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ABSORPTION AND FLUORESCENCE PROPERTIES OF HIGHLY ENRICHED REACTION CENTER PARTICLES OF PHOTOSYSTEM I AND OF ARTIFICIAL SYSTEMS

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Abstract—The absorption and fluorescence characteristics of subchloroplast particles highly enriched in P700 (1 P700 to 10–15 chlorophyll *a* molecules) and of two artificial systems are presented here. The fluorescence characteristics measured were excitation and emission spectra, and the degree of polarization of fluorescence. The model systems studied were chlorophyll *a* and pheophytin *a* in a polystyrene matrix, and a colloidal mixture of these two pigments with bovine serum albumin. Effects of 0.5% sodium dodecyl sulfate on the optical properties of the P700-enriched particles are also described. The importance of pigment–pigment and pigment–protein interactions in determining the fluorescence properties of these particles are discussed. The possible role of pheophytin in these preparations needs further investigation.

INTRODUCTION

The role of photosystem I reaction center (P700) in photosynthesis has been reviewed by Ke (1973). The detailed steps involved in system I photochemistry are little known and must be obtained from purified reaction center preparations as has been possible for bacterial systems (Rockley *et al.*, 1975; Kaufmann *et al.*, 1975). Various attempts to obtain preparations enriched in P700 have been described (Boardman, 1970; Sane and Park, 1970; Vernon *et al.*, 1971; Nelson and Racker, 1972; Wessels and Brochert, 1974; Ke *et al.*, 1975; Thornber, 1975). To date, the most enriched preparation is that of Ikegami and Katoh (1975) with a P700:chlorophyll *a* ratio of 1:8.

Another approach has been to study *in vitro* models for reaction center chlorophylls (Norris *et al.*, 1971; Katz and Norris, 1973; Fong, 1975; Shipman *et al.*, 1976; Boxer and Closs, 1976). The reaction center complex is suggested to be a "special" dimeric adduct of two chlorophyll *a* molecules with two water molecules. The role of pheophytin *a* has not yet been considered here, although bacteriopheophytin has been implicated as the "primary" electron acceptor in bacterial systems (Fajer *et al.*, 1975; Kaufmann *et al.*, 1976). The possible involvement of protein entities in the reaction center complex has been suggested by Shipman *et al.* (1976). Characterization of the protein moiety associated with P700 has been published by Bengis and Nelson (1975). It appears that the structure of the reaction center complex must be uniquely built for its primary photoprocess; the two most important features determining its operation should be the pigment–pigment and the pigment–protein interactions.

To explore the importance of the interactions of pigment and protein components in the operation of the reaction center complex, we measured the optical properties of subchloroplast particles highly enriched in P700 (1/15 chlorophyll *a* molecules), prepared according to Ikegami and Katoh (1975), and of two artificial systems (chlorophyll *a* and pheophytin *a* in polystyrene matrix, and a colloidal mixture of both pigments with bovine serum albumin). Data are presented on absorption spectra, excitation and emission spectra, and the degree of polarization of chlorophyll *a* fluorescence.

MATERIALS AND METHODS

Photosystem I particles were prepared by digitonin treatment of spinach chloroplasts according to the method of Anderson and Boardman (1966) with 10 m*M* $MgCl_2$ present in the buffer media. Detergent solubilized chlorophyll was washed out by resuspending and repelleting the D-144 pellet in distilled water; this process was repeated once. The particles were then extracted with diethyl ether (saturated with water and kept at 4°C for 24 h) according to Ikegami and Katoh (1975) using 10 mℓ ether per 10 mg of lyophilized material. The light-green particles were separated by low speed centrifugation (International Clinical Centrifuge, Model CL) and the pellet quickly dried with nitrogen. The final preparation was stored in powder form. As mentioned by Ikegami and Katoh (1975), the photochemical activity of dried P700-enriched particles remained unchanged for several months.

The polymer foils were prepared according to Vacek *et al.* (1977). Chromatographically purified chlorophyll *a* and pheophytin *a* were prepared according to Skorkovská and Vavřinec (1973). Polystyrene was obtained from Merck. High grade acetone was redistilled prior to use as solvent. The polymer foils were prepared according to Vacek *et al.* (1977); the pigment orientation was checked by small angle light scattering with a He–Ne laser. Polystyrene foils with randomly distributed pigment molecules were used in the present study. In this case, the concen-

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tration dependence of pigment aggregation was similar to that in nonpolar solvents; at chlorophyll *a* concentrations of 10^{-6}–10^{-5} *M*, the "monomeric" form dominates (fluorescence maximum, 675 nm), and at 10^{-3}–10^{-2} *M*, the "aggregated" forms dominate (fluorescence maximum of aggregates, 730 nm). Other characteristics have been described by Vacek *et al.* (1974) and Vacek *et al.* (1977). Švábová (1975) has described the concentration dependence of the fluorescence polarization spectrum of chlorophyll *a* in polymer foils.

