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Documentation of Significant Electron Transport Defects on the Reducing Side of Photosystem II upon Removal of the PsbP and PsbQ Extrinsic Proteins[†]

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ABSTRACT: The Photosystem II extrinsic proteins PsbO, PsbP, and PsbQ are required for efficient oxygen-evolving activity under physiological conditions. In this study, we have used fluorescence decay kinetics to quantitatively probe Photosystem II electron transport upon depletion of these components by standard salt washing protocols. Our results indicate that in addition to the expected oxidizing-side defects, removal of PsbP and PsbQ with 2 M NaCl significantly slows the rate of electron transfer from Q_A^- to Q_B. Electron transfer from Q_A^- to Q_B in Photosystem II reaction centers with an occupied Q_B site was slowed by a factor of 12, while electron transport from Q_A^- to Q_B in centers with an unoccupied Q_B site was slowed by a factor of 6. Subsequent removal of the PsbO protein by treatment with 200 mM NaCl and 2.6 M urea did not induce further reducing-side alterations. Our results demonstrate that studies attributing defects observed upon PsbP and PsbQ removal solely to the oxidizing side must be viewed with caution.

Photosystem II (PS II)[1] is the membrane protein complex found in the thylakoid membranes of cyanobacteria and chloroplasts that performs the light-driven oxidation of water to molecular oxygen (*1*, *2*). At least six intrinsic proteins are required for oxygen-evolving activity. These are the CP47, CP43, D1, and D2 proteins and the α and *β* subunits of cytochrome b_{559} (*3*−*5*). PS II complexes containing only these subunits evolve oxygen at low rates (25−40% relative to the control) even in the presence of high, nonphysiological concentrations of calcium and chloride (*3*, *4*).

In higher plants and green algae, three lumenal extrinsic proteins are associated with the oxygen-evolving complex. These are the PsbO, PsbP, and PsbQ components (*6*, *7*). These interact with the intrinsic membrane proteins and with each other to yield fully functional oxygen-evolving complexes, shielding the Mn_4-Ca_1Cl_{1-2} cluster from exogenous reductants while allowing efficient turnover of the water oxidation complex under physiological ionic conditions. The PsbO protein appears to play a central role in the stabilization of the manganese cluster during exposure to low chloride concentrations or to exogenous reductants and is essential for efficient and stable oxygen evolution (*4*). The PsbP and PsbQ proteins appear to modulate the calcium and chloride requirements for optimal oxygen evolution. Biochemical studies have shown that removal of the PsbP and PsbQ proteins from PS II membranes using high NaCl concentrations (> 1 M) results in dramatically lower oxygen-evolving activity (*8*−*10*). However, this lost oxygen evolution capability can largely be restored either by reconstitution with the PsbP and PsbQ proteins or by the addition of millimolar concentrations of calcium and chloride (*8*).

Recent genetic studies from our laboratory and others have demonstrated that the PsbO and PsbP proteins are required for photoautotrophic growth and PS II assembly in higher plants propagated under normal growth conditions (*11*−*15*). The PsbQ protein was shown to be essential for photoautotrophic growth under low light conditions (*16*). Surprisingly, RNAi suppression of PsbP expression demonstrated that, in vivo, the principal defects observed are on the reducing side of the photosystem (*13*, *14*), are attributable to the loss of PsbP and not to the concomitant loss of PsbQ (*16*), and are accompanied by dramatic alterations in thylakoid membrane organization (*15*). The electron transport defects observed in vivo could be a direct consequence of the loss of the PsbP protein. Alternatively, these could be due to secondary effects induced by more rapid turnover of PS II reaction centers or by defective PS II assembly. Other investigators have observed alterations to the reducing side of the photosystem that correlate with the removal of the PsbP and PsbQ proteins. Binding of atrazine to the Q_B site is enhanced upon removal of the PsbP and PsbQ components by washing PS II membranes with high concentrations of NaCl (*17*), and the period-two oscillation normally associated with Q_A to Q_B electron transfer (*18*) is also modified. Removal of the PsbP and PsbQ components leads to the loss of calcium from the oxygen-evolving site with concomitant electron transport defects being observed on both the oxidizing and reducing sides of the photosystem (*19*).

