The F_A and F_B electron acceptors in Photosystem I (PS I) are [4Fe-4S] clusters ligated by cysteines provided by PsaC. In a previous study (Mehari, T., Qiao, F., Scott, M. F., Nellis, D., Zhao, J., Bryant, D., and Golbeck, J. H. (1995) J. Biol. Chem. 270, 28108–28117), we showed that when cysteines 14 and 51 were replaced with serine or alanine, the free proteins contained a S = 1/2, [4Fe-4S] cluster at the unmodified site and a mixed population of S = 1/2, [3Fe-4S] and S = 3/2, [4Fe-4S] clusters at the modified site. We show here that these mutant PsaC proteins can be rebound to P700-F_X cores, resulting in modified site.

We demonstrate that these mutant PsaC proteins to rebind to PS I and to function in forward electron transfer.

Yean-Sung Jung‡, Ilya R. Vassiliev‡, Fengyu Qiao‡, Fan Yang‡, Donald A. Bryant§, and John H. Golbeck¶

From the ‡Department of Biochemistry, and Center for Biological Chemistry, George W. Beadle Center, University of Nebraska, Lincoln, Nebraska 68588-0664 and the §Department of Biochemistry and Molecular Biology and Center for Biomolecular Structure and Function, The Pennsylvania State University, University Park, Pennsylvania 16802

The F_A and F_B electron acceptors in Photosystem I (PS I) are [4Fe-4S] clusters ligated by cysteines provided by PsaC. In a previous study (Mehari, T., Qiao, F., Scott, M. F., Nellis, D., Zhao, J., Bryant, D., and Golbeck, J. H. (1995) J. Biol. Chem. 270, 28108–28117), we showed that when cysteines 14 and 51 were replaced with serine or alanine, the free proteins contained a S = 1/2, [4Fe-4S] cluster at the unmodified site and a mixed population of S = 1/2, [3Fe-4S] and S = 3/2, [4Fe-4S] clusters at the modified site. We show here that these mutant PsaC proteins can be rebound to P700-F_X cores, resulting in fully functional PS I complexes. The low temperature EPR spectra of the C14G PsaC and C51G PsaC PS I complexes (where X = S, A, or G) show the photoreduction of a wild-type F_A cluster and a modified F_B cluster, the latter with g values of 2.115, 1.899, and 1.852 and linewidths of 110, 70, and 85 MHz. Since neither alanine nor glycine contains a suitable side group, an external thiolate provided by β-mercaptoethanol has likely been recruited to supply the requisite ligand to the [4Fe-4S] cluster. The EPR spectrum of the C51G PsaC PS I complex differs from that of the C51A PsaC PS I or C51G PsaC PS I complexes by the presence of an additional set of resonances, which may be derived from the serine oxygen-ligated cluster. In all other mutant PS I complexes, a wild-type spin-coupled interaction spectrum appears when F_A and F_B are simultaneously reduced. Single turnover flash studies indicate ~50% efficient electron transfer to F_A/F_B in the C14G PsaC PS I, C51A PsaC PS I, C14G PsaC PS I, and C51G PsaC PS I mutants and less than 40% in the C14A PsaC PS I and C51A PsaC PS I mutants, compared with ~76% in the PS I core reconstructed with wild-type PsaC. These data are consistent with the measurements of the rates of cytochrome c_553 (cytochrome c_553) reductase activity, indicating lower rates in the alanine mutants. It is proposed that the chemical rescue of a [4Fe-4S] cluster with a recruited external thiolate at the modified site allows the mutant PsaC proteins to rebind to PS I and to function in forward electron transfer.

Electron transfer proteins of the class "ferredoxins" typically contain iron-sulfur clusters ligated to cysteine thiolates supplied by the polypeptide backbone. The most common representatives of this protein class incorporate the widely distributed [2Fe-2S], [3Fe-4S], and [4Fe-4S] clusters (1, 2), but newly discovered configurations, such as the P-clusters of nitrogense (3), are also known to exist. Exceptions to the cysteine-only motif include histidine ligands to the [2Fe-2S] clusters in the Rieske proteins of bacteria (4), mitochondria (5), and chloroplasts (6); the proposed serine ligand at the iron site of the nitrogense cluster (3); and the interconvertible [3Fe-4S] and [4Fe-4S] clusters that likely contain aspartate ligands in ferredoxins from Desulfovibrio africanus (7) and Pyrococcus furiosus (8).

PsaC is a ferredoxin-like Photosystem I (PS I) protein that ligates two [4Fe-4S] clusters, F_A and F_B, which function as intermediates in electron transfer from F_X to soluble ferredoxin or flavodoxin. The sequence and the kinetics of electron transfer between the F_X, F_B, and F_A iron-sulfur clusters and to the [2Fe-2S] soluble ferredoxin are not well understood. One of the major experimental difficulties is the near-identity of the optical signatures of F_A, F_B, and ferredoxin, and the attendant problem of correlating a given acceptor with observed electron transfer kinetics. Although F_A, F_B, and ferredoxin are distinguishable by low temperature EPR spectroscopy, electron exchange among these redox carriers typically occurs within the rise time of the spectrometer. One approach to differentiating between the iron-sulfur clusters is to modify the kinetic, thermodynamic, and/or spectroscopic properties by altering a cysteine ligand to one of the cubane irons. The most radical change would be to convert a [4Fe-4S] cluster to a [3Fe-4S] cluster, with the consequence that the reduction potential of the cluster should be driven more electropositive (9, 10). Based on precedent with proteins of known structure (8, 11), this change should occur when the second cysteine of each CXXXCSXXX motif is changed to aspartic acid.

The amino acid sequence of PsaC has sufficient similarity with the [4Fe-4S] ferredoxins from Peptococcus aerogenes and Clostridium pasteurianum to predict a similar three-dimensional backbone structure, especially in the region surrounding the iron-sulfur clusters (12, 13). PsaC can be removed from the PS I reaction center with chaotropic agents and replaced with

* This research was supported by National Science Foundation Grants MCB-9205756 (to J. H. G.) and MCB-9206851 (to D. A. B.). This paper is Journal Series 11555 of the University of Nebraska Agricultural Research Division, and is the third article in the series “Modified Ligands to F_A and F_B in Photosystem I.” The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Present address: Dept. of Biochemistry and Molecular Biology, S310 Frear Bldg., The Pennsylvania State University, University Park, PA. Tel.: 814-865-1163; Fax: 814-863-7024; E-mail: jhgg5@psu.edu.

