Light-harvesting Pigment-Proteins of Photosystem I in Maize

SUBUNIT COMPOSITION AND BIOGENESIS*

Alexander Vainstein‡, Camille C. Peterson, and J. Philip Thornber
From the Department of Biology and Molecular Biology Institute, University of California, Los Angeles, California 90024

Three different pigment-binding proteins of the light-harvesting complex (LHC I) of maize photosystem I (PS I) have been isolated. Absorption and fluorescence excitation spectral analyses showed that each pigment-protein can transfer absorbed energy from its carotenoid and/or chlorophyll b components to chlorophyll a. Their apoproteins with apparent sizes of 24 (LHC Ia), 21 (LHC Ib), and 17 (LHC Ic) kDa have been purified to homogeneity. Differences in their pigment and amino acid compositions and in their reactions with antibodies demonstrate that the two smaller pigment-proteins are not proteolytically derived from the largest one. LHC Ib's apoprotein is particularly enriched in cysteine residues. None of the three apoproteins cross-reacted with antibodies raised against the major light-harvesting chlorophyll a/b-protein of photosystem II (LHC IIb) or against the PS I core complex (CC) subunits. Studies of the biogenesis of PS I during greening of etiolated plants showed that all of the CC I subunits accumulated to a detectable level prior to the appearance of the 17-kDa subunit of LHC I, the accumulation of which preceded those of the 24- and 21-kDa subunits of LHC I. In addition, subunit VI of CC I is shown to be differentially expressed in mesophyll and bundle sheath cells; a slightly larger form of it accumulates in mesophyll than in bundle sheath thylakoids during plastid development.

The photosynthetic apparatus in higher plant thylakoids consists of two photosystems, PS I and PS II, each of which can be thought of as containing two pigmented parts: a light-harvesting complex (LHC) and a core complex (CC) (cf. Ref. 1). Both complexes contain polypeptides, some, but not necessarily all, of which are associated with pigment. The LHC is primarily responsible for absorbing incident radiant energy; however, its presence is not essential for photosynthetic electron transfer to take place, which occurs in the CC component.

In the last 20 years, PS I has been isolated by a variety of procedures, almost all of which involve a step in which thylakoid membranes are solubilized by surfactants (e.g. Refs. 2–15). Gentle solubilizations, for example those using minimal Triton concentrations or glycosidic surfactants, retain LHC I with CC I, and more than 7, and in some instances as many as 16, polypeptides are contained in the PS I product (e.g. Refs. 2, 3, 7, 11, and 12). Treatments with higher concentrations of Triton X-100 or SDS generally yield PS I preparations containing only the CC I polypeptides. Thus, preparations made using 2–4% Triton contain only the seven (six in maize) CC I polypeptide subunits (14, 15), whereas PS I material obtained by the use of SDS can have as few as one polypeptide (subunit I of CC I, the P700-chlorophyll a-apolypeptide of ~70 kDa) (cf. Refs. 1 and 10). Those polypeptides in PS I preparations made from gently solubilized membranes that are additional to the seven in CC I, can be LHC I apoproteins, and/or CC I subunits that are more easily removed from the core than the other seven, and/or contaminants, particularly LHC II polypeptides (cf. Refs. 11). Recently, Bruce and Malkin (11) have shown that a PS I complex purified from Lemna gibba contains a single copy of each of the two biochemically very similar largest polypeptides (subunit I of CC I) and one copy each of most of the smaller polypeptides (subunits II–VII of CC I). Subunits II–VII are not associated with pigments, and their functions have not been unequivocally established.

The emphasis in this paper is on the LHC I pigment-proteins. Mullet et al. (2) first reported a detailed polypeptide composition of a holo-PS I complex obtained from pea leaves and proposed that polypeptides in the size range of 20.5 to 24.5 kDa are the apoproteins of LHC I; later, Haworth et al. (12) showed that pea LHC I contains four polypeptides of that size. Lam et al. (13) and others (3, 7) have extended the seminal observations to characterize partially one LHC I pigment-protein (termed LHC Ib in this paper) that has a 20–21-kDa subunit and a 77 K emission maximum at 730 nm. There was indirect evidence from these previous studies that other LHC I pigment-proteins with apoproteins of 24, 23, and 17 kDa might exist, but an unequivocal demonstration that pigment was specifically associated with any one of these polypeptide(s) was lacking (however, see Ref. 7). Concurrently, nuclear genes encoding two different purported LHC I polypeptides have been cloned (16, 17), but as yet it is impossible to pinpoint unequivocally which, if any, of the LHC I polypeptides the cloned genes encode. The research described here on the LHC I of maize should ultimately help to resolve the situation.

