$Y_z^{\bullet}$ Reduction Kinetics in the Absence of the Manganese-Stabilizing Protein of Photosystem II[†]

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Received January 21, 1997; Revised Manuscript Received September 9, 1997[⊗]

ABSTRACT: Decay of Signal II_{vf} of photosystem II (PSII), under repetitive flash conditions, was examined in whole cells of wild-type *Synechocystis* sp. PCC6803 and in cells of an engineered strain, ΔpsbO, which lacks the extrinsic 33 kDa manganese-stabilizing protein (MSP). Previous polarographic analysis had shown that O_2 release during the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition of the catalytic cycle is significantly retarded in the ΔpsbO strain relative to the wild-type [Burnap et al. (1992) *Biochemistry 31*, 7404−7410]. The present experiments provide evidence that a parallel retardation in the rate of reduction of photooxidized Y_z by the H_2O oxidation complex is due to the absence of MSP. The half-time of the Signal II_{vf} component, corresponding to $Y_z^{\bullet}$ reduction during the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition, was estimated to be 1.2 and 6.0 ms in the wild-type and ΔpsbO cells, respectively.

Photosynthetic H_2O oxidation in plants, algae, and cyanobacteria is catalyzed by the photosystem II (PSII)[1] reaction center complex. During H_2O oxidation, four electrons are extracted from two molecules of H_2O, yielding O_2 as a byproduct [for reviews, see Britt (1996), Debus (1992), and Diner and Babcock (1996)]. During the catalytic cycle of H_2O oxidation, the enzyme passes through a series of intermediate oxidation states termed S states (S_i, where $i = 0-4$), which correspond, at least in part, to the stepwise oxidation of a cluster of four Mn ions, $(Mn)_4$, believed to form the catalytic center of the H_2O-splitting reaction. O_2 derived from the decomposition of substrate H_2O is released during the transition from the S_3 state to the S_0 state (the S_4 state being a metastable intermediate). Recent evidence shows that one of the two substrate H_2O is already tightly bound upon reaching the S_3 state, whereas the second substrate H_2O is readily exchangeable with the enzyme poised in this state (Messinger et al., 1995).

Coupling of photochemical charge separation to the sequential photooxidation of the H_2O-splitting enzyme involves a redox-active tyrosine residue, termed Y_z. Y_z is one of two redox-active tyrosines of the PSII reaction center capable of donating electrons to the strongly oxidizing $P680^+$ ($E_{m7} \cong +1200$ mV). The second redox-active tyrosine, Y_D, is also capable of being photooxidized via the primary donor, yet electron donation by Y_D is insignificant during steady-state electron transfer due to the absence of an efficient electron donor to its photooxidized form. Y_z is oxidized by $P680^+$ in the submicrosecond time frame and occurs with a concomitant deprotonation, thereby generating the electroneutral radical, termed $Y_z^{\bullet}$, which is itself a strong oxidant ($E_{m7} \cong +1000$ to $+1100$ mV). This radical forms the reactive interface with the H_2O oxidation complex, and the kinetics of $Y_z^{\bullet}$ reduction can be equated with the kinetics of S-state advancement according to: $S_i + Y_z^{\bullet} \rightarrow S_{i+1} + Y_z$. The kinetics of $Y_z^{\bullet}$ reduction in an intact H_2O oxidation complex occur in the microsecond to millisecond to ms time range, depending upon the S state: the removal of electrons becomes progressively slower as the higher, more oxidized states are achieved. This contrasts with the comparatively slow re-reduction kinetics of the $Y_D^{\bullet}$ radical (termed Signal II_S), which may be observed by EPR minutes to hours, depending on the type of sample, following illumination and return to darkness.

