Subunit Stoichiometry of the Chloroplast Photosystem I Complex*

Barry D. Bruce† and Richard Malkin

From the Division of Molecular Plant Biology, University of California, Berkeley, California 94720

A native photosystem I (PS I) complex and a PS I core complex depleted of antenna subunits has been isolated from the uniformly $^{14}$C-labeled aquatic higher plant, Lemna. These complexes have been analyzed for their subunit stoichiometry by quantitative sodium dodecyl sulfate-polyacrylamide gel electrophoresis methods. The results for both preparations indicate that one copy of each high molecular mass subunit is present per PS I complex and that a single copy of most low molecular mass subunits is also present. These results suggest that iron-sulfur center X, an early PS I electron acceptor proposed to bind to the high molecular mass subunits, contains a single [4Fe-4S] cluster which is bound to a dimeric structure of high molecular mass subunits, each providing 2 cysteine residues to coordinate this cluster.

In oxygenic photosynthesis, two photochemical systems cooperate in the transfer of electrons from water to NADP. One of these, PS I, catalyzes a light-dependent electron transfer from reduced plastocyanin to ferredoxin (1). PS I is an integral membrane protein complex which contains P$_{700}$ as its reaction center chlorophyll. During photooxidation, the electron released from P$_{700}$ is transferred through a series of low potential bound electron acceptors, denoted A$_{0}$, A$_{1}$, Fe$^{-}$S$_{X}$, Fe$^{-}$S$_{A}$, and Fe$^{-}$S$_{B}$ (1, 2). The latter three acceptors contain bound Fe-S centers which have been identified on the basis of low-temperature EPR spectra (3–5). Recent results indicate vitamin K$_{1}$ may function as A$_{0}$ (6–10) and a monomeric form of chlorophyll a as A$_{1}$ (11, 12).

While a great deal of spectroscopic characterization of the PS I primary reactants is now available (reviewed in Ref. 2), structural and functional aspects of the PS I complex have received less attention, due, in part, to the unavailability of well-resolved PS I complexes free from contaminating electron carriers and polypeptide subunits from other chloroplast membrane complexes. The isolation and characterization of a "native" PS I complex which retains the structural and functional organizations of PS I in vivo (13, 14) has led to renewed interest in the nature of the polypeptide subunits in this complex and the role of PS I subunits in specific functions. The native PS I complex, isolated from chloroplast membranes with low concentrations of Triton X-100, has a chlorophyll/P$_{700}$ ratio of approximately 200 and contains at least 10 polypeptide subunits (14, 15).

Two chlorophyll-protein complexes can be resolved from the native PS I complex: CP1 and LHCP I (15). CP1 contains two high molecular mass subunits (approximately 62 and 58 kDa) by SDS-PAGE analysis (16, 17) which are known to bind P$_{700}$ (18), and recent results suggest several early electron acceptors are also present in this complex (5, 12). LHCP I consists of several subunits in the 22–25-kDa molecular mass range which bind chlorophyll a and b and serve as an antenna for the reaction center (19–21). The function of the remaining low molecular mass PS I subunits (less than 22 kDa) remains to be defined.

In addition to these recent results relating to the isolation of resolved PS I complexes, another major advance concerning the structure of PS I is the isolation of several PS I genes and the report of the derived amino acid sequences of these protein subunits. Thus, sequences for the two high molecular mass subunits, identified as products of the psaA and psaB genes, are known for several sources (22–29). Additionally, a low molecular mass subunit of PS I has tentatively been identified as the frxA or psaC gene product (30–32) and the sequence of this protein suggests it may be an Fe-S apoprotein, probably involved in binding Fe$^{-}$S$_{X}$ and Fe$^{-}$S$_{A}$.

