Subunit Stoichiometry of the Chloroplast Photosystem II Antenna System and Aggregation State of the Component Chlorophyll a/b Binding Proteins*

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Photosystem (PS) II membranes, obtained by the method of Berthold et al. (Berthold, D. A., Babcock, G. T., and Yocum, C. F. (1981) FEBS Lett. 134, 231–234), have been fractionated by a sucrose gradient ultracentrifugation method which allows the quantitative separation of the three major chlorophyll binding complexes in these membranes: the chlorophyll (chl) a binding PSII reaction center core, the major light-harvesting complex II, and the minor chl a/b proteins called CP26, CP29, and CP24. Each fraction has been analyzed for its subunit stoichiometry by quantitative sodium dodecyl sulfate-polyacrylamide gel electrophoresis methods. The results show that 12 mol of light-harvesting complex II and 1.5 mol of each of the minor chl a/b proteins are present per mol of the PSII reaction center complex in PSII membranes. These data suggest a dimeric organization of PSII, in agreement with a recent crystallographic study (Bassi, R., Ghiretti Magaldi, A., Tognon, G., Giacometti, G. M., and Miller, K. (1989) Eur. J. Cell Biol. 50, 84–93) and imply that such a dimeric complex is served by antenna chl a/b proteins whose minimal aggregation state includes three polypeptides. This was confirmed by covalent cross-linking of purified antenna complexes.

In oxygenic photosynthesis, two photochemical systems cooperate in the transfer of electrons from water to NADP⁺. In both cases the light reactions are driven by the excitation energy absorbed by the antenna pigments and transferred to the reaction centers (RC). Although the primary chromophore responsible for light absorption in higher plants and algae is chlorophyll a, accessory pigments such as chlorophyll b and carotenoids, which are bound to antenna proteins, extend the spectral range of light absorption and transfer energy to chl a.

In higher plants as many as 210 molecules of chlorophyll are associated with photosystem (PS) I (1) and 230 molecules with PSII (2), but little information is available on the organization of the chlorophyll-proteins of reaction centers and antenna proteins within photosynthetic units.

The PSII core complex is composed of a pigment-protein complex which contains 4–5 chlorophyll a molecules, including the photoactive pigment P680, bound to the D1 and D2 polypeptides (3) and of two partially homologous chlorophyll-proteins, CP47 and CP43, each binding 25 chl a molecules (4). Although these two proteins, mainly CP47, have been indicated as the site of P680, they are now thought to have an inner antenna function (for a review see Ref. 5). Although the above mentioned proteins are coded by the chloroplast genome, the remaining 180 chlorophyll molecules are bound to a number of chl a/b binding proteins coded by an extended multigene family in the nuclear genome (6–8). Three of them, called CP29, CP26, and CP24, are present in small amounts in PSII membranes, whereas a fourth component, the major light-harvesting complex II (LHCII), is the most abundant thylakoid protein (for a review see Ref. 9).

Previous work with higher plants has shown that CP43 is the PSII core subunit which mediates the binding of both LHCII and the minor chl a/b proteins and topological models have been proposed for the organization of chlorophyll a/b proteins (10, 11).

The PSII antenna system is an highly complex structure capable of many physiological mechanisms for the adaptation of the light-harvesting function to the environmental conditions such as state I-state II transitions (12), heat stress (13), and cold stress (14). The understanding of the molecular mechanisms underlying these physiological adaptations requires more information about the composition and supramolecular organization of the antenna system.

Several basic aspects of the system are, however, unknown. As an example, information about the relative abundance of the components of the antenna system as well as their aggregation state and their pigment complement is very imprecise. In this study, we have determined the stoichiometry of the different chl a/b proteins in PSII membranes, as well as the number of chlorophyll molecules they bind and their aggregation state. On the basis of these data and consistent with information obtained from the analysis of two-dimensional crystals of the PSII complex (15), we propose a dimeric organization for PSII reaction center. Our data indicate that each dimer is served by a common antenna system which is composed of trimeric chl a/b proteins.

MATERIALS AND METHODS

Preparation of Thylakoid Membranes—Zea mays seedlings (cv Dekalb DF280) were grown for 2-3 weeks in a growth chamber at 25/21 °C day/night at a light intensity of 10,000 lux and 80% humidity. Leaves from 2- to 3-week-old plants were harvested at the end of a 12 h dark period and thylakoids from mesophyll chloroplasts were obtained as described previously (10). PSII membranes were obtained...
according to the method of Berthold et al. (16) using the modifications described in (17). Aliquots were resuspended in 25 mM Hepes, pH 7.6, 5 mM MgCl₂, 10 mM NaCl, 0.2 M sorbitol, and frozen at −80 °C until required.

