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P-700 CONTENT AND POLYPEPTIDE PROFILE OF CHLOROPHYLL-PROTEIN COMPLEXES OF SPINACH AND BARLEY THYLAKOIDS

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Summary

Of the six chlorophyll-protein complexes of spinach and barley resolved by mild gel electrophoresis, two were chlorophyll *a*-protein complexes of PS I, namely CP1a and CP1, which accounted for up to 30% of the total chlorophyll. Both of these complexes had one *P*-700 per 120 chlorophyll *a* molecules. Since spinach and barley thylakoids have some 400 chlorophyll molecules per *P*-700, these complexes may not have lost any of the chlorophyll associated with them in vivo. This may account for CP1a and CP1 having the characteristic low-temperature fluorescence normally associated with PS I in vivo, which is not found in complexes with low chlorophyll/*P*-700 ratios.

Two-dimensional electrophoresis showed that all of the chlorophyll *a* and *P*-700 of CP1 was bound to 70 kilodalton polypeptides. The PS I reaction centre complex of lowest mobility, CP1a, contained CP1 and four additional low molecular weight polypeptides. The three light-harvesting complexes resolved had major 25 and 23 kilodalton polypeptides. The presumed reaction centre complex of PS II contained major 50 and 47 kilodalton polypeptides.

Introduction

That chlorophyll is associated with protein in vivo was demonstrated in 1966 when two chlorophyll-protein complexes were resolved by SDS-polyacrylamide

Abbreviations: Chl, chlorophyll; CP1, chlorophyll-protein complex 1; LHCP, light-harvesting chlorophyll *a*/*b*-protein complex; PS I, photosystem I; PS II, photosystem II; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

gel electrophoresis [1,2]. These were chlorophyll-protein complex 1 of PS I (10—15% of the total chlorophyll) and the light-harvesting chlorophyll *a/b*-protein complex (40—60% of the total chlorophyll) [3,4]. With improved electrophoretic procedures, less chlorophyll is dissociated from the protein and new chlorophyll-protein complexes have been resolved and characterized [5—13]. The pigment compositions and spectral analyses of these complexes have been presented [5—13] and the distribution of the chlorophyll amongst the three main chlorophyll-protein complexes, the reaction centre complexes of PS I and PS II, and the light-harvesting complex, is beginning to be understood for some plant species. However, less attention has been directed to the polypeptide composition of the chlorophyll-protein complexes [5,7,11,12] and the question as to which polypeptides bind the chlorophyll is only partly resolved.

We have examined the *P*-700 content and polypeptide profiles of the six chlorophyll-protein complexes resolved from spinach and barley thylakoids by a milder electrophoretic method which allows most of the chlorophyll to remain associated with protein. This study shows that the reaction centre complex of PS I was resolved as two chlorophyll *a*-proteins, each of which show reversible *P*-700 photooxidation and have one *P*-700 per 120 chlorophyll *a* molecules. Mild conditions of two-dimensional electrophoresis demonstrated that both the chlorophyll *a* and *P*-700 were bound to 70 kilodalton polypeptides in the PS I complexes. The three light-harvesting chlorophyll *a/b*-proteins resolved had 25 and 23 kilodalton polypeptides, whereas the chlorophyll *a*-protein complex of the presumed reaction centre of PS II had 50 and 47 kilodalton polypeptides.

Methods

Chloroplasts were isolated from spinach (water-culture), normal barley and a chlorophyll *b*-deficient mutant barley [14] as described previously [15]. Thylakoids were washed once in glass-distilled water, 1 mM EDTA (pH 8.0) and twice in 50 mM Tricine (pH 8.0), resuspended in 50 mM Tricine (pH 8.0, 2—4 mg Chl/ml) and stored in liquid N_2. Total chlorophyll and Chl *a*/Chl *b* ratios were determined in 80% acetone [16]. Protein was determined by the method of Lowry et al. [17].

Two polyacrylamide gel electrophoresis methods were used: tube gels based on the procedure of Anderson et al. [9] were used to separate and isolate chlorophyll-protein complexes in the first dimension and slab gels based on the procedure of Laemmli [18] were used to analyze thylakoid membrane polypeptides in the second dimension.

For tube gels, thylakoids were solubilized at 4°C without prior lipid extraction in 0.3 M Tris-HCl (pH 8.8) containing 13% glycerol and 0.5% SDS (instead of 1% SDS [9]) to give an SDS/Chl weight ratio of 10 : 1. The tube gels (0.5 × 10 cm) consisted of a stacking gel containing 4% acrylamide, 56 mM Tris-H_2SO_4 (pH 6.14), 0.1% SDS, 0.1% ammonium persulphate and 0.05% (v/v) *N,N,N',N'*,-tetraethylmethylenediamine (TEMED) and a resolving gel containing 8% acrylamide, 0.22 or 0.43 M Tris-HCl (pH 9.35), 0.1% ammonium persulphate and 0.05% (v/v) TEMED. The upper reservoir buffer (pH 8.64)

