Core Antenna Complexes, CP43 and CP47, of Higher Plant Photosystem II. Spectral Properties, Pigment Stoichiometry, and Amino Acid Composition†

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ABSTRACT: The core antenna complexes of photosystem II, CP43 and CP47, were purified from two higher plants by anion-exchange chromatography, using a combination of the chaotropic agent $LiClO_4$ and the nonionic detergent β-dodecyl maltoside. The Q_y transition was resolved at 48 K into two main bands near 682.3 and 671.5 nm for CP43, while the CP47 spectrum showed a more complex structure with main bands at 688, 681.2, 676, 670, 667, and 661 nm. Emission bands (77 K) were detected at 683 and 695 nm for CP43 and CP47, respectively. Fluorescence excitation spectra showed high efficiency of energy transfer between the different transitions of the chlorophylls and a somewhat lower efficiency from β-carotene. The circular dichroism spectrum of CP47 indicated the presence of excitonic interactions between some chlorophylls. In contrast, CP43 showed a single negative circular dichroism band at 670 nm. The pigment content of the complexes was determined by both spectroscopic measurements and HPLC. Contents of 18 chlorophylls *a* and 5 β-carotenes per CP43 polypeptide and 19 chlorophylls *a* and 3 β-carotenes per CP47 polypeptide were found, using the methods of Lowry or Bradford for protein quantitation. When the protein concentration was determined from the amino acid analysis, 20 chlorophylls *a* and 5 β-carotenes per CP43 and 21–22 chlorophylls *a* and 4 β-carotenes per CP47 were obtained. Thus, a content of 46–48 chlorophylls *a* was obtained for the core complex, assuming 4–6 chlorophylls per reaction center, in agreement with the composition obtained experimentally using a highly purified oxygen-evolving core complex. This suggested that no pigments were lost during the purification procedure. Moreover, the amino acid analysis of the purified complexes revealed a high homology with the amino acid composition derived from the gene sequences reported for other higher plants.

Photosystem (PS[1]) II is a membrane protein complex present in all oxygenic photosynthetic organisms. The smaller particle of PSII isolated so far, which is able to retain the primary processes of photosynthesis, i.e., charge separation, quinone reduction, and oxygen evolution, is a particle called the oxygen-evolving core complex (OECC) (Ghanotakis & Yocum, 1986). This particle consists of eight main polypeptides, i.e., D_1 and D_2, the α- and β-subunits of cytochrome (Cyt) b_{559}, the psbI gene product of the reaction center (RC), the core antennae CP43 and CP47, and the 33-kDa extrinsic protein. Many cofactors are also bound to this complex, including chlorophyll (Chl) *a*, pheophytin (Pheo) *a*, β-carotene, quinones, and microelements such as Fe, Mn, Cl, and Ca. Closely associated with the RC are the core antenna complexes, called CP47 and CP43 after their apparent molecular masses determined by polyacrylamide gel electrophoresis (Camm & Green, 1980). These Chl *a*-containing proteins funnel the excitation energy to the RC, although other structural functions have also been ascribed to these proteins. For instance, CP47 was reported to be associated with the extrinsic 33-kDa polypeptide, protecting it from tryptic attack or labeling by the amino group reagent NHS–biotin (Bricker et al., 1988; Bricker, 1990). Mutation of His residues in the CP47 complex led to the destabilization of PSII (Shen et al., 1993). On the other hand, Petersen et al. (1990) demonstrated that the loss of CP43 destabilized the association of Q_A to the CP47–D_1–D_2–Cyt b_{559} complex.

The pigment stoichiometry of the core antenna complexes, CP43 and CP47, is a subject of controversy. Our knowledge of the chromophore content is necessary for precise spectroscopic, biochemical, and functional analyses. Several groups have reported methods leading to the purification of these proteins (van Dorssen et al., 1987; Akabori et al., 1988; Ghanotakis et al., 1989), although different Chl *a* contents were reported for such preparations. Barbato et al. (1991) claimed 9–11 Chl *a* for CP43 and 9–13 for CP47, and Akabori et al. (1988) calculated 11 and 13 Chl *a* for CP43 and CP47, respectively. The possibility of pigment removal during the purification steps of pigment–protein complexes has been

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[1] Abbreviations: Bis-Tris, [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane; Chl, chlorophyll; CD, circular dichroism; DEAE, (diethylamino)ethyl; DM, β-dodecyl maltoside; HPLC, high-performance liquid chromatography; MES, 2-morpholinoethanesulfonic acid; OECC, oxygen-evolving core complex; OD, optical density; Pheo, pheophytin; PS, photosystem; PAGE, polyacrylamide gel electrophoresis; D_1 and D_2, polypeptides of the photosystem II reaction center; RC, reaction center; Q_A, secondary quinone acceptor; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; Cyt, cytochrome.

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documented (Camm & Green, 1980; Akabori et al., 1988; Montoya et al., 1991, 1993). A different approach was used by de Vitry et al. (1984), who employed radiactive labeling techniques in the *Chlamydomonas reindhartii* PSI-less mutant 14.M18; these authors proposed a content of 20–25 Chl *a* for each CP43 and CP47. However, no data are available for β-carotene stoichiometry.

