A psaC deletion mutant of the unicellular cyanobacterium Synechocystis sp. PCC 6803 was utilized to incorporate site-specific amino acid substitutions in the cysteine residues that ligate the $F_A$ and $F_B$ iron-sulfur clusters in Photosystem I (PS I). Cysteines 14 and 51 of PsaC were changed to aspartic acid (C14D/PsaC, C51D/PsaC), serine (C14S/PsaC, C51S/PsaC), and alanine (C14A/PsaC, C51A/PsaC), and the properties of $F_A$ and $F_B$ were characterized by electron paramagnetic resonance spectroscopy and time-resolved optical spectroscopy. The C14D/PsaC-PS I and C14S/PsaC-PS I complexes showed high levels of photoreduction of $F_A$ with $g$ values of 2.045, 1.944, and 1.852 after illumination at 15 K, but there was no evidence of reduced $F_B$ in the $g = 2$ region. The C51D/PsaC-PS I and C51S/PsaC-PS I complexes showed low levels of photoreduction of $F_A$ with $g$ values of 2.067, 1.931, and 1.881 after illumination at 15 K, but there was no evidence of reduced $F_B$ in the $g = 2$ region. The presence of $F_B$ was inferred in C14D/PsaC-PS I and C14S/PsaC-PS I, and the presence of $F_B$ was inferred in C51D/PsaC-PS I and C51S/PsaC-PS I by magnetic interaction in the photoacquired spectra and by the equal spin concentration of the irreversible P700$^+$ cation generated by illumination at 77 K. Flash-induced optical absorbance changes at 298 K in the presence of a fast electron donor indicate that two electron acceptors function after $F_B$ in the four mutant PS I complexes at room temperature. These data suggest that a mixed-ligand [4Fe-4S] cluster is present in the mutant sites of C14X-PS I and C51X-PS I ($X = D$ or $S$), but that the proposed spin state of $S = 3/2$ renders the resonances undetectable in the $g = 2$ region. The C14A/PsaC-PS I, C51A/PsaC-PS I and C14D/C51D/PsaC-PS I complexes show only the photoreduction of $F_X$ consistent with the absence of PsaC. These results show that only those PsaC proteins that contain two [4Fe-4S] clusters are capable of assembling onto PS I cores in vivo.

There are several exceptions to the nearly universal occurrence of cysteine thiolate ligands to iron-sulfur clusters in the ferredoxin class of electron transfer proteins (1). Examples include the N-ligands from histidine residues in the Rieske subclass of iron-sulfur proteins (2–4), $O$-ligands from (most likely) aspartate in Fd III from Desulfovibrio africanus (5), and a proposed O-ligand from serine to the pentacoordinated iron in the P-cluster of nitrogenase (6). Recently, oxygen ligands have been introduced in lieu of cysteine thiolates by site-directed mutagenesis in proteins that contain [2Fe-2S], [3Fe-4S], and/or [4Fe-4S] clusters. Examples include the introduction of serine for cysteine in the interglycopeptide $F_X$ cluster of Photosystem I (PS I) (7, 8) and the [4Fe-4S] cluster of Escherichia coli fumarate reductase (9). The consequence of these changes include a change in the EPR spectrum and a decreased electron transfer efficiency in the case of serine-ligated $F_X$ and a change in the cluster midpoint potential and intercluster spin interaction in the case of E. coli fumarate reductase.

One instance of a functional [4Fe-4S] cluster derived from an aspartate-for-cysteine change is the modified PsaC subunit of PS I. In a previous study, E. coli expressed mutant PsaC proteins were reconstituted onto P700-$F_X$ cores, and the assignment of the ligands for the two terminal electron acceptors was determined by EPR spectroscopy (10). The substitution of aspartate for cysteine in position 14 of PsaC led to the retention of a $S = 1/2$, [4Fe-4S] cluster at the unmodified site with $g$ values characteristic of $F_A$. Similarly, the substitution of aspartate for cysteine in position 51 of PsaC led to the retention of a $S = 1/2$, [4Fe-4S] cluster at the unmodified site with $g$ values characteristic of $F_B$. Since the pattern of cysteine ligation in PsaC is expected to be identical to that of ferredoxins with two [4Fe-4S] clusters whose structures have been determined, it follows that $F_B$ is ligated by cysteines 11, 14, 17, and 58 and $F_A$ is ligated by cysteines 21, 48, 51, and 54 (10).

The in vitro reconstitution experiments led to several hypotheses regarding alternative ligands to iron-sulfur clusters: 1) unbound PsaC refolds only in the presence of one [3Fe-4S] and one [4Fe-4S] or two [4Fe-4S] clusters when cysteine is replaced in positions 14 and 51 by aspartate, serine, and alanine (11, 12). According to these results, a stable three-dimensional structure requires the presence of two iron-sulfur clusters, one of which must be a cubane. 2) The failure to observe a

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The abbreviations used are: PS I, Photosystem I; $F_X$, the secondary electron acceptor in Photosystem I, a phylloquinone; DCPIP, 2,6-dichlorophenol-indophenol; DM-PS I, Photosystem I complex (containing $F_X$, $F_A$, and iron-sulfur clusters) isolated using n-dodecyl-β-maltoside; P700, the primary electron donor in Photosystem I, a chlorophyll a dimer; $\Delta A_{632}$, photoinduced absorbance change at 632 nm; C14X/PsaC or C51X/PsaC (where $X = A$, $D$, or $S$), unbound mutant PsaC protein; C14X/PsaC-PS I or C51X/PsaC-PS I (where $X = A$, $D$, or $S$), PS I complex incorporating mutant PsaC protein; W, milliwatts; mT, milliteslas; PMS, phenazine methosulfate; MES, 4-morpholineethanesulfonic acid.

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Strains of Synechocystis sp. PCC 6803 with Altered PsaC

II. EPR AND OPTICAL SPECTROSCOPIC PROPERTIES OF $F_A$ AND $F_B$ IN ASPARTATE, SERINE, AND ALANINE REPLACEMENTS OF CYSTEINES 14 AND 51*

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In Vivo Mutagenesis of F_A and F_B in PS I

3Fe-4S] cluster in the *in vitro* reconstituted C14PsaC-PS I or C51X_PsaC-PS I complexes (where X = D, A, or S) indicates that the binding of PsaC onto P700-FX cores requires the presence of two [4Fe-4S] clusters. If rigorously true, it may be difficult to introduce a [3Fe-4S] cluster motif into PsaC *in vivo*. 3) An oxygen ligand to the [4Fe-4S] cluster in the mutant site of the C14D_PsaC-PS I and the C51D_PsaC-PS I complexes leads to a high-spin state (likely S = 3/2).