With the recent proposal that one Fe-S center (Fe$^{-}$S$_{X}$) may be bound to the two high molecular mass subunits of CP1 (5, 33), attention has focused on the subunit stoichiometry of the PS I complex since this stoichiometry relates directly to models for the organization of the high molecular mass subunits in binding a putative Fe-S cluster and as well to the functional organization of chlorophyll among PS I subunits. The first reported stoichiometry values for a PS I complex were for a Swiss chard preparation (34), based on densitometric scans of stained SDS-polyacrylamide gels. Approximately two high molecular mass subunits/PS I complex were found and this value was confirmed for an algal preparation from Chlamydomonas utilizing a uniformly $^{14}$C-labeled complex (35). However, an analysis of a resolved PS I complex from the cyanobacterium, Synechococcus, using the $^{14}$C labeling procedure, reported a tetrameric structure with four high molecular mass subunits/PS I complex (36). Because of the importance of the PS I subunit stoichiometry in any detailed description of the complex, we have investigated this problem utilizing an aquatic higher plant, Lemna, which can be conveniently radiolabeled with $^{14}$C to obtain a uniformly labeled PS I complex suitable for accurate determination of subunit stoichiometry. Our results indicate the higher plant native PS I complex and an antenna-depleted PS I complex contain a single copy of each high molecular mass subunit and one copy of most of the low molecular mass subunits. These results are considered in terms of current models for pigment and prosthetic group binding in PS I.

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‡ The abbreviations used are: PS I; photosystem I; CP1, chlorophyll protein complex I; LHCP I, light harvesting chlorophyll protein I; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EGTA, [ethylenbis(oxyethylenenitrito)]tetraacetic acid; LHCP II, light harvesting chlorophyll protein II; Tricine, N-[2-hydroxy-1,1-bia(hydroxymethyl)ethyl]glycine.
EXPERIMENTAL PROCEDURES

Growth of Lemna gibba L. G-3

Lemna fronds were grown phototrophically under aerobic conditions on 0.5% glucose in E medium (37) at 25 °C using heat-filtered incandescent lights. Fernbach flasks containing 1900 ml of medium were inoculated with 1–10 individual plants and allowed to reach a density of two to three plants deep over a period of 4–5 weeks. In vivo 14C labeling was done in a similar fashion with the addition of 1–2 μCi of uniformly labeled glucose with a specific activity of 160–250 μCi/mm (ICN).

Preparation of a Native PS I Complex (PS I-100)

The fronds were harvested by filtration and washed with distilled water. All the following procedures were carried out at 4 °C except where noted. Washed Lemna fronds were homogenized in a Waring blender three times for 30 s in ice-cold STNM (0.3 M sucrose, 0.05 M Tris-HCl (pH 7.8), 0.01 M NaCl, 0.005 M MgCl2). Prior to blending, the protease inhibitors aminocaproic acid (Sigma), p-amino benzamide (Sigma), and phenylmethylsulfonyl fluoride (Sigma) were added to the blending solution to a final concentration of 2 mM. Fresh phenylmethylsulfonyl fluoride (2 mM) was included in all solutions through four layers of nylon mesh and the chloroplasts were pelleted from this filtrate by centrifugation at 12,000 × g for 10 min. The chloroplasts were lysed by resuspension in 150 mM NaCl for 10 min and resuspended in STNM containing 2 M NaBr for 30 min, diluted with a 1:1 volume of 0.5% distilled water and centrifuged at 12,000 × g for 10 min. The NaBr wash step was repeated once. The pelleted membranes were resuspended in STNM and centrifuged at 12,000 × g for 10 min to remove residual NaBr. The washed membrane pellet was resuspended in STNM to a chlorophyll concentration of 3 mg/ml. An equal volume of STNM containing 10% ammonium sulfate, 60 mM octyl glucoside (Sigma), and 1% sodium cholate was added to the membrane suspension and the suspension was allowed to incubate for 30 min on ice. The solubilized membranes were then centrifuged at 250,000 × g for 1 h in a Spincio Ti 60 rotor. The supernatant contained the cytochrome b-f complex but very little chlorophyll. The chlorophyll-containing pellet was resuspended in distilled water to a chlorophyll concentration of 0.8–1.0 mg/ml. Triton X-100 (Sigma) from a 20% (w/v) stock solution was added to a final concentration of 0.1% Triton X-100 and the suspension was allowed to incubate at 25 °C for 1 h with gentle rocking. After the incubation, the unsolubilized membranes and starch granules were removed by centrifugation at 48,000 × g for 20 min. The supernatant, which contained the solubilized membranes, was loaded onto a 1–0.1 M sucrose gradient containing 0.2% Triton X-100 with a 2 M sucrose cushion and centrifuged at 90,000 × g for 2 h in a Spincio SW-28 rotor at 4 °C. The narrow dark green band at the cushion interface contained the native PS I complex. The preparation was stored at -20 °C prior to use.