The pigment–protein sample was prepared by mixing equal volumes of chlorophyll *a* in acetone (concentration, 6 m*M* or 160 μM), pheophytin *a* in acetone (concentration, 70 μM), and an aqueous solution of bovine serum albumin (32 μM).

Absorption spectra were made on a Cary 14 recording spectrophotometer; the reference cuvette contained an appropriate concentration of a scattering suspension of $BaSO_4$ freshly made by mixing solutions of Na_2SO_4 (1.3 *M*) and $BaCl_2$ (0.88 *M*) (see Latimer, 1956).

Emission spectra were measured with a spectrofluorometer described by Shimony *et al.* (1967). Front surface fluorescence was detected with an EMI 9558 B photomultiplier through a Corning CS 2-58 glass filter and a Bausch and Lomb monochromator (33–86–45; blazed at 700 nm; half-bandwidth, 6.6 nm). All spectra presented were corrected for photomultiplier sensitivity and monochromator characteristics.

Polarization measurements were made with vertically polarized excitation. Fluorescence was measured at right angles to the exciting beam (633 or 640 nm) with an EMI 9558B photomultiplier through two cut off filters, Schott RG 8 and RG 10 (2 mm each), and a 730 nm interference filter (half band width, 8.4 nm). The degree of polarization of fluorescence (p) was calculated as $(F_v - F_h)/(F_v + F_h)$, where F_v is the vertically polarized fluorescence component and F_h is the horizontally polarized component after correction for systematic instrumental errors.

Excitation spectra of fluorescence were obtained using vertically polarized light. Fluorescence intensities, converted into relative numbers of quanta, were calculated as $F_v + 2F_h$.

The photochemical activity of P700 was determined from the light-induced absorbance decrease at 703 nm (half-bandwidth, 10 nm) using the difference spectrophotometer described by Sybesma and Fowler (1968); for other details, see Wong and Govindjee (1976). The actinic blue light was obtained by passing the white light from a 750 W tungsten filament lamp through two heat filters and a 5 mm Corning CS 4-96 glass filter. Sodium ascorbate (2 m*M*) was added to the sample prior to assay. The difference absorption spectrum between the chemically oxidized and reduced P700-enriched particles was obtained with an Aminco DW-2 spectrophotometer.

RESULTS

Photochemical activity and chlorophyll a/P700 *ratio of P700-enriched particles*

The amount of P700 was calculated from the light-induced absorbance decrease at 703 nm (Fig. 1A), using the difference extinction coefficient of 64 $mM^{-1}\,cm^{-1}$ (Hiyama and Ke, 1972). The total chlorophyll *a* was obtained from the absorbance at the red peak of the P700-enriched particles and an assumed extinction coefficient of 70 $mM^{-1}\,cm^{-1}$. The ratio of chlorophyll *a* to P700 varied between 10 and 15 in different preparations. All results presented in this paper are for preparations with chlorophyll *a* to P700 of ~15.

Addition of 0.5% sodium dodecyl sulfate eliminated the photoinduced absorbance change at 703 nm in agreement with the data of Shiozawa *et al.* (1974) for their P700-chlorophyll *a*-protein complex. The loss of photoinduced P700 activity does not imply that it was destroyed by sodium dodecyl sulfate, as Bengis and Nelson (1975) have reported for a chemically-induced absorbance difference at 697 nm between the ascorbate-reduced and ferricyanide-oxidized samples. We obtained the same results in our detergent treated preparations.

