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Polypeptide Composition of Chlorophyll-Protein Complexes from Romaine Lettuce[1]

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ABSTRACT

The protein moiety of the two major chlorophyll-protein complexes associated with chloroplast membranes of outer, dark green leaves of a romaine lettuce shoot (*Lactuca sativa* L. var. Romana) has been analyzed by discontinuous sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis. Complex II, also termed light-harvesting chlorophyll-protein complex, is shown to consist of a major polypeptide of 25 kilodaltons (kD) and two minor ones of 27.5 and 23 kD. The 25 kD subunit is the single largest polypeptide component of the chloroplast membranes, accounting for about 25% of their total protein. Complex I contains only high molecular weight subunits, the major one being at 67 kD, these subunits representing only a small percentage of the chloroplast membrane total protein.

These data, suggesting an oligomeric nature for the apoprotein of these two chlorophyll-protein complexes, are difficult to reconcile with the estimated molecular weights of the native complexes and raise some intriguing questions as to the types of interactions among the components of these major lipoproteins of the photosynthetic membranes.

Two major Chl-protein complexes are associated with the chloroplast internal membranes of higher plants (14). These two complexes, commonly referred to as complex I (CPI)[2] and complex II (CPII) differ in total amounts of bound pigment, in relative content of Chl and carotenoids, in their amino acid composition and apparent mol wt, as well as in their postulated roles in the photosynthetic process (14). CPI, which appears to contain only Chl *a*, comprises a small fraction of the total membrane protein and is thought to represent part of PSI. CPII, in turn, is the single largest component of the chloroplast membranes, accounting for about 50% of the Chl and about 25% of the total membrane protein. This complex functions mainly as a light-harvesting antenna, channeling its excitation energy into the reaction centers of both PSI and PSII (3).

Because the two Chl-protein complexes account for the majority of the chloroplast lamellar mass, the characterization of their protein moiety and its relationship to individual polypeptides seen in SDS-polyacrylamide gels of solubilized chloroplast membranes are of obvious interest. We recently analyzed chloroplasts from photosynthetically competent inner and outer leaves of a romaine lettuce shoot. Chloroplast membranes from outer dark green leaves contained a group of three polypeptides, with mol wt in the range of 25 kD, which are absent in chloroplast membranes of leaves more to the interior of the shoot, depleted in Chl (7). Since these polypeptides are not required for electron transport or for any other essential reactions of the photosynthetic process, we decided to investigate their possible relationship with accessory Chl-proteins of the thylakoid membrane. The data presented here show that these three polypeptides at 27.5, 25, and 23 kD are associated with the light-harvesting CPII; we have similarly investigated the polypeptide composition of CPI from chloroplasts of outer dark green leaves of lettuce and the results are also presented here. Both complexes are shown to be multipeptide units, composed of nonidentical subunits and the implications of these observations are discussed.

MATERIALS AND METHODS

Dark green leaves from the outer region of romaine lettuce shoot (*Lactuca sativa* L. var. Romana) were used in this work.

Chloroplast Isolation. Chloroplasts were isolated as described by Sane *et al.* (12); after isolation, the chloroplast pellet was washed twice with 1 mM EDTA (pH 8) and once with 0.1 M NaCl-0.05 M tris (pH 8) (9). Total Chl and Chl *a* to *b* ratio were determined by Arnon's method (2).

Membrane Solubilization and Fractionation. Washed chloroplast membranes were solubilized in a SDS-containing buffer at a detergent to Chl ratio of 10:1 (w/w), following the procedure of Kan and Thornber (9). Hydroxylapatite (purchased from Bio-Rad Laboratories, Richmond, Calif.) chromatography of the solubilized membranes also followed their procedures (9) using a column (6 × 3 cm). Material eluted at 0.2 M and 0.3 M sodium phosphate was collected, absorption spectra and Chl content were measured, and the material was analyzed electrophoretically. The 0.2 M eluate, although showing Chl *a* to *b* ratios close to 1, was heavily contaminated with uncolored protein and is not described here; the 0.3 M eluate is the subject of this report.

