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THE P700–CHLOROPHYLL *a*–PROTEIN OF A BLUE-GREEN ALGA*

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SUMMARY

The previously isolated chlorophyll *a*–protein of blue-green algae has been shown to contain P700 in a ratio of 1 reaction center molecule per 100 light-harvesting chlorophyll molecules. One-fifth of the molecules in the preparation contain P700 together with some 20 light-harvesting molecules, whereas the other molecules contain bulk chlorophyll only. Both pigment–protein entities are considered to be essentially the same and cannot be fractionated. An aggregate containing both types probably makes up the photochemical portion of the algal Photosystem I *in vivo*. The absorption and emission spectra of the pigment–protein are reported, as well as the spectral changes associated with the photochemical reaction. In addition to chlorophyll, carotenoid and protein the complex contains a quinone, which is not a plastoquinone. This unidentified quinone appears to participate in secondary electron transfer reactions occurring in the complex. Horse cytochrome *c* can be bound to the complex and will donate electrons to $P^{+}700$ upon illumination. Current hypotheses for the identity of the primary electron acceptor were tested. It appears unlikely that flavins, pteridines or iron fill this role.

INTRODUCTION

Knowledge of the photochemical processes in plants and bacteria is currently being extended by studies on isolated fractions of the photosynthetic apparatus. THORNBER AND OLSON[1], VERNON AND OGAWA[2] and BOARDMAN[3] have recently summarized some of the contributions to that knowledge which have come from research on complexes of chlorophyll and protein isolated from detergent-dissociated photosynthetic membranes of plants. One such complex is a chlorophyll *a*-protein which accounts for at least 75% of the chlorophyll of a blue-green alga[4]. This complex represents the light-harvesting component of the algal Photosystem I. The original report[4] also showed that the chlorophyll *a*-protein was chemically analogous to the System I chlorophyll–protein of higher plants, and to the bacteriochlorophyll

Abbreviation: PMS, phenazine methosulfate.

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a–protein of green bacteria. Data on the blue-green algal chlorophyll *a*–protein presented in this paper reveal that some, but not all, of the isolated and essentially homogeneous pigment–protein molecules contain the reaction center chlorophyll, P700, as well as some light-harvesting chlorophyll. A description of P700, together with other spectral characteristics of the complex, form the bulk of the information presented in this paper. In addition, the complex has been analyzed for the presence of low-molecular-weight constituents such as flavins, pteridines and iron, which have been implicated[5–7] as the primary electron acceptor of the photochemical reactions in plants and bacteria.

MATERIALS AND METHODS

The chlorophyll *a*–protein complex was prepared from cells of *Phormidium luridum* var. *olivaceae* grown photoautotrophically on KRATZ AND MYERS'[8] medium C or D in 150-l drums[9] or in 15-l carboys. If alum had been added to the cells to facilitate their being harvested, the cells were exhaustively washed with 50 mM Tris–HCl, pH 8.0, prior to use. Photosynthetic membranes were isolated from cells which had been sonicated (10 kcycles/sec, 1 A, 5 min) in 50 mM Tris–HCl, pH 8.0. The sonicated solution was centrifuged at 10000 × *g* for 15 min and the membranes isolated from the supernatant by centrifugation at 37000 × *g* for 30 min. The pellet was resuspended in buffer, washed once and used.

The original isolation procedure[4] was modified: improved yields of the complex were obtained by using a step-wise elution of the hydroxylapatite[10] column rather than a continuous gradient; steps of 0.01 M, 0.1 M, 0.2 M and 0.3 M sodium phosphate, pH 7.0, were used. A solution of 35 % (w/v) $(NH_4)_2SO_4$ was added to the 0.3 M sodium phosphate eluate until a cloudiness developed, and the precipitate was isolated by centrifugation. The yellow-green supernatant was discarded and the precipitate was dissolved in 50 mM Tris–HCl, pH 8.0. If the $(NH_4)_2SO_4$ addition was performed slowly while the mixture was stirred, and if it was stopped immediately a cloudiness was observed, then a pure chlorophyll *a*-protein preparation, as judged by polyacrylamide gel electrophoresis, was obtained, and Step 4 in ref. 4 was not necessary. Samples of the chlorophyll *a*–protein were usually stored at 4° or −15° prior to use; some samples were also lyophilized following brief dialysis against 1 mM Tris–HCl, pH 8.0.