1 The abbreviations used are: PS I, Photosystem I; A_p, the secondary electron acceptor in Photosystem I, a phylloquinone; Chl, chlorophyll; DCPIP, 2,6-dichlorophenol indophenol; P700, the primary electron donor in Photosystem I; a chlorophyll a dimer; t, life time of exponential decay component; 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.

This paper is available online at http://www-jbc.stanford.edu/jbc/
Further complicating the issue was that the C14APsaC and alanine, an amino acid that contains a methyl side group, support a cubane cluster, this result was quite unexpected for modified sites in the unbound PsaC proteins. While serine, an amino acid sequence analysis as described (16). Nostoc sp. strain PCC 8009 PsaD was purified from solubilized inclusion bodies isolated from *E. coli* cells (23). The refolded protein was purified by chromatography on CM-Sepharose CL-6B using a linear gradient of sodium chloride (50–1000 mM). *Synechococcus* sp. strain PCC 7002 PsaE was purified from the solubilized inclusion body by chromatography on DEAE-Sepharose CL-6B with a linear gradient of sodium chloride (10–200 mM at pH 8.0. The reassembly of the Photosystem I complex was accomplished simultaneously with the insertion of the iron-sulfur clusters into the wild-type and mutant PsaC apoproteins as described in Ref. 24. All PS I complexes were reconstituted in the presence of β-mercaptoethanol unless otherwise noted. Photoreductase activities of the wild-type and mutant PS I complexes were measured according to the methods described in (15). Rates of flavodoxin photoreduction were measured by monitoring the rate of change in the absorption of flavodoxin at 467 nm. Flavodoxin was purified by DEAE-Sepharose and Sephadex G-75 chromatography from a strain of *E. coli* containing the *Synechococcus* sp. strain PCC 7002 isiB gene (25). Rates of flavodoxin-mediated or ferredoxin-mediated NADP\(^+\) photoreduction were measured as described (15, 26).

Chlorophyll was determined after extraction into 80% acetone (27); protein concentration was determined using a dye-binding method (28).

**RESULTS**

### Electron Paramagnetic Resonance Spectroscopy—Electron paramagnetic resonance (EPR) studies were performed using a Bruker ECS-106 X-band spectrometer equipped with either a dual-mode (DM/4116) or standard perpendicular mode (ER/4102ST) resonator. The experimental protocol involves either freezing the sample in darkness and illuminating at low temperature to transfer one electron to the acceptor system, or freezing under continuous illumination to photoaccumulate two or more electrons in the acceptor system. Samples contained either 0.5 mg ml\(^{-1}\) (wild-type) or 1 mg ml\(^{-1}\) (mutants) Chl, 1 mM sodium ascorbate, 30 μM DCPIP in 50 mM Tris-HCl, pH 8.3. For chemical reduction of the F\(_{\alpha}\) and F\(_{\beta}\) clusters, 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 dithionate. All data manipulations and graphics were performed using IGOR Pro 3.0. Simulations of the *S* = 1/2 EPR spectra were performed using the EPRsim XOP (provided by Dr. John Boswell, Oregon Graduate Institute), an adaptation of the program QPOW (30–32).

### Materials and Methods

**Biochemical Protocols**—The construction of mutant PS I complexes containing serine, alanine, or glycine in positions 14 and 51 of PsaC was accomplished using a three-step procedure: (i) biochemical resolution of a P700-\(F_{\alpha}\)/\(F_{\beta}\) complex into P700-\(F_{\alpha}\) cores; (ii) production of the C14Xpapc- and C51Xpapc- (X = S, A, G, or H) holoproteins by overexpressing the site-modified papsX genes in *E. coli*, purifying the apoproteins, and in *vitro* reinsertion of the iron-sulfur clusters with iron, sulfide, and -mercaptoethanol (or dithiothreitol); and (iii) rebinding of the C14Xpapc holoproteins to P700-\(F_{\alpha}\) cores in the presence of PsaD and PsaE (summarized in Ref. 20).

Site-directed mutagenesis was performed as described previously (21, 22); overproduction and purification of wild-type PsaC and mutant C14Xpapc- and C51Xpapc- (X = S, A, G, or H) proteins were performed as described (16). Mutations were verified by subjecting apoproteins (or tryptic peptides) to amino acid sequence analysis as described (16). Nostoc sp. strain PCC 8009 PsaD was purified from solubilized inclusion bodies isolated from *E. coli* cells (23).
be attributed to a reduced $S = 2$, [3Fe-4S]$^0$ cluster (data not shown). Rather, two [4Fe-4S] clusters were found in all C14X$_{PsaC}$-PS I and C51X$_{PsaC}$-PS I complexes. The EPR characteristics of the individual mutant PS I complexes are described in detail below.

C14A$_{PsaC}$-PS I Complex—Illumination of a dark-frozen C14A$_{PsaC}$-PS I complex at 15 K leads to the appearance of resonances characteristic of $F_A$ with $g$ values of 2.045, 1.943, and 1.856 (Fig. 1A). Similar to the wild-type cyanobacterial PS I complex, for which microwave power saturation renders the $F_A$ resonances maximum at $g = 9$ (in 10 dB microwave power; data not shown), the amplitude of the $F_A$ resonances steadily decreases in amplitude as the temperature is lowered from 12 K (Fig. 1B), becoming maximal between 9 K (Fig. 1C) and 6 K (Fig. 1D), and declining at lower temperatures (data not shown). At 12 K, a second set of resonances become prominent (Fig. 1B, arrows), with a low field $g$ value of 2.115, and unresolved mid- and high field resonances falling between 355 and 375 mT. This signal, termed $F_B$, reaches a maximum amplitude at 9 K (Fig. 1C, arrows) and decreases in amplitude at 6 K (Fig. 1D) and lower temperatures (data not shown). When the C14A$_{PsaC}$-PS I complex is frozen during illumination, a spectrum appears with $g$ values of 2.047, 1.937, 1.919, and 1.884 (Fig. 1, E–H), which is nearly identical to the interaction spectrum seen in the wild-type PS I complex. Unlike the latter, where the temperature optimum is $-18$ K (in 10 dB microwave power), the interaction spectrum derived from reduced $F_A$ and $F_B$ increases in amplitude as the temperature is lowered from 18 K to 6 K (Fig. 1, F–H) and declines at lower temperatures (data not shown). The minor resonance at $g = 1.756$ (Fig. 1H) indicates that a small amount of $F_X$ becomes reduced under conditions of photoaccumulation.