In this paper, these hypotheses were tested through the generation of a series of *in vivo* mutations in F_A and F_B. The choices to replace cysteine residues with aspartic acid, serine, and alanine were made because aspartate is known to support [3Fe-4S] and [4Fe-4S] clusters in naturally occurring ferrodoxins (13, 14), serine can support [3Fe-4S] and [4Fe-4S] clusters in the FX binding site of PS I (7) and in the *Azotobacter vine-landii* hydrogenase small subunit (15), and alanine, with the absence of a suitable ligand, should support only [3Fe-4S] clusters. This paper summarizes the EPR spectral characteristics and electron transfer properties of aspartate (C14D_PsaC, C51D_PsaC, C14D/C51D_PsaC), alanine (C14A_PsaC, C51A_PsaC), and serine (C14S_PsaC, C51S_PsaC) mutations in PsaC in *Synechocystis* sp. PCC 6803. The premise tested is that [4Fe-4S] clusters will only be assembled in those mutants where oxygen ligands are available from the side chains of the replacement amino acids. A companion paper (16) describes the genetics, physiology, and electron transfer efficiency on the acceptor side in these *in vivo* PsaC mutants.

**EXPERIMENTAL PROCEDURES**

**Isolation of Thylakoid Membranes and Purification of DM-PS I Complexes**—The protocols for the isolation of the cyanobacterial thylakoid membranes and the purification of the n-dodecyl β-D-maltoside PS I complexes are described in Ref. 16.

**Electron Paramagnetic Resonance Spectroscopy**—Electron paramagnetic resonance (EPR) studies were performed using a Bruker ECS-106 X-band spectrometer equipped with either a standard resonator (ER4102 ST) or a dual-mode resonator (DM/4116). Samples contained either 0.5 mg ml⁻¹ (wild type) or 1 mg ml⁻¹ (mutants) chlorophyll, 1 mM sodium ascorbate, 30 μM DCPIP in 50 mM Tris-HCl, pH 8.3. For chemical reduction, samples were suspended at a chlorophyll concentration of either 0.5 mg ml⁻¹ (wild type) or 1 mg ml⁻¹ (mutants) in 250 mM glycine, pH 10, with 50 mM sodium dithionite. For analysis of the P700⁺ cation, samples were suspended at a chlorophyll concentration of 0.47 mg ml⁻¹ in 50 mM Tris, pH 8.3, 30 μM DCPIP, and 200 μM sodium ascorbate. The spectra were taken at 77 K and at microwave power levels that were in the square-law region and well below saturation. The charge separation between P700 and F_A/F_B is irreversible at this temperature during the time scale of the measurement (17). All data manipulations and graphics were performed using IGOR Pro (WaveMetrics, Lake Oswego, OR). Simulations of the S = 1/2 EPR spectra were performed using the EPRSIM program (18).

**Time-resolved Optical Absorption Spectroscopy**—The number of electron acceptors functioning after A₁ was determined at room temperature using a train of appropriately spaced sequential single turnover flashes similar to that described in (19). In this protocol, reduction of P700⁺ by reduced phenazine methosulfate (PMS) overrules the faster reaction(s) involving F_A and/or A₁ on the first and second flashes, when the electrons are consequently stabilized on both F_A and F_B. On the third and fourth flashes, the faster back reactions from F_A and A₁ with lifetimes shorter than 1 ms become dominant. Hence, when two acceptors function after F_A, the kinetics for the second flash is similar to that for the first, and the kinetics for a fourth flash is similar to that for the third. In practice, the kinetics on the second flash may contain some contribution from F_X due to double hits on the 10-μs duration xenon flash. This protocol, originally introduced by Sauer et al. (20) to determine the number of electrons acceptors in PS I, has been used to characterize PS I complexes with F_B destroyed by HgCl₂ treatment (21, 22) in *in vitro* mutants in the ligands to F_X and F_B in PsaC (23, 24) and *in vivo* mutants in the ligands to F_X and F_B in PsaC (25).

The measurements were performed using the same spectrometer as in Ref. 26 except for a replacement of the measuring beam source with a 832-nm laser diode (PMT285, Power Technology Inc., Mabelvale, AR). The output power was 25 mW, and due to a higher stability of the output, the reference channel was not used. Repetitive flashes at 15-ms intervals were provided by a model 6100E-72 xenon flash lamp (Photoc-chemical Research Associates, London, Ontario, Canada) at full-width half-maximum of 10 μs and flash energy of ~5 mJ. A fiber optic light guide was used to direct the actinic beam to the cuvette in direction perpendicular to that of the measuring beam. The saturating intensity of the flash was confirmed by using the neutral density filters and by monitoring the ΔAₕₜₐₜ signal. The samples were suspended in 20 mM MES buffer, pH 6.3, at a chlorophyll concentration of 25 μg ml⁻¹ with 0.05% β-DM, and PMS and sodium dithionite from freshly prepared solutions were added to concentrations of 10 μM and 2 mM, respectively. The concentration of PMS was chosen to assure re-reduction of P700⁺ prior to recombination with F_A and/or F_B. Sodium dithionite was used to completely reduce the PMS to prevent the oxidized form from functioning as an electron acceptor. The optical path length for the measuring light was 1 cm. The samples were preincubated in the dark in the spectrometer for 4 min prior to flash excitation.

For numeric analysis of the data we have made a simultaneous global fit of all four kinetics to the sum of two exponentials with common lifetimes and common initial amplitude (assuming that the amount of P700⁺ formed on each flash should be the same upon almost complete relaxation of the absorbance change produced by the preceding flash). For each set of four kinetics we determined the initial amplitude of the absorbance change, the lifetime of the slow component accounting for P700⁺ reduction by reduced PMS, the lifetime of the fast component accounting for P700⁺ reduction by F_X and A₁, and a set of four amplitudes of the slow component. In general, this model gave a high quality fit in the millisecond time domain (where the kinetics is mostly governed by the slower PMS component), but in some cases the deviations in the sub-millisecond time domain could be higher due to the presence of two exponentially decaying components in this time domain.

**RESULTS**

**Cysteine → Aspartate: C14D_PsaC-PS I**—When PS I complexes from a mutant expressing C14D_PsaC were frozen in darkness and illuminated at 15 K, the EPR spectrum showed a S = 1/2 ground state iron-sulfur cluster with g values of 2.047, 1.945, and 1.851 (Fig. 1, left column). The resonances, which are diagnostic of F_A, do not diminish in intensity in a subsequent period of darkness. The temperature dependence of the resonances at 18 K (not shown), 15 K (Fig. 1A), 12 K (Fig. 1B), 9 K (Fig. 1C) to 6 K (Fig. 1D) indicates a maximum signal intensity at 15 K, similar to the wild type, although the resonances are present over a much broader and lower range of temperatures. (The trough between 365 and 370 mT may represent the mid-field resonance of F_X; the low-field trough around 390 mT is barely visible). There is no indication of a S = 1/2 ground state iron-sulfur cluster characteristic of reduced F_B in the g = 2 spectral region.

