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Absence of the PsbQ Protein Results in Destabilization of the PsbV Protein and Decreased Oxygen Evolution Activity in Cyanobacterial Photosystem II*

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We have previously reported that cyanobacterial photosystem II (PS II) contains a protein homologous to PsbQ, the extrinsic 17-kDa protein found in higher plant and green algal PS II (Kashino, Y., Lauber, W. M., Carroll, J. A., Wang, Q., Whitmarsh, J., Satoh, K., and Pakrasi, H. B. (2002) Biochemistry 41, 8004–8012) and that it has regulatory role(s) on the water oxidation machinery (Thornton, L. E., Ohkawa, H., Roose, J. L., Kashino, Y., Keren, N., Satoh, K., and Pakrasi, H. B. (2004) Plant Cell 16, 2164–2175). In this work, the localization and the function of PsbQ were assessed using the cyanobacterium Synechocystis sp. PCC 6803. From the predicted sequence, cyanobacterial PsbQ is expected to be a lipoprotein on the luminal side of the thylakoid membrane. Indeed, experiments in this work show that upon Triton X-114 fractionation of thylakoid membranes, PsbQ partitioned in the hydrophobic phase, and trypsin digestion revealed that PsbQ was highly exposed to the luminal space of thylakoid membranes. Detailed functional assays were conducted on the psbQ deletion mutant (ΔpsbQ) to analyze its water oxidation machinery. PS II complexes purified from ΔpsbQ mutant cells had impaired oxygen evolution activity and were remarkably sensitive to NH4OH, which indicates destabilization of the water oxidation machinery. Additionally, the cytochrome b559 (PsbV) protein partially dissociated from purified ΔpsbQ PS II complexes, suggesting that PsbQ contributes to the stability of PsbV in cyanobacterial PS II. Therefore, we conclude that the major function of PsbQ is to stabilize the PsbV protein, thereby contributing to the protection of the catalytic Mn4Ca1Cl1 cluster of the water oxidation machinery.

Photosystem II (PS II)2 evolves molecular oxygen by oxidizing water molecules using the driving force of light energy (1).

This water-splitting reaction is catalyzed by an inorganic Mn4Ca1Cl1 cluster and assisted by several extrinsic proteins located on the luminal surface of PS II complex (2). The subunit composition of these extrinsic proteins differs between cyanobacterial PS II and that from higher plants and green algae. PS II of green algae and higher plants contains three luminal extrinsic proteins: PsbO (33-kDa manganese-stabilizing protein), PsbP (24-kDa protein), and PsbQ (17-kDa protein). In contrast, cyanobacterial PS II complexes contain the extrinsic proteins PsbO, PsbU (12-kDa protein), and PsbV (cytochrome c550) (2–4), in which only PsbO is common to both systems. More recently, PsbP and PsbQ have been identified in cyanobacterial PS II (5, 6).

Biochemical removal and reconstitution experiments have aided in our understanding of the roles of the plant PS II extrinsic proteins. The PsbO protein stabilizes the manganese cluster and is crucial for PS II oxygen evolution activity, whereas PsbP and PsbQ are necessary for oxygen evolution at physiological Ca2+ and Cl− concentrations (2, 7–10). Specifically, reconstitution of PsbP to PS II complexes containing PsbO reduces the Ca2+ requirement, while further reconstitution of PsbQ restored oxygen evolution activity at a moderate rate at low concentrations of Cl− (9, 11). These removal/reconstitution experiments indicate that PsbP and PsbQ offer the binding sites of Ca2+ and Cl− ions or stabilize these ions within the PS II complex (2, 7–9). Together the three extrinsic proteins, PsbO, PsbP, and PsbQ, form a barrier around the manganese cluster protecting the catalytic center from small reductants, such as NH4OH (12).