EPR analyses of the S-state dependence of $Y_z^{\bullet}$ reduction kinetics indicated half-times of ≤100, ≤100, 400, and 1000 μs for the $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, and $S_3 \rightarrow [S_4] \rightarrow S_0$ transitions, respectively (Babcock et al., 1976). Measurements using mainly optical techniques have yielded results generally consistent with these time scales [see Razeghifard et al. (1997) for a summary]. Recently, Razeghifard et al. (1997) have shown from kinetic EPR studies on Signal II_{vf}, in which the individual S-state contributions were isolated by a deconvolution procedure, that the first three transitions have similar, fast kinetics, with the final transition ($S_3 \rightarrow [S_4] \rightarrow S_0$) being much slower. In thylakoids, the average early S-state $t_{1/2}$ was ∼100 μs with a value of 750 μs for the final transition. Thus, the approximately 1 ms half-time for $Y_z^{\bullet}$ decay during the O_2-releasing $S_3 \rightarrow [S_4] \rightarrow S_0$ transition coincides with the kinetics of overall PSII relaxation (Bouges-

[†] This work was supported by a fellowship (R.L.B.) from the Research School of Biological Sciences, The Australian National University, and by the Australian Research Council.

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[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1997.

[1] Abbreviations: Chl, chlorophyll; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, inhibits electron transport between Q_A and Q_B; HA, hydroxylamine (NH_2OH); Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MSP, manganese-stabilizing protein, extrinsic 33 kDa PSII protein; Q_A, primary plastoquinone electron acceptor; Q_B, secondary, exchangeable plastoquinone electron acceptor; P680, photooxidizable chlorophyll species acting as primary electron donor of the reaction center; PSII, photosystem II; *psbO*, gene encoding the manganese-stabilizing protein.

S0006-2960(97)00116-5 CCC: $14.00

Bocquet, 1973; Diner, 1973) and O_2 release (Jursinic & Dennenberg, 1990; Lavorel, 1992; Meunier & Popovic, 1991). This correlation in rates led to the suggestion that H_2O oxidation chemistry is limited by removal of electrons from the water-splitting enzyme by the secondary donor, now known to be the $Y_z^{\bullet}$ radical (Babcock et al., 1976).

A slowing of the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition from a 1.3 ms to a 5 ms half-time upon biochemical removal of the 23 and 18 kDa PSII extrinsic proteins was observed using ultraviolet spectroscopy, which presumably follows Mn redox changes (Dekker et al., 1984). The same study revealed that $Y_z^{\bullet}$ reduction, measured under repetitive flash conditions, also was slowed to approximately 5 ms. Amperometric measurements of the emission of O_2 from spinach PSII particles indicate that biochemical depletion of the extrinsic 33 kDa "manganese-stabilizing" protein (MSP) results in a pronounced slowing of the O_2 release step (Miyao et al., 1987). Genetic removal of MSP in *Synechocystis* sp. PCC6803 results in a retardation of the oxygen, yielding an $S_3 \rightarrow [S_4] \rightarrow S_0$ transition and an increased miss probability (Burnap et al., 1992, 1995). Quite similar results are observed in site-directed mutants with specific amino acid substitution mutants in the CP47 (Putnam-Evans et al., 1996) and D1 proteins (Qian, Burnap, and Debus, unpublished results). The genetically modified *Synechocystis* sp. PCC6803 strain lacking MSP (designated ΔpsbO) exhibits a slowing of the O_2 release to about 10 ms (Burnap et al., 1992, 1995).

The slowed appearance of O_2 in the absence of MSP raises the question as to whether or not this is a consequence of an underlying slowdown in the rate at which electrons are extracted from the water-splitting enzyme. Alternatively, it can be envisaged that electron transfer from the water-splitting enzyme proceeds at a normal rate, but the absence of MSP causes the oxidant, now trapped on the enzyme, to be utilized for the oxidation of H_2O at a reduced rate. In this study, we employ EPR detection of $Y_z^{\bullet}$ decay in wild-type and ΔpsbO cells under repetitive flash light conditions and show that the absence of MSP results in a slowing of $Y_z^{\bullet}$ reduction to a rate closely corresponding to the retarded rate of O_2 release in this mutant. We conclude that MSP optimizes the kinetic efficiency of the O_2-releasing $S_3 \rightarrow [S_4] \rightarrow S_0$ transition at the level of the $Y_z^{\bullet}$ reduction reaction.