When dithiothreitol was used in the reconstitution protocol in lieu of β-mercaptoethanol, the EPR spectral results were identical to that shown in Fig. 1 (A–H) (data not shown).

C14G$_{PsaC}$-PS I Complex—Like alanine, glycine is incapable of providing a ligand to an iron-sulfur cluster. Yet, the $g$ values, linewidths, and temperature dependence of $F_A$ and $F_B$ in the C14G$_{PsaC}$-PS I complex (see Fig. 3D for the 9 K spectrum) were identical to those in the C14A$_{PsaC}$-PS I complex. Photoaccumulation of the C14G$_{PsaC}$-PS I complex (data not shown) leads to an interaction spectrum from reduced $F_A$ and $F_B$ identical to the wild-type PS I complex.

C14S$_{PsaC}$-PS I Complex—In contrast, serine is capable of providing an oxygen ligand to an iron-sulfur cluster, and based on precedent with similar site-directed mutations (33, 34), could support the presence of a [4Fe-4S] cluster. When the dark-frozen C14S$_{PsaC}$-PS I complex is illuminated at 15 K, a set of resonances appear at $g = 2.045, 1.943$, and 1.856 characteristic of $F_A$ (Fig. 2A). The temperature dependence (Fig. 2, B–D) is identical to that of $F_A$ in the C14A$_{PsaC}$-PS I complex (see Fig. 1). Similarly, a low field resonance at $g = 2.115$, and mid- and high field resonances falling between 355 and 375 mT (Fig. 2, B and C, arrows) are present, which have the same spectral
Modified Ligands to FA and FB in Photosystem I

appearances and temperature dependences as \( F_n' \) in the C14APsaC-PS I complex. An interaction spectrum produced by photoaccumulation (Fig. 2, E–H) is also similar to that for the C14APsaC-PS I and wild-type PS I complexes. The only difference from the EPR properties of C14APsaC-PS I is the relatively intense set of resonances at \( g = 2.119, 1.858, \) and 1.756 due to reduced \( F_x \) (Fig. 2H).

An identical set of resonances were found in the dithiothreitol-reconstituted C14SPsaC-PS I complex (data not shown). The amount reduced \( F_x \) is smaller under conditions of photoaccumulation, probably indicating a higher degree of overall reconstitution with dithiothreitol.

**EPR Spectrum of \( F_n' \)**—The absence of magnetic interaction between redox centers when PS I complexes are frozen in the dark and illuminated at low temperatures allows the EPR spectrum of \( F_n' \) to be extracted from the admixture of \( F_A \) and \( F_B \). The \( F_n' \) spectrum, obtained by subtracting a simulated \( F_A \) spectrum (\( g \) values of 2.045, 1.943, and 1.855, and linewidths of 15, 21, and 35 MHz) from the composite spectrum, shows a distinctive low field resonance with mid- and high field features as a partially merged set of resonances in both the C14APsaC-PS I (Fig. 3A) and C14SPsaC-PS I (Fig. 3B) complexes. Within the limits imposed by the S/N, the \( F_n' \) spectrum can be simulated using \( g \) values of 2.115, 1.899, and 1.852, and linewidths of 110, 70, and 85 MHz (Fig. 3C, solid line). The identical EPR spectra for \( F_n' \) in the C14DPSaC-PS I (14), C14APsaC-PS I (Fig. 3A), C14GPsaC-PS I (see Fig. 3D) and C14SPsaC-PS I (Fig. 3B) complexes are consistent with a similar ligand environment surrounding the [4Fe-4S] clusters at the mutant sites. The EPR spectral properties of the \( F_n' \) cluster in the C14APsaC-PS I, C14GPsaC-PS I and C14SPsaC-PS I complexes reconstituted with dithiothreitol (data not shown) are identical to the \( \beta \)-mercaptoethanol-reconstituted PS I complexes. This was the expected result, since dithiothreitol is the equivalent of two tail-to-tail \( \beta \)-mercaptoethanol molecules. The accuracy of the simulations is illustrated in Fig. 3D, which compares an additive composite of \( F_A \) and \( F_n' \) (Fig. 3D, solid line) with the experimental spectrum of the C14GPsaC-PS I complex (Fig. 3D, dotted line). Taking into account the different spin populations at their respective temperature optima of 6 K and at 9 K, double integration of the simulated \( F_A \) and \( F_n' \) spectra show that they are photoreduced in a ratio of \( 1:2 \). In contrast, \( F_A \) and \( F_n' \) are reduced at a ratio of \( 3:2:1 \) in wild-type PS I complexes under similar conditions (data not shown).

**C51A_PsaC-PS I Complex**—The C51A_PsaC-PS I complex shows a set of EPR signals, which are not as easily interpreted as those in the C14APsaC-PS I complex. Illumination of a dark-frozen C51A_PsaC-PS I complex at 15 K leads to the appearance of resonances at \( g = 2.069, 1.930, \) and 1.880 characteristic of \( F_B \) (Fig. 4A). As the temperature is lowered from 12 K to 6 K (Fig. 4, B–D), the \( F_B \) resonances diminish in amplitude due to the onset of microwave power saturation, behavior that is similar to the \( F_B \) cluster in the wild-type and the C51APsaC-PS I complex (15). A second set of resonances derived from an iron-sulfur cluster is visible at 15 K (Fig. 4A) with \( g \) values of 2.045, 1.943, and 1.856 characteristic of \( F_B \). As the temperature is lowered from 12 K to 6 K (Fig. 4, B–D), the amplitude of these resonances increases, consistent with the behavior of \( F_A \) in the wild-type cyanobacterial PS I complex. When the C51A_PsaC-PS I complex is frozen during illumination, a complex set of resonances is found, which can be separated into two temperature-dependent sets. The first set is best observed at 6 K (Fig. 4G) and below (data not shown) and resembles the spin-coupled, interaction spectrum of \( F_A \) and \( F_B \) seen in wild-type PS I complexes. The second set is superimposed on these resonances and is best seen at 15 K (Fig. 4E). The extracted \( g \) values of 2.062, 1.929, and 1.900, obtained by subtracting the 6 K spectrum from the 15 K spectrum after suitable scaling, show that this set of resonances is derived from a single, non-interacting \( F_B \) cluster.

