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THE LIGHT-HARVESTING CHLOROPHYLL *a/b*-PROTEIN COMPLEX FROM BARLEY THYLAKOID MEMBRANES

POLYPEPTIDE COMPOSITION AND CHARACTERIZATION OF AN OLIGOMER

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Summary

Electrophoretic analysis by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis showed that the light-harvesting chlorophyll *a/b*-protein complex of barley thylakoids contains only one polypeptide of apparent molecular weight 26 000. The barley mutant, deficient in chlorophyll *b* and this light-harvesting complex, lacks this polypeptide.

The addition of a nonionic detergent, Triton X-100, to the sodium dodecyl solubilization buffer prior to SDS polyacrylamide tube gel electrophoresis, allowed separation of a relatively stable complex, characterized as an oligomeric form of the light-harvesting complex. The oligomer also contained a polypeptide with an apparent molecular weight of 26 000. The absorption and fluorescence spectral properties of the oligomer are similar to those of the monomer. It is suggested that the oligomer of the light-harvesting chlorophyll *a/b*-protein is closer to the in vivo form rather than the monomer.

Introduction

Most, if not all, of the thylakoid membrane chlorophyll exists as chlorophyll-protein complexes [1—4]. Two major complexes are observed after polyacrylamide gel electrophoresis of thylakoid membranes solubilized in SDS; these are chlorophyll-protein complex I (CPI), which contains the reaction centre *P*700 for photosystem I, and the light-harvesting chlorophyll *a/b*-protein

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Abbreviations: CPI, chlorophyll-protein complex I; LHCP, light-harvesting chlorophyll *a/b*-protein complex; SDS, sodium dodecyl sulphate.

complex (LHCP) which has no apparent photosynthetic activity [1—4]. Particles enriched in LHCP have been extracted from thylakoid membranes by digitonin [5,6] and Triton X-100 [7—9]; however, the more usual procedure for preparation of LHCP has been hydroxylapatite chromatography [10,11] or gel electrophoresis of SDS extracts [12—19].

The properties of LHCP have been described [1,4,6] but there is disagreement as to whether there are one [13,19], two [10,14,16,18,20,21] or more [6,8,17] polypeptides associated with the complex. In attempts to determine which polypeptides are associated with LHCP, thylakoid membranes from a barley mutant which contains no chlorophyll *b* or LHCP have been used for comparison with thylakoid membranes from normal barley. The results suggest that either one [13,22,24], two [20,21] or more [23] polypeptides are missing in the barley mutant membrane.

The confusing and often contradictory data on the polypeptide composition of LHCP is due to the diversity of detergents and procedures used to prepare fractions enriched in LHCP, the probability of minor contaminants being present, the marked difference in resolving power of the various polyacrylamide gel procedures used, and the probability that species differences occur. The thylakoid polypeptide composition of normal barley mutant and a chlorophyll *b*-deficient barley mutant has therefore been re-examined under both dissociating and non-dissociating conditions. Our results indicate that LHCP contains only an apoprotein of apparent molecular weight 26 000. This polypeptide is absent in the barley mutant.

In addition to the two main chlorophyll-protein complexes, several minor chlorophyll-containing complexes have been detected by some SDS polyacrylamide gel electrophoresis methods [17,19,25—27]. One of these has been isolated from tobacco thylakoids by preparative polyacrylamide gels by Remy et al. [19] and characterized as a dimer of LHCP; the tobacco dimer contained only a polypeptide of apparent molecular weight 24 000 [19]. In the green alga, *Acetabularia mediterranea*, Apel [16,28,29] has shown that the light-harvesting complex has a higher molecular weight of 67 000 and is composed of two subunits of 23 000 and 21 000. We have demonstrated by SDS polyacrylamide tube gel electrophoresis at 4°C that an oligomer of LHCP also exists in normal barley thylakoids. The addition of the nonionic detergent, Triton X-100, during the solubilization of thylakoid membranes in SDS, enhances the content of oligomeric LHCP relative to that of LHCP.

Materials and Methods

Normal and mutant strains of barley (*Hordeum vulgare* L.) [30] was grown in pots for 18—25 days in a glass house at 20°C. Leaves were collected on ice, midribs were removed, and 10-g lots were chopped with a razor blade before homogenizing in a Servall Omnimix (5 s at 80 V, 10 s at 180 V) with ice-cold buffer (100 ml) containing 50 mM potassium phosphate (pH 7.2), 10 mM KCl and 0.3 M sucrose; all subsequent steps were performed at 4°C. The brei was filtered through two layers of Miracloth (Chicopee Mills Inc., N.Y.) and the filtrate was centrifuged at 2000 × *g* for 10 min. The pellet was resuspended in a buffer (45 ml) comprising 50 mM potassium phosphate (pH 7.2) and 10 mM

KCl. After centrifugation at 4000 X *g* for 10 min, the membrane pellet was then washed by centrifugation and resuspension with 25 ml of glass distilled water (4000 X *g* for 10 min), 25 ml of 1 mM sodium EDTA (pH 8; 10 000 X *g* for 10 min) and 15 ml of 50 mM Tricine (pH 8; 10 000 X *g* for 10 min). Washed thylakoid membranes were resuspended in 50 mM Tricine (pH 8), (1—2 mg chlorophyll/ml) and were stored in liquid N_2. The protein to chlorophyll ratio (mg/mg) of the washed thylakoid membranes was 5.4 for normal barley and 8.3 for mutant barley.

