PsaD Is Required for the Stable Binding of PsaC to the Photosystem I Core Protein of *Synechococcus* sp. PCC 6301†

Ning Li,‡ Jindong Zhao,§ Patrick V. Warren,‡ Joseph T. Warden,∥ Donald A. Bryant,§ and John H. Golbeck*,‡

Department of Biochemistry, University of Nebraska, Lincoln, Nebraska 68583-0718, Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, and Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York 12180-3590

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ABSTRACT: The *psaC* gene product from *Synechococcus* sp. PCC 7002 and the *psaD* gene product from *Nostoc* sp. PCC 8009 were synthesized in *Escherichia coli* and purified to homogeneity. Incubation of the PsaC apoprotein with the *Synechoccus* sp. PCC 6301 photosystem I core protein in the presence of $FeCl_3$, Na_2S, and β-mercaptoethanol resulted in a time-dependent transition in the flash-induced absorption change from a 1.2-ms, $P700^+$ F_X^- back-reaction to a long-lived, $P700^+$ $[F_A/F_B]^-$ back-reaction. ESR studies showed that F_B and F_A were photoreduced about equally at 19 K, and while the resonances were shifted upfield, they remained as broad as in the free PsaC holoprotein. When the reconstituted complex was purified in a sucrose gradient containing 0.1% Triton X-100, most of the optical absorption transient reverted to that characteristic of the $P700^+$ F_X^- back-reaction. Addition of purified PsaD to the incubation mixture led to a greater extent of recovery of electron flow to F_A/F_B for any given concentration of PsaC. ESR studies showed that F_A, rather than F_B, became the preferred electron acceptor at 19 K; moreover, the resonances moved upfield and sharpened to become nearly identical with those of a control photosystem I complex. When the sample was purified in a sucrose gradient containing 0.1% Triton X-100, the long-lived $P700^+$ $[F_A/F_B]^-$ optical transient remained stable. Analysis by denaturing polyacrylamide gel electrophoresis showed that the PsaC and PsaD proteins had rebound to the photosystem I core. The data indicate that although PsaC can bind loosely, the presence of PsaD leads to a stable, isolatable photosystem I complex which is spectroscopically indistinguishable from the native complex. Since a PsaC1 fusion protein which contains an amino-terminal extension of five amino acids (MEHSM...) does not bind in the absence of PsaD [Zhao, J., et al. (1990) *FEBS Lett. 276*, 175–180], the N-terminus of the PsaC protein could provide a site of interaction with the photosystem I core. We propose that the binding of PsaC to the PsaA/PsaB heterodimer is potentiated by insertion of the F_A/F_B clusters into PsaC, and stabilized by the presence of PsaD.

The photosystem I reaction center in cyanobacteria and green plants is a membrane-bound, multiprotein complex which catalyzes the oxidation of plastocyanin (or cytochrome *c*-553) and the reduction of ferredoxin on the lumenal and stromal sides of the thylakoid membrane, respectively. The photochemical reaction center is composed of a heterodimer of homologous polypeptides, denoted PsaA and PsaB, which carries ~100 core antenna chlorophylls; the primary donor chlorophyll, P700; the primary electron acceptor chlorophyll, A_0; an intermediate quinone acceptor, A_1; and an interpolypeptide [4Fe–4S] cluster, F_X [for reviews, see Malkin (1987), Lagoutte and Mathis (1989), and Golbeck and Bryant (1991)]. The absorption of a photon by an antenna chlorophyll results in the migration of an exciton to the trapping center and charge separation between the primary reactants, P700 and A_0. The chemical free energy is stabilized by rapid exchange of the electron between A_0^-, A_1, and (probably) F_X. The electron is then transferred out of the reaction center to one of two iron–sulfur clusters, F_A and F_B, which are located on a separate 8.9-kDa polypeptide denoted PsaC (Oh-Oka et al., 1988a,b; Wynn & Malkin, 1988; Høj et al., 1987; Dunn & Gray, 1988). The photosystem I holocomplex from higher plants contains at least nine additional polypeptides, labeled PsaD through PsaL, and four light-harvesting chlorophyll proteins, the products of the *cab*-6A/6B, *cab*-7, *cab*-8, and *cab*-11/12 genes (Golbeck & Bryant, 1991; Bryant, 1991). The cyanobacterial photosystem I complex is somewhat simpler; it contains a minimum of seven additional polypeptides (PsaG and PsaH have not been found), and it lacks the entire complement of light-harvesting chlorophyll proteins.

In the last few years, techniques have been developed to dissociate the F_A/F_B-containing, PsaC protein from the photosystem I complex without affecting electron flow from P700 to F_X. One method uses high concentrations of chaotropic agents, such as 6.8 M urea or 2 M NaI, to remove the PsaC, PsaD, and PsaE proteins from the *Synechococcus* sp. PCC 6301 photosystem I complex (Golbeck et al., 1988b; Parrett et al., 1989; Li et al., 1991). Other methods employ 1% lithium dodecyl sulfate (Golbeck & Cornelius, 1986; Warden & Golbeck, 1986; Golbeck et al., 1987) and hot ethylene glycol (Hoshina et al., 1989) to dissociate low molecular mass polypeptides from the spinach photosystem I complex. In all instances, there is a transition from a flash-induced 30-ms back-reaction between $P700^+$ and $[F_A/F_B]^-$ to a 1.2-ms back-reaction between $P700^+$ and F_X^-. This result can be explained by the removal of the PsaC polypeptide from the photosystem I complex, thereby allowing the F_X iron–sulfur cluster to serve as the terminal electron acceptor. When the photosystem I complex from *Synechococcus* sp PCC 6301 is treated with chaotropic agents and ultrafiltered over a 100-kaDa cutoff membrane, the retentate contains the PsaA/PsaB

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* Correspondence should be addressed to this author.
‡ University of Nebraska.
§ The Pennsylvania State University.
∥ Rensselaer Polytechnic Institute.

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heterodimer and the PsaL, PsaF, and PsaK proteins, but is devoid of the PsaC, PsaD, and PsaE proteins [at least three low molecular mass polypeptides are also retained which may include PsaJ and PsaI (Li et al., 1991)]. This preparation has been termed the photosystem I core protein to reflect the fact that the functional electron acceptors, A_0, A_1, and F_X, are resident on the P700-containing PsaA/PsaB heterodimer. The PsaC, PsaD, and PsaE proteins can be recovered in the YM-100 filtrate, but the F_A and F_B iron–sulfur clusters in PsaC are destroyed by the chaotrope treatment. The addition of $FeCl_3$, Na_2S, and β-mercaptoethanol (β-ME)[1] to the mixture of PsaC, PsaD, and PsaE proteins in the YM-100 filtrate yields the PsaC holoprotein, which can be rebound to the photosystem I core protein to reconstitute electron flow from P700 to the F_A/F_B clusters (Parrett et al., 1990; Mehari et al., 1991). The PsaD and PsaE proteins also rebind to the photosystem I core protein, but only when the PsaC protein has been reconstituted with iron–sulfur clusters (Li et al., 1991).

While these studies showed that the PsaC holoprotein is necessary for the binding of the PsaD and PsaE polypeptides to the photosystem I core, they did not address the related question of whether the PsaC holoprotein can rebind in the absence of PsaD and PsaE. A previous study with PsaC1, which contains an amino-terminal extension of five amino acids (MEHSM...), indicated that the presence of the PsaD protein was an absolute requirement for its binding to the *Synechococcus* sp. PCC 6301 core protein (Zhao et al., 1990). Studies with the authentic PsaC protein were needed, and while it may have been possible to purify PsaC, PsaD, and PsaE from the *Synechococcus* sp. PCC 6301 photosystem I complex, a co-purifying protease is present which cleaves PsaD at multiple sites near the C-terminus (Li et al., 1991). The large quantities of PsaC and PsaD protein necessary for these studies also mitigated against their isolation from the thylakoid membrane. We chose, therefore, to produce the cyanobacterial PsaC, PsaD, and PsaE proteins in *Escherichia coli*, and to investigate the individual binding of PsaC and the effect of the PsaD protein on the binding of PsaC to the photosystem I core protein. The strategy employed was to overproduce the *psaC* gene product from *Synechococcus* sp. PCC 7002 in *E. coli*, purify the PsaC apoprotein to homogeneity, and reconstitute the F_A/F_B iron–sulfur clusters in vitro to produce a competent PsaC holoprotein. The *psaD* gene from *Nostoc* sp. PCC 8009 was similarly overexpressed in *E. coli*, and the PsaD protein was purified to homogeneity. The isolation and purification of PsaC and PsaD were aided by the finding that both formed inclusion bodies within the cells of *E. coli* which constituted a large percentage of the total cellular protein. The restoration of electron flow from P700 to F_A/F_B was determined by using optical and ESR spectroscopy, and the binding of the polypeptides to the photosystem I core protein was determined by SDS–PAGE.