The cyanobacterial extrinsic proteins PsbO, PsbU, and PsbV can be removed by high salt wash (e.g. by 1 M CaCl2, but not by 1 M NaCl) (3) and reconstituted to PS II core complexes (4). Upon removal of these proteins, the Ca2+ and/or Cl− requirements are significantly increased for optimal oxygen evolution activity in vitro (4). Because the cyanobacterium Synechocystis sp. PCC 6803 is especially amenable to genetic manipulation, directed mutant studies have provided a large amount of functional information on the cyanobacterial PS II extrinsic proteins. Burnap and Sherman (13) reported that the psbO deletion mutant (ΔpsbO) is photoautotrophic, and therefore cyanobacterial PsbO is not essential for oxygen evolution activity, unlike its plant counterpart (13). In a study of psbU and psbV deletion mutants (ΔpsbU and ΔpsbV, respectively), Shen et al. (14) suggested that PsbU maintained the maximum affinity of PS II for...


**EXPERIMENTAL PROCEDURES**

Cyanobacterial Culture Conditions—Wild type, ΔpsbQ, and ΔpsbV (6) strains of *Synechocystis* 6803 were grown in normal BG 11 medium (28). The HT3 strain of *Synechocystis* 6803 and ΔpsbQHT3 mutant (6) were grown in BG 11 medium supplemented with 2 mM glucose and 50 μg/ml kanamycin (for HT3) or spectinomycin (for ΔpsbQ) at 30 °C under 50 μmol of photons m⁻² s⁻¹. HT3 strain was kindly provided by Dr. T. Bricker (29).

Isolation of Thylakoid Membranes and Purification of PS II Complexes—Thylakoid membrane and PS II complexes were isolated as described in Ref. 5 and resuspended in MMCG solution, containing 50 mM MES-NaOH (pH 6.0), 10 mM MgCl₂, 5 mM CaCl₂, and 25% glycerol.

Isolation of Right-side-out and Inside-out Vesicles—To isolate thylakoid membranes containing predominantly right side-out vesicles (30), cells in MMCG solution were broken as described in Ref. 5 and collected at 36,000 × g after the removal of unbroken cells. For the isolation of thylakoid membranes containing mostly inside-out vesicles (30), cells suspended in 50 mM MES-NaOH (pH 6.0), 10 mM MgCl₂, 5 mM CaCl₂ were passed through a French pressure cell twice at 100 megapascals. After removal of unbroken cells by centrifugation at 3,000 × g, the resulting vesicles were collected at 160,000 × g. Both types of vesicles were suspended in MMCG solution in the final step of preparation. No protease inhibitors were added during these isolations. The lysozyme treatment was omitted to avoid possible proteolytic degradation. Instead, the samples were thoroughly kept cool and processed quickly.

Fractionation of Thylakoid Membrane—Triton X-114 phase partitioning of thylakoid membrane was performed essentially as described in Ref. 31 at 100 μg of Chl/ml. Triton X-114 was purchased from Sigma.

Protease Treatment—Stock solution of trypsin (6,750 units/ml; Sigma; pancreatic type II crude) was added to 1 ml of thylakoid membrane suspension (100 μg of Chl/ml) to 0, 34, 68, and 135 units/100 μg of Chl (equivalent to around 4 mg of protein). The mixtures were incubated at 30 °C for 10 min at pH 6.0. Note that because the pH was not optimal for trypsin activity, the digestion was performed at 30 °C. The reactions were
stopped by adding one-third volume of denaturing solution (20 mM EDTA, 5% lithium dodecyl sulfate, 40 mM dithiothreitol, 172 mM Tris, pH 6.8, and 0.5 M sucrose) and cooling to 0 °C.

**SDS-PAGE and Protein Detection**—Electrophoresis and immunodetection were performed as described in Ref. 32, using a 18–24% gradient acrylamide, 6 M urea SDS-PAGE system. For immunodetection, PsbQ was detected using specific antisera against cyanobacterial PsbQ (6). Bands were visualized using enhanced chemiluminescence reagents (WestPico; Pierce) on a Fujifilm LAS-1000 Plus imager (Fujifilm, Stamford, CT). The c-type heme in cytochrome $c_{550}$ was also detected using WestFemto (Pierce) on a LAS-1000 Plus imager after the transfer of proteins onto polyvinylidene fluoride (PVDF) membrane (Immobilon P; Millipore, Billerica, MA) (33).

