

Isolation of Photosystem I Complexes from Octyl Glucoside/Sodium Dodecyl Sulfate Solubilized Spinach Thylakoids¹

CHARACTERIZATION AND RECONSTITUTION INTO LIPOSOMES

Received for publication December 17, 1984 and in revised form March 8, 1985

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ABSTRACT

We have used the nonionic detergent octyl- β -D-glucopyranoside in combination with sodium dodecyl sulfate to isolate two novel Photosystem I (PSI) complexes from spinach (*Spinacea oleracea* L.) thylakoid membranes. These complexes have been characterized as to their spectral properties, content of PSI reaction center chlorophyll P₇₀₀, and protein composition. PSI-B, purified from solubilized membranes by sucrose density gradient centrifugation, is a putative native PSI complex. PSI-B contains four polypeptides between 21 and 25 kilodaltons in addition to the components of the PSI antenna complex (LHCI); three of these polypeptides have not previously been associated with PSI. A second complex, CPI*, is purified from octyl glucoside/sodium dodecyl sulfate solubilized thylakoids by two cycles of preparative gel electrophoresis under mildly denaturing conditions. Electrophoresis under these conditions releases a discrete set of polypeptides from PSI producing a complex composed only of the PSI reaction center and the LHCI antenna.

In addition, the PSI reaction center complex CPI isolated from preparative gels and PSI-B were reconstituted into lecithin liposomes for structural analysis using freeze-fracture electron microscopy. The results suggest that the native PSI complex produces 12- to 13-nanometer particles, while the PSI reaction center, depleted of LHCI and peripheral proteins, produces particles with an average diameter of 10 nanometers.

The technique used to isolate PSI from higher plant thylakoids usually involves detergent solubilization of the membranes followed by separation of the photosynthetic components by either: (a) sucrose density gradient centrifugation and/or ion-exchange chromatography (1, 4, 20, 25, 28, 32, 35); or (b) electrophoresis under mildly denaturing conditions (1, 6, 7, 18, 23, 34). Based on peptide composition and Chl/P₇₀₀² ratios, it is possible to classify the different PSI preparations into several levels of structural complexity.

PSI particles produced by nonelectrophoretic methods are usually isolated in one of two forms, best represented by the PSI-

110 and PSI-65 particles of Mullet *et al.* (25). PSI-110 is isolated by sucrose density gradient centrifugation of Triton-solubilized thylakoid membranes and is presumed to represent the 'native' PSI complex. This complex is composed of: (a) the P₇₀₀-binding apoprotein of the PSI reaction center (60-70 kD); (b) five or six polypeptides between 10 and 22 kD of unknown function, but which presumably include primary and secondary electron acceptors of PSI; and (c) the PSI-specific antenna LHCI which is composed of three or four polypeptides between 21 and 24 kD. Argyroudi-Akoyunoglou (1) reports the isolation of a similar PSI complex using SDS. Reported Chl/P₇₀₀ ratios for PSI complexes with this polypeptide composition range from 100 to 200 (1, 20, 25).

Further extraction of PSI-110 with Triton (25) or with charged detergents (11) releases the LHCI antenna from the PSI complex to produce PSI-65, which contains only the reaction center apoprotein and the six low mol wt polypeptides. Similar antenna-depleted PSI complexes are produced when detergent-solubilized PSI fractions collected by sucrose gradient centrifugation are subsequently subjected to ion-exchange chromatography (4, 32, 35). The Chl/P₇₀₀ ratios are between 30 and 65.

For completeness, it should be noted that PSI particles with a Chl/P₇₀₀ ratio as low as six have been obtained by ether extraction of digitonin solubilized PSI preparations (13).

PSI can also be isolated from detergent-solubilized thylakoid membranes on mildly denaturing gels in the form of the high mol wt green bands CPI and CPIa (1, 7, 34). There is general agreement that CPI contains 30 to 60 Chl per P₇₀₀ and is composed solely of the 60- to 70-kD reaction center polypeptides (1, 16, 20, 34). The higher mol wt PSI complex CPIa, isolated by mild SDS-PAGE, was recently claimed to be equivalent to PSI-110 (1). Lagoutte *et al.* (18) also reported the isolation of a PSI complex similar to PSI-110 by deoxycholate electrophoresis of digitonin solubilized spinach thylakoids. However, Borchert *et al.* (6) have previously described the isolation of three PSI complexes on Triton/SDS gels with polypeptide compositions similar to PSI-65 and variable Chl/P₇₀₀ ratios of 60 to 115.

We report here on the characterization of PSI complexes isolated from spinach thylakoids using the nonionic detergent octyl glucoside in combination with SDS. Separation of solubilized membranes by sucrose density gradient centrifugation produces a PSI fraction, PSI-B, more complex than has been previously described. We have also isolated a high mol wt PSI complex, CPI*, by two cycles of preparative gel electrophoresis under mild conditions. This procedure results in a novel PSI complex which contains the reaction center and PSI-specific antenna LHCI, but which lacks several proteins found in 'native' PSI preparations. A model is presented which suggests that the set of PSI components preserved by a particular isolation procedure is

¹ Supported by National Institutes of Health Grant GM 22912 to L. A. S.

² Abbreviations: P₇₀₀, reaction center Chl of PSI which exhibits maximum absorbance at 700 nm; octyl glucoside; *n*-octyl- β -D-glucopyranoside; LHCI, chlorophyll *a/b*-light harvesting complex primarily associated with PSII; CPI, the P₇₀₀ Chl *a* protein; CPI^{LH}, CPI light harvesting; PF, protoplasmic face of freeze-fractured membranes (see Ref. 30).

highly dependent on the detergents and electrophoretic conditions used.

Our laboratory is also interested in the relationship of the different PSI complexes to the morphological units of thylakoid membranes as seen in freeze-fracture EM. To this end, we reconstituted CPI isolated by preparative gel electrophoresis, and the PSI sucrose gradient fraction PSI-B, into lecithin liposomes for structural analysis. The results are discussed with regard to previous reports of the role of PSI in thylakoid morphology.

MATERIALS AND METHODS

Isolation of Chl-Protein Complexes. Preparative Gel Electrophoresis. Thylakoid membranes were isolated from supermarket spinach as described by Camm and Green (8) with the addition of the protease inhibitors 5 mM ϵ -aminocaproic acid and 1 mM benzamidine-HCl to all buffers. Chl-protein complexes were isolated by preparative gel electrophoresis using a procedure modified from Camm and Green (7). Isolated thylakoid membranes were washed twice with cold 2 mM Tris-maleate, pH 7.0 (buffer I), then suspended in 0.88% (w/v) octyl glucoside/0.22% (w/v) SDS/10% (v/v) glycerol in buffer I such that the ratio of octyl glucoside/SDS/Chl was 20/5/1. The membranes were stirred on ice for 5 min and unsolubilized membranes removed by centrifugation at 30,000g for 10 min. The supernatant was loaded onto 3.0-mm-thick preparative gels (10% acrylamide) prepared according to the method of Kirchanski and Park (15) except that SDS was omitted from the gels. The gels were run at 4°C for 3 to 4 h at a constant current of 20 or 30 mamp. The pigmented bands were then cut from the preparative gels, pooled, and stored in 0.88% octyl glucoside/buffer I at -70°C.