METHODS

Growth and maintenance of the glucose-tolerant strain of *Synechocystis* sp. PCC6803 (subsequently referred to as the wild-type) and the derivative strain ΔpsbO were performed using established procedures (Williams, 1988). Construction and characterization of ΔpsbO, which completely lacks the extrinsic Mn-stabilizing protein, have been described (Burnap et al., 1992; Burnap & Sherman, 1991). Wild-type and mutant strains were grown in 5 L batch cultures in the presence of 5 mM glucose with air-bubbling and under constant illumination (approximately 80 μE m^{-2} s^{-1}). Cells were harvested during the late logarithmic phase of growth and gently resuspended to a chlorophyll concentration of approximately 1.5 mg of Chl mL^{-1} in HN buffer consisting of 10 mM Hepes−NaOH, 30 mM NaCl adjusted to pH 7.2. Resuspended cells (typically 30 mL) were maintained in a loosely covered 250 mL Erlenmeyer flask on a rotary shaker (approximately 100 rpm) under photoactivating conditions by illumination (approximately 50 μE m^{-2} s^{-1}). Illumination

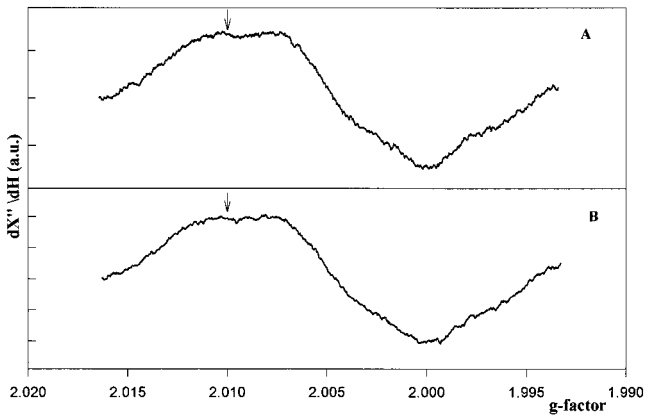

FIGURE 1: Signal II_s spectra of wild-type (A) and ΔpsbO cells (B). Arrows indicate the position at which Signal II_{vf} was monitored. Instrumental settings: 100 kHz modulation frequency, 100 mW microwave power, and 4.0 G modulation amplitude.

of cells is especially critical for the ΔpsbO strain due to the dark-lability of the H_2O oxidation complex (Burnap et al., 1996; Engels et al., 1994). Rates of O_2 evolution, following photoactivation, were determined using 600 μM DCBQ and 5 mM $K_3Fe(CN)_6$ as the artificial electron acceptor system before and after each EPR experiment. Under these conditions, the rate of O_2 evolution was determined to be 495 ± 44 (100%) and 153 ± 23 (31%) μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}, for wild-type and ΔpsbO, respectively.

EPR measurements were conducted at room temperature with a Bruker ESP 300E spectrometer using 100 kHz modulation frequency, 100 mW microwave power, and 4.0 G modulation amplitude. The spectrometer is equipped with a TM011 cavity and a Suprasil quartz flat sample cell. Actinic flash illumination was provided by saturating 10 μs flashes from an EG&G electrooptics discharge xenon lamp. All measurements were performed at room temperature with fresh cells obtained from suspensions maintained under fully photoactivated conditions, as described above. The dark-stable signal IIs spectra ($Y_D^{\bullet}$) shown in Figure 1A,B were obtained after illuminated samples were allowed to dark-adapt approximately 3 min. The amplitudes of Signal IIs spectra were observed to vary 10−20% from batch to batch on a per Chl basis presumably due to changes in the PSI/PSII ratio in different aged cultures. However, for the experiments depicted in Figure 1, the cells were harvested at similar cell densities, and the amplitudes of the Signal IIs spectra were found to be equivalent in the mutant and wild-type samples on a per chlorophyll basis. Subsequent $Y_z^{\bullet}$ reduction kinetic signals were normalized to the corresponding Signal IIs spectrum. $Y_z^{\bullet}$ reduction kinetics were determined by following the decay of Signal II_{vf} under repetitive flash illumination conditions. This was accomplished by setting the spectrometer to the low-field resonance peak of Signal IIs ($g = 2.010$) as indicated in Figure 1A,B. Timing and control of flash illumination and data acquisition were implemented using the EPR spectrometer computer, which was also used for data manipulation and analysis. Signal II_{vf} transients from some sets of 2000 flashes were averaged, summing the digitized data of each flash-induced transient and dividing by the flash number. A field-independent artifact contribution was obtained by performing the equivalent flash sequence at $g = 1.99$, and this was subtracted from the Signal II_{vf} data acquired at $g = 2.010$. A fresh sample aliquot was used for each of 2000