**Oxygen Evolution Assay**—Steady state oxygen evolution was measured on a Clark-type electrode in the presence of 1 mM potassium ferricyanide and 0.5 mM 2,6-dichlorobenzoquinone as electron acceptors at 2°C of Chl/ml in MMCS buffer containing 50 mM MES-NaOH, (pH 6.0), 10 mM MgCl$_2$, 5 or 20 mM CaCl$_2$ and 0.5 M sucrose. To assess the effect of NH$_2$OH, oxygen evolution was measured after PS II complexes (2.0 g of Chl/ml in MMCG buffer, 20 mM CaCl$_2$) were incubated for 1 h on ice in the dark in the presence of variable concentrations of NH$_2$OH (34). Flash oxygen yield measurements were measured using a bare platinum electrode as described in Ref. 26.

**Fluorescence Kinetics**—QA reoxidation kinetics was measured at room temperature using a double-modulation fluorometer, FL-3320 (Photon System Instruments, Brno, Czech Republic) with FluorWin software (version 3.6.3.3). The cell concentration for each sample was adjusted to OD$_{280}$ = 0.08 (2 μg of Chl/ml) measured on a DW2000 spectrophotometer (SLM-Aminco, Urbana, IL). The samples were dark-adapted for 3 min prior to measuring.

**Optical Measurements**—Chl $a$ concentration was determined by the method of Porra et al. (35).

### RESULTS

**Cyanobacterial PsbQ Is a Luminal Protein with a Putative Lipid Anchor**—We have previously postulated that PsbQ in cyanobacteria is a luminally targeted lipoprotein (6). Indeed, cyanobacterial PsbQ was not removed from PS II by biochemical treatments (1 M CaCl$_2$ or 1 M Tris-HCl, pH 8.0) that removed the other extrinsic proteins (PsbO, PsbU, and PsbV) (5). This result was quite different from the characteristics of chloroplast PsbQ, which is removed by high salt treatment (36).

Triton X-114 partitioning, a well established method to separate hydrophobic and hydrophilic proteins (37), was used to assess the hydrophobicity of the mature cyanobacterial PsbQ protein. Thylakoid membranes were subjected to Triton X-114 partitioning, and the proteins were fractionated into a hydrophobic fraction (Triton X-114 fraction; Fig. 1, lane 3), an aqueous fraction (lane 4), and an insoluble fraction (very hydrophilic; lane 5). Upon Coomassie staining, the separate fractions showed different polypeptide profiles. As expected, the hydrophilic phycobiliproteins partitioned into the aqueous and insoluble fractions (Fig. 1, Coomassie staining and no staining, lanes 4 and 5). Heme staining was used to detect PsbV (cytochrome $c_{550}$ apparent molecular mass of 22 kDa), which fractionated into the aqueous phase (Fig. 1, heme staining, lane 4). This result is in agreement with previous reports that PsbV is removed by high salt treatment (4, 5). Unlike these hydrophilic proteins, PsbQ partitioned into the hydrophobic fraction (Fig. 1, PsbQ, lane 3).

Although PsbQ partitioned into the hydrophobic phase (Fig. 1), this protein has no predicted hydrophobic domain and is expected to be exposed on one side of the thylakoid membrane.
Right side-out and inside-out thylakoid membranes were prepared and subjected to limited digestion by trypsin to assess the topology of PsbQ (Fig. 2). PsbQ was not digested in the right side-out membrane samples but was digested in the inside-out membrane samples, similar to the digestion pattern for the luminal protein PsbV (cytochrome $c_{550}$). This result demonstrates that PsbQ is located on the luminal side of thylakoid membrane. Altogether, the data from the Triton X-114 partitioning and the trypsin digestion experiments are consistent with the previous prediction that PsbQ is targeted to the thylakoid lumen and cleaved by signal peptidase II to yield an N-terminal cysteine, which is modified with a lipid anchor (6).

