Structural and Functional Properties of the Cyanobacterial Photosystem I Complex†

R. Max Wynn, John Omaha, and Richard Malkin*

Division of Molecular Plant Biology, 309 Hilgard Hall, University of California, Berkeley, Berkeley, California 94720

Received January 6, 1989; Revised Manuscript Received March 3, 1989

ABSTRACT: Photosystem I (PSI) complexes have been isolated from two cyanobacterial strains, *Synechococcus* sp. PCC 7002 and 6301. These complexes contain six to seven low molecular mass subunits in addition to the two high molecular mass subunits previously shown to bind the primary reaction center components. Chemical cross-linking of ferredoxin to the complex identified a 17.5-kDa subunit as the ferredoxin-binding protein in the *Synechococcus* sp. PCC 6301–PSI complex. The amino acid sequence of this subunit, deduced from the DNA sequence of the gene, confirmed its identity as the *psaD* gene product. A 17-kDa subunit cross-links to the electron donor, cytochrome *c*-553, in a manner analogous to the cross-linking of plastocyanin to the higher plant PSI complex. Using antibodies raised against the spinach *psaC* gene product (a 9-kDa subunit which binds Fe–S centers A and B), we identified an analogous protein in the cyanobacterial PSI complex.

All oxygen-evolving prokaryotic and eukaryotic organisms have two photochemical reaction centers, referred to as photosystems I and II (PSI and PSII),[1] both of which occur as multisubunit integral membrane protein complexes. PSI contains a special chlorophyll, the reaction center chlorophyll, P700, which undergoes photooxidation and thereby initiates the conversion of light energy into stable chemical products. The electron released from P700 is transferred through a series of low-potential intrinsic electron acceptors: A_0, A_1, Fe–S_X, Fe–S_B, and Fe–S_A (Rutherford & Heathcote, 1985; Malkin, 1987). Oxidized P700 is reduced by a mobile electron carrier: the copper-containing protein plastocyanin serves as this donor in higher plants while a *c*-type cytochrome has a similar function in some cyanobacteria. On the electron acceptor side of the PSI reaction center, electrons are transferred from the bound acceptors to soluble ferredoxin. Thus, PSI transports electrons from reduced plastocyanin or a *c*-type cytochrome to ferredoxin in a light-dependent manner.

In addition to the intrinsic PSI electron carriers, all detergent-isolated preparations studied to date contain numerous polypeptide subunits (Bengis & Nelson, 1975; Mullet et al., 1980; Ortiz et al., 1984; Bassi & Simpson, 1985; Lundell et al., 1985; Nechushtai et al., 1987). Only a few of these polypeptides have been implicated in a functional manner that relates to the activity of the complex, and almost all of this work has been done with the PSI complex isolated from the thylakoids of higher plants. Recently, several PSI "core" polypeptides have been related to the function of PSI. The two subunits of approximately 84 kDa, products of the *psaA* and *psaB* genes, have been shown to bind P700, A_0, A_1, and Fe–S_X (Mathis et al., 1978; Hoj & Moller, 1986; Golbeck & Cornelius, 1986; Golbeck et al., 1988). The Fe–S_A and Fe–S_B clusters of PSI are bound to a single polypeptide of 9 kDa (Hoj et al., 1987; Oh-oka et al., 1987; Wynn & Malkin, 1988a). This polypeptide is chloroplast-encoded by the *psaC* gene and appears to be highly conserved (Hayashida et al., 1987). A 22-kDa polypeptide from spinach PSI cross-links to ferredoxin and has been proposed to form part of the binding site for this soluble electron carrier on the PSI complex (Zanetti & Merati, 1987; Zilber & Malkin, 1988). The gene for this protein has been referred to as *psaD*. Similarly, a 19-kDa polypeptide from spinach PSI cross-links to plastocyanin and has been proposed to form part of the binding site for plastocyanin on the donor side of the PSI complex [Wynn & Malkin, 1988b; see also Bengis and Nelson (1977)]. Both of these PSI polypeptides are nuclear-encoded, and until recently, the amino acid sequences of the respective proteins were not known. However, in the past year, sequences of at least four *psaD* gene products have been reported (Dunn et al., 1988; Hoffman et al., 1988; Lagoutte, 1988; Scheller et al., 1988). These sequences all show good homology, especially throughout the middle of the polypeptide. Only partial amino acid sequence data are available for the 19-kDa subunit from spinach (Wynn and Malkin, unpublished results). The gene for this polypeptide has recently been cloned from spinach (Steppuhn et al., 1988) and designated as *psaF*. The derived amino acid sequence gives a molecular mass of 17.3 kDa, and this sequence corresponds to a 17-kDa PSI subunit from pea (Dunn et al., 1988) and to the N-terminal sequence of the subunit in spinach PSI which cross-links to plastocyanin (Wynn and Malkin, unpublished results). Rhiel and Bryant (1988) have also named a cyanobacterial gene, *psaF*, and this gene encodes a protein of approximately 17 kDa, but the cyanobacterial protein does not have an N-terminal amino acid sequence which is homologous to any subunit from the higher plant PSI complex. In addition, Rhiel and Bryant (1988) have identified a gene for a 10-kDa subunit in the cyanobacterial complex which they have designated *psaE*, and the partial sequence of this gene does not correspond to any sequence of a known subunit or gene from higher plant PSI. In addition to these sequences, several groups (Okkels et al., 1988; Dunn et al., 1988; Munch et al., 1988) have reported derived amino acid sequences for a PSI subunit of approximately 10 kDa, and these sequences show a high degree of homology although the

† Supported in part by a grant from the National Science Foundation (DMB-8711206). J.O. was supported by the McKnight Foundation.

* To whom correspondence should be addressed.

[1] Abbreviations: PSI, photosystem I; EDC, *N*-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; TMBZ, tetramethylbenzidine; DCPIP, dichlorophenolindophenol; PCC, Pasteur culture collection.

0006-2960/89/0428-5554$01.50/0

functional role of this subunit is not known and its relationship to subunits in the cyanobacterial PSI complex is unclear.