**Sucrose Gradient Ultracentrifugation**—PSII membranes were washed twice in EDTA pH 7.6, then resuspended in 1% DM. Solubilized membranes were spun 2 h at 15,000 rpm in a 0.1-1 M sucrose gradient containing 10 mM Hepes, pH 7.6, and 0.06% DM. The gradient was then spun on a Beckman SW41 rotor at 39,000 rpm for 23 h at 4 °C. For quantitative determinations, the gradient was fractionated from the top into 250-μl aliquots that were analyzed by SDS-PAGE, absorption spectra, and for their protein and pigment content. Alternatively individual green bands were harvested with a syringe.

**SDS-PAGE and Immunoblotting**—Analytical SDS-PAGE was performed with gradient gels (12-18% acrylamide, 350 x 350 x 1 mm) containing 6 M urea and run at 10 mA for 3 days using the buffer system described previously (10). Alternatively, a high Tris buffer system without urea (12-18% acrylamide gradient) was used (18). For immunoblot assay, samples were separated by one of the gel systems described above and transferred to a nitrocellulose filter (Millipore). The filters were then assayed with antibodies and antibody binding was detected by using alkaline phosphatase-coupled anti-rabbit IgG (Sigma). Antibodies were prepared in rabbits and characterized as described previously (19).

**Purification of Proteins**—Chlorophyll a/b proteins were purified from DM-solubilized PSII membranes by preparative IEF in the pH range 3.5-5 as described previously (20). OEE1 and OEE2 were also purified by preparative IEF but a pH gradient from 3.5 to 10 was used.

**Amino Acid Analysis**—Solutions containing purified proteins were analyzed by quantitative aminoacid assay. Proteins were hydrolyzed in 6 M HCl for 24 h at 110 °C. Samples were then vacuum-dried and treated with phenylisothiocyanate to obtain phenylthiocarbamoyl amino acids following the Pico-Tag procedure (Millipore-Waters) as suggested by the manufacturer. Phenylthiocarbamoyl amino acids were then fractionated by HPLC by using a reverse phase C18 column (Millipore) and a linear gradient from 100% A to 35% B. Phenylthiocarbamoyl amino acids were revealed and quantified by their absorbance at 269 nm. Values of threonine and serine were corrected assuming, respectively, 13 and 17% decomposition during the hydrolysis. A known amount (1000 pmol) of norleucine was added as concentration reference.

**Quantitation of Coomassie-stained Proteins in SDS-PAGE Gels**—Samples from sucrose gradient bands were analyzed by both the SDS-PAGE methods described above. After running, the gel was fixed in 5% TCA and stained with 10% trichloroacetic acid, then stained in 0.125% Coomassie Brilliant Blue R-250 in 40% methanol overnight. The gels were destained twice in 10% methanol and 10% acetic acid for 8 h and then in 7.5% acetic acid. Gel pieces containing a stained protein band were excised with a razor blade, weighted, and placed in an Eppendorf tube with 1 ml of 3% SDS in 50% isopropanol. Gel pieces with the same weight, cut from the interlane areas, were used for background subtraction. The eluted dye was quantified spectrophotometrically at 585 nm (21). The specific binding of Coomassie to individual proteins (CP29, CP26, CP24, LHCII, OEE1, and OEE2) was estimated by loading increasing amounts of purified proteins on a SDS-PAGE gel and determining the bound dye as described above. Alternatively, Coomassie binding was measured by densitometry by using a Shimadzu chromatocanner with a program allowing base-line correction. The two methods gave consistent results, although we found it necessary to stain the gels overnight rather than for 2 h as reported in Ball (21).

**Cross-linking**—For cross-linking experiments, chlorophyll-proteins were isolated as reported above and tested for their aggregation state by sucrose gradient ultracentrifugation in 0.06% DM. Green bands from the sucrose gradient were diluted to 10 μg chl/ml in 10 mM Hepes, pH 7.6, 0.05% DM and treated with 1% glutaraldehyde for 5′ at 20 °C. The reaction was blocked by addition of 0.025 volumes of 2 M NaOH dissolved in 0.1 M NaOH; this reduces and stabilizes the cross-linked products, and untreated glutaraldehyde is inactivated by reduction. The samples were analyzed for their migration in sucrose gradient versus untreated samples to ensure that higher aggregation states were not induced by the treatment. Cold acetone was then added to 80% and the protein precipitated by centrifugation at 15,000 × g for 5′. The pellet was dried and resuspended in 1% SDS, 1% mercaptoethanol, 0.125 M Tris sulfate, pH 9.0, and analyzed by SDS-PAGE (22). A better resolution of the high molecular mass cross-linking products was obtained by using long runs in SDS-urea-PAGE as shown in Fig. 6.