Chemical oxidation–reduction of P700-enriched particles

The chemically induced difference spectrum was obtained as follows: 2.5 m*M* sodium ascorbate was added to a sample suspension to reduce all P700, and it was then divided into equal portions in two cuvettes which were placed in the sample and reference positions close to the photomultiplier of the Aminco DW-2 spectrophotometer, operated in the split-beam mode. Small volumes of ferricyanide (up to 12 m*M*) were added in steps to the sample cuvette. Both the main chlorophyll *a* absorption band and the P700 were chemically oxidized (bleached). Sodium ascorbate (>10 m*M*) was then added to check for reversibility of the absorption change. The P700 change was completely reversible, but the oxidation of the bulk chlorophyll *a* was not. This allowed us to plot the reversible change by taking the absorbance difference between the oxidized and the reduced spectra (Fig. 1B); this is qualitatively similar to the P700 oxidized-minus-reduced absorption spectrum, and has a peak at 697 nm. The estimated amount of P700 from this chemical difference spectrum was the same as that obtained from the photo-induced signals (1 P700 to 10–15 chlorophyll *a*).

Absorption spectra and excitation spectra of fluorescence of P700-enriched particles

The absorption spectrum in the 400–750 nm range, after correction for strong light scattering, and an excitation spectrum for chlorophyll *a* fluorescence at 730 nm in the 400–690 nm range are shown in Fig. 2A. Identical spectra were found for particles suspended in water or 70:30 glycerol–water mixture. A good agreement between the absorption (solid line, Fig. 2A) and the excitation (dashed line) spectra in the red region is observed. The absence of bands at 650 nm and 480 nm in both these spectra shows that chlorophyll *b* is absent. The absorption peaks of chlorophyll *a* are at 435 and 673 nm. The peak at 420–425 nm in both the absorption and the excitation spectra of fluorescence is suggested here to be due to pheophytin *a* (see results on artificial systems, below). Shiozawa *et al.* (1974) has associated a peak at 420 nm to cytochromes. Our absorption spectra are similar to the one published by Ikegami and Katoh (1975) except that their 416 nm peak is at 420 nm.

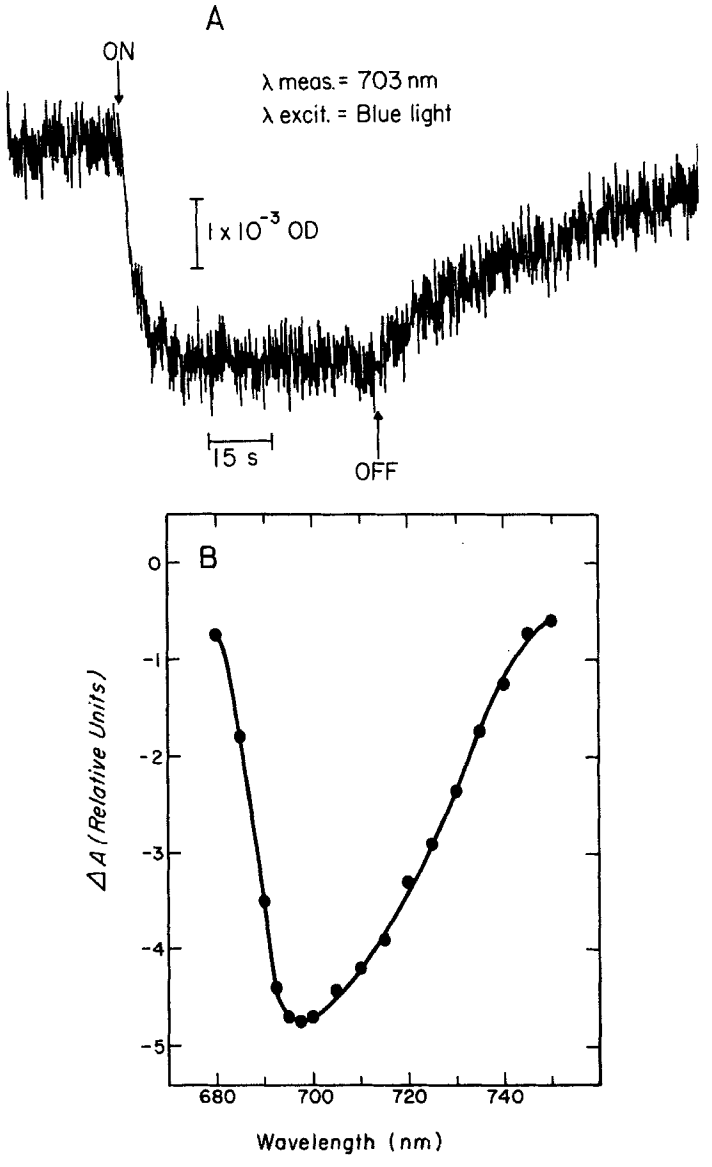


Figure 1. (A) Light-induced absorption change at 703 nm for the P700-enriched particles with 2 m*M* ascorbate (chlorophyll *a* concentration, 6 μM); optical pathlength = 1 mm. (B) Chemically-induced difference absorption spectrum of subchloroplast particles enriched in P700. See text for details.