Gel Electrophoresis. SDS-acrylamide disc gel electrophoresis was performed according to Laemmli's procedure (10) as described before (8). A 0.6-cm-long 5% stacking gel and a 9-cm-long 9% separating gel were used. Gels were scanned at 650 nm and 670 nm for Chl-proteins and 560 nm for Coomassie blue-stained polypeptides. For analysis of their protein moiety, the green, Chl-containing bands were cut out of the gels, the slices finely dispersed, extracted for a 2-hr period with Laemmli's dissolving buffer (10) and reelectrophoresed. Mol wt estimations were obtained by comparison of relative mobility of membrane polypeptides with proteins of known mol wt, according to Weber and Osborn (16).

Absorption Spectra. Absorption spectra of eluted fractions and gel slices and Chl determinations were performed with a Cary 14 spectrophotometer, equipped with a light-scattering device.

RESULTS

Solubilization of chloroplast membranes of lettuce leaves at

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[2] Abbreviations: CPI: chlorophyll-protein complex I; CPII: chlorophyll-protein complex II; FP: free pigment; kD: kilodalton.

relatively low concentrations of the anionic detergent SDS (9) followed by polyacrylamide gel electrophoresis, yields the pattern shown in Figure 1. This pattern is identical to those reported for chloroplast membranes of a variety of other higher plants (14) and comprises two Chl-protein complexes (CPI and CPII) and a smaller band of free pigment (FP). Under our electrophoretic conditions a large fraction of carotenoids, as well as some Chl, fails to leave the buffer front, giving rise to the sharp band, marked "f," below the FP zone. Absorption spectra of gel slices containing the upper (CPI) green band showed a single maximum at 672 nm while the middle (CPII) band possessed a doublet at 669 and 654 nm, as expected for these two chloroplast membrane complexes (14). When the gels are overloaded at least two additional green bands can be observed between CPI and CPII, whose nature and relationship, if any, with the two major Chl-proteins has not yet been fully investigated (5).

Protein staining of the unextracted chloroplast membranes yields a rather more complex densitometric profile (Fig. 2), with a relatively large number of bands migrating independently of the two Chl-proteins. Because the presence of lipids is known to change the mobility of membrane protein components (4), mol wt cannot be estimated under these conditions and, thus, major bands in this profile are marked by numbers. Figure 3 shows the polypeptide profile of solubilized chloroplast membranes from lettuce leaves, after extensive lipid extraction with chloroform-methanol; comparison of this profile with that of Figure 2 reveals a number of differences, the major ones being the complete disappearance of the CPI band and the split of the broad CPII band into three individual polypeptides, with mol wt of 27.5, 25, and 23 kD in the lipid-free material. In addition, bands 2 and 3,

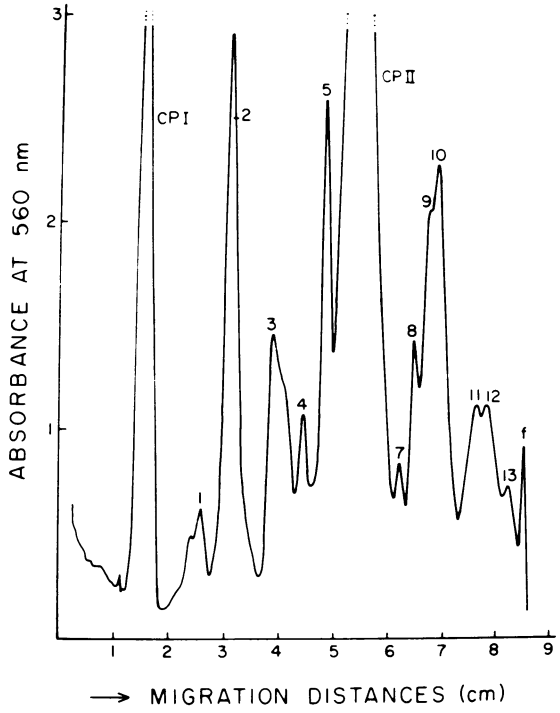

FIG. 2. Scan of Coomassie blue-stained unextracted chloroplast membranes of romaine lettuce leaves, after SDS-acrylamide disc gel electrophoresis; chloroplast membranes were solubilized with SDS, in the absence of β-mercaptoethanol.