Resonances characteristic of F_X were also present in the g = 2 spectral region when PS I complexes from a mutant expressing C14D_PsaC were frozen during illumination (Fig. 1, right column). Under these conditions, more than one electron is promoted to the acceptor system. The intensity of the F_X resonances increase from 15 K (Fig. 1E), to 12 K (Fig. 1F), to 9 K (Fig. 1G), and become maximum at ~6 K (Fig. 1H). This is a temperature behavior different from conditions where only one electron is promoted (compare Fig. 1, B and D with F and H), indicating that the F_X cluster is relieved of power saturation, most likely by the presence of a nearby spin system. One candidate is reduced F_X, which is observed at temperatures <9 K (see Fig. 1, G and H) as a broad, rhombic signal with mid-field and high-field resonances at g = 2.852 and 1.755 (the low-field peak is obscured by the g = 2.044 resonance of F_a). However, it has been shown in a PS I complex which lacks F_a, that reduced F_X does not influence the microwave power or temperature dependence of F_X (27). Assuming that no changes are introduced other than creating an empty site in F_B, these data indicate that F_X does not influence the spin relaxation process.
properties of \( F_A \). The implication is that the enhanced relaxation rate of \( F_A \) is due to the presence of reduced \( F_B \), which we propose is present as a high-spin (likely \( S^{3/2} \)) \([4Fe-4S]\) cluster in the mutant site. At this time, it is only possible to infer the presence of \( F_B \), since we have not located a low-field resonance around \( g = 5.5 \) which could be attributed to a highly rhombic \( S^{3/2} \) spin system. High-spin iron-sulfur clusters are difficult to observe when the \( g \) anisotropy is distributed over a large spectral range. Note also that there is also an apparent broadening of the \( g = 1.852 \) resonance at 6 K (Fig. 1H), but this is probably due to overlap from the midfield resonance of \( F_X \).

**Cysteine \( \rightarrow \) Serine:** C14S\textsubscript{PsaC}-PS I—The PS I complexes from the C14S\textsubscript{PsaC} mutant have EPR spectral properties that are similar, although not identical, to those for the C14D\textsubscript{PsaC}-PS I complex. When the PS I complexes from a mutant expressing the C14S\textsubscript{PsaC} mutant were frozen during illumination (Fig. 2, right column), the \( F_X \) cluster is visible in the 9 K (Fig. 2G) and 6 K spectra (Fig. 2H), with a high-field resonance at \( g = 1.761 \) and a midfield feature around 365 mT. The latter is likely to be responsible for some, but not all, of the features around 360 mT in the 6 K spectrum. There is evidence at 12 K (Fig. 2F, arrows) and 9 K (Fig. 2G, arrows) for additional resonances at \( g = 2.102 \) and 1.883, and for a broader line width of the high-field resonance at \( g = 1.846 \). Except for the temperature dependence, these resonances are strikingly similar to those observed in the PS I complexes containing C51S\textsubscript{PsaC} (see below).

**Cysteine \( \rightarrow \) Aspartic Acid:** C51D\textsubscript{PsaC}-PS I—When PS I complexes from a mutant expressing C51D\textsubscript{PsaC} were frozen in darkness and illuminated at 15 K, the EPR spectrum showed a set of resonances characteristic of \( F_B \), with \( g \) values of 2.067, 1.931, and 1.880 (Fig. 3, left column). There is no indication of reduced \( F_Y \) in the \( g = 2 \) spectral region. The \( F_A \) resonances are present over a narrower range of temperatures than in C14D\textsubscript{PsaC}-PS I, increasing in intensity from 15 K (Fig. 2A), becoming maximum at 12 K (Fig. 2B) and decreasing from 9 K (Fig. 2C) through 6 K (Fig. 2D). This temperature behavior is similar to that of \( F_A \) in the wild type cyanobacterial PS I complex, indicative of an enhanced spin relaxation mechanism at this site.

Resonances characteristic of \( F_A \) are found with the same temperature behavior when PS I complexes from the mutant C14S\textsubscript{PsaC} are frozen during illumination (Fig. 2, right column). The \( F_X \) cluster is visible in the 9 K (Fig. 2G) and 6 K spectra (Fig. 2H), with a high-field resonance at \( g = 1.761 \) and a midfield feature around 365 mT. The latter is likely to be responsible for some, but not all, of the features around 360 mT in the 6 K spectrum. There is evidence at 12 K (Fig. 2F, arrows) and 9 K (Fig. 2G, arrows) for additional resonances at \( g = 2.102 \) and 1.883, and for a broader line width of the high-field resonance at \( g = 1.846 \). Except for the temperature dependence, these resonances are strikingly similar to those observed in the PS I complexes containing C51S\textsubscript{PsaC} (see below).

**Cysteine \( \rightarrow \) Aspartic Acid:** C51D\textsubscript{PsaC}-PS I—When PS I complexes from a mutant expressing C51D\textsubscript{PsaC} were frozen in darkness and illuminated at 15 K, the EPR spectrum showed a set of resonances characteristic of \( F_B \), with \( g \) values of 2.067, 1.931, and 1.880 (Fig. 3, left column). There is no indication of reduced \( F_Y \) in the \( g = 2 \) spectral region. The \( F_A \) resonances are present over a narrower range of temperatures than in C14D\textsubscript{PsaC}-PS I, increasing in intensity from 15 K (Fig. 2A), becoming maximum at 12 K (Fig. 2B) and decreasing from 9 K (Fig. 2C) through 6 K (Fig. 2D). This temperature behavior is similar to that of \( F_A \) in the wild type cyanobacterial PS I complex, indicative of an enhanced spin relaxation mechanism at this site.

**FIG. 1.** EPR spectroscopic properties of the \textit{in vivo} C14D\textsubscript{PsaC}-PS I complex. The 250-\( \mu l \) sample contained 1 mg/ml chlorophyll, 300 \( \mu M \) DCPIP, and 1 mM sodium ascorbate in 50 mM Tris buffer, pH 8.3. The sample was frozen in darkness and illuminated at 15 and measured at 15 K (A), 12 K (B), 9 K (C), and 6 K (D). Sample was frozen during illumination and measured at 15 K (E), 12 K (F), 9 K (G), and 6 K (H). Spectrometer conditions: microwave power, 20 mW; microwave frequency, 9.6471 GHz; modulation amplitude, 10 G at 100 KHz; four scans averaged.