An interesting aspect of the core antenna complexes is the presence of different pools of Chl *a*. Some spectroscopic characteristics of CP47 were described by van Dorssen et al. (1987) and van Kan et al. (1992), using low-temperature absorption and fluorescence spectroscopy, and by de Paula et al. (1990, 1994), on the basis of resonance Raman spectroscopy. Linear dichroism experiments performed by Tapie et al. (1986) on oriented PII membranes and by van Dorssen et al. (1987) on oriented isolated CP47 complex suggested that the 695-nm emission band originated from a transition dipole perpendicular to the plane of the membrane. van Dorssen et al. (1987) also suggested that the Chl responsible for both the linear dichroism signal at 691 nm and the emission band at 693 nm was due to a minor component absorbing at 690 nm.

In the present work, we report the purification of CP43 and CP47 from *Glycine max* and *Beta vulgaris* by a single chromatographic step, using the method of Ghanotakis et al. (1989) with some modifications. Much effort was devoted throughout the work to determine precisely the chemical compositions of the isolated complexes. Absorption, fluorescence, and circular dichroism (CD) spectra were collected to further characterize the preparations.

MATERIALS AND METHODS

Isolation of the Oxygen-Evolving Core Complex. Beta vulgaris L. (v. Monohill) and *Glycine max* (v. Williams) were grown hydroponically in half-Hoagland solution in a growth chamber with 350 μE m^{-2} s^{-1} of light intensity and 70% relative humidity of 24 °C. PSII-enriched membranes were prepared according to Berthold et al. (1981) and solubilized with the nonionic detergent *n*-octyl β-D-glucopyranoside to obtain OECC (Ghanotakis & Yocum, 1986). As we will mention later, the OECC obtained in this way (with a maximum near 674 nm) was often contaminated with CP29, another pigment–protein complex of PSII. Thus, for certain specific experiments CP29 was removed, introducing an additional purification step. To do that, OECC at a Chl *a* concentration of 1.5 mg/mL was mixed with an equal volume of 20 mM Bis-Tris, pH 6.0, and 1% β-dodecyl maltoside (DM), incubated for 20 min, and then subjected to gel-filtration chromatography on a Sephadex G-100 column (1.6 × 105 cm) equilibrated with a buffer containing 20 mM Bis-Tris, pH 6.0, and 0.05% DM. The sample was eluted with the same buffer.

Purification of CP43 and CP47. OECC was first treated as discussed in Ghanotakis et al. (1989) to remove the 33-kDa extrinsic protein. The final pellet was resuspended in 50 mM MES, pH 6.0, 150 mM NaCl, and 400 mM sucrose at 1.5 mg of Chl/mL, mixed with an equal volume of 20 mM Bis-Tris, pH 6.0, 4 M $LiClO_4$, and 15% DM, and incubated at 4 °C for 15 min. After that, the preparation was dialyzed against 20 mM Bis-Tris, pH 6.0, for 2 h. The complex was loaded onto a DEAE-Fractogel TSK 650S anion-exchange column (1.6 × 7.5 cm) previously equilibrated with 20 mM Bis-Tris, pH 6.0, and 0.05% DM. The fraction that eluted first, which did not bind to the column, corresponded to the CP43 complex. When the eluate was colorless, the column was subjected to a 0–175 mM $LiClO_4$ linear gradient at a flow rate of 0.5 mL/min to elute the other fractions. When necessary, the material was concentrated using an Amicon cell unit to an OD of approximately 2 units in the red absorption peak and kept frozen at −80 °C until use.

Spectrophotometric Quantitation of Chl a. Total pigments were extracted with 80% cold acetone at 4 °C in the dark, sonicated for 3 min to favor pigment release from proteins, and centrifuged for 5 min in a microfuge. The Chl concentration in the extract was determined using an extinction coefficient of 75.0 mM^{-1} cm^{-1} at 663 nm (Dawson et al., 1986).

Chl a and β-Carotene Quantitation by HPLC. The concentrations of Chl *a* and β-carotene were determined by the method of de las Rivas et al. (1989). Pigments were extracted with 80% cold acetone and sonicated for 3 min, and the extract was centrifuged for 5 min in a microfuge. All steps were carried out at 4 °C in the dark. Pigments were analyzed by HPLC (Beckman Instruments) using a C-18 reversed-phase Ultrasphere-ODS (5-μm particle size) column (4.6 × 250 mm) (Beckman Instruments). The chromatograms were monitored at 450 nm.