Materials and Methods

Materials and Escherichia coli Strains. Restriction enzymes, DNA modifying enzymes, and T4 DNA ligase were purchased from Bethesda Research Laboratories (Gaithersburg, MD), New England BioLabs (Beverly, MA), or Boehringer-Mannheim Biochemicals (Indianapolis, IN) and were used according to the specifications of the manufacturer. The strain of *Escherichia coli* used for routine genetic manipulations was strain DH5α (Bethesda Research Laboratories, Gaithersburg, MD). *E. coli* strain BL21(DE3) was employed for high-level synthesis of proteins directed by the T7 RNA polymerase (Studier et al., 1990).

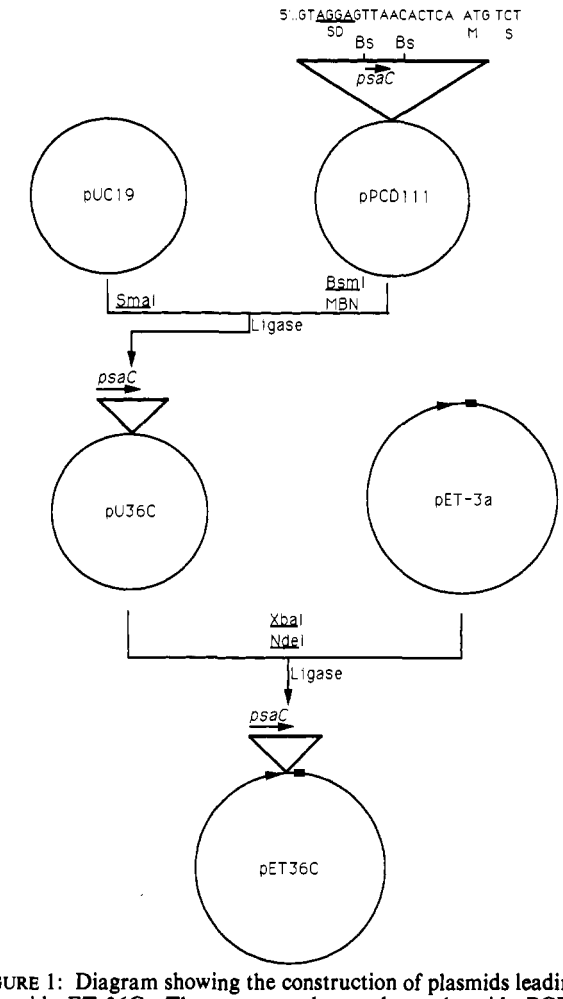

FIGURE 1: Diagram showing the construction of plasmids leading to plasmid pET-36C. The sequence shown above plasmid pPCD111 shows the position of the Shine–Dalgarno (SD), ribosome binding site and first two codons of the *psaC* gene of *Synechococcus* sp. PCC 7002. The arrowhead on plasmid pET-3a indicates the position of the T7 promoter, and the rectangle indicates a transcription terminator. Restriction endonucleases employed in the constructions are indicated. MBN, mung bean nuclease.

Plasmid Constructions. Routine recombinant DNA procedures were performed as described (Ausubel et al., 1987; Maniatis et al., 1982; Sambrook et al., 1989). The cloning of the *psaC* gene of *Synechococcus* sp. PCC 7002 and the *psaD* gene of *Nostoc* sp. PCC 8009 was previously reported (Bryant et al., 1990). The construction of plasmid pET-36C for the expression of the *psaC* gene of *Synechococcus* sp. PCC 7002 is shown diagrammatically in Figure 1. A 441 bp *Bsm*I fragment encoding the PsaC protein was made blunt-ended by treatment with mung bean nuclease and cloned into the *Sma*I site of plasmid pUC19 (Yanisch-Perron et al., 1985). The *psaC* gene was excised from plasmid pU36C on an *Xba*I–*Nde*I fragment and directionally cloned in plasmid pET-3a (Studier et al., 1990) which had been restricted with *Xba*I and *Nde*I. This construction, in which the *psaC* gene was oriented downstream from the T7 promoter of plasmid pET-3a, was denoted pET-36C and was introduced by transformation into *E. coli* strain BL21(DE3). The construction of plasmid pET-3a/D for the expression of the *psaD* gene of *Nostoc* sp. PCC 8009 in *E. coli* is shown diagrammatically in Figure 2. A 1.65 kbp *Nhe*I fragment encoding

[1] Abbreviations: Chl, chlorophyll; ESR, electron spin resonance; CP1, chlorophyll protein 1; PAGE, polyacrylamide gel electrophoresis; PVDF, poly(vinylidene difluoride); DCPIP, dichlorophenolindophenol; β-ME, β-mercaptoethanol; SDS, sodium dodecyl sulfate.

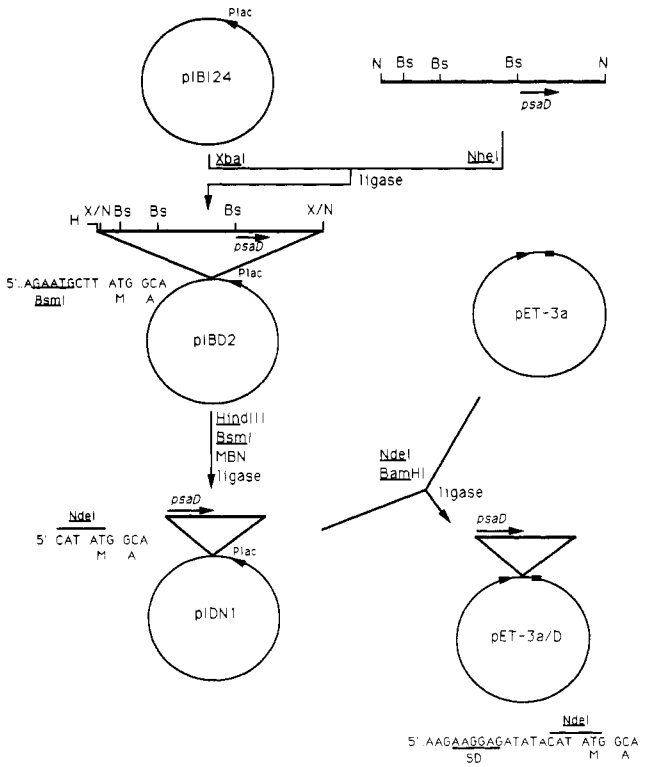

FIGURE 2: Diagram showing the construction of plasmids leading to plasmid pET-3a/D. The 1.65 kbp *Nhe*I fragment encoding the *Nostoc* sp. PCC 8009 *psaD* gene was isolated from plasmid pMAC2 as shown and cloned into the *Xba*I site of plasmid pIBI24. The sequence adjacent to plasmid pIBD2 shows a portion of the original Shine–Dalgarno sequence and the first two codons of the *psaD* gene. The sequence adjacent to plasmid pET-3a/D shows the Shine–Dalgarno (SD) sequence engineered into the expression plasmid and the first two codons of the gene. The arrowheads in plasmids pET-3a and and pET-3a/D indicate the position of the T promoter, and the rectangles indicate transcription termination signals. The restriction endonucleases employed at each step of the construction are indicated. MBN, mung bean nuclease.

the *psaD* gene was cloned into plasmid pIBI24 obtained from International Biotechnologies, Inc. (New Haven, CT) to produce plasmid pIBD2 as shown in Figure 2. Digestion of this plasmid with *Hin*dIII and *Bsm*I, followed by treatment with mung bean nuclease and DNA ligase, caused the deletion of upstream sequences and created plasmid pIDN1 in which a new *Nde*I site overlapped the initiation codon of the *psaD* gene as shown in Figure 2. An *Nde*I–*Bam*HI fragment of pIDN1 including the *psaD* gene was directionally cloned into plasmid pET-3a to produce plasmid pET-3a/D in which the *Nostoc* sp. PCC 8009 *psaD* gene lies downstream from the T7 promoter as shown in Figure 2. Plasmid pET-3a/D was transformed into strain BL21(DE3).