**FIG. 2.** EPR spectroscopic properties of the \textit{in vivo} C14S\textsubscript{PsaC}-PS I complex. The 250-\( \mu l \) sample contained 1 mg/ml chlorophyll, 300 \( \mu M \) DCPIP, and 1 mM sodium ascorbate in 50 mM Tris buffer, pH 8.3. The sample was frozen in darkness and illuminated at 15 and measured at 15 K (A), 12 K (B), 9 K (C), and 6 K (D). The sample was frozen during illumination and measured at 15 K (E), 12 K (F), 9 K (G), and 6 K (H). Spectrometer conditions were the same as in Fig. 1, except for a microwave frequency of 9.6470.
right column). As the temperature is lowered from 15 K (Fig. 3E) to 12 K (Fig. 3F), the F_B resonances remain dominant with a temperature optimum identical to that of a sample illuminated at 15 K. At temperatures of 9 K (Fig. 3G) and 6 K (Fig. 3H), F_B diminishes due to microwave power saturation, but at 6 K a broad peak at g = 2.096, a change in the slope of the mid-field feature at g = 1.925, and a high-field trough at g = 1.843 become visible (Fig. 3H, arrows). These features are assumed to be derived from a mixed-ligand cluster, termed F_A', in the modified site. The maximum signal intensity of the modified F_A' cluster occurs at temperatures between 4.2 and 6 K, a value which is lower than the 15 K optimum of F_A in a wild type complex. The F_X cluster is also seen at 6 K, with its high-field trough at g = 1.76 and a mid-field resonance between 350 and 360 mT, which is obscured by the presence of resonances from F_A and/or F_B.

Cysteine → Serine: C51S_PsaC-PS I—When PS I complexes from a mutant expressing C51S_PsaC were frozen in darkness and illuminated at 15 K, the EPR spectrum was characteristic of F_B, with g values of 2.067, 1.930, and 1.879 (Fig. 4, left column). There is no indication of reduced F_A in the S = 1/2 spectral region. The intensity of the F_B resonances are also weak, but again, are formed irreversibly. The maximum signal intensity occurs at 15 K (Fig. 4A), becoming progressively weaker at 12 K (Fig. 4B), 9 K (Fig. 4C), and 6 K (Fig. 4D). The maximum at 15 K is similar to that of F_B in the wild type complex. Because of the large amount of P700⁺ radical generated, we again surmise that the F_A cluster is present and functional, but that it is not visible in the g = 2 region when F_B is oxidized in the same reaction center. In terms of g values, temperature dependence and spectral lineshape, the F_B cluster in the C51S_PsaC-PS I mutant is identical to that in the C51D_PsaC-PS I mutant.

A stronger set of resonances characteristic of F_B are found when the C51S_PsaC-PS I complex is frozen during illumination (Fig. 4, right column). The signal attains maximum intensity at 15 K (Fig. 4E) and decreases at 12 K (Fig. 4F) due to the onset of microwave power saturation; below 9 K (Fig. 4, G and H), the resonances are within the noise. At 9 K (Fig. 4G) and 6 K (Fig. 4H) high-field resonance of the F_X cluster can be observed at g = 1.756, but the low-field and mid-field resonances are obscured by other peaks. At temperatures of 6 K and lower (Fig. 4H), a new set of features at g = 2.117, 1.916, and 1.837 become visible, these are attributed to a mixed-ligand F_A' cluster in the modified site. Their maximum signal intensity occurs at ≤ 4.2 K, a temperature lower than that of F_A in the wild type PS I complex. The spectral g values and lineshapes are similar to those the in vivo C51D_PsaC-PS I complex; a set of common features includes the temperature dependence of the F_B resonances, the appearance of new resonances at very low temperatures, and the apparent lack of magnetic interaction between the F_B cluster observed at 15 K and the new spin system(s) observed at lower temperatures.

Relative P700⁺ Spin Concentrations in Wild Type and Mu-
tant PS I Complexes—if F_B in the C14D_PsaC-PS I and C14S_PsaC-PS I complexes and if F_A in the C51D_PsaC-PS I and C51S_PsaC-PS I complexes are present as a high spin iron-sulfur clusters, then the relative spin concentrations of F_A and F_B in the non-mutant sites should be equivalent to that in the wild type. One obvious consequence is that the digital summation of the C14X_PsaC-PS I spectrum and the C51X_PsaC-PS I spectrum (where X = D or S) should yield a wild type spectrum. This is shown in Fig. 5A for C14D_PsaC-PS I (data from Fig. 1A) and C51D_PsaC-PS I (data from Fig. 3A), where, under conditions where there is no magnetic interaction between F_A and F_B, the resulting admixture of F_A and F_B is identical to a wild type PS I complex (Fig. 5B). The relative spin concentrations of F_A and F_B in the C14D_PsaC-PS I and C51D_PsaC-PS I complexes were determined by double integration of the simulated spectra. As shown in Fig. 5C, F_A (dotted line) can be accurately simulated assuming g values of 2.047, 1.945, and 1.851 and line widths of 33, 21, and 17 MHz and F_B (dashed line) can be simulated assuming g values of 2.067, 1.931, and 1.880 and line widths of 50, 32, and 33 MHz. Using these parameters, the composite spectrum of F_A/F_B in the wild type and in the digitally summed mutant spectra can be reproduced assuming a 3.17 ratio of F_A to F_B (solid line).

A second consequence is that the P700⁺ cation radical should also be equivalent in spin concentration in the wild type, C14X_PsaC-PS I, and C51X_PsaC-PS I complexes (where X = D or S), regardless of the spin concentration of F_A or F_B seen in the g = 2 region. Fig. 6 shows the P700⁺ radical in a sample after freezing in darkness and illumination at 77 K (dotted line) and in a subsequent period of darkness (solid line). The former corresponds to the charge separated state of P700⁺ (F_B/F_A plus F_X), and the latter represents the permanent charge separated state of P700⁺ (F_B/F_A). In wild type PS I complexes, charge separation is not quantitative, but is progressively induced in a series of laser flashes up to a maximum of two-thirds of the reaction centers (28). Hence, the amount of irreversibly formed P700⁺ radical paired with F_A and F_B in the wild type PS I complex is less than the amount seen on continuous illumination. As shown in Fig. 6, the amount of irreversible P700⁺ generated in the C14X_PsaC-PS I and C51X_PsaC-PS I complexes is roughly equivalent, yet the spin concentration of F_B is only 24% that of F_A (Fig. 5C). This strongly implies that the majority of irreversible P700⁺ is paired with a missing spin system in the C51D_PsaC-PS I complex, presumably the high-spin F_A cluster. Similarly, there is a smaller amount of irreversible P700⁺ paired with a missing spin system in the C14D_PsaC-PS I complex, presumably the high-spin F_B cluster. One final detail is that there is a greater amount of irreversible P700⁺ formed in the C14S_PsaC-PS I and C51S_PsaC-PS I complexes than in the wild type and aspartate mutants, but this could be due to a greater efficiency of forward electron transfer from F_X to F_A or F_B at cryogenic temperatures in the serine mutants.