was 41 mM Tris adjusted with boric acid and 0.1% SDS, and the lower reservoir buffer (pH 9.35) was 0.43 M Tris adjusted with HCl. The stock acrylamide contained 29.2% acrylamide and 0.8% *N,N'*-methylenebisacrylamide. Electrophoresis at 4°C was carried out as described previously [9]. The relative distribution of chlorophyll on these gels was estimated by scanning unstained gels at 675 and 650 nm on a Varian 635 spectrophotometer equipped with a gel scanning attachment [9]. Gel segments or horizontal slices of complete gels containing chlorophyll-protein complexes were cut out and placed in slots of slab gels for re-electrophoresis; the gels were sometimes stored frozen in 125 mM Tris-HCl (pH 6.8) containing 2% SDS at −20°C.

The discontinuous polyacrylamide gel electrophoresis system as described by Laemmli [18] was used for single concentration slab gels and that of O'Farrell [21] for concentration gradient slab gels. In each case, the slabs (20 × 16 × 0.15 cm) contained a 4.4% (w/v) polyacrylamide stacking gel (4 cm high) and either a 14% (w/v) acrylamide resolving gel (12 cm high) or a gradient gel with concentration ranges of 10—16% or 12—16% (w/v) (12 cm high). The acrylamide/bisacrylamide weight ratio was 30 : 0.8. When thylakoid membrane extracts or excised gel segments from the tube gels were applied to slab gels, 12 inserts were made in the stacking gel (2.5 × 0.7 cm). Similar segments from three or four gels were placed in each slot. When a horizontal slice from a complete tube gel was being run in the second dimension, one glass plate with a 45° bevelled edge above the stacking gel was required to accommodate the first dimension tube gel in a horizontal position. Thylakoid membranes and standards were solubilized in an equal volume of solubilizing buffer [18] containing 0.25% (v/v) mercaptoethanol. If required, gels, gel segments (6 min) or samples (2 min) were placed in a boiling water bath for 2 min before electrophoresis. A current of 15 mA (80—100 V) was applied to each gel. Gels were stained in Coomassie brilliant blue (0.25% w/v) in ethanol/acetic acid/water (25 : 7 : 68, v/v/v) and destained in the same solvent mixture. Apparent molecular weights were obtained by comparison with known standards: bovine serum albumin (68 000), ovalbumin (45 000), chymotrypsinogen (25 000) and cytochrome *c* (12 500).

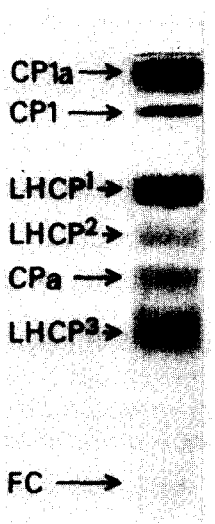
For *P*-700 measurements, excised chlorophyll-containing gel segments were forced through disposable syringes (5-ml) without needles, homogenized in 50 mM Tricine (pH 8.0) and the extracts centrifuged at 7000 × *g* for 15 min. *P*-700 concentration was estimated by two methods using a millimolar extinction coefficient of 64 cm^{-1} [19]. (a) Chemical method: by the ferricyanide-oxidized minus ascorbate-reduced difference spectrum of samples in 50 mM Tricine (pH 8.0) as described in Ref. 20. (b) Photochemical method: the light-induced absorbance change at 698 nm (740 nm reference wavelength) was measured in a Chance Aminco dual-wavelength spectrophotometer as described in Ref. 2. Samples were in 50 mM Tricine (pH 8.0) containing 3 mM sodium ascorbate and 166 μM methyl viologen.

Results

One-dimensional tube SDS electrophoresis

When spinach or barley thylakoids were solubilized at 4°C at an SDS/

chlorophyll weight ratio of 10 : 1 and a final SDS concentration of 0.5%, and fractionated using a mild SDS discontinuous polyacrylamide electrophoretic method, seven chlorophyll-containing zones were resolved and six of these also contained protein (Fig. 1). Only 9% of the total chlorophyll was located in the free pigment zone with spinach thylakoids. The chlorophyll composition of the six chlorophyll-protein complexes have been characterized by pigment and spectral analyses [9]. Their designations are shown on the left-hand side of the gel (Fig. 1) and the relative distribution of chlorophyll amongst the pigment complexes is shown on the right-hand side. Two chlorophyll *a*-proteins, CP1a and CP1, belong to the PS I reaction centre complex and account for 30% of the total chlorophyll with spinach thylakoids (Fig. 1). The other chlorophyll *a*-protein, CPa, which was originally detected by Hayden and Hopkins [6] is the presumed reaction centre complex of PS II [6,7,9–12]; it accounts for 10% of the total chlorophyll. Three chlorophyll *a*/*b*-proteins were resolved, $LHCP^1$ and $LHCP^2$ being termed oligomers of $LHCP^3$, because of their similar spectral properties. The apparent molecular weights of the pigment complexes are also indicated on the right-hand side of the gel, in order to compare band positions resolved on second-dimension gels. However, it must be emphasized that these apparent molecular weights bear no relation to the actual molecular weights of the complexes. Firstly, 1.4 g SDS are bound only to each g protein for polypeptides which are completely denatured, which is not the case for the complexes. Secondly, since the polypeptides of the complexes are all intrinsic proteins [3,4] they are hydrophobic proteins and these often show anomalous SDS binding [22]. Thirdly, Ferguson plots show that the apparent molecular weights of both CP1 and LHCP vary with gel concentrations [23,24].