Chl-protein complexes were isolated from the gel slices by homogenization of the bands in ice-cold 0.88% octyl glucoside/buffer I using a Virtis homogenizer. The acrylamide was removed by centrifugation, and the green supernatant concentrated using an Amicon ultrafiltration device (Amicon Corp., Lexington, MA) equipped with a PM10 filter. The concentrated sample was then reconstituted into liposomes for structural analysis (see below), or re-electrophoresed and re-isolated from 8% preparative gels to ensure purity of the complexes prior to biochemical analysis.

Sucrose Density Gradient Centrifugation. Broken chloroplasts were first isolated from spinach by grinding deveined leaves in a Waring Blendor in buffer B-1 (0.4 M NaCl, 2 mM MgCl₂, 0.2% (w/v) BSA, 20 mM Tricine-NaOH [pH 8.0]) (Table I in 9). The homogenate was filtered through four layers of cheesecloth, centrifuged at 300g to remove debris, and the chloroplasts pelleted at 4000g for 10 min. The pellet was washed once in buffer B-2 (0.15 M NaCl, 5 mM MgCl₂, 0.2% BSA, 20 mM Tricine-NaOH [pH 8.0]) and then suspended in 0.88% octyl glucoside, 0.22% SDS, 15 mM NaCl, 5 mM MgCl₂, 2 mM Tris-maleate (pH 8.0) such that the ratio of octyl glucoside/SDS/Chl was 20/5/1. The chloroplasts were stirred for 10 min on ice, then centrifuged at 40,000g for 10 min. The supernatant was loaded onto 0.1 to 0.8 M linear sucrose gradients containing 0.88% octyl glucoside, 0.1% SDS, 2 mM Tris-maleate (pH 8.0) which had been poured over a 2 M sucrose cushion. The gradients were centrifuged at 100,000g for 15 h at 4°C. One- or 2-ml fractions were collected from the gradients, assayed for Chl content, and analyzed on mildly denaturing and fully denaturing polyacrylamide gels.

Analytical Gel Electrophoresis. The polypeptide composition of isolated complexes was determined using the gel system of Laemmli (17) modified by the addition of 4 M urea to the resolving gel. The isolated complexes (50 to 400 μ g Chl/ml) were diluted 1/1 with 2 \times solubilization buffer (4% SDS, 4% 2-mercaptoethanol, 20% glycerol in 1.3 M Tris-HCl, pH 6.8) and incubated for 15 min at 50°C prior to loading onto 12.5% gels. Alternatively, pigmented gel bands were cut from preparative

nondenaturing gels, soaked in solubilization buffer for 20 min at 50°C, then placed directly into the wells of a Laemmli/urea gel. The gels were stained with Coomassie brilliant blue R250 and destained by standard procedures or stained with silver using the procedure of Merrill *et al.* (22).

Spectroscopy. Room temperature absorption spectra, Chl concentration, and P₇₀₀ concentration were determined using a Perkin-Elmer 330 spectrophotometer. Chl concentration was determined as described by Arnon (3). P₇₀₀ concentration was determined using chemical oxidation/reduction as described by Mullet *et al.* (25), using an extinction coefficient of 64 mm⁻¹ cm⁻¹ (12).

77K fluorescence spectra were obtained using a Perkin-Elmer MPF-43A Fluorescence Spectrophotometer adapted for use at liquid N₂ temperature. The spectra were determined for dilute Chl-protein complexes (<5 μ g Chl/ml) rapidly frozen onto a glass rod, or directly from green bands excised from the gels and rapidly frozen in liquid N₂.

Reconstitution and Freeze-Fracture EM. For reconstitution of Chl-protein complexes, liposomes were prepared from crude vegetable lecithin (ICN Nutritional Biochemicals) in buffer I (10 mg lipid/ml) as described by McDonnel and Staehelin (21). Liposomes were added to the concentrated Chl-protein samples (0.2 to 1.0 mg Chl/ml such that the lipid/Chl ratio was 10:1, and the samples were then dialyzed overnight against buffer I at room temperature. The dialyzed material was subjected to three cycles of rapid freezing and thawing and briefly sonicated. The proteoliposomes were glycerinated to 35%, collected by centrifugation, and frozen on copper supports in liquid N₂-cooled Freon 12. The samples were fractured and replicated with platinum/carbon in a Balzers BA 360 freeze-etch apparatus (Balzers Corp., Nashua, NH) by standard procedures. The replicas were examined in a JEOL 100C electron microscope. Particle size measurements were performed as described by Staehelin (30).

RESULTS

Isolation of PSI Complexes. Preparative Gel Electrophoresis. Figure 1 (lane 1) shows the Chl-protein complexes isolated from spinach by mildly denaturing PAGE as described in "Materials and Methods." The procedure of Camm and Green (7) was modified by the addition of 0.22% SDS to the solubilization buffer to increase the yield of solubilized complexes; the percentage of Chl extracted from the membranes increased from less than 10% with octyl glucoside alone to 75 to 90% with added SDS. Under these conditions, most of the Chl remains bound in Chl-protein complexes, with less than 30% migrating as free pigment-detergent micelles.

PSI has been correlated with the two slowest migrating green bands on mildly denaturing gels; these bands are usually referred to as CPI and CPI_a (1, 20). For reasons which will be made clear below, we have chosen a different term, CPI*, for the high mol wt PSI complex isolated on our gel system, using a modification of the terminology of McDonnel and Staehelin (21).

CPI* and CPI were isolated from 10% preparative gels, then re-electrophoresed on mildly denaturing 8% gels to further purify the complexes (Fig. 1, lanes 2 and 3). The most striking observation was the presence of a new green band associated with CPI* that was not present in CPI. This new complex comigrates with CP43 and was thus previously undetected (23). Based on spectral properties and peptide composition (see below), this band was identified as the PSI-specific antenna complex LHCI which was initially described by Mullet *et al.* (25) and recently isolated and characterized by several other laboratories (1, 11, 20, 23). In keeping with the 'CP' nomenclature for Chl-protein complexes isolated by gel electrophoresis, we propose the name CPI^{LH} for this new green band.