Total chlorophyll and chlorophyll *a/b* ratios were determined in 80% acetone by a modification [31] of the procedure of Mackinney [32]. Protein was determined by the procedure of Lowry et al. [33]; since chlorophyll interferes with the absorption at 600 nm, samples were read at 740 nm which gives accurate values for the protein content of chloroplast samples. The modification of Dulley and Grieve [34] was used to overcome precipitation problems when protein samples contained concentrations of Triton X-100 above 0.1% (v/v). Absorption spectra were recorded at room temperature with a Cary model 14R spectrophotometer. Fluorescence emission and absorption spectra were recorded at 77 K by Dr. S.W. Thorne as previously described [35].

Two polyacrylamide gel electrophoresis procedures have been used; the tube gels based on the procedures of Thornber [22,36] were used to separate and isolate chlorophyll-protein complexes, while the slab gels based on the procedure of Laemmli [37] were used to analyse thylakoid membrane polypeptides. Tube gels (0.6 X 5 cm) contained finally 8.7% (w/v) acrylamide, 0.232% (w/v) *N,N'*-methylenebisacrylamide, 0.1% SDS, 0.0005% (v/v) *N,N,N',N'*-tetramethylethylenediamine, 0.1% (w/v) ammonium persulphate and 50 mM Tris-HCl (pH 8). Gels were overlayered with 50 mM Tris (pH 8) containing 0.1% (w/v) SDS and were polymerized at room temperature. The overlayering buffer was also used in the upper and lower electrode chambers and gels were pre-equilibrated by electrophoresis at 5 mA per gel for 15 min at 4°C. Thylakoid membranes (20 μg chlorophyll) were solubilized on ice in a solution (20—100 μl) which contained finally 50 mM Tris (pH 8), 10% (v/v) glycerol and sufficient SDS to give an SDS/chlorophyll ratio of either 5 or 10/1 (w/w). Samples were applied immediately to the gels which were run at 4°C with a starting current of 0.5 mA/gel for 15 min and then 5 mA/gel for the next 30—60 min. Gels were scanned at 675 and 650 nm on a Varian 635 spectrophotometer with a gel scanning attachment. An estimated relative distribution of chlorophyll on the gels was determined by measuring the area under each peak with the planimeter, averaging the values obtained at 675 and 650 nm, and expressing the area for each peak as a percentage of the total area for all peaks [13]. Chlorophyll-containing bands were excised from gels and absorption spectra determined directly with a Cary 14R spectrophotometer. Fluorescence absorption and emission spectra were obtained from intact gel slices after impregnation with 66% (v/v) glycerol under vacuum. Chlorophyll-protein complexes were electrophoretically eluted from gels by placing chlorophyll-containing gel slices into tubes filled with electrophoresis buffer and sealed at the bottom with a dialysis membrane.

The discontinuous polyacrylamide gel electrophoresis system described by Laemmli [37] was used for slab gels. The slabs (20 X 16 X 0.2 cm) comprised

a resolving gel (15 cm high) of either 10.5% (w/v) or 14% (w/v) acrylamide and a stacking gel (5 cm high with 12 inserts (2.5 X 0.7 cm)) of 4.4% (w/v) acrylamide. Thylakoid membranes (50 μg protein) were solubilized in an equal volume (10—50 μl) of solubilizing buffer [37] containing 0.25% (v/v) 2-mercaptoethanol. If needed, samples were placed in a boiling water bath for 2 min before electrophoresis. Two gels were run overnight at 40 mA (approx. 150–200 V) and 4°C until the bromophenol dye reached the bottom. Gels were stained for 1 h in Coomassie Brilliant Blue (0.25%, w/v) in ethanol/acetic acid/water (25 : 7 : 68, v/v) on a shaking water-bath at 50°C. The gels were destained at 50°C using the same solvent mixture and when the background gel was clear they were soaked in 30% methanol at room temperature for 30 min, and finally were dried under vacuum [38]. Approximate molecular weights of polypeptides were determined from a plot of the logarithm of molecular weight versus distance migrated, obtained with the following standards; bovine serum albumin (68 000), catalase (60 000), aldolase (40 000), chymotrypsinogen (25 700) and cytochrome *c* (11 700).