	Chlorophyll (%)	Apparent Mol. Wt. ($\times 10^{-3}$)
CP1a	21	200
CP1	9	130
$LHCP^1$	25	70
$LHCP^2$	10	48
CPa	10	39
$LHCP^3$	16	27
FC	9	

Fig. 1. Chlorophyll-protein complexes of spinach thylakoids resolved by a mild SDS-polyacrylamide gel electrophoresis method. The designation of the complexes resolved are shown on the left-hand side of the gel. The relative distribution of chlorophyll amongst the chlorophyll-protein complexes and their apparent molecular weights ($\times 10^{-3}$) are indicated on the right-hand side of the gel. FC, free chlorophyll.

P-700 content of chlorophyll-protein complexes

Chlorophyll-protein complexes resolved on preparative tube gels were rapidly eluted with 50 mM Tricine (pH 8.0) and tested for *P*-700 oxidation by chemical and light-induced absorbance change methods. With the chemical method, the ferricyanide-oxidized minus ascorbate-reduced difference spectrum of CP1a from spinach and barley thylakoids showed a minor peak at 698 nm and a major peak at 683 nm (Fig. 2). With time, the 683 nm peak then increased greatly in intensity until it had maximal absorbance of 0.18 after 1 h. Consequently, the initial peak at 698 nm due to *P*-700 oxidation was masked after 10 min. This effect was also observed with CP1 from spinach and barley thylakoids, although the peaks at 683 and 698 nm were equal in the initial spectrum. The final maximum absorbance at 683 nm in the chemical difference spectra of CP1a and CP1 from spinach thylakoids represented 35 and 38% of the total absorbance at their red absorption maximum (678 nm), respectively. Thus, as well as the specific oxidation of *P*-700, some of the antenna chlorophyll *a* molecules of both CP1a and CP1 with a λ_{max} at 683 nm were being oxidized by potassium ferricyanide.

No such problem occurred with the light-induced absorbance change of *P*-700, since reversible photooxidation of *P*-700 was seen. Sodium ascorbate and methyl viologen were added to increase the rate of *P*-700 oxidation and to promote the dark recovery. The number of chlorophyll molecules associated with each *P*-700 molecule is given for spinach and barley CP1a and CP1 in Table I. No *P*-700 was detected in either CPa or any of the LHCPs from spinach and barley thylakoids. In all cases, the values for CP1a are slightly higher than those of CP1 (Table I). Comparable values to those shown in Table I were obtained for the oxidation of *P*-700 in CP1a and CP1 induced by blue light [25].

The chlorophyll/*P*-700 ratios reported for the electrophoretic complexes,

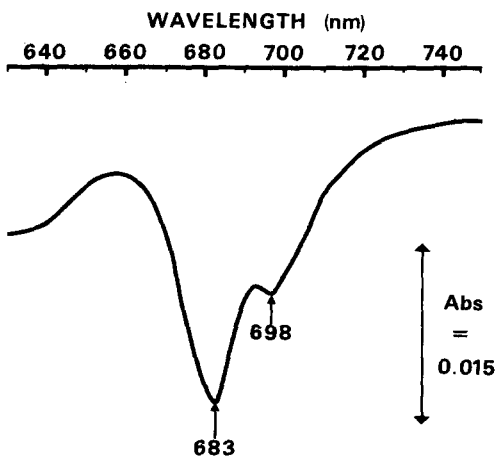


Fig. 2. The ferricyanide-oxidized minus ascorbate-reduced difference spectrum of CP1a from spinach thylakoids resolved by SDS discontinuous polyacrylamide gel electrophoresis. The spectrum was recorded immediately after the addition of ferricyanide and ascorbate. The chlorophyll concentration was 5.7 μg Chl/ml.

TABLE I

CHLOROPHYLL/*P*-700 RATIOS OF PS I REACTION CENTRE COMPLEXES

P-700 was estimated by the light-induced change at 698 nm of chlorophyll-protein complexes, CP1a and CP1, which had been resolved by SDS discontinuous polyacrylamide gel electrophoresis and eluted from gel segments. The values are the average of at least 15 experiments.