Re-electrophoresis of the complexes also showed that a small

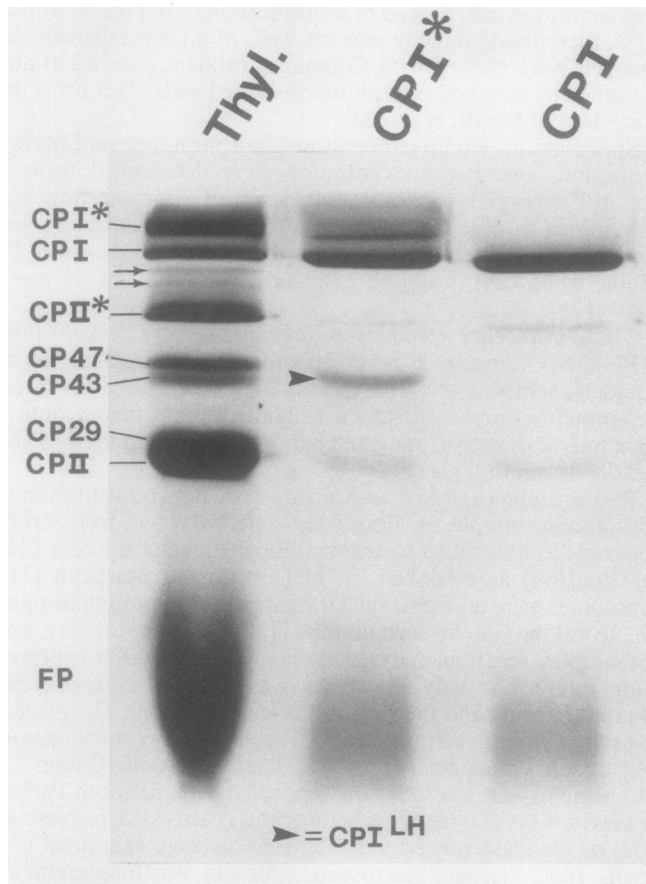


FIG. 1. Unstained, mildly denaturing polyacrylamide gel (8%) showing the Chl-protein complexes of spinach thylakoid membranes (lane 1) and re-electrophoresis of CPI* and CPI isolated from 10% gels. CP47 and CP43 represent the reaction center and primary antenna of PSII (27). CPII* and CPII are oligomeric and monomeric forms of LHCII (7, 21), and CP29 is a Chl *a/b* complex distinct from LHCII of unknown function (7). The bands marked with small arrows are probably additional oligomeric forms of LHCII often seen on heavily loaded gels. The nomenclature used here is adapted from Camm and Green (7) and McDonnell and Staehelin (21). FP = free pigment.

amount of LHCII, as CPII or CPII*, is associated with both of the PSI complexes separated by the electrophoretic method described here. However, it is unlikely that this association reflects the relationship of the complexes *in vivo*. LHCII accounts for up to 60% of the Chl in thylakoid membranes (33) and could randomly associate with the other Chl-proteins during membrane solubilization and electrophoresis.

Except where indicated, all of the following experiments involving CPI*, CPI, and CPI^{LH} were performed using complexes purified by two cycles of electrophoresis as described above, which removed virtually all contaminating CPII* or CPII material.

Sucrose Density Gradient Centrifugation. A PSI fraction was isolated from octyl glucoside/SDS solubilized thylakoids by sucrose density gradient centrifugation as described in "Materials and Methods." Two pigmented regions were clearly resolved, a dark green fraction (A) at the top and a paler green fraction (B) midway down the tube (Fig. 2A). Analysis by gel electrophoresis (Fig. 2B) showed that fraction A contained the PSII bands CP43 and CP47 plus LHCII (CPII and CPII*) and CP29; fraction B contained only the PSI components CPI*, CPI, and CPI^{LH}. Fraction B was occasionally resolved as two bands, with the

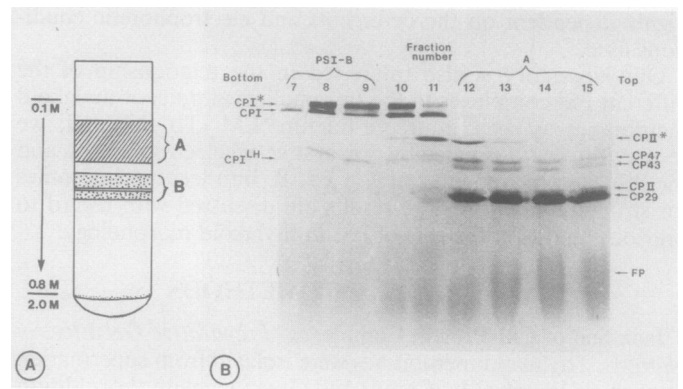


FIG. 2. A, Diagram showing the fractionation of octyl glucoside/SDS solubilized thylakoids by sucrose density gradient centrifugation. B, Analysis of the fractions on mildly denaturing gels. Two-ml aliquots were removed from the gradient and samples containing approximately 6 μ g Chl were placed directly into the wells of a 10% gel. Fraction A contains the components of PSII and LHCII; fraction B contains only the PSI components CPI*, CPI, and CPI^{LH}. Fractions 7 to 9 were pooled and designated PSI-B.

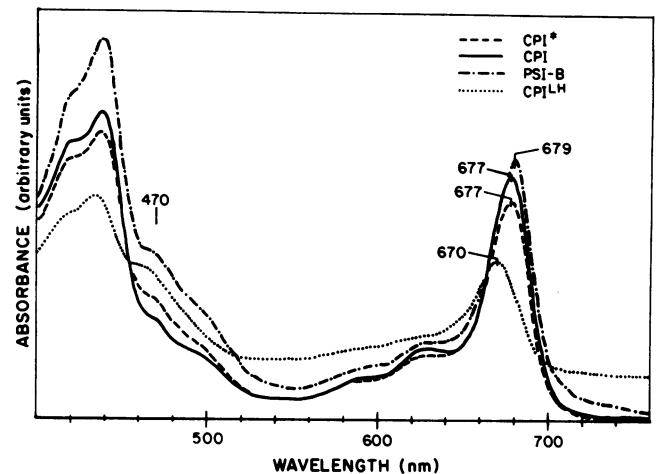


FIG. 3. Room temperature absorption spectra of isolated PSI complexes PSI-B, CPI*, CPI, and CPI^{LH} (LHCI).

upper band enriched in CPI and the lower enriched in CPI*. For the current study, these two subfractions were pooled and the combined fractions are referred to here as PSI-B.

Characterization of Isolated Complexes. The amount of P₇₀₀ present in CPI*, CPI, and PSI-B was calculated from the chemically induced difference spectra of the samples. The ratios of Chl/P₇₀₀ were found to be 72 for CPI* and 45 for CPI (the average of eight measurements on three different preparations for each sample). The Chl/P₇₀₀ ratio of PSI-B was calculated to be 76, the average of seven measurements on pooled PSI-B fractions from three separate experiments.

The room temperature absorption spectra of the isolated complexes are shown in Figure 3. PSI-B, CPI*, and CPI contain primarily Chl *a* and exhibit red absorption maxima at 677 to 679 nm characteristic of preparations enriched in P₇₀₀ (25, 28). CPI^{LH} exhibits an absorption maximum at 670 nm and a significant Chl *b* shoulder at 470 nm. The spectra of PSI-B and CPI* exhibit slight shoulders at 470 nm due to the presence of this antenna complex. The Chl *a/b* ratio for isolated CPI^{LH} was 3.5 to 4.0. The Chl *a/b* ratios could not be determined for CPI*, CPI, or PSI-B because the method used (3) is not accurate for ratios greater than six (5).