Absorption spectra and fluorescence spectra were measured at room and liquid-nitrogen temperatures in the same manner as previously described[11]. Light-induced absorbance changes were measured with a Cary 14R spectrophotometer, the sample chamber of which was adapted for cross-illumination by a 500-W slide projector.

The chlorophyll concentration in a solution of the complex was measured from the absorption spectrum, using $\varepsilon = 60\ mM^{-1} \cdot cm^{-1}$ at 677 nm[4].

P700 concentration was estimated from a light-oxidized *vs.* reduced difference spectrum using a differential extinction coefficient for P700 of 100 $mM^{-1} \cdot cm^{-1}$ at 697 nm. To ensure that P700 was fully reduced in samples which were to be oxidized by light, the chlorophyll *a*–protein solution was stored in the dark for 24 h, or alternatively, sodium ascorbate was added to the solution, which was then exhaustively dialyzed in the dark to remove any excess reductant. Sodium ascorbate (1 mM) and phenazine methosulfate (PMS) (5 μM) were added to the chlorophyll *a*–protein solution in the reference cuvette to maintain P700 in a reduced state.

The redox titration of P700 was done anaerobically in a 1-cm² Pyrex cuvette fitted with a cylindrical upper section (total volume, 7 ml) in which a Radiometer P101 platinum electrode was immersed. A Teflon stopper, containing holes for the electrode, for gas (HP Argon) inlet and exhaust, and for oxidant or reductant injection, was inserted. Potentials were read on a Radiometer pH meter (PHM 22) connected to a scale expander. Reversible light-induced absorbance changes at 697 nm were measured at pH 8.0 in the presence of 10 mM ferri–ferrocyanide ($E_{m,8} = +0.42$ V) redox buffer, and the concentration of reduced P700 estimated from the change in absorbance at 697 nm caused by illumination with saturating blue light (Corning filter 5-57).

Oxygen uptake was measured by a Clark electrode (Yellow Springs Instrument Co., Ohio) contained in a 1-ml cuvette which was vigorously stirred and which was illuminated from the side by a 500-W slide projector. Because of the geometry of the cuvette, the intensity of the actinic light did not saturate this reaction.

Polyacrylamide gel electrophoresis was carried out as previously described[11].

Pteridines were extracted with either 1 % NH_4OH or 0.1 % β-mercaptoethanol, pH 10, for 1 h at room temperature in the dark[12]. Following extraction, the chlorophyll–protein was removed from low molecular weight material by filtration through a Sephadex G-25 column or by $(NH_4)_2SO_4$ precipitation. The chlorophyll *a*–protein-free extract was concentrated *in vacuo* or oxidized by the procedure of MACLEAN *et al.*[13]. The concentrated extract finally was chromatographed on thin-layer plates of cellulose[14] and the pteridines were located by their fluorescence under ultraviolet light.

The flavin content of the chlorophyll *a*-protein was estimated by the method of APPAJI-RAO *et al.*[15]. Iron content was determined by a modification of LANDERS AND ZAK's[16] colorimetric bathophenanthroline method; the determinations were carried out by Mr. S. J. Tassanari, Brookhaven National Laboratory, to whom we are grateful.

Total lipid extractions were done in the dark at room temperature using isopropanol–isooctane–water (1:1:1, by vol.) mixtures. After the organic phase had been separated, it was evaporated *in vacuo* and the residue was taken up in either light petroleum or isooctane. Aliquots were subjected to two-dimensional thin-layer chromatography; the developed plates were sprayed to detect lipids, phosphate and amino groups using the reagents described by BUNN *et al.*[17]. Similar extracts were subjected to one-dimensional chromatography on silica gel GHR plates and developed in benzene, chloroform, or benzene–heptane (85:15 by vol.). Quinones were detected by spraying with leucomethylene blue[18].