Preparation of PS I Core Complex (PS I-100)

PS I prepared as described above, was further fractionated into its core components (PS I-100) and the antenna LHCP I. The PS I-200 from above was diluted with 5 volumes of 20 mM Tris-HCl buffer (pH 7.6) and centrifuged at 250,000 × g for 1 h. This pellet was resuspended in water to a chlorophyll concentration of 0.5 mg/ml. To the suspension solid Zwittergent 16 (Behring Diagnostics) and dodecyl-β-D-maltoside (Behring Diagnostics) were added to a final concentration of 0.2 and 0.15%, respectively. This suspension was gently stirred on ice for 1 h. The suspension was then loaded onto a 1–0.1 M sucrose density gradient in 20 mM Tricine-NaOH buffer (pH 7.8) containing 0.1% Triton X-100 and centrifuged at 90,000 × g for 4 h. The lower green band containing the PS I complex and the top band contained LHCP I and some LHCP II.

Polyacrylamide Gel Electrophoresis and Immunoblotting

Analytical SDS-PAGE of PS I complexes were done at 23 °C with a slightly modified Laemmli buffer system (38) using a 1.5-mm slab gel with a 4% stacking gel and a 13–21% gradient resolving gel. The two high molecular mass subunits of the PS I complex were separated using a 6% slab gel. Prior to electrophoresis, samples were solubilized with 100 μl of 1% SDS, 10 mM Tris-HCl buffer (pH 8.3) at 25 °C for 3 h or 50 °C for 15 min. Electrophoresis was carried out at 25 °C with a constant current of 15 mA for 12–14 h. After electrophoresis, the gel was fixed and stained with Coomassie Brilliant Blue.

Western blotting was carried out as described in the Bio-Rad Alkaline Phosphatase instruction manual with the following modifications: 0.1-μm nitrocellulose paper (Schleicher and Schuell) was used, 0.5% SDS was included in the transfer buffer, Carnation non-fat dry milk (1%) was used as a blocking reagent, and 3H-protein A (ICN) and autoradiography were used as the visualization process. Antibodies to the PS I subunit, LHCP Ib (~20 kDa), were obtained as previously described (19). Antibodies to the 21-kDa subunit were obtained from Dr. R. Nechustai (University of California, Los Angeles).

Quantitation of 14C-Labeled Proteins in Polyacrylamide Gels

Due to the lack of an accepted procedure to accurately quantitate the amount of 14C contained in polypeptides resolved by SDS-PAGE, three different procedures were used to determine the amount of 14C contained in each PS I subunit.

Autoradiography of Stained and Dried Slab Gels—The labeled in vivo complex was resolved into individual subunits by the SDS-PAGE procedure described above. A range of at least an order of magnitude in concentration of chlorophyll was electrophoresed so that the linearity of the autoradiography could be tested. The gel was rinsed in distilled water and stained in 0.125% Coomassie Brilliant Blue in 40% methanol and 10% acetic acid. The gel was destained first in 40% methanol and 10% acetic acid. The gel was then dried onto Whatman 3MM filter paper using a Hoeffer gel dryer. The dried gel was covered with Saran Wrap and exposed to Amersham Hyperfilm-βmax, without an intensifying screen for 5–10 days. After development, the autoradiogram was scanned using a scanning densitometer. The resulting densitometric traces were then digitized on a digitization pad connected to a Hewlett-Packard 85B computer. The peak areas were determined using a program which allowed use of an operator defined base line. Only sample lanes which were shown to be within the linear range of the film were used for analysis.

