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MEMBRANE POLYPEPTIDES OF SOME HIGHER PLANT CHLOROPLASTS

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SUMMARY

Sodium dodecylsulfate–polyacrylamide gel electrophoresis of membrane polypeptides of the mesophyll cell chloroplasts of barley, pea, and maize show similar profiles, with the polypeptides falling into two major groups: those associated with a membrane fraction enriched in Photosystem I (called Group I polypeptides) and those associated with a membrane fraction enriched in Photosystem II (called Group II polypeptides a, b, and c). In contrast to these profiles, the polypeptides from the extensively unstacked membranes of chloroplasts from the chlorophyll-deficient mutant strains of barley and pea as well as those obtained from the agranal bundle sheath cell chloroplasts of maize are deficient in the Group II polypeptides b and c. It is proposed that these polypeptides are required for membrane stacking in higher plant chloroplasts.

These Group II polypeptides b and c are not required for Photosystem II activity since both the barley and pea mutant chloroplasts and the maize bundle sheath chloroplasts possess Photosystem II activities.

INTRODUCTION

The structural organization of the membranes of higher plant chloroplasts is characterized by two well defined regions: regions of stacked grana membranes and regions of unstacked stroma membranes [1]. This unique organization of the chloroplast membranes can be modified in certain instances by mutation or by marked variations in growth conditions [1]. Since the structural and functional organization of biological membranes depends to a large extent on their protein components, we have compared the membrane polypeptides of chloroplasts from the same species which have different amounts of stacking.

We have examined the chloroplast membrane polypeptides from a chlorophyll-deficient mutant strain of barley and of pea. The chloroplast membranes are mostly unstacked in both of these species, and they have fewer membranes per granum and fewer grana per chloroplast compared to their respective wild-type chloroplasts [2, 3]. The mutant strain of barley is 30% deficient in total chlorophyll and totally lacking

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in chlorophyll *b*, yet it has good photosynthetic activity [4–6]. The mutant strain of pea is about 50% deficient in total chlorophyll and has a higher chlorophyll *a*/chlorophyll *b* ratio (10 to 18) compared to the wild-type strain ratio of 3; it also shows good photosynthetic activity [3].

The leaves of plants with the C_4-dicarboxylic acid pathway of photosynthetic carbon dioxide fixation [7], possess two morphologically distinct classes of chloroplasts. One class, found in mesophyll cells, possesses grana; the second class is found in the bundle sheath cells and the chloroplast membranes show varying degrees of membrane stacking depending on the species [8]. Maize is well suited for the study of dimorphic chloroplasts since the bundle sheath cell chloroplasts are agranal [9] and a method is available for the isolation of intact chloroplasts [10]. Accordingly we have compared the chloroplast membrane polypeptides obtained from the two types of chloroplasts isolated from the same plant.

Results reported here show that the profiles of chloroplast membranes of the chlorophyll *b*-less barley mutant, the chlorophyll-deficient mutant pea strain, and the agranal maize bundle sheath chloroplasts exhibit marked reductions in the amounts of the Group II polypeptides b and c. These findings are in agreement with those made in *C. reinhardi* [11] and support the view that some or all of the Group II polypeptides must be present in order for the membranes to form stacks [11].

METHODS

The two strains of barley (*Hordeum vulgare* L.) used were kindly provided by Dr Harry R. Highkin and have been described previously [4, 5]. The pea strains (*Pisum sativum* L., var. Greenfeast) came from CSIRO, Canberra, the mutant strain being derived from the commercial variety as a spontaneous mutation [3]. Barley and pea strains were grown in vermiculite in a growth chamber (22–25 °C) with a light period of 16 h for 18–25 days. Seedlings of maize (*Zea mays* L., var. NES 1002) were grown in vermiculite in a glass-house (21 °C) for 14–21 days. Spinach was obtained from the local market.

For the isolation of chloroplasts from barley, pea, and spinach, leaf tissue (15 g), in 90 ml of 0.05 M phosphate buffer (pH 7.2) containing 0.3 M sucrose and 0.01 M KCl, was blended in a Servall Omnimixer for 6 s at 120 V. The breis were filtered through two layers of Miracloth (Chicopee Mills Inc., New York), and the chloroplasts were sedimented by centrifugation at $1000 \times g$ for 10 min. The sediments were resuspended in the sucrose–phosphate buffer and centrifuged again to give once-washed chloroplasts.