Protein Determination. Protein concentration was determined using the method of Markwell et al. (1981), which is a modification of the method of Lowry et al. (1951), and the method of a Bradford dye reagent protein assay. In both cases, bovine serum albumin was used as a standard. When the method of Markwell et al. (1981) was used, interference of the assay reagents with the Bis-Tris was detected. To eliminate this problem, the sample was dialyzed against 50 mM MES, pH 6.0, and 0.05% DM for 24 h with two buffer changes. No interference of this new buffer with the assay was detected. The Bradford method (Bio-Rad) was used for comparison with the results obtained with the procedure of Markwell et al. because no interference with the buffer Bis-Tris plus DM was detected. To further compare the results, a third method based on the amino acid composition data was used to calculate the protein content. For these calculations, the molecular masses of CP43 and CP47 were estimated to be 52 and 56 kDa, respectively. These molecular masses were taken from the average amino acid compositions derived from the gene sequences of spinach, tobacco, pea, maize, and wheat for CP43 and those of spinach, marchantia, and tobacco for CP47 (Shinozaki et al., 1986; Bricker, 1990).

Determination of Cytochrome Content. Cytochrome was quantified from the dithionite-reduced minus ferricyanide-oxidized absorption spectra using an extinction coefficient of 21 mM^{-1} cm^{-1} at 559 nm (Stewart & Bendall, 1981).

Low-Temperature Spectroscopy. Optical absorption measurements at 48 K were made with a Hitachi U3400 spectrophotometer, equipped with a CTi cryogenic cooler, in 0.3-cm path length cuvettes. Samples were mixed with glycerol (70% (v/v) final concentration) before cooling. For low-temperature emission measurements, samples were prepared in the same way and kept at 77 K with liquid N_2. Samples were excited at 430 nm, and the emission spectra were recorded in a Hitachi F4500 spectrofluorimeter. Fluorescence excitation spectra were carried out at room temperature using rhodamine-B as the photon counter to correct differences in the excitation intensity.

Circular Dichroism. Circular dichroism (CD) spectra were obtained at room temperature with a Jobin-Yvon Mark III dichrograph, fitted with a 250-W xenon lamp. Spectra were recorded at a 0.2 nm/s scanning speed. Samples were in 1-cm path length cuvettes in 20 mM Bis-Tris, pH 6.0, and 0.05% DM.

Amino Acid Composition. Proteins were dialyzed for 48 h in deonized water, lyophilized, and hydrolyzed at 105 °C for 24 and 96 h with 5.7 N HCl containing 0.1% (w/v) phenol and 20 nmol/mL 2-aminohexanoic acid as internal standard, in sealed tubes under vacuum. Hydrolysates were analyzed on a Beckman System 6300 amino acid analyzer with an analogue interface module of the System Gold software. For each hydrolysis time, at least two separate determinations were performed. Half-cystine was determined as cysteic acid after oxidation of the protein with performic acid (Hirs, 1967). Tryptophan was determined spectrophotometrically (Beaven & Holiday, 1952).

Electrophoresis. SDS–PAGE was carried out as described in Laemmli (1970) in a 12.5% acrylamide gel containing 6 M urea in both the resolving and stacking gels. Samples were denatured at room temperature for 1 h in a solution containing 0.05 M Tris-HCl, pH 7.2, 2% SDS, 2 M urea, and 40 mM dithiothreitol. Gels were stained with Coomassie Brilliant Blue.

RESULTS

Purification. Several pigmented fractions were eluted off the anion-exchange column loaded with solubilized OECC (Figure 1). The CP43 complex, which did not bind to the column, eluted first. When the eluate became colorless, the column was subjected to a 0–175 mM $LiClO_4$ linear gradient at 0.5 mL/min, eluting in the following order: CP29, CP47, and a mixture of CP47/RC (Figure 1A). Figure 1B shows the polypeptide content of each chromatographic band.

Absorption and Fluorescence Spectroscopy. The low-temperature absorption spectrum of CP43 at 48 K is shown in Figure 2A. This spectrum presents maxima at 671, 625, 592, 438, and 416 nm due to Chl *a*. A prominent shoulder at 682 nm was also detected. The inset shows a more detailed view of the red spectral region. The presence of two main components in the red region at 682 and 671 nm was also confirmed in the second derivative of the spectrum (Figure 2B). The carotenoid region was well resolved at this temperature, with two peaks at 495 and 466 nm.

The low-temperature absorption spectrum of CP47 at 48 K showed maxima at 675, 625, 592, 438, and 416 nm due to Chl *a* (Figure 3A). It also showed peaks at 503 and 468 nm due to carotenoids. Upon close analysis, a complex structure of the Chl Q_y transition was observed at this temperature, consisting of several overlapping absorption bands, as shown in the inset. The second derivative of the spectrum allows one to differentiate several pools of Chl *a* with maxima at 681.2, 676, 670, 667, and 661 nm (Figure 3B). Using Gaussian deconvolution analysis (Figure 4), the spectrum was fit to several components with maxima at 688, 681.2, 676, 669, 661, and 652.7 nm. Note the absence of the Pheo Q_x transition band near 542 nm from both absorption spectra, as expected for purified antenna complexes.