Scintillation Counting of Gel Slices—Destained gels were washed twice with distilled water and cut into individual lanes with a razor blade. The gel strips were cut into 130–190 1-mm slices using a Hoeffer gel slicer. Each slice was put into a gas tight scintillation vial containing 0.2 ml of ice-cold 70% perchloric acid and 0.4 ml of ice-cold 30% H2O2 and quickly sealed. These vials were then incubated at 65–70 °C overnight in the dark with gentle rocking. Before opening, the vials were chilled to 4 °C for several hours in the dark. Amersham PCS scintillation fluid (6 ml) was quickly added to each tube followed by several inversions to assure complete mixing. The tubes were then placed in a Beckman LS-1801 scintillation counter and allowed to come to thermal equilibrium in the dark. To exclude random counts originating from internal chemiluminescence, window channels were used which allowed detection of only authentic 14C isotope decay, channel 200–700. Care was also taken to check that non-protein contamination of gel slices was at a base-line level throughout the gel. Counting was performed until each sample reached a 2 S level of 5%. No background subtraction was performed. The counts in counts/min were plotted versus slice number. This plot was then digitized and peak areas were determined as described above.

Scintillation Counting of Single Coomassie Blue Bands—After de-staining and washing, single Coomassie Brilliant Blue staining bands corresponding to subunits were excised with a razor blade taking only the visibly blue region. These larger acrylamide slices were treated in an identical fashion to the 1-mm slices above. However, since no digitization was required, each subunit was represented as its total counts/min instead of total area.

Other Methods

Chlorophyll determinations were made using 80% acetone with the extinction coefficients of Arnon (39). P680 measurements were made optically using an Aminco DW-2 spectrophotometer using the extinction coefficient of Hiyuma and Ke (40). EPR spectra at liquid helium temperatures were recorded with an X-band Bruker spectrometer as previously described (41).

RESULTS

Native PS I Complex Isolation and Characterization—The isolation procedure used in this work is shown in Fig. 1. This procedure allows the isolation of both the cytochrome b-f
The exact molecular mass of these proteins is difficult to determine with accuracy. However, using molecular mass standards (Bio-Rad), their estimated apparent masses are 12.0, 10.5, 9.0, and 8.5 kDa. Immunoblotting has identified the 23-kDa protein as equivalent to the 20-kDa LHCP Ib subunit of spinach (19) and the 21-kDa protein equivalent to the “19”-kDa from spinach (data not shown). Immunoblotting has also shown that only peptides in the 27-30-kDa range are related to LHCP II (data not shown).

In Vivo Labeling—In vivo labeling of Lemma with uniformly labeled [14C]glucose was carried out with two different cultures. PS I isolated from these two different labelings had specific activities of 3200 and 4500 cpm/μg chlorophyll, respectively. A Coomassie-stained gel of PS I-200 isolated from in vivo-labeled plants and its corresponding autoradiogram is shown in Fig. 2.

Subunit Stoichiometry of the Native PS I Complex—Fig. 3 shows an example of data from gel slicing and scintillation counting of an in vivo labeled PS I-200 complex. Fig. 4 shows a densitometric trace of an autoradiogram from a SDS-PAGE gel of a similar preparation. The results of the three different methods of quantitation are summarized in Table I. To generate this table, the actual peak area or the total counts/min measured for each protein band has been divided by the molecular mass of each subunit. This results in the amount of label per subunit with units of area or total counts/min/kDa protein. This correction requires several assumptions relating to the molecular masses of these proteins. Molecular masses of membrane proteins based on SDS-PAGE can be very anomalous. In the case of the L-subunit of the reaction centers of *Rhodopseudomonas capsulata*, sequence data has shown the SDS-PAGE molecular mass value to be underestimated by over 50% (42). However, with the exception of the high molecular mass subunits of CP1 and the frxA gene product, no molecular masses determined from amino acid sequences are available. In the case of CP1, derived amino acid sequences give a molecular mass of approximately 84 kDa even though a value of ~60 kDa is obtained from SDS-PAGE analysis. This former value assumes that there is no post-translational processing (22) and that there are no significant differences between maize and *Lemna* CP1 apoproteins.