When the C51A_PsaC-PS I complex is reconstituted using dithiothreitol, a smaller spin population of \( F_B \) is found when the sample is illuminated at 15 K. A spin-coupled, interaction spectrum is present with resonances at \( g = 2.047, 1.937, 1.919, \) and 1.884, similar to that in the wild-type PS I complex, and there is no evidence for the presence of a non-interacting \( F_B \).
cluster (data not shown). Hence, the non-interacting population of FB under conditions of photoaccumulation correlates with the amount of FB that is visible under conditions of illumination at 15 K (Fig. 4A).

**C51GPsaCz PS I Complex**—The EPR spectra and the temperature dependence of FA and FB in the C51GPsaCz PS I complex are similar to those of the C51APsaCz PS I complex. One minor difference is that when the sample is illuminated at low temperature, the spin concentration of the two clusters and that of P700 is lower than expected (data not shown). The EPR spectrum of the C51GPsaCz PS I complex shows an interaction spectrum similar to the wild-type (data not shown).

**C51SPsaCz PS I Complex**—When the dark-frozen C51SPsaCz PS I complex is illuminated at 15 K (Fig. 5A), there is evidence for two independent spin systems, represented by FA and FB in the C51GPsaCz PS I complex and similar to those of the C51S PsaCz PS I complex (data not shown). One minor difference is that when the sample is illuminated at low temperature, the spin concentration of the two clusters and that of P700 is lower than expected (data not shown). The optical study shows an interaction spectrum similar to the wild-type (data not shown).

**C51SPsaCz PS I Complex**—When the dark-frozen C51SPsaCz PS I complex is illuminated at 15 K (Fig. 5A), there is evidence for two independent spin systems, represented by FA and FB in the C51SPsaCz PS I complex. One minor difference is that when the sample is illuminated at low temperature, the spin concentration of the two clusters and that of P700 is lower than expected (data not shown). The EPR spectrum of the C51SPsaCz PS I complex shows an interaction spectrum similar to the wild-type (data not shown).

**Optical Studies**

**Optical Kinetic Spectroscopy of the Reconstituted Photosystem I Complexes**—Because charge separation between P700 and [F/A]/[F/B] is irreversible at cryogenic temperatures, the EPR data obtained by continuous illumination provide little information on the ability of the mixed-ligand iron-sulfur clusters to function as efficient electron carriers. The latter can be inferred by measuring flash-induced absorbance changes at 820 nm, where the time constants derived from the kinetic transients identify backreactions of the various electron acceptors with P700. A complicating feature is that the backreaction kinetics of most electron acceptors in PS I are multiphasic. To accom-
plish a meaningful analysis that encompasses all of the electron acceptors, the kinetic decomposition is carried out globally over 6 orders of magnitude of time. This allows monitoring of a continuum of kinetic phases corresponding to P700\(^-\) backreactions, as well as those arising from reactions of P700\(^-\) with redox agents in the media. The approximate lifetimes of charge recombination from different components of the PS I acceptor side to P700\(^-\) range from 35 ns for \(A_0\) (35); 10 and 110 ms for \(A_1\) (36); 500 ms and 3 ms for \(FX\) (26); and 15 and 80 ms for \(F_AF_B\) (29).

Wild-type PS I Complex and P700-FX Core—In wild-type PS I complexes, the quantum yield of electron transfer approaches 1.0; hence, the majority of the backreactions should be derived from \(FA/FB\). In n-dodecyl-\(\beta\)-D-maltoside PS I particles from Synechococcus sp. PCC 6301, 41% of the recombination kinetics are derived from the 17-ms and 65-ms components attributed to P700\(^-\) \([F_AF_B]\) recombination (data not shown; see Ref. 29). An additional 35% are derived from slower phases with lifetimes of 282 ms and 2.8 s, resulting in a 76% efficient transfer to \(F_AF_B\) (in thylakoid membranes the efficiency of electron transfer to \(F_AF_B\) is 94%). The slowest kinetic phases are due to exogenous donors undergoing redox reactions with P700\(^-\) and come about in reaction centers where \([F_AF_B]\) has become oxidized by one or more exogenous electron acceptors in the medium. The sum contribution of earlier acceptors, including \(FX\) and \(A_1\), is 24% of the total absorption change. In P700-FX cores, the \([F_AF_B]\) backreaction is replaced with one or more faster components arising from earlier electron acceptors (Fig. 6A). The 19-ms transient representing the unresolved \([F_AF_B]\) backreaction contributes only about 3%, and those with slower phases representing exogenous donors to P700\(^-\) contribute an additional 16% of the total absorption change, resulting in an overall transfer efficiency of 76% to FA/FB. The relative contribution of the FX\(^-\) backreaction, represented by the 441- and 3.2-ms kinetic phases, has declined to about 14% of the total absorption change, and 10% of the absorption change occurs with a lifetime of 35 ms, which may represent an unresolved \(A_1\) backreaction. Hence, the yield of reconstitution of PsaC onto P700-FX cores is reflected in the large contribution of long-lived charge separation.

C14A\(_{pasc}\)PS I and C51A\(_{pasc}\)PS I Complexes—The kinetics of the reconstituted C14A\(_{pasc}\)PS I complex show that only a fraction of the wild-type kinetics is recovered (Fig. 6C). The \([F_AF_B]\) backreaction, with lifetime components of 19 and 73 ms contributes 41% to the total absorption change; an additional 35% is contributed by the slower donation to P700\(^-\) by external donors, leading to an overall transfer efficiency of 76% to \(F_AF_B\). The relative contribution of the FX\(^-\) backreaction, represented by the 441-\(\mu\)s and 3.2-ms kinetic phases, has declined to about 14% of the total absorption change, and 10% of the absorption change occurs with a lifetime of 35 \(\mu\)s, which may represent an unresolved \(A_1\) backreaction. Hence, the yield of reconstitution of PsaC onto P700-FX cores is reflected in the large contribution of long-lived charge separation.

C14A\(_{pasc}\)PS I and C51A\(_{pasc}\)PS I Complexes—The kinetics of the reconstituted C14A\(_{pasc}\)PS I complex show that only a fraction of the wild-type kinetics is recovered (Fig. 6C). The \([F_AF_B]\) backreaction, with lifetime components of 19 and 73 ms contributes 41% to the total absorption change; an additional 35% is contributed by the slower donation to P700\(^-\) by external donors, leading to an overall transfer efficiency of 76% to \(F_AF_B\). The relative contribution of the FX\(^-\) backreaction, represented by the 441-\(\mu\)s and 3.2-ms kinetic phases, has declined to about 14% of the total absorption change, and 10% of the absorption change occurs with a lifetime of 35 \(\mu\)s, which may represent an unresolved \(A_1\) backreaction. Hence, the yield of reconstitution of PsaC onto P700-FX cores is reflected in the large contribution of long-lived charge separation.
of 7.2 and 78 ms contributes 15% to the total absorption change; an additional 13% is contributed by the slower donation to P700 by external donors, resulting in a transfer efficiency of 28% to FB. Some of the backreaction occurs from components with lifetimes of 413 ms (22%) and 1.3 ms (10.5%) derived from FX, but ~40% occurs from a component or components with a lifetime of 39 μs.