Cysteine → Alanine: C14A_PsaC-PS I and C51A_PsaC-PS I—Western blots showed the absence of PsaC (as well as PsaD and PsaE) on the C14A_PsaC-PS I and C51A_PsaC-PS I complexes (16). From this result, it was anticipated that when these complexes were frozen in darkness and illuminated at 9 K, the EPR spectrum would be devoid of any resonances characteristic of F_A or F_B. As expected, a set of broad resonances characteristic of F_X and not F_A or F_B were found with g values of 2.070, 1.877, and 1.773 and at a temperature optimum of 9 K (data not shown). When the C14A_PsaC-PS I and C51A_PsaC-PS I complexes were frozen under illumination, the F_X resonances are present at a ~2-fold higher spin concentration (Fig. 7, A and B).

**FIG. 5. Comparisons of C14S_PsaC-PS I plus C51S_PsaC-PS I complex (A), wild type PS I complex (B), and simulation of F_A and F_B (C).** The spectrum of the C14D_PsaC-PS I complex (Fig. 1A) was added to the spectrum of the C51D_PsaC-PS I complex (Fig. 3A), and the result was smoothed once to reduce noise using the Gaussian filter within IGOR Pro. The wild type sample was a Triton X-100-prepared PS I complex from *Synechocystis* sp. PCC 6803. The 250-μl sample contained 1 mg/ml chlorophyll, 300 μM DCPIP, and 1 mM sodium ascorbate in 50 mM Tris buffer, pH 8.3. The sample was frozen in darkness and illuminated at 15 K spectrometer conditions: microwave power, 10 mW; microwave frequency, 9.458 GHz; modulation amplitude, 10 G at 100 KHz; four scans averaged. The temperature was 15 K. The simulated spectrum shows F_A (dotted line) with g values of 2.047, 1.945, and 1.851 and line widths of 33, 21, and 17 MHz and F_B (dashed line) with g values of 2.067, 1.931, and 1.880 and line widths of 50, 32, and 33 MHz. The composite spectrum of F_A/F_B (solid line) was constructed assuming a 3.17 ratio of F_A to F_B.
Cysteines 14 and 51 to Aspartic Acid: C14D/C51D PsaC-PS I

A double replacement, with aspartic acid in positions 14 and 51, was generated in an attempt to localize two mixed-ligand [4Fe-4S] clusters in PsaC, one in the FA site and the other in the FB site. The prediction, based on the in vitro reconstitutions of PsaC onto P700-F X cores (12), was that the C14D/C51D PsaC-PS I double mutant would not contain two [4Fe-4S] clusters; rather it would contain one [3Fe-4S] cluster and one low-spin [4Fe-4S] cluster in a nearly stoichiometric ratio (data not shown). Hence, the absence of two [4Fe-4S] clusters should preclude binding of PsaC in the in vivo mutant. Indeed, immunoblots showed that no PsaC was assembled in the in vivo C14D/C51D PsaC-PS I mutant complex (16). The light-induced EPR spectrum showed reduced F X, with g values of 2.054, 1.873, and 1.779 when a dark-frozen C14D/C51D PsaC-PS I complex was illuminated at 9 K (not shown). When the double mutant was frozen under illumination, the F X resonances are present at a higher spin concentration (Fig. 7C).

**Number of Acceptors Functioning in the C14X PsaC-PS I and C51X PsaC-PS I Complexes**—EPR studies provide indirect evidence for the existence of two acceptors, F A and F B, functioning at low temperature in the C14X PsaC-PS I and C51X PsaC-PS I complexes (where X = D or S). To investigate the number of electron acceptors functioning at room temperature, the kinetics of P700 + reduction were monitored at 832 nm upon excitation with trains of four consecutive flashes in the presence of a fast donor, PMS. To establish the validity of the experiment, a wild type and a HgCl2-treated PS I complex were first analyzed. The HgCl2-treated PS I complex (isolated with Triton X-100) has been studied by low-temperature EPR spectroscopy and NADP+ reduction protocols and shown to be 95% depleted in F B while retaining F A and F X. As a control for the mutants we have studied the DM-PS I complex from Synechocystis sp. 6803, which gave virtually the same results with respect to the derived fit parameters and their dependencies on the flash
number as the Triton X-100-PS I from Synechococcus sp. 6301 (26). A matrix of conditions showed that a flash interval of 15 ms is optimal for all samples; for the control sample a 10-s interval between flash trains is sufficient for complete recovery of the four-flash kinetic pattern, but in the case of HgCl₂-treated sample even a longer interval is insufficient, leading to a less distinct difference between the first and second kinetics.

When this protocol is applied to the wild type PS I complex (Fig. 8A and Table I), a slow component with a lifetime of 3.3 ms was found which constitutes almost 100% of initial amplitude on the first flash and about 71% on the second flash, whereas on the third and the fourth flashes its contribution is less than 40%. When applied to the HgCl₂-treated sample, the contribution of the slow component on the first flash is only 36%, which is consistent with a lower efficiency of forward electron transfer from Fₓ in the absence of Fᵧ (see Ref. 26). The amplitudes of the slow component in the HgCl₂-treated sample on the second, third, and fourth flashes are remarkably similar, contributing ~23% to the total amplitude of the absorbance change. In qualitative compliance with previous findings (21), the equality of the second and third kinetics of P700⁻ recovery upon repetitive flash excitation is considered an important criterion (along with the EPR data) for the absence of one cluster on PsaC.

The kinetics on a single flash in the presence of the slow donor, reduced DCPIP, showed that three of the PsaC complexes (C14DPsaC-PS I, C51D_PsaC-PS I, and C14SPsaC-PS I) have a distinct backreaction from reduced [Fₓ/Fᵧ] in the tens-of-milliseconds time scale, whereas C51SPsaC-PS I has a very low amplitude of the [Fₓ/Fᵧ] backreaction, and C14APsaC-PS I, C51APsaC-PS I, and C14D/C51D_PsaC-PS I are devoid of this component (16). Hence, we performed the multiple flash experiments only with the former four mutant PS I complexes. As seen from Fig. 8, C–F and Table I, all four PS I mutant complexes show a significant difference between the amplitudes of the slow component appearing on the second and the third flash. Namely, the percentage of the third amplitude relative to the second makes up 75, 58, 70, and 80% for C14DPsaC-PS I, C51D_PsaC-PS I, C14SPsaC-PS I, and C51SPsaC-PS I, respectively. The same parameter makes up 52% for the wild type and 97% for the HgCl₂-treated sample. This makes the mutant PS I complexes distinctly different from the Fᵧ-less HgCl₂ sample and demonstrates the availability of two functional electron acceptors in these complexes.