To determine the number of subunits per complex we have defined the 21-kDa subunit to be present in one copy per complex. Therefore, the other polypeptides are present with a copy number relative to this value. The values obtained with this procedure are quite close to integer values. The three procedures used for the analysis of the labeled in vivo complex agree well with one another. From a consensus of the three different procedures, it can be concluded that the ratio of the high molecular mass subunits to the lower molecular mass subunits is 2:1, with the exception of LHCP Ib and the 16.5-kDa subunits which are present in two copies per complex. These results represent the average of at least four measurements for each of the three different procedures and uses preparations from two independent labelings. No effort has been made to determine the subunit stoichiometry of the individual LHCP I polypeptides.

The accuracy of quantitation of the low molecular mass proteins (below 14 kDa) is more difficult due to both the lower amount of radioactivity associated with these bands and the co-migration of non-proteinaceous labeled in vivo material such as chlorophyll, carotenoids, and lipids. It is therefore difficult to determine the absolute numbers of these subunits. All of these subunits appear to be present in at least one copy per complex and it appears that the 8.5-kDa subunit,
**FIG. 2.** A, Coomassie-stained gel of labeled in vivo native PS I complex and LHCP II from *Lemna*. The numbers shown represent the amount of chlorophyll in each lane. B, in vivo labeling was carried out by the addition of 1 mCi of $[^14C]$glucose (uniformly labeled) to the growth medium. Single plants were added to this medium and allowed to grow vegetatively for 3-5 weeks. The native PS I complex was isolated as shown in Fig. 1 and had a specific activity of 3200 cpm/Mg chlorophyll. The numbers above represent the amount of chlorophyll applied to each lane. Autoradiography was carried out on the dried gel and exposed for 21 days at $-80^\circ$C without an intensifying screen.

**FIG. 3.** Scintillation counting of polyacrylamide gel slices was performed as follows: a Coomassie-stained gel was sliced into 1-mm slices and incubated with 30% hydrogen peroxide and 70% perchloric acid overnight at 70 °C; to this 7 ml of Amersham PCS scintillation fluid was added, and the sample was cooled at 4 °C in the dark overnight. Each slice was then counted to a 2% value of 5% with windows of 200-700 on a Beckman 1801 scintillation counter. The amount of $^{14}$C/slice was then plotted and the area of the resulting peaks determined by digitization.

**TABLE I**

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<th>Subunit $M_s$</th>
<th>Gel slicing</th>
<th>Autoradiography</th>
<th>Single band</th>
<th>Consensus</th>
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<td>PS I complex</td>
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<td>1.0</td>
<td>1.0</td>
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<tr>
<td></td>
<td>18,100</td>
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<td>8,500</td>
<td>1.1 ± 0.1</td>
<td>1.8</td>
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**FIG. 4.** Densitometric trace of an autoradiogram from a SDS-PAGE gel of a native PS I complex from *Lemna*. The smallest of the low molecular mass proteins, may be present at two copies per complex. It is possible that the two copies of the 8.5 and 16.5 subunits detected per PSI complex actually represent two different protein subunits which co-migrate under our electrophoretic conditions. Further experimentation will be required to test this question.

**PS I Core Complex Isolation and Characterization**—The core complex produced by the procedure shown in Fig. 1 and described above has a chlorophyll/P$_{700}$ ratio of 125–135. The chlorophyll $a/b$ ratio is greater than 11 as calculated by the procedure of Arnon (39). This complex is lacking both the ensemble of proteins at 23–27 kDa (LHCP I) and the ensemble at 27–30 (LHCP II). However, it still shows P$_{700}$ photoox-
idation and the ability to photoreduce the centers Fe-S_A and Fe-S_B. A Coomassie-stained gel and the corresponding autoradiogram of a labeled in vivo preparation is shown in Fig. 5, A and B.

Subunit Stoichiometry of PS I Core Complex—The results of the three different methods of quantitation of the PS I core complex are shown in Table II. The procedure for determining these values was the same as used for the native complex. The Coomassie-stained gel of the core complex isolated from in vivo labeled plants and its corresponding autoradiogram are shown in Fig. 5. The polypeptides corresponding to LHCP I and LHCP II are missing from this preparation, and there is the total loss of one of the low molecular mass polypeptides, that of 12.0 kDa. The 18.1-kDa polypeptide is also partially lost with small (<50%) but variable amounts being retained in the core complex. However, the subunit composition of the remaining core polypeptides is the same in that two copies of the high molecular mass subunits are present per complex.