Synthesis and Purification of the PsaC and PsaD Proteins. *E. coli* strains harboring pET-36C and pET-3a/D were grown in medium NYZCM (Sambrook et al., 1989) except that magnesium sulfate was omitted. Additionally, the medium for cells synthesizing PsaC was supplemented with 50 mM ferric ammonium citrate (Zhao et al., 1990). Expression was performed essentially as described previously (Studier et al., 1990) and was initiated by the addition of 0.5 mM IPTG to the growth medium. After 1 h, 20 μg/mL rifamycin was added to the medium, and expression was continued for an additional period of 5–7 h. Cells were harvested by centrifugation and washed once with TS buffer (TS buffer = 20 mM Tris-HCl, pH 8.0, and 10 mM NaCl). Cells were resuspended in TS buffer containing 2 mM dithiothreitol and 2 mg/mL DNase and disrupted by two passes through a French pressure cell at 18 000 psi at 4 °C. Inclusion bodies were collected by centrifugation of the whole cell extract at 7650*g* for 5 min at 4 °C. The inclusion bodies were resuspended in TS buffer containing 2 mM dithiothreitol and aliquots overlayed of 15 mL of 10% (w/v) sucrose in TS buffer. The inclusion bodies were then collected by centrifugation at 14460*g* for 20 min at 4 °C. Typically, more than 100 mg of protein as inclusion bodies was isolated from a 1-L culture of *E. coli*. Further purification of the PsaC protein was achieved by chromatography on DEAE-Sephacel at pH 8.0. The PsaC inclusion bodies were solubilized in TS buffer containing 6.5 M urea and 2 mM dithiothreitol. The solubilized proteins were applied to the column which was washed with TS buffer containing 2 mM dithiothreitol. The column was then developed with a linear gradient of sodium chloride (50–1000 mM NaCl); the PsaC protein was eluted at approximately 350 mM NaCl. Further purification of the PsaD protein was achieved by chromatography on CM-Sepharose CL-6B. The inclusion bodies were solubilized in TS buffer containing 7.0 M urea and applied to the column. The column was developed with a linear gradient of NaCl (50–1000 mM NaCl) in 50 mM Tris-HCl buffer, pH 8.0. The PsaD protein was eluted at approximately 250 mM NaCl.

Polypeptide Analysis. N-Terminal amino acid sequencing of the PsaC and PsaD proteins was performed with a Milligen/Biosearch 6600 ProSequencer at the University of Nebraska Protein Core Facility. Protein samples were dialyzed against water, and aliquots were subjected to automated Edman degradation. Samples were immobilized either on diisothiocyanate (DITC) or on arylamine membrane disks (Milligen/Biosearch). The PsaD protein was diluted 2-fold with 50% 2-propanol/2% *N*-methylmorpholine (v/v), applied to DITC disks for coupling, and allowed to dry at 55 °C. The PsaC protein was first diluted with 50% ethanol/2% acetic acid (v/v) and dried on arylamine (Sequelon-AA) disks at 55 °C. Covalent attachment to the membrane was performed by using water-soluble carbodiimide in MES buffer at pH 5.0. Both DITC and arylamine disks containing covalently attached protein were washed with methanol, water, and methanol again prior to sequencing.

Preparation of the Photosystem I Core Protein. The photosystem I complex from *Synchococcus* sp. PCC 6301 was isolated by treating the thylakoid membranes with 1% Triton X-100 followed by two sucrose density ultracentrifugation steps, the first in the presence of 0.1% Triton X-100 and the second in its absence (Li et al., 1991). The photosystem I core protein was isolated from a *Synechococcus* sp. PCC 6301 photosystem I complex with 6.8 M urea, concentrated by ultrafiltration over a YM-100 membrane, and purified by sucrose density gradient ultracentrifugtion in 0.1% Triton X-100 (Golbeck et al., 1988b; Parrett et al., 1989).

Rebinding of the PsaC and PsaD Proteins to the Photosystem I Core Protein. For optical studies, reconstitution of the F_A/F_B iron–sulfur clusters and rebinding of the PsaC and PsaD polypeptides to the photosystem I core protein were performed according to the following protocol: (i) a solution of 50 mM Tris-HCl, pH 8.3, was purged with oxygen-free N_2 in a closed reaction vessel; (ii) after 2 h, β-ME was added through a septum to a final concentration of 0.5%; (iii) the photosystem I core protein (final concentration of 50 μg/mL Chl) and the PsaC and/or PsaD proteins (at the molar ratios given in the text) were added and purged with oxygen-free N_2 at a low flow rate; (iv) after 10 min at room temperature, an aliquot of 30 mM $FeCl_3$ (dissolved in oxygen-free water) was added to a final concentration of 150 μM; (v) after 5 min, an

aliquot of 30 mM Na_2S (dissolved in oxygen-free water) was added slowly to a final concentration of 150 μM. This solution was allowed to incubate in the dark at room temperature for 18 h. The reaction vessel was then uncapped, and the solution was transferred to an ultrafiltration cell (Amicon 8050) equipped with a YM-100 membrane, concentrated to near-dryness, and washed twice with 50 mM Tris buffer, pH 8.3, containing 0.04% Triton X-100. The reconstituted photosystem I complex was further purified by ultracentrifugation in a sucrose gradient containing 0.1% Triton X-100 in 50 mM Tris buffer, pH 8.3. The reconstituted photosystem I complex was recovered, dialyzed against 50 mM Tris buffer, pH 8.3, concentrated by ultrafiltration to 1.0 mg/mL Chl, and stored at −80 °C. The efficiency of the reconstitutions were confirmed by optical flash spectroscopy prior to ESR spectroscopy and analysis by SDS–PAGE.

SDS–PAGE. Polypeptides were analyzed on 120 mm × 100 mm × 1.5 mm polyacrylamide slab gels (16.5% T, 6% C separating gel; 4% T, 3% C stacking gel; 10% T, 3% C spacer gel) according to the method of Schägger and van Jagow (1987). Protein samples were incubated in 62.5 mM Tris buffer, pH 6.8, 2% SDS, 10% glycerol, and 5% β-ME for 3 min at 90 °C. Electrophoresis was carried out at room temperature at a constant current of 30 mA for 8–10 h. Gels were stained with 0.125% Coomassie Brilliant Blue and destained first with 50% methanol and 10% acetic acid and then with 5% methanol and 7% acetic acid. The gels were dried with BioGel Wrap (Biodesign product G101) over an incandescent lamp for 3–4 h. The transparent gel was scanned with an LKB 2202 laser densitometer and digitized with a Keithley voltmeter (Model 195A) and a Macintosh IIci computer using software written in-house. The chemicals used in preparing the gels were Bio-Rad (Richmond, CA) electrophoresis-purity reagents.

Electron-Spin Resonance Spectroscopy. Electron spin resonance studies were performed with a Varian E-9 spectrometer interfaced to a PDP-11/23A minicomputer operating under Berkeley Software Distribution UnixTM (BSD2.9) compiled with real-time process extensions. Cryogenic temperatures were obtained with an Air Products LTD-3-100 Heli-Tran cryostat. Sample tempertures were monitored by a calibrated thermocouple situated beneath the 3-mm i.d. quartz sample tube. Actinic illumination of the sample (400–700 nm at 6000 W m^{-2}) was provided by a 1000-W tungsten–halogen source (Oriel). Simulations of the ESR spectra of the F_A/F_B clusters were performed with either a Sun Microsystems 3/50 or a Sun Sparcstation 1 using a protocol based on solution of the spin Hamiltonian for an anisotropic, spin = $^1/_2$ system possessing rhombic symmetry ($g_x \neq g_y \neq g_z$). Line-width anisotropy was approximated as an unresolved, first-order hyperfine interaction with principal axes collinear with those for the **g** tensor. The Gaussian line-shape function was used for all simulations. Relative cluster concentrations were determined by using a procedure in which the composite spectra of F_A and F_B in reaction centers exhibiting partial or total reduction of F_B in the presence of F_A- were constructed via weighted convolution of the individual, spin-normalized simulations for each center (Golbeck & Warden, 1982).