The photosynthetic apparatus of maize differs from that of C3 plants. Maize, a C4 plant, has two photosynthetically active cell types, namely mesophyll (M) and bundle sheath (BS), that differ structurally and functionally. While scientific data agree upon the existence of PS I in both cell types, the presence of PS II in BS is controversial (1, 18–21). The majority view is that BS cells show greatly reduced PS II activity and much smaller amounts of LHC II when compared to M cells (cf. Ref. 22). Thus, BS cells are naturally enriched in PS I and therefore are advantageous material in which to

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§ The abbreviations used are: PS, photosystem; LHC, light-harvesting complex; CC, core complex; M, mesophyll; BS, bundle sheath; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
study PS I. In the present study, maize BS cells were used as a source from which we obtained homogenous preparations of some LHC I chlorophyll proteins. We show that maize LHC I contains at least three pigment-binding proteins with apoproteins of apparent sizes, 24, 21, and 17 kDa. Spectral and immunological properties, as well as the amino acid composition, of each chlorophyll protein are presented. The biogenesis of some LHC I and CC I subunits is also studied.

**Experimental Procedures**

**Plant Materials**

*Zea mays* W273 seeds (Wisconsin-certified) were soaked in water overnight and then grown in vermiculite in a greenhouse for 2 weeks (photoperiods of approximately 12 h light, 12 h dark). Tips, 3 cm in length, of secondary and tertiary leaves were used for preparation of BS and M thylakoids.

**Preparation of M and BS Thylakoid Membranes**

**M Thylakoids**—Ice-cold, water-washed leaf material was cut with a razor blade into 10-mm transverse sections. Sections were ground in a razor blade blender (23) for three 1-s pulses in a buffer containing 0.4 M sorbitol, 0.01 M NaCl, and 0.02 M Tricine-NaOH, pH 7.6. The suspension was filtered through two layers of Miracloth, and the filtrate was centrifuged at 4,000 × g for 1 min. The pellet was homogenized in 1 mM EDTA and 25 mM Tricine-NaOH, pH 7.6, in a glass homogenizer and repelleted by centrifugation at 20,000 × g for 5 min. To prepare NaBr-washed thylakoids, EDTA-washed membranes were suspended in distilled water to give a chlorophyll concentration of 1 mg/ml and then incubated at 4°C for 20 min in buffered 0.4 M sorbitol, 0.01 M NaCl, and 0.02 M Tricine-NaOH, pH 7.6. The suspension was filtered through two layers of Miracloth. The residue was resuspended and blended once more. The resulting BS strands (residues) were homogenized in 1 mM EDTA and 25 mM Tricine-NaOH, pH 7.6, by centrifugation at 20,000 × g for 5 min. The final pellet was homogenized in 50 mM glycine, 6 mM Tris-HCl, pH 8.3, and 10% glycerol to give a chlorophyll concentration of 1.1 mg/ml.

**BS Thylakoids**—Separation of BS thylakoids was done according to Chollet and Ogren (24) with some modifications. Secondary and tertiary leaves were washed in ice-cold water and then cut with a razor blade into 5-mm transverse segments. The segments were ground for 1 min in a razor blade blender (23) in 0.6 M sorbitol, 10 mM Hepes-KOH, pH 7.5, and 2.5 mM CaCl₂. The suspension was filtered through two layers of Miracloth. The residue was resuspended and ground, as above, four more times. The homogenate was filtered through 20- and 35-mesh sieves, and the filtrate was then blended for 30 s in a Polytron disintegrator (Brinkmann). The resultant suspension was filtered through an 80-μm nylon net. The residue was resuspended and blended once more. The resulting BS strands (residue of the last filtration) had a chlorophyll a/b ratio of ~3.5 and, as judged by light microscopy, were not contaminated with M cells. The final residue was ground in a razor blade blender for three 1-s pulses, filtered through two layers of Miracloth, and BS thylakoids were pelleted from the extract by centrifugation at 4000 × g for 30 s. The pellet was resuspended in 1 mM EDTA and 25 mM Tricine-NaOH, pH 7.6, and then processed as described for the M thylakoid preparation.