The fluorescence spectra of CP43 and CP47 at 77 K upon excitation at 430 nm displayed maxima at 683 and 695 nm, respectively (Figure 5). A vibrational sub-band at 763 nm was also observed in CP47, which is similar to that reported earlier (van Dorssen et al., 1987). Fluorescence excitation spectra for both antenna complexes are shown in Figure 6. A comparison between absorption and fluorescence excitation spectra revealed a high efficiency of energy transfer for all Chl transitions in both complexes. However, a similar comparison in the carotenoid region indicated good energy transfer from β-carotene in the case of CP47 but a somewhat

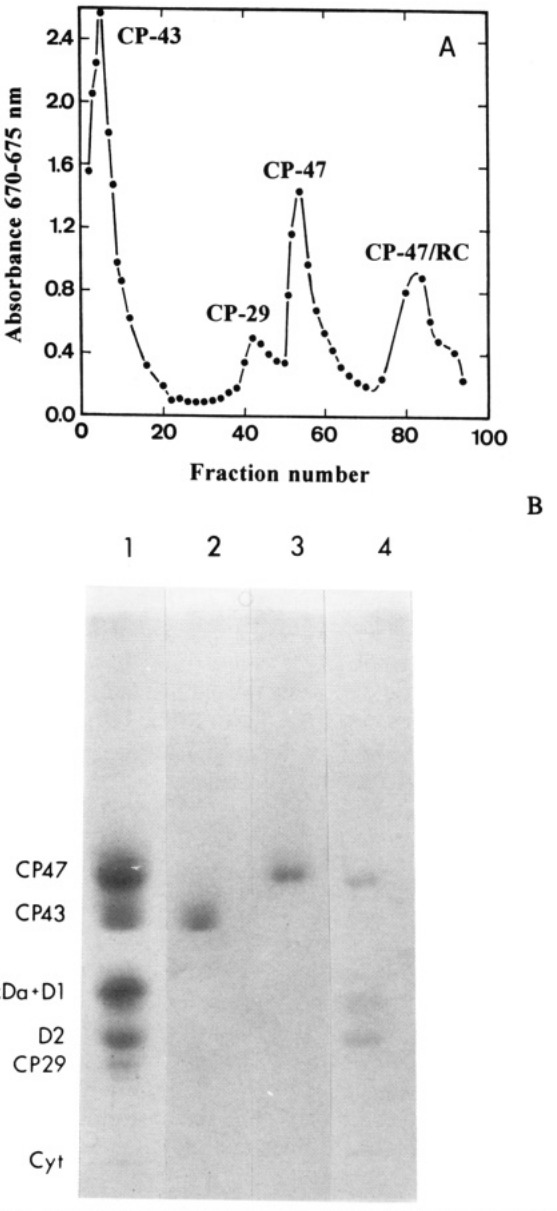

FIGURE 1: (A) Elution profile of anion-exchange chromatography monitored at 671 (CP43), 676 (CP29), and 675 (CP47 and CP47/RC) nm. (B) Electrophoretic pattern of the fractions off the chromatography column in a 12.5% acrylamide gel containing 6 M urea: (1) OECC; (2) CP43; (3) CP47; (4) CP47/RC. Proteins were stained with Coomassie Brilliant Blue.

lower energy transfer in the case of the CP43 complex. The reasons for these differences remain to be tested.

Circular Dichroism. CD spectra of CP43 and CP47 preparations measured at room temperature are shown in Figure 7. The spectrum from the CP43 complex showed a strong negative band at 670 nm, which corresponded to the main Chl *a* absorption band in the red region. Contributions to the spectrum at 438, 405, and 380 nm by Chl *a* and at 456 nm by carotenoids were also observed. In contrast, the CD spectrum of CP47 showed a doublet with negative and positive signs at 680 and 666 nm, respectively. Contributions to the spectrum at 434 and 405 nm by Chl *a* were also detected. However, not much contribution to the spectrum by carotenoids was detected.

Pigment Stoichiometry of CP43 and CP47. Total pigments from OECC and the CP43 and CP47 complexes were extracted and analyzed using reversed-phase HPLC. As shown in Figure 8, the chromatogram for OECC exhibited four main peaks

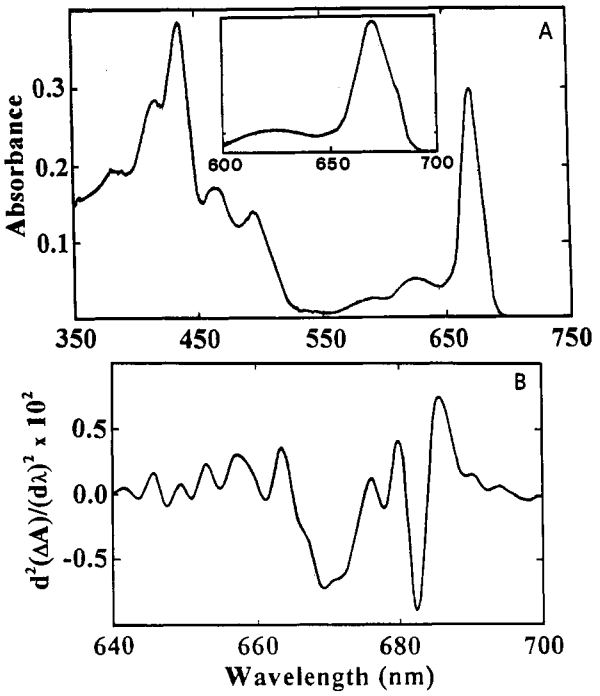

FIGURE 2: (A) Low-temperature (48 K) absorption spectrum of CP43 in 20 mM Bis-Tris, pH 6.0, plus 0.05% DM. The inset shows the 600–700-nm spectral region in more detail. (B) Second derivative of the absorption spectrum in the 640–700-nm range. Samples were mixed with glycerol (70% (v/v) final concentration) before cooling.