To decrease the scattering of light by the sample we added Triton X-100, cholate, lauryl dimethylamine oxide or sodium dodecyl sulfate; the last one produced the greatest decrease in light scattering. Addition of 0.5% sodium dodecyl sulfate caused (1) the turbidity of the suspension to be much reduced; (2) the red absorption band to be blue-shifted by 2 nm (to 671 nm); and (3) the double peak in the Soret region at 420 and 435 nm to be changed to a single peak at 414 nm. A correlation between the absorption and the excitation peaks (for fluorescence at 730 nm) was again observed (Fig. 2B).

Absorption spectra of artificial systems

The absorption spectrum for chlorophyll *a* in polystyrene was reported by Vacek *et al.* (1977) and Cerna (1972). The absorption spectrum of a mixture of chlorophyll *a* (0.2 m*M*) and pheophytin *a* (0.25 m*M*) in polystyrene matrix shows peaks at 432, 578, 619, and 668.5 nm (chlorophyll *a*) and at 417, 508, and 539.5 nm (pheophytin *a*) (Fig. 3). The chlorophyll *a* peaks in polystyrene are blue-shifted by 3 to 4.5 nm compared to those in P700-enriched particles. Assuming an equivalent shift in pheophytin *a*, the 420 nm band observed in the particles would correspond to the 417 nm band observed in polystyrene—supporting the idea that the 420 nm peak is due to pheophytin *a*.

Emission spectra of P700-enriched particles

The fluorescence spectra of these particles in the solid state (powder form) and in glycerol–water suspension, normalized to the same value at 678 nm, are

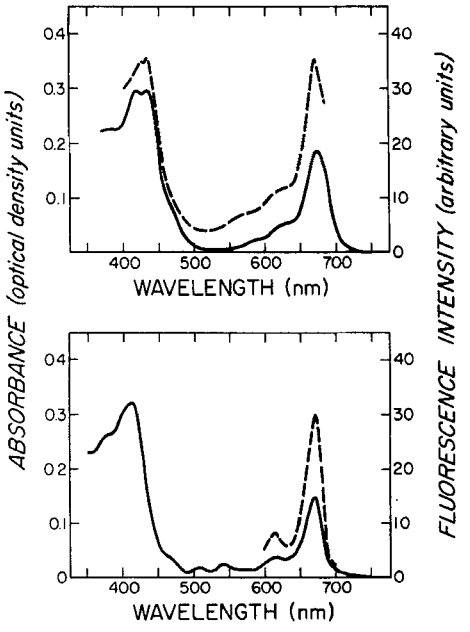


Figure 2. Absorption spectrum (——) and excitation spectrum of fluorescence at 730 nm (– – –) of subchloroplast particles enriched in P700 (Chl *a*/P700 = 15) in aqueous suspension at 297 K. Chlorophyll concentration, 2.7 μM, (A) in the absence and (B) in the presence of 0.5% sodium dodecyl sulfate. All measurements were corrected for scattering artifacts.

presented in Fig. 4A. Exact locations and the intensity ratios of the peaks under the various conditions are given in Table 1.

P700-enriched particles, in powder form, show emission peaks of about equal intensities at 676, 704 and 729 nm; the peak at 704 nm may be comparable to a peak at 705 nm observed in dehydrated *Chlorella* cells (see p. 79; Cho, 1969). The locations and relative intensities of the emission bands show a strong dependence on the suspension medium: In 70:30 glycerol–water mixture, they are 677.5, 688 and 723 nm in the ratio of 2:1.8:1 (Fig. 4A), but in water, at 675 and 737.5 nm in the ratio of 4.3:1 (Fig. 4B). The species responsible for the 704 nm band (in powder) must