C14GPsaCPS I and C51GPsaCPS I Complexes—The reconstituted C14GPsaCPS I and C51GPsaCPS I complex show a greater degree of recovery of wild-type kinetics. In the C14GPsaCPS I complex, the [F_A/F_B] backreaction, with lifetime components of 34 and 209 ms, contributes 56% to the total absorption change; an additional 11% is contributed by the slower donation to P700 by external donors, leading to a 67% efficient transfer to FA/FB (Fig. 7A). A smaller percentage of the backreaction occurs from components with lifetimes of 586 ms (11%) and 6.2 ms (9%) derived from FX, and only 15% occurs from a component with a lifetime of 63 μs. In the C51GPsaCPS I complex, the [F_A/F_B] backreaction, with lifetime components of 26 and 119 ms, contributes 36% to the total absorption change (Fig. 7B). An additional 15% is contributed by the slower donation to P700 by external donors, leading to a 51% efficient transfer to FA/FB. Some of the backreaction occurs from components with lifetimes of 783 ms (12%) and 4.6 ms (9%) derived from FX, and 17% occurs from a component or components with a lifetime of 106 μs.

C14SPsaCPS I and C51SPsaCPS I Complexes—When the reconstituted C14SPsaCPS I complex is analyzed (Fig. 7C), 31% of the total absorption change is derived from 16-ms and 58-ms lifetime components attributed to [F_A/F_B]. An additional 19% is due to slower donation to P700 from exogenous electron donors, leading to a 50% efficient transfer to F_A/F_B. The relative contribution of the FX backreaction, judged by the 550-μs and 2.1-ms kinetic phases, equals 22% of the total absorption change, a value higher than that found with wild-type PsAC. About 28% of the total absorption change is derived from the 33-μs component related to the backreaction from A_1. Electron transfer in the C51SPsaCPS I complex (Fig. 7D) appears slightly more efficient, with 37% of the absorption change contributed by the 17-ms and 51-ms components derived from [F_A/F_B] and an additional 19% derived from slower donation to P700 from exogenous electron donors, leading to a 56% efficient transfer to F_A/F_B. About 23% of the total absorption change is due to FX, with lifetime components of 501 μs and 5.2 ms, and about 21% is due to the 31-μs component discussed above.

Cytochrome c-NADP⁺ Reductase Activity of the Reconstituted PS I Complexes—While single turnover flashes provide data on the efficiency of electron transfer to the mixed-ligand iron-sulfur clusters, it does not indicate whether donation of electrons occurs from the clusters to the physiologically relevant electron acceptors ferredoxin and flavodoxin. Table I shows rates of NADP⁺ photoreduction mediated by flavodoxin and ferredoxin in a wild-type PS I complex, a P700-F_X core, and in PS I complexes reconstituted with PsAC, C14APsaC, C51APsaC, C14SPsaC, and C51SPsaC. In wild-type complexes, the reduction of flavodoxin (i) and the reduction of NADP⁺ mediated by flavodoxin (ii) or ferredoxin (iii), occurs at rates of 900, 930, and 820 μmol (mg Chl)⁻¹ h⁻¹, respectively. The comparable rates in P700-F_X cores are only 7% (i), 17% (ii), and 6% (iii), respectively, of the wild-type rates. The residual rates are probably derived from a small amount of retained PsAC, indicating that FX is unable to transfer electrons efficiently to either flavodoxin or ferredoxin. When the P700-F_X cores are reconstituted with PsAC, the comparable rates are 53% (i), 62% (ii), and 60% (iii), respectively, of the control rates. The mutant

**Fig. 7.** Kinetics of absorbance changes at 820 nm measured at room temperature in mutant PS I preparations. A, PS I complex reconstituted with C14GPsaC; B, PS I complex reconstituted with C51GPsaC; C, PS I complex reconstituted with C14SPsaC; D, PS I complex reconstituted with C51SPsaC. Conditions are the same as for Fig. 6.
C14A_{PsaC}-PS I complex supports rates that are 17% (i), 29% (ii), and 24% (iii), respectively, of the control rates, and the mutant C51A_{PsaC}-PS I complex supports rates that are 25% (i), 34% (ii), and 35% (iii) of the control rates, respectively. These low steady-state rates are consistent with the relatively inefficient electron transfer rates on a single flash for the alanine mutants. When C14S_{PsaC}-PS I is used in the reconstitution protocol, the recovery is 82% (i), 56% (ii), and 61% (iii) of the wild-type rates, and when C51S_{PsaC}-PS I is used in the reconstitution protocol, the recovery is 82% (i), 70% (ii), and 76% (iii) of the wild-type rates. Note that when compared with the PsaC-reconstituted complex, both C14S_{PsaC}-PS I and C51S_{PsaC}-PS I support electron transfer rates to flavodoxin and ferredoxin that are equivalent to those rates. The rates for the C14D_{PsaC}-PS I and C51D_{PsaC}-PS I complexes have been published elsewhere (14, 15) and are reproduced in Table I to show that they fall between the alanine and serine sets (the glycine mutants were not studied). The most noteworthy features of these results are that a mixed-ligand iron-sulfur clusters in the C14X_{PsaC}-PS I and C51X_{PsaC}-PS I complexes (where X = D or S) can be as effective as all-cysteine [4Fe-4S] clusters in the PsaC-PS I complexes in establishing electron throughput from P700 to NADP⁺.

