Functional Analysis of Photosystem II in a PsbO-1-Deficient Mutant in *Arabidopsis thaliana*†

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ABSTRACT: The *Arabidopsis thaliana* mutant *psbo1* (formerly the mutant LE18-30), which contains a point mutation in the *psb*O-1 gene leading to defective expression of the PsbO-1 protein, has recently been described [Murakami, R. et al. (2002) *FEBS Lett. 523*, 138−142]. This mutant completely lacks the PsbO-1 protein and overexpresses the PsbO-2 protein. To further study the effect of PsbO-1 deficiency on the function of photosystem II, the polyphasic chlorophyll *a* fluorescence rise and flash fluorescence induction and decay of the relative fluorescence quantum yield were measured in whole leaves from wild type and the *psbo1* mutant. Additionally, flash oxygen yield experiments were performed on thylakoid membranes isolated from wild type and the *psbo1* mutant. The results obtained indicate that during fluorescence induction the *psbo1* gene exhibited an enhanced O to P transition. Additionally, while the J to I transition in wild type accounted for more than 30% of the total fluorescence yield, in the mutant it accounted for less than 2% rise in the total. Analysis of the flash-induced fluorescence rise in the presence of DCMU indicated that in wild type the ratio of PS II_α to PS II_β reaction centers was ∼1.2 while in the mutant the ratio was ∼0.3. Fluorescence decay kinetics in the absence of DCMU indicated that electron transfer to Q_B was significantly altered in the mutant. Fluorescence decay kinetics in the presence of DCMU indicated that the charge recombination between Q_A^- and the S_2 state of the oxygen-evolving complex was retarded. Furthermore, flash oxygen yield analysis indicated that both the S_2 and S_3 states exhibited significantly longer lifetimes in the *psbo1* mutant than in wild type. Our data indicate that while PsbO-1-deficient plants can grow photoautotrophically (although at a reduced growth rate) the photochemistry of PS II is significantly altered.

Photosystem II (PS II)[1] functions as a light-driven, water-plastoquinone oxidoreductase. In higher plants and cyanobacteria, at least six intrinsic proteins appear to be required for O_2 evolution (*1*−*3*). These are CP47, CP43, the D1 protein, the D2 protein, and the α and *β* subunits of cytochrome b_{559}. Additionally, in higher plants, three extrinsic proteins, with apparent molecular masses of 33 (PsbO), 24 (PsbP), and 16 kDa (PsbQ), are also required for maximal rates of O_2 evolution at physiological inorganic cofactor concentrations. Of these three proteins, the PsbO protein appears to play a central role in the stabilization of the manganese cluster and is essential for efficient and stable O_2 evolution.

In *Arabidopsis thaliana*, two genes which encode PsbO (*psb*O-1, At5g66570, and *psb*O-2, At3g50820) are expressed under normal growth conditions, yielding two different PsbO proteins (PsbO-1 and PsbO-2, respectively). These have been identified on two-dimensional gels of lumenally localized proteins (*4*,*5*) and are also resolved via one-dimensional SDS−PAGE (*6*). It is unclear what advantage the plant would accrue from two copies of the PsbO component. A high fluorescence mutant, *psbo1*, was recently identified in which a stop codon had been introduced into the *psb*O-1 gene by EMS mutagenesis at amino acid residue 74 of the mature PsbO protein (^{74}Gln → Stop), which led to the loss of this component (*6*). The mutant exhibited a lower F_V/F_M, lower rates of steady state O_2 evolution, and retarded growth. It was demonstrated that PsbO-1 is the major isoform in wild type under normal growth conditions and that in the *psbo1* mutant the PsbO-2 protein is upregulated in a compensatory manner. Interestingly, the increased expression level of the PsbO-2 in the mutant appeared to be less than the total amount of PsbO-1 and PsbO-2 in wild type, at least in young leaf tissue. The mechanism which leads to the increased level of expression of the PsbO-2 protein in the *psbo1* mutant is unclear at this time. While these authors concluded that in mature leaves the total amount of PsbOs in the *psbo1* mutant is smaller than in wild type, analysis of the data presented in Figure 2B of ref *7* indicates that no statistically significant difference exists. Domain- swapping analysis followed by in vitro reconstitution experiments were interpreted as indicating that three amino acid differences between the

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[1] Abbreviations: BSA, bovine serum albumin; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EDTA, ethylenediaminetetraacetic acid; EMS, ethane methyl sulfonate; MS media, Murashige and Skoog media; PS II, photosystem II; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; Tricine, *N*-tris(hydroxymethyl)-methylglycine.