In addition to the high molecular mass subunits (*psaA* and *psaB* gene products), cyanobacterial PSI complexes contain several small polypeptides (Nechushtai et al., 1983; Takahashi & Katoh, 1982; Lundell et al., 1985; Bullerjahn et al., 1985; Alhadeff et al., 1988), but their functions are as yet undefined. One of these is antigenically related to the higher plant *psaD* gene product (Nechushtai et al., 1983) although a functional similarity has not been shown. This work examines the functional role of several small subunits in the cyanobacterial PSI complex using chemical cross-linking techniques and immunoblotting procedures as well as comparison of relevant amino acid sequences. A 17.5-kDa subunit in cyanobacterial PSI cross-links to ferredoxin in a comparable manner to a 22-kDa subunit from spinach PSI. The complete amino acid sequence of this cyanobacterial protein is presented. We have identified a 17-kDa subunit which cross-links to cytochrome *c*-553 in a manner which is analogous to the cross-linking of plastocyanin to a 19-kDa subunit from spinach PSI. Finally, we provide conclusive evidence for the unambiguous identification of the *psaC* gene product from cyanobacteria.

Materials and Methods

Materials. EDC, TMBZ, NADP, and Triton X-100 were obtained from Sigma. Horseradish peroxidase conjugated IgG was purchased from Bio-Rad. All other chemicals were of reagent grade. Spinach proteins (plastocyanin, ferredoxin, and ferredoxin–NADP reductase) were prepared in our laboratory and were generous gifts of Richard Chain. Cytochrome *c*-553 and ferredoxin from *Synechococcus* sp. PCC 7002 were purified to homogeneity by the procedures of Ho and Krogmann (1984) and Hutson et al. (1980). Ferredoxin from the cyanobacterium *Nostoc muscorum* (strain 7119) was a generous gift of Dr. Michel Droux of our laboratory. Antibodies to the spinach PSI *psaD* gene product (22-kDa subunit) and to the spinach 19-kDa subunit were prepared in our laboratory using rabbits. Antibodies to spinach ferredoxin were prepared in collaboration with Dr. S. Mayfield (Scripps Research Clinic, La Jolla) using ferredoxin cross-linked to keyhole limpet hemocyanin. Antibodies against the *psaC* gene product (9-kDa subunit) from spinach were obtained from Professor H. Matsubara (Osaka University). Greenhouse-grown spinach was used for all preparations from this source.

Cell Material. The unicellular cyanobacteria *Synechococcus* sp. PCC 6301 and 7002 were used for the preparation of PSI, cytochrome *c*-553, and ferredoxin. Strain PCC 6301 was grown in 10-L carboys on the BG11 medium (Rippka et al., 1979) at 35 °C in warm fluorescent light at 300 $\mu E/(m^2 \cdot s)$ to a density of 10–15 g per carboy. Strain PCC 7002 was grown under similar conditions in medium A with 1 mg/mL $NaNO_3$ as described by Stevens and Porter (1980).

Purification of Cyanobacterial PSI. Synechococcus sp. PCC 6301 and 7002 cells (approximately 20 g wet weight), suspended in 50 mM sodium phosphate buffer (pH 7.0) to a volume of 100 mL, were broken at 4 °C by using a Branson sonicator at a power setting of 8 for a total of 8 min. Cycles of 1 min on and 1 min off were used. The suspension was centrifuged at 5000*g* for 5 min to remove unbroken cells, and the resulting supernatant solution was centrifuged at 200000*g* at 4 °C in a Spinco Ti60 rotor for 45 min to pellet the membrane fragments. The pelleted membranes were resuspended in 30–40 mL of a solution containing 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, and 0.1% Triton X-100. This suspension was incubated at 4 °C for 15 min and then centrifuged in the Ti60 rotor as before. The pelleted membranes were resuspended to a final volume of approximately 30 mL in 50 mM sodium phosphate buffer (pH 7.0) and 2% Triton X-100 to give a final chlorophyll concentration of 1 mg/mL. The suspension was stirred for 30 min at room temperature and centrifuged at 200000*g* at 4 °C in the Ti60 rotor for 30 min. The supernatant solution was applied to sucrose gradients prepared in Spinco SW27 swinging-bucket centrifuge tubes. The gradients were from 0.2 to 1.0 M and contained no Triton X-100. The gradients were centrifuged in the SW27 rotor at 90000*g* for 16 h at 4 °C. The green pellets were collected from each tube and washed with 50 mM sodium phosphate buffer (pH 7.0) by recentrifugation at 200000*g* in the Ti60 rotor for 30 min. The pellets were resuspended in 2–3 mL of the same buffer containing 0.5% Triton X-100, the solution was applied to a linear 5–20% sucrose gradient containing 0.1% Triton X-100 in Spinco SW41 tubes, and the gradients were centrifuged at 170000*g* for 16 h at 4 °C. The lower green band contained the PSI complex, and this material was collected and stored in aliquots at −20 °C.

Cross-Linking Ferredoxin to Synechococcus sp. PCC 6301–PSI Using EDC. PSI (approximately 20 μg of chlorophyll/mL), isolated from *Synechococcus* sp. PCC 6301, was treated in the presence and absence of 10 μM ferredoxin with 3 mM EDC in 20 mM MOPS buffer (pH 6.5) and 10 mM $MgCl_2$. The reaction mixture was incubated for 30 min at 25 °C, followed by quenching the reaction with the addition of solubilization buffer used for SDS–PAGE.

Cross-Linking Cytochrome c-553 to Synechococcus sp. PCC 7002–PSI Using EDC. PSI (approximately 20 μg of chlorophyll/mL), isolated from *Synechococcus* sp. PCC 7002, was treated in the presence and absence of 10 μM cytochrome *c*-553 as described for the ferredoxin treatment.

Polyacrylamide Gel Electrophoresis and Immunoblotting. SDS–PAGE analyses of PSI complexes and of EDC-cross-linked PSI complexes were performed at 25 °C with a slight modification of the Laemmli buffer system (Laemmli, 1970): the concentrations of Tris and glycine were increased to 0.033 and 0.25 M, respectively. A 1.5-mm slab gel with a 4% stacking and a 10–20% gradient resolving gel was used. Prior to electrophoresis, samples were solubilized with 200 mM DTT, 2% SDS, and 20 mM Tris-HCl buffer (pH 8.3) at 55 °C for 20 min. In order to achieve the highest electrophoretic resolution, it was found necessary to use the highest electrophoresis-grade reagents. Electrophoresis was conducted at a constant current of 20 mA for 6–8 h. Following electrophoresis, the gels were either stained with Coomassie Brilliant Blue, stained for heme with TMBZ/H_2O_2 (Thomas et al., 1976), or electroblotted onto nitrocellulose as described by Towbin et al. (1978) for antibody probing. For quantitation of stained gels, gels were scanned by using a Hoefer scanning densitometer.