RESULTS

Absorption spectrum

The spectrum of the chlorophyll *a*–protein in the visible range (Fig. 1) is dominated by the red and the Soret peaks of chlorophyll *a* at 677 and 437 nm, respectively. The broad 490–495-nm-shoulder is due mainly to residual carotenoids, mostly β-carotene[4]. The prominent shoulders at 420 and 380 nm as well as the minor peaks in the red region are also due to chlorophyll *a* (*cf.* HOUSSIER AND SAUER[19]). As the complex ages or the pH of the suspending medium is lowered,

pheophytinization causes the 420-nm-peak to become the dominant band in the Soret region. Degradation of the chlorophyll also causes the 677-nm-peak to decrease in height and shift to shorter wavelength; additionally, a small peak at 530 nm appears. These changes occasionally occur during the preparation of the complex, in which case the chlorophyll *a*–protein preparation should be discarded.

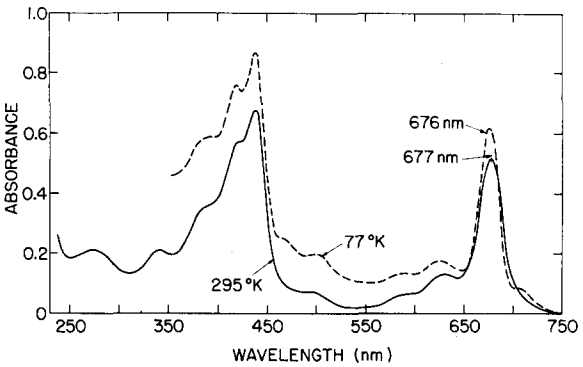

Fig. 1. Absorption spectrum of chlorophyll–protein complex in 50% glycerol at room temperature (———) and at 77°K (– – – –). Chlorophyll concentration, 7.5 μM.

The ultraviolet region shows two small peaks (270 nm and 340 nm). The 340-nm-peak is almost certainly due to the presence of chlorophyll *a* (*cf.* ref. 19), whereas the 270-nm-peak is due to both lipid-soluble material as well as protein, since chlorophyll *a* in ether has a peak at approx. 250 nm[19], and secondly, a prominent peak at 280 nm remains after extraction of the lipid-soluble material from the protein with butanol (Fig. 2). The absorption peaks at 415 and 665 nm (Fig. 2) indicate the presence of small amounts of chlorophyll or its degradation products in the extracted protein. The spectrum of the butanol-extracted chlorophyll–protein is similar, except in the cytochrome region, to chloroplast lamellar proteins prepared by other investigators[20–22].

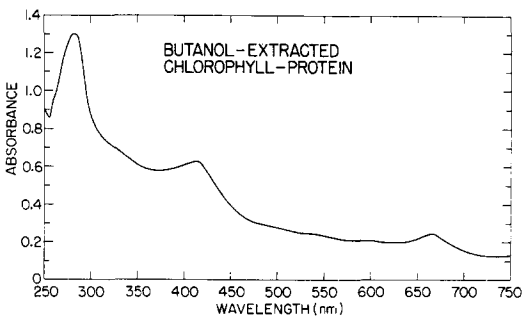

Fig. 2. Absorption spectrum of chlorophyll–protein complex after butanol extraction (3 ×) and dialysis against 0.5% sodium dodecyl sulfate. Protein concentration, 2 mg/ml.

At 77°K, in addition to sharpening of the main peak, a small shoulder of the red chlorophyll peak is resolved at 710 nm (Fig. 1). The major red peak is shifted to 676 nm. The 710-nm-chlorophyll does not seem to be a redox component since the addition of oxidizing and reducing agents prior to freezing caused no detectable change

in the magnitude of its absorbance. Isolated Phormidium membranes show a similar spectrum at 77°K, but exhibit an additional asymmetry indicating the presence of a shorter wavelength form of chlorophyll *a* (*cf.* ref. 23); since this third spectral form is not seen in the spectrum of the chlorophyll *a*–protein, it may represent a form of chlorophyll *a* in the membranes which is associated with a different photosystem.