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PsbO-1 and PsbO-2 components ($^{186}V \rightarrow {}^{186}S$, $^{204}V \rightarrow {}^{204}I$, and $^{246}L \rightarrow {}^{246}I$) could explain the functional differences between the two PsbO proteins (*7*). These authors concluded that both the lower expression level of the PsbO-2 protein and inherent functional defects of this component are responsible for the phenotype observed in the mutant *psbo1*.

Although data from these studies demonstrated that lower O_2 evolution activity occurs upon the binding of PsbO-2 in vivo (*6*) and in vitro (*7*), the mechanistic details of the mutants' photochemistry are unclear. In this study, we have used fluorescence induction and decay experiments and flash oxygen yield analysis to characterize the effects of the loss of the PsbO-1 protein on PS II function in the *psbo1* mutant. Our findings indicate that significant alterations in PS II photochemistry result from the loss of the PsbO-1 protein.

MATERIALS AND METHODS

Plant Materials. Surface-sterilized seeds of wild type *A. thaliana* (var. *Landsberg erecta*) and the mutant *psbo1* were germinated on solid MS medium (*8*) containing 0.7% (w/v) agar after cold treatment for 24 h at 4 °C. The seedlings were transferred to soil 10 days later and grown at 22 ± 0.5 °C under 50−80 μmol photons m^{-2} s^{-1} of white light. Only fully expanded rosette leaves of 4−6 week-old plants were used. The leaves were dark-adapted for at least 5 min prior to measurements.

Fluorescence Experiments. Fluorescence induction was monitored with a Photon Systems Instruments (PSI) FL3000 dual modulation kinetic fluorometer (commercial version of the instrument described in ref *9*). Both measuring and saturating flashes are provided by computer-controlled photodiode arrays. The flash profile exhibited a square shape for low power measuring flashes and only deviated 5% from an ideal square shape for saturating actinic flashes. For all of the fluorescence experiments, single leaves from wild type and *psbo1* were excised and dark-incubated for 5 min before initiation of the experiments. In the standard fluorescence induction experiments (Kautsky experiments), data were collected in a logarithmic time series between 1 ms and 4 s after the onset of strong actinic light. In the flash fluorescence induction experiments, the kinetics of the rapid fluorescence rise following a single saturating flash delivered by light-emitting diodes were examined for 50 μs with a time resolution of 1 μs in the presence of DCMU. Data were collected at a frequency of 10 MHz with 12 bit resolution. Proportions of PS II_α and PS II_β centers were calculated using proprietary PSI software (*9*). In the fluorescence decay experiments, the kinetics of the transfer of an electron between Q_A^- and Q_B were examined in the absence of DCMU, while the recombination reactions of Q_A^- with PS II donor-side components were examined in the presence of DCMU. For these experiments, data were collected between 150 μs and 60 s following a single saturating flash. Data were analyzed using the equations outlined in ref *10*. In this mathematical treatment, three exponential decay components and a long-lived (essentially nondecaying) residual component were included. In the DCMU treatment experiments, the leaves were immersed in 40 μM DCMU and 0.1% Tween 20 in water for 30 min prior to performance of the fluorescence experiments. Data were analyzed using Origin version 6.1 and proprietary software provided by Photon Systems Instruments.

Flash Oxygen Yield Experiments. These studies were performed on thylakoid membranes isolated from wild type and *psbo1* plants. Thylakoids were isolated by grinding two to five leaves in a glass homogenizer with 1.0 mL of a buffer containing 0.45 M sorbitol, 10 mM EDTA, 0.1% BSA, 1% polyvinylpyrrolidone, and 20 mM Tricine-NaOH (pH 8.4). The homogenate was filtered through one layer of Miracloth, and the thylakoids pelleted by centrifugation at 4 °C and 16000 × *g* in a microcentrifuge. Flash oxygen yield measurements were performed on a bare platinum electrode (Artesian Scientific Co., Urbana, IL). Flashes were supplied by an integrated, computer-controlled xenon flash lamp (20 μs). For the measurements of S-state distributions and S-state parameters, thylakoids were pelleted and applied to the electrode as a thin paste. The thylakoids were then incubated for 5 min in the dark; the electrode was polarized at 0.73 V for 10 s, and a series of saturating flashes was applied. Data points were collected at 500 μs intervals during the duration of the flash train. The data were analyzed using a four-state, homogeneous model (*11*). Five- and six-state models which incorporated either an S_{-1} state or S_{-1} and S_{-2} states, respectively, uniformly failed to fit the data acquired from either wild type or mutant thylakoids.