**DISCUSSION**

**Number of Electron Acceptors Present in the C14X_PsaC-PS I and C51X_PsaC-PS I Mutants**—The following generalizations can be made about the C14X_PsaC-PS I and C51X_PsaC-PS I mutants (where X = D, S, and A). First, only [4Fe-4S] clusters are found in the in vivo mutant PS I complexes, even though the unbound PsaC mutants contain [3Fe-4S] and mixed-ligand [4Fe-4S] clusters in the altered site (11, 12). The PsaC protein does not assemble in the in vivo mutants for which cysteines 14 and 51 are substituted with alanine, C14APsaC, and C51APsaC, most likely because a [4Fe-4S] cluster cannot assemble in the treated PS I complex from wild type Synechocystis sp. PCC 6803, an average of two four-flash trains applied to different samples; C, C14D_PsaC-PS I complex; D, C51D_PsaC-PS I complex; E, C14S_PsaC-PS I complex; F, C51S_PsaC-PS I complex. Samples C–F involve an average of two four-flash trains applied to different samples. The experimental protocol involved excitation of PS I complexes with trains of four consecutive flashes in the presence of 10 μM PMS and 2 mM sodium dithionite and two-exponential fits of the kinetics. The experimental data are depicted as the dotted line; the multieponential fit is overlaid as a solid line. The parameters of the fit are summarized in Table I.
absence of a suitable ligand. The absence of an oxygen or sulfur ligand from the replacement amino acid, alamine, and the resulting destabilizing effect of a [3Fe-4S] cluster on PsaC binding to the P700-F$_X$ heterodimer are apparently the reasons for the inability of the C14A$_{psaC}$ and C51A$_{psaC}$ mutants to bind to PS I. Second, all mutant PS I complexes that contain mixed-ligand [4Fe-4S] clusters are capable of electron transfer to F$_X$ and F$_B$ at 15 K. These mutant complexes are also capable of supporting electron throughput to NADP$^+$ at room temperature. This is also true for PS I complexes reconstituted in vitro with C14D$_{psaC}$ (23) and C51D$_{psaC}$ (24). Third, the results of the in vivo experiments agree with the cysteine ligand assignments to F$_X$ and F$_B$ made using in vitro reconstitution of E. coli expressed proteins onto P700-F$_X$ cores (10).

Although the optical results provide a qualitative, rather than quantitative, estimate of the efficiency of the F$_X$ and F$_B$ photoreduction, it is clear that both acceptors are photochemically active in C14D$_{psaC}$-PSI, C51D$_{psaC}$-PSI, C14S$_{psaC}$-PSI, and C51S$_{psaC}$-PSI complexes and that they may operate with lower quantum efficiencies than in the wild type. However, heterogeneity due to incomplete binding of PsaC would give rise to the same kinetics in single turnover experiments as does forward electron transfer inefficiency. To this end, we have taken care to isolate near-homogeneous PS I complexes from the thylakoids based on the different densities of PS I reaction centers with and without PsaC, PsaD, PsaE, and PsaL. Nevertheless, low-temperature optical kinetic measurements, single-turnover EPR experiments, and quantitative enzyme-linked immunosorbent assays are being pursued to unambiguously distinguish between inefficient electron transfer and sample heterogeneity. The lower amount of F$_X$/F$_B$ photoreduction is also manifest in lower contribution of the P700$^+$ reduction from reduced PMS to the overall $\Delta A_{380}$ relative to the back-reaction(s) from F$_X$ and F$_B$, even on the first flash. We note, however, that the C51S$_{psaC}$ mutant shows both a decreased contribution of the F$_X$/F$_B$ backreaction on a single flash in the presence of a slow electron donor (16) and a less distinct difference between the kinetics upon multiple flash excitation in the presence of a fast donor (Table I).

Comparison with In Vitro Reconstituted C14D$_{psaC}$-PS I and C51D$_{psaC}$-PS I Mutant Complexes—The similarities and the differences between the in vivo engineered and in vitro reconstituted C14D$_{psaC}$-PS I and C51D$_{psaC}$-PS I complexes depend on whether the mutation is in the F$_A$ or the F$_B$ site. Under conditions of photoaccumulation, the in vivo C51D$_{psaC}$-PS I complex is similar in spectral appearance to E. coli-expressed C51D$_{psaC}$-PsaC reconstituted in vitro onto P700-F$_X$ cores (23). These similarities indicate that oxygen from aspartate (alternatively, water or OH$^-$) provide the ligands to F$_B$ in the modified site. The slight differences in the g values between the in vivo and in vitro PS I complexes may be related to species differences (29), since the in vivo C51D$_{psaC}$-PS I mutant PS I complexes were derived from Synechocystis sp. PCC 6803, whereas the in vitro mutant PS I complexes were hybrids, composed of a Synechococcus sp. PCC 6301 P700-F$_X$ core, PsaC and PsaE derived from Synechococcus sp. PCC 7002, and a PsaD protein derived from Nostoc sp. PCC 8009 (30). Under conditions where only one electron was promoted to the acceptor side, only a low spin concentration of the F$_B$ cluster was observed in the in vivo and in vitro mutants; yet the size of the P700$^+$ radical indicates that the majority of the electrons were promoted to the proposed high-spin F$_B$ cluster. However, a very low spin concentration of F$_A$ was also observed in the in vitro mutant, which may have been derived from a minority population of a S = 1/2 cluster. One candidate is a sulfur thiolate ligand derived from carryover of the $\beta$-mercaptoethanol used in the reconstitution protocol as suggested in Ref. 31.

In contrast, the in vivo C14D$_{psaC}$-PS I complex differs substantially from the in vitro reconstituted C14D$_{psaC}$-PS I complex. In the in vivo mutant, the F$_B$ cluster is not observed in the g = 2 region, but is inferred from the size of the P700$^+$ radical and the presence of new EPR resonances at very low temperatures which may be derived from intercluster spin interaction between F$_A$ and F$_B$. The proposal is that the F$_B$ cluster is present as a high-spin system (likely S = 3/2). A search for the expected g = 5 to 6 low-field resonance of a S = 3/2 cluster failed; however, because of the broad anisotropy of a highly rhombic spin system, along with its detection as a first derivative, the resonances may be too weak to be detected at these sample concentrations. In the in vitro mutant, the F$_B$ cluster was observed as a ground state S = 1/2 spin system with g values of 2.118, 1.911, and 1.883. The difference in the in vivo engineered and in vitro assembled C14D$_{psaC}$-PS I complexes is that only oxygen ligands are available in the former, whereas sulfur thiolate ligands may also be available in the latter. The $\beta$-mercaptoethanol used in the in vitro iron-sulfur reinsertion protocol may have been recruited as a ligand at the mutated site of the in vitro complex (31). It is likely that sulfur provides a better ligand to an iron-sulfur cluster, replacing some or all of the oxygen-ligated cluster in the in vitro experiments. In this mutant, F$_B$ may be present as a mixed population of sulfur-ligated S = 1/2 clusters visible in the g = 2 region, and oxygen-ligated S = 3/2 clusters invisible in the g = 2 region. The fraction of sulfur- and oxygen-ligated clusters may have more to do with steric hindrance or accessibility to solvent than with inherent differences in the C14D$_{psaC}$ and C51D$_{psaC}$ sites of PsaC.