Emission spectrum

The room temperature fluorescence spectrum (Fig. 3a) of the isolated complex shows a maximum emission at 680 nm, and a minor longer wavelength component at approx. 705 nm, which is seen as an asymmetry of the major peak; it was assumed that the shape of the long wavelength side of the peak should be a mirror image of the short wavelength side. Oxidation or reduction of the sample (Fig. 3a) results in a decrease or an increase, respectively, of the fluorescence yield of both fluorescing forms; however, the ratio between the heights of the two fluorescent components is unaffected at room temperature (Table I). At 77°K (Fig. 3b) the fluorescence maximum is at approx. 717 nm (F717), and a minor component in the spectrum is observed at 690 nm (F690). Reduction increases the fluorescence yield of both species (Fig. 3b) as well as the ratio of F717/F690 at 77°K (Table I). In the fluorescence

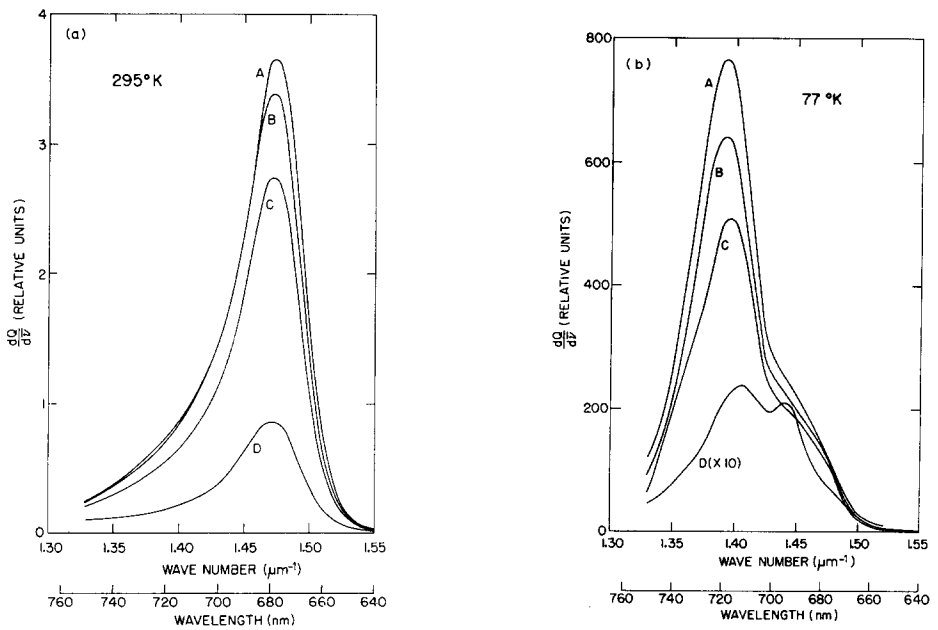

Fig. 3. Fluorescence emission spectra of the chlorophyll–protein complex measured at (a) 295°K and (b) 77°K. Curves A, B, C, and D show spectra of samples which were reduced with sodium dithionite, reduced with sodium ascorbate (10 mM), untreated and oxidized with potassium ferricyanide (10 mM), respectively. Chlorophyll concentration, 15 μM.

spectrum of isolated membranes it is observed that at both temperatures the maxima are located 6–10 nm towards longer wavelengths (Table I); however, the response of the fluorescence yield of membranes to oxidation and reduction is very similar to that of the isolated complex (Table I).

TABLE I

EFFECT OF THE ADDITION OF OXIDIZING AND REDUCING AGENTS ON THE FLUORESCENCE OF *P. luridum* CHLOROPHYLL–PROTEIN

Addition	*Chlorophyll–protein*		*Membranes*	
	*705/680** (*298° K*)	*717/690** (*77° K*)	*715/686** (*298° K*)	*730/693** (*77° K*)
Untreated	0.25	3.1	0.30	5.3
Potassium ferricyanide (10 mM)**	0.27	2.7	—	3.0
Sodium dithionite***	0.29	8.8	0.48	9.0
Sodium ascorbate (10 mM)**	0.28	8.5	0.28	7.4

* Ratio of the fluorescence intensity at the two wavelengths.
** Concentrations given represent the final strength of the additive.
*** A few crystals of sodium dithionite were added to the cuvette.