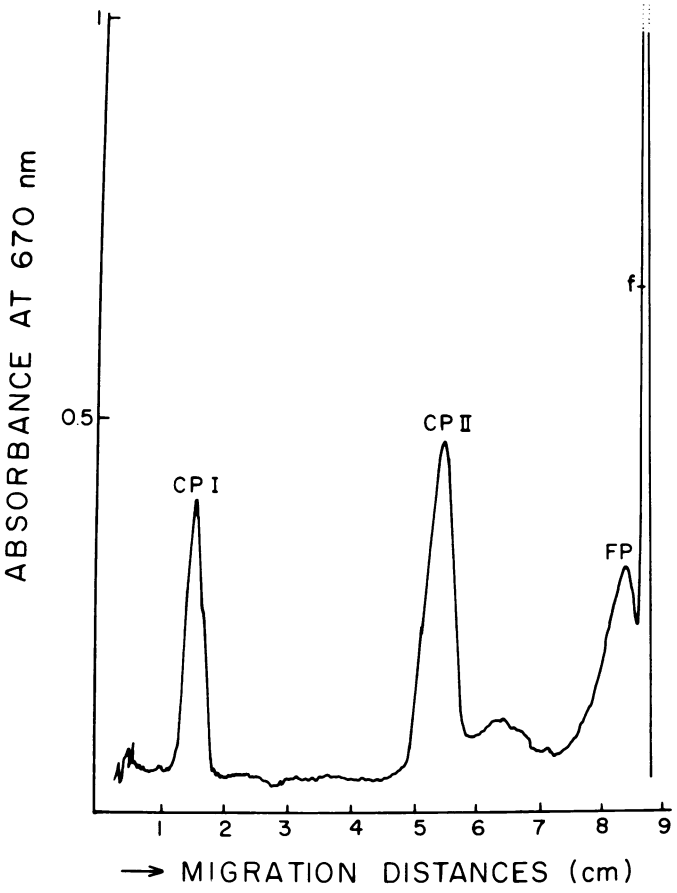

FIG. 1. Scan of Chl-containing bands after SDS-acrylamide disc gel electrophoresis; chloroplast membranes from romaine lettuce leaves were solubilized with SDS, in the absence of β-mercaptoethanol.

which also bind Chl, are similarly decreased in lipid-extracted membrane preparations.

The material eluted from an hydroxylapatite column by 0.3 M sodium phosphate displayed a single Chl-protein band (CPII) and the FP zone (Fig. 4). The CPI complex is completely absent, as shown in this gel tracing and also expected from the low Chl *a* to *b* ratio of this material (1 ± 0.1) contrasted to the much higher Chl *a* to *b* ratio of starting membrane material (2.6 ± 0.2). Protein staining of the unextracted 0.3 M eluate yields the profile shown in Figure 5. Apart from the almost complete absence of the CPI band, and rather to our surprise, we found this profile to be remarkably similar, both qualitatively and quantitatively, to that of whole solubilized membranes (Fig. 2). A noticeable quantitative difference between the two profiles resides in the much higher amounts of the band 1, immediately ahead of the minor CPI peak, in the 0.3 M eluate, which seems to be a dimer of CPII complex. These results contrast with the data of Kan and Thornber (9) for *Chlamydomonas* which show that the unextracted 0.3 M eluate contains only a single polypeptide with the same electrophoretic mobility of the native CPII complex. Although these authors were analyzing algal and not higher plant chloroplasts, they contend that *Chlamydomonas* chloroplast membranes are homologous to those in higher plants. Accepting the validity of this conclusion, we have a major experimental disagreement since the electrophoretic conditions used by those authors fail to show any other polypeptides, besides those associated with the Chl, in whole chloroplast membrane preparations whereas we show here, for lettuce, at least a dozen distinct components not associated with the pigments (Fig. 2). It appears that hydroxylapatite chromatography, although convenient for isolation of the CPII complex from other major Chl-proteins is, in our hands, not suitable for separation of that green complex from uncolored membrane components in lettuce.

The green CPII band obtained from the 0.3 M eluate fraction was cut out of gels and the individual components were extracted