Sequence Analysis of the psaD Gene Product in Synechococcus sp. PCC 6301. The *psaD* gene from *Synechococcus* sp. PCC 6301 was cloned by using oligonucleotide probes prepared in collaboration with Professor A. Glazer (Department of Microbiology, University of California, Berkeley). These probes were prepared on the basis of the N-terminal amino acid sequence of the 17.5-kDa PSI subunit isolated from the *Synechococcus* sp. PCC 6301–PSI complex (Alhadeff et al., 1988). Complete details of the analysis of this gene will be presented in a subsequent report.

Other Methods. Chlorophyll concentrations were measured by using an 80:20 (v/v) acetone/water solution and the extinction coefficients of Arnon (1949). P700 measurements

Table I: NADP Photoreduction Activity of a PSI Complex from *Synechococcus* sp. PCC 7002[a]

electron acceptor	electron donor	activity
Syn. 7002 ferredoxin	DCPIP	22 ± 3
Syn. 7002 ferredoxin	*Syn.* 7002 cyt *c*-553	38 ± 4
Syn. 7002 ferredoxin	*Syn.* 7002 cyt *c*-553 + DCPIP	80 ± 5
Syn. 7002 ferredoxin	spinach plastocyanin + DCPIP	28 ± 4
spinach ferredoxin	*Syn.* 7002 cyt *c*-553 + DCPIP	76 ± 4
Nostoc ferredoxin	*Syn.* 7002 cyt *c*-553 + DCPIP	82 ± 4

[a] PSI preparations from *Synechococcus* (*Syn.*) sp. PCC 7002, containing 25–50 μg of chlorophyll/mL, were incubated with 20 μg of the indicated ferredoxin, 30 μg of spinach plastocyanin, 20 μg of spinach ferredoxin–NADP reductase, 30 μg of *Synechococcus* sp. PCC 7002 cytochrome *c*-553, 5 mM ascorbate, and 0.2 mM DCPIP, where present, in 50 mM Tris-HCl buffer (pH 7.5). Initial rates of NADP reduction were measured in saturating red light. Activity is expressed as micromoles of NADP reduced per milligram of chlorophyll per hour.

were made optically at room temperature with steady-state actinic illumination in an Aminco DW-2A spectrophotometer and the extinction coefficients of Hiyama and Ke (1972) used to estimate concentrations. NADP photoreduction was measured in a Gilford spectrophotometer modified for side actinic illumination (McSwain & Arnon, 1968).

Results

Photochemical Activities of the Cyanobacterial PSI Complexes. The PSI complexes prepared from cyanobacteria as described under Materials and Methods contained approximately 130 chlorophyll molecules per P700. This value is comparable to that reported by Lundell et al. (1985) for a cyanobacterial PSI complex which retains the structural and functional organization of the in vivo PSI complex.

As shown in Table I, the PSI complex isolated from *Synechococcus* sp. PCC 7002 was active in the photoreduction of NADP when supplemented with ferredoxin and an electron donor system. Activity of the preparation was observed when cytochrome *c*-553 isolated from *Synechococcus* sp. PCC 7002 was used as the electron donor while the analogous protein from higher plants, plastocyanin, was ineffective as an electron donor. Maximum activity required the presence of DCPIP in addition to cytochrome *c*-553. Rates of NADP photoreduction for these preparations were not as high as those reported for a higher plant PSI complex (Wynn & Malkin, 1988b), but this may be related to the higher concentration of Triton X-100 used in the preparation of the cyanobacterial complexes [see also Ratajczak et al. (1988)]. In contrast to this specificity on the electron donor side of the complex, ferredoxins from various sources, including higher plants and cyanobacteria, were interchangable as electron acceptors.

Polypeptide Composition of Cyanobacterial PSI Complexes. All PSI complexes are characterized by the presence of two subunits of apparent mass of 60 kDa, as shown in Figure 1 for cyanobacterial (lanes 1 and 2) and a higher plant PSI complex (lane 3). These two bands are unresolved under the present SDS–PAGE conditions but are known to run anomalously (Vierling & Alberte, 1983; Fish & Bogorad, 1986; Bruce & Malkin, 1988). Additionally, these complexes contain a number of low molecular mass subunits, as shown in this figure. Unlike the previously reported PSI complex from cyanobacteria (Lundell et al., 1985; Alhadeff et al., 1988), in which SDS–PAGE analysis under denaturing conditions indicated the presence of only four low molecular mass subunits, the results shown in Figure 1 give better resolution below 20 kDa. At least six distinct bands can be seen in the complex isolated from *Synechococcus* sp. PCC 7002 (17.5, 17, 16, 10, 8, and 6 kDa). The preparation from *Synechococcus* sp. PCC 6301 also shows numerous low molecular mass subunits, and in this case, it appears seven subunits are present although the resolution is not as good for the lowest of these. Also shown in this figure (lane 3) is the polypeptide composition of a PSI complex isolated from higher plants (PSI-200 from spinach) to indicate the more complicated subunit composition of this preparation.

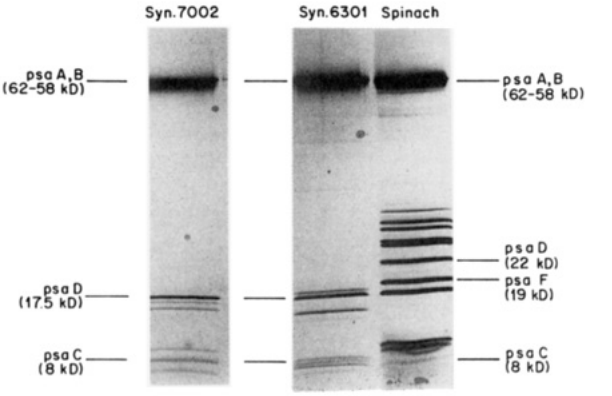

FIGURE 1: SDS–PAGE analysis of cyanobacterial and spinach PSI preparations. PSI–200 isolated from spinach membranes by the method of Mullet et al. (1980) contained 20 μg of chlorophyll per lane while the cyanobacterial PSI preparations contained 10 μg of chlorophyll per lane. Lane 1, *Synechococcus* sp. PCC 7002–PSI; lane 2, *Synechococcus* sp. PCC 6301–PSI; lane 3, spinach PSI–200. Molecular weight standards were phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme.