Photochemical activity

The light-*minus*-dark difference spectrum of the chlorophyll *a*–protein indicates a reversible bleaching due to P700. The light induced, reversible absorbance changes between 400 and 900 nm are shown in Fig. 4. The major decreases in absorbance are at 697 and 430 nm. Greater detail in the red region of the difference spectrum is given in ref. 24. The difference spectrum (Fig. 4) shows spectral changes similar to those observed by KOK[25] in the original report of P700. VERNON *et al.*[26–28], using Photosystem I fractions isolated from Triton X-100-treated chloroplasts and algae, have observed an additional bleaching at 680 nm which they related to changes in the state of P700. In the chlorophyll *a*–protein of Phormidium we have found that a bleaching at 680 nm can be demonstrated in light-*minus*-dark and chemically oxidized-*minus*-reduced difference spectra, but the change is irreversible and is therefore absent in Fig. 4. The decrease in absorbance at 680 nm which we see appears to be due to nonspecific bleaching of light-harvesting chlorophyll, and not related to P700 changes.

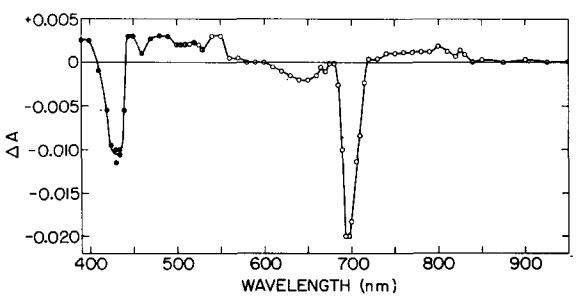

Fig. 4. Light-induced reversible absorbance changes of the chlorophyll–protein complex at 26° in the presence of 10 mM sodium ascorbate, 100 μM methyl viologen, and 50 mM Tris–HCl, pH 8.0. Chlorophyll concentration, 30 μM. ○, measurements of the absorbance changes when saturating blue light was used for bringing about the oxidation of P700; ●, red light was used.

In the ultraviolet region (Fig. 5), a large light-induced absorbance decrease around 260–270 nm was observed as well as a small, broad increase in absorbance at 310 nm. The difference spectrum (Fig. 5) is similar to the slow light-induced absorption change observed by WITT[29] and attributed by him to the reduction of plastoquinone. The

oxidized-*minus*-reduced difference spectrum of chlorophyll *a in vitro*[30] is also similar in placement of absorption maxima and minima, but the magnitude per chlorophyll molecule of the absorbance differences is smaller and the ratio of the positive to negative deflections is closer to unity.

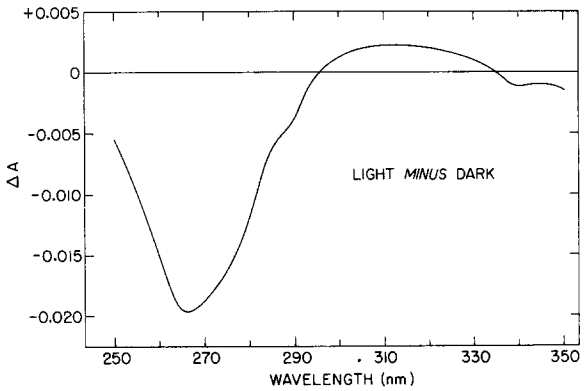

Fig. 5. Light-*minus*-dark UV difference spectrum of the chlorophyll–protein complex. Chlorophyl concentration, 20 μM.

The concentration of P700 relative to bulk chlorophyll was estimated as described under MATERIALS AND METHODS. These conditions for measuring P700's concentration were the most satisfactory since the rate of P^+700 reduction was minimal thereby enabling P700 to be essentially completely oxidized by light, and nonspecific bleaching due to the use of harsh oxidants and reductants was avoided. One P700 molecule per 84 chlorophyll molecules was determined as the average ratio for 12 different preparations of the complex; the ratio (1:60 to 1:130) varied from preparation to preparation.

The reversible photobleaching of the reaction center was quite stable in preparations stored at 4°, and decayed at a rate of approx. 5 % per month. It was noted, however, that degradation of the bulk chlorophyll occurred at a faster but more variable rate; P700 activity remained in preparations in which over half of the chlorophyll molecules had pheophytinized. No changes were observed in P700 activity or in the forms of bulk chlorophyll present if the chlorophyll *a*–protein was stored in a frozen (−15°) or lyophilized state.