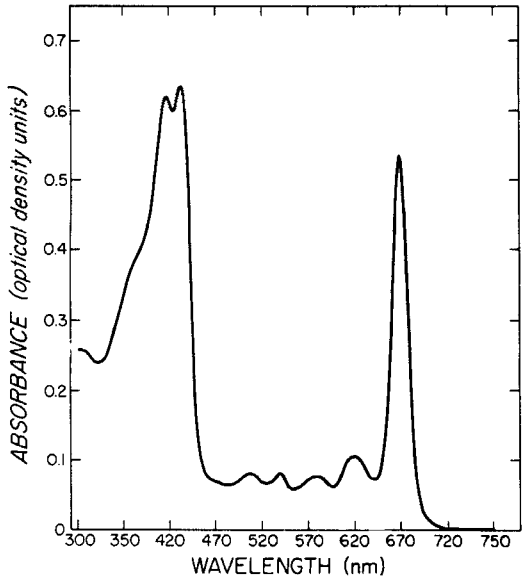
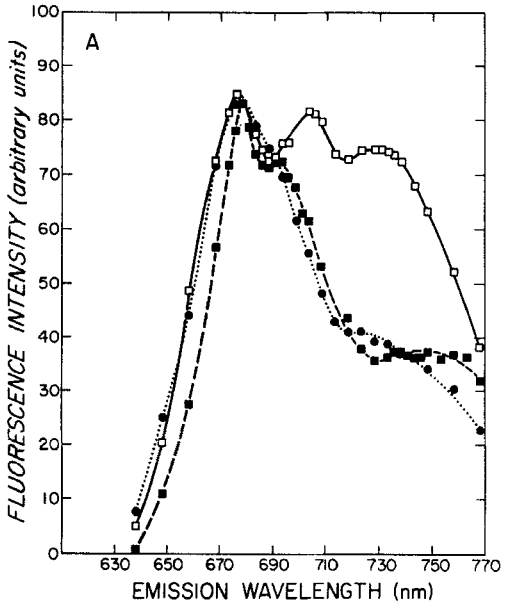


Figure 3. Absorption spectrum of mixture of chlorophyll *a* (0.2 mM) and pheophytin *a* (0.23 mM) in polystyrene at 297 K.



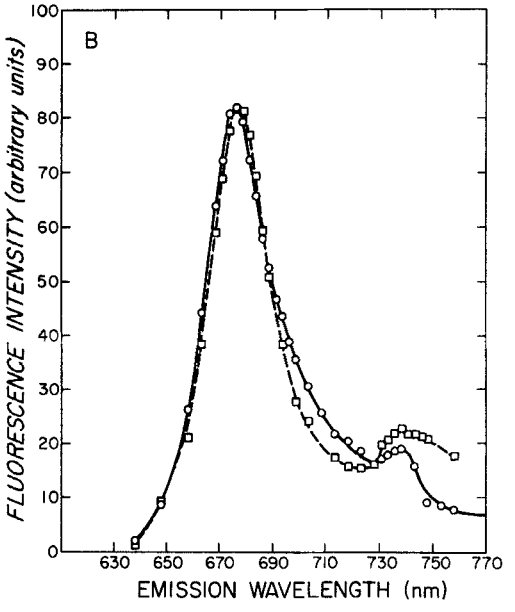


Figure 4. Fluorescence spectra of subchloroplast particles enriched in P700 (Chl *a*/P700 = 15) excited at 435 nm. In powder form at 297 K (——), and in glycerol-water (70:30) at 297 K (...) and at 77 K (– – –). Chlorophyll concentration, 2.7 μM; the curves were normalized to the same value at 678 nm. (B) Fluorescence spectra of subchloroplast particles enriched in P700 (Chl *a*/P700 = 15) in water in the absence of (——) and in the presence of 0.5% sodium dodecyl sulfate (– – –) at 297 K. The spectra were normalized to the same peak height by multiplying all points on the emission spectrum for P700-enriched particles in water without detergent by 5.

Table 1. Locations and relative intensities of the emission peaks of subchloroplast particles enriched in P700 (Chl *a*/P700 = 15) under different conditions. All results reported, unless specified, are for 297 K. The final concentrations of chlorophyll *a* in all the suspensions were 2.7 μM. Excitation at 435 nm (bandwidth, 6.6 nm) was with light obtained from a 100 W tungsten ribbon lamp passed through a Bausch and Lomb monochromator (see Materials and Methods). Front surface fluorescence from the sample was passed through a Corning CS 2-58 glass cut-off filter and another Bausch and Lomb monochromator with a bandwidth of 6.6 nm. Detection was with an EMI 9558 B photomultiplier

Condition	Fluorescence maxima, nm			Ratios of fluorescence intensities	
	A	B	C	Ratio A/C	Ratio B/C
Powder	676	704	729	1.1	1.1
In H_2O	675	—	737.5	4.3	—
In H_2O + 0.5% Sodium dodecyl sulfate	676.5	—	738.5	3.6	—
In 70:30 glycerol–water	677.5	688	723	2.0	1.8
In 70:30 glycerol–water water at 77 K	678	692	740	2.2	1.9

have undergone an extensive change when in suspension; perhaps, a large blue shift occurred leading to a broadening of the 675 nm band. The ratio of the short to the long wavelength bands seems to increase with increasing concentration of water.