The 77K fluorescence spectra of PSI-B, CPI*, CPI, and CPI^{LH}

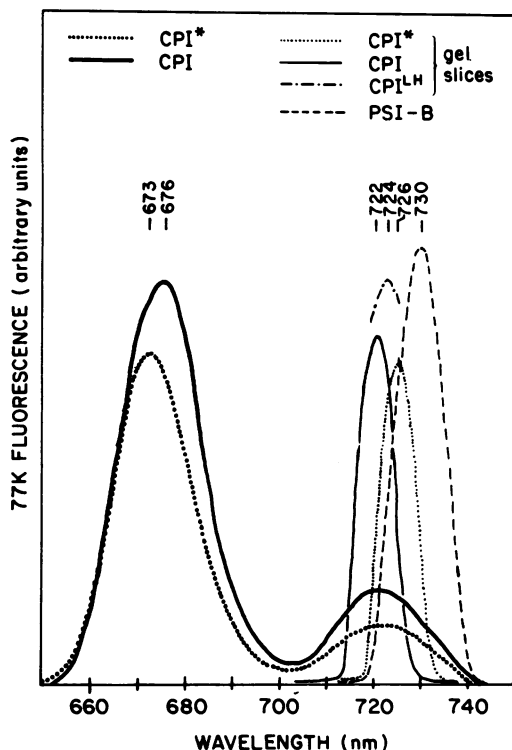


FIG. 4. 77K fluorescence emission spectra of octyl glucoside/SDS solubilized PSI complexes recorded for either dilute isolated complexes or directly from the green bands excised from polyacrylamide gels. See text for discussion.

are shown in Figure 4. PSI-B exhibits long wavelength fluorescence at 730 nm characteristic of the native PSI complex (25). In contrast, CPI* and CPI produce fluorescence emission maxima at 726 and 722 nm, respectively, when the spectra are recorded directly from gel bands frozen in liquid N₂. If the complexes are isolated from the gels, the peaks at 722 to 726 nm are reduced, and fluorescence emission peaks between 673 and 676 nm become dominant. The 676-nm peak is probably the result of detergent-induced dissociation of the Chl-protein complexes during isolation and storage (1). The fluorescence emission peak for CPI^{LH}, recorded from the gel band, was at 724 nm. LHCI is responsible for long wavelength fluorescence (736 nm) of PSI at 77K (25), and LHCI isolated from PSI-110 fluoresces at 730 nm (11). However, recent investigations of LHCI isolated by mild SDS gel electrophoresis have shown 77K fluorescence emission peaks for the complex of 729 to 730 nm (16) or variable emission peaks at 681, 691, or 717 nm (1); if the complex is eluted from the gels, the fluorescence maximum is at 681 nm (20). These variations suggest that LHCI is unstable and differences in the fluorescence maxima probably reflect various degrees of disruption of the native complex.

Figure 5 shows the polypeptide compositions of the isolated, 2X-purified complexes as determined by electrophoresis under fully denaturing conditions. The major band in CPI*, CPI, and PSI-B represents the apoprotein of the PSI reaction center which migrates between 55 to 60 kD in our gel system. This band has been shown to consist of two immunologically related polypeptides which apparently result from posttranslational modification of a single gene product (34, 35). In addition to the reaction center band, PSI-B contains four polypeptide bands between 21 and 25 kD, a doublet at 17 to 18 kD, and three low mol wt bands between 10 and 12 kD. The band at about 50 kD is a contaminant which was found in only one of three PSI-B preparations. In contrast, CPI* contains the reaction center polypeptides, peptides at 10 and 12 kD, and three polypeptides between

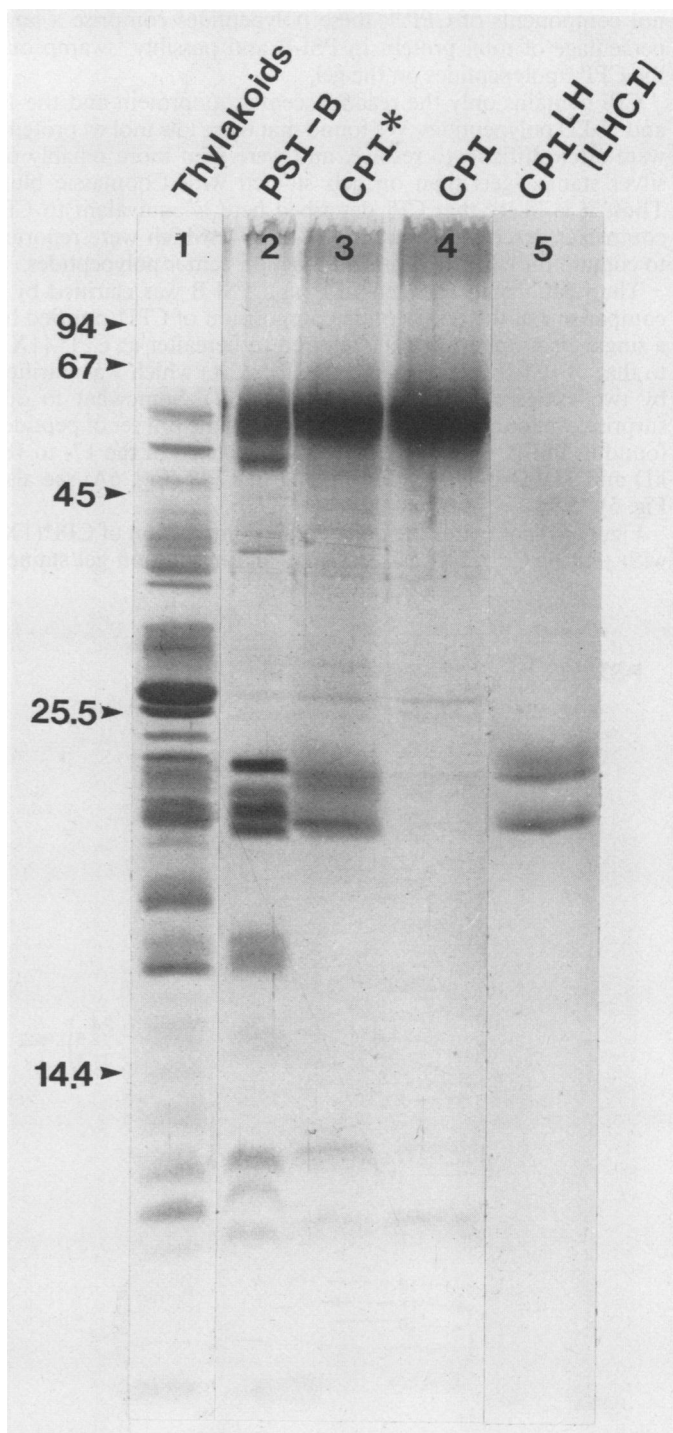


FIG. 5. Silver-stained 12.5% Laemmli/urea gel showing the polypeptide composition of isolated PSI complexes. The band migrating just below the reaction center polypeptide in PSI-B (at approximately 50 kD) is a contaminant which was found in only one of three PSI-B preparations. The small arrows indicate the position and size (in kD) of the mol wt markers. The marker proteins used were, from top to bottom, phosphorylase a, BSA, ovalbumin, chymotrypsinogen, and lysozyme.

21 and 24 which comigrate with the LHCI proteins found in CPI^{LH} (lane 5). Unexpectedly, close comparison of lanes 3 and 5 with lane 2 showed that the CPI^{LH} polypeptides do not comigrate with the PSI-B peptides in the same size range, although PSI-B clearly contains CPI^{LH} (Fig. 2b). Thus, PSI-B apparently contains four additional polypeptides between 21 and 25 kD which are

not components of CPI^{LH} ; these polypeptides comprise a large percentage of total protein in PSI-B and possibly 'swamp out' the CPI^{LH} polypeptides on the gel.

CPI contains only the reaction center apoprotein and the 10 and 12 kD polypeptides. We found that these low mol wt proteins were often difficult to resolve, and were seen more reliably on silver stained gels than on gels stained with Coomassie blue. Thus, it is likely that CPI described here is equivalent to CPI complexes described elsewhere (6, 20, 34) which were reported to contain only the high mol wt reaction center polypeptides.