**DISCUSSION**

Two intact [4Fe-4S] Clusters Are Required for PsaC Binding to P700-F₇ Complexes—Similar to the aspartate series of PS I mutants (14, 15), neither S = 1/2, [3Fe-4S]¹⁺ clusters (under oxidizing conditions), nor S = 2, [3Fe-4S]⁰ clusters (under reducing conditions) could be found in the reconstituted C14X_{PsaC}-PS I and C51X_{PsaC}-PS I complexes (where X = A, G, or S). In the unbound C14X_{PsaC} and C51X_{PsaC} proteins, the altered site is presumed to be occupied by a mixed population of [3Fe-4S] and S = 3/2, [4Fe-4S] clusters (16). The data show that only those proteins containing two [4Fe-4S] clusters are capable of binding to P700-Fₓ cores. The presence of two intact [4Fe-4S] clusters in the mutant complexes is inferred in the C14X_{PsaC}-PS I and C51X_{PsaC}-PS I complexes by the presence of a spin-coupled EPR spectrum observed when Fₐ and Fₐ are simultaneously reduced within the same reaction center. The g values and linewidths are strikingly similar to the Fₓ/Fₐ interaction spectrum in wild-type PS I complexes. The presence of occupied Fₐ and Fₐ sites in in vitro C14D_{PsaC}-PS I (14) and C51D_{PsaC}-PS I (15) complexes was demonstrated by successive-flash experiments in which two electrons must be promoted before the backreactions proceed from Fₓ⁻. The multiple flash study shows that there is no missing cluster in the modified site of the in vitro aspartate mutants as has been intimated in an in vivo cysteine 13 (equivalent to cysteine 14 in this study) to aspartate change in PsaC of Anabaena variabilis (39). In support of this conclusion, a study of in vivo mutations to introduce C14X_{PsaC} and C51X_{PsaC} (where X = D, S, or A) into Synechocystis sp. PCC 6803 (40) showed the complete absence of PsaC in the C14A_{PsaC} and C51A_{PsaC} PS I complexes, implying that PsaC containing only a [3Fe-4S] cluster in the mutant site (16) is incapable of binding to PS I in vivo.

Proposed Identity of the Ligand to the Rescued [4Fe-4S] Clusters in C14X_{PsaC}-PS I Complexes—The g values, linewidths, and temperature optima of Fₐ in the reconstituted C14X_{PsaC}-PS I complexes are identical to those of the Fₐ in the wild-type PS I complex. The Fₐ clusters differed from the wild-type, but nevertheless all mutant complexes showed identical EPR spectra, with g values of 2.115, 1.899, and 1.852 and linewidths of 110, 70, and 85 MHz. Since alanine and glycine are incapable of supplying the requisite ligand to an iron-sulfur cluster, one candidate to occupy the open coordination site of the [4Fe-4S] cluster is oxygen provided by OH⁻ or H₂O from the solvent. However, when these mutations were introduced in vivo in Synechocystis sp. PCC 6803, the alanine substitution did not lead to the accumulation of a PsaC on the PS I complex, and the serine and aspartate substitutions led to a presumed population of S = 3/2 ground state [4Fe-4S] clusters which were not detectable in the g = 2 region (40). Hence, the presence of an oxygen-ligated, iron-sulfur cluster at the C14 position is not supported by the evidence. A second candidate is the β-mercaptoethanol used in the iron-sulfur insertion protocol of the mutant PsaC proteins. Unlike the presumed S = 3/2 spin state of an oxygen-ligated cluster at position 14, the spin state of a thiolate-ligated cluster is S = 1/2 when the PsaC protein is rebound to P700-Fₓ cores. Since the spin state of the thiolate-ligated cluster is S = 3/2 in the unbound mutant PsaC proteins, a cross-over must occur to S = 1/2 when the mutant PsaC protein is rebound. The factors that lead to a change of spin state have not been systematically studied in iron-sulfur proteins and the energetics of the cross-over are likely to be subtle and hence difficult to predict.

Proposed Identity of the Ligand to the Rescued [4Fe-4S] Cluster in C51X_{PsaC}-PS I Complexes—In contrast to the uniformity of the C14X_{PsaC}-PS I complexes, there are spectral differences among the various C51X_{PsaC}-PS I complexes, which make the interpretation of the EPR spectra challenging. First, most complexes show wild-type resonances, which resemble those of wild-type Fₐ and Fₐ, but the relative intensities of these resonances are low compared with those in the C14X_{PsaC}-PS I complexes. The Fₐ cluster occupies the non-mutant site in the C51X_{PsaC}-PS I complex and by analogy with the wild-type might be expected to show a low level of photooxidation at 15 K. The low spin concentration of Fₐ is therefore surprising, especially since the spin concentration of irreversible P700⁺ is identical to that for the wild-type and to the C14X_{PsaC}-PS I complexes. We suggest that this is due to the presence of a [4Fe-4S] cluster in the mutant site that cannot be observed in the g = 2 region. The missing spins are likely represented by a higher spin state, presumably S = 3/2, for the Fₐ cluster. The proposed existence of S = 3/2 [4Fe-4S] clusters in the mixed-ligand site of the unbound C51A_{PsaC}, C51S_{PsaC}, C51D_{PsaC} and C51S_{PsaC} Proteins is consistent with this suggestion (16). While no evidence for signals derived from a S = 3/2 spin pair was found in the region of g = 5–6, these resonances would have been difficult, if not impossible, to detect at the sample concentrations employed in this study.

The presence of two [4Fe-4S] clusters in the mutant C51X_{PsaC}-PS I complexes was demonstrated by the presence of a spin-coupled spectrum observed when both Fₐ and Fₐ are simultaneously reduced within the same reaction center. How-
ever, there are spectral differences between the mutant PS I complexes, which make interpretation of the EPR spectra difficult. The C51A_pauC–PS I complex is the simplest, but even here, there are two sets of temperature-dependent resonances. Since the non-interacting population of F_B observed at 15 K under conditions of photoaccumulation correlates with the amount of F_B that is visible when the sample is dark-frozen and illuminated at 15 K (Fig. 4A), this population may come about in reaction centers where F_B is a S = 1/2 ground state [4Fe-4S] cluster; for unknown reasons, interaction with reduced F_B does not result in a spin-coupled, interaction spectrum. The spectrum observed at 6 K and lower has g values and linewidths similar to the F_A/F_B interaction spectrum in wild-type PS I complexes. This spectrum may come about in reaction centers where F_B is a S = 3/2 ground state [4Fe-4S] cluster. Hence, it is necessary to postulate a mixed population of S = 1/2 ground state and S = 3/2 ground state [4Fe-4S] clusters in the modified F_B site to explain the data. The serine mutant was different; in addition to a distorted interaction spectrum observed at 15 K, there is a second set of resonances at g = 2.115, 1.919, and 1.840 observed at 6 K and below. The C51D_pauC–PS I complex reported in Ref. 15 also showed an additional set of resonances at g = 2.110, 1.934, and 1.847 with a temperature maximum at 6 K or lower. We suggest that these new spectra, present only in photoaccumulated PS I complexes, results from the crossing-over of a S = 3/2, mixed-ligand (3 sulfur, 1 oxygen) [4Fe-4S] cluster to the S = 1/2 spin state. This idea is supported by the presence of an identical EPR spectrum in the in vivo C51X_pauC mutants (where X = D or S) of Synechocystis sp. PCC 6803 (40).