The quantum requirement for the P700 reaction measured in the presence of 4 mM sodium ascorbate, 35 μM methyl viologen and 3.5 μM 2,3′,6-trichlorophenolindophenol was found to be 10 $\pm$ 2 quanta per electron transferred. Actinic wavelengths used were 699, 675 and 660 nm, all of which gave the same value. Because of the very slow back-reaction of $P^+700 \rightarrow$ P700 in the untreated chlorophyll *a*-protein (see later), substrate concentrations of sodium ascorbate were needed in these measurements. Thus the rate of P^+700 reduction was high; the initial back rate was approx. 45 % of the initial forward rate. The quantum requirement that was measured must therefore be considered a maximal value and is very probably much lower.

Redox titration of the chlorophyll *a*-protein with the ferro–ferricyanide couple gave an average E'_0 (pH 8) of + 405 mV; three different preparations had midpoint potentials of +380, +403, and +432 mV. The average value is at least 40 mV lower

than has been found in other preparations[25,26], and may reflect the high degree of membrane comminution as well as the presence of a strongly ionic detergent on the molecule's surface.

Oxygen uptake by the chlorophyll–protein

HONEYCUTT AND KROGMAN[31] have recently provided evidence that the uptake of oxygen by isolated blue-green algal membranes in the presence of an artificial electron donor occurs at a higher rate than in other chloroplast preparations. Furthermore, the reaction in blue-green algae is much less dependent on the addition of an exogenous, autooxidizable electron acceptor than the same reaction in spinach chloroplasts, but nevertheless there is a requirement for an intermediate acceptor molecule. The rates of oxygen uptake for the Phormidium chlorophyll *a*–protein (Table II) show an absolute requirement, under the conditions employed, for an electron donor but none for an intermediate acceptor, methyl viologen. This implies that the source of electrons reacting with O_2 is more available in the chlorophyll *a*–protein than in intact membranes.

TABLE II

LIGHT-DRIVEN O_2 UPTAKE BY *P. luridum* CHLOROPHYLL–PROTEIN

The illumination was provided by a 500-W slide projector, the output of which was filtered through 2 cm of water. The intensity of the white light striking the sample was $7.5 \cdot 10^5$ ergs $\cdot cm^{-2} \cdot sec^{-1}$.

	mmoles O_2/min per mole chlorophyll
Dark	38
Light	41
Light, sodium ascorbate (1 mM)	870
Light, sodium ascorbate (1 mM), methyl viologen (50 μM)	865
Light, sodium ascorbate (1 mM), methyl viologen (100 μM)	875
Light, methyl viologen (50 μM)	53

Minor constituents of the chlorophyll–protein

The amino acid, chlorophyll and carotenoid composition of the complex has been reported[4]. Together with the detergent necessary to maintain the chlorophyll *a*–protein in solution, the known components account for most of the material in the molecule. However, constituents of low molecular weight for which analysis had not been performed previously, could also occur in the complex, and they could be involved in the primary and secondary electron transfer reactions. Hence, a careful examination was made for such components.

Organic solvent extracts show traces of phospholipids (*e.g.* phosphatidyl ethanolamine and phosphatidic acid) in addition to carotenoid and chlorophyll. The phospholipids are present in small amounts and their content varies from preparation to preparation. It is thought that they represent lipid not successfully replaced by sodium dodecyl sulfate during isolation of the complex. Because of the similarity of the ultraviolet difference spectrum (Fig. 5) to that attributed to plastoquinone reduction[29], total lipid extracts and heptane extracts of lyophilized chlorophyll *a*–protein

were analyzed for the presence of quinones. Cochromatography with spinach chloroplast extracts showed that plastoquinone was absent in the chlorophyll *a*–protein. However, another quinone-like component was present; its chromatographic behavior suggests a similarity to tocophorylquinones[17] or to the polar naphthoquinone which has been detected in the blue-green algae[32]. Spectral analysis of this substance after elution from the silica gel plates has been unsuccessful.

Non-covalently bound flavins were not present in the complex. Similarly, neither pteridines nor their oxidation products were detected in the chlorophyll *a*–protein, but they were observed in washed algal membranes. Samples of chlorophyll *a*–protein which were reisolated after treatment with 1 % NH_4OH for 24 h (a procedure which should remove pteridines from the complex) showed an unimpaired photooxidation of P700, thereby further demonstrating that pteridines are unlikely to be the primary electron acceptor for P700.