In this study, we have analyzed the fluorescence decay kinetics of PS II membranes containing different extrinsic protein compositions to gather more precise information about the PS II defects induced by the removal of these proteins. The intact PS II membranes analyzed in this study contained all three extrinsic proteins. The NaCl-washed membranes were depleted of the PsbP and PsbQ proteins but retained the PsbO protein. Finally, the NaCl-urea-washed membranes lacked all three extrinsic

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[1]Abbreviations: chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MES, 2-(*N*-morpholino)ethanesulfonic acid; PS II, Photosystem II; SD, standard deviation.

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Table 1: Photosynthetic Parameters for Thylakoids and the Various PS II Membrane Preparations Used in This Study (n = 3−5, ±1.0 SD)

membrane type	parameter	SMN	SMN + $CaCl_2$[a]
thylakoid membranes	F_V/F_M	0.54 ± 0.01	0.63 ± 0.01
	O_2 evolution[b]	ND[c]	ND[c]
PS II membranes	F_V/F_M	0.56 ± 0.01	0.66 ± 0.00
	O_2 evolution[b]	490 ± 81	507 ± 103
NaCl-washed PS II membranes	F_V/F_M	0.31 ± 0.03	0.44 ± 0.01
	O_2 evolution[b]	85 ± 10	356 ± 98
NaCl-urea-washed PS II membranes	F_V/F_M	0.24 ± 0.01	0.43 ± 0.01
	O_2 evolution[b]	24 ± 11[d]	78 ± 27[d]

[a]Assayed in the presence of 10 mM $CaCl_2$. [b]In micromoles of O_2 per milligram of chl per hour. [c]Not determined, since these buffers are suboptimal for the assay of thylakoids (*22*). In 400 mM sucrose, 20 mM NaCl, 5 mM $MgCl_2$, and 50 mM HEPES-NaOH (pH 7.5), a rate of 196 ± 19 μmol of O_2 (mg chl)$^{-1}$ h^{-1} was observed (n = 3). [d]Assayed in the presence of 100 mM NaCl to maintain intact manganese clusters.

components. The kinetics of fluorescence decay after a single saturating flash provided information about electron transfer characteristics of the PS II complex. When measured in the absence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the fluorescence decay reflects forward electron transfer from Q_A^- to Q_B and highlights differences on the reducing side of PS II. In the presence of DCMU, forward electron transfer is blocked and the fluorescence decay represents charge recombination events with oxidizing-side components of the photosystem. Our findings indicate that removal of the PsbP and PsbQ components results in a dramatic slowing of electron transfer from Q_A^- to Q_B.

MATERIALS AND METHODS

Preparation of PS II Membranes. Chloroplasts were isolated from spinach bought at a local market (*20*). The chl concentration was measured by the method of Arnon (*21*). Oxygen-evolving PS II membranes were prepared by the method of Berthold et al. (*22*), with the modifications described by Ghanotakis and Babcock (*23*). Typical preparations had a chl *a*/chl *b* ratio of 1.9−2.0 and oxygen evolution rates in excess of 450 μmol O_2 (mg chl)$^{-1}$ h^{-1}. NaCl-washed PSII membranes lacking the PsbP and PsbQ proteins were obtained by washing PSII membranes at a chl concentration of 1.5 mg/mL twice with a buffer containing 2 M NaCl, 300 mM sucrose, 10 mM $MgCl_2$, and 50 mM MES-NaOH (pH 6.0) for 1 h on ice in the dark followed by centrifugation at 38000*g* for 25 min. NaCl-washed membranes were given a final wash in a buffer containing 400 mM sucrose, 15 mM NaCl, and 50 mM MES-NaOH (pH 6.0) (SMN buffer) and resuspended in the same buffer. NaCl-urea-washed PSII membranes, which lack the PsbO protein, were prepared by treatment of the NaCl-washed PSII membranes at a chl concentration of 0.5 mg/mL with a buffer containing 200 mM NaCl, 2.6 M urea, 300 mM sucrose, 10 mM $MgCl_2$, and 50 mM MES-NaOH (pH 6.0) for 30 min on ice in the dark, followed by centrifugation at 38000*g* for 25 min. NaCl-urea-washed PSII membranes were given a final wash in a buffer containing 400 mM sucrose, 200 mM NaCl, and 50 mM MES-NaOH (pH 6.0) and resuspended in the same buffer. The inclusion of high concentrations of NaCl in this buffer was required to maintain functional manganese clusters.