Results

Characterization of thylakoid membranes by polyacrylamide gel electrophoresis. Thylakoid membranes from normal and mutant barley were solubilized in SDS and fractionated by polyacrylamide tube gel electrophoresis at 4°C. In normal barley, three major and two minor chlorophyll-containing peaks were observed (Fig. 1). Peaks marked CPI and LHCP represent the two complexes which have been studied in detail [1—4] (CPI is chlorophyll-protein

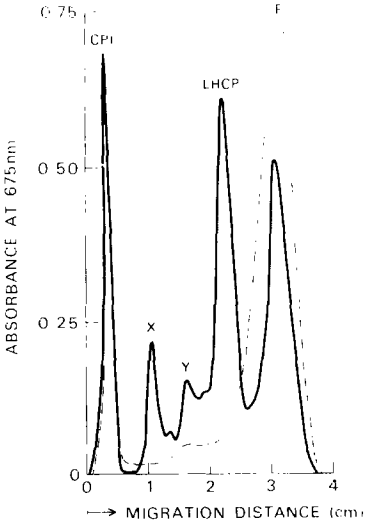

Fig. 1. Polyacrylamide tube gel electrophoresis at 4°C of washed thylakoid membranes from normal (———) and mutant (- - - - -) barley. The SDS/chlorophyll ratio was 5/1 (w/w). The gels have not been stained for protein and the peaks that are observed at 675 nm are chlorophyll-containing bands: CPI, X and Y (minor chlorophyll-containing complexes) LHCP and F (free chlorophyll). As estimated percent distribution of chlorophyll under each peak is detailed in Table I.

complex I) and peak F represents free chlorophyll; peaks X and Y are both chlorophyll-protein complexes which will be referred to as complex X and complex Y, respectively. Reference to a chlorophyll-containing peak on a tube gel as a chlorophyll-protein complex does not imply that only one polypeptide is present under that peak and for clarity the protein(s) which actually bind chlorophyll will be referred to as the apoprotein(s) of the complex. The barley mutant which lacks chlorophyll *b* has neither complex X nor LHCP (Fig. 1). A minor green peak with the same mobility as complex Y was seen with the mutant membranes but it was not further studied due to its variable reproducibility. The estimated relative distributions of chlorophyll in the complexes of normal and mutant barley are compared in Table I.

Thylakoid membranes from normal barley were run on polyacrylamide slab gels to determine their polypeptide composition (Fig. 2). The conditions used for slab gels are harsher than those used for tube gels since the electrophoresis time is 16 h instead of 1 h, and greater dissociation of the chlorophyll-protein complexes to apoproteins and free chlorophyll occurs. When samples were not heated before electrophoresis only traces of CPI were seen, but the apoprotein of this complex (Cp in Fig. 2) was clearly visible. This protein was identified as the apoprotein by re-electrophoresis of a dissociated sample of CPI obtained from a tube gel (Fig. 5). In contrast to CPI, LHCP was not dissociated completely. The diffuse green band corresponding to this complex is seen in the region of polypeptides 6, Lp and 7 on a 14% acrylamide gel, and just above polypeptide 8 and well below polypeptides 6 and Lp on a 10.5% acrylamide gel. The variation of mobility of LHCP with respect to other thylakoid polypeptides

TABLE I

THE EFFECT OF TRITON X-100 ON THE DISTRIBUTION OF CHLOROPHYLL IN POLYACRYLAMIDE TUBE CELLS

Thylakoid membranes from normal or mutant barley were dissolved in tube gel solubilizing buffer at a SDS/chlorophyll ratio of 5 or 10 and various ratios (v/w) of Triton X-100/chlorophyll. The relative distribution of chlorophyll was estimated as described in Materials and Methods.

SDS / Chlorophyll	Triton X-100 / Chlorophyll	Number of gels	Average percent of chlorophyll estimated in complexes *					
			CPI	X	Y	LHCP	F	X plus L
			Normal thylakoids					
5	0	8	12.7	8.2	4.6	44.2	33.8	52.4
5	2.5	3	10.7	12.9	2.2	36.8	38.2	49.7
5	5	4	10.8	13.2	5.5	29.4	45.2	42.6
5	10	4	11.8	15.1	6.0	23.6	46.5	38.7
5	20	8	11.9	17.4	4.9	12.9	58.7	31.3
10	0	13	12.1	7.2	4.2	37.5	37.4	44.7
10	20	11	13.7	19.2	4.3	15.0	49.0	34.2
			Mutant thylakoids					
10	0	4	20.0	—	—	—	79.8	—
10	20	4	20.9	—	—	—	80.9	—

* CPI, X, Y, LHCP and F as for fig. 1.