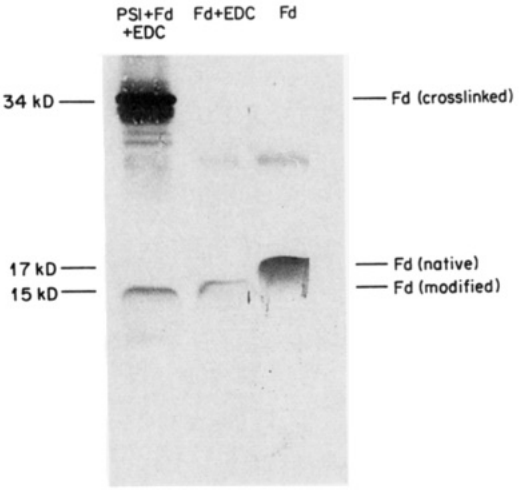

FIGURE 2: Immunoblot analysis of EDC-cross-linked PSI preparations from *Synechococcus* sp. PCC 6301 using an antibody raised against spinach ferredoxin. Chlorophyll concentrations were 20 μg of chlorophyll per lane. Lane 1, PSI + ferredoxin + EDC (3 mM); lane 2, ferredoxin + EDC (3 mM); lane 3, ferredoxin.

Chemical Cross-Linking of Ferredoxin to Synechococcus sp. PCC 6301–PSI. The results of a study in which ferredoxin has been cross-linked to the PSI complex from *Synechococcus* sp. PCC 6301 are shown in Figure 2. In this figure is an analysis of products using an immunoblotting procedure with an antibody raised against ferredoxin. Lane 1 shows that in a complete reaction mixture (PSI + ferredoxin + EDC), unreacted ferredoxin (apparent SDS–PAGE molecular mass of 15 kDa; see below) cross-reacts with the antibody and that a major product of approximately 34 kDa is present as well as two products of slightly lower molecular masses. Ferredoxin runs anomalously in this gel system, presumably due to its high negative charge, and ferredoxin which has been treated with EDC shows a change in migration, and this yields the dif-

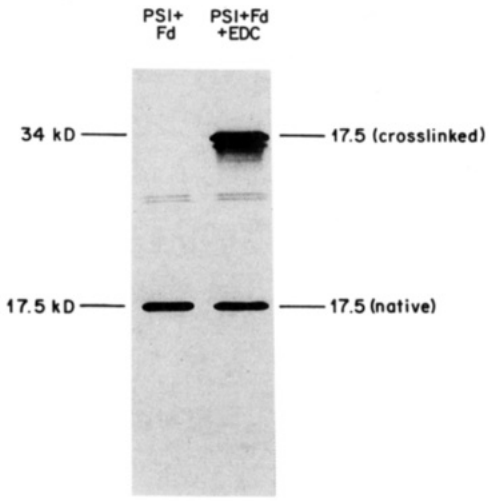

FIGURE 3: Immunoblot analysis of EDC-cross-linked PSI preparations from *Synechococcus* sp. PCC 6301 with an antibody raised against the 22-kDa subunit from spinach PSI. Chlorophyll concentrations were 20 μg of chlorophyll per lane. Lane 1, PSI + ferredoxin; lane 2, PSI + ferredoxin + EDC (3 mM).

ferences in migration pattern for this protein seen in lanes 1 and 2 versus lane 3. Densitometric scans of the lanes containing the cross-linked product indicate that approximately 40% of the cross-linked product has been obtained (data not shown). In two control samples (lanes 2 and 3), it can be observed that the high molecular mass products are only detected when all the components of the reaction mixture are present. An analogous experiment using an antibody raised against the spinach *psaD* gene product (22-kDa subunit in spinach PSI) is shown in Figure 3. As shown in lane 1, this antibody cross-reacts with a PSI subunit of 17.5 kDa in the *Synechococcus* sp. PCC 6301–PSI complex, and no higher molecular mass products are present even in the presence of ferredoxin. In the complete reaction mixture containing the PSI complex, ferredoxin, and EDC, a product of approximately 34 kDa is detected with this antibody. These results would argue for the assignment of the 34-kDa product seen in the presence of PSI, ferredoxin, and EDC as a cross-linked product between a cyanobacterial subunit of molecular mass 17.5 kDa and ferredoxin. Similar results to these have been previously obtained upon treating higher plant PSI complexes with EDC in the presence of ferredoxin and using antibodies to ferredoxin and/or the *psaD* gene product to detect cross-linked products (Zanetti & Merati, 1987; Zilber & Malkin, 1988).

Chemical Cross-Linking of Cytochrome c-553 to Synechococcus sp. PCC 7002–PSI. As previously shown in Table I, cytochrome *c*-553 is an effective electron donor to the cyanobacterial PSI complexes used in this work. The results of a chemical cross-linking study with cytochrome *c*-553 and *Synechococcus* sp. PCC 7002–PSI are shown in Figure 4. In this experiment, TMBZ heme staining has been used to detect cross-linked products containing the *c*-type heme of the cytochrome. Lane 1 shows the results with a complete reaction mixture containing the *Synechococcus* sp. PCC 7002–PSI complex, cytochrome *c*-553, and EDC. Densitometric scans of the lanes containing the cross-linked product indicate that approximately 40% of the cross-linked product is obtained (data not shown). In addition to unreacted cytochrome *c*-553 (11.5 kDa), a 29-kDa product is present which is absent in lanes 2 and 3 which are appropriate control samples. On the basis of the molecular mass of this product, we would anticipate that the 16–17.5-kDa subunits could be involved as the reaction partner with cytochrome *c*-553 to form the cross-