Fluorescence Measurements. The fluorescence decay after a single saturating flash was monitored with a Photon Systems Instruments FL3000 dual modulation kinetic fluorometer (commercial version of the instrument described in ref *24*). Both measuring and saturating flashes were provided by computer-controlled photodiode arrays. Samples were assayed at a chl concentration of 10 μg/mL in an assay buffer [0.4 M sucrose, 50 mM MES-NaOH (pH 6.0), 10 mM $CaCl_2$, and 80 mM NaCl] in the presence or absence of 10 μM DCMU. All samples were dark-incubated for 5 min prior to measurement. Data were fit to a three-component exponential decay equation after normalization, as described previously (*25*), using Origin version 6.1. F_0, F_M, and F_V values for the determination of PS II quantum yields were obtained from unnormalized flash data collected in the absence of DCMU. The fluorescence intensity recorded 50 μs after the saturating actinic flash was collected as the F_M value, while the fluorescence intensity collected immediately before the flash was collected as the F_0 value.

Oxygen Evolution Measurements. Steady-state oxygen evolution was assessed with a Hansatech Instruments Oxy-Lab poloragraphic electrode at a chl concentration of 10 μg/mL with 300 μM 2,6-dichloro-*p*-benzoquinone added as an electron acceptor. The assay was performed in SMN buffer in the case of the untreated and NaCl-washed membranes and SMN with 100 mM NaCl in the case of the NaCl-urea-washed membranes. In some experiments, 10 mM $CaCl_2$ was included. Saturating light was provided from a photodiode array with a light intensity of 4000 μmol of photons m^{-2} s^{-1}.

RESULTS AND DISCUSSION

Four types of membrane preparations were used in these studies: isolated thylakoid membranes, intact PS II membranes that contain a full complement of extrinsic protein components (PsbO, PsbP, and PsbQ), 2 M NaCl-washed PS II membranes that contain only the PsbO extrinsic protein, and 200 mM NaCl-2.6 M urea-washed membranes that lack all three extrinsic components. In Table 1, some photosynthetic parameters for these membrane preparations are presented. The steady-state oxygen evolution rates for the NaCl-washed PS II membrane preparations indicate that removal of the PsbP and PsbQ components strongly inhibits oxygen evolution and that inclusion of $CaCl_2$ during the assay, in large measure, reverses this inhibition. The NaCl-urea-washed membranes, which were also lacking the PsbO component, exhibited extremely low levels of oxygen evolution in the absence of added $CaCl_2$. In the presence of $CaCl_2$, oxygen evolution rates of ∼15% of control values were observed. These results are essentially identical to those obtained previously (*4*, *9*, *26*, *27*). The quantum yield for PS II excitation trapping (F_V/F_M) was also examined. Thylakoid membranes and untreated PS II membranes exhibited almost identical values either in the absence or in the presence of $CaCl_2$, indicating that no perturbation in trapping efficiency is induced per se by PS II membrane protein isolation with TX-100. In the

