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Involvement of Histidine 190 on the D1 Protein in Electron/Proton Transfer Reactions on the Donor Side of Photosystem II†

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ABSTRACT: Flash-induced chlorophyll fluorescence kinetics from photosystem II in thylakoids from the dark-grown wild type and two site-directed mutants of the D1 protein His190 residue (D1-H190) in *Chlamydomonas reinhardtii* have been characterized. Induction of the chlorophyll fluorescence on the first flash, reflecting electron transport from Y_Z to $P680^+$, exhibited a strong pH dependence with a p*K* of 7.6 in the dark-grown wild type which lacks the Mn cluster. The chlorophyll fluorescence decay, measured in the presence of DCMU, which reflects recombination between Q_A^- and Y_Z^{ox}, was also pH-dependent with a similar p*K* of 7.5. These results indicate participation by the same base, which is suggested to be D1-H190, in oxidation and reduction of Y_Z in forward electron transfer and recombination pathways, respectively. This hypothesis was tested in the D1-H190 mutants. Induction of chlorophyll fluorescence in these H190 mutants has been observed to be inefficient due to slow electron transfer from Y_Z to $P680^+$ [Roffey, R. A., et al. (1994) *Biochim. Biophys. Acta 1185*, 257−270]. We show that this reaction is pH-dependent, with a p*K* of 8.1, and at pH ≥9, the fluorescence induction is efficient in the H190 mutants, suggesting direct titration of Y_Z. The efficient oxidation of Y_Z (≈70% at pH 9.0) at high pH was confirmed by kinetic EPR measurements. In contrast to the wild type, the H190 mutants show little or no observable fluorescence decay. Our data suggest that H190 is an essential component in the electron transfer reactions in photosystem II and acts as a proton acceptor upon Y_Z oxidation. In the H190 mutants, this reaction is inefficient and Y_Z oxidation only occurs at elevated pHs when Y_Z itself probably is deprotonated. We also propose that H190 is able to return a proton to Y_Z^{ox} during electron recombination from Q_A^- in a reaction which does not take place in the D1-H190 mutants.

Photosystem II (PSII)[1] catalyzes the light-driven oxidation of water and the reduction of plastoquinone (*1*−*4*). The PSII reaction center is made up of at least six polypeptides, including D1, D2, cytochrome b_{559}, and some low-molecular weight subunits. The structurally similar D1 and D2 membrane-spanning polypeptides bind the primary donor, P680, the pheophytin acceptor, and the first and second quinone acceptors, Q_A and Q_B, respectively. After a charge separation, forming $P680^+$ and $Pheo^-$, these radical species are stabilized by the oxidation of $Pheo^-$ by a fixed plastoquinone molecule, Q_A, and the reduction of $P680^+$ (40−280 ns) by Y_Z [Tyr161 on the D1 protein, which acts as a neutral radical, (*4*)]. Charge recombination between Q_A^- and Y_Z^{ox} is prevented by rapid electron transport to Q_B, and when plastoquinol is formed, it diffuses out of the Q_B site on the D1 protein. On the donor side of PSII, Y_Z^{ox} is reduced by the nearby Mn cluster within 30−1300 μs, depending on the oxidation state of the Mn cluster (*3*−*5*).

The Mn cluster is the catalytic site for water oxidation and is generally thought to contain four manganese atoms. During catalysis, it cycles through five S states, S_0−S_4, and molecular oxygen is released in the $S_3 \rightarrow S_4 \rightarrow S_0$ transition (*2*, *3*, *6*, *7*). The Mn cluster also contains calcium and chloride ions which are essential for oxygen evolution (see refs *2*, *3*, and *7* for a detailed discussion). Most likely, the D1 and D2 proteins contribute ligands to the manganese, calcium, and probably also to the chloride ions (*3*), although CP43, CP47, and cytochrome b_{559} might also be involved in cofactor binding (*8*).

A number of potentially manganese-coordinating amino acid residues have been studied by site-directed mutagenesis in D1 protein mutants of *Synechocystis* sp. PCC 6803 (*9*−

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[1] Abbreviations: PSII, photosystem II; Chl, chlorophyll; P680, primary donor of PSII; P700, primary donor of PSI; Pheo, pheophytin; Q_A and Q_B, primary and secondary quinone acceptors, respectively; Y_Z and Y_D, redox active tyrosines, secondary donors in PSII; D1 and D2, reaction center polypeptides of PSII; CP43 and CP47, chlorophyll-containing polypeptides in PSII; H190, histidine 190 on the D1 protein; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; EPR, electron paramagnetic resonance; F_o, initial level of fluorescence before actinic flash; F'_{max}, maximal level of fluorescence after actinic flash; F_{max}, total maximal level of fluorescence, measured in the presence of 10 mM dithionite; F'_v, variable fluorescence after actinic flash ($F'_{max} - F_o$); F_v, total variable fluorescence ($F_{max} - F_o$); F_{final}, final level of fluorescence after decay reactions are completed.

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23) and *Chlamydomonas reinhardtii* (*24*−*27*). D1-H190 is a conserved, important residue on the lumenal side of the D1 protein which is located in the vicinity of P680 and Y_Z (*28*−*30*). H190 has been suggested to have several functions such as being an active component of the water-splitting complex (*31*), a manganese ligand (*16*, *21*, *24*, *32*, *33*), and providing a hydrogen bond to Y_Z (*16*, *18*, *28*, *29*).

The two latter ideas are particularly interesting with respect to the recently proposed hydrogen atom abstraction mechanism for water oxidation involving metalloradical chemistry (*7*, *34*−*38*). In this model, Y_Z^{ox} abstracts hydrogen atoms from Mn-coordinated water molecules during the S state transitions. During oxidation of Y_Z by $P680^+$, the phenolic proton on Y_Z is rapidly released, leaving a neutral tyrosine radical on Y_Z. Y_Z^{ox} would then move and extract a hydrogen atom from a nearby water molecule coordinated to Mn. This model is supported by the observed flexibility of Y_Z^{ox} (*35*, *39*−*42*), the proposed short distance between Y_Z and the Mn cluster (*7*, *41*, *43*), and the favorable energetics (*44*). However, it is challenged by the small isotope effects on the reactions involved (*45*−*47*; see however ref *48*) and the proposal that the proton expelled from Y_Z upon its oxidation remains in the vicinity of P680. The latter hypothesis is derived from proton release and electrochromic shift measurements (*47*, *49*, *50*) and transient absorption change measurements (*47*, *50*, *51*).

Regardless of the mechanism for water oxidation, the oxidation of Y_Z involves movement of a proton from the oxidized tyrosine residue to a nearby base, and characterization of the proton release from Y_Z is very important for our understanding of this process. From computer modeling studies, H190 has been suggested to lie close to (3−4 Å) Y_Z on the D1 protein (*28*, *29*). It was, however, pointed out in ref *28* that the distance in the computer model between Y_Z and H190 is too long to promote a typical hydrogen bond, and recent spectroscopic evidence suggests there are at least two partners for hydrogen bonding to Y_Z, one of them probably H190 (*43*).

In this study, we have performed electron transfer measurements in mutants in the D1-H190 residue to further assess the partner for forming a hydrogen bond to Y_Z. Mutants in D1-H190 (from *C. reinhardtii* or *Synechocystis* 6803) are unable to assemble the Mn cluster, making them unable to oxidize water (*24*−*26*). In addition, and very importantly, the electron transfer from Y_Z to $P680^+$ is very slow in the mutants (*16*, *21*, *24*), leading to inefficient induction of the EPR spectrum from Y_Z^{ox}. Nevertheless, the EPR spectrum of Y_Z^{ox} is unchanged (*25*), indicating that the structural changes in the vicinity of Y_Z are not very large. Here we have applied detailed fluorescence studies in the dark-grown wild type and two mutants in H190. It is found that the efficiency of electron donation from Y_Z varies greatly with pH. At elevated pH, both forward electron transfer and back reactions are influenced differently in the mutants and the wild type, permitting new conclusions to be drawn concerning the involvement of D1-H190 in the hydrogen bonding to Y_Z.

MATERIALS AND METHODS

Site-Directed Mutagenesis. Site-directed mutations in PSII of the green algae *C. reinhardtii* were introduced into the D1 protein at the H190 position (*24*). H190F contains a histidine to phenylalanine mutation as a result of the CAC to TTC substitution in exon 4 in the *psbA* gene. H190Y, a histidine to tyrosine mutant, was generated by a CAC to TAC substitution at the H190 (D1) codon. The construction, verification, and molecular characterization of the site-directed mutants are described elsewhere (*24*).

Growth Conditions. Cells of the *C. reinhardtii* wild type CC-2137 strain that becomes green in the dark, and both mutants, were grown heterotrophically in Tris-acetate phosphate medium (*52*) in a shaking incubator at 25 °C in total darkness (except for the light-grown wild type). Darkness was used to avoid photoligation of the Mn cluster in PSII in the wild type and to eliminate photoinhibitory effects during cell growth. The latter is especially important since the mutants are very sensitive to light (*24*−*26*). Cells were grown for 7−10 days before harvesting near the end of the logarithmic phase at a concentration of about 10^7 cells/mL. The cell density was determined using a light microscope and a hemeocytometer.