Subunit Stoichiometry of CPI—An autoradiogram of a 6% SDS-PAGE gel of PS I-200 is shown in Fig. 6A. This figure shows that the solubilization conditions affect the separation of the two high molecular mass subunits. When the solubilization is done at 25 °C, a chlorophyll-containing band with an apparent molecular mass of 110 kDa can be observed. Under these conditions (6% resolving gel) all other PS I subunits migrate together near the dye front and free pigment.

However, when the solubilization is done at 50 °C, this chlorophyll pigment protein dissociates within 20 min. As the time of treatment is increased at 50 °C, there is a degradation of the 62-kDa subunit. This is apparent both by its gradual loss and by the appearance of a lower diffuse band at about 48 kDa. This degradation during solubilization could not be prevented by the addition of the following protease inhibitors: EDTA (5 mM), EGTA (5 mM), aminocaproic acid (2 mM), p-aminobenzamidine (2 mM), phenylmethylsulfonyl fluoride (2 mM), aprotinin (20 µg/ml), trypsin inhibitor (100 µg/ml), and pepstatin (60 µg/ml). To determine the stoichiometry of the two high molecular subunits, it is important to have them completely dissociated from the 110-kDa form yet still free from any degradation artifacts. By solubilizing the native complex for 20 min at 50 °C, we meet these criteria, and a densitometric trace of this lane from the autoradiogram is shown in Fig. 6B. From this trace we can calculate by the procedures described above that the two subunits are present in a 1:1 ratio.

**DISCUSSION**

Initial efforts to isolate a native PS I complex by a procedure previously described (13) produced PS I preparations which had either low chlorophyll a/b and chlorophyll/P_700 ratios or preparations which had many contaminating polypeptides in the region of 30–65 kDa as determined by SDS-PAGE. We have therefore developed an alternative procedure which yields a better resolved PS I preparation. PS I isolated by this procedure appears similar to preparations from other organisms in its chlorophyll a/b and chlorophyll/P_700 ratios.
The apoprotein for center Fe-Sx is or at least is very closely related to the PS I complex and not to an artifact of the isolation procedure or a compositional difference in * Lemma*. The function of these low molecular mass proteins is unknown. However, recent work of Moller et al. and others (30–32) has identified a chloroplast-encoded protein from PS I with an approximate molecular mass of 9 kDa as a putative 2 [4Fe-4S] protein. It has been suggested that this protein is the apoprotein for Fe-Sx and Fe-Sb (30–32).

The chlorophylls associated with the light harvesting component of PS I, LHCP I, have been shown to exist in a complex distinct from the P700-containing core complex (14, 43). This complex has a chlorophyll a/b ratio of 3.5–4.0 and is responsible for binding all the chlorophyll b associated with PS I (14, 43). Approximately 100 chlorophyll molecules are present in LHCP I (14). The LHCP I complex of * Lemma* and other higher plants has been shown to contain at least 5 subunits in the 22–27-kDa range (14, 43–45). The assignment of which subunits actually bind chlorophyll is a current area of controversy. Work by Lam et al. (19) demonstrated that spinach LHCP I can be further resolved into at least two chlorophyll-containing fractions: LHCP Ia, which contained apoproteins of 22 and 23 kDa, and LHCP Ib, which contained only a 21-kDa apoprotein. These two chlorophyll protein fractions differed in their fluorescence and absorbance properties. Bassi and Simpson (44) also report two different chlorophyll-binding complexes in LHCP I from barley. However, the recent work by Nechustai and co-workers (45) suggests that LHCP I of * Lemma* contains only a single subunit with a molecular mass of 20 kDa. This LHCP I protein contains both chlorophyll a and b and was shown to be immunologically related to the 21-kDa chlorophyll protein isolated by Lam et al. (19) from spinach. Nechustai et al. (45) argue strongly for there being only one chlorophyll-containing subunit in LHCP I. Our results show that there are only two copies of the LHCP I subunit. This presents the problem of how two copies of a 23-kDa protein per complex can bind the ~100 chlorophyll molecules associated with LHCP I. This problem is exacerbated by the fact that this protein contains only 1 histidine residue (45). Assuming this protein binds 12–13 molecules of chlorophyll, as has been proposed for LHCP II (46–48), it could only account for one-fourth of the chlorophyll known to be associated with LHCP I. Therefore, based on the stoichiometry of the 23-kDa subunit, our data suggests that there must be additional chlorophyll-binding proteins associated with LHCP I.