Flash-Induced Absorption Changes. Flash-induced absorption transients were determined at 698 nm by using a single-beam spectrophotometer described previously (Parrett et al., 1989).

Biochemical Assays. Chlorophyll *a* was determined in 80% acetone (Arnon, 1949). Protein concentrations were determined routinely by using a dye binding method (Bradford, 1976) after suitable correction based on the quantitative amino acid analysis of acid-stable residues in the PsaC and PsaD proteins. The complete amino acid sequences, and hence compositions, of the *Synechococcus* sp. PCC 7002 PsaC protein and the *Nostoc* sp. PCC 8009 PsaD protein are known [see Golbeck and Bryant (1991)]. Quantitative amino acid analyses of the PsaC and PsaD proteins were determined at the Protein Structure Laboratory (University of California, Davis, CA). The proteins were dialyzed exhaustively against twice-distilled water, lyophilized, and subjected to acid hydrolysis with HCl. When the amino acid analysis data are used as standard, the *Synechococcus* sp. PCC 7002 PsaC apoprotein has a calculated molar extinction coefficient at 280 nm of 13.2 mM^{-1} cm^{-1}, and the *Nostoc* sp. PCC 8009 PsaD protein has a calculated molar extinction coefficient at 280 nm of 12.1 mM^{-1} cm^{-1}. Given a mass of 8813 daltons for the PsaC apoprotein and 15 223 daltons for the PsaD protein, 1.0 ODU at 280 nm corresponds to a protein concentration of 0.67 mg/mL for PsaC and 1.26 mg/mL for PsaD. The Bradford dye binding assay, when based on bovine γ-globulin as standard, overestimates the amount of PsaC apoprotein by a factor of 1.43 and PsaD protein by a factor of 1.85.

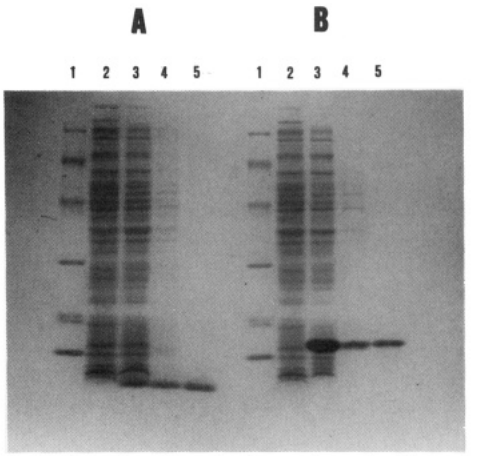

FIGURE 3: Polyacrylamide gel electrophoresis of proteins in the presence of sodium dodecyl sulfate. (Panel A) Synthesis of the *Synechococcus* sp. PCC 7002 PsaC protein in *E. coli*: lane 1, molecular mass markers, phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa); lane 2, whole cell extract from *E. coli* cells; lane 3, whole cell extract from *E. coli* cells harboring plasmid pET-36C 8 h after induction with IPTG; lane 4, inclusion bodies of the PsaC protein after being pelleted through a 10% sucrose solution; lane 5, PsaC protein after chromatography on DEAE-cellulose. (Panel B) Synthesis of the *Nostoc* sp. PCC 8009 PsaD protein in *E. coli*: lane 1, molecular mass markers as for lane 1 of panel A; lane 2, whole cell extract from *E. coli* cells harboring plasmid pET-3a/D in the absence of IPTG; lane 3, whole cell extract from *E. coli* cells harboring plasmid pET-3a/D in the absence of IPTG; lane 3, whole cell extract from *E. coli* cells harboring plasmid pET-3a/D 8 h after induction with IPTG; lane 4, purified PsaD inclusion bodies after being pelleted through a 10% sucrose solution; lane 5, PsaD protein after purification to homogeneity by chromatography on CM-Sepharose CL-6B.

RESULTS

Synthesis of the PsaC and PsaD Proteins in Escherichia coli. Plasmid pET-3a (Figure 1) directed the synthesis of high levels of the PsaC protein of *Synechococcus* sp. PCC 7002 in *E. coli*. Although some PsaC protein appeared to be synthesized in *E. coli* in the absence of the inducr IPTG (Figure 3A, lane 2), addition of IPTG caused the accumulation of the PsaC protein as inclusion bodies that represented as much as 20% of the total cell protein in some experiments. *E. coli* cells

synthesizing the PsaC protein and the PsaC inclusion bodies were dark brown in color, suggesting that the protein was chelating large amounts of iron. Previous studies had shown that the yields of a mutant form of the PsaC protein, denoted PsaC1, were greater when the medium was supplemented with iron (Zhao et al., 1990). Although the PsaC inclusion bodies were relatively free from contaminating proteins, the protein could be purified to electrophoretic homogeneity by chromatography on DEAE-Sephacel in the presence of 2 mM dithiothreitol (Figure 3A, lane 5). The N-terminal amino acid sequence of the PsaC inclusion body protein, as well as that of the purified protein, was determined. In both cases, the sequence, SHSVKIY..., was identical with that of the authentic protein of the *Synechococcus* sp. PCC 7002 photosystem I complex (N. Li, D. A. Bryant, G. Frank, and J. H. Golbeck, unpublished results) and was identical with that predicted by the gene except for the absence of the initiator methionine residue (Bryant et al., 1990).

Plasmid pET-3a/D (Figure 2) allowed very high levels of the PsaD protein of *Nostoc* sp. PCC 8009 to be produced in *E. coli*. This protein routinely accounted for about 30% of the total cellular protein in extracts prepared from *E. coli* cells about 6–8 h after IPTG induction (Figure 3B, lane 3). Although the purified inclusion bodies were largely free from contaminating proteins, the PsaD protein could be purified to electrophoretic homogeneity by chromatography on CM-Sepharose CL-6B (Figure 3B, lane 5). The N-terminal sequence of the purified protein was determined to be AEQLSGKTPL.... This sequence is identical with that of the PsaD protein isolated from the photosystem I complex of *Nostoc* sp. PCC 8009 (Rhiel & Bryant, 1988) and is identical with that predicted from the *psaD* gene except for the absence of the initiator methionine residue (Bryant et al., 1990).

Optical Characterization of the Reconstituted Photosystem I Complex. The reconstitution of the photosystem I complex was studied after incubation of the purified *Synechococcus* sp. PCC 7002 PsaC apoprotein with the *Synechococcus* sp. PCC 6301 photosystem I core protein in the presence of $FeCl_3$, Na_2S, and β-ME. The extent of the reconstitution was monitored at room temperature by observing the transition of the 1.2-ms, $P700^+$ F_X^- back-reaction to the long-lived, $P700^+$ $[F_A/F_B]^-$ back-reaction. The time course of the flash-induced absorption change was determined at various molar ratios of PsaC to the photosystem I core protein. At molar ratios of 3 PsaC:1 core, 7 PsaC:1 core, and 15 PsaC:1 core, the reconstitution is, respectively, 19%, 58%, and 81% complete after 1 h of incubation, and there is no further recovery in time periods extending to 18 h (Figure 4, top). The iron–sulfur reconstitution reagents alone in the absence of the PsaC apoprotein had a negligible effect on the $P700^+$ F_X^- back-reaction kinetics at these concentrations. Because of the high degree of reconstitution at relatively low molar concentrations of PsaC, it is unlikely that diffusion-mediated electron transfer to F_A or F_B accounts for the transition to a long-lived absorption transient. More likely, PsaC has been rebound the photosystem I core protein, thereby allowing F_A and/or F_B to serve as the terminal electron acceptor(s). When the 1-h sample of 15 PsaC:1 core is subjected to repeated dilution and ultrafiltration over a YM-100 membrane in the absence of Triton X-100, there is little change in the extent of reconstitution. However, when the PsaC-containing sample is ultracentrifuged in a sucrose gradient containing 0.1% Triton X-100, the majority of the reconstituted photosystem I reaction centers revert to the 1.2-ms transient characteristic of the $P700^+$ F_X^- back-reaction (Figure 4, top).