Thylakoid Preparation. All preparation procedures were carried out in total darkness, and the samples were kept on ice throughout the process. Cells were harvested by centrifugation for 5 min at 5000*g* and washed once in a buffer containing 25 mM Hepes-NaOH (pH 7.0) and 15 mM NaCl. The resulting pellet was resuspended in the same buffer with the addition of 5 mM EDTA, and the cells were thereafter broken by repeated sonication for 2 × 10 s in a Branson Sonifier B-30 sonicator. Unbroken cells and cell debris were removed by centrifugation for 15 min at 400*g*. The thylakoid membranes were then harvested by centrifugation for 10 min at 20000*g* and washed once in the same EDTA-containing buffer. After EDTA washing, the thylakoids were resuspended in a buffer containing 25 mM Hepes-NaOH (pH 7.0), 400 mM sucrose, and 15 mM NaCl and washed twice to remove the EDTA. The final preparation of thylakoids was frozen in liquid nitrogen and stored at −80 °C at 2.5−3.0 mg of Chl/mL.

Chlorophyll a Fluorescence Measurements. Chlorophyll *a* fluorescence was detected with a PAM 100, pulse amplitude modulated fluorimeter (Walz, Effeltrich, Germany). The monitoring measuring light pulses (650 nm) with a 1 μs duration were applied at a frequency of 1.6 kHz, except as noted. Single saturating actinic flashes with an approximately 8 μs width at maximum were provided by a Walz XST-103 xenon flash lamp. The fluorescence detection began 120 μs after the flash. Data were collected and analyzed using the Q_A-FIP program (version 4.3, Q_A-Data Oy, Turku, Finland). The thylakoid concentration was 20 μg of Chl/mL in the presence of 40 μM DCMU. Before the measurement, each sample was dark adapted for 1 min after the addition of DCMU.

EPR Spectroscopy. X-band EPR spectra at room temperature and at the temperature of liquid helium were recorded with a Bruker ESP380E spectrometer equipped with an Oxford Instruments cryostat and temperature controller. Signal II_{slow} (Y_D^{ox}) was generated by continuous illumination at room temperature with a 1000 W projector lamp filtered through 5 cm of copper sulfate solution. For kinetic measurements, saturating laser flashes (6 ns, 300 mJ at 532 nm) from a Nd:YAG laser (Spectra Physics NRF-05) were directed into the EPR cavity. The thylakoid suspension was

Table 1: Characterization of PSII in Thylakoids from the Light-Grown (LG) and Dark-Grown (DG) Wild Type and H190 Mutants of *C. reinhardtii*

	oxygen evolution[a]	PSII content[b]	F_v/F_o[c]	Mn content[d]
LG WT	205	100	0.60	5.19
DG WT	0	41	0.44	1.3
DG H190F	0	33	0.34	1.1
DG H190Y	0	39	0.35	0.9

[a] Micromoles of O_2 per milligram of Chl per hour (measured with a Clark electrode with 2 mM ferricyanide and 0.5 mM DCBQ as electron acceptors and 3 μg/mL of gramicidin at 20 °C). [b] The relative PSII content was determined on the basis of the maximal inducible size of EPR signal II_{slow}, measured on a Chl basis (%). [c] Measured in the presence of 40 μM DCMU and 10 mM dithionite. [d] Analysis of the Mn content was done on the Spectra AA 10 Varian atomic absorption spectrophotometer. The Mn content is given as the number of Mn ions per 200 Chl molecules.

contained in a flat cell, and the triggering of the laser and the EPR recording was carried out by a home-built triggering device. The spectra were evaluated using ESP300 and Bruker Win-EPR software.

RESULTS

Photosystem II Characterization

In the presence of an exogenous carbon source, cells of *C. reinhardtii* can be grown in complete darkness. Under such conditions, PSII is assembled and the PSII complex is able to perform primary charge separation and light-driven electron transport from the donor side to the quinone acceptors. However, PSII from dark-grown cells is unable to oxidize water because the assembly of a functional Mn cluster is a light-dependent process (*53*−*56*). Table 1 summarizes some of the characteristics concerning the PSII content and activity of the preparations used in this study. It is clear that PSII from dark-grown cells contains no or very little Mn. In addition, both the EPR data and the F_v/F_o ratio indicate that the PSII content is significantly lower in the dark-grown membranes.

EPR Measurements. Figure 1 shows the EPR spectra of Y_D^{ox} (signal II_{slow}) in thylakoid membranes from the wild type and the H190 mutants of *C. reinhardtii*. The EPR spectrum of Y_D^{ox} is similar in the different samples and shows a *g* value of 2.0045−2.0048 and an overall peak to trough line width of 2 mT which is normal for Y_D^{ox} (*57*−*59*). It should also be noted that, under our measuring conditions (pH 7.0), Y_D^{ox} was more difficult to induce in the dark-grown wild type and H190 mutants than in the light-grown wild type. Light saturation experiments (not shown) revealed that illumination for 8 min at room temperature was necessary to induce maximal Y_D^{ox} in the dark-grown material compared to illumination for 2 min in the light-grown wild type. The relative amount of PSII was calculated on the basis of the maximal EPR signal size from Y_D^{ox} (Figure 1) which corresponds to one radical per PSII reaction center. Interestingly, the PSII content in the dark-grown wild type was only 41% of the PSII content in the light-grown wild type. The PSII content in the dark-grown cells was almost similar in the wild type and the H190 mutants (33 and 39% for the H190F and H190Y mutants, respectively, Table 1). It should be noted that we earlier estimated the amount of Y_D^{ox} in these mutants to be lower [≈15% (*25*)]. The reason

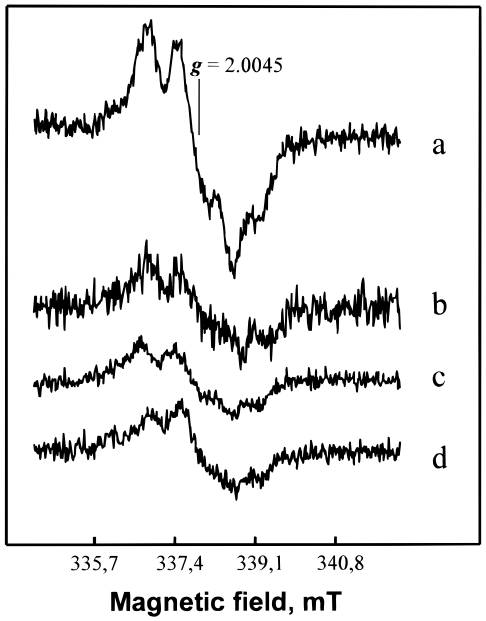


FIGURE 1: EPR spectra of Y_D^{ox} in thylakoid membranes from *C. reinhardtii* in the light-grown wild type (a), the dark-grown wild type (b), the dark-grown D1-H190F mutant (c), and the dark-grown D1-H190Y mutant (d). Spectra were normalized to the same concentration of Chl. The radicals were induced by illumination for 2 min at room temperature for spectrum a and for 8 min for spectra b−d. The samples were dark adapted for 5 min prior to the measurements. EPR conditions were as follows: microwave frequency, 9.47 GHz; microwave power, 1.15 μW; modulation amplitude, 0.32 mT; time constant, 20.48 ms; and conversion time, 81.92 ms. The spectra were recorded at 15 K. The different signal-to-noise ratio reflects the fact that eight scans were added in spectra a and b while 16 scans were added in spectra c and d. The thylakoid concentrations were 1.5−2.5 mg of Chl/mL.

for this discrepancy is that Y_D^{ox} is difficult to induce in these mutants. We were not aware of this in our earlier study and used too little light to achieve maximal Y_D^{ox} induction.

Estimation of F_v Fluorescence. A versatile tool for analyzing the function of PSII is to measure Chl *a* fluorescence, and the variable fluorescence, F_v, is an important indicator of PSII activity (*60*, *61*). To determine whether there were any subtle differences between the PSII activity in the dark-grown wild type and the H190 mutants, we measured the Chl *a* fluorescence rise and decay after single-turnover flash excitation of the thylakoid membranes. Initially, the F_v/F_o ratio representing the maximal F_v was determined in the presence of 40 μM DCMU and 10 mM dithionite to completely reduce Q_A. In thylakoids from the light-grown wild type, the F_v/F_o ratio was 0.60 (Table 1) which is unexpectedly low and probably reflects our growing and preparation conditions. It should be pointed out, however, that the high rate of oxygen evolution (Table 1) indicates that the seemingly low F_v/F_o is not due to a large fraction of inactive PSII centers. In the dark-grown wild type, F_v/F_o was found to be 0.44, probably reflecting the lower PSII content in the dark-grown cells. Furthermore, the F_v/F_o determined in the presence of dithionite was found not to be pH-dependent. The flash-induced F_v in the H190F mutant (in the presence of DCMU at pH 7.5) was reported to be extremely low [about 10% of that in the wild type (*24*)]. Our measurements confirm these observations (see below). In contrast, the determination of the total F_v (not only from the first flash) in the presence of 10 mM dithionite

shows a F_v/F_o value of 0.34−0.35 in the two H190 mutants. Thus, the determination of the total F_v indicates that the content of functional (except for water oxidation) PSII centers is similar in the H190 mutants and the dark-grown wild type, consistent with our EPR results (Figure 1 and Table 1).

Flash-Induced Variable Fluorescence Decay Kinetics

An assay sensitive to the functional properties of the donor side in PSII is based on measuring the kinetics of the fluorescence decay after a single flash in the presence of the inhibitor DCMU (*11*, *13*, *14*, *19*, *20*, *22*, *62)*. The flash-generated charge separation results in formation of Q_A^- which is considered to be related to the yield of variable fluorescence (fluorescence rise from the F_o level to F'_{max}). In the presence of DCMU, forward electron transfer from Q_A^- to Q_B is blocked. Instead, recombination reactions to the donor side of PSII will dominate in the decay of the variable fluorescence. Consequently, this assay is sensitive to the properties and oxidation state of the redox components on the donor side of PSII. It might therefore be useful to study electron transfer in mutants in the vicinity of Y_Z like the H190 mutants.