The ratio of the fluorescence at 675–680 nm to that at 720–740 nm (at 77 K) is very high (about 2) in suspensions of P700-enriched particles in glycerol–water mixture as compared to that observed in system I particles (Boardman *et al.*, 1966; Gasanov and Govindjee, 1974). This is partly due to the low concentrations of chlorophyll *a* (2.7 μM) and to very few chlorophyll *a* molecules per particle, which reduce the reabsorption of short wavelength fluorescence. However, we cannot unequivocally exclude the possibility of (a) a disruption in energy transfer from the short to the long wavelength absorbing chlorophyll *a* molecules and (b) a slight contamination of detergent solubilized chlorophyll *a* fluorescing strongly at 675 nm. As we shall see later, decrease in energy transfer is likely in our preparations.

The presence of an emission band at about 688 nm in the P700-enriched particles confirms the existence of a chlorophyll *a* complex (fluorescing at this wavelength) which must be tightly associated with reaction center I (P700). Cooling the suspension (in 70:30 glycerol–water) to 77 K produced mainly a narrowing of the 675 nm band (peak at 677.5 nm) and 4 and 17 nm red shifts in the 690 and 730 nm bands, respectively, but only slight changes in the ratio of these bands; this is also in contrast to the results with system I particles. A slight increase in fluorescence intensity was accompanied by an increase in lifetime of fluorescence from 1.4 ns (at room temperature) to 1.8 ns (at 77 K) confirming that this was a true change in quantum yield of fluorescence (Vacek, Wong, Merkelo and Govindjee, unpublished).

Addition of 0.5% sodium dodecyl sulfate to water suspension of P700-enriched particles resulted in an increase in fluorescence intensities at all wavelengths (Fig. 4B), with a slight change, from 4.3 to 3.6, in the ratio of the short to long wavelength emission bands. Sodium dodecyl sulfate caused a relative decrease in the 710 nm and an increase in the 730 nm region. The five-fold increase in the fluorescence intensity was accompanied by an almost equivalent increase in the lifetime of chlorophyll *a* fluorescence (from 1.4 to 5.4 ns; Vacek, Wong, Merkelo and Govindjee, unpublished) confirming that it is a true change in the quantum yield of fluorescence.

Emission spectra of artificial systems

The fluorescence spectra of chlorophyll *a* and of pheophytin *a* in polystyrene at 77 K show two emission peaks in the 675 and 730 nm regions (Fig. 5). A colloidal mixture of chlorophyll *a*, pheophytin *a* and bovine serum albumin at 77 K, however, shows three peaks at about 670, 710 and 730 nm. Summary of the locations and relative fluorescence intensities of the short to long wavelength emission peaks are

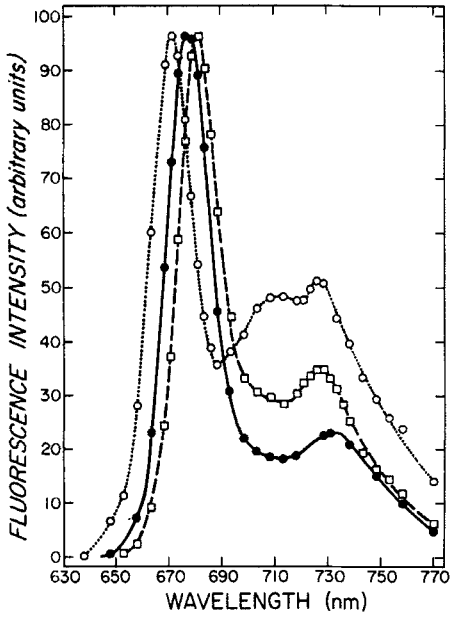


Figure 5. Fluorescence spectra of artificial systems at 77 K. Chlorophyll *a* (0.77 mM) in polystyrene (——); pheophytin *a* (1.23 mM) in polystyrene (– – –); chlorophyll *a* (6 mM), pheophytin *a* (70 μM) and bovine serum albumin (30 μM) in acetone–water (2:1) (. . .). All spectra were normalized to the same peak-height.