CP1 has been shown to contain approximately 30% of the 12–14 acid-labile sulfide molecules associated with the native PS I complex (32). Earlier work by Høe and Moller (33) has also shown that CP1 contains 4.3 iron atoms/molecule of P700. The optical measurements of Golbeck and Cornelius (49) using lithium dodecyl sulfate-treated subchloroplast particles depleted of low molecular mass proteins has suggested that the apoprotein for center Fe-Sx is or at least is very closely associated with CP1. Although these results strongly suggest CP1 to be the apoprotein of the center Fe-Sx, they cannot distinguish whether Fe-Sx contains a single [4Fe-4S] center or two [2Fe-2S] centers.

Earlier work by Evans et al. (50) had suggested that center X was a [4Fe-4S] center based on Mossbauer spectroscopy. However, the recent work of McDermott et al. (51) using extended x-ray absorption fine structure measurements have fit their data to a model in which PS I contains both [4Fe-4S] centers and at least one [2Fe-2S] center. Their model is supported by core extrusion experiments which also identify both [4Fe-4S] centers and [2Fe-2S] centers in PS I (52). Since centers Fe-Sa and Fe-Sb are widely accepted to be [4Fe-4S] centers (1, 2), these spectroscopic results suggest that Fe-Sx contains two [2Fe-2S] centers.

CP1 is composed of two subunits as shown in this work and by others. The apoproteins of both subunits are chloroplast-encoded and share a high degree of homology (16). Their genes psaA and psaB have been sequenced in maize (22), tobacco (23), pea (24), spinach (25), liverwort (28), *Chlamydomonas* (27), *Euglena* (28), and *Synechococcus* (29). In the four higher plants the psaA gene contains 4 cysteines while the psaB gene contains only 2 cysteines. In all eight organisms, the 12-amino acid sequence that contains 2 of the 4 cysteines in the psaA gene product is totally conserved with a 12-amino acid region that contains the 2 cysteines found in the psaA product (32). The 2 remaining cysteines in the psaA product occur in regions that are less conserved. These results suggest that the cysteines found outside the conserved 12-amino acid region in the psaA product have a less critical role in the structure and function of CP1 and are probably not involved in the coordination to a putative Fe-S center.

If the center, Fe-Sx, associated with CP1, is to bind the 4 acid-labile sulfides and the 4 iron atoms found in CP1, it must be either a single [4Fe-4S] center or two [2Fe-2S] centers. If Fe-Sx is a [4Fe-4S] center, this would require the coordination of 4 cysteines and these could be provided by one copy of each of the high molecular mass subunits. However, if Fe-Sx is composed of two [2Fe-2S] centers, Fe-Sx cluster formation requires 8 cysteine residues, and this could be provided in a tetrameric structure of 4 high molecular mass subunits. Since our analysis of the stoichiometry of the two CP1 subunits indicates the presence of only 1 copy each of the psaA gene product and the psaB gene product, there are only 4 cysteines available to coordinate the center Fe-Sx. We would propose that Fe-Sx is coordinated by the 2 conserved cysteine residues from 1 copy of the psaB gene product and 2 conserved cysteine residues from a single copy of the psaA gene product. Whether Fe-Sx is a single [2Fe-2S] center or a single [4Fe-4S] center cannot be determined from our data. Clearly more work must be done to clarify the nature of the Fe-S cluster associated with CP1.

Our dimeric model for PS I is in good agreement with the recent crystallization data of PS I reaction centers from the cyanobacteria *Phormidium* and *Synechococcus* (53, 54). Both of these preliminary reports suggest that PS I may exist in a trimeric complex with each monomer having an approximate molecular mass of 150 or 230 kDa, respectively. A reaction center complex with a molecular mass of ~200 kDa can only include a single copy of each of the high molecular mass subunits plus the low molecular mass subunits and pigments.

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REFERENCES


