Heterogeneity and Pigment Composition of Isolated Photosystem II Reaction Centers[†]

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ABSTRACT: Photosystem II reaction centers (RC) isolated from peas (*Pisum sativum* L) purified by ion-exchange chromatography were shown, by high-performance liquid chromatography (HPLC) size-exclusion analyses, to consist of a mixture of monomers (180 ± 20 kDa) and dimers (390 ± 35 kDa). Both fractions were resolved and purified by sucrose density gradient centrifugation and their homogeneity was demonstrated in size-exclusion HPLC elution profiles. Also present in the nonresolved preparation and the monomeric fraction were low levels of CP47 apoprotein (1.8% and 0.9% apoprotein of that found in a CP47-RC preparation). This CP47 contamination could maximally account for 0.41 and 0.22 Chl/RC, respectively, based on 22 chlorophylls being bound to each CP47 protein. The level of CP47 apoprotein was undetectable in the dimeric fractions. Pigment analysis using reverse-phase HPLC confirmed that contamination by chlorophyll bound to the CP47 apoprotein in the nonresolved RC preparation was low and that the ratio of chlorophyll *a* to pheophytin *a* remained 6 when the preparation was separated into its monomeric and dimeric components. We conclude, in agreement with earlier work, that the reaction center of PSII, when isolated using mild detergents and ion-exchange chromatography, contains 6 chlorophyll *a*/2 pheophytin *a*. We therefore do not concur with the recent published work of Pueyo et al. [(1995) *Biochemistry 34*, 15214−15218) that this type of preparation contains 4 chlorophyll *a*/2 pheophytin *a* and that the remaining 2 chlorophyll *a* are due to contamination by CP47.

The reaction center (RC)[1] of photosystem II (PSII), consisting of the D1 and D2 polypeptides, α- and β-subunits of cytochrome b_{559}, and the *psb*I gene product, has been isolated from spinach (Nanba & Satoh, 1987), pea (Barber et al., 1987), wheat (Ikeuchi & Inoue, 1988), *Synechocystis* 6803 (Giorgi et al., 1996), *Beta vulgaris* (Montoya et al., 1991), and *Chlamydomonas reinhardtii* (Alizadeh et al., 1995). Despite substantial interest in the structure and function of the isolated PSII RC, its pigment composition is still a matter of controversy. A correct knowledge of the pigment composition within the complex is indispensable in discussing the mechanism of PSII photochemistry. In addition, such knowledge would shed light on the fundamental question of the extent of homology at the molecular level which exists between PSII and the RC of purple photosynthetic bacteria (Michel & Deisenhofer, 1988).

Two main sources of confusion regarding the pigment stoichiometry of the isolated PSII RC may be due to the use of different preparation procedures (especially the employment of different detergents) and pigment quantification methods. Nanba and Satoh (1987) and Barber et al. (1987), using reverse-phase HPLC, reported the chlorophyll *a* (Chl *a*):β-carotene (β-Car):pheophytin *a* (Pheo *a*) molar ratio of the isolated complex to be 4−5:1:2. These early preparations of the reaction centers, isolated and suspended in the presence of the nonionic detergent Triton X-100, were, however, unstable, showing a high susceptibility to photoinduced damage (Chapman et al., 1988; Seibert et al., 1988). The approaches used to overcome this problem of instability included exchanging the Triton X-100 after the purification of the PSII RC with milder detergents, e.g., *n*-dodecyl β-D-maltoside (DM) (Chapman et al., 1989; Seibert, 1993). These stable PSII preparations were found to contain the molar ratio 6 Chl *a*:2 β-Car:2 Pheo *a* (Kobayashi et al., 1990; Gounaris et al., 1990; Montoya et al., 1991; Eijckelhoff & Dekker, 1995). However, it has also been reported that the chromophore stoichiometry of RC preparations depends on the extent of the washing procedure during preparation. Montoya et al. (1991) obtained two types of RC preparations with Chl *a*:β-Car:Pheo *a* ratios 6:2:2 after short washing and 4:1:2 after long washing with a buffer containing Triton X-100. The authors suggested that the extra pigments (2 Chl *a* and 1 β-Car) removed by the extensive washing corresponded to those located on or close to the surface of the protein complex and thus were more easily removed by the more vigorous washing procedure. The removal of 2 Chl molecules by this washing procedure was not confirmed in other laboratories (Eijckelhoff & Dekker, 1995; our unpublished results) although the removal of one β-Car was. Analyzing more recently the "6 Chl" RC, isolated from three different species by sieve chromatography, Aured et al. (1994) found them to be heterogeneous, containing three fractions with Chl *a*:2 Pheo *a* ratios of 9.9, 4, and 4.1, respectively. It was concluded from these results that the RC itself is associated with 4 Chl *a*/2 Pheo *a* molecules and