Table 2. Locations and relative intensities of fluorescent emission peaks in artificial systems at 77 K. For other details, see the legend of Table 1.

System	Fluorescence maxima, nm A	B	C	Fluorescence intensities Ratio A/C
Chlorophyll *a* (0.77 m*M*) in polystyrene	677	—	733	4
Pheophytin *a* (1.23 m*M*) in polystyrene	680	—	727	2.8
Chlorophyll *a* (6 m*M*), pheophytin *a* (70 μM), and bovine serum albumin (30 μM) in acetone–water (2:1)	670.5	711	726.5	1.9

given in Table 2; this ratio is between 1.9 and 4 depending upon the system.

Degree of polarization of fluorescence P700-enriched particles and of artificial systems

P700-enriched particles have a relatively high degree of polarization, 13.9% (λ excitation, 640 nm), which is further increased by the addition of 0.5% sodium dodecyl sulfate to 16.7% (Table 3). Similar changes were noted for excitation wavelengths ranging from 620 to 690 nm. The pigment concentrations in the artificial systems were about three orders of magnitude higher than in the case of particles (1 m*M* vs 1 μM). Owing to extensive energy migration, these systems show a lower degree of polarization (1.4% for chlorophyll *a* + pheophytin *a* + bovine serum albumin colloid; and 7.8% for chlorophyll *a* in polystyrene).

DISCUSSION

Optical properties of the subchloroplast particles highly enriched in photoactive reaction center I (P700)

The characteristics of the P700-enriched particles are: (1) the chlorophyll *a* to P700 ratio is 10–15 (Fig. 1A); (2) the chemical oxidized-minus-reduced difference absorption spectrum has a peak at 697 nm (Fig. 1B); (3) chlorophyll *b* is absent (Fig. 2); (4) the bulk chlorophyll *a* absorption bands are at 435 and 673 nm and they match the excitation peaks of chlorophyll *a* fluorescence at 730 nm (Fig. 2); (5) a band at 420–425 nm, both in the absorption spectrum and the excitation spectrum of fluorescence (Fig. 2), is suggested to be due to pheophytin *a* [by comparison with the spectrum of pheophytin *a* in polystyrene matrix (Fig. 3)]; (6) in powder form, the fragments show emission peaks of equal heights at 676, 704, 729 nm (Fig. 4A); a suspension in water gives bands at 675 and 738 nm in the ratio of ~4 to 1, and in glycerol–water at 678, 688, and 723 nm in the ratio of 2:1.8:1 (Figs. 4A); and (7) the degree of polarization of fluorescence at 730 nm (excitation, 640 nm) is ~14% (Table 3). Finally, unpublished data of K. Vacek, D. Wong, H. Merkelo, and Govindjee show that the lifetime of chlorophyll *a* fluorescence is 1.4 ns, and thus the quantum yield of chlorophyll *a* fluorescence is ~10%.

The best evidence that our particles contain *some* native complexes is obvious from the extremely high activity of P700. However, we do not suggest that the entire complex is in its native form. If these particles are structurally altered by the experimental procedures, then the pigment–pigment and pigment–protein interactions in them are different from those in chloroplasts or in system I particles. This is particularly clear from the blue-shifts in both fluorescence and absorption peaks, increased degree of polarization of fluorescence, and the high quantum yield of fluorescence. The physico–chemical characteristics of our preparation are, however, sufficient for efficient

Table 3. Degree of polarization of fluorescence at 730 nm of subchloroplast fragments enriched in P700 (Chl *a*/P700 = 15) and some artificial systems. The P700-enriched particles were excited at 640 nm and the artificial systems at 633 nm (bandwidth, 6.6 nm). This difference in excitation caused no significant effects on the polarization of fluorescence at 730 nm; for example, excitation of P700-enriched particles in water with 0.5% sodium dodecyl sulfate gave a value of polarization of 17.7% when excited at 633 nm. Fluorescence was detected at right angles to the direction of the excitation beam through Schott RG 8 and RG 10 cut-off filters and a 730 nm interference filter with a half-bandwidth of 8.4 nm. All measurements were made at room temperature