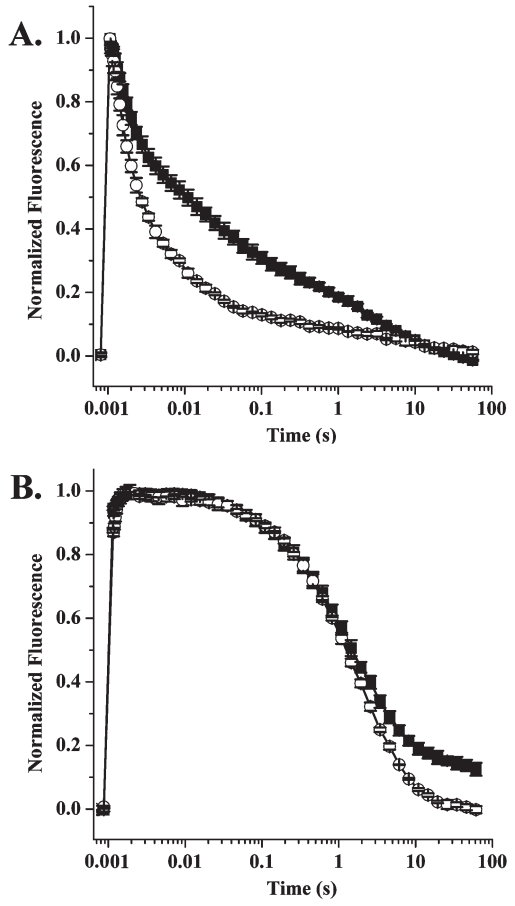

FIGURE 1: Comparison of Q_A^- reoxidation kinetics after a single saturating flash in isolated thylakoids and in untreated PS II membranes. Data were collected after dark incubation for 5 min. (A) Fluorescence decay in the absence of DCMU. (B) Fluorescence decay in the presence of 10 μM DCMU. Thylakoids (○) and PS II membranes (■). $n = 5$; error bars denote ±1.0 SD. In some cases, the error bars are smaller than the symbols.

absence of $CaCl_2$, removal of the PsbP and PsbQ components by NaCl-washing reduced the quantum yield to ~50% of control values. Subsequent removal of the PsbO protein via treatment with NaCl and urea further decreased the quantum yield to ~40% of control. In the presence of $CaCl_2$, however, both NaCl-washed and NaCl-urea-washed membranes exhibited quantum yields that were ~66% of the control values. These results indicate that while the PS II quantum efficiency is certainly affected by treatments that remove the extrinsic components, substantial PS II functionality is still present, both in the presence and in the absence of $CaCl_2$. The original data used to calculate the F_V/F_M are listed in Table S1 of the Supporting Information.

In our initial fluorescence decay experiments, we compared isolated thylakoid membranes with PS II membranes to examine any possible alterations in electron transfer kinetics induced by the TX-100 treatment used during PS II membrane isolation. Figure 1 compares the fluorescence decay observed in thylakoids and untreated PS II membranes both in the absence (Figure 1A) and in the presence (Figure 1B) of 10 μM DCMU. These experiments were performed in the presence of 10 mM $CaCl_2$ and 80 mM NaCl. Earlier, it had been shown that calcium depletion could affect electron transfer on both the oxidizing and reducing sides of the photosystem (*19*). Qualitatively, in the absence of DCMU, the fluorescence decay appeared slower in the PS II membranes than in the isolated thylakoid membranes. In the presence of DCMU, however, the fluorescence decay observed for both thylakoids and PS II membranes appeared quite similar. Quantitative evaluation of these decay curves was performed by fitting to a three-exponential component decay equation (*25*). These results are listed in Table 2.

In the absence of DCMU, the fastest decay component represents electron transfer from Q_A^- to Q_B (*28*, *29*). The intermediate decay component is associated with Q_A^- to Q_B electron transfer in reaction centers where plastoquinone must first be bound to the Q_B site prior to oxidation of Q_A^- (*30*). The

Table 2: Kinetic Parameters for Q_A^- Reoxidation after a Single Flash in Thylakoids and PS II Membranes in the Absence or Presence of DCMU ($n = 5$, ±1.0 SD)

	parameter[a]	thylakoid membranes	PS II membranes	NaCl-washed PS II membranes	NaCl-urea-washed PS II membranes
			Without DCMU		
fast phase	τ (ms)	0.9 ± 0.06	1.2 ± 0.1	14 ± 2[b]	8 ± 2[b,c]
	%	81 ± 1[b]	64 ± 3	34 ± 2[b]	29 ± 1[b]
intermediate phase	τ (ms)	16 ± 1[b]	40 ± 4	240 ± 30[b]	130 ± 40[b,c]
	%	13.7 ± 0.6[b]	19.5 ± 1.0	29.8 ± 0.4[b]	28 ± 2[b]
slow phase	τ (s)	3 ± 1	3.4 ± 0.2	5.0 ± 0.1[b]	13 ± 3[b,c]
	%	4.2 ± 0.5[b]	16 ± 2	33 ± 1[b]	31 ± 3[b]
residual	%	1.2 ± 0.1	1.0 ± 0.1	3.5 ± 0.6[b]	12 ± 1[b,c]
			With DCMU		
fast phase	τ (ms)	170 ± 10	150 ± 80	100 ± 4	22 ± 3[b,c]
	%	1 ± 5[b]	14 ± 4	20 ± 3	15 ± 3
intermediate phase	τ (s)	1.3 ± 0.3	1.4 ± 0.4	1.2 ± 0.3	0.7 ± 0.3
	%	51 ± 2	49 ± 5	28 ± 3[b]	12 ± 3[b,c]
slow phase	τ (s)	5.5 ± 0.6	9 ± 4	35 ± 7[b]	39 ± 19[b]
	%	37 ± 8	22 ± 9	14 ± 2[b]	30 ± 5
residual	%	1.4 ± 0.8[b]	14 ± 4	38 ± 3[b]	43 ± 8[b]