RESULTS AND DISCUSSION

Fluorescence Induction. Figure 1 shows the chlorophyll *a* fluorescence rise observed in the *A. thaliana* (*Landsberg*) wild type and in the *psbo1* mutant leaves. The normalized fluorescence yield is shown. Using a logarithmic timing series, a polyphasic fluorescence rise exhibiting the O-J-I-P transients was observed for wild type sample (*12*, *13*). An inspection of the curves shown in Figure 1A reveals several interesting features. First, the O−J transition occurs with a higher fluorescence yield in the *psbo1* mutant than in wild type. The initial O−J transition constitutes the photochemical phase of the chlorophyll *a* fluorescence rise. This result indicates that electron transfer from Q_A^- to Q_B is slowed in the mutant. This would result in an increased level of accumulation of Q_A^- and consequently an increased fluorescence yield. This result is similar to, but less extreme than the effects of treatment with DCMU on the fluorescence induction curve (*14*). Second, in wild type the J and I transients appeared at 4 and 30−50 ms, respectively; however, in *psbo1* there was no clearly distinct I transient. In wild type, the J−I transition (4−30 ms) accounted for 30% of the total fluorescence yield. However, in *psbo1* during this same time period, the fluorescence yield accounted for less than 2% of the total fluorescence signal. This result may indicate a defect in the oxygen-evolving complex. A similar decrease in magnitude of the J−I transition has been observed in treatments which damage the oxygen-evolving complex, such as mild Tris or heat treatment (*15*). Alternatively, the absence of the J−I transition may indicate a defect in the ability to form the $Q_A^-Q_B^-$ state (*13*). It should be noted that these two explanations are not mutually exclusive and both could, in principle, contribute to the loss of the J−I transition in the mutant. In both wild type and *psbo1*, the magnitude of the fluorescence signal started to rise at 30 ms, with both strains reaching the P level in 100−200 ms. Overall, the total fluorescence yield of the thermal phase (J−P transition) is reduced in *psbo1* (32% in *psbo1* vs 48% in wild type), while the photochemical

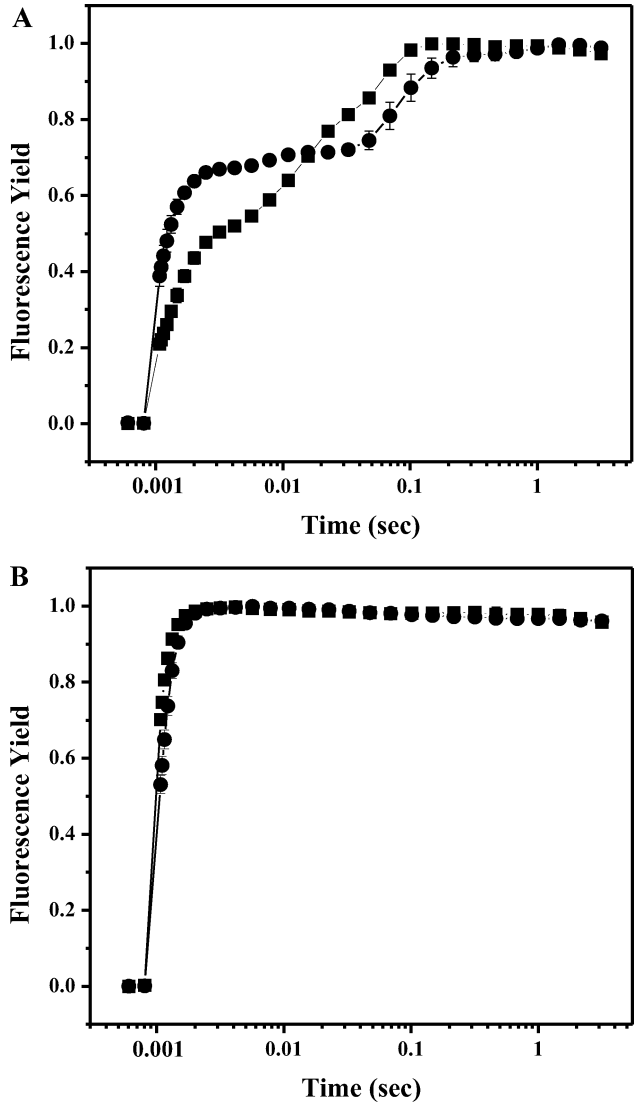

FIGURE 1: Fluorescence induction of wild type and *psbo1* plants. Data were collected after dark incubation for 5 min. (A) Fluorescence induction in the absence of DCMU. (B) Fluorescence induction in the presence of DCMU: (■) wild type and (●) *psbo1*. $n = 3$ (error bars, ±1.0 standard deviation); in some instances, the error bars are smaller than the symbols.