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[1] Abbreviations: BBYs, photosystem II enriched membranes isolated according to Berthold et al. (1981); β-Car, β-carotene; Chl, chlorophyll; DM, *n*-dodecyl β-D-maltoside; HPLC, high-performance liquid chromatography; Pheo, pheophytin; PSII; photosystem II; RC, reaction center(s); SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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that the higher pigment ratio reported from other laboratories is due to a contamination of the preparation. Using immunoaffinity chromatography to purify further the "6 Chl" RC, Pueyo et al. (1995) suggested that the source of chlorophyll contamination is CP47, a Chl *a*-containing protein very closely associated with PSII RC. As a result, the conclusion has been drawn that the pigment stoichiometry of 6 Chl *a*:2 Pheo *a*, published by several groups (Kobayashi et al., 1990; Gounaris et al., 1990; Montoya et al., 1991; Eijckelhoff & Dekker, 1995) is incorrect and that this invalidates the interpretation of many studies of PSII photochemistry using this isolated complex.

In this report we present detailed analyses of the heterogeneity and pigment composition of PSII RC isolated from pea (*Pisum sativum* L.) using ion-exchange chromatography by the method of Chapman et al. (199). We show that the immunodetected CP47 contamination of these RC could not contribute to the two extra chlorophylls as is claimed by Pueyo et al. (1995) and that the preparation consists of a mixture of monomeric and dimeric complexes.

MATERIALS AND METHODS

Preparation of PSII RC and Monomeric and Dimeric RC. PSII RC complexes were isolated from chamber-grown peas (*Pisum sativum* L.) by ion-exchange chromatography (Chapman et al., 1991). This preparation was resolved to monomeric and dimeric RC by sucrose density gradient centrifugation. To prepare sucrose gradients, centrifuge tubes were filled with a sucrose gradient mix solution (50 mM Tris/HCl, pH 7.2, 0.5 M sucrose, and 2 mM DM) and frozen at −20 °C. Slow thawing at 4 °C resulted in the formation of a sucrose density gradient. The isolated RC (typically 50 μg of Chl) were then loaded onto the gradients and centrifuged overnight at 35 000 rpm and 4 °C in a Beckman SW41 swing rotor.

Room-temperature absorption spectra were measured using an SLM Aminco (Urbana, IL) Model DW2000 spectrophotometer.

Preparation of CP47−RC Complex. CP47−RC complex was isolated from BBY-type PSII enriched membranes (Berthold et al., 1981). BBYs (1 mg of Chl/mL) were solubilized with DM (1:10 w/w) and heptyl thioglycopyranoside (2.7%) at room temperature for 30 min. For purification the solubilized BBYs were loaded on a Fractogel ion-exchange column (DEAE-Toyopearl 650S, TSK) which had been equilibrated with 50 mM Tris/HCl (pH 7.4) and 2 mM DM. The sample was washed with 50 mM Tris/HCl (pH 7.4), 2 mM DM, and 30 mM NaCl and was eluted with the same buffer but using a 30−150 mM NaCl gradient. The CP47−RC complex eluted at about 70 mM and its purity was checked by the absorption spectrum and by SDS−PAGE.

Reverse-Phase HPLC Pigment Analysis. Pigments were extracted into 80% (v/v) acetone:H_2O at 4 °C under dim light conditions. The samples were vortexed for 30 s, centrifuged for 2 min in a microfuge in order to be depleted of proteinaceous materials, and filtered through a 0.2-μm (pore size) membrane [poly(vinylidene difluoride), Whatman] before injection (injection volume was 20 μL). The pigments were resolved using an ODS-1 Spherisorb column (Anachem) and isocratic elution with methanol/ethyl acetate/water 68:30:2 (vol/vol/vol), at a flow rate of 1 mL/min. We used a variable-wavelength detector (Kontron 30), detecting simultaneously at 663 nm (for Chl *a* and Pheo *a*) and 453 nm (for β-Car). Chl *a* and β-Car quantification was performed after calibration of the corresponding peaks with pigment standards (purchased from Sigma), whose concentrations were determined using extinction coefficients 76.79 mM^{-1} cm^{-1} at 663.6 nm in 80% acetone for Chl *a* (Porra et al., 1989) and 139 mM^{-1} cm^{-1} at 452 nm in 100% hexane for β-Car (Zechmeister & Polgar, 1943). The Pheo *a* standard used for calibration was produced by acidifying the Chl *a* standard with 2 mM HCl. Alternatively, the Chl *a*:Pheo *a* ratio was determined by measuring the Pheo *a* peak area before and after acidification of the 80% acetone extract of RC pigments (approximately 11 μg of Chl *a*/mL) with 2 mM HCl.