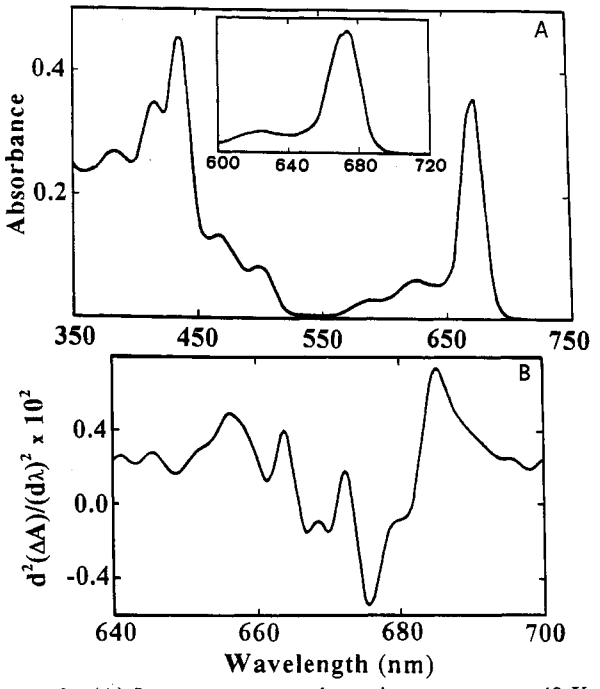

FIGURE 3: (A) Low-temperature absorption spectrum at 48 K of CP47 in 20 mM Bis-Tris, pH 6.0, plus 0.05% DM. The inset shows the 600–720-nm spectral region in more detail. (B) Second derivative of the spectrum in the 640–700-nm range. Samples were mixed with glycerol (70% (v/v) final concentration) before cooling.

attributable to Chl *b*, Chl *a*, Pheo *a*, and β-carotene. A small amount of lutein was also detected. In contrast, the chromatograms from CP43 and CP47 showed only peaks corresponding to Chl *a* and β-carotene. These results agree with the spectroscopic analysis, indicating that Chl *a* and β-carotene are the only pigment species bound to the core antenna polypeptides. The small amounts of Chl *b* and lutein detected in OECC should come from the CP29 complex, a common contaminant of OECC preparations.

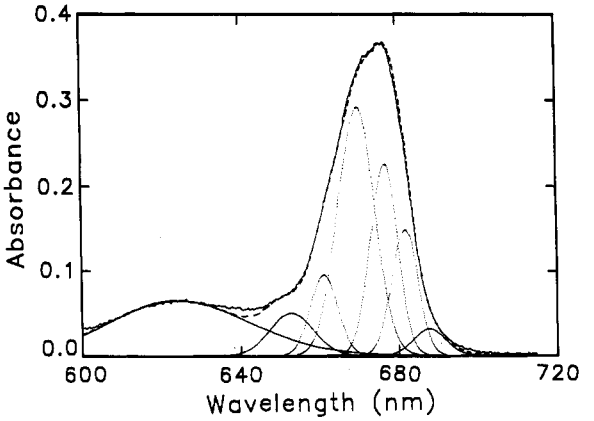

FIGURE 4: Gaussian deconvolution of the red absorption band at 48 K of CP47: solid line, experimental data; dotted lines, deconvoluted Gaussian components and sum of the Gaussian components.

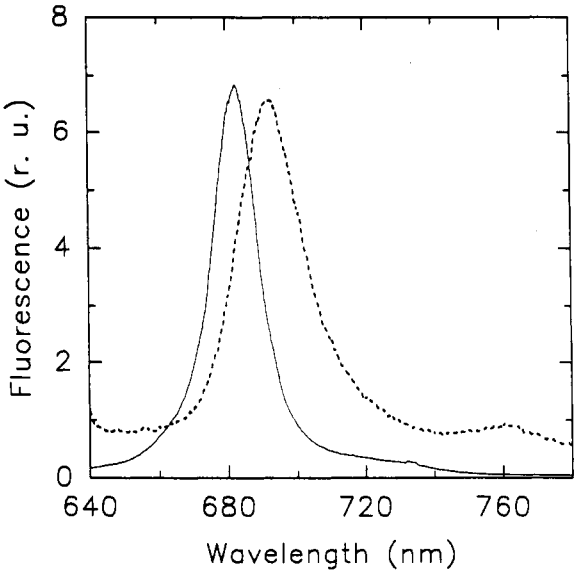

FIGURE 5: Fluorescence spectra of the CP43 and CP47 complexes at 77 K with excitation at 430 nm: solid line, CP43; dotted line, CP47. Samples were prepared in the same way as for low-temperature absorption measurements at a Chl concentration for an OD of approximately 0.1 unit in the red absorption peak.