**Electron Transfer Inefficiency Versus Sample Heterogeneity**—Using optical kinetic spectroscopy as a probe of electron transfer into the modified clusters, we found that the reconstituted C14X_pauC–PS I and C51X_pauC–PS I complexes function as relatively efficient electron carriers at room temperature. The results of room temperature measurements of P700− decay kinetics indicate a high efficiency of F_A/F_B photoreduction comparable with that of a PS I complex reconstructed with wild-type PsaC in all but the alanine mutants. It is suggested that the presence of hydrophobic methyl group in the alanine mutants prevents a high degree of external thiolate access to the iron atom, thus leading to a lower yield of reconstitution with the mutant PsaC proteins. It is also likely that the alanine mutant samples are heterogeneous, containing a subpopulation of the non-reconstructed P700-F_X cores. However, a complication arises because sample heterogeneity due to incomplete reconstitution gives rise to the same kinetics in single turnover experiments as does forward electron transfer inefficiency.

In performing the optical measurements, an interesting difference in the C14S_pauC–PS I and C51S_pauC–PS I complexes was noted; of the two kinetic phases that are attributed to the backreaction from the F_X and F_B clusters, the shorter phase (15–18 ms) is predominant in the reconstituted C51S_pauC–PS I complex, whereas in the wild-type, the PsaC-reconstituted and the C14S_pauC–PS I complex, the longer kinetic phase (50–80 ms) is predominant. However, the significance of the two kinetic phases derived from the [F_A/F_B] backreaction is not clear; both phases are present in H2O-treated PS I complexes, which lack F_B, implying that neither can be assigned uniquely to either terminal electron acceptor (29).

**Comparison with Other Cysteine to Serine Mutations in [4Fe-4S] Ferredoxins**—It is interesting that mixed-ligand iron-sulfur clusters are very rarely found in naturally occurring soluble ferredoxins or in other complex iron-sulfur proteins. It may be that sulfur is a much better ligand than oxygen in supporting biogenesis of iron-sulfur proteins in vivo, and that assembly considerations are more important than electron-transport considerations in dictating the selection of ligands during evolution of iron-sulfur proteins. With the exception of the nitrogenase cluster (3), the few examples of cubane clusters supported by 3 cysteines and 1 serine have been engineered by site-directed mutagenesis. Fumarate reductase with Cys570 → Ser, Cys210 → Ser, and Cys214 → Ser mutations result in enzymes with negligible activity and with subunits that have dissociated from the membrane (43, 44). One of the [4Fe-4S] clusters of dimethyl sulfoxide reductase (45) has been changed to a [3Fe-4S] cluster by converting cysteine 102 to serine. There is one example of a modified protein containing a [4Fe-4S] cluster supported by 3 cysteines and 1 serine: the genetically engineered C565S_pauB and C585S_pauB mutants in the F_X cluster of Synechocystis sp. PCC 6803. This is a rare example of a interpoly peptide [4Fe-4S] cluster with cysteine ligands contributed by two separate polypeptides (there are several homologs of NiFH such as the dark protochlorophyllide reductase of plants, which, on the basis of sequence homology, are also presumed to have interpoly peptide iron-sulfur clusters). The C565S_pauB and C585S_pauB mutants were capable of functioning as an electron transfer carrier from the intermediate electron acceptor, A_1−, to the terminal iron-sulfur clusters, F_X and F_B (37); however, the efficiency of forward electron transfer was lower than the wild-type. The working hypothesis, yet to be tested, is that the reduction potential of the F_X cluster is driven more electronegative by the change from cysteine to serine. In the present work we show that such a mixed-ligand [4Fe-4S] cluster in PsaC can be sufficiently functional to mediate electron transfer from F_X to ferredoxin and flavodoxin.

**Conclusions**—Mutant PS I complexes reconstituted in vitro contain two [4Fe-4S] clusters regardless of whether the mutation at C14 or C51 is aspartate (14, 15), alanine, glycine, or serine (this work). The identical EPR spectra of the F_X cluster in the C14D_pauC–PS I, C14A_pauC–PS I, C14G_pauC–PS I, and C14S_pauC–PS I complexes, along with the inability of alanine and glycine to supply a suitable ligand, suggest that the fourth coordination site is occupied by an external thiolate. The thiolate would likely be supplied by a β-mercaptoethanol or dithiothreitol retained from the cysteine exchange reaction between the inorganic [4Fe-4S] clusters and the mutant PsaC apoproteins. The EPR spectrum of the F_X cluster in the C51S_pauC–PS I complex differs from the C51A_pauC–PS I or C51G_pauC–PS I complexes, implying that serine has displaced the β-mercaptoethanol at this site. This may be a consequence of steric hindrance at this site that comes about when the holoprotein is bound to the P700-F_X core. One generalization that follows from this work is that the mixed-ligand iron-sulfur cluster in the free mutant PsaC exists in a high spin state (probably S = 3/2) when either oxygen from serine (or aspartate) or the thiolate from β-mercaptoethanol (or dithiothreitol) provides the ligand to the open coordination site. The spin state changes to S = 1/2 when the thiolate-ligated cluster, but not the oxygen-ligated cluster, is bound to the P700-F_X core. A spin-coupled, interaction spectrum identical to that seen in a wild-type PS I complex is observed for all but the PS I-LC51S complex; the latter showed an additional set of resonances that may be derived from the serine oxygen-ligated [4Fe-4S] cluster. The proposed chemical rescue of a [4Fe-4S] cluster in the site-modified proteins using external thiolates thus appears to be the critical feature that allows some mutant PsaC proteins to rebind to PS I. These studies indicate that mixed-ligand (3 cysteine, 1 serine) and chemically rescued (3 cysteine, 1 presumed external thiolate) [4Fe-4S] clusters are capable of participating as an electron carrier in a physiologically relevant setting, i.e., within a functional PS I complex.
REFERENCES

1. Moura, J. J. G., Macedo, A. L., and Palma, P. N. (1994) in Inorganic Microbial Sulfur Metabolism (Peck, H. D., and Legall, J., eds) Vol. 243, pp. 165–188, Academic Press Inc., San Diego.