Thylakoids	Chlorophyll/*P*-700	
	CP1a	CP1
Spinach	130	115
Normal barley	140	124

CP1a and CP1, are much higher than those of the *P*-700-chlorophyll *a*-protein complex of PS I isolated by hydroxyapatite chromatography after Triton X-100 fragmentation of various thylakoids [3,4] which have about 40 chlorophylls per *P*-700. In certain cases, complexes with even greater enrichment of *P*-700 have been isolated after detergent chromatography [26—28] or solvent extraction [29—31]. With earlier SDS gel procedures, no *P*-700 was detected in CP1 [3,4]. Wessels and Borchert [7] found 40—50 chlorophylls per *P*-700 in their PS I complex. The values in Table I are more comparable to those of Remy and Hoarau (11) who resolved three CP1s from tobacco which together accounted for 27% of the total chlorophyll. The varying amplitudes of the light-induced absorbance changes at 698 nm (in absorbance units $\times 10^{-4}$) were 48, 78 and 88 for the three CP1s compared to 27 for chloroplasts [11].

Re-electrophoresis of gel segments containing resolved chlorophyll-protein complexes

Since the pigment and spectral composition of the various chlorophyll-protein complex complexes resolved with improved methods are not always identical and polypeptides not necessarily belonging to the chlorophyll-protein complexes comigrate with them in the first dimension, it is important to identify the polypeptides which belong to the pigment-protein complexes. Therefore, the chlorophyll-containing zones were excised from tubes gels (Fig. 1) and subjected to re-electrophoresis in the second dimension on 14% acrylamide or concentration gradient slab gels. The gel segments were placed (either directly or after heating to denature the complexes) in the slots of slab gels, rather than using complexes eluted from the gels prior to re-electrophoresis. We confirmed the finding of Wessels and Borchert [7] that re-electrophoresis of complexes directly from gel segments was a milder procedure. The apparent molecular weights of the polypeptides found in each gel segment from spinach thylakoids are shown in Table II. Some of these will be extraneous polypeptides which comigrate with the complexes in the first dimension. CP1a and CP1 both gave a faint green band with an apparent molecular weight of 130 000 (and migrating with the same mobility as CP1 in the first dimension) and an intense non-pigmented band of 70 kilodaltons. In addition to the 70 kilodalton polypeptide, which binds the chlorophyll of CP1, CP1a possessed several other low molecular weight polypeptides of 24,

TABLE II

APPARENT MOLECULAR WEIGHTS OF POLYPEPTIDES PRESENT IN GEL SEGMENTS CONTAINING CHLOROPHYLL-PROTEIN COMPLEXES

The six chlorophyll-protein complexes of spinach and barley thylakoids were resolved on SDS tube gels (Fig. 1) and three or four segments containing each complex were run on SDS slab gels in the second dimension. Polypeptides which aggregate on heating are underlined.

Chlorophyll-protein complex	CPIa	CPI	LHCP[1]	LHCP[2]	CPa	LHCP[3]
Apparent molecular weight ($\times 10^{-3}$)	130	130	65	57	56	37
	70	70	27	—	50	31.5
	24	—	25	25	47	27.5
	22	—	—	—	45	25
	20	—	23	23	43	24
	14	—	—	—	41.5	23
	—	—	—	—	—	21

22, 20 and 14 kilodaltons (Table II). Heating of CP1a or delipidation of the eluted complex did not affect the mobilities of the low molecular weight polypeptides. Heating either CP1a or CP1 gel segments prior to re-electrophoresis, resulted in little or no observable 70 kilodalton polypeptide; aggregated polypeptides were seen at the gel top. The aggregation of the 70 kilodalton polypeptide of CP1 on heating, delipidation of thylakoids or eluted complexes, or ageing is well-documented [8,16,25,26].

LHCP[1] and LHCP[2] were classified as oligomers of LHCP[3] on the basis of similar pigment composition and spectral characteristics [9]. Indeed, the three LHCPs contained two major polypeptides of apparent molecular weights of 25 000 and 23 000 as well as additional polypeptides (Table II). Traces of a 27 kilodalton polypeptide were seen with LHCP[1] and LHCP[2]. Heat did not alter the mobility of any of these polypeptides.

Re-electrophoresis of gel segments of CPa yielded polypeptides of 56, 50, 47, 45, 43 and 41.5 kilodaltons (Table II), none of which retained chlorophyll. Most of these polypeptides tended to aggregate on heating, but sometimes traces remained at similar positions on the gels (Table II). The 50 and 47 kilodalton polypeptides were the major ones. CPa did not have the 27, 25 or 23 kilodalton polypeptides of LHCP.