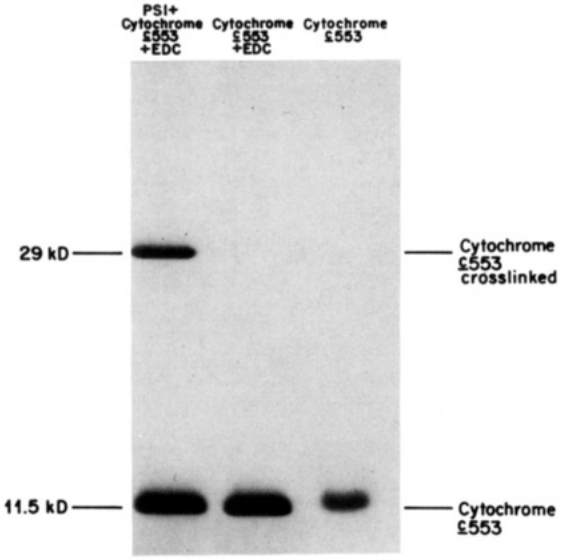

FIGURE 4: Heme-stained analysis of SDS–PAGE of EDC-cross-linked PSI preparations from *Synechococcus* sp. PCC 7002 with cytochrome *c*-553. Chlorophyll concentrations were 20 μg of chlorophyll per lane. Lane 1, PSI + cytochrome *c*-553 + EDC (3 mM); lane 2, cytochrome *c*-553 + EDC (3 mM); lane 3, cytochrome *c*-553.

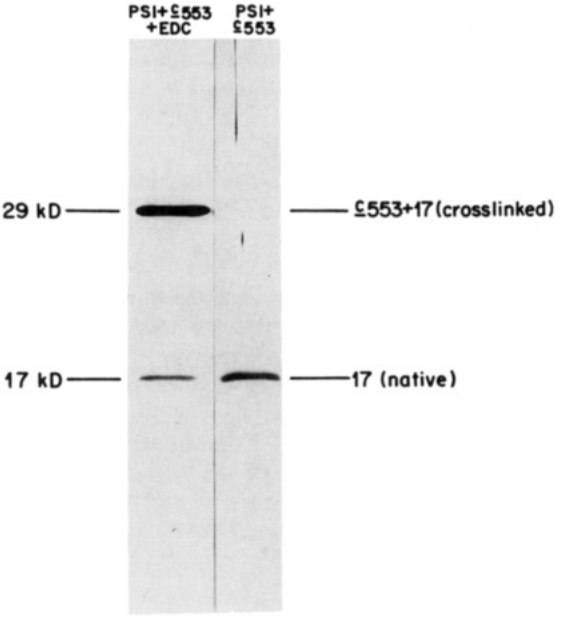

FIGURE 5: Immunoblot analysis of EDC-cross-linked PSI preparations from *Synechococcus* sp. PCC 7002 with an antibody raised against the 19-kDa subunit of spinach PSI. Lane 1, PSI + cytochrome *c*-553 + EDC (3 mM); lane 2, PSI + cytochrome *c*-553. The chlorophyll concentrations were 20 μg per lane.

linked product. When an antibody against the 22-kDa subunit from spinach (*psaD* gene product) was used as a probe in this experiment, only a single cross-reacting band at 17.5 kDa was detected even in the samples which contained the PSI complex, cytochrome *c*-553, and EDC, and this shows that the *psaD* gene product in the cyanobacterial PSI complex is not responsible for forming the cross-linked product with cytochrome *c*-553. An analogous experiment using an antibody raised against the spinach *psaF* gene product (19 kDa in the spinach PSI complex) is shown in Figure 5. As shown in lane 2, this antibody cross-reacts with a PSI subunit of approximately 17 kDa in the *Synechococcus* sp. PCC 7002–PSI complex, and no high molecular mass products are present even in the presence of cytochrome *c*-553. In the complete reaction

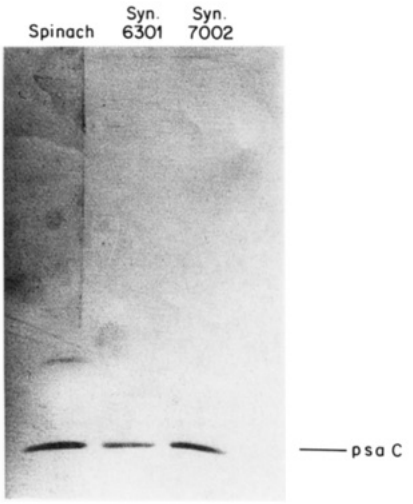

FIGURE 6: Immunoblot analysis of spinach and cyanobacterial PSI preparations with an antibody raised against the spinach 9-kDa subunit (*psaC* gene product). Approximately 20 μg of chlorophyll was present in each of the indicated lanes.

mixture containing the PSI complex, cytochrome *c*-553, and EDC (lane 1), a product of 29 kDa is detected with this antibody. These results would argue for the assignment of the 29-kDa product seen in the presence of PSI, cytochrome *c*-553, and EDC as a cross-linked product between a subunit of 17 kDa and cytochrome *c*-553. These results complement the TMBZ heme staining results which demonstrated the presence of a heme staining band of 29 kDa in the complete reaction mixture.

Identification of the Cyanobacterial psaC Gene Product. The results of Figure 6 show an immunoblot of *Synechococcus* sp. PCC 7002–PSI, *Synechococcus* sp. PCC 6301–PSI, and spinach PSI complexes probed with an antibody raised against the spinach *psaC* gene product (9-kDa subunit). The cross-reaction among these three complexes is similar, suggesting the amino acid sequences of these three proteins may be similar. Recently, the *psaC* gene was cloned from *Synechococcus* sp. PCC 7002, and the deduced amino acid sequence has been shown to be highly conserved when compared with the sequence for the spinach *psaC* gene product: only seven conservative amino acid replacements were detected for the entire polypeptide sequence (D. Bryant, personal communication).

Discussion

The present results demonstrate that the cyanobacterial PSI complex contains several low molecular mass subunits which are functionally analogous to subunits in the higher plant PSI complex. Previous comparison of the deduced amino acid sequence for the two high molecular mass subunits (*psaA* and *psaB* gene products) from *Synechococcus* sp. PCC 7002 with sequences from higher plants indicated a high degree of sequence conservation in these subunits (Cantrell & Bryant, 1987). We have now identified three additional cyanobacterial PSI subunits which are functionally related to subunits in the higher plant PSI complex.