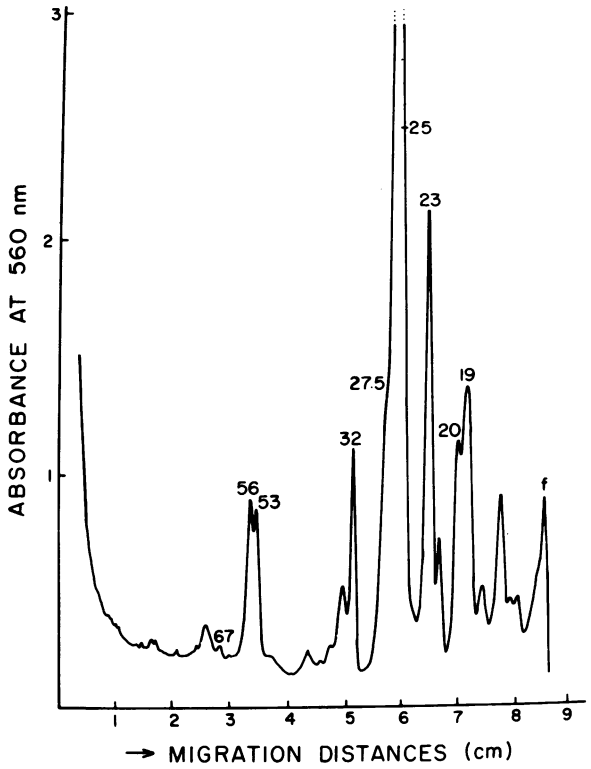

FIG. 3. SDS-acrylamide disc gel electrophoretic polypeptide profile of chloroplast membranes of romaine lettuce leaves; chloroplast membranes were lipid-extracted and dissociated with SDS and β-mercaptoethanol.

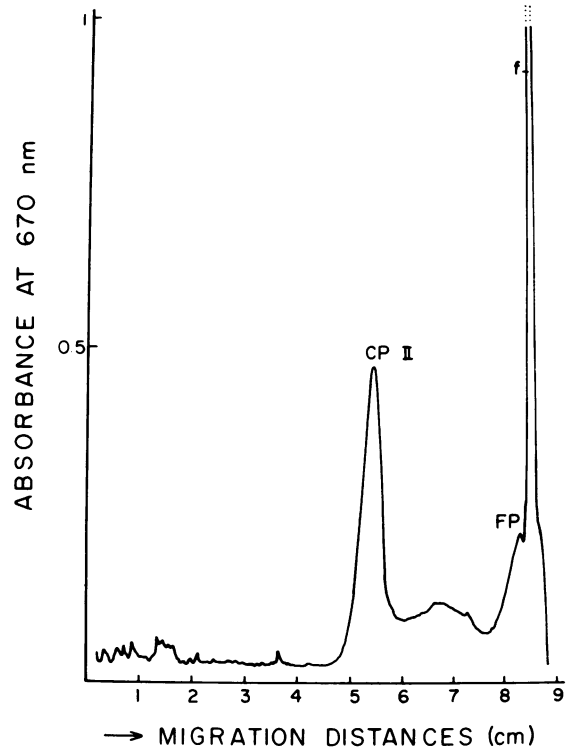

FIG. 4. Scan of Chl-containing bands of chloroplast membrane material eluted with 0.3 M sodium phosphate from an hydroxylapatite column.

by homogenization under dissociating conditions. Reelectrophoresis of this material showed three major polypeptides with mol wt of 27.5, 25, and 23 kD (Fig. 6) and a few minor peaks of high mol wt which we assume to be polymerization products of the complex subunits.

CPI complex green band was cut out of gels in which solubilized whole lettuce chloroplast membranes had been separated and the individual components were extracted in a way similar to CPII; polypeptide analysis of the denatured CPI apoprotein revealed a major component of 67 kD and two minor peaks at 61 and 58 kD (Fig. 7). In control experiments where no protein was loaded into the gels, we have observed the presence of two Coomassie blue-staining bands with mobilities similar to those of the 61- and 58-kD peaks. This observation suggests that the relative amounts of the CPI polypeptides might not be accurately depicted in Figure 7, the 67-kD band being possibly underrepresented. It is also of interest that the protein moiety of the CPI complex appears to comprise a very minor fraction of the whole membrane mass (Fig. 3); this might be due, in part, to the high affinity of the apoprotein of this complex for Chl (14), or to a low affinity of the apoprotein for the Coomassie blue stain used here.

DISCUSSION

We have used the procedure recently described by Kan and Thornber (9) to isolate the CPII complex from chloroplast membranes of lettuce leaves. After chromatography on hydroxylapatite, followed by two gel electrophoretic separations, the CPII complex exhibited three subunits with estimated mol wt of 27.5, 25, and 23 kD. Interestingly, these three peptides are the same we previously reported (7) to be present in chloroplast membranes of the dark green leaves of the outer region of romaine lettuce shoot, which contain normal amounts of the CPII com-