Two-dimensional electrophoresis

In order to demonstrate more conclusively which polypeptides belong to the pigment-complexes resolved in tube gels, a horizontal slice from a complete tube gel, resolved as usual in the first dimension, was placed onto a slab SDS concentration gradient gel and resolved in the second dimension. Components belonging to the complexes will then be located anywhere on the vertical lines below each complex, whilst extraneous polypeptides which comigrated with a complex will appear on the diagonal displayed by those thylakoid polypeptides running with unaltered mobilities in the second dimension. Since certain of the polypeptides of the complexes aggregate on heating (Table II), the tube gels were not heated. As seen in Fig. 3a with spinach thylakoids, both CP1a and CP1 itself gave traces of faint green polypeptides of greater than

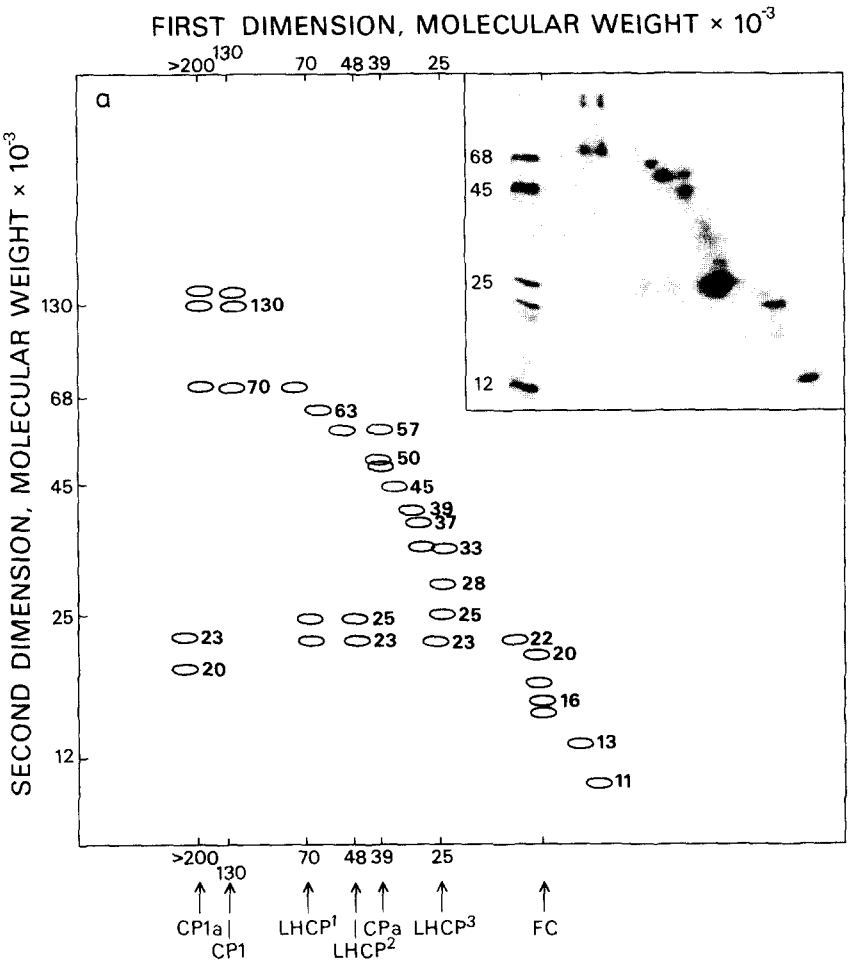


Fig. 3a.

200 and 130 kilodaltons (showing that some CP1a and CP1 remained undissociated on re-electrophoresis), as well as the apoprotein of CP1 at 70 kilodaltons. Faint traces of the low molecular weight polypeptides characterized by tube gels (Table II) were also detected under CP1a. As expected from the results with gel segments, the three LHCPs each showed two major polypeptides off and below the diagonal with apparent molecular weights of 25 000 and 23 000. Trace amounts of the minor 27 kilodalton polypeptide was sometimes observed with LHCP[1]. Some distortion always occurred in the region below LHCP[3] but 28.5, 25, 23 and 21.7 kilodalton polypeptides were seen below the diagonal. In contrast to the polypeptides of the LHCPs which were located only below the diagonal, CPa had three or four polypeptides all off and above the diagonal. Thus, the 56, 50 and 47 kilodalton polypeptides were migrating with lower mobilities than CPa. No chlorophyll was associated with any of these polypeptides.