2. Meyer, T. E. (1994) in Inorganic Microbial Sulfur Metabolism (Peck, H. D., and Legall, J., eds) Vol. 243, pp. 435–447, Academic Press Inc., San Diego.

3. Mesuesa, J. M., Noodelman, L., and Case, D. A. (1994) Inorg. Chem. 33, 4819–4830.

4. Davidson, E., Ohnishi, T., Atta-Asafo-Adjei, E., and Daldal, F. (1992) Biochemistry 31, 3342–3351.

5. Shergill, J. K., and Cammack, R. (1994) Biochem. Biophys. Acta 1185, 35–42.

6. Beach, K. M., Sauer, K., Klein, M. P., Knaff, D. B., Kriauciunas, A., Yu, C.-A., Yu, L., and Malkin, R. (1991) Biochemistry 30, 1892–1901.

7. Thomson, A., Breton, J., Butt, J., Hatchikian, E., and Armstrong, F. (1992) J. Inorg. Biochem. 47, 197–207.

8. Conover, R. C., Kowal, A. T., Fu, W., Park, J.-B., Aono, S., Adams, M. W. W., Song, P. S., and Horspool, W., eds) pp. 1407–1419, CRC Press, Boca Raton, FL.

9. Beinert, H., and Kennedy, M. C. (1989) Eur. J. Biochem. 186, 5–15.

10. Beinert, H., and Thomason, A. J. (1993) Arch. Biochem. Biophys. 222, 333–361.

11. Thomson, A., Breton, J., George, S., Butt, J., Armstrong, F., and Hatchikian, E. (1991) Biochem. Soc. Trans. 19, 594–599.

12. Oh-Oka, H., Takahashi, Y., and Matsubara, H. (1989) Plant Cell Physiol. 30, 869–875.

13. Dunn, P. P. J., Packman, L. C., Pappin, D., and Gray, J. C. (1988) FEBS Lett. 228, 157–161.

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

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

16. Mejia, T., Qiao, F., Scott, M. P., Nellis, D. F., Zhao, J., Bryant, D. A., and Golbeck, J. H. (1995) J. Biol. Chem. 270, 28108–28117.

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

18. Kojima, Y., Hiyama, T., and Sakurai, H. (1987) in Progress in Photosynthesis Research (Biggins, J. ed) Vol. 2, pp. 57–60, Martinus Nijhoff, Dordrecht, Netherlands.

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

20. Golbeck, J. H. (1995) in CRC Handbook of Organic Photochemistry and Photobiology (Song, P. S., and Horspool, W., eds) pp. 1407–1419, CRC Press, Boca Raton, FL.

21. Kriksci, T. A., Roberts, J. D., and Zahour, R. A. (1987) Methods Enzymol. 154, 367–382.

22. Zhao, J., Li, N., Warren, P. V., Golbeck, J. H., and Bryant, D. A. (1992) Biochemistry 31, 5093–5099.

23. Li, N., Zhao, J., Warren, P. V., Warden, J. T., Bryant, D. A., and Golbeck, J. H. (1991) Biochemistry 30, 7863–7872.

24. Zhao, J., Warren, P. V., Li, N., Bryant, D. A., and Golbeck, J. H. (1990) FEBS Lett. 276, 175–180.

25. Mühlenhoff, U., Zhao, J., and Bryant, D. A. (1996) Eur. J. Biochem. 235, 324–331.

26. Yu, J., Smart, L. B., Jung, Y.-S., Golbeck, J., and McIntosh, L. (1995) Plant Mol. Biol. 29, 311–342.

27. Arnon, D. I. (1949) Plant Physiol. 24, 1–15.

28. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254.

29. Vassiliev, I., Jung, Y.-S., Mamedov, M. D., Semenov, A. Y., and Golbeck, J. H. (1996) Biophys. J., in press.

30. Belford, R. L., and Nilges, M. J. (1979) in EPR Symposium, 21st Rocky Mountain Conference, August, 1979, Denver, Colorado.

31. Nilges, M. J. (1979) Electron Paramagnetic Resonance Studies of Low Symmetry Nickel(I) and Molybdenum(V) Complexes. Ph.D. thesis, University of Illinois, Urbana, IL.

32. Altman, T. E. (1981) Analysis of Nuclear Quadrupole Coupling in EPR Spectra. Ph.D. thesis, University of Illinois, Urbana, IL.

33. Warren, P. V., Smart, L. B., McIntosh, L., and Golbeck, J. H. (1993) Biochemistry 32, 4411–4419.

34. Kowal, A. T., Werth, M. T., Manodori, A., Cecchini, G., Schroder, I., Gunsalus, R. P., and Johnson, M. K. (1995) Biochemistry 34, 12284–12293.

35. Mathis, P., Ikegami, I., and Sétil, P. (1988) Photosynth. Res. 16, 203–210.

36. Brettel, K., and Golbeck, J. H. (1995) Photosynth. Res. 45, 183–193.

37. Vassiliev, I., Jung, Y.-S., Smart, L. B., Schulz, R., McIntosh, L., and Golbeck, J. H. (1995) Biophys. J. 69, 1544–1553.

38. Brettel, K., and Golbeck, J. H. (1995) Photosynth. Res. 45, 183–193.

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

40. Jung, Y.-S., Yu, J., Yu, L., Zhao, J., Bryant, D., McIntosh, L., and Golbeck, J. H. (1995) in Photosynthesis: From Light to Biosphere (Mathis, P. A., ed) Vol. II, pp. 127–130, Kluwer Academic Publishers, Dordrecht, Netherlands.

41. Golbeck, J. H. (1993) Curr. Opin. Struct. Biol. 3, 508–514.

42. Krauss, N., Hinrichs, W., Witt, I., Fromme, P., Pratikow, W., Dauter, Z., Betzel, C., Wilson, K. S., Witt, H. T., and Saenger, W. (1993) Nature 361, 326–331.

43. Werth, M. T., Cecchini, G., Manodori, A., Acherell, B. A. C., Schroder, I., Gunsalus, R. P., and Johnson, M. K. (1990) Proc. Natl. Acad. Sci., U. S. A. 87, 8965–8969.

44. Manodori, A., Cecchini, G., Schroder, I., Gunsalus, R., Werth, M., and Johnson, M. (1992) Biochemistry 31, 2703–2712.

45. Rothery, R., and Weiner, J. (1991) Biochemistry 30, 8296–8305.