[a] τ is the exponential decay time constant, and % is the fraction of the total fluorescence decay attributable to each decay phase. [b] Comparison to intact PS II membranes; significantly different with a p of < 0.01 using the Student's t test. [c] Comparison between NaCl-washed and NaCl-urea-washed membranes; significantly different with a p of < 0.01 using the Student's t test.

slow decay component reflects Q_A^- charge recombination with oxidizing-side components (*31*). Finally, a residual fraction of long-lived fluorescence may be due to the equilibrium between Q_A^- and Q_B (*25*, *32*). In our experiments, the time constant for the fast phase was nearly identical in both thylakoids and PS II membranes, although the proportion of the fluorescence decay attributable to the fast phase was lower in the intact PS II membranes than in thylakoid membranes. This indicates that the rate of electron transfer from Q_A^- to Q_B, in PS II reaction centers containing plastoquinone in the Q_B site, was unaffected by the isolation of the PS II membranes. However, the occupancy of the Q_B site with plastoquinone may be lowered. It should be noted that the time constant that we observe for the fast decay phase is somewhat slower than that usually reported. This is due to the fluorescence decay experiment being performed at pH 6.0 in the presence of 100 mM NaCl. Both of these conditions have been shown to slow the rate of electron transfer from Q_A^- to Q_B (*33*−*35*). Both the time constant for the intermediate phase and the proportion of fluorescence decay attributable to this component increased significantly in the PS II membranes. This was fully expected, as thylakoid membranes contain ~7 photoreducible plastoquinones per PS II reaction center (*36*), while PS II membranes contain only ~2.5 (*37*). Consequently, fewer Q_B sites are occupied at the time of the flash by plastoquinone in the PS II membranes, and the time required for plastoquinone to diffuse to the Q_B site is also apparently increased. No increase in the time constant for the slow decay phase was observed, although the proportion of PS II reaction centers exhibiting the slow decay component did increase significantly, possibly indicating some modification on the oxidizing side of the photosystem. Finally, no alteration was observed in the residual fluorescence decay component. This indicates that the equilibrium between Q_A^- and Q_B was not affected by PS II membrane isolation. In large measure, these results are congruent with earlier studies.

In the presence of DCMU, which blocks electron transfer from Q_A^- to Q_B, fluorescence decay monitors charge recombination between Q_A^- and oxidizing-side components of the photosystem. Qualitatively, the initial stages of the fluorescence decay of the isolated thylakoid membranes were very similar to that of the PS II membranes. However, the latter portion of the decay curve for the PS II membranes indicates that there was some modification of the oxidizing side of the photosystem. For the quantitative evaluation of these fluorescence decay curves, the same three-exponential component decay model was used as described above (*25*). This was chosen, rather than alternative two-exponential decay models, because under some conditions, and for some mutants, the two-exponential decay model may be inadequate (T. M. Bricker, unpublished data). In this case, the fast component represents the fraction of PS II centers that lack a functional $Mn_4Ca_1Cl_{1-2}$ cluster, in which Q_A^- recombines with oxidized Y_z (*38*). The slow phase is associated with charge recombination between Q_A^- and the S_2 state (*39*), while the origin of the middle component is unclear (see ref *25* for a discussion). Finally, the residual component represents very slow charge recombination between Q_A^- and unidentified oxidizing-side components. These results are also listed in Table 2. No change was observed in either the time constants or the proportion of fluorescence decay associated with the intermediate and slow decay phases in the PS II membranes. The latter indicates that the charge recombination between Q_A^- and the S_2 state of the oxygen-evolving complex remains unchanged. No change in the time constant was observed for the fast phase of PS II membranes, although the proportion of the fluorescence decay associated with the fast phase did increase significantly. This indicates that the $Mn_4Ca_1Cl_{1-2}$ cluster may have been damaged in a proportion of the PS II reaction centers. Finally, there was a significant increase in the proportion of the residual fluorescence signal. These latter two observations indicate that the isolation of PS II membranes leads to a modest modification of the oxidizing side of the photosystem.