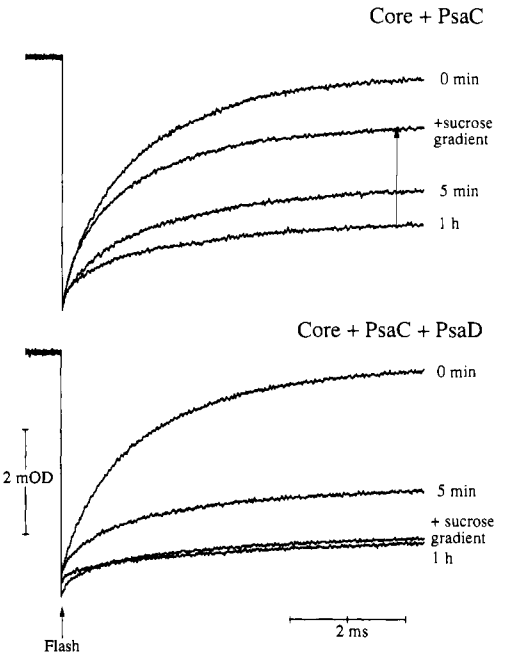

FIGURE 4: Flash-induced absorption transient at 698 nm after addition of the PsaC and PsaD protein derived from *Synechococcus* sp. PCC 7002 and *Nostoc* sp. PCC 8009 to a photosystem I core protein isolated from *Synechococcus* sp. PCC 6301 in the presence of $FeCl_3$, Na_2S, and β-ME. Time course of the reconstitution performed at a ratio of 15 PsaC to 1 photosystem I core (top) and at a ratio of 7 PsaC to 5 PsaD to 1 photosystem I core (bottom). The 1-h samples were concentrated by ultrafiltration over a YM-100 membrane and ultracentrifuged in a sucrose gradient containing 0.1% Triton X-100. All measurements were performed at 5 $\mu g\ mL^{-1}$ chlorophyll *a* in 50 mM Tris-HCl buffer, pH 8.3, containing 1.7 mM ascorbate and 33 μM DCPIP under anaerobic conditions.

The reconstitution was also studied after incubation of the purified PsaC apoprotein with the PsaD protein of *Nostoc* sp. PCC 8009 and the *Synechococcus* sp. PCC 6301 photosystem I core protein in the presence of $FeCl_3$, Na_2S, and β-ME. The flash-induced $P700^+$ absorption transient was determined for various molar ratios of PsaC and PsaD to photosystem I core protein. At molar ratios of 3 PsaC:5 PsaD:1 core, 7 PsaC:5 PsaD:1 core, and 7 PsaC:15 PsaD:1 core, the reconstitution is, respectively, 25%, 89%, and 100% complete after 1 h of incubation, and there is no further recovery in time periods extending to 18 h (Figure 4, bottom). These data indicate that the presence of PsaD allows the reconstitution to proceed to a greater degree of completion for any given concentration of PsaC. The presence of PsaD does not seem to affect the rate at which the PsaC protein rebinds to the photosystem I core protein. Similar to the situation with PsaC alone, when the 1-h sample of 7 PsaC:5 PsaD:1 core is subjected to repeated ultrafiltration over a YM-100 membrane, there is no change in the degree of reconstitution. Furthermore, when the reconstituted complex is ultracentrifuged in a sucrose gradient containing 0.1% Triton X-100, the long-lived charge separation remains stable (Figure 4, bottom).

ESR Spectral Characterization of the Reconstituted Photosystem I Complex. The ESR spectrum of the PsaC protein derived from *Synechococcus* sp. PCC 7002 and expressed in *E. coli* is shown in Figure 5. The iron–sulfur clusters were reinserted in vitro, and the holoprotein was chemically reduced with dithionite at pH 10.0 prior to freezing (Mehari et al., 1991). When measured under these conditions, the resonances in the unbound PsaC protein are broad (Figure 5A), and with the exception of a broadened midfield feature at 3390 G, the spectrum is similar to that of the spinach PsaC protein assayed

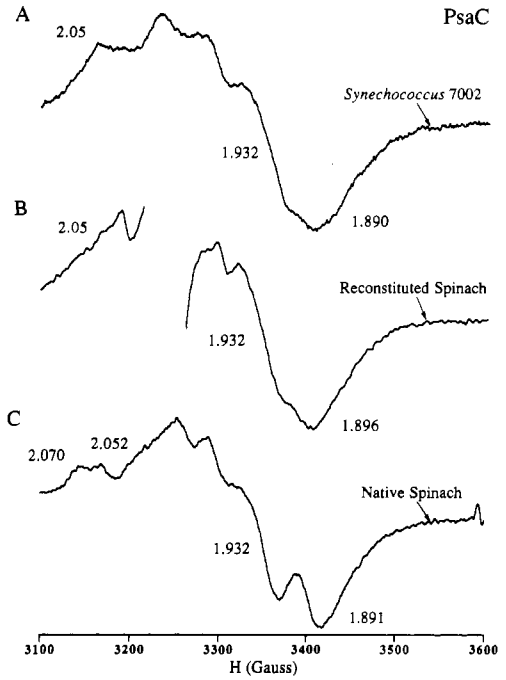

FIGURE 5: ESR spectrum of the chemically reduced, free PsaC protein. (A) PsaC protein derived from *Synechococcus* sp. PCC 7002 after reconstitution with $FeCl_3$, Na_2S, and β-mercaptoethanol. (B) PsaC protein isolated from spinach thylakoid membranes after denaturation with 2 M urea and 5 mM potassium ferricyanide followed by reconstitution with $FeCl_3$, Na_2S, and β-mercaptoethanol. (C) PsaC holoprotein isolated fresh from spinach thylakoid membranes. The samples were treated directly in the ESR sample tube with excess sodium dithionite and 33 μM methyl viologen in 0.1 M glycine, pH 10. The spectra were resolved by subtracting the oxidized spectrum from the chemically reduced spectrum and amplifying the difference 5-fold in software. Spectrometer conditions: temperature, 19 K; microwave power, 20 mW; microwave frequency, 9.128 GHz; receiver gain, 5.0×10^3; modulation amplitude, 10 G at 100 kHz.

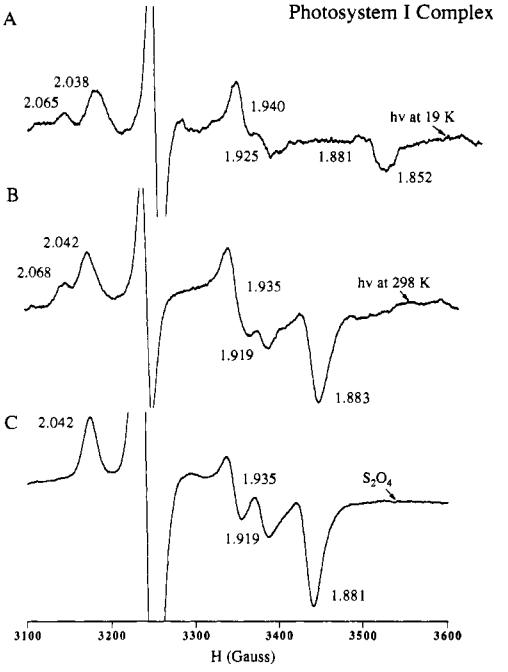

FIGURE 6: ESR spectra of the control photosystem I complex isolated from *Synechococcus* sp. PCC 6301. Spectrum (A) after freezing in darkness and illumination at 19 K, (B) after illumination during freezing to 19 K, and (C) after chemical reduction with sodium dithionite and freezing in darkness. The resonances were resolved by subtracting the light-off (before light on) from the light-on spectrum. All samples were suspended in 50 mM Tris buffer, pH 8.3, containing 1 mM sodium ascorbate and 30 μM DCPIP at 300 μg of Chl/mL. Spectrometer conditions identical with those in Figure 5.

shortly after isolation from thylakoid membranes (Figure 5C). However, the midfield feature of the spinach PsaC protein is also broadened if the iron–sulfur clusters are oxidatively denatured with 2 M urea and 5 mM ferricyanide, and then renatured with $FeCl_3$, Na_2S, and β-ME (Figure 5B). Simulation of the data in Figure 5 reveals that the spectrum for the unbound PsaC protein represents the superposition of two rhombic spectra, one exhibiting g factors of 1.890, 1.932, and 2.05 and the second with values of 1.896, 1.962, and 2.03. The data shown here indicate that although there are discernible differences in the g factors of the F_A/F_B clusters between higher plant and cyanobacterial PsaC proteins when they are bound to the photosystem I complex (Mehari et al., 1991), the spectra are virtually identical when the PsaC protein is in the free state. There were no discernible changes in the line shapes or g factors of the F_A or F_B resonances when the PsaC protein was incubated with stoichiometric amounts of PsaD alone (not shown).