The iron content of the complex is initially about one-tenth that of magnesium. Following dialysis against 1 mM EDTA, or after $(NH_4)_2SO_4$ precipitation of the protein from 1 mM EDTA solution, the iron content becomes about the same as that of P700 (*i.e.* one-tenth of its original value). However, if the dialyzed or precipitated protein is then further dialyzed against deionized water, the concentration of iron decreases to about one-third that of the remaining P700, which would preclude iron being the electron acceptor in the photosynthetic system studied. The iron analysis data are considered to be preliminary since a sizable portion of the chlorophyll *a*–protein denatures upon dialysis against EDTA solutions or deionized water; however, support for the tentative conclusion of the iron analyses is provided by the observation that bleaching of P700 is unimpaired in the presence of 0.1 mM *o*-phenanthroline.

Other electron transfer reactions in the complex

Some of the P700 molecules are in an oxidized state immediately after the complex has been isolated, since no precautions are taken to exclude light during the isolation procedure. Difference spectra show that this P^+700 is reduced very slowly in the dark, complete reduction often taking several hours. The rate of the reduction is hastened by addition of sodium ascorbate (100 μM); 1 μM 2,3′,6-trichlorophenolindophenol further accelerates the back-reaction. PMS (5 μM) in the absence of any exogenous electron donor causes the rapid and complete reduction of any photooxidized P700. This last result implies the presence of a pool of low potential electrons in the chlorophyll *a*–protein which can be coupled to P^+700 by PMS.

The chlorophyll *a*–protein contains no cytochrome but horse heart cytochrome *c* can be made to bind to the complex in 10 mM Tris–HCl, pH 8.0. Passage of the mixture through a 30-cm Sephadex G-75 column separated the chlorophyll *a*–protein–cytochrome complex from free cytochrome. 4 to 5 molecules of cytochrome *c* were bound per molecule of chlorophyll *a*–protein. After reduction of the bound cytochrome by sodium ascorbate, a portion (29–36 %) can be photochemically oxidized, presumably by donating electrons to P^+700. If it is assumed that each chlorophyll *a*–protein molecule binds the same number of cytochrome molecules, the proportion of cytochrome undergoing photooxidation is about twice the expected amount. This result suggests that the distribution of the cytochrome on the chlorophyll *a*–protein molecules was either less than random or that intermolecular electron transfer was made more efficient by the cytochrome bound to the chlorophyll *a*–protein.

DISCUSSION

Our data indicate that the use of the anionic detergent, sodium dodecyl sulfate, along with a procedure which removes sodium dodecyl sulfate-solubilized chlorophyll and carotenoids, allow the isolation of an essentially homogeneous component which exhibits several properties of the intact photosynthetic membrane. The overall similarity of the absorption and emission spectra between the *in vivo* and the *in vitro* states, as well as the presence of P700 in the isolated complex argues strongly that no significant conformational changes occur within the pigment–protein during solubilization and subsequent chromatography. Those differences noted between the intact membrane and the isolated complex could be a result of the noncondensed state of the complex, as well as of the interaction of sodium dodecyl sulfate with the protein. Some differences are due to the fact that the membrane contains that chlorophyll (chl a_{II}, ref. 33) associated with Photosystem II, whereas the complex does not. The absence of a shorter wavelength spectral form in the red peak of the chlorophyll *a*–protein is probably explained by this latter point. The chlorophyll *a*–protein contains several spectral forms of chlorophyll *a*[34]. The longest wavelength form (710 nm, Fig. 1) observed at 77°K is of interest. This form has been identified with P700 (refs. 35, 36); however, we observed no change in absorbance at 710 nm if P700 was chemically oxidized prior to freezing. Hence we believe chlorophyll 710 nm is a different entity than P700. Whether this 710-nm-spectral form or some other form (*e.g.* chlorophyll *a* 695) gives rise to the strong fluorescence at 717 nm remains to be determined, as does the meaning of the change in fluorescence yield as a function of redox potential.