HPLC Size-Exclusion Analysis. HPLC size-exclusion analysis was carried out using a Zorbax GF-450 column 9.4/250 mm (Jones Chromatography). The mobile phase consisted of 50 mM Tris/HCl, pH 7.2, 0.3 M sucrose, and 0.03% *n*-dodecyl β-D-maltoside (DM) and was passed through the column at a rate of 0.5 mL/min. The temperature was kept at 4 °C using a column chiller (Jones Chromatography). The 20-μL samples contained 5 μg of Chl *a*. The elution profiles were monitored at 418 and 280 nm.

SDS−PAGE and Western Blotting. The polypeptide composition of the isolated PSII RC preparations was analyzed by gradients SDS−PAGE (10−17% polyacrylamide) containing 6 M urea, essentially using the method of Laemmli (1970). The gels were stained with Coomassie R-250.

The protein profiles resolved by SDS−PAGE were transferred onto nitrocellulose (Dunn, 1986) and immunolabeled with a CP47-specific antibody raised against electrophoretically purified spinach CP47 (a kind gift from Dr. R. Barbato) or C-terminal D1-specific antibody (Dupont 304) raised against a synthetic polypeptide, homologous to the 29 amino acids of the C-terminal pea D1 precursor (a kind gift from Dr. P. Nixon). Biotinylated anti-rabbit IgG was used as a secondary antibody and was labelled with Extravidine−alkaline phosphatase conjugate (Sigma). The chromogenic substrates were 5-bromo-4-chloroindolyl phosphate *p*-toluidine salt and nitro-blue tetrazolium chloride. The gels and immunoblots were scanned and the density of the bands was determined using a Hirschmann ELS 400 V2.0 densitometer (Scanning Program OSU 406 v1.4, 2 D evaluation software V2.7).

RESULTS

Heterogeneity and Protein Composition of Isolated PSII Reaction Centers. RC complexes, isolated from pea PSII-enriched membranes by ion-exchange chromatography (Chapman et al., 1991) were subjected to gel-permeation chromatography and shown to consist of two populations (Figure 1A). Using a gel calibration kit (Pharmacia) the molecular masses of these two populations of particles were calculated to be 180 ± 20 kDa and 390 ± 35 kDa based on the analyses of samples derived from at least three independent preparations. The two fractions were resolved and purified by sucrose density gradient centrifugation (Figure 2) and their homogeneity demonstrated by size-exclusion HPLC elution profiles (Figure 1B,C). The SDS−PAGE protein profiles of the two populations (Figure 3, lanes 3 and 4) were very

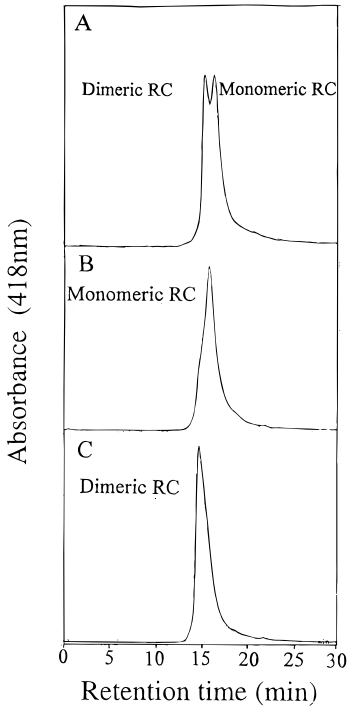

FIGURE 1: Size-exclusion HPLC elution profiles of (A) isolated PSII RC, (B) monomeric RC, and (C) dimeric RC.