Using the analytical methods described in the Materials and Methods section, the pigment stoichiometry of these pigment–protein complexes was obtained. Table 1 gives a summary of the results for both plants studied in this work. Using the Lowry and Bradford methods for protein quantitation, contents of 18 Chl's *a* and 5 β-carotenes per CP43 and 19 Chl's *a* and 3 β-carotenes per CP47 were obtained. To do the calculations, molecular masses of 52 and 56 kDa were used for CP43 and CP47, respectively. These molecular masses, which significantly differed from those of 43 and 47 kDa obtained by SDS–PAGE, were obtained from the amino acid compositions derived from the *psbC* (CP43) and *psbB* (CP47) gene sequences from several higher plants, as mentioned in the Materials and Methods section.

Using a different approach, protein content was calculated from the amino acid analysis of each polypeptide. This method eliminates any potential colorimetric interference with the reagents, and thus a more accurate protein determination should, in principle, by obtained. Values of 20 Chl's *a* and 5 β-carotenes for CP43 and 21–22 Chl's *a* and 4 β-carotenes for CP47 were calculated according to this method. This indicated that both the Lowry and the Bradford methods have quite good accuracy in protein quantitation for CP43 and

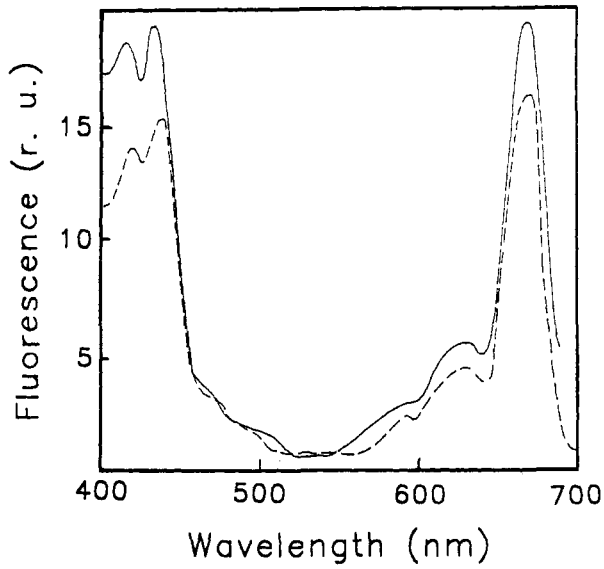

FIGURE 6: (A) Fluorescence excitation spectra of the CP43 and CP47 complexes at room temperature: solid line, CP43; dotted line, CP47. The concentration of Chl used corresponded to that of an OD of 0.1 unit in the red maximum peak. The detection was at 695 nm for CP43 and at 710 nm for CP47.

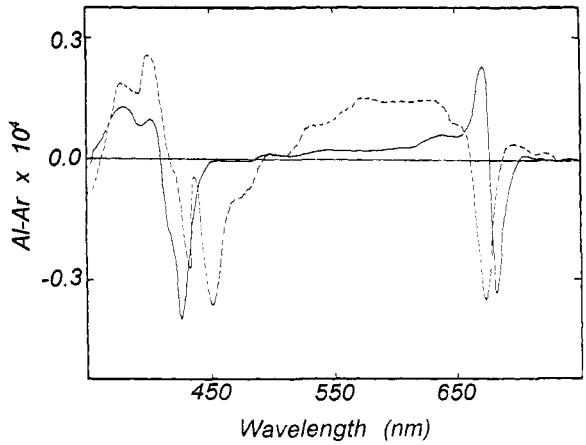

FIGURE 7: Circular dichroism spectra of CP43 and CP47 at room temperature: solid line, CP47; dotted line, CP43. Samples were resuspended in 10 mM Bis-Tris, pH 6.0, and 0.05% DM buffer at a Chl concentration corresponding to an OD of 1.0 unit at the red maximum peak.

CP47 samples, even though a certain overestimation was apparent. From these data, and assuming 4–6 Chl's *a* per RC, we calculated about 46–48 Chl's *a* per OECC, which are very similar to the values reported earlier based on Q_A reduction or cytochrome content (van Dorssen et al., 1987; Bowlby et al., 1988; Barbato et al., 1991). To confirm these data, we further purified OECC to eliminate any contamination by CP29, as described in the Materials and Methods section. A value of 46 Chl's *a* per Cyt b_{559} was obtained in this highly purified particle.

Amino Acid Composition. Table 2 gives the mole percent amino acid composition for both core antenna polypeptides purified from *Glycine max* and the composition derived from the gene sequences of spinach. The results showed good matching between both sets of data, indicating the existence of a high degree of similarity for these antenna complexes in higher plants.