The relationship between CPI^* and PSI-B was clarified by a comparison of the polypeptide composition of CPI^* purified by a single electrophoretic run (referred to hereafter as $\text{CPI}^* [1\text{X}]$) to that of PSI-B (Fig. 6A) and to $\text{CPI}^* (2\text{X})$ which was purified by two cycles of electrophoresis (Fig. 6B). Somewhat to our surprise, we found that $\text{CPI}^* (1\text{X})$ contains the full set of peptides found in PSI-B, although $\text{CPI}^* (1\text{X})$ is depleted in the 17- to 18-kD and 11-kD polypeptides missing in CPI^* (Fig. 6A; see also Fig. 5).

Figure 6B compares the polypeptide composition of $\text{CPI}^* (1\text{X})$ with that of $\text{CPI}^* (2\text{X})$ as seen on a high-resolution gel stained

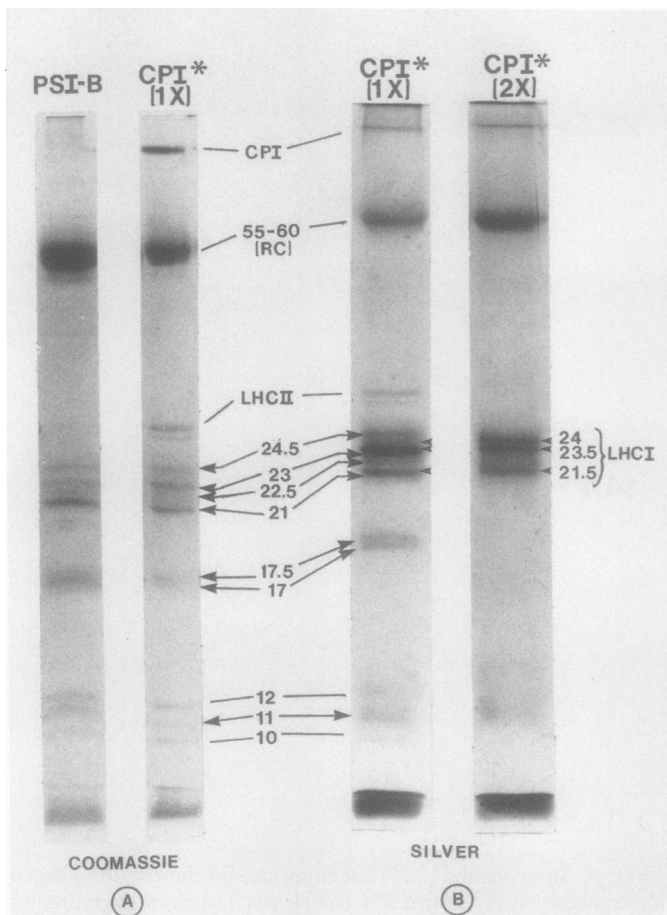


FIG. 6. Polypeptide composition of CPI^* purified by a single electrophoretic run ($\text{CPI}^* [1\text{X}]$) as compared to PSI-B and $\text{CPI}^* (2\text{X})$. A, Coomassie blue stained gel showing that $\text{CPI}^* (1\text{X})$ contains the same set of polypeptides present in PSI-B, although $\text{CPI}^* (1\text{X})$ is contaminated somewhat by LHCII (Fig. 1) and depleted in the 11 kD and 17- to 18-kD polypeptides. B, Protein composition of $\text{CPI}^* (1\text{X})$ compared to that of $\text{CPI}^* (2\text{X})$; stained with silver. The LHCI proteins are marked with small arrowheads; the bands marked with large arrows are lost from $\text{CPI}^* (1\text{X})$ during the second electrophoretic run. (On this particular gel, the 10- and 12-kD peptides were not resolved in $\text{CPI}^* (2\text{X})$; see Fig. 5 for comparison.) RC = apoprotein of the PSI reaction center.

with silver. Of particular interest is the 21- to 25-kD region containing the CPI^{LH} polypeptides. $\text{CPI}^* (1\text{X})$ contains the CPI^{LH} polypeptides (small arrowheads) plus four additional proteins between 21 and 25 kD (arrows); the two widest bands in this region are actually doublets. These four non- CPI^{LH} polypeptides are lost from CPI^* during the second electrophoretic run, along with the 17-, 17.5-, and 11-kD polypeptides (see also Figs. 5 and 6A).

Reconstitution and Freeze-Fracture EM. We have previously shown that both CPI and CPI^* complexes which have been isolated from polyacrylamide gels as described here, as well as the LHCII oligomer CPII^* , are capable of forming particles upon reconstitution into lecithin liposomes (31). We were interested in whether we could detect size differences between the native PSI preparation and the purified reaction center. To this end, CPI and PSI-B were reconstituted into lecithin liposomes and analyzed by freeze-fracture EM.

Both complexes efficiently formed particles in liposomes (Fig.

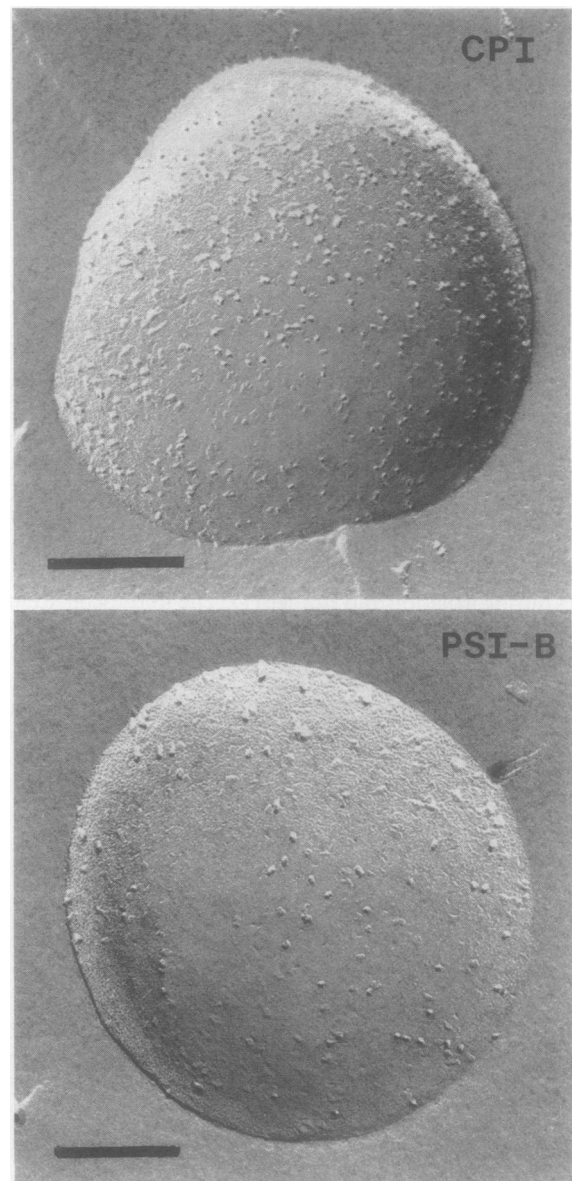


FIG. 7. Freeze-fracture electron micrographs of CPI and PSI-B reconstituted into lecithin liposomes. Bar = 0.2 μm . Magnifications: CPI = $\times 80,000$; PSI-B = $\times 90,000$.