**RESULTS**

**Fractionation of PSII Membranes by Sucrose Gradient Ultracentrifugation**—Photosystem II membranes obtained by the procedure of Berthold et al. (16) were solubilized with 1% DM and fractionated in a 0.1-1 M sucrose gradient containing 0.06% DM. The separation pattern is shown in Fig. 1A, whereas the absorption spectra of the major bands are shown in Fig. 1B. The polypeptide composition of the green bands is shown in Fig. 1C. Since the apparent molecular mass of the chlorophyll a/b binding apoproteins is very similar, we have assayed blots with antibodies raised against purified complexes as described previously (Dainese et al., 1990) to identify...
TABLE I
Characteristics of the green bands obtained by sucrose gradient ultracentrifugation of dodecyl maltoside-solubilized PSII membranes

Values are the average of five independent experiments.

| Chl | Chl a/β ratio | Chl/carotenoid ratio | Protein | Chl a peak |
|-----|---------------|---------------------|---------|------------|
| %   | %, w          | mm                  |         |            |
| B1  | 1.9           | 3.8                 | 1.0     | 3.0         | 669       |
| B2  | 16.4          | 2.2                 | 5.5     | 17.9       | 677       |
| B3  | 51.9          | 1.4                 | 7.0     | 42.2       | 674.5     |
| B4  | 6.7           | 1.6                 | 6.3     | 6.1        | 676       |
| B5  | 45            | >20                 | 6.8     | 6.0        | 674       |
| B6  | 21            | >20                 | 7.0     | 3.8        | 676       |
| B7  | 16.6          | >50                 | 7.7     | 21.0       | 674       |

*OEE polypeptides dissociated from PSII-RC migrates at this level.

![Fig. 2. Polypeptide composition (A) and immunoblot analysis (B) of chl α containing sucrose gradient bands (5 to 7).](image)

The characteristics of the green bands above described are summarized in Table I, which shows that 23% of the chlorophyll and 31% of the protein content of grana membranes are due to the chl a reaction center core complex, whereas the remaining 77% (69% in protein) is due to chl a/b antenna complexes.

**Stoichiometry of Chlorophyll a/b Binding Proteins**—To determine the quantitative relations between chl a/b binding proteins, we analyzed sucrose gradient bands 2–4 for their polypeptide composition by using two different SDS-PAGE gel systems to avoid comigration. The individual Coomassie-stained bands were identified by immunoblot with specific antibodies and quantified either by densitometry or by excising the bands and measuring the eluted dye (21). As an example, the results obtained by the analysis of sucrose band 4 are shown in Fig. 3. The specific binding of Coomassie R-250 to individual chl a/b apoproteins as well as the two major extrinsic OEE polypeptide which are often contaminants of sucrose bands 1–4 was evaluated by loading different amounts of purified proteins obtained by preparative isoelectric focusing on gels and determining the amount of bound dye. The results obtained by the analysis of sucrose band 4 are shown in Fig. 3 as an example. There were differences in the stainability of Cab proteins as shown in Fig. 4, and the data were corrected according to the specific binding of Coomassie to isolated proteins. The same samples were also analyzed for their chlorophyll and carotenoid content and the pigment to protein ratios were reported in Table II. These values were obtained with complexes purified by a single IEF step. Alternative procedures including several purification steps or mild SDS-PAGE consistently yielded lower chlorophyll to protein ratios while chl a/b ratios were higher for CP29 and CP26 and lower for CP24 and LHCII. The results of quantifying Cab apoproteins are summarized in Table III and allow the determination of the stoichiometry of Cab proteins in granal membranes. Minor chl a/b proteins are therefore present in grana mem-