A cross-linked product between ferredoxin and a 17.5-kDa subunit has been identified in the cyanobacterial complex after reaction with EDC. The complete amino acid sequence, derived from sequencing of the gene, is shown for this protein from *Synechococcus* sp. PCC 6301 in Figure 7 and is compared with several sequences for the 22-kDa subunit (the *psaD* gene product) recently reported for several higher plant subunits. The basic amino acids, lysine and arginine, have been represented in boldface, and these residues show a high degree

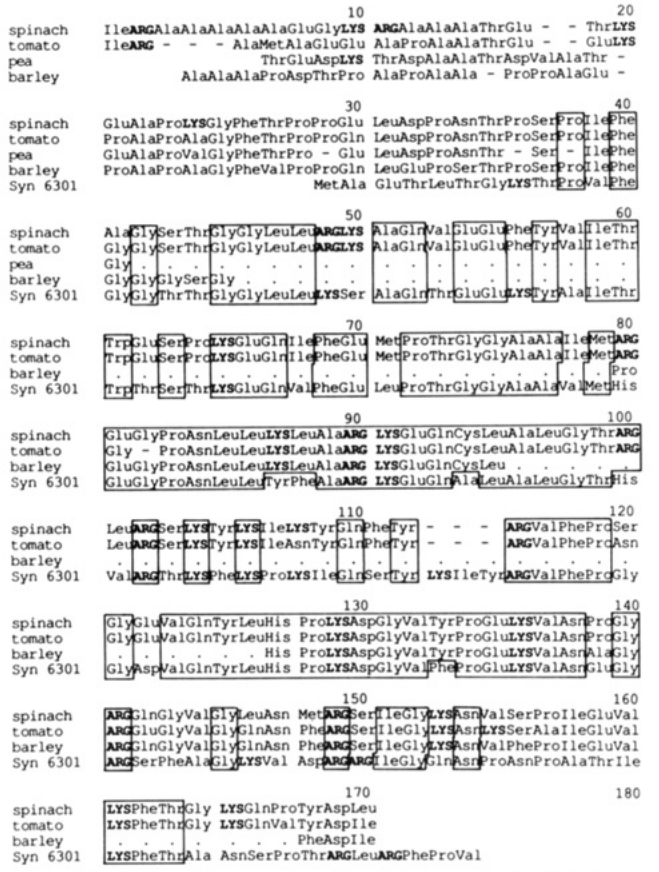

FIGURE 7: Amino acid sequences of *psaD* gene products. The boxes represent conserved sequence regions. Dots represent an unknown sequence while dashes were used for sequence alignment. The sequence of the spinach protein is from Lagoutte (1988), the tomato sequence from Hoffman et al. (1988), and the pea sequence from Dunn et al. (1988).

of conservation in all these sequences. Of the 15 lysine residues in the spinach protein, 8 are conserved in the cyanobacterial protein. The arginine residues are conserved to a lesser degree. For all the sequences shown, residues 80–160 are highly conserved, and this is the portion of the polypeptide which contains the greatest number of conserved lysine residues. A comparison of these sequences indicates that the cyanobacterial PSI subunit is 54% identical with the corresponding spinach PSI subunit although the degree of conservation is considerably higher in the middle and near the C-terminus of the subunits. On the basis of this sequence and functional homology, we would suggest the identification of the cyanobacterial 17.5-kDa PSI subunit as the *psaD* gene product. A conclusion similar to this has recently been reported by Reilly et al. (1988), who have cloned and sequenced the *psaD* gene from the cyanobacterium *Synechocystis* sp. PCC 6803 and have shown that this gene encodes a subunit of approximately 16 kDa. We would postulate that the C-terminus portion of this protein may be involved in the interaction with the acidic protein, ferredoxin, and we would further suggest that the conserved basic amino acid residues are involved in electrostatic interactions that play a role in stabilizing the interaction of PSI and ferredoxin. Presumably, one or more of these conserved basic amino acids are involved in the formation of the cross-linked product between ferredoxin and this subunit. Since ferredoxin sequences are also highly conserved (Matsubara & Hase, 1983), it is easy to understand how a ferredoxin from one organism might be capable of substituting for another ferredoxin as a PSI electron acceptor. The data from Table I certainly reflect this similarity in structure.

The results of cross-linking cytochrome *c*-553 to cyanobacterial PSI also identify a specific PSI subunit which interacts with the electron donor. On the basis of the molecular mass of the cross-linked product (29 kDa) and cytochrome *c*-553 (11.5 kDa), the data are most interpretable if the 17-kDa subunit participates in forming the cross-linked product. This appears to be the case, based on the immunological results shown in Figure 5. N-Terminal amino acid sequencing of the 17-kDa subunit of *Synechococcus* sp. PCC 7002–PSI by Rhiel and Bryant (1988) indicates the presence of 5 lysine residues within the first 40 amino acids. This sequence is not homologous to the N-terminal sequence of the spinach PSI subunit which has been shown to cross-link to plastocyanin (Wynn & Malkin, 1988b). This would suggest that the cyanobacterial PSI complex contains a functionally analogous subunit of 17 kDa which interacts with cytochrome *c*-553 through ionic bonds. Despite the lack of sequence homology at the N-terminus with its analogue in the higher plant PSI complex, an antibody raised against the spinach 19-kDa subunit recognizes the cyanobacterial 17-kDa subunit. This lack of structural conservation would agree with the results of Table I showing specificity of the cyanobacterial PSI complex with respect to electron donors. The ability of cytochrome *c*-553 to donate electrons to PSI is documented in the NADP photoreduction assays (Table I), but it is interesting that spinach plastocyanin is unable to donate electrons to the cyanobacterial PSI complex, even though this protein and the soluble *c*-type cytochrome have similar p*I* values of approximately 4.5 (Ho & Krogmann, 1980). It would be of interest to look at a cyanobacterial system which could synthesize both plastocyanin and cytochrome *c*-553 to see if various growth conditions would favor different subunit components in the PSI complex. The complete sequence of the 17-kDa subunit of the cyanobacterial complex would be of interest in relation to the higher plant *psaF* gene product sequence which has recently been reported (Steppuhn et al., 1988).