These broad resonances in the unbound PsaC protein should be compared with the narrow resonances seen in the ESR spectrum of a control photosystem I complex from *Synechococcus* sp. PCC 6301 (Figure 6). When the photosystem I complex is frozen in darkness and illuminated at 19 K, only one electron is transferred from P700 to the terminal iron–sulfur clusters in any given reaction center; thus, either F_A or F_B becomes photoreduced, but not both (Figure 6A). Under these conditions, about 85% of F_A normally becomes photoreduced (g factors of 2.038, 1.940, and 1.852), and 15% of F_B becomes photoreduced (g factors of 2.065, 1.925, and 1.881). When the photosystem I complex is illuminated during freezing (Figure 6B), more than one electron can be transferred from the donor side of photosystem I, resulting in the quantitative reduction of F_A (g factors of 2.042, 1.935, and 1.883) and the nearly quantitative reduction of F_B (g factors of 2.068, 1.919, and 1.883). When both F_A and F_B are reduced in each reaction center, the 1.85 resonance of F_A moves downfield to merge with the 1.88 resonance of F_B, and the 2.07 resonance of F_B moves upfield to merge with the 2.04 resonance of F_A. Also, the midfield resonance of F_A moves upfield from $g = 1.940$ to 1.935, and the midfield resonance of F_B moves upfield from $g = 1.925$ to 1.919. We have observed a similar upfield shift of the midfield resonances of F_A and F_B in spinach, tobacco, *Synechococcus* sp. PCC 6301, *Synechococcus* sp. PCC 7002, *Synechocystis* sp. PCC 6803, and the thermophilic *Synechococcus* sp. photosystem I complexes (not shown). After chemical reduction with sodium dithionite at pH 10.0, F_A and F_B are quantitatively reduced (Figure 6C), resulting in g factors similar to those observed when the sample is frozen during illumination (Figure 6B). There is no additional effect of light at 19 K, thus indicating the chemical reduction of F_A and F_B is complete (not shown). When the PsaC, PsaD, and PsaE proteins are removed from the *Synechococcus* sp. PCC 6301 photosystem I complex with the use of chaotropes, the photosystem I core protein is devoid of photochemically or chemically reducible F_A or F_B clusters. F_X is present (Golbeck et al., 1988b), but it cannot be observed at the experimental conditions (temperature, microwave power, and modulation amplitude) optimal for F_A and F_B.

The reconstitution of the photosystem I complex was carried out by incubating the *Synechococcus* sp. PCC 7002 PsaC protein with the photosystem I core protein at a ratio of 15 PsaC:1 core in the presence of $FeCl_3$, Na_2S, and β-ME. After incubation for 18 h at room temperature, the low molecular

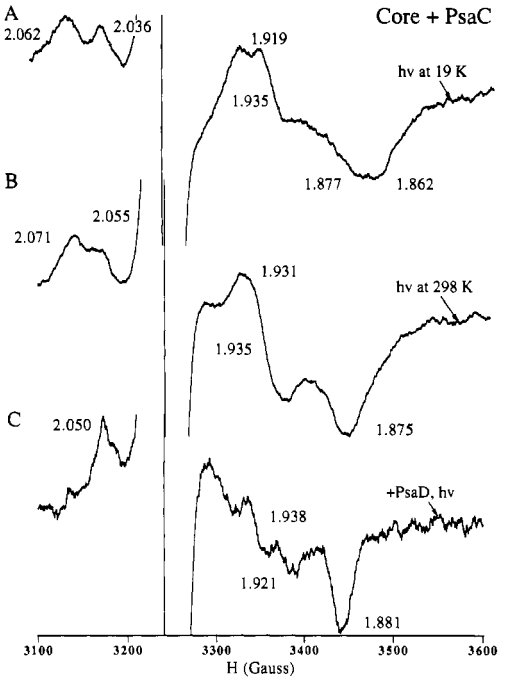

FIGURE 7: ESR spectra of the photosystem I complex reconstituted with the *Synechococcus* sp. PCC 7002 PsaC protein and the *Synechococcus* sp. PCC 6301 photosystem I core protein in the presence of $FeCl_3$, Na_2S, and β-mercaptoethanol. Spectrum (A) after freezing in darkness and illumination at 19 K, (B) after illumination during freezing to 19 K, and (C) after addition of PsaD and incubation for 1 h at room temperature. The resonances were resolved by subtracting the light-off (before light on) from the light-on spectrum. Sample and spectrometer conditions identical with those in Figure 6.

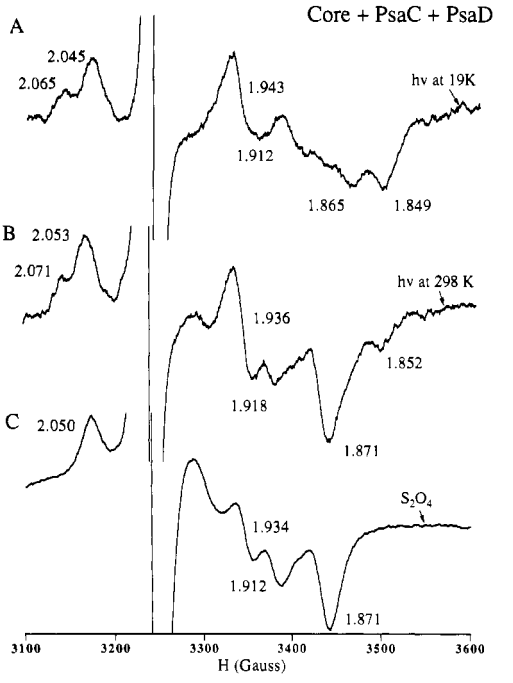

FIGURE 8: ESR spectra of the photosystem I complex reconstituted with the *Synechococcus* sp. PCC 7002 PsaC protein, the *Nostoc* sp. PCC 8009 PsaD protein, and the *Synechococcus* sp. PCC 6301 photosystem I core protein in the presence of $FeCl_3$, Na_2S, and β-mercaptoethanol. (A) Spectrum after freezing in darkness and illumination at 19 K. (B) Spectrum after illumination during freezing to 19 K. (C) Spectrum after chemical reduction with sodium dithionite and freezing in darkness. The resonances were resolved by subtracting the light-off (before light on) from the light-on spectrum; in (B), the resonances were measured directly. Samples and spectrometer conditions identical with those in Figure 6.

mass cofactors, including unbound PsaC protein and iron–sulfur reconstitution agents, were removed by repeated ultrafiltration of the sample over a YM-100 membrane. When the reconstituted sample was frozen in darkness and illuminated at 19 K, appreciable reduction of the iron–sulfur clusters was observed (Figure 7A). In contrast to the control sample, the photoreduced signals are very broad, and there is significant photoreduction of F_B as indicated by resonances at g = 2.062 and 1.919 in addition to photoreduction of F_A as indicated by resonances at g = 2.036 and g = 1.935. The broadness of the spectrum is also seen by the fact that the highfield g = 1.862 resonance of F_A is not resolved from the g = 1.877 resonance of F_B. When the composite spectrum is fit by the procedure described previously (Golbeck & Warden, 1982), the ratio of photochemically reduced F_A to F_B is about 1.5:1. A sample frozen during illumination shows photoreduction of both F_A and F_B (Figure 7B), and, although the g_{mid} resonances have shifted upfield to approximately the g factors observed in the control photosystem I complex (compared in Figure 6B), they are still of similar breadth as those of the unbound PsaC protein (ca. 50 G). Hence, the midfield resonances of F_A and F_B are nearly merged. In contrast to the shifts observed in the g_{mid} and g_{max} features that accompany the simultaneous reduction of both F_A and F_B, the lowfield resonances (g_{min}) of F_A and F_B can still be distinguished with only a minor shift of g_{min} for the F_A cluster observed. Although the highfield and lowfield resonances are broader than their counterparts in the control complex, these peaks are distinctly narrower in line width compared to unbound PsaC (ca. 45 G vs 70 G for g_{max}).