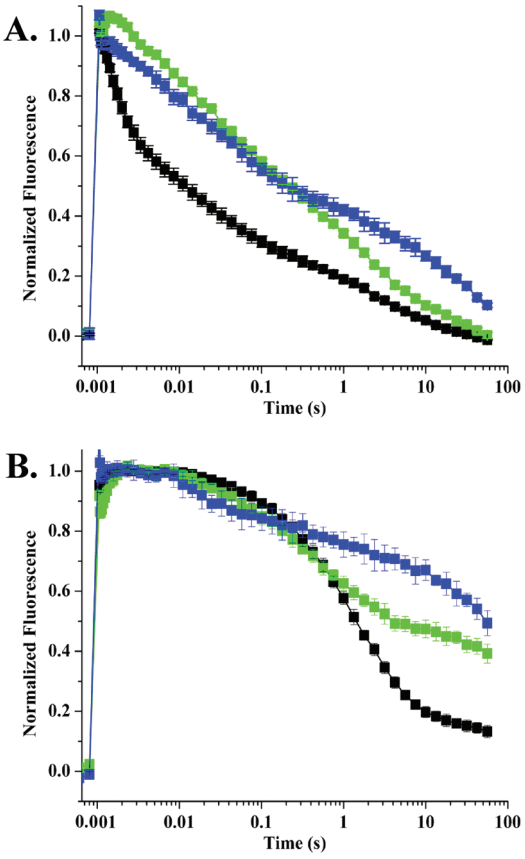

FIGURE 2: Q_A^- reoxidation kinetics after a single saturating flash in intact and salt-washed PSII membranes. Data were collected after dark incubation for 5 min. (A) Fluorescence decay in the absence of DCMU. (B) Fluorescence decay in the presence of 10 μM DCMU. Intact PSII membranes (black), NaCl-washed membranes (green), NaCl-urea-washed (blue). $n = 5$; error bars denote ± 1.0 SD. In some cases, the error bars are smaller than the symbols.

Treatment of PS II membranes with high concentrations of NaCl (> 1 M) removes the PsbP and PsbQ components (*40*), while treatment with 200 mM NaCl-2.6 M urea removes the PsbO, PsbP, and PsbQ components (*41*). The consequences of these treatments on the oxidizing side of PS II have been well-documented (for reviews, see refs *6*, *7*, *42*, and *43*). The effects of these treatments on the Q_A^- reoxidation kinetics are shown in Figure 2. In the absence of DCMU, qualitatively both the NaCl-treated membranes and the NaCl-urea-treated membranes exhibited fluorescence decay characteristics markedly slower than those observed for untreated membranes (Figure 2A). Quantitatively, the treated membranes exhibited significantly slower time constants for the fast and intermediate phases relative to those of the intact membranes, indicating slower electron transfer from Q_A^- to Q_B upon removal of the extrinsic proteins (Table 2). The fraction of the fluorescence signal associated with the fast decay decreased significantly in the treated samples, while the fraction exhibiting intermediate decay increased significantly.

These results were somewhat unexpected and indicate substantial alterations on the reducing side of the photosystem. Both the NaCl-washed membranes (lacking PsbP and PsbQ) and NaCl-urea-washed membranes (lacking PsbO, PsbP, and PsbQ) exhibited very similar decay characteristics for the fast and intermediate phases. This indicates that the removal of the PsbP and PsbQ proteins was responsible for the observed defects in the fluorescence decay. The treated membranes also exhibited an increased proportion of the slow phase decay component relative to the intact membranes, indicating significant defects on the oxidizing side of PS II in these samples, a result that was expected. Of the treated membranes, the NaCl-urea-washed membranes exhibited the largest time constant for this slow phase. The residual fluorescence signal increased significantly in the NaCl-washed and, in particular, in the NaCl-urea-washed membranes. This may indicate an alteration in the equilibrium between Q_A^- and Q_B in favor of the Q_A^- state.