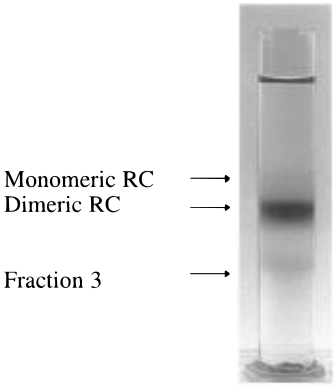

FIGURE 2: Sucrose density gradient of PSII RC, isolated by the method of Chapman et al. (1991). The details of the sucrose density gradient centrifugation are given in the Materials and Methods section.

similar, consisting of D1, D2, cytochrome b_{559}, and the D1/D2 heterodimer. The subunit composition and molecular mass determination suggests that the 390- and 180-kDa complexes correspond to dimeric and monomeric RC, respectively. A similar value for the molecular mass of monomeric RCs has been reported by Tang et al. (1990).

A very low proportion of the total chlorophyll in the sucrose gradient was associated with a third fraction (fraction 3, Figure 2) having a mass greater than that of the dimer and only detected under high loading conditions. The protein composition of this fraction is shown in Figure 3, lane 5. The size-exclusion HPLC profile of this population showed that it was not homogenous, consisting of a CP47−RC complex, monomeric and dimeric RC, and several fractions with smaller molecular weights (data not shown). In addition, fraction 3 was not retained by Amicon membrane with a 100-kDa cutoff. These data suggest that fraction 3 is formed by aggregation of different particles during the preparation procedure and is a very minor contaminant in the nonseparated RC preparation.

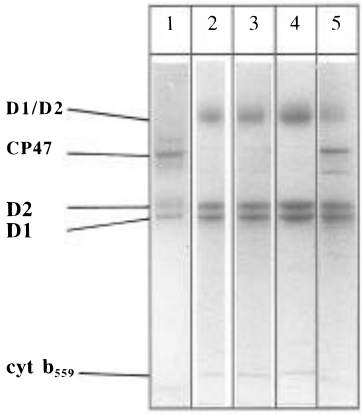

FIGURE 3: SDS−PAGE protein profiles of (lane 1) CP47−RC complex, (lane 2) isolated nonseparated PSII RC, (lane 3) monomeric RC, (lane 4) dimeric RC, (lane 5) fraction 3. Loading amount was 2.5 μg of Chl. The gels were stained with Coomassie R-250.

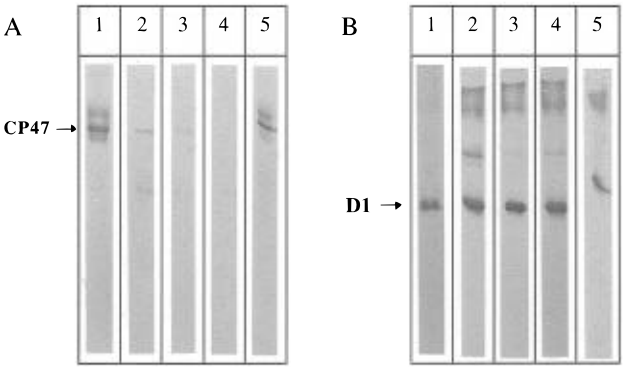

FIGURE 4: CP47 (A) and D1 (B) immunoblots of (lanes 1) CP47−RC complex, (lanes 2) isolated nonseparated PSII RC, (lanes 3) monomeric RC, (lanes 4) dimeric RC, (and lanes 5) fraction 3. Loading amount was 1 μg of Chl.

Quantification of CP47 Contamination of the Isolated PSII RC. As can be seen in Figure 3, no CP47 contamination could be detected by Coomassie staining after SDS−PAGE of the nonseparated preparation or in the dimeric and monomeric fractions. In contrast, CP47 contamination was observable in fraction 3 when it was loaded on an SDS gel with a chlorophyll level comparable to that of the RC preparations. Lane 1 in Figure 3 gives a reference for staining of CP47 relative to the D1 and D2 proteins in the isolated CP47−RC complex. When immunolabeling was carried out, a very faint band of CP47 was, however, detected in nonseparated PSII RC preparations (Figure 4A, lane 2) and in the monomeric fraction (Figure 4A, lane 3), indicating some contamination by this protein. As expected, CP47 was detectable in fraction 3 (Figure 4A, lane 5) when it was loaded with Chl and D1/D2 protein levels equivalent to that for the RC lanes (see Figure 4B).