flash sequences. Flashes were given to cells at a frequency of 10 Hz as opposed to longer flash intervals as a compromise to minimize sample degradation, yet still observe possible transients that may have half-times in the tens of milliseconds. Furthermore, flashes given at 10 Hz effectively maintain otherwise labile mutant cells in a fully photoactivated state (Burnap et al., 1996). During the course of an experiment, no systematic variation in the kinetic response of individual sample aliquots was observed. These checks indicated that photoactivation and photoinactivation processes were negligible under the defined experimental conditions.

The Signal II_{vf} kinetics were fitted with eqs 1 and 2 in which $I(t)$ is the total signal amplitude at time t:

for $t < 0$

$$I(t) = 0 \quad (1)$$

for $t > 0$

$$I(t) = I_a\{0.75[(e^{(-t/\tau_e)} - e^{(-t/\tau_r)})/(\tau_r - \tau_e)] + 0.25[(e^{(-t/\tau_f)} - e^{(-t/\tau_r)})/(\tau_r - \tau_f)]\} + I_{slow}[(e^{(-t/\tau_{slow})} - e^{(-t/\tau_r)})/(\tau_r - \tau_{slow})] \quad (2)$$

where τ_r is the instrument time constant, τ_f is the decay time constant for the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition, τ_e is the average decay time constant for the three earlier transitions, and τ_{slow} is the decay time constant for the slow component observed in the mutant. I_a and I_{slow} are the signal amplitudes for active centers and centers giving rise to the slow component, respectively. It is assumed that the rise and fall kinetics of the $Y_z^\bullet$ in each S state are monoexponential, with the rise time governed by the spectrometer response time.

RESULTS

Figure 2 shows the decay kinetics of Signal II_{vf} in wild-type (panel A) and ΔpsbO (panel B) cells, respectively, under repetitive (10 Hz) flash illumination conditions. Addition of DCMU, which blocks PSII electron transfer from Q_A^- to Q_B, completely abolished these signals (not shown), whereas Signal I, due to $P700^+$ of PSI, was entirely unaffected by the addition of DCMU (not shown). Given the 100 ms interval that the repetitive flashes were applied, the loss of the $Y_z^\bullet$ transients from PSII containing intact $(Mn)_4$ in the presence of DCMU is expected since Q_A^- should photoaccumulate due to the fact that charge recombination between Q_A^- and the S_2 state of the H_2O oxidation complex should occur with a $t_{1/2} \sim 1$ s. On the other hand, DCMU-inhibited PSII centers devoid of active-site Mn will form primarily $Y_z^\bullet P680Q_A^-$ with charge recombination between Q_A^- and the donor side (primarily $Y_z^\bullet$) occurring much more rapidly (approximately 15−20 ms) than in the case of centers with intact $(Mn)_4$ (Nixon & Diner, 1992). Therefore, charge recombination restoring the initial state, $Y_z^\bullet P680Q_A$, should be largely complete during the 100 ms flash interval, and thus transients should be observable after each flash during the sequence provided that alternate donors to $Y_z^\bullet$ do not trap the centers in the $Y_zP680Q_A^-$ state. However, $Y_z^\bullet$ transients are not observed in the DCMU-inhibited samples, indicating that either (1) the majority of centers capable of oxidizing Y_z have a long-lived (>100 ms) charge-separated state [e.g., due to the presence of an intact $(Mn)_4$] or (2) alternative donors capable of trapping the $Y_zP680Q_A^-$ state are present.