The ratio of P700 to total chlorophyll in the preparation is about 1:84. In light of KE *et al.*[37] recent determination of 120 $mM^{-1} \cdot cm^{-1}$ for the differential absorption coefficient of P700, our ratio should probably be changed to one P700 per 100 chlorophyll molecules. In view of this ratio, of the previously determined[4] twenty chlorophylls per molecule of chlorophyll *a*–protein, and of the uniform quantum requirement for P700 oxidation over the red absorbance peak of the complex, the chlorophyll *a*–protein preparation is envisaged to contain two slightly different entities: one, representing 20% of the molecules, has P700 and light-harvesting chlorophyll of essentially the same spectral forms that occur in the other 80% of the molecules, but the latter entity lacks P700. That the two entities are very similar has been deduced from our failure to separate them even partially by ultracentrifugation or chromatography[4]. Furthermore, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, an excellent fractionation technique for proteins in which minor differences in molecular weight occur, was tried without success; consecutive slices through the chlorophyll *a*–protein zone in an electrophoresed gel showed no significant variation in the P700/chlorophyll ratio. The quantum requirement data are explained by postulating that energy transfer does not occur between individual chlorophyll *a*–protein molecules when they are in solution, hence the quantum requirement for P700 bleaching would be one-fifth of that determined, *i.e.* two; this value is a maximum value (see RESULTS) and therefore it is most likely that in the P700–chlorophyll *a*–protein molecule, one absorbed photon transfers one electron. It is thought that *in vivo* the two slightly different entities of the chlorophyll *a*–protein form an aggregate which gives rise, when associated with cytochrome *f*, *etc.*, to the photosynthetic unit

of System I. The number of chlorophyll *a*–protein molecules in a unit probably varies depending upon growth conditions; such a change would account for the variation observed in P700/chlorophyll ratios in different preparations of the chlorophyll *a*–protein. The P700 content of the chlorophyll *a*–protein represents only a slight enrichment over that of the intact membrane[38], and is slightly less than that obtained by VERNON *et al.*[2,26–28] for their HP700 fractions. A recalculation of the P700 content of the HP700 fraction using KE *et al.*[37] differential extinction coefficient gives one P700 molecule per 66 chlorophyll molecules. The chlorophyll *a*–protein and the HP700 fraction must of necessity be closely related. The two preparations do, however, differ not only in P700 content, but also in a 680-nm bleaching associated with P700 oxidation in the HP700 fraction, and in the relative intensity of the long wave fluorescence. The P700–chlorophyll *a*–protein molecule appears more stable than the other chlorophyll *a*–protein molecules in the preparation; thus it might be possible to obtain a P700/chlorophyll ratio of 1/20, or perhaps 1/5 (*cf.* ref. 1) by using this property. By obtaining a fraction which is greatly enriched in P700, then even more information may be derived about the reaction center and its function; discrepancies should become resolvable, and the chance of identifying the primary electron acceptor should be improved. The studies reported here on the chlorophyll *a*–protein of *P. luridum* yield results which should be generally applicable to the Photosystem I of all plants[1].

Light-induced reduction of the polar quinone-like material may be responsible for most of the absorbance change observed in the ultraviolet region (Fig. 5); the oxidation of P700 probably accounts for only a small fraction of this ultraviolet change (see RESULTS). This unidentified quinone may also comprise that electron pool which feeds electrons to P^+700 slowly in the dark and rapidly in the presence of PMS. The reactions thus envisaged to occur in the isolated chlorophyll *a*–protein are: reduction of the primary acceptor by P700 in the light, and a light-independent transfer of these electrons from the acceptor to a pool; in the presence of PMS, an additional electron flow occurs from the pool to P^+700. The absence from the chlorophyll *a*–protein of natural cycling cofactors, which may have been dissociated by sodium dodecyl sulfate treatment, explains why the recovery of the reaction-center bleaching occurs slowly after illumination. A similar reasoning may explain the effect of PMS or methylene blue addition on the rate of electron transfer reactions in the reaction center preparations from *Rps. viridis*[39] and *Chromatium*[11,40]. *In vivo* cytochrome and/or other components probably provide this coupling. Oxygen must interact with a component of lower redox potential than the electron pool, however, possibly the primary acceptor. The identity of the primary electron acceptor remains unknown. Flavins or pteridines, unless covalently bound, do not appear to fill the role. Although our experiments make iron an unlikely candidate for the primary acceptor for P700 in the blue-green algae, it cannot yet be completely eliminated.

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