Comparison with PS I Mutants from Chlamydomonas reinhardtii and A. variabilis—When the psaC gene is interrupted in C. reinhardtii, neither the PS I reaction center subunits nor the small polypeptides accumulate in the thylakoid membranes of the transformants (32). In this eukaryotic organism, PsaC appears to be an essential component for the stable assembly of PS I.

When the psaC gene is interrupted in A. variabilis, the PS I reaction center accumulates (33), but PsaC, PsaD, and PsaE

| Fit parameter | Wild type | HgCl$_2$-treated | C14D$_{psaC}$ | C51D$_{psaC}$ | C14S$_{psaC}$ | C51S$_{psaC}$ |
|---------------|-----------|------------------|--------------|--------------|--------------|--------------|
| $n_1$ (ms)    | 3.27 ± 0.01| 5.87 ± 0.03      | 3.47 ± 0.03  | 3.75 ± 0.03  | 3.05 ± 0.04  | 4.78 ± 0.06  |
| $n_2$ (μs)    | 490 ± 5   | 359 ± 4          | 407 ± 7      | 500 ± 13     | 437 ± 9      | 590 ± 10     |
| $A_0$         | 0.808 ± 0.003| 1.603 ± 0.008    | 0.913 ± 0.010| 0.722 ± 0.009| 0.969 ± 0.012| 1.077 ± 0.009|
| $a_1$         | 0.825 ± 0.002| 0.361 ± 0.002    | 0.430 ± 0.004| 0.370 ± 0.005| 0.574 ± 0.006| 0.387 ± 0.005|
| $a_2$         | 0.757 ± 0.002| 0.240 ± 0.002    | 0.284 ± 0.004| 0.324 ± 0.004| 0.250 ± 0.005| 0.303 ± 0.005|
| $a_3$         | 0.303 ± 0.002| 0.237 ± 0.001    | 0.175 ± 0.003| 0.244 ± 0.004| 0.245 ± 0.005| 0.242 ± 0.004|
| $a_4$         | 0.227 ± 0.002| 0.225 ± 0.001    | 0.160 ± 0.003| 0.132 ± 0.004| 0.195 ± 0.005| 0.184 ± 0.004|

In Vivo Mutagenesis of F$_A$ and F$_B$ in PS I
are missing (34). Using site-directed mutagenesis, cysteine 13 (equivalent to Cys-14 in Synechocystis sp. PCC 6803) and cysteine 50 (equivalent to Cys-51 in Synechocystis sp. PCC 6803) were changed in vivo to aspartic acid in A. variabilis (25). The authors of this work argue that since the former mutant lacks an EPR spectrum characteristic of F_A, yet the organism grows at wild type rates and reduces NADP⁺, this cluster is dispensable. A comparison of the respective EPR spectra, however, shows striking similarities between the mutants in A. variabilis and Synechocystis sp. PCC 6803. In both organisms, under conditions of photoaccumulation, the F_A cluster in the C14D_PsaC mutants appears identical to the wild type, while the F_B cluster is not observed. Under similar conditions, the F_B cluster in the C51D_PsaC mutant also appears identical to the wild type; however, the F_A cluster can be observed at low temperatures. The F_C cluster is strikingly similar in the two organisms, showing a large midfield derivative resonance around g = 1.94 consisting of two closely spaced resonances (best visible in Synechocystis sp. PCC 6803) and possibly derived from magnetic coupling between F_B and F_A. We argue from the spin relaxation data and from the spin concentration of P700⁺ generated irreversibly at low temperatures that the F_B cluster in the C14D_PsaC mutant is present, but that a spin state of S = 3/2 renders it undetectable in the g = 2 region.

Whereas the optical results in Synechocystis sp. PCC 6803 concerning the availability of two electron acceptors in the C51D_PsaC mutant (C50D in A. variabilis) agree with (25), results on the C14D_PsaC mutant (C13D in A. variabilis) do not. In the A. variabilis mutant, the contribution of the slow (PMS-driven) component to the absorbance change on the third flash was considered to be relatively close to that on the second flash; the difference between the second and third traces was attributed to a variable amount of F_A⁻ oxidation during the relatively long intervals between the flashes (50 ms). However, this interval is quite comparable with the intrinsic lifetime of P700⁺ [F_A/F_B⁻], making it difficult to determine whether this small difference is solely the result of F_A⁻ oxidation or the result of a second photochemically active cluster on PsaC. It is obvious that the C13D mutant has a very high contribution of a phase decaying faster that 500 µs, which may be related to either an inefficiency in the electron transfer from F_A to the PsaC-bound cluster(s) or heterogeneity of the sample. Our C14D_PsaC-PS I complex (16) shows a similar inefficiency (or a heterogeneous population), making it necessary to optimize the PMS concentration and flash and dark intervals to differentiate the kinetics between the successive flashes.

The inability to engineer a PsaC protein with either a missing cluster or a [3Fe-4S] cluster in vitro in PsaC from Synechocystis sp. PCC 7002 (12) or in vivo in Synechocystis sp. PCC 6803 (this work) is further evidence that two [4Fe-4S] clusters must be present for PsaC to bind to the PS I core. This result is consistent with in vitro reconstitutions of E. coli-expressed mutant PsaC proteins, where only those proteins with two intact [4Fe-4S] clusters could be reconstituted onto P700-Fx cores (31). There could, of course, be species-dependent differences in the behaviors of the filamentous A. variabilis and the unicellular Synechocystis sp. PCC 6803, but the similarity in the amino acid sequence of PsaC in the two organisms argues against this interpretation.

Magnetic Interaction between Mixed-ligand and All-cysteine F_A and F_B Iron-Sulfur Clusters—The photoaccumulated spectra of the mutant PsaC complexes contain additional features which raise several interesting issues. In the C14D_PsaC-PS I complex, the temperature behavior implies the presence of an independent spin system which is not visible in the g = 2 region. In the C14D_PsaC-PS I complex, additional spectral features appear at very low temperatures, which imply that this second missing spin system may have crossed over from the proposed S = 3/2 state to the S = 1/2 state. Yet, this new spin system does not appear to be magenically coupled to the F_A resonances which are seen at higher temperatures. In the C51D_PsaC-PS I complex, only the F_B resonances are seen at temperatures of 15 K and higher, but additional spectral features become apparent (Fig. 3H, arrows) at very low temperatures, which do not appear to be spin-coupled to F_B. Yet, the spectrum is complex, including the presence of two resonances in the mid-field region around 350 mT, suggesting that this may represent spin-spin interaction between F_A⁻ and F_B⁻. Another possibility is that altered g values of the modified cluster might lead to a spectrum that appears similar to an interaction spectrum. Nevertheless, the presence of new spectral features at very low temperatures in the C515_PsaC-PS I complex indicates the presence of a second spin system in addition to F_A. The presence of the missing spin system under conditions where only one electron is promoted (Figs. 1–4, left column) becomes manifest under conditions where more than one electron is promoted (Figs. 1–4, right column). The salient issue is that there are more experimental features than can be accounted for than by a straightforward magnetic interaction of F_A⁻ and F_B⁻.