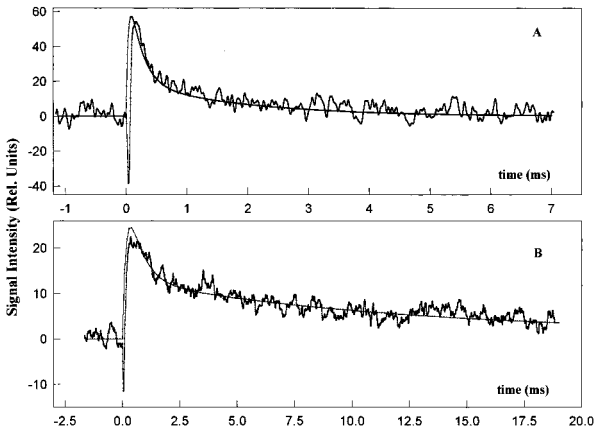

FIGURE 2: EPR detection of $Y_z^\bullet$ decay in the wild-type (A) and ΔpsbO mutant (B) cells under repetitive (10 Hz) flashing light conditions. The signal amplitudes are divided into number averages, 20 000 for (A) and 16 000 for (B). The negative spike arises from the residual contribution of the PSI spin-polarized signal. The solid smooth curve is the least-squares fit of eqs 1 and 2 to the data points. Instrumental settings: 100 kHz modulation frequency, 100 mW microwave power, and 4.0 G modulation amplitude; time constant = 40 μs and 160 μs for the wild-type and mutant, respectively.

In the absence of DCMU, the amplitude of Signal II_{vf} in the mutant is approximately 40% that of the wild-type normalized to the corresponding amplitudes of Signal IIs in these samples (Figure 1). It should be noted that the amplitude of Signal IIs was equivalent in the mutant and wild-type on a per chlorophyll basis, and therefore we conclude that the number of PSII centers producing detectable $Y_z^\bullet$ transients under the 10 Hz repetitive flashes is decreased by approximately 60%, although the total number of PSII centers capable of forming Y_D is unaffected by the absence of MSP. Although not utilized for signal-to-noise considerations, initial experiments employing data acquisitions with a faster 40 μs time constant did not reveal a signal amplitude greater than the 40% of wild-type value, indicating that faster components of decay escaping detection are unlikely. The decreased amplitude of the mutant Signal II_{vf} signal is consistent with estimates that ΔpsbO cells have approximately 40−60% the number of O_2-evolving centers on the basis of flash O_2 yield (Burnap et al., 1992; Vass et al., 1992), flash fluorescence yield (Philbrick et al., 1991), and thermoluminescence yield measurements (Burnap et al., 1992; Vass et al., 1992). On this basis, we tentatively conclude that the observed Signal II_{vf} in the ΔpsbO mutant corresponds primarily to $Y_z^\bullet$ in O_2-evolving centers. The loss of 60% of Signal II_{vf} in the mutant could, in principal, be due to (1) non-O_2-evolving centers in which re-reduction of $Y_z^\bullet$ is slow and thus does not occur significantly during the 100 ms flash interval or (2) photoinhibitory processes resulting in damage to Y_z or the uncoupling of its oxidation by $P680^+$.

Figure 2 shows that the decay of Signal II_{vf} in the ΔpsbO mutant is significantly slower than the wild-type (note scale difference on time axes). Due to the fact that the Signal II_{vf} transients were obtained under repetitive flash conditions, the kinetics of decay are assumed to reflect approximately equal contributions of $Y_z^\bullet$ decay during each of the four

Table 1: EPR and Kinetic Properties of Wild-Type and Mutant *Synechocystis* sp. PCC6803

strain	signal II_{vf} amplitude[a]	$VO_{2,max}$[b]	decay of signal II_{vf} (μs)[c]
wild-type	1.0	1.0	140 (0.75) 1200 (0.25)
ΔpsbO	0.38	0.31	400 (0.64) 6000 (0.21) 25000 (0.15)[d]