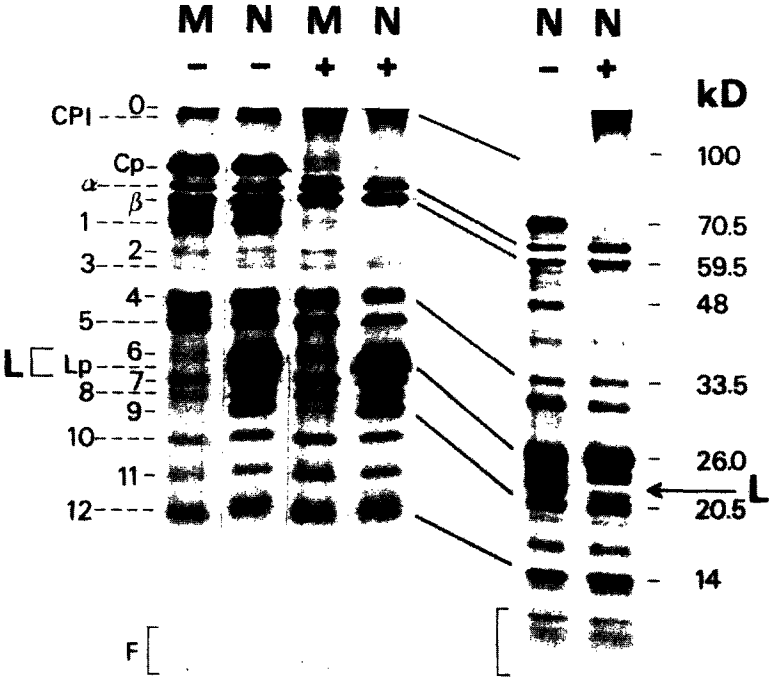

Fig. 2. Polyacrylamide slab gel electrophoresis at 4°C of washed thylakoid membranes from normal (N) and mutant (M) barley. Solubilized membranes were either heated in a boiling-water bath for 2 min (+) or were applied directly to gels without heating (—). Gels were composed of either 14% (gels 1—4) or 10.5% acrylamide (gels 5, 6) and were stained for protein with Coomassie Blue. The positions of CPI, LHCP, and their derived polypeptides, Cp and Lp, respectively, free chlorophyll (F) and the α and β subunits of the chloroplast coupling factor are indicated. Other major polypeptides are numbered 1—12. The correspondence between equivalent polypeptides on the two gel systems is indicated by the solid lines, and the approximate molecular weight of selected polypeptides is indicated on the right hand side.

at different gel concentrations has been observed previously [18,39]. No chlorophyll-containing bands equivalent to complexes X and Y seen in tube gels were visible on the slab gels, even though they were run at 4°C. Heating thylakoid membranes from normal barley before electrophoresis causes complete dissociation of CPI and LHCP. When LHCP was dissociated by heating there was a concomitant increase in the intensity of polypeptide Lp (Fig. 2), indicating that polypeptide Lp is the apoprotein of LHCP. This was confirmed by reelectrophoresis of a sample of dissociated LHCP obtained from the tube gel procedure (Fig. 5). Heating also caused the disappearance of polypeptides Cp and 1, as well as the diffuse band which appears immediately below polypeptide β. A strongly-staining protein band was seen at the origin of the gel after heating suggesting that the polypeptides had aggregated and no longer entered the resolving gel. This phenomenon has previously been observed with *Chlamydomonas* polypeptide Cp [18,39]. We found that polypeptides Cp and 1 were also markedly decreased in content relative to other polypeptides, after delipidation of thylakoid membranes by acetone extraction prior to solubilization with SDS, and aggregates were again seen at the top of the gel.

Barley mutant thylakoid membranes lacked polypeptide Lp, and only traces

of polypeptide 9 were detected compared to the normal barley; otherwise the polypeptide profiles were similar. The mutant thylakoid membranes have polypeptides 6 and 7 which almost comigrate with LHCP and polypeptide Lp, on the 14% acrylamide gel. Aggregation of polypeptides Cp and 1 with heating and acetone extraction were also seen with mutant barley (Fig. 2). The identity of the other thylakoid-membrane polypeptides is generally unknown, but electrophoretic examination of washed membranes which had been further treated with 2 M NaBr [40] indicated that significant quantities of the chloroplast coupling factor polypeptides were still present, even though the membranes had been washed with EDTA during preparation. Polypeptides α and β of molecular weights approx. 63 000 and 59 500 (Fig. 2) were shown by electrophoresis of the NaBr extract to be the α and β subunits, respectively, of the chloroplast coupling factor.

Characterization of complex X as an oligomer of LHCP. Various Triton X-100 solubilized thylakoid fractions were analysed by SDS polyacrylamide gel electrophoresis in an attempt to determine the amount of chlorophyll still in the form of chlorophyll-protein complexes. As the concentration of Triton X-100 was increased, there was a decrease in the proportion of chlorophyll in LHCP, and an increase in complex X and free chlorophyll, while the amount of chlorophyll associated with CPI was unchanged (results not shown). This unexpected increase in the amount of chlorophyll associated with complex X in Triton X-100 was further examined. When thylakoid membranes were solubilized directly in the SDS solubilization buffer, together with Triton X-100, there was a significant increase in the proportion of chlorophyll in complex X (Table I); e.g. a Triton X-100/chlorophyll ratio of 20 and a SDS/chlorophyll ratio of 10, resulted in a doubling of the chlorophyll in complex X, an increase in free chlorophyll, a decrease in LHCP, and CPI was unchanged while the minor complex Y was variable. The addition of Triton X-100 had no effect with the barley mutant as expected, since both complex X and LHCP are absent. Similar effects to those seen in Table I were observed with spinach thylakoid membranes. Other nonionic detergents, at the same concentrations as Triton X-100, such as nonidet P40, emasol, Tween 40, digitonin and saponin, as well as the ionic detergent, deoxycholate, all increased the concentration of chlorophyll in complex X, relative to electrophoresis with SDS alone. Addition of 200 mM NaCl to the solubilizing buffer decreased the concentration of complex X in the presence of SDS with or without Triton X-100. In all cases the decrease in chlorophyll associated with LHCP was accounted for by the increase in proportion of chlorophyll in complex X and free chlorophyll.