DISCUSSION

Other methods leading to the purification of PSII RC and antenna complexes from higher plants and photosynthetic

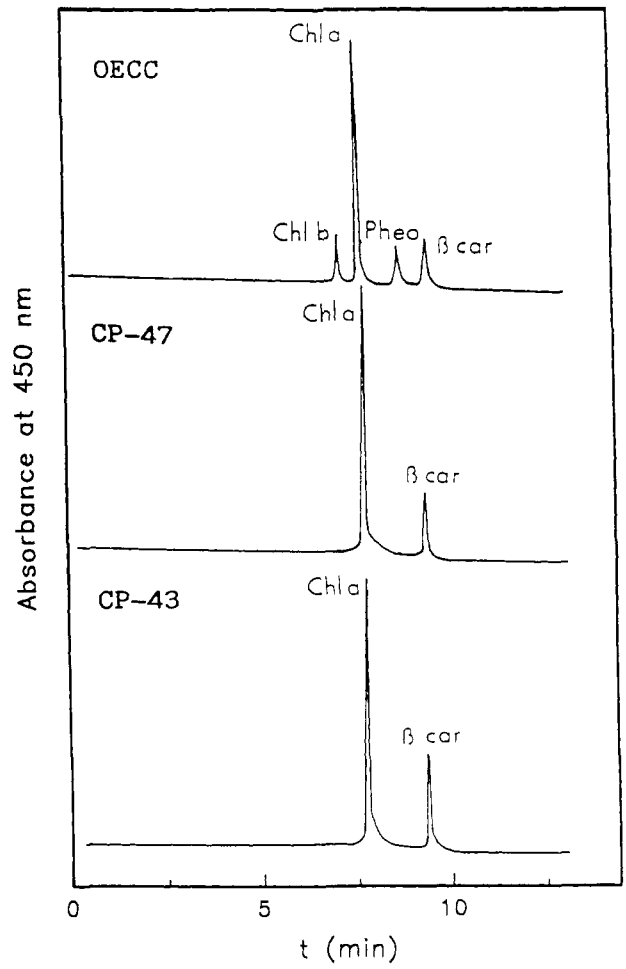

FIGURE 8: HPLC chromatograms of pigment extracts from OECC, CP47, and CP43 samples. Peaks were detected at 450 nm. For more details, see the Materials and Methods section.

microorganisms have been reported using heptyl thioglucoside (van Dorssen et al., 1987), Triton X-100 (Nanba & Satoh, 1987), or a combination of octyl β-D-glucopyranoside and octyl β-D-thioglucopyranoside (Akabori et al., 1988). Ghanotakis et al. (1989) reported a method using the detergent DM that allowed the purification of PSII RC and CP47 complexes. This nonionic detergent is known to give more stable preparations than Triton X-100 (Ghanotakis et al., 1989; McTavish et al., 1989; Braun et al., 1990). We used a one-step chromatographic procedure in the presence of DM to purify CP43 and CP47. Besides, the method also allowed the purification of CP29, another pigment–protein complex of PSII. In order to determine whether this purification procedure caused some absorption spectral modifications of CP43 or CP47 compared to that *in vivo*, we simulated an OECC absorption spectrum, taking into account the spectra of isolated PSII RC and antenna complexes and the pigment content of each complex. Using a stoichiometry of 20 and 22 Chl's per CP43 and CP47, respectively, and 4–6 Chl's per RC complex (Kobayashi et al., 1990; Gounaris et al., 1990; Montoya et al., 1991; Yruela et al., 1994), we obtained the simulated spectrum depicted by a solid line in Figure 9, which looks like the spectrum of OECC obtained experimentally and represented by a dotted line in Figure 9. The second derivatives of both the experimental and simulated spectra are shown in the inset of this figure. The data suggest that the absorption spectral properties of core antenna complexes did not change significantly upon their purification. This is a prerequisite for any detailed structural or functional studies.

Table 1: Pigment Stoichiometry of the Core Antenna Complexes, CP43 and CP47, from *Glycine max* and *Beta vulgaris*[a]

	sample	Chl a/polypeptide Lowry	Bradford	AA analysis	Chl a/β-carotene HPLC	β-carotene/polypeptide
CP-43	*Beta vulgaris*	18.22 ± 0.88	18.66 ± 2.21		3.96	4.66
	Glycine max	17.88 ± 0.21	18.18 ± 0.81	20.03 ± 0.72	3.77	4.78
CP-47	*Beta vulgaris*	19.01 ± 1.37	19.59 ± 1.79		6.16	3.13
	Glycine max	19.19 ± 1.71	17.93 ± 0.44	21.63 ± 0.87	5.44	3.41

[a] Values represent means ± SE (n = 5).