Two-dimensional electrophoresis of the chlorophyll-protein complexes of

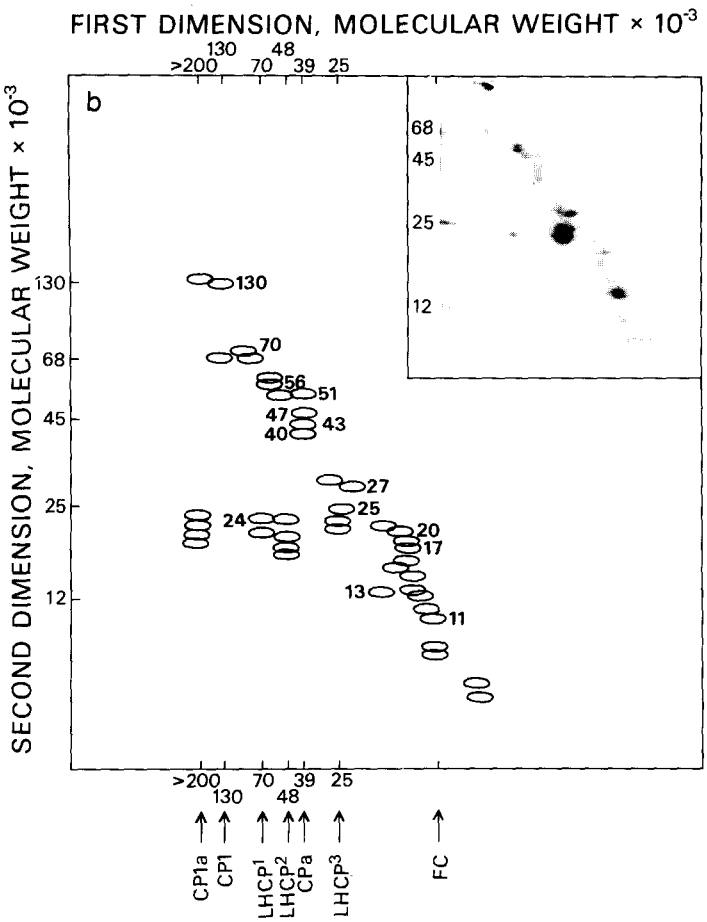


Fig. 3. Schematic diagram of the two-dimensional electrophoretic map of the chlorophyll-protein complexes of (a) spinach and (b) barley thylakoids. The off-diagonal polypeptides represent polypeptides of the dissociated chlorophyll-protein complexes.

normal barley (Fig. 3b) showed a somewhat similar polypeptide profile to that obtained with spinach thylakoids (Fig. 3a), although no CP1a remained on the second dimension. The low molecular weight components of CP1a were found at somewhat different positions compared to those of spinach CP1a (Fig. 3a). Once again, the similar polypeptide composition of LHCP[1] and LHCP[2] show that these complexes are oligomers of LHCP[3].

Two-dimensional electrophoresis of the chlorophyll *b*-deficient mutant barley (Fig. 4) which has no LHCP [3,4] was included for comparison with normal barley and spinach. The low molecular weight polypeptides of CP1a were not detected due to the low amount of PS I reaction centre complex resolved as CP1a. The polypeptides associated with CPa were detected above and off the diagonal at 53, 50, 47 and 45 kilodaltons (Fig. 4). One main polypeptide was seen below the diagonal: this migrated with an apparent molecular weight of 14 500. The origin of this polypeptide is not clear.

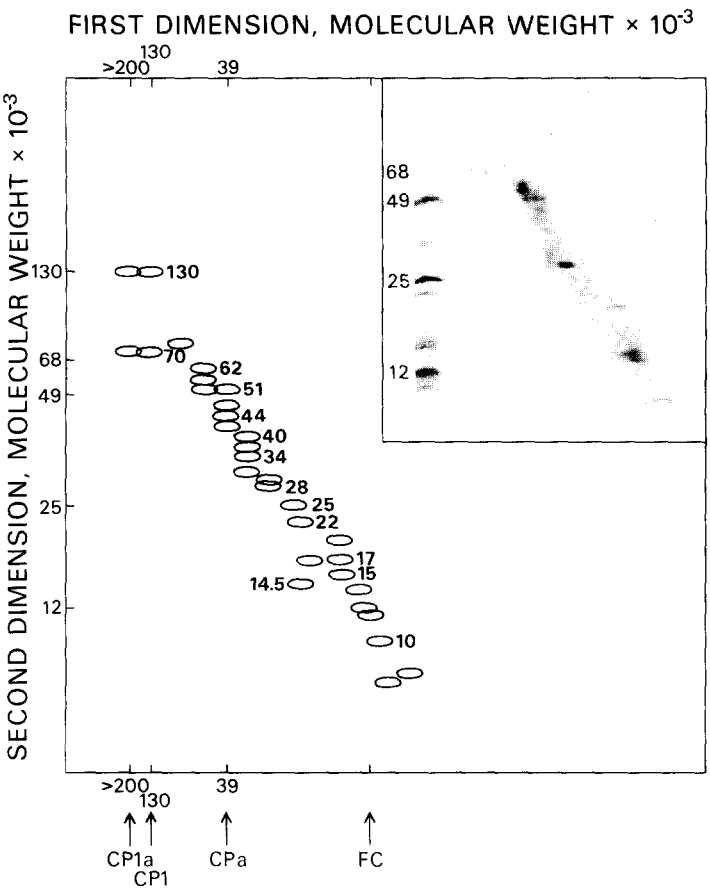


Fig. 4. Schematic diagram of the two-dimensional electrophoretic map of the chlorophyll-protein complexes of the chlorophyll *b*-deficient mutant barley. The off-diagonal polypeptides represent polypeptides of the dissociated chlorophyll-protein complexes.

Despite the low amounts of polypeptides able to be resolved by two-dimensional gel electrophoresis using a complete tube gel, it is evident that polypeptides located off the diagonal (whether above or below it) belong to dissociated complexes. It has been shown that CP1a and CP1 previously classified on chlorophyll spectral data possess a 70 kilodalton polypeptide and that the three LHCPs do indeed share at least two polypeptides of 25 and 23 kilodaltons and CPa has at least three polypeptides.