To quantify the CP47 contamination of our preparations, the CP47/D1 stoichiometry was calculated using the stain density of the bands of CP47 and D1 in the immunoblots (Figure 4 and Table 1). Comparing the CP47:D1 ratio of PSII, RC, monomeric, and dimeric RC, and CP47−RC complex, we conclude that the nonseparated PSII RC (referred to as PSII RC in Table 1) preparation contains 1.8% of CP47 associated with the CP47−RC complex (assuming CP47:D1 a stoichiometry of 1:1 in this isolated complex).

Room-Temperature Absorption Spectra and Pigment Stoichiometry. Figure 5 shows the room-temperature absorption

Table 1: Comparison of CP47:D1 Ratios in Various Preparations[a]

preparation	CP47:D1 × 1000 (density units)	% of CP47:D1 of CP47 RC complex
CP47 RC	43.00	100.0
PSII RC	0.79	1.8
monomeric RC	0.39	0.9
dimeric RC	0	0
fraction 3	19	44.0

[a] The ratios were calculated from the density of the bands detected in CP47 and D1 (C-terminal) immunoblots (Figure 4).

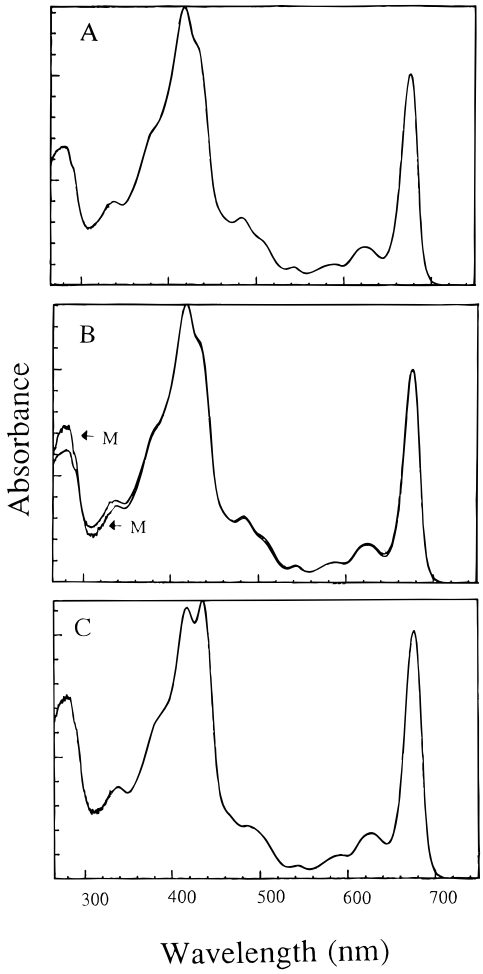

FIGURE 5: Absorption spectra of (A) isolated nonseparated PSII RC, (B) monomeric (M) and dimeric RC, and (C) fraction 3.

spectra of (A) nonseparated PSII RC, (B) monomeric (indicated by M) and dimeric RC, and (C) fraction 3. The spectra of nonseparated PSII RC and monomeric and dimeric RC look almost identical with the red band peaking at 675.5 nm (Q_y transitions of Chl *a* and Pheo *a*), a small band at 543 nm (Q_x transition of Pheo *a*), β-Car absorption in the 510−450-nm region, a band at 435 nm (Soret transition of Chl), and a band at 416 nm (the Chl *a* vibronic band and the Soret transition of Pheo *a* and oxidized cytochrome b_{559}). A very small difference in the β-Car absorption region was observed between the spectra of monomeric and dimeric RC, which shows a slightly lower β-Car content in the monomeric than in dimeric RC (Figure 5B). The absorption spectra of monomeric and dimeric RC have A_{418}/A_{280} ratios 1.8 and 2.1, respectively. This difference reflects a higher level of proteins not binding pigments in the monomeric fraction, most probably degradation products. The absorption spectrum of fraction 3 (Figure 5C) possesses a distinct and pronounced 435-nm band, reflecting a higher Chl *a* content due to the presence of CP47 (Figure 3, lane 5; Figure 4, lane 5; Table 1). However, we have to emphasize that fraction 3 contained a very low level of the total chlorophyll of nonseparated PSII RC loaded onto the sucrose gradients (Figure 2). By calculating what the contribution of CP47 contamination is to the pigment stoichiometrics of the nonseparated PSII RC we conclude that it could maximally increase the Chl *a*:2 Pheo *a* ratio by 0.26 or 0.41, depending on whether CP47 contains 14 (Akabori et al., 1988; Kwa et al., 1992; Chang et al., 1994; our unpublished data) or 22 molecules of Chl *a* (Alfonso et al., 1994), respectively, and that all these chlorophyll molecules are bound to the apoprotein detected in the immunoblots. These calculations indicate that the CP47 Chl *a* contamination of the nonseparated PSII RC can maximally be 0.41 by Chl of 6.07 Chl and is in agreement with the analytical data obtained by reverse-phase HPLC pigment analyses (Table 2).