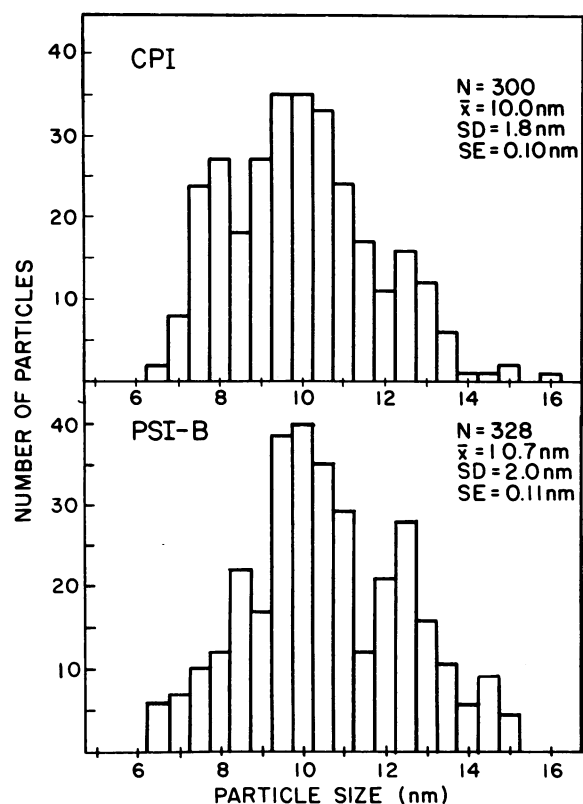


FIG. 8. Histograms showing the distribution of particles sizes produced by the reconstituted PSI complexes.

7), although PSI-B produced fewer particles than CPI when reconstituted using equivalent lipid/Chl ratios. Particle size measurements (Fig. 8) revealed that the average size for each complex was 10.0 ± 1.8 nm for CPI and 10.7 ± 2.0 nm for PSI-B. Note the increase in the number of large (12 to 13 nm) particles observed in PSI-B proteoliposomes as compared to the reconstituted CPI complexes.

DISCUSSION

We have isolated two unique PSI Chl-protein complexes from octyl glucoside/SDS solubilized spinach thylakoid membranes. The differences between PSI-B and CPI* as described here, and PSI complexes isolated by other investigators are summarized diagrammatically in Figure 9. We propose that the 'native' PSI complex is actually more structurally complicated than previously described (1, 4, 18, 25); three of the four 21- to 25-kD polypeptides found in PSI-B and missing from CPI*(2X) have not previously been reported to be components of PSI. These three polypeptides were isolated with octyl glucoside/SDS fractions purified both on sucrose gradients (PSI-B) and by mild electrophoresis [CPI*(1X)]. Thus, these proteins are probably not copurifying contaminants but rather are peripheral PSI components whose association with PSI is stabilized by octyl glucoside.

CPI*(2X), purified by two cycles of electrophoresis, is also unique in that, to our knowledge, this is the first report of a PSI complex which retains the reaction center and LHCI antenna, but which lacks the 11-, 17- to 18-, and 21-kD polypeptides found in PSI-110-type complexes. The association of the components of the PSI complex is probably the result of a complex mixture of hydrophobic and charge interactions. Thus, it is not surprising that the use of different detergents and isolation procedures would stabilize PSI preparations with varying compositions.

The interpretation of these data is further complicated by the

fact that little is known about the function of any of the polypeptides commonly isolated with the PSI reaction center complex. Bengis and Nelson (4) provided evidence that a 20-kD subunit from Swiss chard was involved in the donation of electrons from reduced plastocyanin to P_{700} . However, a recent report from Takabe *et al.* (32) suggests that plastocyanin interacts directly with P_{700} . There is also evidence which indicates that the low mol wt proteins (<12 kD) are Fe-S proteins which function as primary or secondary electron acceptors for PSI (10, 18). The work presented here, unfortunately, complicates the picture further, particularly with respect to the proteins between 21 and 25 kD. The observation that up to seven polypeptides associated with PSI can now be identified in this region on high-resolution gels indicates that care should be taken in the interpretation of analyses of PSI complexes isolated by various techniques.

The work presented here confirms recent reports by several laboratories (1, 16, 20, 23) that the PSI specific antenna LHCI can be isolated as a unique green band on polyacrylamide gels. Several recent papers dealing with the characterization of LHCI have generated a controversy in the literature concerning the number of polypeptides composing the LHCI complex. The original identification (25) and isolation (11) of the PSI antenna indicated that the complex is composed of three or four polypeptides in the range of 21 to 24 kD (the 21-kD band is sometimes seen as a doublet), and possibly a minor component of 10 kD as well. The LHCI complexes isolated from SDS gels as described by Argyroudi-Akoyunoglou (1) and Lam *et al.* (20) were composed of only a single polypeptide of about 21 kD. Our data and those of Metz *et al.* (23) show the LHCI band to contain three peptides in the 21- to 26-kD range. Recently, Lam *et al.* (19) showed that LHCI isolated by extraction of PSI-110 as described by Haworth *et al.* (11) could be further fractionated into two pigmented complexes termed LHCPIa and LHCPIb. LHCPIa is enriched in the 23- and 24-kD polypeptides and LHCPIb in the 21-kD polypeptide of LHCI; both fractions contain Chl *a* and Chl *b*. LHCPIa was found to be more unstable than LHCPIb in the presence of detergents. Thus, it appears that mild conditions (Triton/sucrose gradient centrifugation or octyl glucoside solubilization and mild electrophoretic conditions) preserve an intact PSI antenna complex with three or four polypeptide components. Harsher conditions (solubilization and electrophoresis with SDS alone) disrupt the LHCPIa component of LHCI and result in an LHCI band containing only the 20 to 21 kD protein.

The fact that LHCI is apparently composed of two unique components is interesting in light of recent studies on the organization of PSI components in the green alga *Chlamydomonas reinhardtii*. In *Chlamydomonas*, a complex similar to CPI¹⁻¹¹, (termed CPO) was identified which migrates on gels as a green band between CPIa and CPI (14, 36). By analysis of a photoautotrophic mutant, Ish-Shalom and Ohad (14) demonstrated that CPO is composed of two Chl-proteins: a peripheral antenna (one polypeptide) plus a connecting antenna (three polypeptides) necessary for efficient energy transfer from the peripheral antenna to the reaction center. The evidence presented above suggests an analogous situation in higher plants, in that the harsher isolation conditions could be preserving only a portion of the native PSI antenna complex.

PSI has been correlated with 10- to 13-nm PF freeze-fracture particles in mature thylakoid membranes *in vivo* by analysis of PSI-deficient mutants (24, 29). In contrast, immature thylakoids from plants grown in intermittent light do not contain Chl *b* or LHCI (26) and exhibit only 6- to 8-nm PF particles (2).

We interpret our results as follows. CPI, representing the mature PSI reaction center, produces particles with an average diameter of 10 nm. The 6- to 8-nm PF particles found in intermittent light thylakoids *in vivo* are probably immature reaction center particles that contain fewer internal antenna Chl.