The average chlorophyll *a/b* ratio of complex X isolated from preparative tube gels by electrophoretic elution was 1.26. This value is similar to that of LHCP in runs where LHCP was clearly separated from free chlorophyll. The absorption spectrum of complex X at 25°C clearly shows the high content of chlorophyll *b* in the complex with absorption maxima at 473 and 652 nm (Fig. 3) and a carotenoid shoulder is seen at 481 nm. This spectrum is similar to that of LHCP. The fluorescence emission and excitation (Fig. 4) spectra of tube gel pieces containing complex X were essentially the same as those of LHCP with respect to absorption maxima and fluorescence intensity. Chlorophyll *b* efficiently transfers its absorbed energy to chlorophyll *a* as indicated by

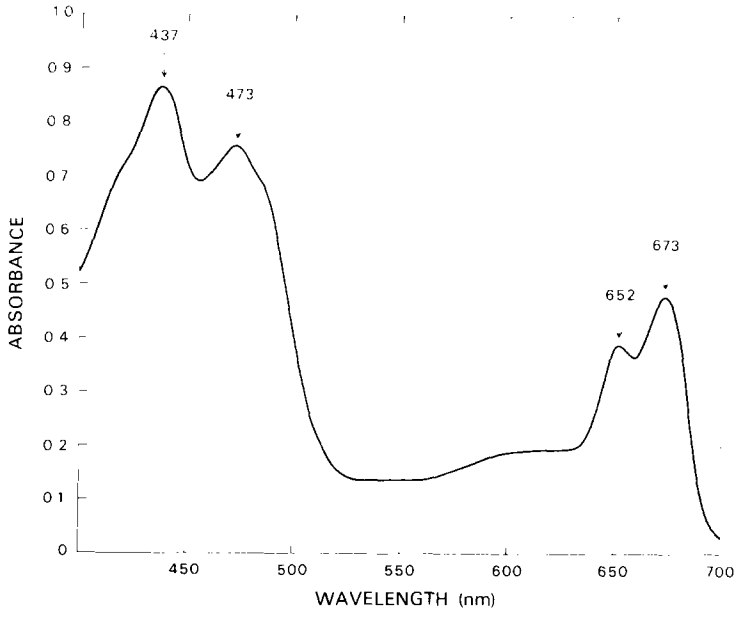

Fig. 3. Absorption spectrum of a polyacrylamide tube gel piece containing complex X obtained at 25°C.

the large peak in the excitation spectrum at 470 nm. Moreover, the shoulder at 480 nm in the excitation spectrum suggests that the carotenoid associated with complex X is also transferring its energy to chlorophyll *a* and it may therefore be an integral part of complex X.

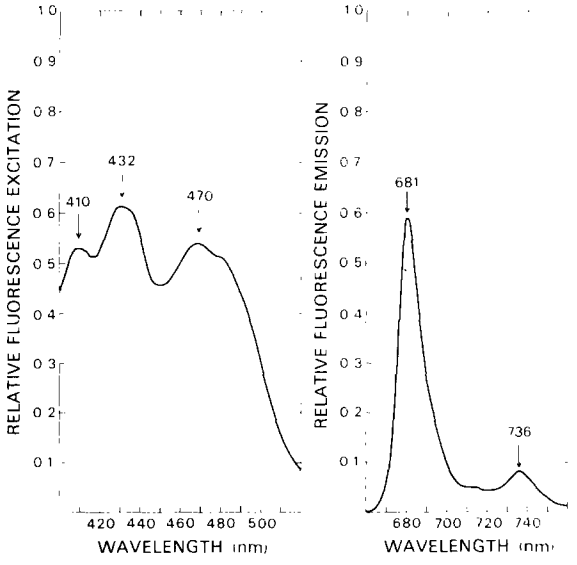

Fig. 4. Fluorescence excitation and emission spectra of a gel piece containing complex X obtained at 77 K. The excitation wavelength for the emission spectra was 440 nm and the emission wavelength for the excitation spectra was 678 nm.

The apparent molecular weight of complex X (68 000) was more than twice that of LHCP (25 000). These values are similar to those found by Remy et al. [19] who classified the higher molecular weight complex as a dimer of LHCP. However, the apparent molecular weights of chlorophyll-protein complexes are clearly anomalous [3] and since it is impossible to obtain true molecular weight by this method, we have termed complex X an oligomer of LHCP.