Table 2: Pigment Stoichiometry Obtained by Reverse-Phase HPLC[a]

preparation	Chl *a*:β-Car:Pheo *a* (using HPLC calibration)			Chl *a*:2 Pheo *a* (using "areas" method)
	Chl *a*	β-Car	Pheo *a*	
PSII RC	6.01 ± 0.23	1.75 ± 0.24	2	6.07 ± 0.28
monomeric RC	5.77 ± 0.19	1.20 ± 0.27	2	5.78 ± 0.23
dimeric RC	5.73 ± 0.15	1.75 ± 0.17	2	5.72 ± 0.19
fraction 3	9.20 ± 0.46	1.65 ± 0.23	2	9.50 ± 0.37

[a] Values are based on the analyses of samples taken from three independent RC preparations.

The monomeric and dimeric RC, purified by sucrose density gradient centrifugation, have a slightly lower Chl *a*:2 Pheo *a* ratio than the mixed PSII RC preparations from which they were isolated. This is due to the separation of fraction 3 which contains most of the contaminating CP47. However, there is no difference between the Chl *a*:2 Pheo *a* ratios of monomeric and dimeric RC in spite of a very small amount of CP47 apoprotein detected in the monomeric RC.

As the extinction coefficients used could influence significantly the estimation of the pigment stoichiometry, we determined the Chl *a*:2 Pheo *a* ratio by a method independent of any extinction coefficients. The Chl *a* associated with the isolated PSII RC was completely pheophytinized by acidification with 2 mM HCl (Figure 6) and the ratio between Pheo *a* peak areas before and after pheophytinization was used to calculate the Chl *a*:2 Pheo *a* stoichiometry. For all the preparations discussed here this ratio is close to 4 and the Chl *a*:Pheo *a* ratio is 6:2 (see Figure 6). The data obtained by using this method (referred to as the "areas" method) are presented in the last column of Table 2. The correlation between HPLC peak areas at 663 nm of different concentrations of standard Chl *a* and the corresponding peak areas of Pheo *a*, obtained after Chl *a* pheophytinization and monitored at the same wavelength, was linear which indicates that the "areas" method is reliable for determining the Chl *a*:2 Pheo *a* ratio. A comparison of the data calculated using HPLC calibration and the "areas" method (Table 2) shows that the difference is less than 1%.

DISCUSSION

Despite the claims of others (Pueyo et al., 1995), our work confirms earlier (Kobayashi et al., 1990; Gounaris et al.,

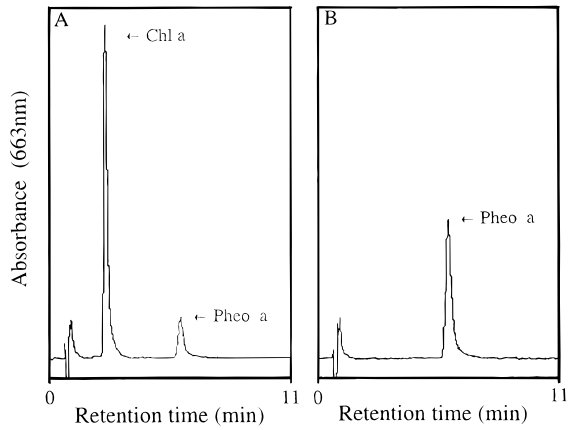

FIGURE 6: Reverse-phase HPLC elution profiles, monitored at 663 nm, of (A) nonseparated PSII RC pigment extract in 80% acetone and (B) PSII RC pigment extract in 80% acetone and pheophytinized with 2 mM HCl.