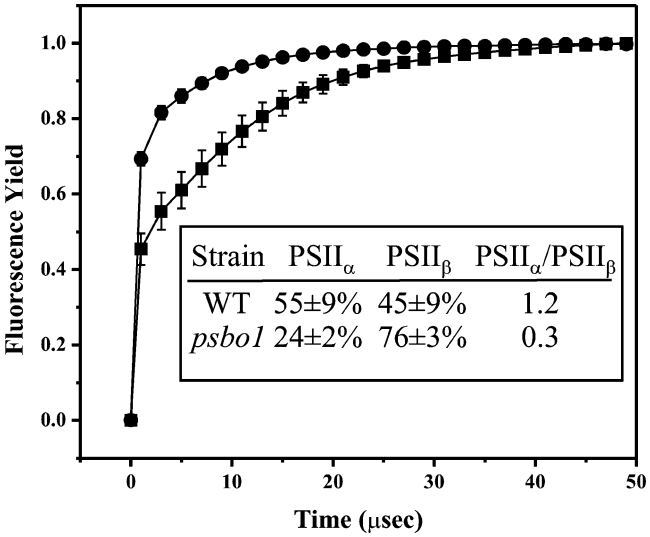

FIGURE 2: Fluorescence induction following a single saturating flash in the presence of DCMU. Data were collected after dark incubation for 5 min. Please note the different time scales used in this figure vs that in Figure 1B. The inset shows the amounts of PS II$_\alpha$ and PS II$_\beta$ reaction centers present in the wild type (WT) and *psbo1* based on this flash fluorescence induction data: (■) wild type and (●) *psbo1*. $n = 3$ (error bars, ±1.0 standard deviation); in some instances, the error bars are smaller than the symbols.

phase (O−J transition) is increased in the mutant (68% in *psbo1* vs 52% in wild type). In the presence of DCMU, both wild type and *psbo1* reached maximal fluorescence at approximately the same position as the J transient in the sample without DCMU (Figure 1B).

Flash Fluorescence Induction. Figure 2 shows the flash-induced fluorescence induction at a 1 μs time resolution in the presence of DCMU. The normalized variable fluorescence yield is shown. In this experiment, electron transfer between Q_A^- and Q_B is abolished by the presence of DCMU. The observed fluorescence rise is the result of the re-reduction by the oxygen-evolving complex of Y_Z, which is in equilibrium with the primary electron donor P_{680}^+ (*40*). The shape of this fluorescence rise contains information bearing on the amount of PS II$_\alpha$ and PS II$_\beta$ reaction centers present in the sample. Exponential fluorescence rise kinetics indicate that the antennae of individual PS II centers are not coupled (i.e., a characteristic of PS II$_\beta$ centers), while sigmoidal fluorescence rise kinetics indicate a high degree of interconnectivity between the antennae of PS II centers (i.e., a characteristic of PS II$_\alpha$ centers) (*41*). This is true under conditions of both continuous relatively weak light illumination (slow fluorescence induction) and single saturating flash conditions (fast fluorescence induction) as demonstrated by Nedbal et al. (*9*).

This experiment allows the determination of the relative proportions of PS II$_\alpha$ and PS II$_\beta$ reaction centers (*9*) (Figure 2, inset). In addition to the differences noted above, PS II$_\beta$ centers have a smaller antenna size, are enriched in chlorophyll *a*, and are depleted of the light-harvesting chlorophyll proteins. They appear to be principally located in the stromal thylakoid membranes (*16*) and at the grana margins (*17*). Additionally, PS II$_\beta$ centers appear to be defective in their ability to transfer electrons from Q_A^- to Q_B (*18*). In wild type, we find the ratio of PS II$_\alpha$ to PS II$_\beta$ to be 1.2. This value is similar to those obtained in other studies (*19*−*21*). In *psbo1*, however, there is a marked enrichment of PS II$_\beta$ centers, with the PS II$_\alpha$ to PS II$_\beta$ ratio being 0.32. The high proportion of PS II$_\beta$ centers which we observe may indicate an increased rate of metabolic turnover of PS II in the mutant, as conditions which increase the rate of photoinactivation also lead to increases in the amount of PS II$_\beta$ centers that are observed (*18*).