Re-electrophoresis on a tube gel of an electrophoretically eluted sample of complex X gave chlorophyll-containing bands in the position of LHCP and free chlorophyll only, while direct re-electrophoresis of complex X from a tube gel segment on a second tube gel consistently gave chlorophyll in the positions of complex X (25%), LHCP (55%) and free chlorophyll (20%). Re-electrophoresis of LHCP either directly or after electrophoretic elution, always gave only LHCP and free chlorophyll and never any complex X. These results indicate that SDS electrophoresis causes dissociation of complex X to LHCP and free chlorophyll, and LHCP dissociates to free chlorophyll. Re-electrophoresis on polyacrylamide slab gels of samples of CPI, complex X and LHCP obtained from tube gels by electrophoretic elution showed that the main polypeptide of CPI was Cp, and that of both complex X and LHCP was Lp (Fig. 5). Electrophoretic elution of CPI undoubtedly causes some dissociation, the apoprotein Cp shows a marked tendency to aggregate and this accounts for the material seen at the origin of this gel. The α and β subunits of the coupling factor seen with complex X and polypeptide 6 seen with LHCP are contaminants, which have similar electrophoretic mobilities on tube gels to complex X and LHCP respectively. Re-electrophoresis of a tube gel piece directly onto a slab gel

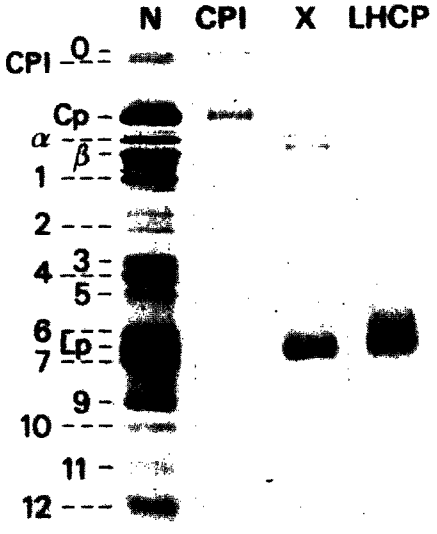

Fig. 5. Polyacrylamide slab gel electrophoresis of chlorophyll-protein complexes which had been eluted from a tube gel. CPI, complex X and LHCP were electrophoretically eluted from the tube gels, concentrated and rerun (25 μg protein) on a 14% polyacrylamide slab gel which was stained for protein with Coomassie Blue. Washed thylakoid membranes from normal barley (N) were run as a standard.

avoided the electrophoretic elution step and the polypeptides all ran at an angle to the origin, but in a straight line, except polypeptides Cp and Lp. Only these two polypeptides were seen out of line because of dissociation of CPI and complex X, respectively, during the second electrophoretic step. Only one polypeptide was ever associated with complex X after re-electrophoresis on gels on either 10.5% or 14% acrylamide. No trace of the 20 500 molecular weight polypeptide (9 in Fig. 2) was seen with complex X, and polypeptides 6 and 7 were also absent.

Discussion

During SDS polyacrylamide gel electrophoresis, the oligomer, complex X, dissociates to LHCP which in turn dissociates to the apoprotein, Lp and free chlorophyll. It is possible, therefore, that under non-dissociating conditions, the apoprotein of LHCP could be seen in more than one position on polyacrylamide gels. Analysis of thylakoid membranes by comparison of both the dissociated and non-dissociated polypeptide patterns is needed to determine the polypeptides likely to be the apoprotein(s) of chlorophyll-protein complexes (Fig. 2 and refs. 19, 20 and 39). However, heating or acetone extraction of thylakoid membranes from normal barley causes aggregation of the apoprotein of CPI [18,39] and a polypeptide of molecular weight 48 000 (Fig. 2), as well as dissociation of complexes X, Y and LHCP. Since the 48 000 molecular weight polypeptide is also aggregated in the barley mutant, which lacks complex X and LHCP, it cannot be the apoprotein of complex X.

Our results indicate that CPI of barley thylakoids contains a single polypeptide of apparent molecular weight 68 000, in agreement with other studies [14,19,39]. Further, we find that barley LHCP has only one apoprotein (Lp) of apparent molecular weight 26 000. First, this polypeptide is missing in the mutant of barley (Fig. 2) which lacks chlorophyll *b*, complex X and LHCP. Second, when LHCP is dissociated prior to electrophoresis, the concentration of polypeptide Lp is always greatly increased (Fig. 2). Third, re-electrophoresis of LHCP shows only one major polypeptide (Lp) and the dissociated oligomer of LHCP also only has polypeptide Lp (Fig. 5). Fourth, in addition a homogeneous fraction of barley LHCP can be isolated in a single step by absorption chromatography on controlled-pore glass [41] and this fraction contains only polypeptide Lp. It is important to note that we find polypeptide Lp always runs as a broad, somewhat diffuse band compared to the other thylakoid polypeptides no matter what concentration of acrylamide is used. It is possible that more than one molecular form of polypeptide Lp is present and the fact that LHCP is partially phosphorylated [42] and contains carbohydrates [27] may be important. Two-dimensional separation of LHCP [43] may resolve this possibility. Remy et al. [19] also conclude that tobacco LHCP has only one polypeptide. Interestingly, Apel and Kloppstech [44] suggest that the protein moiety of barley LHCP contains only one polypeptide since they showed the light-induced appearance of only one mRNA species coding for the apoprotein of LHCP.