Addition of the PsaD derived from *Nostoc* sp. PCC 8009 to the ESR sample tube at a ratio of 15 PsaC:15 PsaD:1 core followed by incubation for 1 h causes the spectrum to sharpen considerable (Figure 7C). Indeed, when this sample was frozen during illumination, the midfield resonances of F_A and F_B are clearly indistinguishable at g = 1.938 and 1.921, and the highfield resonance has a line width approaching that of F_A/F_B in the control photosystem I complex. When the sample is chemically reduced with dithionite at pH 10.0, the spectrum shows some broadening in the g_{mid} region; however, the characteristic resonances of F_A and F_B are clearly visible in contrast to the spectrum from the sample lacking the PsaD protein (data not shown). To study the effect of PsaD further, we reconstituted a photosystem I complex by incubating the PsaC protein and the PsaD protein with the photosystem I core protein at a ratio of 7 PsaC:5 PsaD:1 core in the presence of $FeCl_3$, Na_2S, and β-ME. After incubation for 18 h, the low molecular mass cofactors and the unbound PsaC and PsaD proteins were removed by repeated ultrafiltration of the sample over a YM-100 membrane. When the reconstituted photosystem I complex is frozen in darkness and illuminated at 19 K, the line widths of the F_A and F_B resonances appear very similar to those in the control photosystem I complex (Figure 8A). Under these conditions, about 80% of F_A becomes photoreduced (g = 2.045, 1.943, and 1.849), and 20% of F_B becomes photoreduced (g = 2.065, 1.912, and 1.865). When the reconstituted photosystem I complex is illuminated during freezing (Figure 8B), the spectrum becomes identical with the control photosystem I complex (compare Figure 6B). Chemical reduction with dithionite at pH 10 (Figure 8C) quantitatively reduces the F_A/F_B clusters and, except for the appearance of a resonance at g = 1.963 of unknown origin (see also Figure 8B), produces a spectrum which is also identical with the control photosystem I complex. There is no additional effect of light at 19 K, indicating the chemical reduction of F_A and F_B is complete (not shown).

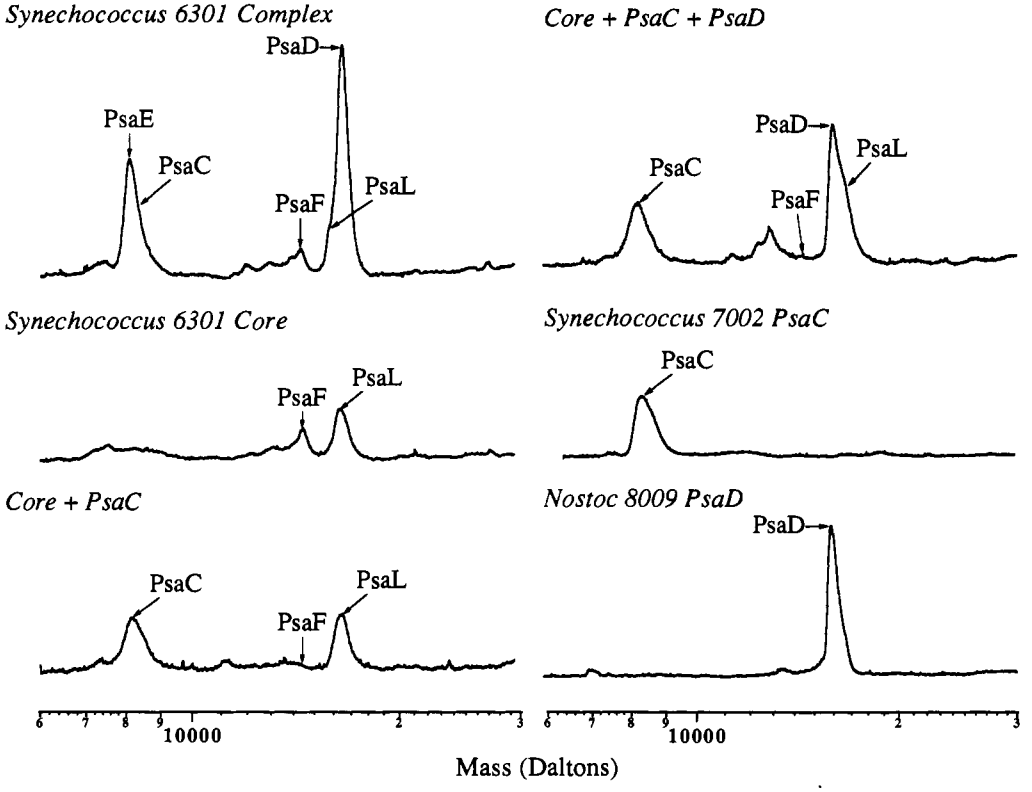

FIGURE 9: Densitometric tracings of polyacrylamide gels in the presence of sodium dodecyl sulfate. The low molecular mass polypeptides present in the *Synechococcus* sp. PCC 6301 photosystem complex (top left), the *Synechococcus* sp. PCC 6301 photosystem I core protein (middle left), and the *Synechococcus* sp. PCC 6301 photosystem I complex reconstituted with the *Synechococcus* sp. PCC 7002 PsaC protein in the presence of $FeCl_3$, Na_2S, and β-mercaptoethanol (bottom left). The latter sample was purified by ultrafiltration over a YM-100 membrane and ultracentrifugation in a sucrose gradient containing 0.1% Triton X-100. The low molecular mass polypeptides present in the photosystem I complex reconstituted with the *Synechococcus* sp. PCC 7002 PsaC protein, the *Nostoc* sp. PCC 8009 PsaD protein, and the *Synechococcus* sp. PCC 6301 photosystem I core protein in the presence of $FeCl_3$, Na_2S, and β-mercaptoethanol (top right). The sample was purified by ultrafiltration over a YM-100 membrane and ultracentrifugation in a sucrose gradient containing 0.1% Triton X-100. The 64-kDa PsaA/PsaB heterodimer and the polypeptides <6 kDa are not shown for any of the above tracings. The purified *Synechococcus* sp. PCC 7002 PsaC apoprotein (middle right) and the purified *Nostoc* sp. PCC 8009 PsaD protein (bottom right).

SDS–PAGE of the Reconstituted Photosystem I Complex. The polypeptide composition of the *Synechococcus* sp. PCC 6301 photosystem I complex, which is competent in electron transfer from P700 to F_A/F_B, has been determined (Li et al., 1991). The complex contains the PsaA and PsaB proteins (not shown), the PsaC, PsaD, PsaE, PsaF, PsaK, and PsaL proteins (Figure 9, top left), and three low molecular mass polypeptides which probably correspond to the PsaJ, "4.8-kDa", and PsaI proteins (not resolved in this gel). The photosystem I core protein, which is competent in electron transfer from P700 to F_X, is lacking the PsaC, PsaD, and PsaE proteins (Figure 9, middle left).

When the reconstitution is carried out at a ratio of 15 PsaC:1 core, the majority of the flash-induced absorption change reverts to a 1.2-ms transient characteristic of $P700^+$ F_X^- after sucrose density ultracentrifugation (Figure 3A). The polypeptide composition of this preparation is shown after SDS–PAGE in Figure 9 (bottom left). The PsaD protein is absent, and only the PsaL protein is seen at 15.8 kDa. The PsaF protein is almost completely removed, a result consistent with our earlier observation that extended exposure to Triton X-100 leads to its loss from the photosystem I core protein (Parrett et al., 1990; Li et al., 1991). However, the PsaC protein binds to the photosystem I core protein in about the amount expected in the photosystem I complex. Clearly, although the F_A/F_B clusters are no longer acting as electron acceptors, the PsaC protein (or apoprotein) still remains bound to the photosystem I core. The addition of the PsaC apoprotein to the photosystem I core protein in the absence of the iron–sulfur reconstitution agents also resulted in the rebinding of PsaC (not shown). The addition of $FeCl_3$, Na_2S, and β-ME to either sample did not result in restoration of electron flow to the F_A/F_B iron–sulfur clusters. The addition of PsaD to the photosystem I core protein in the absence of PsaC did not result in the rebinding of PsaD (data not shown).