Dark-Grown Wild Type. In thylakoid membranes from dark-grown cells, several flashes were needed to induce maximal F_v (Figure 2A−C). After five flashes, the fluorescence level reached its maximum, indicating the accumulation of Q_A^- in all PSII centers. This flash-dependent increase of F'_v was clearly pH-dependent and much more efficient at higher pH. At pH 5.5, the observed F'_v produced by the first flash was only half of that produced by the second flash, and five flashes were needed to reach the total F_v (Figures 2A and 3A). At pH 7.0, the F'_v produced by the first flash was equal to the F'_v produced by the second flash, and two or three flashes were needed to reach the total F_v. At pH 9.0, almost 90% of the total F_v was produced by the first flash (Figures 2B,C and 3A). The latter result shows that the flashes were saturating, and we can conclude that all PSII centers could be excited by the flash.

The low F'_v in the first flash at pH 5.5 (about 45% of the total F_v, Figures 2A and 3A) then suggests that a large fraction of the Q_A^- formed by the flash never resulted in a high fluorescence state. In the presence of the DCMU, this probably reflects recombination between Q_A^- and $P680^+$ in a large fraction of the centers. The remaining F'_v, however, represents centers in which $P680^+$ was reduced from Y_Z. Thus, it seems that, in PSII from the dark-grown wild type, the electron donation to $P680^+$ from Y_Z (nanosecond time scale in PSII capable of oxidizing water) after the first flash is slower at low pH values, resulting in low F'_v. The more efficient F'_v induction at higher pH values indicates that the electron transfer from Y_Z to $P680^+$ becomes faster and suggests a protonation step at this stage. It should be noted that the flash-induced induction of F'_v in thylakoids from light-grown cells was more efficient at all pH values compared to that of the dark-grown material (not shown).

We also studied the decay of the flash-induced F_v in thylakoids from dark-grown wild type cells (Figure 2). The results are shown in Table 2. The half-time for the fluorescence decay again shows considerable dependence on the pH at which the fluorescence was measured. At pH 5.5, it was found to be 9.3 ms, and this value increased with pH

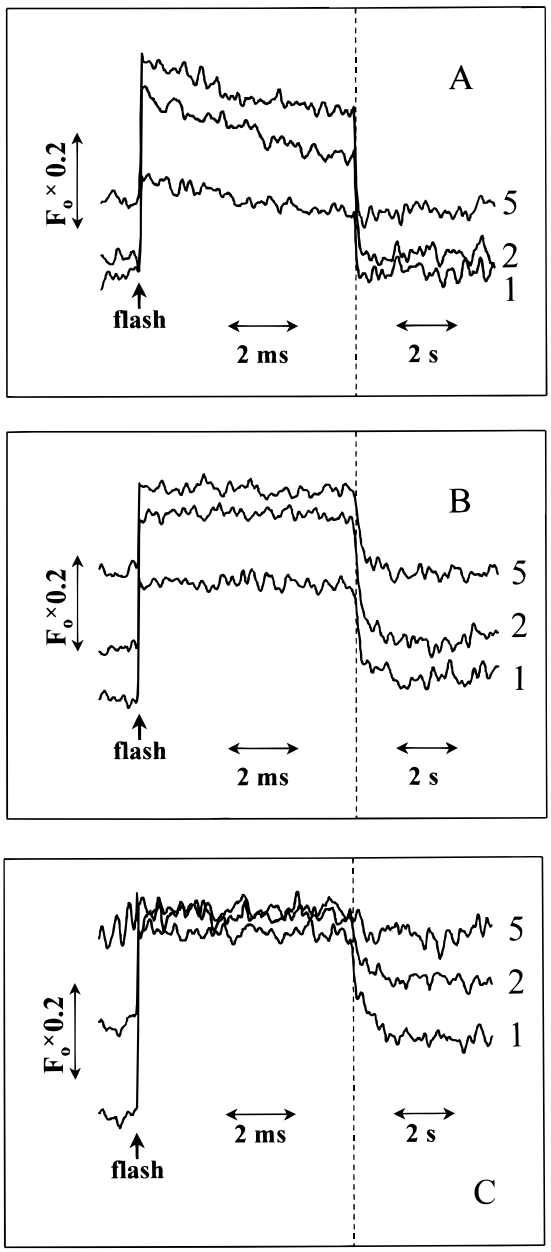


FIGURE 2: Single flash-induced fluorescence kinetics of charge recombination between Q_A^- and the donor side of PSII in thylakoids from dark-grown cells of the wild type. The numbers on the right refer to the flash number in a train of actinic flashes. Measurement conditions were as follows: 19 °C, 30 μg of Chl in 1.5 mL of a buffer containing 400 mM sucrose, 15 mM NaCl, and 40 μM DCMU at (A) pH 5.5 (25 mM Mes-NaOH), (B) pH 7.0 (25 mM Hepes-NaOH), and (C) pH 9.0 (25 mM Tris-HCl). The first 10 ms of the decay was measured at a modulation frequency of 100 kHz, beginning 2 ms before the actinic flash was administered. The slower time scale was measured at a modulation frequency of 1.6 kHz. The actinic flashes were given at 5 s intervals. Each trace represents the average of 10 measurements in independent samples.

to 43 ms at pH 7.0 and 131 ms at pH 9.0. These half-times are in the millisecond time range and probably reflect recombination between Q_A^- and Y_Z^{ox} which occurs in this time regime (*11*, *13*, *14*, *20*, *62*−*64)*. Importantly, the faster recombination rate between Q_A^- and Y_Z^{ox} at lower pH values also suggests the involvement of a protonatable group at this stage.

Another observation in these measurements was that, at high pH values, the final level of the fluorescence (F_{final})

Table 2: pH Dependence of the Decay Half-Time of the Flash-Induced Fluorescence, Obtained Using a Single-Exponential Curve Fit, in Thylakoids from Dark-Grown Wild Type *C. reinhardtii* Measured in the Presence of 40 μM DCMU

pH	$t_{1/2}$ (ms)	pH	$t_{1/2}$ (ms)	pH	$t_{1/2}$ (ms)
5.5	9.3	7.0	43	8.5	103
6.0	17	7.5	73	9.0	131
6.5	31	8.0	87	9.5	123

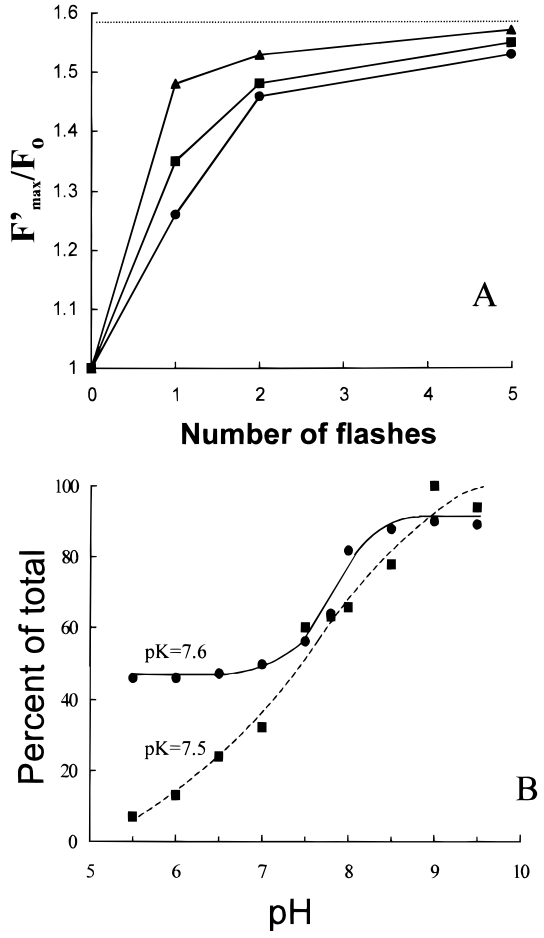


FIGURE 3: (A) Flash number dependence on the accumulation of the high fluorescence state (F'_{max}) in the thylakoids from dark-grown wild type cells of *C. reinhardtii*. The fluorescence yield was measured essentially as in Figure 2 at pH 5.5 (●), 7.0 (■), and 9.0 (▲). The F_{max} level, reached in the presence of 10 mM dithionite, is indicated by the dotted line. (B) pH dependence and fitting of the experimental data of the first flash amplitude (●) and the half-time of fluorescence decay (■) as a percentage of total F_v and fluorescence decay at pH 9.0.

after the first flash was higher than the initial F_o. At pH 9.0, F_{final} remains at half of the F_v value which indicates that the electron remains on Q_A^- in a substantial fraction of the centers (Figure 2C). This phenomenon has been previously addressed (*62*−*64*), and the stable F_{final} was attributed to the presence of an unknown donor in this type of material (*16*, *24*).

The pH dependence of the flash-induced fluorescence amplitudes is shown in Figure 3B. The increase of the first flash amplitude with pH, i.e., the efficiency of the electron donation to $P680^+$, correlates with the decrease of the second flash amplitude (not shown). Fitting the experimental data with a Henderson−Hasselbalch equation results in a p*K* of 7.6 for this phenomenon. It is interesting to note that a similar pH dependence was reported for the induction of Y_D^{ox} [pK = 7.3 (*65*)]. In this case, D2-H190 was suggested to be involved in the protonation and deprotonation reactions of Y_D during its oxidation. It is likely that similar interactions might also be important in the oxidation of Y_Z on the D1 protein, since the phenolic proton must leave the Y_Z side chain after oxidation [it is known that the Y_Z^{ox} EPR spectrum represents a deprotonated but hydrogen-bonded tyrosine radical (*35*, *39*, *40*)].