**Separation of Pigment-Protein Complexes from Thylakoid Membranes**

M and BS thylakoids (30 μg of chlorophyll) were solubilized with 3.6 μl of 10% octyl glucoside, 1.8 μl of 10% nonyl glucoside, 2.6 μl of 10% SDS, and 3 μl of 20% Triton X-100. Thylakoids were solubilized for 5 min at 20 °C, and the extracts were then centrifuged for 2 min in a Microfuge at room temperature. The supernatant was electrophoresed on a nondenaturing Deriphat-polyacrylamide gel (25) at 100 V for 1 h.

**Purification of the Apoproteins from Pigmented Bands on the Deriphat-Gel**

Colored bands of interest were excised from the nondenaturing gel, and, after incubation in 3% SDS, 1 mM diithiothreitol, 5 mM EDTA, 100 mM Tris-HCl, pH 6.8, for 30 min at room temperature, the slices were re-electrophoresed on a fully denaturing 10–17% linear gradient SDS-polyacrylamide gel (10). Gels were fixed and stained as described previously (10). The resultant apoprotein bands were excised and electroeluted in a sample concentrator (ISCO) (26). After repeating the electrophoresis on an SDS-polyacrylamide gel and the electrophoretic run, the amino acid compositions of the purified LHC Ia, -b, and -c apoproteins were determined after performic acid oxidation (27).

**Analytical Methods**

Chlorophyll was determined according to Ref. 28. Absorbance spectra were recorded on an Aminco DW-2 spectrophotometer. Fluorescence excitation/emission spectra were recorded on an Aminco SPF-500 fluorometer. Electrortransfer from polyacrylamide gel to nitrocellulose paper and immunodetection with antibodies was performed using alkaline phosphatase and 5-bromo-4-chloro-3-indolyl phosphate to locate antibody-antigen complexes (29). The antibodies used were prepared either by Dr. R. Nechushtai of The Hebrew University of Jerusalem, for LHC I (cf. Ref. 30), or Dr. E. M. Tokin, UCLA, for LHC II (cf.Refs. 3 and 25). Polyclonal antibodies against each of the denatured thylakoid apoproteins (LHC Ia, -b, -c) were prepared by us as described in Bulinski and Borsy (31), and affinity-purified using nitrocellulose filters (32).

**Chemicals**

Octyl glucoside and nonyl glucoside were purchased from Calbiochem Behring Diagnostics. SDS was from Boehringer Mannheim. Deriphat (disodium N-dodecyl-β-mimidopropionate) was from Henkel Corp. (Hoboken, NJ). All the chemicals used for protein separation and sequencing were purchased from Bio-Rad. Other chemicals were purchased from Sigma.

**Results**

Thylakoid membranes of BS plastids were solubilized in a mixture of octyl and nonyl glucosides plus SDS and Triton X-100 (see "Experimental Procedures") and then fractionated into their pigment-protein complexes by electrophoresis through nondenaturing Deriphat-polyacrylamide gel (Fig. 1A). These treatments largely disrupted photosystem I into its components: only a small amount of holo-PS (i.e. CC I plus LHC I) remained (band 1 in the gel (Fig. 1A)), whereas the bulk of the PS I material was found as the CC I and LHC I components (Fig. 1A). The polypeptide compositions of

![FIG. 1. A, B, and C Thylakoid membranes of BS plastids were solubilized in a mixture of octyl and nonyl glucosides plus SDS and Triton X-100 (see "Experimental Procedures") and then fractionated into their pigment-protein complexes by electrophoresis through nondenaturing Deriphat-polyacrylamide gel (Fig. 1A). These treatments largely disrupted photosystem I into its components: only a small amount of holo-PS (i.e. CC I plus LHC I) remained (band 1 in the gel (Fig. 1A)), whereas the bulk of the PS I material was found as the CC I and LHC I components (Fig. 1A). The polypeptide compositions of](https://example.com/fig1.png)
holo-PS I and CC I, determined by SDS-PAGE, are shown in Fig. 1B. There are several prominent polypeptides of >15 kDa which are present in the PS I band, but are absent from the CC I band. The two fractions analyzed in Fig. 1B were obtained from thylakoids solubilized in octyl and nonyl glucosides, but lacking the Triton X-100 and half of the SDS used to solubilize the thylakoids in the gel shown in Fig. 1A. This gentler solubilization retained more of those polypeptides specifically associated with PS I, but lacking in CC I and, of course, reduced the amounts of the putative LHC I pigment-proteins on the gel.