Fluorescence Decay Kinetics. Additional information concerning the electron transfer characteristics on both the reducing and oxidizing sides of the photosystem was obtained by examining Q_A^- reoxidation kinetics in either the absence or the presence of DCMU. These results are shown in Figure 3. In the absence of DCMU (Figure 3A), the fluorescence decay after a single saturating flash can be resolved into three exponential components (*10*). The fastest (and dominant) exponential decay component which we observed for wild type is related to the transfer of an electron from Q_A^- to Q_B (220 μs, 46%) (see Table 1). The middle exponential decay component (0.88 ms, 25%) is associated with transfer of an electron from Q_A^- to Q_B in reaction centers which have to bind plastoquinone to the Q_B site before Q_A^- oxidation can

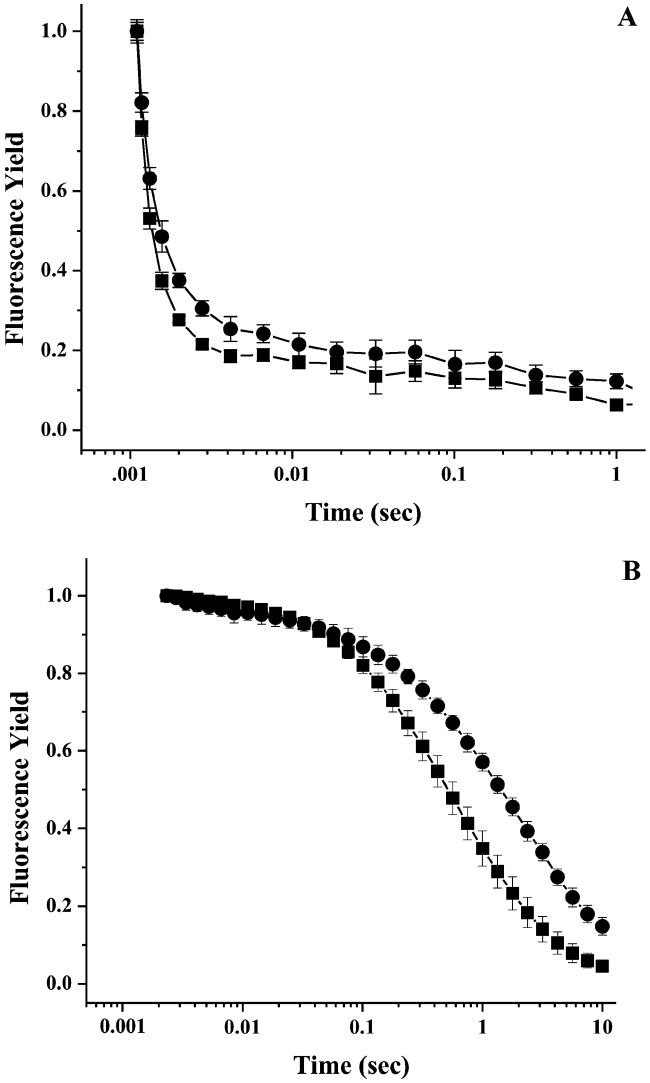

FIGURE 3: Q_A^- reoxidation kinetics following a single saturating flash of wild type and *psbo1* plants. Data were collected after dark incubation for 5 min. (A) Fluorescence decay in the absence of DCMU. (B) Fluorescence decay in the presence of 40 μM DCMU. Please note the different time scales used in panels A and B. (■) wild type and (●) *psbo1*. $n = 6-8$ (error bars, ±1.0 standard deviation); in some instances, the error bars are smaller than the symbols.

Table 1: Kinetic Parameters of Q_A^- Reoxidation after a Single Flash in Untreated Leaves from Wild Type and the *psbo1* Mutant of *A. thaliana*

kinetic component[a]	wild type	*psbo1*
fast phase [τ (μs)/% amplitude]	$220 \pm 25^{b,c}/46 \pm 4^c$	$401 \pm 36^c/54 \pm 4^c$
middle phase [τ (ms)/% amplitude]	$0.88 \pm 0.12^c/25 \pm 3^c$	$3.9 \pm 1.6^c/13 \pm 3^c$
slow phase [τ (s)/% amplitude]	$0.69 \pm 0.06/18 \pm 0.9^c$	$0.76 \pm 0.06/20 \pm 1^c$
residual [τ (s)/% amplitude]	$>10/11 \pm 1^c$	$>10/15 \pm 2^c$

[a] $n = 6-8$. [b] Error, ±1.0 standard deviation. [c] $p < 0.01$ (Student's *t*-test).