The PsbV Protein Partially Dissociates from $\Delta$psbQHT3 PS II Complexes—The polypeptide profiles of HT3 and $\Delta$psbQHT3 PS II complexes are shown in Fig. 3. As expected, the PsbQ protein is absent from the $\Delta$psbQHT3 PS II complexes. Also, PsbV was partially lost in $\Delta$psbQHT3 PS II, indicating that, in the absence of PsbQ, the structure of the water oxidation machinery becomes labile. The fact that PsbV, a luminal PS II protein, is affected by the absence of PsbQ is also consistent with the luminal localization of PsbQ determined during the preceding experiments.

The $\Delta$psbQ Mutant Has Defects in PS II Water Oxidation—To address the contribution of PsbQ to the water oxidation process, fluorescence kinetics measurements were performed using cells grown in normal BG11. Because PsbV became labile in PS II upon the absence of PsbQ, the $\Delta$psbV mutant was also assayed for comparison. For samples at the same chlorophyll concentration, the $F_o$ value for $\Delta$psbQ cells was essentially the same as that of wild type cells ($0.735 \pm 0.0802$ versus $0.715 \pm 0.0805$; S.D., $n = 5–6$). This is consistent with previous data showing no significant difference in the relative amounts of PS II between wild type and $\Delta$psbQ cells (6). Whereas the $F_o$ value of $\Delta$psbV cells is significantly increased relative to wild type ($1.08 \pm 0.136$; S.D., $n = 5–6$), indicating an increase in impaired PS II complexes. The normalized variable fluorescence yield in $\Delta$psbQ was also comparable with that in wild type ($0.742 \pm 0.0864$ versus $0.694 \pm 0.0379$; S.D., $n = 5–6$), whereas that of $\Delta$psbV was much lower ($0.307 \pm 0.121$; S.D., $n = 5–6$). The fluorescence decay kinetics for cells after a single saturating flash, which represents the reoxidation kinetics of QA in PS II, are shown in Fig. 4. Qualitatively, the $\Delta$psbQ mutant has a decay curve intermediate to that of wild type and the $\Delta$psbV mutant. To quantitatively compare the fluorescence decay curves for all strains, they were fit with three components of different decay half-times (Table 1). The first phase reports on the electron transfer from QA to QB (38–40). The second phase represents the turnover of plastoquinone molecules at the QB site (41). The last component with the longest half-time is the oxidation of QA by PS I (42). It is noteworthy that the decay half-time of the
FIGURE 4. Kinetics of Q_A reoxidation. Fluorescence was measured on samples at OD_{730} = 0.08 (~2 μg of Chl/ml) at room temperature after 3 min of dark incubation. Solid line, wild type; dashed line, ΔpsbQ; dotted line, ΔpsbV.

TABLE 1
Exponential decay components in Q_A reoxidation kinetics

| Component | Decay half-time (ms) | Relative amount (%) |
|-----------|----------------------|---------------------|
| Wild type | 307 ± 21.1 | 94 ± 8.7 |
| ΔpsbQ     | 3.28 ± 0.346 | 4.7 ± 0.59 |
| ΔpsbV     | 9.63 ± 1.18 | 1.3 ± 0.30 |
| ΔpsbV     | 244 ± 14.3 | 97 ± 0.49 |
| ΔpsbV     | 3.29 ± 0.704 | 2.3 ± 0.36 |
| ΔpsbV     | 13.5 ± 1.87 | 0.64 ± 0.15 |
| ΔpsbV     | 250 ± 17.5 | 97 ± 0.57 |
| ΔpsbV     | 3.17 ± 0.544 | 2.7 ± 0.46 |
| ΔpsbV     | 14.9 ± 5.41 | 0.25 ± 0.13 |

fastest component in ΔpsbQ was 20% faster than that in wild type; furthermore, the relative amounts of each component in ΔpsbQ differed from those in wild type. This result is consistent with that observed in the ΔpsbV mutant in which the water oxidation process is highly modified.

Flash oxygen yield measurements were also conducted to assay the function of the water oxidation machinery in the absence of PsbQ. ΔpsbQ and ΔpsbV cells showed a characteristic but somewhat modified period four oscillation. The relative oxygen yields after first and second flashes were slightly larger in ΔpsbQ cells than in wild type cells but smaller than in ΔpsbV (data not shown).