The subunit compositions of green bands other than PS I and CC I were examined (Fig. 1C). Green band 4 has two major polypeptides of 21 kDa, band 7 has one of 24 kDa, and band 8 has one of 17 kDa. Two green bands (3 and 6) identified by their sizes, spectra, and subunit compositions as LHC IIb pigment-protein complexes, were also present in BS thylakoids in relatively minor amounts, as expected from our previous work which showed that BS plastids do indeed contain some LHC IIb, contrary to some other reports (cf. Ref. 22). The major polypeptides in bands 4, 7, and 8 are almost certainly the apoproteins of three LHC I components, LHC Ib, -a, and -c, respectively (cf. Ref. 10). We base this view on the fact that these polypeptides are present in PS I and not in CC I, that some polypeptides of these sizes have been shown or implied previously to be LHC I apoproteins (3, 7, 30), that they react with anti-LHC I antibodies (see below), and that each of them is associated with chlorophyll and carotenoid. There are additional minor polypeptides present in the lanes of most of the dissected LHC I bands shown in Fig. 1C. We believe that these polypeptides are not LHC I apoproteins, but rather that they are derived from components migrating in the region of, but not exactly coincident with, the pigmented LHC I bands, since, on a few occasions, these minor polypeptides were absent from the dissected green bands, LHC Ia, -b, and -c, which had essentially the same spectrum regardless of their presence. Note that LHC Ib’s 21-kDa apoprotein(s) appears to be a doublet of polypeptides, which is seen only when 4 M urea and twice the ionic strength of the published (cf. Ref. 33) buffers are used in the SDS-PAGE separating gel. Furthermore, the size of the LHC Ib holocomplex (Fig 1A) is much larger than expected for a pigment-protein containing a 21-kDa subunit, thus LHC Ib must be present in the surfactant extract in an oligomeric, not monomeric, form, and hence it probably occurs in such a form in vivo.

The LHC Ia, -b, and -c apoproteins of approximately 24, 21, and 17 kDa, respectively, have each been purified to homogeneity (Fig. 2) by using a series of separations on nondenaturing and fully denaturing SDS-PAGE (see “Experimental Procedures”). Only the smaller of the doublet of LHC Ib’s apoproteins (Fig. 1C) was purified. As a verification of their identity, the three purified LHC I apoproteins were probed with a mixture of antibodies against LHC II and subunits II, IV, and VI of CC I (Fig. 3A), and with antibodies against LHC I (Fig. 3B). The former antibodies did not cross-react with the putative LHC I apoproteins, but did cross-react with some of the polypeptides in thylakoid membranes (Fig. 3, column A2), whereas the LHC I antibodies cross-reacted with all three LHC I apoproteins (Fig. 3, column B3) and with three polypeptides of equivalent sizes in the thylakoids (Fig. 3, column B2). We made polyclonal antibodies against each of the apoproteins of LHC Ia, -b, and -c and affinity-purified them. The resulting material gave no cross-reaction between any one purified antibody and the other two LHC I apoproteins (data not shown).

Spectral analyses of LHC Ia, -b, and -c (Fig. 4) were made on green bands excised from nondenaturing Deriphat-containing gels similar to that shown in Fig. 1. Relatively large amounts of chlorophyll b are present with chlorophyll a in

![Fig. 2. Purification of LHC I subunits. NaBr-washed thylakoid membranes were solubilized (see "Experimental Procedures") and then subjected to non-denaturing polyacrylamide gel electrophoresis (see Fig. 1A). The pigmented LHC I complexes were excised and re-electrophoresed after denaturation on a fully denaturing SDS-polyacrylamide gel (10). Each apoprotein was then excised and electroeluted from the gel. Column 1, polypeptide pattern of BS thylakoid membranes used for preparation of LHC I components. Columns 2, 3, and 4, samples of the apoproteins of LHC Ic, -b, and -a, respectively, used for determination of their amino acid composition (Table II).](image-url)