occur. The slowest decay component (690 ms, 18%) is related to a charge recombination reaction in which the reoxidation of Q_A^- occurs with donor-side components. Finally, a residual fraction of the fluorescence yield (~11%) is very long-lived and may result from the equilibrium between Q_A^- and Q_B (*22*). For the *psbo1* mutant, the time constant for the fast phase increased from 220 to 401 μs. This indicates that electron transport from Q_A^- to Q_B is somewhat slowed in the mutant. Additionally, the time constant for the middle exponential decay component increased from 0.88 to 3.9 ms in the mutant. This large increase may be related to the large proportion of PS II$_\beta$ reaction centers present in the mutant. PS II$_\beta$ reaction centers are localized principally in the stromal thylakoid membranes. Since the stromal membranes are deficient in photochemically reducible plastoquinone (*23*), binding of free plastoquinone to the Q_B site would be expected to be slowed in the *psbo1* mutant. Additionally, the time constant for the slow decay component increased slightly from 690 to 760 ms. This change was not significant. The residual amplitude (for the decay component(s) with a τ of >10 s) also increased significantly, perhaps indicating a change in the $Q_A^-Q_B$ equilibrium. Overall, these results indicate that the principal modification on the reducing side of the photosystem observed in the mutant, the large increase in the time constant for the middle exponential decay component, can be explained by the high proportion of PS II$_\beta$ centers present in the mutant.

Table 2: Kinetic Parameters of Q_A^- Reoxidation after a Single Flash in DMCU-Treated Leaves from Wild Type and the *psbo1* Mutant of *A. thaliana*

kinetic component[a]	wild type	*psob1*
fast phase [τ (ms)/% amplitude]	$65 \pm 26^{b,c}/3 \pm 1$	$11 \pm 11^c/3 \pm 1$
middle phase [τ (ms)/% amplitude]	$483 \pm 5/38 \pm 4^c$	$398 \pm 133/15 \pm 4^c$
slow phase [τ (s)/% amplitude]	$2.38 \pm 0.35^c/53 \pm 6^c$	$3.6 \pm 0.5^c/65 \pm 3^c$
residual [τ (s)/% amplitude]	$>10/6 \pm 2^c$	$>10/17 \pm 4^c$

[a] $n = 6-8$. [b] Error, ±1.0 standard deviation. [c] $p < 0.01$ (Student's *t*-test).

In the presence of DCMU, which prevents the transfer of an electron from Q_A^- to Q_B, the decay of fluorescence following a saturating flash is dominated by charge recombination between Q_A^- and the oxidizing-side components of the photosystem. Figure 3B illustrates the fluorescence decay kinetics of wild type and *psbo1* in the presence of DCMU. The observed fluorescence decay curves were fit to the same model described above (*24*). Significant alterations are observed in the mutant. It should be noted that alternative models containing two exponential decay components and a hyperbolic component (*24*), or only two exponential decay components, failed to adequately fit the data. Table 2 shows the kinetic parameters obtained for the fluorescence decay in the presence of DCMU. The fastest decaying component observed in wild type exhibited a time constant of 65 ms and is attributed to a small fraction of PS II reaction centers (<5%) that lack a functional manganese cluster (*25*), in which Q_A^- recombines with oxidized Y_Z. The slowest decay component observed for wild type exhibited a time constant of 2.4 s and is attributed to charge recombination between Q_A^- and the S_2, and possibly the S_3, states (*26*). The origin of the intermediate decay component observed in wild type (483 ms) is unclear, although it may represent a subfraction of reaction centers in which the rate of charge recombination between Q_A^- and the S_2 state is

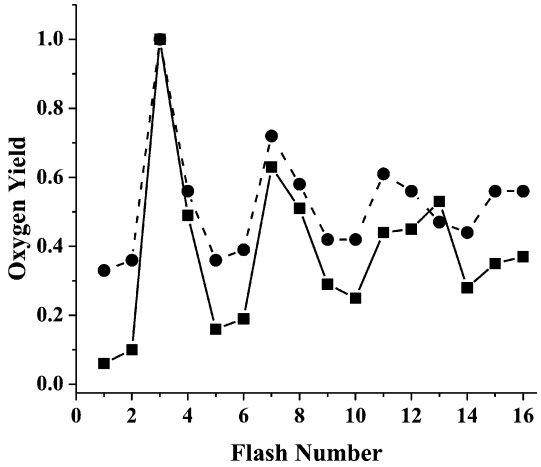

FIGURE 4: Typical flash oxygen yield experiments for wild type and *psbo1* thylakoid membranes. Data were collected after dark incubation for 5 min. Flash oxygen yields were normalized to the oxygen yield observed on the third flash. Note the high oxygen yield observed on the first and second flashes for *psbo1* thylakoid membranes: (■) wild type and (●) *psbo1*. $n = 3$ (error bars, ±1.0 standard deviation); in some instances, the error bars are smaller than the symbols.