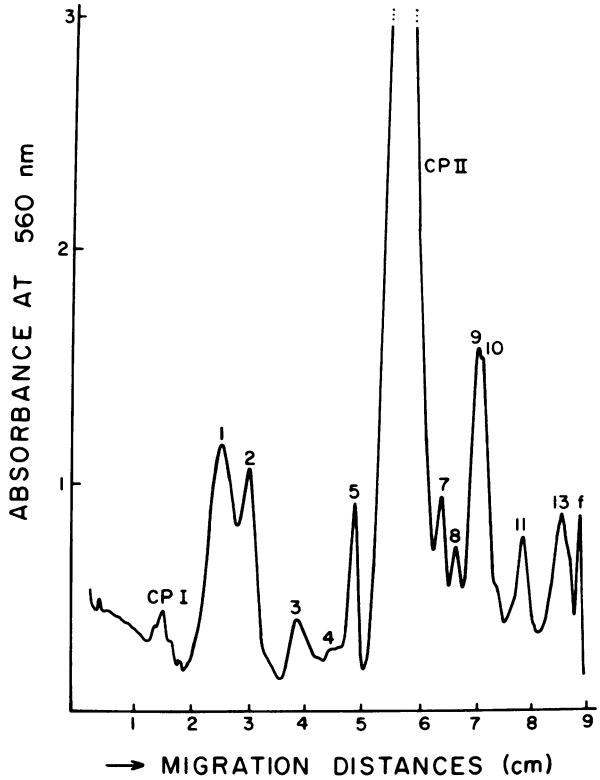

FIG. 5. Scan of Coomassie blue-stained unextracted chloroplast membrane fraction eluted from an hydroxylapatite column with 0.3 M sodium phosphate, and separated by SDS-acrylamide disc gel electrophoresis.

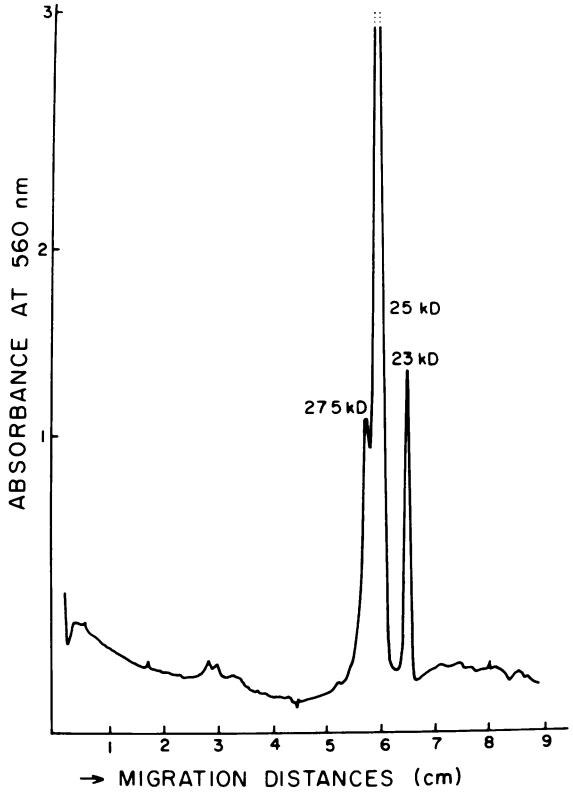

FIG. 6. Polypeptide profile of lettuce chloroplast CPII complex after fractionation by SDS-acrylamide disc gel electrophoresis; apoprotein of CPII complex was dissociated with SDS and β-mercaptoethanol.

plex, but absent from leaves more to the interior of the shoot which are largely depleted in pigment and missing that Chl-protein complex. We have also shown (7) that the absence of these three peptides does not affect the CO_2 fixation ability of chloroplasts from inner leaves of the lettuce head, and thus, have suggested that they constitute the protein moiety of a light-gathering pigment antenna for PSI and PSII. We also found that the CPI complex from lettuce leaves is apparently an oligomeric structure, composed of large mol wt subunits (>55 kD) with the major one at 67 kD. The observation that CPI-associated polypeptides are present in low amounts in thylakoid membrane seems to invalidate previous identification of these subunits with the "group I polypeptides," which are present in relatively much larger amounts of (1); elsewhere (8) we showed that the latter peptides represent, at least in their majority, subunits of coupling factor strongly absorbed to the thylakoid membrane. The low amounts of CPI protein may also explain why most workers reported only one polypeptide component for this complex; the additional minor bands shown in this work and reported before (13) are present in two small an amount to be detected in gels loaded with normal concentrations of membrane protein.