Sample	Degree of polarization (%)
P700-enriched particles suspended in water	13.9 ± 0.5
P700-enriched particles suspended in water + 0.5% sodium dodecyl sulfate	16.7 ± 0.5
Chlorophyll *a* (0.77 m*M*) in polystyrene	7.8 ± 0.5
Chlorophyll *a* (6 m*M*), pheophytin *a* (70 μM), and bovine serum albumin (30 μM) in acetone–water (2:1)	1.4 ± 0.5

photochemistry, and a high 730 nm emission band is not a necessary prerequisite for high P700 activity (see also Ogawa and Vernon, 1970). It seems that a major difference between the highly P700-enriched and system I particles or chloroplasts is the removal of the weakly fluorescent long wavelength chlorophyll *a* complex absorbing at 693 nm at room temperature and fluorescing strongly at 730 nm (at 77 K) (see Das and Govindjee, 1967). This alteration appears to be responsible for most of the fluorescence properties of the P700-enriched particles.

Two explanations for the high degree of polarization of fluorescence measured at 730 nm (13.9%) in P700-enriched particles are: (a) a decreased energy migration due to an increase in average distance between the individual chlorophyll *a* molecules (as most bulk molecules have been removed); and/or (b) a relative enrichment of the chlorophyll *a* complexes with a high degree of orientation in the P700-enriched particles.

Effects of sodium dodecyl sulfate: perturbation of proteins

In order to evaluate, in the first approximation, the importance of the protein components in the P700-enriched particles, we studied the action of 0.5% sodium dodecyl sulfate which was suggested to cause either dissociation of proteins (Bengis and Nelson, 1975) or unwinding or the polypeptides (Shiozawa *et al.*, 1974). Addition of 0.5% sodium dodecyl sulfate caused: (1) the replacement of the 420 and 435 nm absorption bands with a peak at 414 nm (Fig. 2B); (2) an increase of about five-fold in the fluorescence yield at all wavelengths of emission with a slight decrease in the ratio F(675-677)/F(737-739) (Fig. 4B); (3) a loss of photochemical activity as monitored by the light-induced absorbance decrease at 703 nm; and (4) an increase in the polarization of fluorescence from 13.9% to 16.7% at 730 nm (Table 3). (1) and (3) are in agreement with the observations of Shiozawa *et al.* (1974) on their P700-chlorophyll *a* protein complex. The changes in the absorption spectrum may reflect a strong influence of this detergent on the pigment–protein complex of the P700-enriched particle through changes in the protein structure. The loss of photochemical activity (which is not due to a loss of P700 *per se*) suggests the need for some definite structure for the operation of the reaction center. The increase in the fluorescence yield and in the degree of polarization of fluorescence at 730 nm could be taken to imply a probable decrease in pigment–pigment interaction. This could be brought about by a larger separation between pigment molecules (either because of protein dissociation or unfolding) leading to a decrease in intermolecular energy exchange and an increase in quantum yield (and lifetime) of fluorescence.

Comparison with artificial systems

The ratio of short to long wavelength emission (Fig. 5; Table 2) was 4 for chlorophyll *a* in polystyrene matrix, but it was lower (1.9) in the case of a colloidal mixture of chlorophyll *a*, pheophytin *a*, and bovine serum albumin. The latter ratio is similar to that for the P700-enriched particles suspended in a glycerol–water mixture (2.0 at 297 K and 2.2 at 77 K). This could mean that the pigment–protein interactions exert influence on the relative intensities of the emission bands at 680 and 730 nm. However, the ratio of short to long wavelength fluorescence bands for the P700-enriched particle in water (4.3) was similar to that for chlorophyll *a* in polystyrene at 297 K (3.6, not included in Table 2) and at 77 K (4); the replacement of glycerol with water led to this increase. In the solid state (powder) the long wavelength fluorescence band at 730 nm was almost as high as that at 675 nm. These results suggest the obvious: the chemical and physical environments, which may lead to changes in pigment–pigment and pigment–protein interactions, are important in determining the fluorescence characteristics of P700-enriched particles.

A comparison of the absorption spectrum of pheophytin *a* in polystyrene foil with that of the subchloroplast particles enriched in P700 shows that the 417 nm pheophytin *a* peak in polystyrene corresponds to the 420 nm peak in the particles (Fig. 2A), if a 3 to 4.5 nm blue shift of all absorption peaks in the polymer matrix is accepted. Although no chemical evidence is obtained for the existence and role of pheophytin *a* in the P700-enriched particles, it is still tempting to speculate, by analogy to photosynthetic bacteria, that pheophytin may play an important role in the native primary photochemistry of green plants.

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