4−5-fold faster than the 2.4 s component (*10*). In the mutant *psbo1*, the slowest decay component was observed to have a time constant of 3.6 s, and its amplitude increased from 53 to 65%. This result indicates that the S_2 state and possibly the S_3 state are more stable in the *psbo1* mutant than in wild type. Little change was observed for the time constant of the intermediate decay component, although its amplitude decreased from 38% in wild type to 15% in the mutant. The residual decay component ($\tau > 10$ s) increased nearly 3-fold in amplitude. These three decay components accounted for more than 95% of the total fluorescence signal. A large difference was observed for the minor (<5%) rapidly decaying component. The time constant for this decay component decreased from 65 ms in wild type to 11 ms in the mutant. The origin of this 6-fold rate increase is very unclear at this time. The small amplitude of the signal makes it difficult to mathematically extract the kinetic parameters for this component. Consequently, the observed rapid time constant may be an artifact.

Flash Oxygen Yield Experiments. To obtain additional information about the donor side of PS II in the *psbo1* mutant, flash oxygen yield experiments were performed. Figure 4 shows the results of these studies. Wild type *Arabidopsis* thylakoids exhibited a normal flash oxygen yield series with relatively low oxygen yields on the first and second flashes. This indicates that after dark incubation for 5 min relatively few PS II reaction centers were in the S_3 and S_2 states. The large oxygen yield on the third flash indicates that most of the reaction centers were in the dark-stable S_1 state. In the *psbo1* mutant, however, a significantly different flash oxygen yield pattern was observed. Significant amounts of oxygen were released on the first and second flashes. This result indicates qualitatively that an increased number of oxygen-evolving reaction centers were in the S_3 and S_2 states. Analysis of the flash oxygen yield pattern using a four-state homogeneous model (*11*) quantitatively confirmed this observation (Table 3). It should be noted that S-state transition models incorporating S_{-1} or S_{-1} and S_{-2} states (*27*) uniformly failed to fit the data. After dark incubation for 5 min, the mutant exhibited a significantly higher proportion of oxygen-evolving centers in the S_2 and S_3 states and a significantly lower proportion of centers in the S_1 state. These changes were not associated with alterations of the S-state parameters, as no significant differences were observed in single hits, double hits, misses, or deactivations. Our results indicate that the oxygen-evolving apparatus in *psbo1* PS II is altered and that the S_2 and S_3 states are long-lived.

Table 3: S-State Distributions and Parameters for Wild Type and the *psbo1* Mutant of *A. thaliana*

S-state distribution[a]	wild type	psbo1
S_0	17.6 ± 2.4[b]	18.6 ± 1.8
S_1	74.8 ± 10.8[c]	53.5 ± 10.8[c]
S_2	2.9 ± 7.0[c]	13.0 ± 4.2[c]
S_3	3.1 ± 4.8[c]	15.0 ± 6.6[c]
S-state parameter[a]	**%**	**%**
misses	10.2 ± 1.2	9.9 ± 1.4
single hits	85.0 ± 1.9	85.3 ± 1.8
double hits	2.8 ± 0.5	3.0 ± 0.2
deactivations	0.8 ± 0.9	0.4 ± 0.8

[a] $n = 4$. [b] Error, ±1.0 standard deviation. [c] $p < 0.05$ (Student's *t*-test).

In spinach, which possesses only a single PsbO protein isoform, chemical removal of this component from PS II membranes leads to increased lifetimes for the S_2 and S_3 states (*28*). Similar results are obtained for the cyanobacterium *Synechocystis* in mutants from which the PsbO protein had been deleted (*2, 29*) and in mutants which could not bind PsbO normally (*30*). Our observation that the *psbo1* mutant, which lacks PsbO-1, exhibits long-lived S_2 and S_3 states appears to indicate that the PsbO-2 protein which is present is defective in supporting normal S-state turnover. It should be noted that this observed defect does not appear to be the result of the overall smaller amount of the total amount of PsbOs present in the *psbo1* mutant as previously asserted (*7*), at least for mature leaves (such as those used in this study). Examination of Figure 2B of ref *7* demonstrates that there is no statistically significant difference in the total amounts of PsbOs in wild type versus the *psbo1* mutant even though these workers conclude otherwise. In vitro reconstitution of saturating amounts of PsbO-2 protein onto PsbO-depleted PS II membranes restored steady-state oxygen evolution rates to only ~75% of that of wild type (*7*). These workers also demonstrated that both the PsbO-1 and PsbO-2 proteins bound to PS II membranes with similar affinity. In our hands (Figure 1), the fluorescence quantum yield for wild type is 0.8 and for *psbo1* is 0.6 (i.e., the mutant quantum yield is 75% of that exhibited by wild type), which is consistent with the results reported in ref *7*.