Discussion

In contrast to values of 40 chlorophyll *a* molecules per *P*-700 obtained for *P*-700-chlorophyll *a*-protein complexes isolated with Triton X-100 from many different plants and algae [3,4], CP1a and CP1 from spinach thylakoids described here have 120 chlorophyll *a* molecules per *P*-700 and the barley complexes have slightly higher values (Table I). As pointed out by Remy et al. [5,11], a large amount of the free chlorophyll found in earlier gel procedures

came from the dissociation of CP1. With this procedure, some 30% of the total chlorophyll is associated now with the PS I reaction centre complexes, CP1a and CP1, resolved from spinach and barley thylakoids (Fig. 1). Concomitant with this increase in the amount of chlorophyll associated with the reaction centre complex of PS I, both CP1a and CP1 show reversible photooxidation of *P*-700. The single CP1 resolved by earlier gel procedures which had only 10—15% of the total chlorophyll did not possess any light-induced *P*-700 oxidation [3,4]. The photosynthetic unit (defined as the number of chlorophyll molecules for each reaction centre molecule of *P*-680 and *P*-700) of spinach thylakoids possesses about 400 chlorophyll molecules and barley thylakoids about 460 chlorophyll molecules. With 30% of the total chlorophyll associated with the reaction centre complex of PS I in spinach thylakoids (Fig. 1), this would be equivalent to 120 chlorophyll molecules per photosynthetic unit, and it would be expected that CP1a and CP1 should have about 120 chlorophyll molecules per *P*-700. This ratio was indeed measured (Table I), demonstrating that these complexes have lost little or none of the chlorophyll associated with them in vivo.

Significantly, CP1a and CP1 resolved by mild SDS electrophoresis (Fig. 1) show for the first time with SDS-PS I complexes the characteristic low-temperature fluorescence emission at 735 and at 722 nm [9] associated with PS I in vivo. The fluorescence emission spectra at 77 K of higher plant chloroplasts have 3 main peaks at 685, 695 and 735 nm; the 685 and 695 nm emissions have been associated with PS II and that at 735 nm with PS I [3]. Digitonin PS I particles had 735 nm fluorescence [32] and a Chl/*P*-700 ratio of 205 [33]. The highly-purified digitonin PS I particles of Satoh and Butler [34] also have 735 nm fluorescence emission, but no Chl/*P*-700 ratio was reported. In contrast, the *P*-700-chlorophyll *a*-protein complexes isolated by Triton X-100 from higher plants [3,4] and brown algae [35] have fluorescence emission at 681 nm, and only about 40 chlorophyll *a* molecules per *P*-700. Therefore, it is suggested that isolated PS I complexes need more than 40 chlorophyll *a* molecules per *P*-700 for the fluorescence emission to be at 735 nm as in vivo.

Both CP1s of spinach and barley thylakoids possess a 70 kilodalton polypeptide which binds chlorophyll *a* and *P*-700. However, CP1a is not a dimer of CP1, but a reaction centre complex of PS I which includes CP1 and four additional low molecular weight polypeptides (Table II, Fig. 3a). The polypeptide composition of CP1a is somewhat similar to that of the PS I reaction centre complex isolated by Nelson and Notsani [38] which contains the 70 kilodalton polypeptide of CP1 and subunits of 25, 20, 18, 16 and 8 kilodaltons associated with the primary electron acceptors of PS I. Since all of the chlorophyll and *P*-700 of CP1 is bound only to 70 kilodalton polypeptides, and the amount of *P*-700 on a chlorophyll basis is the same as that found in CP1a, which had additional low-molecular weight polypeptides, and CP1a and CP1 together possess the theoretical amount of chlorophyll *a* and *P*-700 expected for a PS I reaction centre complex, it appears that all of the chlorophyll of this complex may be bound only to 70 kilodalton polypeptides, and not to the low molecular weight components of CP1a. However, a direct demonstration is required to show which polypeptides bind the chlorophyll of CP1a.

Many reports have shown that some of the chlorophyll of various *P*-700-chlorophyll *a*-proteins of higher plants and green algae is bound to a single polypeptide which varies from 70 to 64 kilodaltons [5,7,8,15,24,27,39], although there are reports of two polypeptides with lower dissimilar molecular weights [28,40]. However, in view of the loss of chlorophyll from many of the isolated *P*-700-chlorophyll *a* protein complexes, and the fact that other *P*-700-chlorophyll *a* protein complexes contain additional non-chlorophyll-binding low molecular weight polypeptides associated with the heart of PS I (CP1a and the Nelson complex [38]), neither an accurate protein/chlorophyll ratio for the 70 kilodalton polypeptide nor the molecular weight of the *P*-700-chlorophyll *a*-protein of the PS I reaction centre complex in vivo have yet been determined.