The distribution of S-states after 10 min of dark adaptation was somewhat perturbed in ΔpsbQ cells relative to wild type cells. ΔpsbQ cells exhibited a higher fraction of the S_0-state (4.5 ± 0.18 versus 2.3 ± 0.18%; S.E., n = 4–8) and a lower fraction of the S_2-state (32 ± 0.83 versus 37 ± 0.81%; S.E., n = 4–8). The distribution in ΔpsbV cells was more perturbed relative to that in wild type cells with an increased population of S_3- and S_5-states and a lower fraction of S_0- and S_1-states (data not shown). The yield itself was also affected, in that the averaged amplitude after damping (flash number 13–16) in ΔpsbQ cells was typically 90% of that in wild type cells (n = 5) but still larger than that in ΔpsbV cells (24% of that in wild type; n = 6).

Fig. 5 shows the kinetics of oxygen release from cells poised in the S_3-state, as detected using a bare platinum electrode for wild type, ΔpsbQ, and ΔpsbV cells (14, 43). The rise kinetics for wild type and ΔpsbQ cells were identical, giving the same peak time (~17 ms following the third flash), whereas ΔpsbV cells showed a slower rise time and ~7-ms slower peak time than the other strains (Fig. 5). Interestingly, the decay half-time of the oxygen release curve was different for wild type and ΔpsbQ cells (29.5 and 39 ms, respectively). In the ΔpsbQ cells, the decay was decelerated, but not to the extent of that in ΔpsbV cells (62.8 ms). Here again, the ΔpsbQ mutant displays a phenotype intermediate to that of wild type and ΔpsbV. Although this signal has been used previously as an indirect measurement of S_3- to S_5-state advancement (14, 43), other factors, including long oxygen diffusion pathways to the electrode and oxygen consumption by the cells, also contribute to its lifetime.

The Structural Integrity of the PS II Water Oxidation Complex Is Compromised in the Absence of PsbQ—It has been reported that the removal of PsbP and PsbQ from higher plant PS II membrane preparations causes a remarkable decrease in the oxygen evolution rate and an increase in the Ca^2+ and Cl^- requirement for activity and exposes the oxidizing side of PS II to exogenous reductants, such as NH_2OH (12, 44). Table 2 shows the steady state oxygen evolution activities of isolated PS II complexes from HT3 and ΔpsbQHT3. The activity of ΔpsbQHT3 PS II complexes was lower than that of HT3 even in the presence of 20 mM CaCl_2 (60% of HT3 PS II). Whereas HT3 PS II evolved oxygen at the same rate in the presence of 5 and 20 mM CaCl_2, the rate of oxygen evolution by ΔpsbQHT3 PS II
with 5 mM CaCl₂ decreased to 70% of its activity in the presence of 20 mM CaCl₂. Thus, PS II complexes lacking PsbQ are more sensitive to the amount of CaCl₂ in the assay buffer relative to the control.

To further analyze the structural integrity of the isolated PS II complexes, the effect of hydroxylamine on steady state oxygen evolution activity was measured (Fig. 6). In the presence of NH₂OH, the rate of oxygen evolution supported by saturated light was measured in the presence of 1 mM ferricyanide and 0.5 mM 2,6-dichloro-p-benzoquinone. The oxygen evolution activity at 0 μM NH₂OH were 1130 and 464 μmol of O₂ mg of Chl⁻¹ h⁻¹ for HT3 and ΔpsbQHT3 PS II, respectively.

**DISCUSSION**

The current models for PS II extrinsic proteins include five proteins in cyanobacteria (PsbO, PsbU, PsbV, PsbP, and PsbQ) and three proteins in higher plants (PsbO, PsbP, and PsbQ) (6). The PsbO protein, which is shared by both systems, may have a common function in cyanobacteria and plants. However, the PsbU and PsbV proteins have a specialized function in cyanobacteria, whereas PsbP and PsbQ have a modified function between the two systems. Therefore, it is necessary to reevaluate the roles of each of these proteins in the water oxidation reaction. The current study has focused on the localization and function of cyanobacterial PsbQ.