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proteins were run on a SDS-urea gel that was stained with Coomassie R-250. Individual bands were excised from the gel and the bound dye was eluted and spectrophotometrically quantified. A single chlorophyll binding protein having a molecular mass calculations we have considered the PSII-RC core complex as binding proteins of PSII membranes (Table IV). In these branes in a ratio approaching 1:1 and together constitute 25% of the chl a/b proteins. The data in Tables I and III can be used to calculate stoichiometric data for all chlorophyll binding proteins of PSII membranes (Table IV). In these calculations we have considered the PSII-RC core complex as a single chlorophyll binding protein having a molecular mass of 240 kDa based on the sum of the component apoproteins. On the basis of the determined chl to protein ratio, the numbers of chlorophyll molecules were also calculated for individual chlorophyll-proteins. The values obtained were consistent with the experimental data for the chl to protein ratio within the individual sucrose gradient bands (data not shown). We calculate that 53 chl a molecules are bound to the PSII-RC core complex, 131 to the major LHCII, and 15, 14, and 8, respectively, to CP29, CP26, and CP24. In terms of protein stoichiometry, we obtain 12 LHCII moles and approximately 1.5 mol each of the minor chl a/b proteins/mol of PSII-RC core complex.

**Table II**

| Chl a/b ratio | Chl/protein ratio | Carotenoid/protein ratio | Red absorption peak |
|---------------|-------------------|-------------------------|--------------------|
| Mol/mol       | nm                |                         |                    |
| CP24          | 1.6 ± 0.1         | 5.1 ± 0.2               | 11.2 ± 0.05        | 674.5              |
| CP26          | 2.2 ± 0.1         | 9.1 ± 0.4               | 1.8 ± 0.18         | 676               |
| LHCII         | 1.4 ± 0.06        | 12.3 ± 0.4              | 1.8 ± 0.05         | 674.5              |

**Table III**

| Relative protein content (percent) of chl a/b binding polypeptides in sucrose gradient bands 2-4 (containing chlorophyll a/b proteins) from DM-solubilized PSII membranes |
|-------------------------------------------------------------------------------------------------|
| Numbers in parentheses refer to values before correction for Coomassie binding. |

**Table IV**

| Chlorophyll and protein distribution among chlorophyll-proteins in PSII membranes |
|----------------------------------------------------------------------------------|
| Band                              | LHCII | CP26 | CP29 | CP24 |
|-----------------------------------|-------|------|------|------|
| Chl                               | 25.4  | 8.0  | 15.4 | 7.1  |
| Carotenoid                        | 32.4  | 8.2  | 15.4 | 7.1  |
| Red absorption peak               | 674.5 | 675  | 674  | 674  |

*After correction for molecular mass. Values are as follows: LHCII, 28.5 kDa; CP29, 31 kDa; CP26, 28.5 kDa; CP24, 20 kDa (Bassi et al., 1987).*

**Fig. 4.** Quantification of the Coomassie binding to individual chl a/b apoproteins. Different amounts of purified chl a/b proteins were run on a SDS-urea gel that was stained with Coomassie R-250. Individual bands were excised from the gel and the bound dye was eluted and spectrophotometrically quantified.

**Fig. 5.** Sedimentation behavior of purified chlorophyll a/b proteins. The samples, purified by IEF as under "Materials and Methods," were run in a 0.1–1 M sucrose gradient containing 0.06% DM.
results are a clear demonstration that the lower aggregation state of chl a/b proteins includes three polypeptides. This is common to the minor chl a/b proteins and to the lower aggregation state of LHCCI, whereas a higher number of high molecular mass LHCCI cross-linking products is present in the case of the LHCCI preparation migrating in sucrose band 3. It is not possible from our data to determine the exact number of polypeptides in LHCCI due to the increasingly diffuse appearance of the high molecular mass bands produced by cross-linking.

DISCUSSION

We report here on the stoichiometry of chlorophyll binding proteins within the granal membranes of maize chloroplasts and on the aggregation state of polypeptides within purified chl a/b proteins.

Three methods have been particularly important in this work: (i) the development of a sucrose gradient fractionation method which allows the separation of the minor chl a/b proteins, the major LHCCI complex and of the chl a binding PSII-RC core complex; (ii) the use of specific antibodies to identify closely migrating polypeptides in SDS-PAGE gels; and (iii) the use of a recently developed non-denaturing IEF method that allows the purification of unladenated chl binding proteins.

When PSII membranes are separated by SDS-PAGE, the apparent molecular mass of chl a/b apoproteins is very similar, ranging from 20 and 31 kDa. It has therefore been necessary to fractionate the DM-solubilized membranes by sucrose gradient ultracentrifugation. This is a very mild and effective procedure, since less than 2% of the pigments are released and allows the separation of the major LHCCI and the minor chl a/b proteins by a simple procedure. The conditions of solubilization are very critical and can be used to modulate the results of the separation; short incubation time with 1% DM yields a very clean preparation of the minor chl a/b proteins in sucrose band 2, the same procedure with 0.4% DM yields essentially CP26 in band 2, whereas CP29 and CP24 migrate in band 4 with a fraction of LHCCI. Longer incubations in the detergent (>15') decrease the amount of chl associated with sucrose band 4 but also increase the contamination of sucrose band 2 with dissociated LHCCI. We have determined the distribution of the chl among chlorophyll-proteins in each of the above conditions obtaining consistent results.