The increase of the fluorescence decay half-time was also pH-dependent. Simulation shows a p*K* of 7.5 which is very similar to the p*K* of the flash-induced fluorescence rise, possibly indicating the involvement of the same protonatable group in this reaction (Figure 3B). Summarizing our flash-induced fluorescence measurements in thylakoids from the dark-grown wild type, we conclude that both the forward electron transfer from Y_Z to $P680^+$ and the recombination pathways are sharply pH-dependent. Y_Z is more easily oxidized at higher pH values.

Mutants in D1-His190. Fluorescence measurements in D1-H190 mutants in *Synechocystis* sp. PCC 6802 (*16*, *18*, *21*) and from the *C. reinhardtii* H190F mutant (*24*) have been reported earlier. In the H190F mutant, the fluorescence rise was 10^2-fold slower than in the dark-grown wild type and Q_A^- was accumulated with a very reduced quantum yield (*24*).

Figures 4 and 7 show the flash-induced fluorescence kinetics in the two mutants studied here, D1-H190F and D1-H190Y. The kinetics of the fluorescence rise after a single flash are very slow and show a rise half-time of 500−700 μs in thylakoids from the H190F mutant (Figure 4) which is similar to those reported earlier (*24*). This rise was interpreted to reflect slow reduction of $P680^+$ from Y_Z which thus is thought to be a very slow and inefficient donor in H190 mutants (*16*, *21*, *24*). Accumulation of the high fluorescence state, reflecting the presence of Q_A^- in all PSII centers, shows considerable dependence on pH. At low pH values, more than five flashes are required to accumulate Q_A^- (or F_{max}) in all active centers (Figures 4A,B and 5A), as observed previously (*16*, *21*, *24*).

Unexpectedly, at pH 9.0, only two to four flashes were needed, suggesting more efficient electron donation at higher pHs (Figures 4C and 5A). Interestingly, the F_{final} level was stable for a considerable time (minutes) under our measuring conditions, and no decay from the high fluorescence state was observed even at low pH values which is different from the situation in the wild type where the decay occurred quickly at low pH (9.3 ms at pH 5.5, Table 2).

The pH dependence of the fluorescence amplitude after the first flash in the H190F mutant is shown in Figure 5B. Fitting the experimental data produces a p*K* of 8.1 for this induction which is 0.5 pH unit higher than in the wild type (Figure 3B), suggesting that a different titratable side chain on the donor side of PSII controls this reaction (oxidation of Y_Z) in the H190F mutant compared to that for the wild type.

The observation (Figure 4C) that the fluorescence induction is more efficient at elevated pHs in the H190F mutant suggests that a donor to $P680^+$ becomes active. The main candidates for this donor are Y_Z, cytochrome b_{559}, and Y_D. In control experiments, we could exclude cytochrome b_{559} and Y_D (see below). Therefore, we turned to kinetic studies

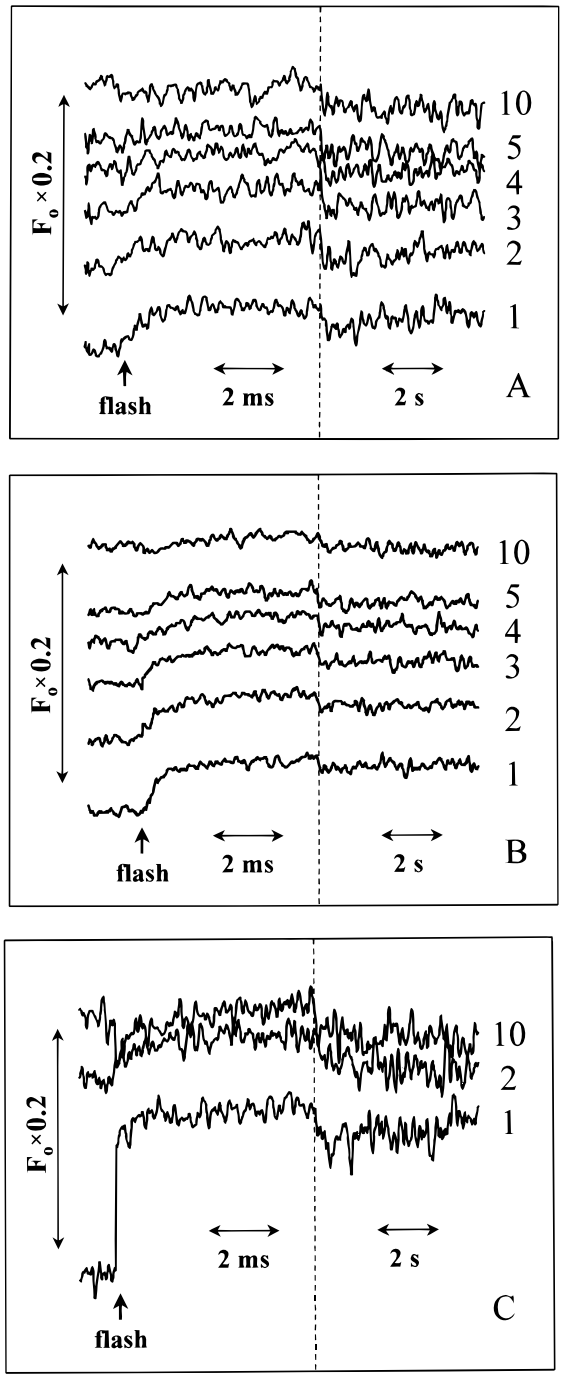


FIGURE 4: Single flash-induced fluorescence kinetics in the D1-H190F mutant. Thylakoids from dark-grown cells were assayed as in Figure 2 at pH 5.5 (A), 7.0 (B), and 9.0 (C). Each trace represents the average from ten (B), five (A), and three (C) samples. Actinic flashes were given at 5 s intervals.

of Y_Z induction at different pH values. Figure 6A shows the results of these experiments. At pH 5.5, we observed a very small induction of the Y_Z^{ox} radical. In contrast, at pH 9.0, the amplitude of the flash-induced Y_Z^{ox} was much bigger. Spin quantification using the maximal amplitude of Y_Z^{ox} in the wild type as the spin standard revealed that as much as $70 \pm 3\%$ of Y_Z was oxidized in a single flash in the mutant. At pH 7.0, about $20 \pm 5\%$ of Y_Z^{ox} was oxidized by the flash, consistent with our earlier studies (*25*) where we observed 10−15% Y_Z oxidation at pH 6.0. It should also be noted that the 10 flashes given to the sample did not result in any measurable oxidation of Y_D (not shown).

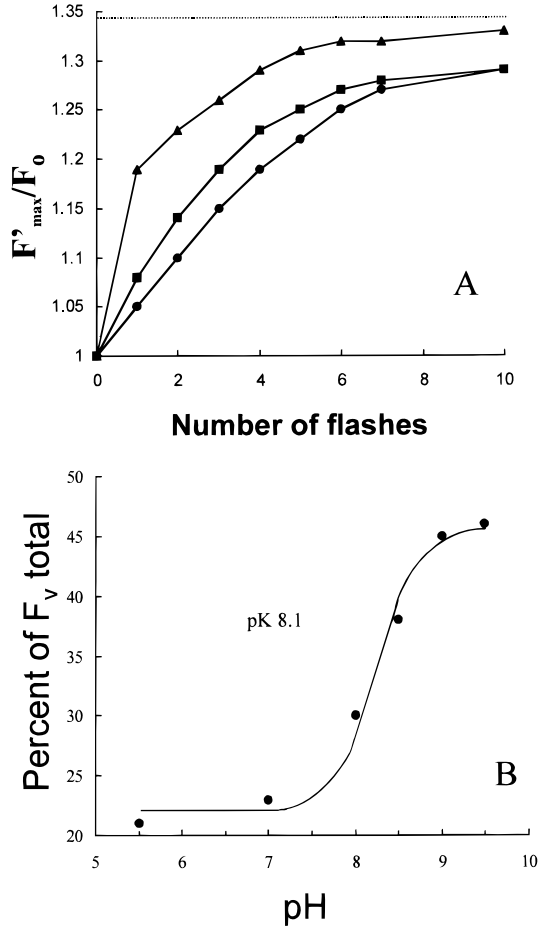


FIGURE 5: (A) Effect of flash number on the accumulation of the high fluorescence state in the H190F mutant. The fluorescence yield was measured essentially as in Figure 2 at pH 5.5 (●), 7.0 (■), and 9.0 (▲). The F_{max} level, reached in the presence of 10 mM dithionite, is indicated by the dotted line. (B) pH dependence and fitting of the experimental data of the first flash amplitude in the H190F mutant.

It is very important to assign the flash-induced radical transients in Figure 6A. At pH 6.0, this was done as previously described (*25*), and transient spectroscopy revealed that the spectral shape of Y_Z^{ox} was not affected in the H190 mutants, although only 10−15% of the radical was induced. At pH 9.0, we have not performed a complete spectral analysis, but in Figure 6A are shown traces recorded at two field positions. The trace recorded at 346.6 mT corresponds to the low-field shoulder of Y_Z^{ox}. The signal is very large and amounts to 70% of Y_Z oxidation. A kinetic trace was also recorded at 347.7 mT. At this field position, we could not observe any signal from a flash-induced radical. This is not surprising since 347.7 mT corresponds to the *g* value ($g = 2.0046$) of Y_Z^{ox}. At the *g* value, a radical species shows no amplitude in the EPR spectrum, and the lack of amplitude is, in fact, support for our argument in favor of assignment of the radical to Y_Z^{ox}. It should also be noted that other potential radicals in PSII (Chl^+, $P680^+$, carotenoid$^+$, etc.) have lower *g* values than Y_Z^{ox} and should show considerable amplitude at 347.7 mT.