Finally, our results provide unambiguous identification of the *psaC* gene product from *Synechococcus* sp. PCC 6301 and 7002. Since the complete amino acid sequence shows only seven conservative replacements between the cyanobacterial and tobacco *psaC* gene product (D. Bryant, personal communication), the results are certainly understandable.

Similar to studies conducted with the spinach PSI complex in relation to the 22-kDa subunit (Zanetti & Merati, 1987; Zilber & Malkin, 1988) and with the 19-kDa subunit (Bengis & Nelson, 1977; Wynn & Malkin, 1988b), it stands to reason that the 17.5- and 17-kDa subunits in cyanobacterial PSI complexes are suface-exposed on opposite sides of the thylakoid membranes. Since Fe–S centers A and B bind to a single subunit of 9 kDa (Hoj et al., 1987; Oh-oka et al., 1987; Wynn & Malkin, 1988a) and these centers presumably donate electrons to ferredoxin, this subunit may be bound in the vicinity of the 17.5-kDa subunit. Further studies are necessary to define the functions of the remaining subunits of the cyanobacterial and higher plant PSI complexes.

The results presented indicate a high degree of similarity between higher plant and cyanobacterial PSI complexes. This parallel translates into a functional similarity for all PSI complexes. This analogy was first proposed at a biophysical level and subsequently at a biochemical level (Glazer, 1983). The present state of technology and the tools of molecular biology enable us to see a certain pattern of molecular architecture for these complexes. The ultimate details of the structure of these complexes will be discovered through X-ray crystallographic analyses, and it is encouraging that two groups have reported the crystallization of cyanobacterial PSI complexes (Witt et al., 1987; Ford et al., 1987, 1988).

Acknowledgments

We thank R. Chain and Dr. M. Droux for some of the purified proteins used in these studies, Professor H. Matsubara for providing an antibody raised against the spinach *psaC* gene product, and April Zilber for help with the alignment of the *psaD* gene sequences. We also thank Dr. Don Bryant for supplying some of his sequence data prior to publication.

References

Alhadeff, M., Lundell, D. J., & Glazer, A. N. (1988) *Arch. Microbiol. 150*, 482–488.
Arnon, D. I. (1949) *Plant Physiol. 14*, 1–14.
Bassi, R., & Simpson, D. (1987) *Eur. J. Biochem. 163*, 221–230.
Bengis, C., & Nelson, N. (1975) *J. Biol. Chem. 250*, 2783–2788.
Bengis, C., & Nelson, N. (1977) *J. Biol. Chem. 252*, 4564–4569.
Bruce, B. D., & Malkin, R. (1988) *J. Biol. Chem. 263*, 7302–7308.
Bullerjahn, G. S., Riethman, H. C., & Sherman, L. A. (1985) *Biochim. Biophys. Acta 810*, 148–157.
Cantrell, A., & Bryant, D. A. (1987) *Plant Mol. Biol. 9*, 453–468.
Dunn, P. P. J., Packham, L. C., Pappin, D., & Gray, J. C. (1988) *FEBS Lett. 228*, 157–161.
Fish, L. E., & Bogorad, L. (1986) *J. Biol. Chem. 261*, 8134–8139.
Ford, R. C., Picot, D., & Garavito, R. M. (1987) *EMBO J. 6*, 1581–1586.
Ford, R. C., Pauptit, R. A., & Holzenburg, A. (1988) *FEBS Lett. 238*, 385–389.
Glazer, A. N. (1983) *Annu. Rev. Biochem. 52*, 125–157.
Golbeck, J. H., & Cornelius, J. M. (1986) *Biochim. Biophys. Acta 849*, 16–24.
Golbeck, J. H., Parrett, K. G., Mehari, T., Jones, K. L., & Brand, J. J. (1988) *FEBS Lett. 228*, 268–272.
Hayashida, N., Matsubayashi, T., Shinozaki, K., Sugiura, M., Inoue, K., & Hiyama, T. (1987) *Curr. Genet. 12*, 247–250.
Hiyama, T., & Ke, B. (1972) *Biochim. Biophys. Acta 276*, 160–171.
Ho, K. K., & Krogamn, D. W. (1980) *J. Biol. Chem. 255*, 3855–3861.
Ho, K. K., & Krogman, D. W. (1984) *Biochim. Biophys. Acta 766*, 310–316.
Hoffman, N. E., Pichersky, E., Malik, V. S., Ko, K., & Cashmore, A. R. (1988) *Plant Mol. Biol. 10*, 435–455.
Hoj, P. B., & Moller, B. L. (1986) *J. Biol. Chem. 261*, 14292–14300.
Hoj, P. B., Svendsen, I., Scheller, H. V., & Moller, B. L. (1987) *J. Biol. Chem. 262*, 12676–12684.
Hutson, K. J., Rogers, L. J., Haslett, B. G., & Boulter, D. (1980) *FEMS Microbiol. Lett. 7*, 279–284.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Lagoutte, B. (1988) *FEBS Lett. 232*, 275–280.
Lundell, D. J., Glazer, A. N., Melis, A., & Malkin, R. (1985) *J. Biol. Chem. 260*, 646–654.
Malkin, R. (1987) in *Photosynthesis: The Light Reactions* (Barber, J., Ed.) pp 495–525, Elsevier, Amsterdam.
Mathis, P., Sauer, K., & Remy, R. (1978) *FEBS Lett. 88*, 275–278.
Matsubara, H., & Hase, T. (1983) in *Proteins and Nucleic Acids in Plant Systematics* (Jensen, U., & Fairbrothers,