![Fig. 3. Immunodecoration of electrophoretically resolved maize thylakoid membranes (2) and LHC Ia + b + c (3). 1, polypeptide pattern of BS thylakoid membranes, fixed and stained for protein. Panel A, immunodecoration with a mixture of antibodies raised specifically against CC I subunits II, IV, VI, and LHC II; panel B, antibody against L. gibba LHC I.](image-url)
LHC Ib (chlorophyll $a/b = 2–2.5$); LHC Ia and -c contain much smaller proportions of chlorophyll b, (chlorophyll $a/b = 4–5$ (cf. Fig. 4B). The blue wavelength absorbances are contributed by chlorophyll $a$ (437 nm max), chlorophyll $b$ (470 nm max), and carotenoids. The proportion of carotenoid/chlorophyll in LHC Ia, as judged from the ratio of absorbances in the blue to the red, is much greater than in other higher plant pigment-proteins (Fig. 4B). The wavelength of maximum fluorescence at room or liquid nitrogen temperatures is given for each LHC I component in Table I. As expected from previous work, LHC Ib has a 730-nm emission at 77 K (2, 12). The emission wavelength maxima of the other two pigment-proteins are at relatively short wavelengths. This may be due to surfactant interaction with the pigment-proteins (cf. Ref. 35) and hence the observed maxima may not correctly reflect those in situ. Excitation energy transfer studies (Fig. 4A) show that chlorophyll $b$ transfers its absorbed excitation efficiently to chlorophyll $a$ in LHC Ib, whereas that absorbed by carotenoid is transferred to chlorophyll with low efficiency in all three LHC I components.

The amino acid compositions (Table II) of performic acid-oxidized samples of the purified pigment-proteins of LHC Ia, -b, and -c show that they have similar yet distinct compositions. The analyses are typical of intrinsic membrane proteins in the chloroplast except for the high cysteine content of LHC Ib. They are hydrophobic proteins whose amino acid compositions bear considerable resemblance to those published for other higher plant pigment-proteins (e.g. LHC II) (1). The LHC I component purified by Nechushtai et al. (3) has a composition almost identical with that of maize LHC Ib (Table II). Note the specific distinctions between the compositions of LHC Ia, -b, and -c. Most noticeable are LHC Ib's high cysteine content, an unusually large number of 10 Cys residues occur per polypeptide chain calculated in part from a knowledge of the apparent size of the polypeptide and from the method described in Thornber and Olson (34).

The biogenesis of the three LHC I apoproteins was studied. Maize seedlings were grown in total darkness for 10 days and then harvested after different times of exposure to light. BS thylakoids were prepared from the plants so treated and analyzed by Western blotting (Fig. 5 and 6) using an antibody that cross-reacts with all three LHC I apoproteins (see above). LHC Ia apoprotein is the first of the three to accumulate to a detectable level after an 8-h greening of etiolated plants (Fig. 5). The apoproteins of LHC Ia and LHC Ib appear only.

### Table I

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<th>Pigment-Protein</th>
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<tr>
<td>LHC Ia</td>
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<tr>
<td>LHC Ib</td>
<td>213-9,100</td>
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<tr>
<td>LHC Ic</td>
<td>213-9,100</td>
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* Table II