Although other studies have suggested that more than one polypeptide was associated with LHCP, one major polypeptide was prominent in all LHCP frac-

tions examined with higher plants [6,8,13,15,17,19,22] and the minor polypeptides, molecular weights were smaller or larger by less than 2000. This variability in composition and content of the minor polypeptide constituents of the LHCP fractions throws some doubt on their authenticity as apoproteins. The minor polypeptides found in some studies [15,17,22] may be due to co-electrophoresis of unrelated polypeptides with LHCP in the preparative polyacrylamide gel electrophoresis. The green band of LHCP is broad and covers unrelated polypeptides, such as polypeptides 6 and 7 (Fig. 2). Preparation of LHCP fractions on hydroxylapatite columns [10,17] or by sucrose density gradient centrifugation [5,6,8] does not necessarily eliminate non-chlorophyll-containing polypeptides being isolated with LHCP. With barley thylakoid membranes Anderson and Levine [20,21] suggested that two polypeptides (IIb and IIc) were associated with LHCP; IIb predominated after heating and acetone extraction while IIc predominated before lipid extraction [20]. We now interpret this data to mean that IIb is equivalent to polypeptide Lp (Fig. 2) and IIc is LHCP. The reason that some polypeptide IIc apparently survived heating and acetone extraction was that the lower resolving power of the gels did not separate polypeptides 7, 8 amd 9 (Fig. 2) from LHCP and these polypeptides were unaffected by heating and lipid extraction. Henriques and Park [23] and Machold et al. [24] found that the main difference between normal and mutant barley was the almost complete loss of a polypeptide of approx. molecular weight 25 000 in the mutant. No other differences were observed by Machold et al. [24], but Henriques and Park [23] found minor decreases in mutant barley polypeptides of molecular weights 27 500 and 20 000. The quantitative loss of polypeptides such as those of Henriques and Park [23] and our polypeptide 9 (Fig. 2) might indicate that these polypeptides are closely associated with LHCP but are not involved with binding of the chlorophyll.

In contrast to higher plants, two green algae appear to have two polypeptides as apoproteins of LHCP. With *A. mediterranea* the chlorophyll-protein complex which electrophoresed in the same position as LHCP of higher plants [28] was found to have polypeptides of molecular weight 21 500 and 23 000 associated with it [16,29]. With *Chlamydomonas reinhardtii*, Chua et al. [39] resolved two chlorophyll-containing polypeptides in the position of LHCP by extended electrophoreseis on a highly resolving polyacrylamide gradient gel. Bar-nun et al. [18] also found two polypeptides of molecular weights of 22 000 and 24 000 associated with LHCP.

The addition of Triton X-100 to thylakoid membranes, either prior to or during the SDS solubilization, allows greater amounts of chlorophyll to remain associated with complex X in the subsequent tube gel electrophoresis. This may result if Triton X-100, which is known to preserve the quaternary structure of membrane proteins [45], preferentially replaces the usual boundary lipids surrounding the oligomer, thereby shielding the complex to some extent from dissociation by sodium dodecyl sulphate. This finding has allowed us to isolate and characterize an oligomer of LHCP. Apart from their differing mobilities in electrophoresis, the properties of the monomer and oligomer of LHCP are almost identical. Both have chlorophyll *a/b* ratios of 1.26 and similar absorption spectra. Although aggregation of LHCP by the addition of cations to

LHCP fractions, led to a marked decrease in its fluorescence intensity [6,8], our results show that the fluorescence excitation and emission spectra of the oligomer are the same as those of the monomer, without any alteration in fluorescence intensity. Furthermore, the fluorescence excitation spectra show that a carotenoid is an integral part of both complexes. The oligomeric form of LHCP contains also only a polypeptide of apparent molecular weight 26 000.

Although LHCP is widely quoted as having equimolar proportions of chlorophyll *a* and chlorophyll *b* [1,2,4,9,10] we find that both the oligomer and monomer of LHCP have a chlorophyll *a/b* ratio of 1.26, significantly greater than 1. This is in agreement with values obtained by Remy et al. [19] for tobacco LHCP, and by Arntzen et al. [6,8] for pea LHCP.

The question arises whether LHCP normally exists as a monomer or an oligomer. We suggest that the oligomer is the more usual form rather than the monomer for the following reasons. Prolonged or re-electrophoresis of complex X causes its dissociation to LHCP, which in turn dissociates to free chlorophyll. Small amounts of oligomer are always present in gels run at 4°C (Fig. 1) and relatively more of the 'dimer' has been detected by Remy et al. [19] after rapid solubilization and short times for electrophoresis. Further, oligomer formation is favoured by the use of lower ratios of detergent/chlorophyll [13] and by the addition of Triton X-100 to the SDS. Thus, milder conditions favor the oligomer, suggesting that the monomer is formed by the strongly dissociating conditions of ionic SDS. Whether higher molecular weight aggregates of LHCP occur in vivo cannot be determined at the present time.