CONCLUSIONS

The studies presented here demonstrate that mixed-ligand [4Fe-4S] clusters can assemble in the PsaC protein of PS I in vivo and that PS I complexes containing such mixed-ligand clusters can bind to PS I core complexes and function in electron transfer reactions from P700 to ferredoxin or flavodoxin. The efficiency of iron-sulfur center insertion into the mutant proteins, or the stability of mutant proteins after cluster insertion, varies depending upon the chemical nature of the side group on the replacement amino acid. Differences observed between the spectroscopic properties of PS I complexes containing mutant PsaC proteins formed by in vitro or in vivo methods are most likely due to the chemical nature of the ligands to the [4Fe-4S] clusters. The common denominator is that only those PsaC proteins which contain two [4Fe-4S] clusters are capable of assembling onto P700-Fx cores either in vivo or in vitro.

REFERENCES

1. Moulis, J.-M., Davasse, V., Golinelli, M.-P., Meyer, J., and Quinkal, I. (1996) J. Inorg. Biol. Chem. 1, 2–14
2. Davidson, E., Ohnishi, T., Attaasafoadjei, E., and Daldal, F. (1992) Biochemistry 31, 3342–3351
3. Shergill, J. K., and Cammack, R. (1994) Biochim. Biophys. Acta 1185, 35–42
4. Brit, B. D., Sauer, R., Klein, M. P., Knaff, D. B., Krauschems, A., Yu, C.-A., Yu, L., and Malkin, R. (1991) Biochemistry 30, 1892–1901
5. Teler, J., Smith, E. T., Adams, M. W. W., Conover, R. C., Johnson, M. K., and Hoffman, B. M. (1995) J. Am. Chem. Soc. 117, 5133–5140
6. Moeska, J. M., Noodlemen, L., and Case, D. A. (1994) Inorg. Chem. 33, 4819–4830
7. Warren, P. V., Smart, L. B., McIntosh, L., and Golbeck, J. H. (1993) Biochemistry 32, 4411–4419
8. Vassiliev, I., Jung, Y.-S., Smart, L. B., Schulz, R., McIntosh, L., and Golbeck, J. H. (1995) Biochim. Biophys. Acta 1254, 1534–1535
9. Kowal, A. T., Werth, M. T., Bianchi, G., Schroder, I., Gunsalus, R. P., and Johnson, M. K. (1995) Biochemistry 34, 12284–12293
10. Zhao, J., Li, N., Warren, P., Golbeck, J., and Bryant, D. (1992) Biochemistry 31, 5093–5099
11. Yu, L., Zhao, J. D., Lu, W. P., Bryant, D. A., and Golbeck, J. H. (1993) Biochemistry 32, 8251–8258
12. Mehari, T., Qiao, F., Scott, M. P., Neilis, D. F., Zhao, J., Bryant, D. A., and Golbeck, J. (1995) J. Biol. Chem. 270, 28108–28117
13. Armstrong, F. A., George, S. J., Cammack, R., Hatchickian, E. C., and Thomson, A. J. (1989) Biochim. J. 265, 265–273
14. Conover, P. R., Kowal, A. T., Fu, W., Park, J.-B., Aono, S., Adams, M. W. W., and Johnson, M. K. (1999) J. Biol. Chem. 274, 8533–8541
15. McTavish, H., Sayavedroso, L. A., and Arp, D. J. (1995) J. Bacteriol. 177, 3960–3964
16. Yu, J., Vassiliev, I., Jung, Y.-S., Golbeck, J. H., and McIntosh, L. (1997) J. Biol. Chem. 272, 8032–8039
17. Chamovorsky, S. K., and Cammack, R. (1982) Biochim. Biophys. Acta 679, 146–155
In Vivo Mutagenesis of $F_A$ and $F_B$ in PS I

18. Belford, R. L., and Nüges, M. J. (1979) in *EPR Symposium, 21st Rocky Mountain Conference*, Denver, CO.

19. Bottin, H., and Mathis, P. (1987) *Biochim. Biophys. Acta* **892**, 91–98.

20. Sauer, K., Mathis, P., Acker, S., and Van Best, J. A. (1978) *Biochim. Biophys. Acta* **503**, 120–134.

21. Sakurai, H., Inoue, K., Fujii, T., and Mathis, P. (1991) *Photosynth. Res.* **27**, 65–71.

22. He, W.-Z., and Malkin, R. (1994) *Photosynth. Res.* **41**, 381–388.

23. Yu, L., Bryant, D. A., and Golbeck, J. H. (1995) *Biochemistry* **34**, 7861–7868.

24. Yu, L., Vassiliev, I. R., Jung, Y.-S., Bryant, D. A., and Golbeck, J. H. (1995) *J. Biol. Chem.* **270**, 28118–28125.

25. Mannan, R. M., He, W.-Z., Metzger, S. U., Whitmarsh, J., Malkin, R., and Pakrasi, H. B. (1996) *EMBO J.* **15**, 1826–1833.

26. Vassiliev, I., Jung, Y.-S., Namekawa, M. D., Semenov, A. Y., and Golbeck, J. H. (1996) *Biophys. J.* **72**, 301–315.

27. Jung, Y.-S., Yu, L., and Golbeck, J. H. (1995) *Photosynth. Res.* **46**, 249–255.

28. Sétif, P., Mathis, P., and Vannegaard, T. (1984) *Biochim. Biophys. Acta* **767**, 404–414.

29. Mehari, T., Parrett, K. G., Warren, P. V., and Golbeck, J. H. (1991) *Biochim. Biophys. Acta* **1056**, 139–148.

30. Li, N., Warren, P. V., Golbeck, J. H., Frank, G., Zuber, H., and Bryant, D. A. (1991) *Biochim. Biophys. Acta* **1059**, 215–225.

31. Jung, Y.-S., Vassiliev, I. R., Qiao, F., Yang, F., Bryant, D., and Golbeck, J. H. (1996) *J. Biol. Chem.* **271**, 31135–31144.

32. Takahashi, Y., Goldschmidt-Clermont, M., Seo, S. Y., Franzen, L. G., and Rochaix, J. D. (1991) *EMBO J.* **10**, 2033–2040.

33. Mannan, R. M., Whitmarsh, J., Nyman, P., and Pakrasi, H. B. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 10168–10172.

34. Mannan, R. M., Pakrasi, H. B., and Sonnleitner, K. (1994) *Arch. Biochem. Biophys.* **315**, 68–73.