CONCLUSIONS

The *psbo1* mutant exhibits significant defects in PS II photochemistry. Our fluorescence induction measurements indicate that the photochemical phase of the induction curve is enhanced in the mutant, while the initial component of the thermal phase (i.e., I−J transition) is nearly absent. This indicates a probable defect in the water-oxidizing complex. Fast fluorescence induction measurements in the presence of DCMU indicate that the mutant accumulates significantly more PS II_β reaction centers than wild type does.

Alterations in fluorescence decay kinetics either in the absence or presence of DCMU are also evident. In the absence of DCMU, the time constant of the middle decay component is increased 4-fold when compared to that of the wild type. This indicates that transfer of an electron from Q_A^- to Q_B is retarded in PS II reaction centers in which the Q_B site is initially unoccupied by plastoquinone. This is consistent with the high proportion of PS II_β reaction centers present in the mutant. Because of their stromal thylakoid location, these centers have access to less photochemically reducible plastoquinone than do the granal thylakoid-localized PS II_α reaction centers. The fast components of the fluorescence decay curve are also retarded, although to a degree much smaller than that observed for the middle decay component. The residual amplitude (for the decay component(s) with a τ of >10 s) also increased significantly, perhaps indicating a change in the $Q_A^-Q_B$ equilibrium. In the presence of DCMU, the fast component of the fluorescence decay curve is greatly accelerated (16-fold) compared to that of wild type, although this represents a very minor component of the entire decay curve (3−4%). The cause of this acceleration is unclear at this time. The time constant for the slow component of the decay curve, and its amplitude, is increased in the mutant. Additionally, the residual amplitude (for the decay component(s) with a τ of >10 s) increased nearly 3-fold. These results indicate that charge recombination between Q_A^- and the oxidizing side of the photosystem is significantly retarded.

Finally, our flash oxygen yield experiments demonstrated that after dark incubation a significantly larger number of oxygen-evolving PS II reaction centers in the *psbo1* mutant were in the S_2 and S_3 states when compared to wild type. This indicates that the S_2 and S_3 states are long-lived in the mutant. No differences are observed in the various S-state parameters (single hits, misses, double hits, or deactivations). Our working hypothesis is that the stabilization of the S_2 and S_3 states in the *psbo1* mutant increases the likelihood that damaging oxidizing-side radical(s), most likely P_{680}^+ (*31*) and/or oxidized Y_Z (*32*), will accumulate. Conditions which increase S-state lifetimes appear to uniformly increase the sensitivity to photoinhibition; these include chloride depletion (*32*), calcium depletion (*33*, *34*), and the loss of PsbO (*35*). The presence of long-lived S_2 and S_3 states in *psbo1* would lead to an increased rate of damage to the photosystem and an increased rate of conversion of PS II_α to PS II_β reaction centers as a prelude to photosystem disassembly and subsequent repair (*36*).

It has been suggested that the PsbO-2 protein plays a specific role in regulating the phosphorylation state of PS II reaction center components (*37*). Specifically, it was hypothesized that the presence of PsbO-2 leads to the dephosphorylation of the D1 protein and that this dephosphorylation targets PS II reaction centers for degradation and subsequent repair. These investigators concluded that the PsbO-2 protein regulates the turnover of D1. Our view is that this regulatory role for PsbO-2 is unlikely and that the observed dephosphorylation is a result of an increased level of photodamage to PS II due to the inability of PsbO-2 to support normal S-state advancement in the oxygen-evolving complex.

Since there are very few amino acid differences between PsbO-1 and PsbO-2, what is the molecular basis for the increased S_2- and S_3-state lifetimes in the *psbo1* mutant? This question is difficult to answer at this time. Since the PsbO-1 and PsbO-2 proteins exhibit similar affinities for photosystem II (*7*), it would appear that the increased S-state lifetimes are due to intrinsic features of the PsbO-2 protein and not to defects in the binding of this component to PS II. We have earlier hypothesized that the PsbO protein functions in maintaining the chloride associated with oxygen evolution in the vicinity of the oxygen-evolving site (*38*). This was principally based on the observation that the removal of the PsbO protein, either chemically or by mutagenesis, yielded a phenotype which was very similar to that observed upon chloride depletion. Recently, direct experimental support for this hypothesis has been published (*39*). We hypothesize that the amino acid differences between PsbO-1 and PsbO-2 (i.e., $^{186}V \rightarrow {}^{186}S$, $^{204}V \rightarrow {}^{204}I$, and/or $^{246}L \rightarrow {}^{246}I$), identified previously (*7*) in domain-swapping experiments, may disrupt the ability of the PsbO-2 protein to maintain chloride in the vicinity of the oxygen-evolving site without altering the protein's binding characteristics. We are currently testing this hypothesis.

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