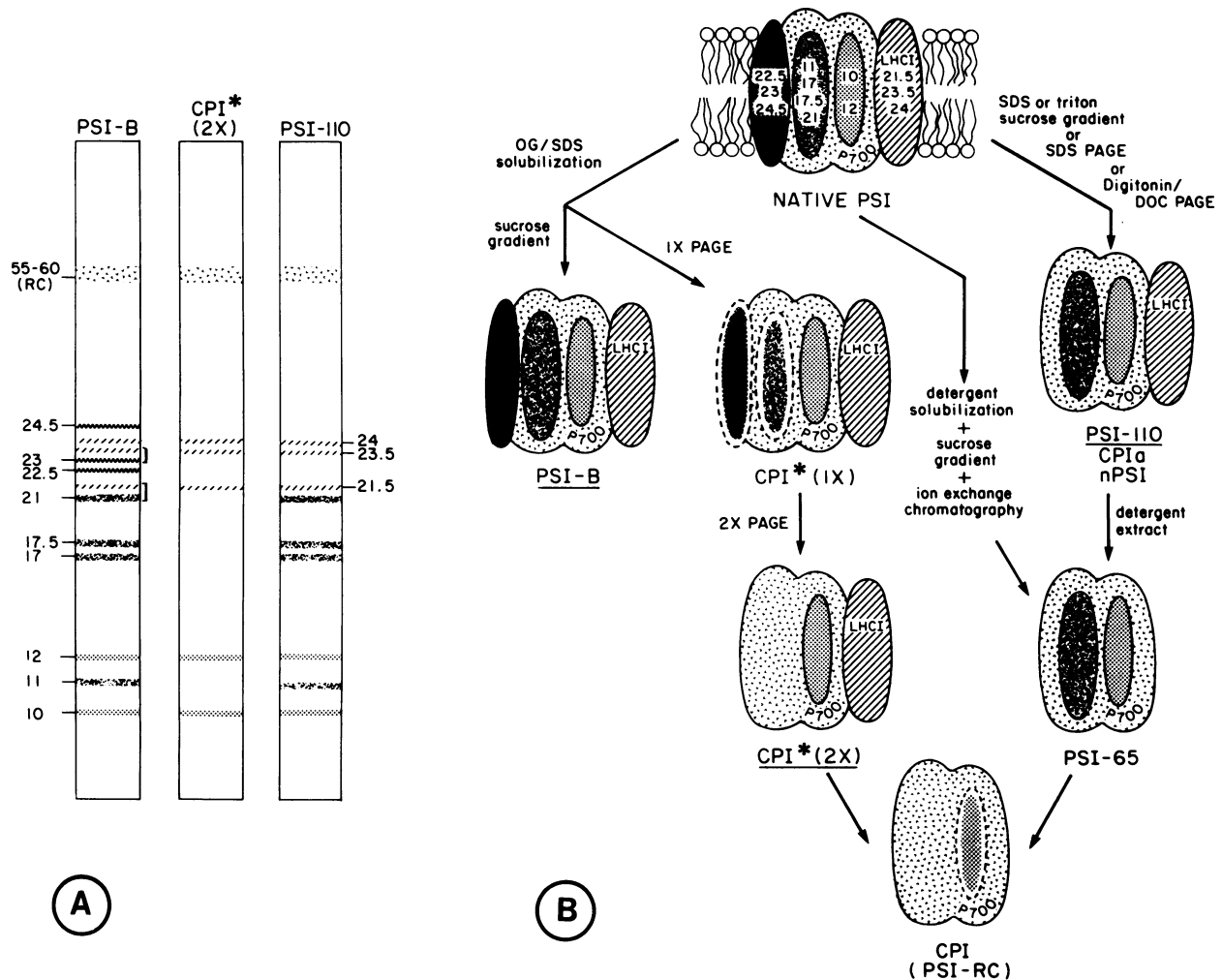


FIG. 9. A, Schematic representation of the polypeptide compositions of PSI-B and CPI*(2X) as compared to PSI-110-type complexes as determined by SDS-PAGE. The composition of PSI-110 was taken from Figure 3 of Reference 25; PSI complexes with similar polypeptide compositions have also been described by Argyroudi-Akoyunoglou (1; CPIa) and Lagoutte *et al.* (18; nPSI). The bands are grouped using different shading patterns here and in Figure 9B according to their behavior in the various isolation protocols and no attempt was made to indicate functional or topographical relationships. The only Chl-binding subunits of PSI are the 55- to 60-kD reaction center apoprotein and the polypeptides associated with LHCI. B, Diagrammatic model of the dissection of PSI using various detergents and isolation conditions. We propose that the 'native' PSI complex contains several more polypeptide components than previously indicated (25). This complete PSI complex can be preserved intact by solubilization of thylakoids in octyl glucoside and SDS followed by sucrose gradient centrifugation (PSI-B); the same complex can be isolated by one cycle of mild electrophoresis (CPI*[1X]), although it is depleted in a subset of polypeptides. A second electrophoretic run completely removes these peptides, leaving only the PSI reaction center and LHCI antenna (CPI*[2X]). In contrast, other detergents and electrophoretic conditions (1, 18, 25) preserve a complex (PSI-110) which has lost three 22- to 25-kD peptides found in PSI-B. Further detergent extraction of PSI-110 releases only LHCI to produce PSI-65 (11, 25). Complexes analogous to PSI-65 can also be isolated from detergent-solubilized thylakoids using a combination of sucrose gradient centrifugation and ion-exchange chromatography (4, 32, 35). CPI represents the PSI reaction center stripped of the LHCI antenna and peripheral proteins; it is unclear if the 10- and 12-kD polypeptides have been lost from CPI preparations described elsewhere (1, 16, 20, 34) or are merely not resolvable or poorly stainable under some electrophoretic conditions. (Note: The P_{700} -binding proteins of the reaction center are depicted here as a dimer based on the model of Bengis and Nelson (4), however the stoichiometry of the reaction center components is still controversial. Vierling and Alberte (34) recently proposed that four to six 60-kD polypeptides are associated with each P_{700} Chl). OG = octyl glucoside; DOC = deoxycholate.

The larger average size for PSI-B particles (10.7 nm as opposed to 10.0 nm for CPI) is due to an increase in 12- to 13-nm particles and a reduction in 7- to 8-nm particles as compared to reconstituted CPI (Fig. 8). We propose that these 12- to 13-nm particles represent the native PSI complex, which is in agreement with the *in vivo* studies (24, 29). Mullet *et al.* (25) showed that PSI-110 produced particles with an average diameter of 10.6 nm when reconstituted into lecithin liposomes, and this value is commonly quoted in discussions of the native PSI complex. However, this report did not include a particle size histogram; thus, it is likely that the 10.6-nm average size reported for PSI-

110, as well as the 10.7-nm average size for PSI-B reported here, actually represents a population of intact PSI complexes (12 to 13 nm), plus 10- to 11-nm PSI reaction center particles from which some LHCI or other components have become detached during the reconstitution protocol.

Acknowledgments—We wish to thank Dr. Sallie Sprague and Ms. Trish Johnston for critical reading of the manuscript and Ms. Jan Logan for her excellent artwork.