The determination of subunit stoichiometry has been mostly carried out with aquatic organisms such as *Chlamydomonas reinhardtii* (26) or *Leucaena minor* (27) in which uniformly radioactively labeled proteins can be obtained by supplying 14C substrates in the growing solution. This is practical since the method does not require the isolation of each protein subunit. Since this procedure is not feasible with *Z. mays*, we have used the Coomassie binding for protein quantitation. The binding of dyes to proteins is variable and, in principle, the possibility that the stainability of different apoproteins is very different cannot be ruled out. We have assayed purified proteins and determined in each case the specific binding of the dye, whereas absolute protein concentration in the purified protein solution was assayed by quantitative amino acid analysis. The measured values are very similar for the different chl a/b binding proteins within either the LHCCI group and the minor chl a/b protein group as could be expected from their belonging to the same multigene family and sharing of epitopes (19, 28). Although the presence of common epitopes could be a problem in the identification of the apoproteins by immunoblot analysis, we have shown that only minor cross-reaction could be detected when native chlorophyll-proteins rather than denatured apoproteins are used as antigens (19).

At the dilution of antisera used in this work, the apoproteins could be easily distinguished. Only the antibody against CP26 cross-reacted with CP29, but the apoproteins could be easily resolved by SDS-urea-PAGE. The isolation of pure chlorophyll-proteins allowed the determination of the stoichiometry of pigment binding to the different chl a/b proteins. The value we obtained for LHCCI (12 chl and 2 carotenoids for 28.5 kDa protein) is in close agreement with the most recent determinations (29, 30), whereas for CP29, CP26, and CP24 we obtained a higher pigment content ranging from 9 to 5 chl molecules for each polypeptide. Chlorophyll has been shown to be bound to the hydrophobic portion of LHCCI buried in the thylakoid membrane (29). Although CP26 and CP24 genes have not been cloned yet, the deduced amino acid sequence for CP24 (31) predicts two trans-membrane a-helices rather than the three indicated for LHCCI, consistent with the lower pigment content. To our knowledge, quantitative determination of pigment binding to CP24 has not been reported before, whereas CP29 and CP26 have been reported to bind 4 and 5 chl molecules with a chl a to b ratio of 4.3 and 2.7, respectively (32). We have obtained lower pigment content than those reported in Table II in the case of preparations obtained by multistep procedures (see "Sucrose Gradient Ultracentrifugation" under "Materials and Methods"). We therefore suggest that the procedure employed by these authors, which includes solubilization with octyl a-D-glucoside in high salt conditions, results in partial removal of pigments from CP26 and CP29.

To generate stoichiometry data, the values for protein content (Table III) have been divided by the molecular mass of each chl a/b binding subunit and corrected for specific Coomassie binding. The correction for molecular mass requires several assumptions relating to the molecular mass of these proteins. Molecular masses of membrane proteins based on SDS-PAGE can be very anomalous. In an extreme case, the 1 subunit of the reaction center of *Rhodopseudomonas capsulata*, sequence data has shown the SDS-PAGE molecular...
mass value to be underestimated by over 50% (33). However, no molecular mass values obtained by deduced amino acid sequences are available for maize chl a/b proteins with the exception of a single c-DNA clone (34) which it is not known to correspond to a particular LHCII apoprotein. We have therefore used for the correction molecular mass values obtained from SDS, 6 M urea-PAGE (10), since the more complete denaturation obtained in these conditions is more likely to favor stoichiometric SDS binding to the unfolded polypeptide and minimize anomalies in migration patterns. In any case, it is likely that such anomalies are similar for all chl a/b proteins, since they belong to the same multigene family and share sequence stretches and secondary structure patterns. It should be remembered, moreover, that quantitation of apoproteins from SDS-PAGE has been used in this work only to assess the relative contributions of the different proteins within each sucrose gradient band, while the absolute protein content was determined in solution by the bicinchoninic acid method (25) which does not have the limitations of the SDS-PAGE/Coomassie stain method and, when required, confirmed by quantitative aminocid analysis.