D. E., Eds.) pp 168-181, Springer-Verlag, Berlin.
McSwain, B. D., & Arnon, D. I. (1968) *Proc. Natl. Acad. Sci. U.S.A. 61*, 989-996.
Mullet, J. E., Burke, J. J., & Arntzen, C. J. (1980) *Plant Physiol. 65*, 814-822.
Munch, S., Ljunberg, U., Steppuhn, J., Schneiderbauer, A., Nechushtai, R., Beyreuther, K., & Herrmann, R. G. (1988) *Curr. Genet. 14*, 511-518.
Nechushtai, R., Muster, P., Binder, A., Liveanu, V., & Nelson, N. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1179-1183.
Nechushtai, R., Peterson, C. C., Peter, G., & Thornber, J. P. (1987) *Eur. J. Biochem. 164*, 345-350.
Oh-oka, H., Takahashi, Y., Wada, K., Matsubara, H., Ohyama, K., & Ozeki, H. (1987) *FEBS Lett. 218*, 52-54.
Okkels, J. S., Jepsen, L. B., Honberg, L. S., Lehmbeck, J., Scheller, H. V., Brandt, P., Hoyer-Hansen, G., Stummann, B., Henningsen, K. W., von Wettstein, D., & Moller, B. L. (1988) *FEBS Lett. 237*, 108-112.
Ortiz, W., Lam, E., Ghirardi, M., & Malkin, R. (1984) *Biochim. Biophys. Acta 776*, 505-509.
Ratajczak, R., Mitchell, R., & Haehnel, W. (1988) *Biochim. Biophys. Acta 933*, 306-318.
Reilly, P., Hulmes, J. D., Pan, Y.-C. E., & Nelson, N. (1988) *J. Biol. Chem. 263*, 17658-17662.
Rhiel, E., & Bryant, D. A. (1988) in *Light Energy Transduction in Photosynthesis: Higher Plants and Bacterial Models* (Bryant, D. A., & Stevens, S., Eds.) pp 274-289, American Society of Plant Physiology, Rockville, MD.
Rippka, R., Pervelles, J., Waterbury, J. B., Herdman, M., & Stanier, R. Y. (1979) *J. Gen. Microbiol. 111*, 1-61.
Rutherford, A. W., & Heathcote, P. (1985) *Photosynthesis Res. 6*, 295-316.
Scheller, H. V., Hoj, P. B., Svensden, I., & Moller, B. L. (1988) *Biochim. Biophys. Acta 933*, 501-505.
Steppuhn, J., Hermans, J., Nechushtai, R., Ljungberg, U., Thummler, F., Lottspeich, F., & Herrmann, R. G. (1988) *FEBS Lett. 237*, 218-224.
Stevens, S. E., Jr., & Porter, R. D. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6052-6056.
Takahashi, Y., & Katoh, S. (1982) *Arch. Biochem. Biophys. 219*, 219-227.
Thomas, D. E., Ryan, D., & Levin, W. (1976) *Anal. Biochem. 75*, 168-176.
Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4749-4753.
Vierling, E., & Alberte, R. S. (1983) *Plant Physiol. 72*, 625-633.
Witt, I., Witt, H. T., Gerken, S., Saenger, W., Dekker, J. P., & Rogner, M. (1987) *FEBS Lett. 221*, 260-264.
Wynn, R. M., & Malkin, R. (1988a) *FEBS Lett. 229*, 293-297.
Wynn, R. M., & Malkin, R. (1988b) *Biochemistry 27*, 5863-5869.
Zanetti, G., & Merati, G. (1987) *Eur. J. Biochem. 169*, 143-146.
Zilber, A., & Malkin, R. (1988) *Plant Physiol. 88*, 810-814.

Manganese-Binding Proteins of the Oxygen-Evolving Complex†

R. Mei,‡ J. P. Green,‡ R. T. Sayre,§ and W. D. Frasch*,‡

The Department of Biology, University of Michigan, Ann Arbor, Michigan 48109-1048, and The Departments of Botany and Biochemistry, The Ohio State University, Columbus, Ohio 43210

Received December 6, 1988; Revised Manuscript Received March 3, 1989

ABSTRACT: The extrinsic 33-kDa protein (P_{33}) was cross-linked covalently to the binding site on P_{33}-depleted PSII preparations which is responsible for reconstitution of photosynthetic water oxidation after PSII preparations have been washed with 1 M $CaCl_2$. Conditions were found in which more than half of the cross-linked protein complexes formed in the PSII preparations retained the ability to catalyze the oxidation of water. The complex is composed of the P_{33} cross-linked to the D_1 and D_2 proteins and a 34-kDa protein, which is present in lower abundance than the other three proteins. After solubilization of the membranes with SDS and purification by preparative SDS-PAGE, the complex retains bound manganese and can catalyze the conversion of H_2O_2 to O_2. Calcium and chloride increased the catalase activity of the purified cross-linked complex while lanthanum or hydroxylamine abolished the activity. By use of the specific activity of the H_2O_2-dependent reaction to follow the extent of purification of the cross-linked complex, the most highly purified complex was determined to contain 0.34 μg of manganese/180 μg of protein. The mole ratio of Mn/protein was calculated to range from 3.6 to 4.5 depending on the assumed stoichiometry of the protein subunits. The results presented here provide direct evidence that one or more of the three proteins that have cross-linked to the P_{33} are responsible for binding the manganese of the oxygen-evolving complex.

The oxygen-evolving complex (OEC)[1] catalyzes the oxidation of water to molecular oxygen in order to supply electrons to the photosystem II (PSII) reaction center. Oxidants generated by the photoreactions of PSII drive the sequential advancement of the five intermediate states (S_0-S_4) of the OEC (Kok et al., 1970; Forbush et al., 1971). Four bound manganese are

† This work was supported by grants from the National Science Foundation (DMB-8604118) to W.D.F. and from the National Institutes of Health (GM 40703) to R.T.S.

* To whom correspondence should be addressed.
‡ University of Michigan.
§ The Ohio State University.

[1] Abbreviations: Chl, chlorophyll; LHC, light-harvesting chlorophyll proteins; BCA, bicinchoninic acid; LHCII*, the 120-kDa aggregate of LHC proteins; D_1, the herbicide-binding protein; D_2, the diffusely staining 34-kDa protein; MES, 4-morpholineethanesulfonic acid; OEC, oxygen-evolving complex; PSII, photosystem II; P_{33}, the extrinsic 33-kDa protein of photosystem II; PAGE, polyacrylamide gel electrophoresis; SADP, succinimidyl [(4-azidophenyl)dithio]propionate; SDS, sodium dodecyl sulfate; SSADP, sulfosuccinimidyl [(4-azidophenyl)dithio]-propionate.

0006-2960/89/0428-5560$01.50/0