LITERATURE CITED

1. ARGYROUDI-AKOYUNOGLU J 1984 The 77K fluorescence spectrum of the Photosystem I pigment-protein complex CPIa. FEBS Lett 171: 47-53

2. ARMOND PA, LA STAEHELIN, CJ ARNTZEN 1977 Spatial relationship of Photosystem I, Photosystem II and the light-harvesting complex in chloroplast membranes. *J Cell Biol* 73: 400-418
3. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* 24: 1-15
4. BENGIS C, N NELSON 1977 Subunit structure of chloroplast Photosystem I reaction center. *J Biol Chem* 252: 4564-4569
5. BOARDMAN NK, SW THORNE 1971 Sensitive fluorescence method for the determination of chlorophyll a/chlorophyll b ratios. *Biochim Biophys Acta* 253: 221-231
6. BORCHERT MT, FWJM SPIJKERBOER, JSC WESSELS 1981 Different photochemical properties and polypeptide composition of electrophoretically prepared P₇₀₀-chlorophyll a-protein complexes from spinach thylakoids. In G Akoynoglou, ed. *Photosynthesis III: Structure and Molecular Organization of the Photosynthetic Apparatus*. Balaban International Science Services, Philadelphia, 595-605
7. CAMM EL, BR GREEN 1980 Fractionation of thylakoid membranes with the nonionic detergent octyl- β -D-glucopyranoside. Resolution of chlorophyll-protein complex II into two chlorophyll-protein complexes. *Plant Physiol* 66: 428-432
8. CAMM EL, BR GREEN 1982 The effects of cations and trypsin on the extraction of chlorophyll-protein complexes by octyl glucoside. *Arch Biochem Biophys* 214: 563-572
9. DUNAHAY TG, LA STAEHELIN, M SEIBERT, PD OGLIVIE, S BERG 1984 Structural, biochemical, and biophysical characterization of four oxygen-evolving Photosystem II preparations from spinach. *Biochim Biophys Acta* 764: 179-193
10. GUIKEMA J, L SHERMAN 1982 Protein composition and architecture of the photosynthetic membranes from the cyanobacterium *Anacystis nidulans* R2. *Biochim Biophys Acta* 681: 440-450
11. HAWORTH P, JL WATSON, CJ ARNTZEN 1983 The detection, isolation, and characterization of a light-harvesting complex which is specifically associated with Photosystem I. *Biochim Biophys Acta* 724: 151-158
12. HIYAMA T, B KE 1972 Difference spectra and extinction coefficients of P₇₀₀. *Biochim Biophys Acta* 267: 160-171
13. IKEGAMI I, S KATOH 1975 Enrichment of Photosystem I reaction center chlorophyll from spinach chloroplasts. *Biochim Biophys Acta* 376: 588-592
14. ISH-SHALOM D, I OHAD 1983 Organization of chlorophyll-protein complexes of Photosystem I in *Chlamydomonas reinhardtii*. *Biochim Biophys Acta* 722: 498-507
15. KIRCHANSKI SJ, RB PARK 1976 Comparative studies of the thylakoid proteins of mesophyll and bundle sheath plastids of *Zea mays*. *Plant Physiol* 58: 345-349
16. KUANG TY, JH ARGYROUDI-AKOYUNOGLU, HY NAKATANI, J WATSON, CJ ARNTZEN 1984 The origin of the long wavelength fluorescence emission band (77K) from Photosystem I. *Plant Physiol* 75: S-114
17. LAEMMLI UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685
18. LAGOUTTE B, P SETIF, J DURANTON 1984 Tentative identification of the apoproteins of iron-sulfur centers of Photosystem I. *FEBS Lett* 174: 24-29
19. LAM E, W ORTIZ, R MALKIN 1984 Chlorophyll a/b proteins of Photosystem I. *FEBS Lett* 168: 10-14
20. LAM E, W ORTIZ, S MAYFIELD, R MALKIN 1984 Isolation and characterization of a light-harvesting chlorophyll a/b protein complex associated with Photosystem I. *Plant Physiol* 74: 650-655
21. MCDONNELL A, LA STAEHELIN 1980 Adhesion between liposomes mediated by the chlorophyll a/b-light harvesting complex isolated from chloroplast membranes. *J Cell Biol* 84: 40-56
22. MERRILL CR, D GOLDMAN, SA SEDMAN, MH EBERT 1981 Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science* 211: 1437-1438
23. METZ JG, RW KRUEGER, D MILES 1984 Chlorophyll-protein complexes of a Photosystem II mutant of maize. Evidence that chlorophyll-protein a-2 and a chlorophyll-protein complex derived from a Photosystem I antenna system comigrate on polyacrylamide gels. *Plant Physiol* 75: 238-241
24. MILLER KR 1980 A chloroplast membrane lacking Photosystem I: changes in unstacked membrane regions. *Biochim Biophys Acta* 592: 143-152
25. MULLET JE, JJ BURKE, CJ ARNTZEN 1980 Chlorophyll-proteins of Photosystem I. *Plant Physiol* 65: 814-822
26. MULLET JE, JJ BURKE, CJ ARNTZEN 1980 A developmental study of Photosystem I peripheral chlorophyll proteins. *Plant Physiol* 65: 823-827
27. NAKATANI HY, B KE, E DOLAN, CJ ARNTZEN 1984 Identity of the Photosystem II reaction center polypeptide. *Biochim Biophys Acta* 765: 347-352
28. SHIOZAWA JA, RS ALBERTE, JP THORNER 1974 The P-700-chlorophyll a-protein. Isolation and some characteristics of the complex in higher plants. *Arch Biochem Biophys* 165: 388-397
29. SIMPSON D 1983 Freeze-fracture studies on barley plastid mutant membranes. VI. Location of the P₇₀₀-chlorophyll a-protein I. *Eur J Cell Biol* 31: 305-314
30. STAEHELIN LA 1976 Reversible particle movements associated with unstacking and restacking of chloroplast membranes *in vitro*. *J Cell Biol* 71: 136-158
31. STAEHELIN LA, SG SPRAGUE, TG DUNAHAY 1984 New methods for making chloroplast lipid liposomes and for reconstituting chlorophyll-protein complexes isolated from SDS polyacrylamide gels. *Photosynth Res* 5: 293-296
32. TAKABE T, H ISHIKAWA, S NIWA, S ITOH 1983 Electron transfer between plastocyanin and P₇₀₀ in highly purified Photosystem I reaction center complex. Effects of pH, cations, and subunit peptide composition. *J Biochem* 94: 1901-1911
33. THORNER JP 1975 Chlorophyll-proteins: light harvesting and reaction center components of plants. *Annu Rev Plant Physiol* 26: 127-158
34. VIERLING E, RS ALBERTE 1983 P₇₀₀ chlorophyll a-protein. Purification, characterization, and antibody preparation. *Plant Physiol* 72: 625-633
35. WESTHOFF P, J ALT, N NELSON, W BOTTOMLY, H BÜNEMANN, RG HERRMANN 1983 Genes and transcripts for the P₇₀₀ chlorophyll a apoprotein and subunit 2 of the Photosystem I reaction center complex from spinach thylakoid membranes. *Plant Mol Biol* 2: 95-107
36. WOLLMAN F-A, P BENNOUN 1982 A new chlorophyll-protein complex related to Photosystem I in *Chlamydomonas reinhardtii*. *Biochim Biophys Acta* 680: 352-360