[a] Relative normalization of Signal IIs amplitude in the sample, setting the value to 1.0 for the wild-type. [b] Light-saturated maximal rates of O_2 evolution with DCBQ and $K_3Fe(CN)_6$ as electron acceptors. Under these conditions, the maximal rate of O_2 evolution was determined to be 495 ± 44 (1.0) and 153 ± 23 (0.31) μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}, for wild-type and ΔpsbO, respectively. [c] Kinetic fits assumed equal contributions from each S-state transition and a 'two-component approximation' model for active centers (see text). Values in parentheses represent the fractional contributions of decay components. [d] Slow component proposed to arise from inactive centers.

S-state transitions, with the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition being the slowest. A two-component kinetic fit of the wild-type data was used as an approximation based upon recent kinetic measurements of the S-state dependence of $Y_z^{\bullet}$ re-reduction in thylakoids which show that the earlier S-state transitions occur with half-times ranging from ~50 to 140 ms, whereas the final $S_3 \rightarrow [S_4] \rightarrow S_0$ transition, at 750 ms, is more than 5-fold slower than the slowest of these (Razeghifard et al., 1997). Kinetic fits (smooth solid lines in Figure 2A,B) to the repetitive $Y_z^{\bullet}$ decays using this 'two-component approximation' as the initial assumption (eqs 1 and 2) yielded the decay times shown in Table 1. The wild-type transient can be well described by two components with half-times of 140 μs (75% of transient) and 1200 μs (25% of transient). The 1200 μs component, which should reflect $Y_z^{\bullet}$ reduction during the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition, is in good agreement with previously established values for the half-time of that transition in other photosynthetic samples. Similarly, the 140 μs half-time component represents the convolution of the three earlier S-state transitions and fits well as an average of these transition half-times according to previous data (Razeghifard et al., 1997).

When a similar analysis was applied to the repetitive $Y_z^{\bullet}$ decay transient obtained from the ΔpsbO mutant, a good fit was not obtained unless an additional third slow (~25 ms) component comprising about 15% of the total signal was included. This slow component is that part of the signal which fails to fully return to the base line during the 20 ms of data acquisition, but returns to the base line during the subsequent 80 ms prior to the succeeding flash. In contrast, virtually all of the signal in the wild-type returns to the base line during the 8 ms of data acquisition following the actinic flash. The relatively slow ~25 ms component in ΔpsbO may originate from damaged or unassembled PSII centers, as noted above. On the other hand, the majority (~85%) of the ΔpsbO Signal II_{vf} transient is well-described by two faster components, with half-times of 400 and 6000 μs (Table 1) assuming relative contributions of 3 to 1, respectively. Accordingly, the 6000 μs component of the repetitive flash signal is interpreted as $Y_z^{\bullet}$ reduction during the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition, whereas the 400 μs component corresponds to the three earlier S-state transitions. Therefore, we conclude that $Y_z^{\bullet}$ reduction during the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition is retarded approximately 5-fold upon genetic removal of MSP. Additionally, the analysis suggests that the absence of MSP also retards at least one of the other earlier S-state transitions. However, the latter assessment is rendered especially tentative since the 160 μs time constant used for the measurement of the kinetics in the mutant compromises detection of transients in this time range.

DISCUSSION

In the present work, an EPR study of the kinetics of $Y_z^{\bullet}$ reduction under repetitive flash conditions was performed in the wild-type cyanobacterium *Synechocystis* sp. PCC6803 and a genetically engineered derivative *Synechocystis* that lacks the extrinsic 33 kDa manganese-stabilizing protein associated with the H_2O oxidation complex of PSII. This is the first reported analysis of Signal II_{vf} $Y_z^{\bullet}$ reduction kinetics in this widely used model organism, and it extends previous observations that the genetic removal of MSP results in a lower accumulation of O_2-evolving PSII centers and those centers that are assembled and evolve O_2 do so with retarded kinetics and increased susceptibility to photodamage.