Seemingly simple, the experiments in Figure 6A turned out to be very prone to misinterpretation. We earlier observed that Y_Z is rapidly inhibited by flashing in the H190 mutants (*25*). At pH 9.0, this photoinhibition is very prominent, and a significant fraction (>25%) of the Y_Z^{ox}

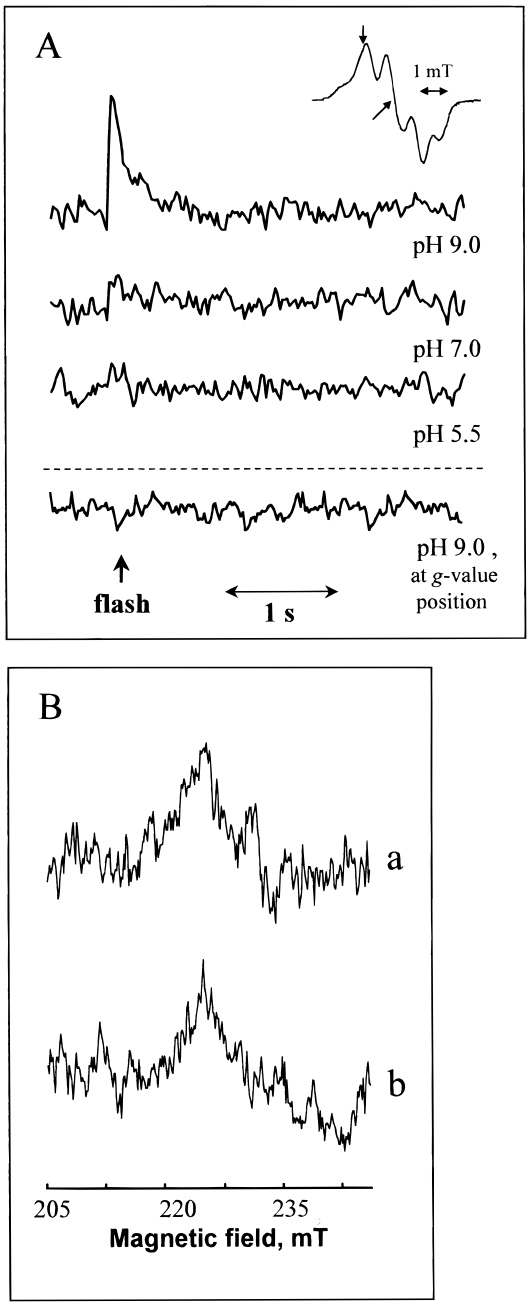


FIGURE 6: (A) Kinetic decay traces of flash-induced Y_Z^{ox} recorded at different pHs in thylakoids from the dark-grown D1-H190F mutant in the presence of 100 mM ferricyanide [to quantitatively oxidize P700 (*25*)]. Each trace represents the average from 10 samples (only 10 flashes with a 30 s interval were applied to each sample to minimize photoinhibition) at a magnetic field 346.6 mT (at the low-field shoulder of Y_Z^{ox}) or 347.7 mT (at the *g* value of Y_Z^{ox}). EPR conditions were as follows: microwave frequency, 9.77 GHz; microwave power, 40 mW; modulation amplitude, 0.5 mT; time constant, 20.48 ms; and conversion time, 20.48 ms. The kinetics were recorded at 294 K. The concentration of the thylakoids was ≈2.5 mg of Chl/mL. The inset shows a spectrum of Y_D^{ox} which is very similar to the spectrum of Y_Z^{ox} (*25*). The two arrows indicate the field positions used at 346.6 (the low-field maximum) and 347.7 mT. The latter position corresponds to the *g* value of Y_Z^{ox} and Y_D^{ox}. (B) EPR spectra of the $g_z \approx 3.0$ peak from oxidized cytochrome b_{559} in the H190F mutant at pH 9.0 from the dark-grown thylakoids prior the flash illumination (top spectrum, a) or after five saturating laser flashes (bottom spectrum, b). EPR conditions were as follows: microwave frequency, 9.47 GHz; microwave power, 5.41 mW; modulation amplitude, 1.58 mT; time constant, 40.96 ms; and conversion time, 81.92 ms. The spectra were recorded at 15 K.

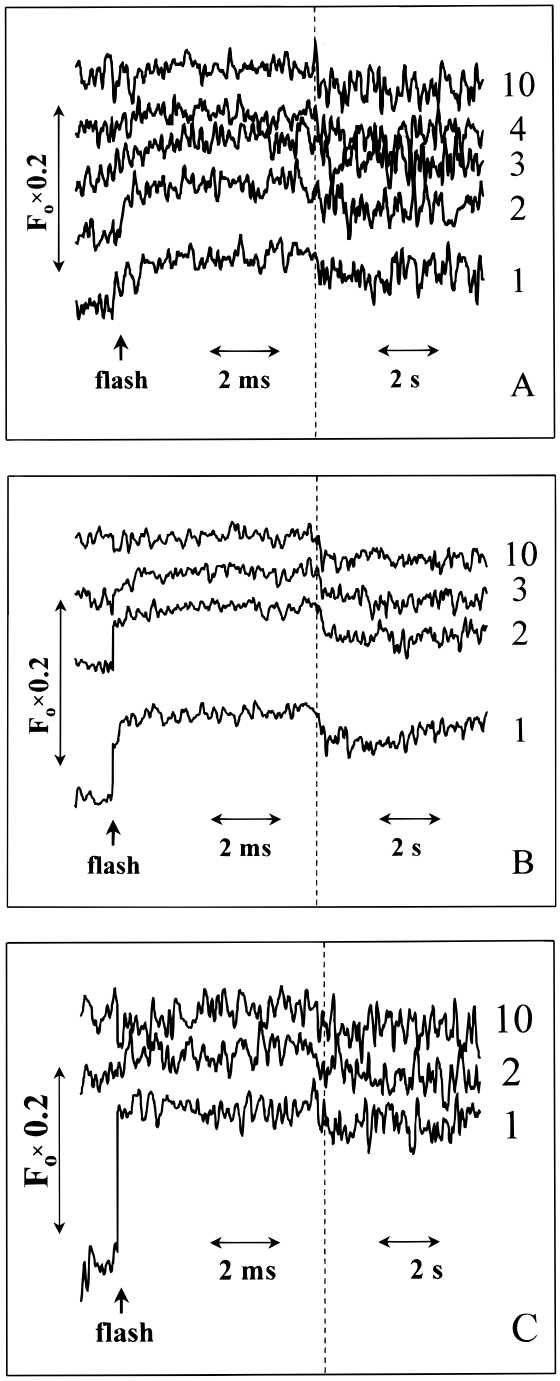


FIGURE 7: Single flash-induced fluorescence kinetics in thylakoids from the dark-grown D1-H190Y mutant. The measuring conditions were as described in Figure 2 at pH 5.5 (A), 7.0 (B), and 9.0 (C). Each trace represents the average of three (A and C) and five (B) samples. Actinic flashes were given at 5 s intervals.

radical is irreversibly photodamaged over only 20 flashes. To minimize this problem, each sample in the traces in Figure 6A was only exposed to 10 laser flashes. We can thus conclude that the 70% flash induction of Y_Z^{ox} at pH 9.0 probably underestimates the true first flash induction of Y_Z. The main conclusion is clear however; Y_Z also is the functional donor to $P680^+$ at high pH in the mutants.

Here we also report flash-induced fluorescence results for the D1-H190Y mutant in *C. reinhardtii* (Figures 7 and 8). The fluorescence rise characteristics at low pH are quite similar to those in the H190F mutant and show slow electron

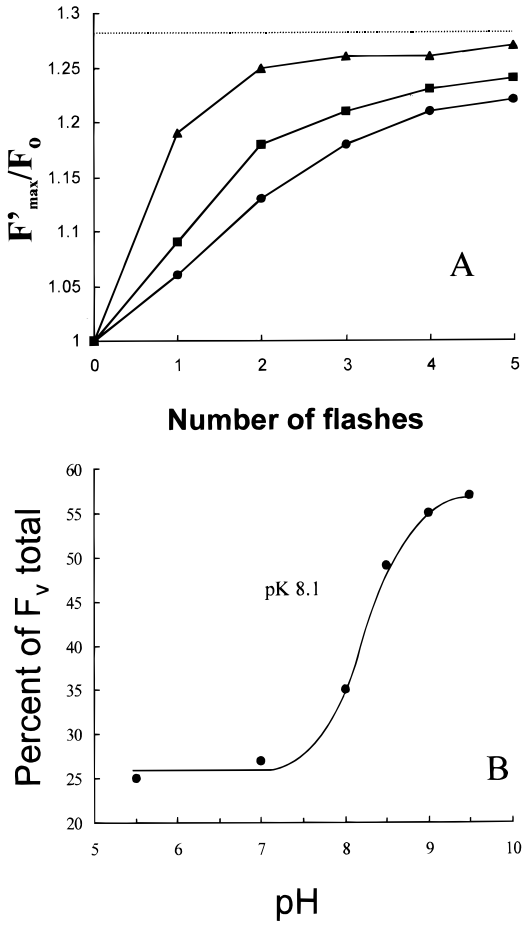


FIGURE 8: (A) Effect of flash number on the accumulation of the high fluorescence state in the H190Y mutant. The fluorescence yield was measured essentially as in Figure 2 at pH 5.5 (●), 7.0 (■), and 9.0 (▲). The F_{max} level, reached in the presence of 10 mM dithionite, is indicated by the dotted line. (B) pH dependence and fitting of the experimental data of the first flash amplitude in the H190Y mutant.

transport to $P680^+$ from Y_Z (rise half-time of about 500 μs at pH 5.5). However, the fluorescence rise kinetics become faster with increasing pH, and at pH 9.0, the rise half-time in the H190Y mutant was less than 100 μs (within our time resolution, Figure 7C). This indicates that the electron transfer from Y_Z to $P680^+$ is accelerated with increasing pH in this mutant. The high fluorescence state is also accumulated significantly more efficiently in the H190Y mutant than in the H190F mutant. Furthermore, the fluorescence level induced by a single flash decays with a half-time of approximately 400 ms in 30−40% of the PSII centers at low pH values (Figure 7A,B) which is different from the very stable fluorescence in the H190F mutant. Together, these data suggest that, although the mutation of H190 to a tyrosine slowed the reduction of $P680^+$ from Y_Z, the donor side is less severely inhibited in H190Y than in the H190F mutant. At higher pH, the donation from Y_Z is quite efficient, suggesting that a neighboring titratable group might accept the proton from Y_Z during its oxidation. Our analysis of the pH effect on the first flash amplitude (Figure 8B) shows a p*K* value for this group of 8.1, very similar to the situation in the H190F mutant (Figure 5B).

