjumeau, France) (33).

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1. Ferreira KN, Iverson TM, Maghlaoui K, Barber J, Iwata S (2004) *Science* 303:1831–1838.
2. Kamiya N, Shen J-R (2003) *Proc Natl Acad Sci USA* 100:98–103.
3. Loll B, Kern J, Saenger W, Zouni A, Biesiadka J (2005) *Nature* 438:1040–1044.
4. Zouni A, Witt HT, Kern J, Fromme P, Krauss N, Saenger W, Orth P (2001) *Nature* 409:739–743.
5. Aro EM, Suorsa M, Rokka A, Allahverdiyeva Y, Paakkarinen V, Saleem A, Battchikova N, Rintamaki E (2005) *J Exp Bot* 56:347–356.
6. Baena-Gonzalez E, Aro EM (2002) *Philos Trans R Soc London B* 357:1451–1459.
7. Kashino Y, Lauber WM, Carroll JA, Wang Q, Whitmarsh J, Satoh K, Pakrasi HB (2002) *Biochemistry* 41:8004–8012.
8. Thornton LE, Ohkawa H, Roose JL, Kashino Y, Keren N, Pakrasi HB (2004) *Plant Cell* 16:2164–2175.
9. Kashino Y, Inoue-Kashino N, Roose JL, Pakrasi HB (2006) *J Biol Chem* 281:20834–20841.
10. Summerfield TC, Shand JA, Bentley FK, Eaton-Rye JJ (2005) *Biochemistry* 44:805–815.
11. Summerfield TC, Winter RT, Eaton-Rye JJ (2005) *Photosynth Res* 84:263–268.
12. Ishikawa Y, Schroder WP, Funk C (2005) *Photosynth Res* 84:257–262.
13. Balsera M, Arellano JB, Revuelta JL, de las Rivas J, Hermoso JA (2005) *J Mol Biol* 350:1051–1060.
14. Ristvejova J, Kopecky V, Jr, Sovova Z, Balsera M, Arellano JB, Green M, Ettrich R (2006) *Biochem Biophys Res Commun* 345:287–291.
15. Kuwabara T, Murata T, Miyao M, Murata N (1986) *Biochim Biophys Acta* 850:146–155.
16. Bricker TM, Morvant J, Masri N, Sutton HM, Frankel LK (1998) *Biochim Biophys Acta* 1409:50–57.
17. Chen H, Zhang D, Guo J, Wu H, Jin M, Lu Q, Lu C, Zhang L (2006) *Plant Mol Biol* 61:567–575.
18. Roose JL, Pakrasi HB (2004) *J Biol Chem* 279:45417–45422.
19. Nowaczyk MM, Hebel R, Schloeder E, Meyer HE, Warscheid B, Rogner M (2006) *Plant Cell* 18:3121–3131.
20. Seidler A (1996) *Biochim Biophys Acta* 1277:35–60.
21. Ghanotakis DF, Topper JN, Yocum CF (1984) *Biochim Biophys Acta* 767:524–531.
22. Nield J, Barber J (2006) *Biochim Biophys Acta* 1757:353–361.
23. Lakshmi KV, Reifler MJ, Chisholm DA, Wang JY, Diner BA, Brudvig GW (2002) *Photosynth Res* 72:175–189.
24. van Wijk KJ, Bingsmark S, Aro EM, Andersson B (1995) *J Biol Chem* 270:25685–25695.
25. Zhang L, Paakkarinen V, van Wijk KJ, Aro EM (1999) *J Biol Chem* 274:16062–16067.
26. Anbudurai PR, Mor TS, Ohad I, Shestakov SV, Pakrasi HB (1994) *Proc Natl Acad Sci USA* 91:8082–8086.
27. Rokka A, Suorsa M, Saleem A, Battchikova N, Aro EM (2005) *Biochem J* 388:159–168.
28. Nixon PJ, Barker M, Boehm M, de Vries R, Komenda J (2005) *J Exp Bot* 56:357–363.
29. Allen MM (1968) *J Phycol* 4:1–4.
30. Kashino Y, Koike H, Satoh K (2001) *Electrophoresis* 22:1004–1007.
31. Porra RJ, Thompson WA, Kriedemann PE (1989) *Biochim Biophys Acta* 975:384–394.
32. Navarro JA, Hervas M, De la Cerda B, De la Rosa MA (1995) *Arch Biochem Biophys* 318:46–52.
33. Zak E, Norling B, Maitra R, Huang F, Andersson B, Pakrasi HB (2001) *Proc Natl Acad Sci USA* 98:13443–13448.