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<th>Amino acid composition of the LHC I pigment-proteins</th>
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<tr>
<td>Amino acid</td>
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<tr>
<td>Aspartic acid</td>
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<td>Glutamic acid</td>
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<td>Phenylalanine</td>
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* Figures in parentheses represent number of residues per polypeptide chain calculated in part from a knowledge of the apparent size of the polypeptide and from the method described in Thornber and Olson (34). * Determined using performic acid-oxidized samples.
were separated using SDS-polyacrylamide gel electrophoresis to determine the polypeptide compositions (Fig. 1B) showed that there are 10 subunits of the C4 plant, maize. Subunit VI appears to exist in two slightly different molecular weight forms, each of which is present at early stages of greening (data not shown). Since the CC I subunits (note that subunit III of CC I is absent from maize thylakoids unlike the situation in C3 plants (15)) accumulate to a detectable level after only 4 h of greening (Fig. 6), our data probably indicate that LHC Ia and -b are more peripheral to CC I than subunit III of CC I is absent from maize thylakoids, whereas at a later stage only the higher molecular weight form accumulates in M thylakoids, whereas at a later stage only the higher molecular weight form accumulates in M thylakoids. The relative rates of accumulation of the LHC I apoprotein and the CC I subunits correlate well with the observations of Breidenkamp and Baker (36), who determined the time of appearance of LHC I during plastid biogenesis in wheat by following the time that 770 nm fluorescence appeared; this long wavelength emission is thought to arise from LHC Ib (Table I). It was of particular interest that there was differential expression between the two cell types of subunit VI of CC I. Subunit VI appears to exist in two slightly different molecular weight forms, each of which is present at early stages of greening in both BS and M thylakoids, whereas at a later stage only the higher molecular weight form accumulates in BS thylakoids and the lower one in M thylakoids (Fig. 6). Thus, some subunits of PS I in the two photosynthetically active cell types in maize are differentially expressed as development of the plant proceeds. Similarly, LHC II has been shown to be qualitatively and quantitatively expressed as development of the plant proceeds.

Studies on maize plastid biogenesis using antibodies to L. gibba LHC I revealed (Fig. 5) that the accumulation of LHC Ic apoprotein after 8 h of greening preceded by several hours the accumulation of either of those of LHC Ia or LHC Ib; use of a higher titer of LHC I antibody did not alter this observation (data not shown). Since the BS I subunits (note that subunit III of CC I is absent from maize thylakoids unlike the situation in C3 plants (15)) accumulate to a detectable level after only 4 h of greening (Fig. 6), then our data probably indicate that LHC Ia and -b are more peripheral to CC I than subunit III of CC I is absent from maize thylakoids, whereas at a later stage only the higher molecular weight form accumulates in M thylakoids, whereas at a later stage only the higher molecular weight form accumulates in M thylakoids. The relative rates of accumulation of the LHC I apoprotein and the CC I subunits correlate well with the observations of Breidenkamp and Baker (36), who determined the time of appearance of LHC I during plastid biogenesis in wheat by following the time that 740 nm 77 K fluorescence appeared; this long wavelength emission is thought to arise from LHC Ib (Table I). It was of particular interest that there was differential expression between the two cell types of subunit VI of CC I. Subunit VI appears to exist in two slightly different molecular weight forms, each of which is present at early stages of greening in both BS and M thylakoids, whereas at a later stage only the higher molecular weight form accumulates in BS thylakoids and the lower one in M thylakoids (Fig. 6). Thus, some subunits of PS I in the two photosynthetically active cell types in maize are differentially expressed as development of the plant proceeds. Similarly, LHC II has been shown to be qualitatively and quantitatively expressed differentially in BS and M cells; a low molecular weight form of it was shown to be absent from BS cells (19, 20, 22).

The light-induced accumulation of the LHC Ia, -b, and -c

Fig. 5. Light-induced synthesis of LHC Ia, LHC Ib, and LHC Ic apoproteins. BS thylakoids were prepared after different times of greening of etiolated leaves. Polypeptides (25 μg per lane) were separated using SDS-polyacrylamide gel electrophoresis (10) and immunodecorated with antibodies against L. gibba LHC I.

Fig. 6. Light-induced synthesis of CC I subunits from BS thylakoids (see Fig. 5). Immunodecorated was performed with antibodies raised against subunits II, IV, VI, and VII of CC I (1). G = fully greened tissue.

Discussion

C4 plants present an interesting system in which to study differential expression of photosystem components because they have two structurally and functionally different photosynthetic cell types (18-21, 24). It has been a majority view to study some PS I components in this paper. We were able to show that most of the additional polypeptides in PS I are apoproteins of the three LHC I pigment-proteins which we had fractionated (Fig. 1A). Unfortunately, for spectral analyses of the LHC I components (Fig. 4 and Table I), the relatively harsh conditions needed to release the pigment-binding LHC I components from PS I probably displace some pigments from the LHC I components, and therefore, their spectral characteristics (Fig. 4) and pigment contents should not be regarded as being absolutely definitive. High efficiency of energy transfer from chlorophyll b to chlorophyll a molecules in LHC Ib, and low efficiency of transfer from carotenoids to chlorophyll in all three are, however, noteworthy.