Determination of the localization of PsbQ within the cyanobacterial PS II complex is critical for understanding the roles of all of the associated extrinsic proteins. Structural studies have not yet resolved the PsbQ protein in PS II (19, 23, 45, 46). Cyanobacterial PsbQ associates with PS II complexes but shows different biochemical properties than higher plant PsbQ, since it is not removed by 1 M CaCl₂ or 1 M Tris (5, 6). In this work, the physical properties of cyanobacterial PsbQ were investigated using Triton X-114 partitioning. Our results show that PsbQ fractionated into the hydrophobic Triton X-114 phase (Fig. 1). This result is consistent with our previous prediction from sequence analysis that PsbQ is a lipoprotein (6). This kind of modification has been reported previously in *Synechocystis* 6803 for the periplasmic protein NrtA (47). Thus, it is reasonable to assume that the N-terminal lipid modification causes PsbQ to partition into the hydrophobic Triton X-114 phase and anchors PsbQ to the membrane such that it is not removed by 1 M CaCl₂ or 1 M Tris (5).

The fact that cyanobacterial PsbQ is a lipoprotein may explain why PsbQ is present in HT3 PS II but not in the current crystallographic model (19, 23, 24). HT3 PS II was purified under mild detergent conditions (33), and it is expected that the lipid bound to the N terminus of PsbQ should remain anchored to the hydrophobic domain of PS II. However, the prevailing procedure to purify membrane protein complexes suitable for crystallization includes a step(s) to remove excess lipids (33, 48–50). Thus, when the lipids around the PS II complex were removed during purification, it is conceivable that PsbQ was also removed or could no longer anchor to the lipid-depleted PS II complex. Tll2057, the PsbQ homologue in *T. elongatus* used for PS II crystallographic studies, (available on the World Wide Web at www.kazusa.or.jp/cyano/Thermo/index.html), is also predicted to contain a lipoprotein signal peptide. The functional significance of the N-terminal lipid anchor in cyanobacterial PsbQ is unclear, since PsbQ in plants lacks this modification.

To confirm the luminal localization of cyanobacterial PsbQ, thylakoid membrane vesicles of opposite orientations were subjected to trypsin digestion. PsbQ was digested by trypsin in the inside-out membrane samples, whereas it was protected from digestion in the right side-out membrane samples, as was the *bona fide* luminal protein PsbV (cytochrome c₅₅₉). We have reported previously that upon trypsin digestion of right side-out membranes prepared in this way, the luminal PsbQ protein is protected from digestion, whereas the cytoplasmically exposed PsaD protein is degraded. The opposite digestion profiles were observed for the inside-out prepared membranes (30). Our current results clearly indicate that PsbQ localizes to the luminal side of thylakoid membrane and excludes the possibility of the presence of a transmembrane domain in cyanobacterial PsbQ. Altogether, these data confirm that PsbQ closely associates to the luminal side of cyanobacterial PS II complexes (Fig. 2) (5), and it can be concluded that PsbQ is a component of the water oxidation complex in cyanobacterial PS II.

Based on the above mentioned localization of PsbQ, the role of PsbQ in relation to the water oxidation reaction was assessed. The variable yield of the fluorescence ((Fₘₕ – Fₐ)/Fₐ) in ΔpsbQ was comparable with that of wild type, whereas the yield in ΔpsbV was much smaller than that of wild type (typically less...
than 45%). The rate of Q$_A$ reoxidation was accelerated in $\Delta$psbQ as well as $\Delta$psbV with the decay half-time of the major, fastest component 20% faster in the mutant cells compared with that of wild type cells (Fig. 4 and Table 1). Although Q$_A$ is localized on the reducing side of PS II, defects on the oxidizing side of PS II directly cause an acceleration of Q$_A$ reoxidation (51). This result indicates that the absence of PsbQ affects the stability of the water oxidation complex.