Numerous studies on Signal II_{vf} have shown that the slowest, most readily resolved transition, $S_3 \rightarrow [S_4] \rightarrow S_0$, has a time scale of 0.7−1.2 ms (Babcock et al., 1976; Cole & Sauer, 1987; Hoganson & Babcock, 1988; Lydakis-Simantiris et al., 1995; Razeghifard et al., 1997; Razeghifard & Pace, 1997). The most recent data further indicate that the earlier S-state transitions, although of distinguishable individual time scales, are sufficiently close kinetically, and sufficiently different from the final, slow transition, that a 'two-rate constant' decomposition of the data from averaged multiple turnovers is appropriate (Razeghifard et al., 1997). Such conditions were recently shown to be satisfied in the situation where a slowdown of the S-state transitions occurs due to perturbations (Razeghifard & Pace, 1997). In the repetitive flash experiments reported here, the decay of Signal II_{vf} contains equal contributions of the $Y_z^{\bullet}$ reduction transient from PSII centers in each of the S states, similar to the initial repetitive flash measurements (Blankenship et al., 1975). The results obtained for wild-type *Synechocystis* cells, which retain MSP, shown in Figure 2 are similar to those observed previously with spinach broken chloroplasts. Analysis of the $Y_z^{\bullet}$ reduction kinetics in wild-type *Synechocystis* cells resolves two kinetic components with half-times of 1200 and 140 μs attributed to $Y_z^{\bullet}$ reduction during the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition and as an average decay rate of $Y_z^{\bullet}$ during the earlier transitions. Importantly, the 1200 μs half-time closely agrees with recent measurements of a 1200 μs half-time of O_2 release and PSII turnover from wild-type *Synechocystis* cells and membranes (Burnap et al., 1995; Qian and Burnap, unpublished experiments). This similarity in the rates of O_2 release and $S_3 \rightarrow [S_4] \rightarrow S_0$ indicates that the $Y_z^{\bullet}$ rate of reduction is rate-limiting in the H_2O oxidation reactions as originally proposed by Babcock et al. (1976).

The central finding of the present experiments is that genetic removal of MSP slows the rate of $Y_z^{\bullet}$ reduction (compare Figure 2A,B) to a rate quite similar to the rate of O_2 release observed amperometrically in this mutant (Burnap et al., 1992, 1995). In contrast to the comparatively fast ~1 ms half-time of O_2 release in unperturbed samples, biochemical and genetic removal of the extrinsic polypeptides of PSII causes a retardation of the S-state transitions. Previous amperometric measurements of PSII turnover indicated that biochemical removal of MSP by NaCl−urea

treatment results in a specific slowdown of the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition. Measurement of O_2 release kinetics in ΔpsbO using similar methods provided a half-time of approximately 10 ms for this transition (Burnap et al., 1992). Improvements of this method using a centrifugal electrode (Burnap et al., 1995; Lavorel, 1992; Nixon & Diner, 1992a; Putnam-Evans et al., 1996), and separate measurements using double-flash techniques (Bouges-Bocquet, 1973), place this value in ΔpsbO at $t_{1/2} \cong 8$ ms (Burnap et al., 1995; Qian and Burnap, unpublished experiments). This is consistent with the approximately 6 ms $Y_z^\bullet$ decay component ascribed here to the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition in the mutant (Figure 2B and Table 1). The slowed kinetics of O_2 release in ΔpsbO therefore appear to be primarily a consequence of a retardation in the reduction of $Y_z^\bullet$. Since O_2 release closely parallels the reduction of $Y_z^\bullet$ kinetically, O═O bond formation and dioxygen release must occur very rapidly following the rate-limiting $S_3Y_z^\bullet \rightarrow S_4Y_z$ transition (Babcock et al., 1976), even in the perturbed enzyme.

ACKNOWLEDGMENT

We thank Dr. Curt Hoganson for useful discussions and Prof. C. Barry Osmond for his support.

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BI970116G