The three LHC I polypeptides of 24, 21, and 17 kDa, which have been purified to homogeneity (Fig. 2) starting with the isolated pigment-protein complex, enabled us to obtain their amino acid compositions (Table II). The most striking results are the presence of the unusually large number of 10 cysteine residues per LHC Ib polypeptide chain, when compared with any other thylakoid membrane protein analysis. The amino-terminal residue of all three LHC I apoproteins is not blocked, and we have been able to obtain the sequence of the first 20 or more residues in each case.2 The NH2-terminal sequences are quite different from each other, as are their amino acid compositions (Table II) and pigment compositions; they also lack cross-reactivity against antibodies affinity-purified against any one of the three polypeptides. Thus, they are certainly different pigment-proteins and not one pigment-protein, the polypeptide of which has undergone proteolysis. It is of interest that the LHC I antibodies we tested (Figs. 3 and 4) showed no cross-reactivity between the LHC I and LHC II components; controversy exists about such relatedness (see Ref. 39 for discussion). The LHC I polypeptides are nuclear-encoded, and genes are presently being cloned using synthetic oligonucleotide probes and an antibody to each polypeptide.

Studies on maize plastid biogenesis using antibodies to L. gibba LHC I revealed (Fig. 5) that the accumulation of LHC Ic apoprotein after 8 h of greening precedes by several hours the accumulation of either of those of LHC Ia or LHC Ib; use of a higher titer of LHC I antibody did not alter this observation (data not shown). Since the BS I subunits (note that subunit III of CC I is absent from maize thylakoids unlike the situation in C3 plants (15)) accumulate to a detectable level after only 4 h of greening (Fig. 6), then our data probably indicate that LHC Ia and -b are more peripheral to CC I than is the 17-kDa pigment-protein. The relative rates of accumulation of the LHC Ic apoprotein and the CC I subunits correlate well with the observations of Breidenkamp and Baker (36), who determined the time of appearance of LHC I during plastid biogenesis in wheat by following the time that 740 nm 77 K fluorescence appeared; this long wavelength emission is thought to arise from LHC Ib (Table I). It was of particular interest that there was differential expression between the two cell types of subunit VI of CC I. Subunit VI appears to exist in two slightly different molecular weight forms, each of which is present at early stages of greening in both BS and M thylakoids, whereas at a later stage only the higher molecular weight form accumulates in BS thylakoids and the lower one in M thylakoids (Fig. 6). Thus, some subunits of PS I in the two photosynthetically active cell types in maize are differentially expressed as development of the plant proceeds. Similarly, LHC II has been shown to be qualitatively and quantitatively expressed differentially in BS and M cells; a low molecular weight form of it was shown to be absent from BS cells (19, 20, 22).

2 A. Vainstein, manuscript in preparation.
apoproteins in maize leaf tissue was studied using an antibody to \textit{L. gibba} LHC I which cross-reacts with all three maize apoproteins (Fig. 3). Recently, Schuster et al. (30) detected only one polypeptide in \textit{Chlamydomonas reinhardtii} thylakoids that cross-reacts with this same antibody, which means that either \textit{C. reinhardtii} does not have the LHC Ia and LHC Ic subunits, or that their primary structures differ considerably from those of higher plants. Our laboratory earlier reported the occurrence of a single LHC I pigment-protein in \textit{L. gibba}. We did indicate, however, that there might be other LHC I pigment-proteins in \textit{L. gibba} (3); furthermore, the antibody that we used in this earlier work (3) was not the one used here but was one that cross-reacted with LHC Ib only (13). Thus, our data on maize do not contradict those on \textit{L. gibba}.

In summary, our observations show unequivocally that earlier suggestions that the 24- and 17-kDa PS I polypeptides are associated with photosynthetic pigment molecules (see introduction to the text) is indeed correct. It is now of much interest to obtain and compare the complete amino acid sequence of these LHC I apoproteins with those already known for other LHC components and to devise gentler procedures for isolating the LHC I pigment-proteins so that their pigment compositions and spectral properties can be more precisely determined.

Acknowledgments—We wish to thank Drs. Elaine Tobin, Rachel Nechushtai, and Gary Peter for providing antibodies and for discussions during the course of this work.

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