Table 2: Amino Acid Composition of CP43 and CP47 from *Glycine max*[a]

	CP43 mol %	CP43 no. of residues	CP43 no. of residues from sequence	CP47 mol %	CP47 no. of residues	CP47 no. of residues from sequence
Asx	7.12	33	29	7.65	37	31
Thr	4.87	23	25	5.55	27	27
Ser	8.70	40	27	6.91	34	29
Glx	6.85	32	31	7.39	36	32
Pro	4.98	23	22	5.00	24	23
Gly	13.57	63	57	14.13	69	57
Ala	8.40	39	40	8.10	40	41
Val	6.53	30	30	7.23	35	32
Met	1.16	6	7	1.23	6	7
Ile	6.05	28	26	5.01	24	26
Leu	11.57	54	56	9.40	56	57
Tyr	3.02	14	14	3.19	16	15
Phe	6.36	29	34	6.62	32	35
His	1.97	9	12	2.59	13	14
Lys	4.01	18	15	4.07	20	16
Arg	4.83	22	22	5.92	29	23
Trp		14	14		9–10	17
Cys	0.58	3	3	0.61	3	3

[a] Number of residues was calculated from the derived amino acid sequence (Bricker, 1990).

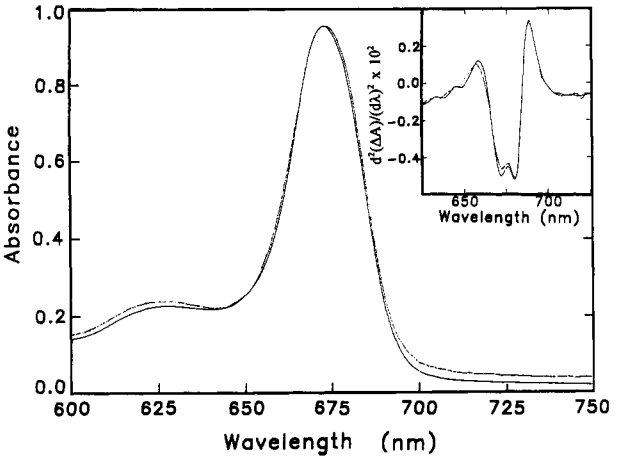

FIGURE 9: Comparison between the experimentally measured absorption spectrum of isolated OECC (dotted line) and the simulated absorption spectrum taking into account the spectral shapes and Chl contents of CP43, CP47, and PSII RC complexes described in the Results section (solid line). Inset: Second derivative of the measured (dotted line) and simulated (solid line) spectra.

The presence in the CP43 complex of a small band around 682 nm and a main one at 671 nm might indicate a partial denaturation of the protein. However, the fluorescence spectrum showed a main single band at 683 nm, which indicated that the Chl absorbing at 671 nm was connected, for energy transfer, to that absorbing at 682 nm. In the case of denaturation of the protein complex, which would produce highly fluorescent unconnected Chl absorbing at around 670 nm, the fluorescence maximum will be near 672 nm (Seibert et al., 1988; Braun et al., 1990; Hoff & Amesz, 1991; G. Montoya, R. Cases, R. Rodríguez, and R. Picorel, submitted to *Biochemistry*). On the other hand, the characteristic CD doublet observed in CP47 indicates that some Chl's are excitonically coupled within the complex. CD activity is very sensitive to small changes in the distance and/or orientation of the chromophores as a consequence of protein structure modification. Indeed, small changes in the protein structure induced the loss of CD activity in the red region in PSII RC (Braun et al., 1990; He et al., 1990; G. Montoya, R. Cases, R. Rodríguez, and R. Picorel, submitted to *Biochemistry*). Thus, CD spectra of the CP47 complex confirm the intactness of the preparation described in the present work.

HPLC pigment analyses established without a doubt that Chl *a* and β-carotene are the only pigment species present in CP43 and CP47. However, Chl *b* and lutein were detected in OECC, most probably due to the presence of variable amounts of CP29, as detected in the ion-exchange chromatography. We have calculated contents of 20 Chl's *a* and 5 β-carotenes for CP43 and 21–22 Chl's *a* and 4 β-carotenes for CP47. This is in agreement with de Vitry et al. (1984) but is at odds with other reports (Akabori et al., 1988; Barbato et al., 1991). As mentioned in the Results section, pigment losses during the purification procedure and/or some error in the protein determination or polypeptide mass estimation might be potential causes for the discrepancies. To be certain of the pigment–protein stoichiometry, we used a protein determination based on amino acid analyses, a polypeptide molecular mass calculation based on gene sequences, and a pigment determination based on HPLC analyses. From our data, a content of 46–48 Chl's *a* per OECC (CP43–CP47–RC complex) is obtained assuming 4–6 Chl's *a* per RC. This was experimentally confirmed in the present work using OECC devoid of any CP29 complex. Thus, the stoichiometry given for CP43 and CP47 is sound.

The amino acid compositions of both CP43 and CP47 presented in this report are in good agreement with the amino acid composition derived from gene sequences in spinach. This proves the high degree of similarity between the core antenna complexes in higher plants and is consistent with a recent report (Kulkarni et al., 1993) that compared the *psbB* gene (coding for CP47) sequence from *Synechococcus* PCC 7942 with those of algae, higher plants, and other cyanobacteria. A degree of conservation higher than 70% was always found. This fact, together with the similar pigment–protein stoichiometries found for *Beta vulgaris* and *Glycine max*, indicates that these two polypeptides are highly conserved in all oxygenic photosynthetic organisms.

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