An unexpected observation was that the high fluorescence state did not decay in the H190 mutants. This was especially pronounced at high pH (Figures 4C and 7C). This was observed previously at neutral pHs and was attributed to the action of an unknown donor (*16*, *24*). We exclude Y_D as a possible donor to $P680^+$ in H190 mutants due to difficulties in inducing Y_D^{ox} even at high pH (see above). Another possible stable donor is cytochrome b_{559} which, at high pH, is converted to its high-potential form (*66*). However, cytochrome b_{559} can also be excluded as our EPR measurements at pH 9.0 showed no changes in the content of oxidized cytochrome b_{559} after five laser flashes in both H190 mutants (Figure 6B, not shown for the H190Y mutant). This flash treatment is enough to fully reduce Q_A (compare Figures 4C and 7C), and if cytochrome b_{559} had been the unknown electron donor, it should have been oxidized by the flashes. Instead, our experiments (Figure 6A) clearly show that Y_Z is the electron donor to $P680^+$. The slow decay of the fluorescence then suggests that Y_Z^{ox} instead is reduced by an electron from a source other than Q_A. Presumably, this comes from the medium as Y_Z^{ox} decays on the hundreds of milliseconds time scale also at high pH (Figure 6A).

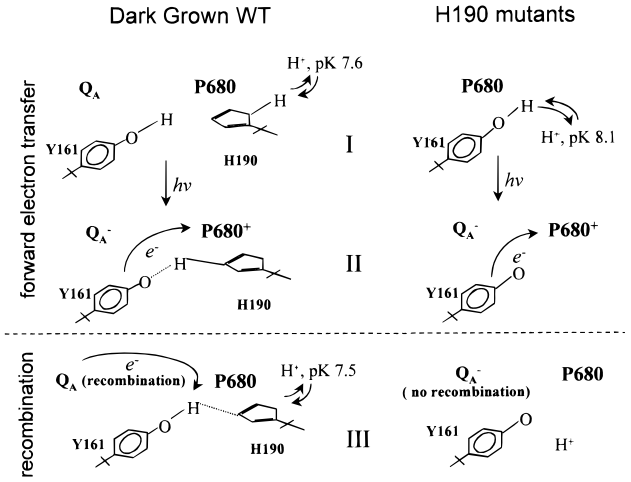
At low pH, we observed some decay in H190Y, but due to the small induction of fluorescence, it was difficult to study with precision. Therefore, we performed an experiment in which the high fluorescence state was induced at pH 9.6 in a weak 5 mM Tris buffer. The pH was then quickly readjusted to 5.5 with 50 mM Mes buffer. This allowed us to observe the decay of the flash-induced fluorescence, and in both mutants, this occurred almost to the F_o level within 1 min of dark incubation.

Thus, at high pH, the electron transport from Y_Z to $P680^+$ is accelerated, leading to efficient accumulation of the high fluorescent state ($P680/Q_A^-$) in the studied D1-H190 mutants and in the wild type. However, the recombination kinetics were severely inhibited in the mutants compared to the wild type which suggests that the D1-H190 residue is important not only for forward electron transfer but also in recombination pathways from Q_A^- to Y_Z^{ox} in PSII. In the H190Y mutant, the recombination rate was intermediate, always much slower than in the wild type but faster that in the H190F mutant.

DISCUSSION

We have characterized PSII reactions in the dark-grown wild type and two site-directed mutants, D1-H190F and D1-H190Y. A common way to study the assembly and function of the donor side of PSII is to remove the Mn cluster and peripheral subunits with biochemical treatments such as Tris washing (*53*−*55*, *67*−*69*) which might affect the amino acid residues around the redox components on the donor side of PSII. Here we have chosen another approach to minimize these problems. *C. reinhardtii*, unlike higher plants, becomes green when it is grown in darkness in the presence of a carbon source. The entire PSII apparatus can be assembled in darkness except for the Mn cluster, and "dark-grown PSII" performs both efficient charge separation and photoinduced electron transport reactions (excluding oxygen evolution). Therefore, thylakoid preparations from dark-grown cells might provide a more suitable material for studying donor side redox components such as Y_Z and Y_D due to a less modified protein environment and a lack of the influence from the Mn cluster than compared to commonly used Mn-extracted preparations.

Scheme 1



We estimate, from EPR and fluorescence measurements, the content of PSII in dark-grown material to about 40−50% of that in the light-grown cells (Table 1) which correlates well with earlier electron transport data in the H190 mutants (*24*, *25*). F_v/F_o was 0.4 in the dark-grown wild type and 0.34−0.35 in the H190 mutants. This F_v/F_o value is rather low but was repeatedly found in material grown under these circumstances. Thus, dark growing conditions produce only approximately half as many PSII centers compared to light growing conditions. In addition, the F_o level was higher in the dark-grown material, indicating that a significant part of the antenna chlorophylls might not be connected to the reaction center.

Our fluorescence measurements show that the accumulation of the high fluorescence state (reduction of Q_A) is sharply dependent on the apparent pH value in the dark-grown material. This does not reflect pH-dependent acceptor side reactions since the experiments were performed in the presence of DCMU which prevents forward electron transfer from Q_A^-. Instead, the accumulation of the high fluorescence state (F_v induction) reflects forward electron transfer from Y_Z to $P680^+$, and our experiments showed that this is more effective at high pH. Our analysis of the first flash amplitude induction indicates that the Y_Z-P680^+ electron transfer kinetics are pH-dependent and accelerated with a p*K* value of 7.6 in the wild type (Figure 3B). The control of this reaction by the local pH was shown earlier in Tris-treated PSII preparations (*70*−*73*). The group responsible for this pH-dependent control of Y_Z oxidation was not identified. However, it is interesting that a similar p*K* (≈7.3−7.5) was reported for the protonation that retards electron transfer from Y_D during its oxidation (*65*), and it was proposed that this p*K* reflected protonation of the nearby H190 on the D2 protein. The involvement of D2-H190 in hydrogen bond formation with Y_D has later been proved by computer modeling (*29*) and advanced spectroscopy (*74*−*77*). We propose here that a similar mechanism controls Y_Z oxidation involving simultaneous proton transfer to D1-H190 and oxidation of Y_Z in the dark-grown PSII (Scheme 1).

A first argument for this proposal comes from the first flash fluorescence induction in the H190 mutants and its pH dependence. At low pH, we hardly observed any oxidation of Y_Z [Figure 6A (*25*)]. Also, the efficiency of the fluorescence induction was much lower than in the wild type at low pH (Figures 3B, 5B, and 8B), and the fluorescence rise was very slow (100−1000-fold slower compared to that for the wild type) which is consistent with previous reports (*18*, *21*, *24*). At pH 9.0, we could observe almost complete oxidation of Y_Z (Figure 6A). In accordance with this, the fluorescence induction was also efficient at high pH in the H190 mutants. Thus, the electron transfer from Y_Z to $P680^+$ in the H190 mutants is controlled by the pH in the medium. In both mutants, the p*K* value for this acceleration was different from the wild type value (8.1 compared to 7.6). Therefore, the removal of H190 made the p*K* for Y_Z oxidation higher.

The question then is, which base controls the Y_Z oxidation in the mutants? A p*K* of 8.1 is basic, and there are few clear potential candidates in the vicinity of Y_Z judging from the structural proposals for the Y_Z environment (*29*). However, one option is that the increase of the pH resulted in deprotonation of Y_Z itself. The effect of Y_Z deprotonation would indeed be that the electron transfer from Y_Z would be facilitated despite the lack of the normal base (H190). Thus, we attribute the pH control (p$K = 8.1$) of Y_Z oxidation in the H190 mutants to direct titration of Y_Z itself. A p*K* of 8.1 is lower than that for free tyrosine (p$K = 10.1$). However, it is not uncommon that amino acid residues show abnormal p*K*s in proteins, and the lower p*K* might reflect local effects around Y_Z induced by the protein.

A second argument for our proposal that H190 is involved in the proton translocation around Y_Z comes from the recombination reactions. They were also found to be pH-dependent in the dark-grown wild type (Figure 2) which is consistent with a recent study on Mn-depleted PSII (*51*). In the wild type, the fluorescence decay was controlled by a base (p$K = 7.5$) which most likely reflects protonation of the same base that controls Y_Z oxidation (p$K = 7.6$). Thus, we suggest that this base is H190 and that the decay results show that $Q_A^-Y_Z^{ox}$ recombination occurs efficiently when H190 can provide a proton (Scheme 1). However, at high pH, when the H^+ is absent, the recombination is slower and does not occur.