A more critical assumption had to be made in the generation of Table IV in which a molecular mass value of 240 kDa has been attributed to the chl a binding PSII-RC core complex by the summation of the molecular masses of the component subunits CP47, CP43, D1, D2, cytochrome b562, OEE1, OEE2, OEE3, and 9-kDa phosphoprotein. This value is commonly accepted (35, 36), and when employed in our calculations yields a pigment complement of 53 chlorophyll molecules for the PSII core complex, a value that is consistent with reported data (3, 4, 35).

To our knowledge there has been no attempt to determine the stoichiometry of chlorophyll binding proteins in the PSII antenna system. We have obtained a value of 12 mol of the major LHCII complex and 1.5 mol of each of the three minor chl a/b proteins CP29, CP26, and CP24/mol of PSII-RC core complex. If we attribute to each chlorophyll-protein the pigment complement determined (Table II), then a value of 232 chl molecules/PSII reaction center is obtained, which compares favorably with the spectrophotometric determination of 230 chlorophylls for the PSII antenna size in isolated grana membranes (2). Since proteins of the antenna system bind chl a and chl b in ratios ranging from 1.4 to ∞, a comparison can be made with chl a/b ratio values determined in isolated complexes. A calculated chl a/b ratio of 2.25 is thus obtained for PSII membranes versus a measured value of 2.3 ± 0.05 by using the same determination method for the membranes and isolated complexes (23).

We assume here that all the PSII complexes in the membrane preparation used have all of the component polypeptides and polypeptide-chlorophyll complexes in the same ratio. This cannot be proven at the present; however, several data support the assumption: (a) PSII has been shown to be heterogeneous with respect to the antenna size and the characteristics of the donor side, but the two PSII types (a and b) were found to be located in different membrane compartments. Thus grana membranes, that we have used in this work, contain mainly PSIIa, whereas PSIIb was found in strongly exposed thylakoids (37); (b) the chlorophyll a PSII core complex appears to be very homogeneous, since 80% of it is contained in sucrose band 7, whereas sucrose band 5 is likely to be a dissociation product of the complex in band 7; (c) the chlorophyll a/b proteins CP24, CP29, and a LHCII subset can be isolated in conditions which preserve their complete association in the supramolecular complex of subcrosse band 4, thus showing that the organization of these antenna complexes is homogeneous in PSII membranes.

The molar ratio of 1.5 obtained for each of the three minor chl a/b proteins and the PSII-RC complex is intriguing, since both whole numbers 1 and 2 are too far from the experimental values to be preferred with respect to 1.5. On this basis, we suggest that PSII-RC is organized in dimers which are served by a common antenna system composed of 24 LHCII polypeptides and three each of the CP29, CP26, and CP24 proteins. Three lines of evidence support our hypothesis: (i) in our sucrose gradient fractionation (Fig. 1) two bands (5 and 7) have PSII-RC complexes containing all the intrinsic membrane subunits of PSII. The complex in band 7 could be an oligomeric (probably dimeric) form of the one in band 5. In most conditions the putative dimeric form is predominant (by 80%) and increases to 100% when the solubilization is carried on in 5 mM MgCl2 (37).

(ii) Image analysis of two-dimensional crystals of PSII-RC (15) show a dimeric structure for the complex. (iii) Electrophoretic and ultracentrifugation studies indicate that LHCII can be present mainly in two aggregation states whose apparent molecular masses differ by a factor of three (30, 38). Minor chl a/b proteins show the same sedimentation rate as the lower molecular mass form of LHCII (20). Although some authors (30, 38) have suggested that the two aggregation states correspond, respectively, to monomer and trimer, in our opinion this has not been proven. In this paper we show by covalent cross-linking that the lower aggregation state of LHCII as well as the three minor chl a/b proteins, CP29, CP26, and CP24, are in fact trimers, whereas the higher aggregation form of LHCII exhibits an even higher oligomeric state. Although our experiments with a cross-linking agent can hardly indicate the number of LHCII polypeptides present in the latter case, we propose that this number is nine based on ultracentrifugation (30), electrophoretic, and spectroscopic data (39). Our results are consistent with previous structural studies with light-harvesting proteins that have been shown to have a trimeric symmetry such as the bacteriochlorophyll-protein complex of Prosthecochloris aestuarii (40), the c-phycocyanine of Mastigocladus laminosus (41), and the purple membrane of Halobacterium halobium (42). The trimeric symmetry appears to be essential for excitation energy delocalization within the antenna protein and therefore for the efficiency of light-harvesting function (43).

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