1990) and more recent (Eijckelhoff & Dekker, 1995) analyses that the pigment content of the reaction center of PSII (D1/D2/cytochrome b_{559} complex) isolated by ion-exchange chromatography using *n*-dodecyl β-D-maltoside (Chapman et al., 1989, 1991) or other methods (Eijckelhoff & Dekker, 1995) has a stoichiometry of 6 Chl *a*:2 β-Car:2 Pheo *a*. A similar conclusion has also recently been reached by Eijcklehoff, van Roon, Groot, van Grondelle, and Dekker (personal communication). We have no evidence to suggest that our preparations contain sufficient contamination of CP47 to invalidate this conclusion. Using size-exclusion chromatography, however, we have shown that the PSII reaction center preparation is heterogeneous, containing monomeric and dimeric populations. Separation of these populations was achieved by sucrose density centrifugation, which also yielded a third fraction containing CP47. Thus a minor amount of CP47 does exist in this type of preparation as argued by Pueyo et al. (1995), but in our preparations contamination by the apoprotein is less than 2% of that found in the CP47−RC preparation and could only account for a maximum of 0.41 Chl *a* molecule/RC. This also assumes that CP47 still retains 22 chlorophylls (Alfonso et al., 1994). Moreover, this contamination by CP47 is undetectable in dimeric PSII RC fractions isolated by sucrose density centrifugation. In agreement with this, direct pigment analyses confirm that the ratio of 6 Chl *a*:2 Pheo *a* holds for monomeric and dimeric fractions as well as for the nonseparated PSII RC. Therefore our conclusion is consistent with earlier rigorous pigment analyses made on this complex (Kobayashi et al., 1990; Gounaris et al., 1990) and by the recent careful reconstruction of the absorption spectrum of the PSII RC by Eijckelhoff and Dekker (1995).

Further support for the 6 Chl *a*:2 Pheo *a* stoichiometry comes from the recent work of Vacha et al. (1995), who devised a procedure for isolating a modified form of a PSII RC which involved the use of a Cu affinity rather than an ion-exchange column. When eluted from this column, the complex was found to have a pigment stoichiometry of 5 Chl *a*:1 β-Car:2 Pheo *a*. The reduction of the pigment level gave expected changes in the room temperature and 77 K absorption spectra. Notably, compared with the 6 Chl *a* preparation, there was a red shift in the Q_y band to about 677 nm at room temperature and a distinct change in the ratio of 670/680-nm absorption peaks at 77 K. These spectral changes are consistent with the removal of 1 Chl *a* molecule absorbing at about 670 nm. The 5 Chl *a*/2 Pheo *a* preparation was photochemically active showing identical kinetics for charge separation to that of the 6 Chl *a*/2 Pheo *a* RC complex. However, when the complex was excited at 665 nm, it was clear that the extent of an energy transfer step attributed to secondary chlorophylls near the periphery of the complex had been halved in the 5 Chl *a*/2 Pheo *a* RC. All these data were consistent with removal of one light-harvesting Chl *a* molecule which absorbs at about 670 nm. Thus, it seems that the 6 Chl *a*:2 Pheo *a* ratio can be lowered in the isolated PSII RC but this reduction in chlorophyll level is not due to removal of contaminating CP47. By comparison of the similarities and differences of the primary structures of the L and M subunits of the reaction center of purple bacteria and the D1 and D2 proteins of PSII, it seems likely that the two additional chlorophylls may be bound to D1 and D2 His118 residues which are fully conserved in these proteins but not found at all in the L and M subunits. These two histidine residues are located in the putative second transmembrane segments of the D1 and D2 proteins (Michel & Deisenhofer, 1988) and thus toward the periphery of the complex. They may, for example, play a role in mediating energy transfer from the secondary antenna complexes CP47 and CP43 to the reaction center.

The finding that isolated reaction centers are predominantly dimeric (see Figures 1 and 2) is intriguing and may indicate that this is a preferred conformational state *in vivo*. Indeed, monomerization of the dimeric RC was observed after treatment with Triton X-100, high DM concentrations (above 5 mM), or exposure to high light intensities (Zheleva et al., 1995). These observations therefore suggest that the monomeric RC originates from a dimeric form of the RC. Interestingly, the 5 Chl *a*/2 Pheo *a* RC complex obtained from Cu affinity chromatography consisted only of monomeric complexes (Zheleva et al., 1995). The relevance of the monomeric/dimeric state of the isolated RC complex to function and stability needs to be addressed further and related to the increasing amount of evidence which suggests that functional PSII may exist as a dimer *in vivo* (Rögner et al., 1996).

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