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References

1 Thornber, J.P. (1975) Annu. Rev. Plant Physiol. 26, 127—158
2 Anderson, J.M. (1975) Biochim. Biophys. Acta 416, 191—235
3 Boardman, N.K., Anderson, J.M. and Goodchild, D.J. (1978) in Current Topics in Bioenergetics (Sanadi, D.R. and Vernon, L.P., eds.), Vol VIII, pp. 35—109
4 Thornber, J.P., Alberte, R.S., Hunter, F.A., Shiozawa, J.A. and Kan, K.-S. (1977) Brookhaven Symp. Biol. 28, 132—148
5 Wessels, J.S.C. and Borchart, M.T. (1974) in Proceedings of the Third International Congress on Photosynthesis (Avron, M., ed.), pp. 473—484, Elsevier, Amsterdam
6 Arntzen, C.J. and Ditto, C.L. (1976) Biochim. Biophys. Acta 449, 259—274
7 Vernon, L.P., Shaw, E.R., Ogawa, T. and Raveed, D. (1971) Photochem. Photobiol. 14, 343—357
8 Burke, J.J., Ditto, C.L. and Arntzen, C.J. (1978) Arch. Biochem. Biophys., in press
9 Kung, S.D. and Thornber, J.P. (1971) Biochim. Biophys. Acta 253, 285—289
10 Kan, K.-S. and Thornber, J.P. (1976) Plant Physiol. 57, 47—52
11 Ogawa, T., Obata, F. and Shibata, K. (1966) Biochim. Biophys. Acta 112, 223—234
12 Thornber, J.P., Gregory, R.P.F., Smith, C.A. and Bailey, J.L. (1967) Biochemistry 6, 391—396
13 Genge, S., Pilger, D. and Hiller, R.G. (1974) Biochim. Biophys. Acta 347, 22—30
14 Machold, O. (1975) Biochim. Biophys. Acta 382, 494—505
15 Süss, K.H., Schmidt, O. and Machold, O. (1976) Biochim. Biophys. Acta 448, 103—113
16 Apel, K., Bogorad, L. and Woodcock, C.L.F. (1975) Biochim. Biophys. Acta 387, 568—579
17 Henriques, F. and Park, R. (1977) Plant Physiol. 60, 64—68
18 Bar-Nun, S., Schantz, R. and Ohad, I. (1977) Biochim. Biophys. Acta 459, 451—467

19 Remy, R., Hoarau, J. and Leclerc, J.C. (1977) Photochem. Photobiol. 26, 151—158
20 Anderson, J.M. and Levine, R.P. (1974) Biochim. Biophys. Acta 357, 118—126
21 Anderson, J.M. and Levine, R.P. (1974) Biochim. Biophys. Acta 333, 378—387
22 Thornber, J.P. and Highkin, H.R. (1974) Eur. J. Biochem. 41, 109—116
23 Henriques, F. and Park, R.B. (1975) Plant Physiol. 55, 763—767
24 Machold, O., Meister, A., Sagromsky, H., Hoyer-Hansen, G. and von Wettstein, D.Y. (1977) Photosynthetica 11, 200—206
25 Hermann, F. and Meister, A. (1972) Photosynthetica 6, 177—182
26 Hiller, R.G., Genge, S. and Pilger, D. (1974) Plant Sci. Lett. 2, 239—242
27 Hayden, D.B. and Hopkins, W.G. (1977) Can. J. Bot. 55, 2525—2529
28 Apel, K. (1977) Biochim. Biophys. Acta 462, 390—402
29 Apel, K., Miller, K.R., Bogorad, L. and Miller, G.J. (1976) J. Cell Biol. 71, 876—893
30 Highkin, H.R. (1950) Plant Physiol. 25, 294—306
31 Arnon, D.I. (1949) Plant Physiol. 24, 1—15
32 Mackinney, G. (1941) J. Biol. Chem. 140, 315—322
33 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
34 Dulley, J.R. and Grieve, P.A. (1975) Anal. Biochem. 64, 136—141
35 Boardman, N.K. and Thorne, S.W. (1968) Biochim. Biophys. Acta 153, 448—458
36 Thornber, J.P. (1970) Biochemistry 9, 2688—2698
37 Laemmli, U.K. (1970) Nature 227, 680—685
38 Fairbanks, Jr., G., Levinthal, C. and Reeder, R.M. (1965) Biochem. Biophys. Res. Commun. 20, 393—399
39 Chua, N.H., Matlin, K. and Bennoun, P. (1975) J. Cell Biol. 67, 361—377
40 Kamienietzky, A. and Nelson, N. (1975) Plant Physiol. 55, 282—287
41 Dunkley, P.R. and Anderson, J.M. (1978) Arch. Biochem. Biophys., in the press
42 Bennett, J. (1977) Nature 269, 344—346
43 Novak-Hofer, I. and Siegenthaler, P.A. (1977) Biochim. Biophys. Acta 468, 461—471
44 Apel, K. and Kloppstech, K. (1978) Eur. J. Biochem. 85, 581—588
45 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29—79