When the reconstitution is carried out at a ratio of 7 PsaC:5 PsaD:1 core, the optical and ESR spectroscopic characteristics of the reconstituted photosystem I complex were virtually indistinguishable from the control (Figures 4 and 8). The polypeptide composition of the reconstituted photosystem I complex is shown in Figure 9 (top right). In addition to the polypeptides present in the photosystem I core protein, the PsaC protein at 8.9 kDa and the PsaD protein at 15.4 kDa have been rebound. The PsaF protein is almost completely absent, and the newly appearing polypeptide bands between 13 and 14 kDa are most likely proteolytic cleavage fragments of PsaD [see Li et al. (1991)]. Similar to the free *Synechococcus* sp. PCC 7002 apoprotein (Figure 9, middle right), the rebound PsaC protein runs as a comparatively broad band, and the area under the peak is about that expected in the photosystem I complex. It should be noted that no PsaE was added, thus explaining the difference in staining intensity of this band with the control complex. Unlike PsaD in the *Synechococcus* sp. PCC 6301 photosystem I complex (Figure 9, top left), the rebound PsaD protein migrates slightly more rapidly than the PsaL protein. However, the *Nostoc* sp. PCC 8009 PsaD protein contains 139 amino acids and has a mass of 15.4 kDa (Golbeck & Bryant, 1991; Bryant, 1991), whereas

the *Synechococcus* sp. PCC 6301 PsaD protein contains 144 amino acids and has a mass of 15.9 kDa (Wynn et al., 1989). Although the mass calculated from the SDS gels differs slightly from the true molecular mass, the difference in the two proteins can be seen clearly in Figure 9 (bottom right), in which the free *Nostoc* sp. PCC 8009 PsaD protein migrates slightly faster than the *Synechococcus* sp. PCC 6301 protein. It should also be noted that the amount of Coomassie bound to the *Nostoc* sp. PCC 8009 PsaD protein is consistently less than that bound to the analogous *Synechococcus* sp. PCC 7002 and *Synechococcus* sp. PCC 6301 proteins.

Discussion

We have shown that the purified PsaC protein, derived from the *Synechococcus* sp. PCC 7002 gene, expressed in *E. coli*, and reconstituted with iron–sulfur clusters, is capable of rebinding to the *Synechococcus* sp. PCC 6301 photosystem I core protein. The PsaC protein alone is effective in restoring low-temperature electron flow from P700 to the F_A/F_B clusters; however, the ESR resonances remain broad, and there is little discrimination between F_A or F_B as the preferred acceptor. That the PsaC peptide is most certainly bound to the PsaA/PsaB heterodimer is indicated by the increase in g anisotropy for the F_A/F_B clusters as compared to that of the free PsaC protein. This increase in rhombicity is most apparent in the significant shift observed for the g_{max} component for both clusters. On the basis of a computer simulation of Figure 3A, the spectrum of the free PsaC protein is best interpreted as a composite of two rhombic signals originating in the magnetically independent clusters F_A and F_B. Significantly, this observation suggests that the two clusters are sufficiently separated (centroid to centroid) in space to preclude the interaction that is observed in the fully reduced control complex. Such a situation would exist if the unbound polypeptide was partially unfolded, resulting in an increase in the intercluster separation beyond ca. 11 Å (Stack et al., 1989). Additionally the values of the principal g tensors for both clusters in the unbound PsaC are similar, in contrast to the situation when the polypeptide is bound to the photosystem I core. One can interpret these data as signifying that both clusters experience a common distribution of microenvironments in the free protein. Indeed, we interpret the broad spectrum of the F_A/F_B clusters in the free PsaC protein to be a superposition of g tensors resulting as a consequence of enhanced conformational flexibility in the free protein. When the PsaC peptide is rebound to the photosystem I core protein, a limited range of docking orientations may be possible, each of which may place either the F_A or the F_B cluster in proximity with the preceding electron donor (presumably F_X). That the resonances remain broad indicates that the rebound PsaC protein still exhibits considerable conformational flexibility in the vicinity of the iron–sulfur clusters. Nevertheless, the reestablishment of the differentiation in g factors between the F_A/F_B clusters as well as the partial expression of the dipolar interaction between the fully reduced clusters upon rebinding of PsaC suggests that the unbound peptide is subjected to an extensive conformational change upon binding to the photosystem I core. The reversion of the $P700^+$ $[F_A/F_B]^-$ back-reaction to the $P700^+$ F_X^- back-reaction on purification in a 0.1% Triton X-100 containing sucrose gradient indicates that the reconstituted photosystem I complex is not stable. The PsaC polypeptide, however, remains bound to the photosystem I core protein under these conditions, indicating that the instability is not due to the physical loss of the protein from the PsaA/PsaB heterodimer. Instead, the F_A/F_B iron–sulfur clusters may have been degraded, in which case the apoprotein continues to remain bound to the photosystem I core protein. Alternately, the PsaC holoprotein may be reassociated with the core in an unusual configuration that does not permit electron flow to either F_A or F_B. Additional studies will be needed to clarify this point.

When PsaD is included in the reconstitution protocol, selective reduction of the F_A rather than F_B cluster is restored during low-temperature illumination, and the ESR resonances of F_A and F_B attain g factors and narrowed line widths nearly indistinguishable from those in the control photosystem I complex. Additionally, the interpolypeptide magnetic interactions accompanying complete reduction of the clusters are reestablished in the reconstituted complex. According to this reasoning, the PsaD protein rather than the PsaA/PsaB heterodimer may be the principal conformational determinant for the PsaC protein, and the sharpened resonances observed in the control photosystem I complex are the consequence. The binding of PsaD must, in essence, orient and lock the PsaC protein into a specific conformation (or limited range of conformations), leading to the photoreduction of F_A rather than F_B. Most significantly, the reconstituted photosystem I complex remains stable to ultracentrifugation in a 0.1% Triton X-100 containing sucrose gradient. The PsaD protein, therefore, appears to be necessary for the correct orientation and stable attachment of PsaC to the *Synechococcus* sp. PCC 6301 photosystem I core protein. This property, in addition to its role as a ferredoxin "docking" protein (Zanetti & Merati, 1987; Zilber & Malkin, 1988), confers a second function to the PsaD protein.

We had shown earlier that a PsaC1 fusion protein, containing an amino-terminal extension of five amino acids (MEHSM...), could also be combined with PsaD and the photosystem I core protein of *Synechococcus* sp. PCC 6301 to reconstitute electron transport from P700 to the terminal F_A/F_B acceptors (Zhao et al., 1990). Unlike the situation with PsaC, reconstitution was found to be absolutely dependent upon the presence of PsaD. The fact that PsaC, but not PsaC1, binds loosely to the photosystem I core protein in the absence of PsaD might indicate that the N-terminus has a site of interaction with the PsaA/PsaB heterodimer. When the N-terminus is extended, as in PsaC1, this site would be altered or eliminated. Alternately, the hypothesis presumes that PsaC and PsaD have sites of interaction in addition to their binding to the PsaA/PsaB heterodimer. However, PsaD does not bind to the photosystem I core protein in the absence of the PsaC holoprotein. There is also no apparent interaction between PsaC and PsaD in the form of a binary complex, although we have only assayed such interaction by the effect of PsaD on the line width and field position of the F_A/F_B resonances in the isolated PsaC protein. Instead, we visualize a point of contact between PsaC and the photosystem I core protein that allows communication between iron–sulfur centers F_X and F_A (or F_B). According to this model, PsaD is not necessary for PsaC binding, but it stabilizes and locks the PsaC protein rigidly onto the photosystem I core. In summary, the studies with both PsaC and PsaC1 indicate that PsaD is required for stable binding of PsaC to the PsaA/PsaB heterodimer.

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