The isolation of a PS I reaction centre complex, resolved as CP1a and CP1 by mild SDS-polyacrylamide gel electrophoresis which shows light-induced *P*-700 oxidation, the characteristic long-wavelength fluorescence emission and the theoretical amount of chlorophyll *a* per *P*-700 (perhaps bound only to 70 kilodalton polypeptides), suggests that the Triton X-100-*P*-700-chlorophyll *a*-protein complexes with only 40 chlorophyll *a* molecules per *P*-700 and that the 'enriched *P*-700-chlorophyll *a*-protein complexes' with some 20 chlorophyll *a* molecules per *P*-700 [26—31] have lost antenna chlorophyll associated with the complex in vivo. The 80 chlorophyll *a* molecules more-loosely associated with CP1 may be bound differently than the more-firmly bound 40 chlorophyll molecules. Perhaps this group of 40 chlorophyll *a* molecules closest to *P*-700 is all buried within the polypeptide chains, as is the case for the bacteriochlorophyll molecules of the water-soluble bacteriochlorophyll complex of a photosynthetic bacterium [38]. The phytyl chains of the 80 less-firmly attached chlorophyll *a* molecules may be associated with the hydrophobic exteriors of the intrinsic polypeptide chains and the porphyrin rings may only bend into the hydrophobic region of the polypeptides of the complex [37].

The two-dimensional approach has shown that some of the polypeptides associated with $LHCP^1$, $LHCP^2$ and $LHCP^3$ are similar (Figs. 3a and b). $LHCP^1$, $LHCP^2$ and $LHCP^3$ possess two main 25 and 23 kilodalton polypeptides, and $LHCP^1$ and $LHCP^3$ both have a minor 27.5 kilodalton polypeptide. The low amount of the minor band of $LCHP^2$ did not allow qualitative differences in the relative amounts of the two main polypeptides to be ascertained. $LHCP^1$ and $LHCP^2$ migrate relatively close to one another and do not necessarily appear to be a dimer and trimer of monomeric $LHCP^1$. The first oliogmers of LHCP detected [5,41] were termed dimers. Later procedures found a minor LHCP band of intermediate mobility [8,9,11,12]. The low amount of chlorophyll found with $LHCP^2$ suggests that $LHCP^2$ is a labile complex, but its relationship to $LHCP^1$ is not clear.

The puzzling questions as to what is the true number of polypeptides associated with the light-harvesting complex and which polypeptide(s) actually binds chlorophyll are unresolved [3,4]. Some groups find one polypeptide [5,11,15] and others two or more polypeptides associated with LHCP [7,8,12, 25,39,42—45]. Here we find two main polypeptides, yet earlier only one [15]. In two cases it has been shown that the 25 and 23 kilodalton polypeptides of LHCP have considerable amino acid sequence homology [39,44]. This study shows that the three LHCPs possess both the 25 and 23 kilodalton polypep-

tide, and that LHCP[1] and LHCP[3] have the minor 27.5 kilodalton polypeptide, but it does not establish which polypeptides bind chlorophyll. Although the chlorophyll content of the LCHPs resolved by mild SDS-polyacrylamide gel electrophoresis reflects the true amount of chlorophyll associated with LHCP in vivo, accurate protein/chlorophyll molar ratios are not yet possible, since additional polypeptides migrate with them (Table II). Furthermore, the resolution of 'monomeric' LHCP of tobacco chloroplasts into two different chlorophyll-protein complexes by Machold and Meister [45] shows that LHCP may not be homogenenous.

Two-dimensional gel electrophoresis (Figs. 3 and 4) demonstrated that CPa, the presumed reaction centre complex of PS II, had two major 53 and 47 kilodalton polypeptide. Polypeptides in the 40—50 kilodalton range have been implicated for the reaction centre complex of PS II. The absence of a certain polypeptide in mutants was correlated with the lack of PS II activity (47 and 46 kilodaltons in *Chlamydomonas* [47] and mutant barley [48], respectively). Henriques and Park [8] and Remy and Hoarau [11] found a major 42 kilodalton component, and Wessels and Borchert [7] found a 47 kilodalton component associated with the chlorophyll *a*-protein presumed to be the reaction centre complex of PS II. Satoh [49] identified 43 and 27 kilodalton polypeptides from a dissociated extensively-purified PS II reaction centre complex. Two minor chlorophyll *a*-proteins, termed complexes III and IV, were resolved by lithium dodecyl sulphate-polyacrylamide gel electrophoresis at 4°C by Delepelaire and Chua [50]. The apoproteins of complexes III and IV had apparent molecular weights of 50 000 and 47 000, respectively, and they were not immunologically cross-reactive. Both complexes were indirectly associated with the primary photochemistry of PS II. The major 53 and 47 kilodalton polypeptides of CPa may be identical to those of the complexes of Delepelaire and Chua [50].

In conclusion, knowledge of the distribution of chlorophyll amongst the three main pigment-protein complexes is becoming clearer, and it has been demonstrated here that all of the *P*-700 and possibly all of the chlorophyll *a* of the PS I reaction centre complex are bound to 70 kilodalton polypeptides. There still are no unequivocal data on either the number of polypeptides in the three main pigment-protein complexes, or which polypeptides actually bind the chlorophyll in the light-harvesting complex or reaction centre complex of PS II.

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