Flash yield oxygen measurements showed a slightly altered S-state distribution for $\Delta$psbQ relative to wild type cells, consistent with the lower oxygen evolution activity observed in $\Delta$psbQ cells (6, 27). The rise kinetics of oxygen release from cells poised in the S$_3$-state in $\Delta$psbQ cells was identical to that of wild type cells (Fig. 5). Although the oxygen signal after the third flash is not a direct measurement of oxygen release from the enzyme, this signal has been correlated to the kinetics of oxygen release from the S$_3$-state, assuming that the diffusion rates of oxygen from different cells to the electrode are similar (14, 43). Whereas $\Delta$psbQ cells behaved similarly to wild type cells for the rise kinetics of oxygen release, the decay kinetics of oxygen release in $\Delta$psbQ cells were slower compared with wild type but not to the extent of that in $\Delta$psbV cells (Fig. 5). The biological significance of the decay part of the oxygen release curve is not known, and additional processes (i.e. oxygen diffusion to the electrode and oxygen consumption by the cells) contribute to this part of the signal.

The absence of PsbQ had specific consequences on the extrinsic protein PsbV (cytochrome c$_{350}$). Whereas the Chl a-normalized amount of PsbV in $\Delta$psbQ cells was comparable with that in wild type cells (measured by heme staining; data not shown), there was a significant decrease in the amount of PsbV associated with PS II complexes purified from the $\Delta$psbQ/H73T mutant (Fig. 3). The absence of PsbQ did not result in the complete removal of PsbV, and this is consistent with previous reports that the $\Delta$psbQ mutant grew faster than $\Delta$psbV mutant in both normal medium and Ca$^{2+}$- or Cl$^{-}$-depleted medium (6). From these results, we conclude that PsbQ is in close proximity to the PsbV protein within PS II and functions to stabilize the extrinsic proteins in the PS II water oxidation complex. These results suggest that a common role for PsbQ in cyanobacteria and plants is to stabilize other PS II extrinsic proteins and modulate the Cl$^{-}$ requirement for oxygen evolution activity. However, the specific effect on PsbV stability in cyanobacterial PS II upon the loss of PsbQ is somewhat surprising, because higher plant PS II complexes do not contain PsbV.

The absence of PsbQ leads to low water oxidation activity in isolated PS II and the higher requirement of Ca$^{2+}$ and Cl$^{-}$ for oxygen evolution (Table 2). This is consistent with the observed effect on PsbV in the $\Delta$psbQ mutant. To further probe the stability of the water oxidation machinery in PS II complexes lacking PsbQ, the effect of NH$_4$OH was measured. Ghanotakis et al. (12) have shown that if PsbP and PsbQ are removed from intact PS II membranes from higher plants, the small redundant NH$_4$OH causes extensive damage to the water-oxidizing side of PS II. Thus, PsbP and PsbQ, along with PsbO, shield the water oxidation machinery of plant PS II from the luminal space, and once these proteins are removed, the accessibility of NH$_4$OH to the Mn$_{4}$-Ca$_{4}$-Cl$_{x}$ cluster increases significantly. The same effect was observed in isolated PS II complexes from the $\Delta$psbQ/H73T mutant. $\Delta$psbQ/H73T PS II was considerably more sensitive to NH$_4$OH, whereas only a limited effect was observed for HT3 PS II (Fig. 6). These results highlight the role of PsbQ in stabilizing the components of the water oxidation machinery in cyanobacterial PS II.

In conclusion, PsbQ associates with the luminal side of cyanobacterial PS II complexes and participates in the water oxidation reaction. PsbQ is important for stabilizing PsbV within the PS II complex, and the majority of the defects described for $\Delta$psbQ can be explained by a partial loss of PsbV. However, PsbQ must have a role beyond that of stabilizing PsbV, because the double deletion mutant $\Delta$psbQ/$\Delta$psbV cannot grow photoautotrophically, whereas the respective single mutants can grow photoautotrophically (27). It is possible that PsbQ contributes to the stabilization of the other extrinsic proteins on the luminal side of PS II, and the absence of both PsbQ and PsbV results in such an instability of the water oxidation machinery that it can no longer support photoautotrophic growth. Thus, PsbQ is an important extrinsic protein in cyanobacterial PS II, which contributes to the protection of the catalytic Mn$_{4}$-Ca$_{4}$-Cl$_{x}$ cluster of the water oxidation machinery.

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