A comparison of these results on the polypeptide composition of the two major Chl-protein complexes with those of other workers (1, 6, 9, 11, 13, 15) is difficult as different workers have used different plant materials and techniques for isolation of membrane complexes, their solubilization, and characterization of their individual components. We strongly believe that one of the main reasons for the disparity in the number of polypeptides reported to be associated with the two major Chl-proteins results from variations in the resolution of peptides separated by different electrophoretic techniques. Separations using electrophoretic systems of lower resolution show fewer components, frequently only one (6, 9, 11, 15), whereas those of higher resolution consistently show that the protein moiety of these complexes is comprised of multiple distinct subunits. One drawback of all techniques which resort only to SDS-acrylamide gel electrophoresis for "purification" of the Chl-protein complexes is that the green bands of each complex may be contaminated with unrelated peptides of the same mobility and such contamination taken as genuine components of the native complexes.

If we assume that more than one polypeptide makes up the protein moiety of CPII complex, we must explain how a complex with a mol wt in the vicinity of 35 kD can accommodate two (1, 9) or probably three distinct polypeptides with individual mol wt close to 25 kD. The simplest explanation for this apparent paradox is that CPII is not a complex at all, but is a band of three closely migrating Chl proteins. Some preliminary evidence tends to support this possibility. First, treatment of thylakoid membranes with low SDS to Chl ratios yields dimers migrating at about 55 kD which can be dissociated by higher SDS concentration to yield the monomeric forms (5, 14). Second, during experiments in which we used the Laemmli's procedure (10) for membrane solubilization and subsequent fractionation, we observed an apparently splitting of CPII complex into closely adjacent green bands; other workers (1) have also reported a composite shape of this complex in SDS-acrylamide gels, comprising a peak and a shoulder. Such electrophoretic behavior of the CPII Chl-protein complex may result from partial dissociation of the nature complex under stronger solubilization conditions and longer running distances; it alternatively might indicate the existence of distinct chl-proteins of close electrophoretic mobility. Third, we have further measured absorption spectra of serial sections of the CPII green band and detected small, but consistent, alterations in the Chl *a* to *b* ratios of successive slices, Chl *a* being retarded relative to Chl *b*, again suggestive of some

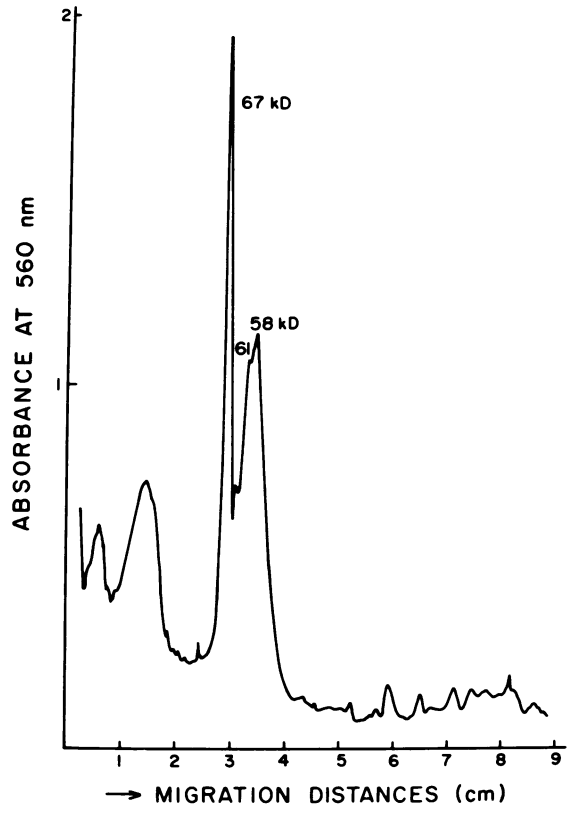

FIG. 7. Polypeptide profile of lettuce chloroplast CPI complex after fractionation by SDS-acrylamide disc gel electrophoresis; apoprotein of CPI complex was dissociated with SDS and β-mercaptoethanol.

heterogeneity in pigment distribution within the broad CPII band. On the other hand, polypeptide analysis of the "CPII dimer" revealed the presence of the 27.5-, 25-, 23-kD polypeptides, implying some sort of association among these three membrane constituents. These observations indicate that the CPII band may, in fact, consist of three dissociated monomers which are combined to form much larger structures in the intact membranes.

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