This conclusion is corroborated by our observations in the H190 mutants where we only observed partial decay of the high fluorescence state in the H190Y mutant at low pH (Figures 4 and 7). In H190F, and at higher pHs in the H190Y mutant, the fluorescence did not decay within minutes. This stable Q_A^- was observed previously (*16*, *24*, *51*, *62*−*64*), and it was suggested that an alternative donor to Y_Z^{ox} prevents charge recombination between Q_A^- and Y_Z^{ox} in the H190 mutants. We have searched for this donor and can exclude Y_D, cytochrome b_{559}, and probably also Mn^{2+} due to the low Mn content (≤1 Mn/PSII). Instead, our experiments show that the situation is conceptually similar in the wild type and H190 mutants. In the wild type, the $Q_A^-Y_Z^{ox}$ recombination occurs with a half-time of 10 ms at low pH (no alternative donor is observed, the decay encompasses all centers). At high pH, the recombination rate was significantly decreased ($t_{1/2} > 100$ ms), and about 40% of the centers showed no recombination (Figure 2C). This can be explained by competing Y_Z^{ox} reduction from electron sources other than Q_A^- which occurs in this time range irrespective of pH (not shown, compare Figure 6A for the H190F mutant). Thus, in the wild type, $Q_A^-Y_Z^{ox}$ recombination is controlled by pH and wins, at lower pHs,

over Y_Z^{ox} reduction from other possible electron donors in this kind of preparation (for a discussion, see ref *51*). In the mutants, however, recombination is slow also at low pH and Y_Z^{ox} is reduced from the medium instead. We propose that the slow recombination is due to the lack of H190 which should have delivered the proton during Y_Z^{ox} reduction.

A third observation supporting our notion that D1-H190 is important for the function of Y_Z is that the recombination differs in the H190F and H190Y mutants. In both mutants, the Q_A^-−Y_Z^{ox} recombination was slow relative to that of the wild type. Q_A^-−Y_Z^{ox} recombination was only observed in the H190Y mutant at low pH where it occurred in a fraction of the PSII centers with a half-time of 400 ms. Thus, the rate of recombination is under all circumstances slower than or similar to the rate of Y_Z^{ox} reduction from other electron donors (*25*; compare also with Figure 5, the decay half-time of Y_Z^{ox} in H190F is 100 ms at pH 9.0). It should also be noted that the very stable high fluorescence state, with no recombination to the PSII donor side in H190 mutants, correlates well with the complete absence of the A_T thermoluminescence band in the H190F mutant (*26*) which probably reflects poor recombination between Q_A^- and Y_Z^{ox} (*3*, *26*). These experiments suggest that the exchange of D1-H190 to phenylalanine impedes Q_A^-−Y_Z^{ox} recombination. We suggest that the electron stays at Q_A^- when H190, the proton abstracting partner for Y_Z, is absent. The partial decay of Q_A^- in the H190Y mutant might, however, suggest that a residue like tyrosine might partially replace histidine while a phenylalanine (H190F mutant) cannot. Debus and co-workers (*16*) also reported partial fast (hundreds of milliseconds) Q_A^- decay in D1-H190 mutants (H190N, H190Q, H190L, and H190Y) from fluorescence studies in whole cells of *Synechocystis* sp. PCC 6803.

Recently, Rappaport and Lavergne (*51*) proposed that Q_A^-−Y_Z^{ox} recombination is controlled by a group "G", with a p*K* of 6.0. This interacts electrostatically with Y_Z^{ox}, while the proton given off during Y_Z oxidation, which in their view does not necessarily occur via a hydrogen bond, is trapped by a nearby residue "A" which may or may not be the same as "G". Very recently Ahlbrink et al. (*47*) suggested that in oxygen-evolving material this base, denoted as "B", is very effective and the rate of proton transfer between Y_Z and "B" is comparable to the rate of electron transfer from Y_Z. In the case of PSII without oxygen evolution, electron transfer from Y_Z to $P680^+$ is controlled by the protonation state of this base with a p*K* of 7.0 (*47*). Our results are in essence very similar to those in refs *47* and *51*. However, we consider that the results from our extension of the study to the H190 mutants are best accommodated in a model where both these reactions, i.e., electron transfer from Y_Z to $P680^+$ and Q_A^-−Y_Z^{ox} recombination, are controlled by the same residue, D1-H190 (i.e., residues A, G, and B represent H190).

Our conclusion is that H190 participates in both forward and backward electron transfer (Scheme 1). In forward electron transfer, H190 participates in proton reshuffling around Y_Z. When the H190 is not protonated (high pH), electron transfer is fast, while at low pH, the electron transfer is slower. In the wild type, Y_Z can always deprotonate to H190 which acts as a weak base and accepts H^+ from Y_Z. In the mutants, H190 is not present to accept the phenolic proton from Y_Z, leading to slower rates of $P680^+$ reduction and a lower F_{max} level following a flash (most of the oxidized primary donor $P680^+$ is lost to fast recombination with Q_A^-). This inhibitory situation can however be abolished at high pH probably by direct titration of Y_Z-OH.

In the Q_A^-−Y_Z^{ox} recombination pathway, the situation is very different and recombination does not occur at all in the H190F mutant and only to a limited extent in the H190Y mutant (see also ref *16*). In the wild type, charge recombination is controlled by a group which has a p*K* similar to the p*K* of the group which regulates Y_Z oxidation. This probably again reflects H190 protonation−deprotonation. At high pH, Y_Z^{ox} can either recombine or be re-reduced by other donors, and both reactions occur on the same time scale. In the mutants, the recombination is always slower and Y_Z^{ox} is mainly reduced by the electrons from the surroundings.

If this model is correct, an interesting question arises. If the presence of a proton in the vicinity of Y_Z^{ox} is necessary for its reduction by the recombination pathway, why is it not necessary for the reduction of Y_Z^{ox} from the surroundings? A possible way to explain this might be related to how Y_Z is normally reduced. It was recently proposed that Y_Z^{ox}, upon its reduction, mediates hydrogen atom transfer from metal-coordinated water (*7*, *34*−*38*). In this mechanism, the reduction of Y_Z^{ox} from the Mn cluster is charge neutral (an electron and a proton are translocated simultaneously) and the proton leaves and comes back to Y_Z via different sites. Therefore, in this model, there are two pathways for the proton to approach Y_Z^{ox}: from substrate water or by reversal of the proton abstraction via H190. One conclusion from our mutant studies is that they support the options for two proton sites with access to Y_Z^{ox}. In the absence of H190, Y_Z oxidation is very inefficient due to the inability of Y_Z to become deprotonated. Thus, H190 seems to be the proton-accepting base in forward electron transfer. However, our studies are performed in the absence of the Mn cluster. In this case, Y_Z^{ox} is never reduced by hydrogen atom transfer from water. Nevertheless, we observe decay kinetics of Y_Z^{ox} (Figure 6A) which are pH-independent and quite normal for Y_Z^{ox} in the absence of the Mn cluster. In addition, both electrons and protons can access Y_Z^{ox} despite the absence of H190, suggesting another proton pathway which is still functional in the mutants. In contrast, the absence of H190 completely prevents the back reaction from Q_A^- to Y_Z^{ox} which suggests that proton translocation occurs via D1-H190 in this reaction. Another conclusion from the results in the H190 mutants, where Y_Z^{ox} reduction from Q_A^- is severely slowed, is also that the reduction of Y_Z^{ox} is a coupled charge neutral reaction. The electron and the proton leave Y_Z simultaneously and have to come back simultaneously independent of pathway.

To conclude, our results support earlier observations that the H190 residue on the D1 protein is an important component in the electron transport reaction from Y_Z to $P680^+$. Our pH-dependent measurements indicate that H190 accepts a proton from Y_Z (*16*, *18*, *29*). In addition, our results rule out the direct involvement of D1-H190 in electron transport from Y_Z to $P680^+$ since Y_Z becomes an efficient donor at elevated pHs in the mutants. We also propose that H190 is able to return a proton to Y_Z^{ox} during the recombination from Q_A^-. In the H190 mutants, no such recombination takes place. Our model for these reactions is outlined in Scheme 1 where we show interactions between Y_Z and the H190 residue in the wild type and the H190

mutants before (I) and after (II) the first flash and during charge recombination (III).