Qualitatively, in the presence of DCMU, the sequential removal of the extrinsic proteins led to progressively slower fluorescence decay (Figure 2B). Quantitatively, the NaCl-urea-washed samples exhibited a large decrease in the time constant for the fast decaying phase (Table 2). This indicates an increased proportion of reaction centers in which the $Mn_4Ca_1Cl_{1-2}$ cluster was not fully functional. Both the NaCl-washed and NaCl-urea-washed membranes exhibited substantially larger time constants for the slow decaying phase and a higher proportion of residual fluorescence. These results indicate that removal of the extrinsic proteins significantly slows charge recombination between Q_A^- and the S_2 state (*9*) and other oxidizing-side components. While removal of the PsbP and PsbQ proteins results in slower fluorescence decay, the absence of all three extrinsic proteins displays the most severely affected fluorescence decay kinetics. These results were expected given the large body of literature documenting the function of the PsbO, PsbP, and PsbQ proteins on the oxidizing side of PS II (*6*, *7*).

Our findings clearly demonstrate significant PS II reducing-side defects upon removal of the PsbP and PsbQ proteins. It is unclear at this time if the reducing-side defects we observed in vitro were due directly to the loss of the PsbP and PsbQ components or result from the salt-washing procedures necessary to remove these components. It must be pointed out, however, that we have observed very similar reducing-side defects, in vivo, upon RNAi suppression of PsbP expression. In these experiments, PS II was never exposed to high salt concentrations. The RNAi-P1 mutant, for instance, contained no detectable PsbP or PsbQ proteins, exhibited an F_V/F_M that was 78% of that observed for the wild type, and exhibited a significant slowing of electron transfer from Q_A^- to Q_B (*13*). The equilibrium between Q_A^- and Q_B was also shifted significantly in favor of Q_A^- in this mutant.

Other investigators have also observed alterations to the reducing side of the photosystem that correlate with the removal of PsbP and PsbQ. First, the Q_B site, which is located on the D1 protein and exposed at the stromal surface of PS II, has been shown to be modified after the removal of the PsbP and PsbQ proteins using a NaCl treatment. The level of atrazine binding at the modified Q_B site was markedly increased in the absence of the PsbP and PsbQ proteins (*17*) but could be partially reversed by millimolar concentrations of calcium. Removal of these components also led to a loss of the period-two oscillations normally associated with Q_A to Q_B electron transfer (*18*). It was also shown that upon removal of PsbP and PsbQ, calcium was readily lost from the oxygen-evolving site (*19*), leading to electron transport defects on both the oxidizing and reducing sides of the photosystem. In this study, a K_d for calcium was determined to be ~50 μM. All of the calcium-related defects on the reducing side of the photosystem were abolished with the addition of ~1 mM calcium. In our studies, large reducing-side defects persisted even in the presence of 10 mM calcium.

Since the PsbP and PsbQ components are located on the lumenal face of PS II, how could their removal affect the Q_A and Q_B sites, which are located on the stromal face of the photosystem? One possibility is that the removal of these extrinsic proteins leads to structural alterations in membrane-spanning components of the photosystem. Indeed, image analysis of PS II supercomplexes of higher plants from which the PsbP and PsbQ components were removed indicated that CP29 moves 1.2 nm toward the central core of the PS II complex (*44*). This suggests that removal of the PsbP and PsbQ components from the lumenal face of the complex may induce transmembrane alterations in the structure of PS II, possibly disrupting the Q_A and/or Q_B sites or modifying the plastoquinone−plastoquinol exchange channel (*45*).

CONCLUSIONS

Removal of the PsbP and PsbQ components by salt washing, in vitro, modifies both the reducing and oxidizing sides of PS II. Similar results from our laboratory had been obtained previously, in vivo, by RNAi suppression of PsbP expression, which leads to the loss of both the PsbP and PsbQ proteins. Our results demonstrate that studies attributing defects observed upon PsbP and PsbQ removal solely to the oxidizing side must be viewed with caution.

SUPPORTING INFORMATION AVAILABLE

One supplementary table. This material is available free of charge via the Internet at http://pubs.acs.org.

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