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REFERENCES

1. Andersson, B., and Styring, S. (1991) in *Current Topics in Bioenergetics* (Lee, C. P., Ed.) Vol. 16, pp 1−81, Academic Press, San Diego, CA.
2. Rutherford, A. W., Zimmermann, J.-L., and Boussac, A. (1992) in *The Photosystems: Structure, Function and Molecular Biology* (Barber, J., Ed.) pp 179−229, Elseiver Science Publishers, B. V., Amsterdam, The Netherlands.
3. Debus, R. J. (1992) *Biochim. Biophys. Acta 1102*, 269−352.
4. Diner, B. A., and Babcock, G. T. (1996) in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yocum, C. F., Eds.) pp 213−247, Kluwer Academic Publishers, Dordrecht, The Netherlands.
5. Renger, G. (1992) in *The Photosystems: Structure, Function and Molecular Biology* (Barber, J., Ed.) pp 45−99, Elseiver Science Publishers, B. V., Amsterdam, The Netherlands.
6. Renger, G. (1993) *Photosynth. Res. 38*, 229−247.
7. Britt, R. D. (1996) in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yocum, C. F., Eds.) pp 137−164, Kluwer Academic Publishers, Dordrecht, The Netherlands.
8. Bricker, T. M., and Ghanotakis, D. F. (1996) in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yocum, C. F., Eds.) pp 113−136, Kluwer Academic Publishers, Dordrecht, The Netherlands.
9. Barry, B. A., Boerner, R. J., and de Paula, J. C. (1994) in *The Molecular Biology of Cyanobacteria* (Bryant, D., Ed.) pp 217−257, Kluwer Academic Publishers, Dordrecht, The Netherlands.
10. Bernard, M. T., MacDonald, M. D., Nguyen, A. P., Debus. R. J., and Barry, B. A. (1995) *J. Biol. Chem. 270*, 1589−1594.
11. Boerner, R. J., Nguyen, A. P., Barry, B. A., and Debus, R. J. (1992) *Biochemistry 31*, 6660−6672.
12. Chu, H.-A., Nguyen, A. P., and Debus, R. J. (1993) *Biophys. J. 64*, A216.
13. Chu, H.-A., Nguyen, A. P., and Debus, R. J. (1994) *Biochemistry 33*, 6137−6149.
14. Chu, H.-A., Nguyen, A. P., and Debus, R. J. (1994) *Biochemistry 33*, 6150−6157.
15. Chu, H.-A., Nguyen, A. P., and Debus, R. J. (1994) *Photochem. Photobiol. 59*, 82S.
16. Chu, H.-A., Nguyen, A. P., and Debus, R. J. (1995) *Biochemistry 34*, 5839−5858.
17. Chu, H.-A., Nguyen, A. P., and Debus, R. J. (1995) *Biochemistry 34*, 5859−5882.
18. Diner, B. A., Nixon, P. J., and Farchaus, J. W. (1991) *Curr. Opin. Struct. Biol. 1*, 546−554.
19. Diner, B. A., and Nixon, P. J. (1992) *Biochim. Biophys. Acta 1101*, 134−138.
20. Nixon, P. J., and Diner, B. A. (1992) *Biochemistry 31*, 942−948.
21. Nixon, P. J., and Diner, B. A. (1994) *Biochem. Soc. Trans. 22*, 338−343.
22. Nixon, P. J., Trost, J. T., and Diner, B. A. (1992) *Biochemistry 31*, 10859−10871.
23. Debus, R. J., Barry, B. A., Babcock, G. T., and McIntosh, L. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 427−430.
24. Roffey, R. A., Kramer, D. M., Govindjee, and Sayre, R. T. (1994) *Biochim. Biophys. Acta 1185*, 257−270.
25. Roffey, R. A., van Wijk, K. J., Sayre, R. T., and Styring, S. (1994) *J. Biol. Chem. 269*, 5115−5121.
26. Kramer, D. M., Roffey, R. A., Govindjee, and Sayre, R. T. (1994) *Biochim. Biophys. Acta 1185*, 228−237.
27. Whitelegge, J. P., Koo, D., Diner, B. A., Domian, I., and Erickson, J. M. (1995) *J. Biol. Chem. 270*, 225−235.
28. Svensson, B., Vass, I., Cedergren, E., and Styring, S. (1990) *EMBO J. 9*, 2051−2059.
29. Svensson, B., Etchebest, C., Tuffery, P., van Kan, P., Smith, J., and Styring, S. (1996) *Biochemistry 35*, 14486−14502.
30. Ruffle, S. V., Donelly, D., Blundell, T. L., and Nugent, J. H. (1992) *Photosynth. Res. 34*, 287−300.
31. Boussac, A., Zimmermann, J. L., Rutherford, A. W., and Lavergne, J. (1990) *Nature 347*, 303−306.
32. Tang, X.-S., Diner, B. A., Larsen, B. S., Gilchrist, M. L., Lorigan, G. A., and Britt, R. D. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 704−708.
33. Tang, X.-S., Nixon, P. J., Britt, R. D., and Diner, B. A. (1994) *Photochem. Photobiol. 59*, 82S−83S.
34. Hoganson, C. W., Lydakis-Simantiris, N., Tang, X.-S., Tommos, C., Warncke, K., Babcock, G. T., Diner, B. A., McCracken, J., and Styring, S. (1995) *Photosynth. Res. 46*, 177−184.
35. Tommos, C., Tang, X.-S., Warncke, K., Hoganson, C. W., Styring, S., McCracken, J., Diner, B. A., and Babcock, G. T. (1995) *J. Am. Chem. Soc. 117*, 10325−10335.
36. Babcock, G. T. (1995) in *Photosynthesis: from Light to Biosphere* (Mathis, P., Ed.) Vol. II, pp 209−215, Kluwer Academic Publishers, Dordrecht, The Netherlands.
37. Hoganson, C. W., and Babcock, G. T. (1997) *Science 277*, 1953−1956.
38. Tommos, C., and Babcock, G. T. (1998) *Acc. Chem. Res. 31*, 18−25.
39. Mino, H., and Kawamori, A. (1994) *Biochim. Biophys. Acta 1185*, 213−220.
40. Force, D. A., Randall, D. W., Britt, R. D., Tang, X.-S., and Diner, B. A. (1995) *J. Am. Chem. Soc. 117*, 12643−12644.
41. Gilchrist, M. L., Ball, J. A., Randall, D. W., and Britt, R. D. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 9545−9549.
42. Tang, X.-S., Zheng, M., Chisholm, D. A., Dismukes, G. C., and Diner, B. A. (1996) *Biochemistry 35*, 1475−1484.
43. Force, D. A., Randall, D. W., and Britt, R. D. (1997) *Biochemistry 36*, 12062−12070.
44. Blomberg, M. R. A., Siegbahn, P. E. M., Styring, S., Babcock, G. T., Åkermark, B., and Korall, P. (1997) *J. Am. Chem. Soc. 119*, 8285−8292.
45. Karge, M., Irrgang, K.-D., and Renger, G. (1997) *Biochemistry 36*, 8904−8913.
46. Haumann, M., Bögershausen, O., Cherepanov, D. A., Ahlbrink, R., and Junge, W. (1997) *Photosynth. Res. 51*, 193−208.
47. Ahlbrink, R., Haumann, M., Cherepanov, D., Bögershausen, O., Mulkidjanian, A., and Junge, W. (1998) *Biochemistry 37*, 1131−1142.
48. Lydakis-Simantiris, N., Ghanotakis, D. F., and Babcock, G. T. (1997) *Biochim. Biophys. Acta 1322*, 129−140.
49. Haumann, M., and Junge, W. (1996) in *Oxygenic Photosynthesis: The Light Reactions* (Ort, D. R., and Yocum, C. F., Eds.) pp 165−192, Kluwer Academic Publishers, Dordrecht, The Netherlands.
50. Haumann, M., and Junge, W. (1994) *Biochemistry 33*, 864−872.
51. Rappaport, F., and Lavergne, J. (1997) *Biochemistry 36*, 15294−15302.
52. Gorman, D. S., and Levine, R. P. (1965) *Proc. Natl. Acad. Sci. U.S.A. 54*, 1665−1669.
53. Tamura, N., and Cheniae, G. M. (1987) *Biochim. Biophys. Acta 890*, 179−194.
54. Blubaugh, D. J., and Cheniae, G. M. (1990) *Biochemistry 29*, 5109−5118.
55. Miyao, M., and Inoue, Y. (1991) *FEBS Lett. 278*, 183−186.
56. Tamura, N., Kamachi, H., Hokari, N., Masumoto, H., and Inoue, H. (1991) *Biochim. Biophys. Acta 1060*, 51−58.
57. Babcock, G. T., and Sauer, K. (1973) *Biochim. Biophys. Acta 325*, 483−503.
58. Barry, B. A., and Babcock, G. T. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7099−7103.

59. Miller, M., and Brudvig, G. W. (1991) *Biochim. Biophys. Acta 1056*, 1−18.
60. Krause, G. H., and Weis, E. (1991) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 42*, 313−349.
61. Dau, H. (1994) *J. Photochem. Photobiol., B 26*, 3−27.
62. Ghirardi, M. L., Lutton, T. W., and Seibert, M. (1996) *Biochemistry 35*, 1820−1828.
63. Dekker, J. P., van Gorkom, H. J., Wensink, J., and Ouwehand, L. (1984) *Biochim. Biophys. Acta 767*, 1−9.
64. Metz, J. G., Nixon, R. G., Rogner, M., Brudvig, G. W., and Diner, B. A. (1989) *Biochemistry 28*, 6960−6969.
65. Vass, I., and Styring, S. (1991) *Biochemistry 30*, 830−839.
66. De Las Rivas, J., Klein, J., and Barber, J. (1995) *Photosynth. Res. 46*, 193−202.
67. Miller, M., and Brudvig, G. W. (1989) *Biochemistry 28*, 8181−8190.
68. Miller, M., and Brudvig, G. W. (1990) *Biochemistry 29*, 1385−1392.
69. Ono, T.-A., and Inoue, Y. (1987) *Plant Cell Physiol. 28*, 1293−1299.
70. Conjeaud, H., and Mathis, P. (1980) *Biochim. Biophys. Acta 590*, 353−359.
71. Conjeaud, H., and Mathis, P. (1986) *Biophys. J. 49*, 1215−1221.
72. Renger, G., and Voelker, M. (1982) *FEBS Lett. 149*, 203−207.
73. Schlodder, E., and Meyer, B. (1987) *Biochim. Biophys. Acta 890*, 23−31.
74. Tommos, C., Davidsson, L., Svensson, B., Madsen, C., Vermaas, W., and Styring, S. (1993) *Biochemistry 32*, 5436−5441.
75. Tang, X.-S., Chilsholm, D. A., Dismukes, G. C., Brudvig, G. W., and Diner, B. A. (1993) *Biochemistry 32*, 13742−13748.
76. Rigby, S. E. J., Nugent, J. H. A., and O'Malley, P. J. (1994) *Biochemistry 33*, 1734−1742.
77. Warncke, K., McCracken, J., and Babcock, G. T. (1994) *J. Am. Chem. Soc. 116*, 7332−7340.

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