Maize mesophyll cell chloroplasts were isolated from chilled leaves by blending in the Servall Omnimixer for 3 s as described previously [12], except that the sucrose–phosphate buffer described above was used for the isolation buffer. Intact maize bundle sheath cell chloroplasts were then isolated from the bundle sheath cell strands by the single-chop procedure [10]. Both types of chloroplasts were washed once in the sucrose–phosphate buffer.

All chloroplast preparations were homogenized in glass-distilled water (10 ml) using a Potter–Elvehjem homogenizer, and the homogenates were centrifuged at $10\,000 \times g$ for 10 min. To remove most of the soluble proteins the water-lysed membranes were washed twice with 20 ml of 0.05 M phosphate buffer (pH 7.2) containing

0.01 M KCl; centrifugations were at 15 000 × *g* for 15 min. Finally, to ensure removal of the coupling factor, the membranes were extracted with 1 mM EDTA (20 ml) and centrifuged at 15 000 × *g* for 30 min.

For digitonin fractionations, once-washed chloroplasts were incubated with 0.5% digitonin for 30 min at 0 °C as described previously [13], and the fractions sedimenting at 10 000 × *g* for 30 min (D-10) and 144 000 × *g* for 60 min (D-144) were retained from the differential centrifugation separation.

Chlorophyll was determined by a modification [14] of the procedure of Mackinney [15] and protein by the method of Lowry et al.]16].

The washed, EDTA-treated chloroplast membranes, and the D-10 and D-144 fractions from the digitonin fractionations were extracted twice with 90% acetone at room temperature and the protein pellets solubilized in a 2% sodium dodecylsulfate–0.5 M urea buffer as described by Hoober [17] except that the proteins were solubilized by heating in a boiling water bath for 90 s. Electrophoresis on sodium dodecyl sulfate–polyacrylamide gels was performed using the procedures of Hoober [17] with a pulsed power supply operating at 160 V, 25 mA, and 105 pulses/s; the temperature was 4 °C.

RESULTS

Fig. 1 compares the profiles of polypeptides obtained from the purified chloroplast membranes of the wild-type and the chlorophyll *b*-less strains of barley. Comparison of the wild-type barley profile (Fig. 1A) with that of spinach (Fig. 2)

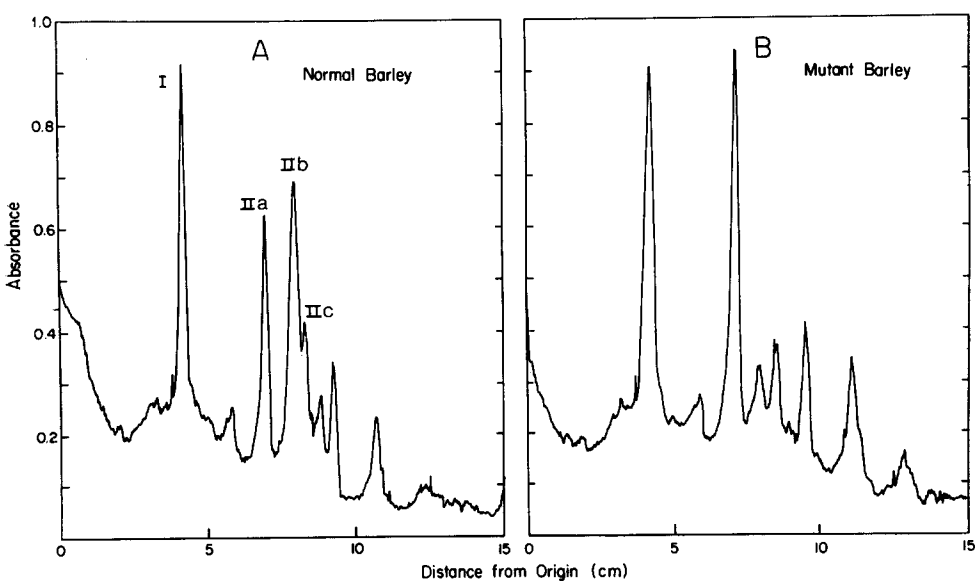

Fig. 1. Electrophoretic separation of chloroplast membrane polypeptides isolated from barley. A. Wild-type. B. Chlorophyll *b*-less mutant strain. Sodium dodecylsulphate–polyacrylamide gel electrophoresis was performed as described by Hoober [17] except that the pulsed power supply was operated at 160 V, 25 mA and 105 pulses/s. Between 50–75 μg protein was loaded on each gel and the gels were stained with Coomassie blue and scanned at a wavelength between 530 to 550 nm.

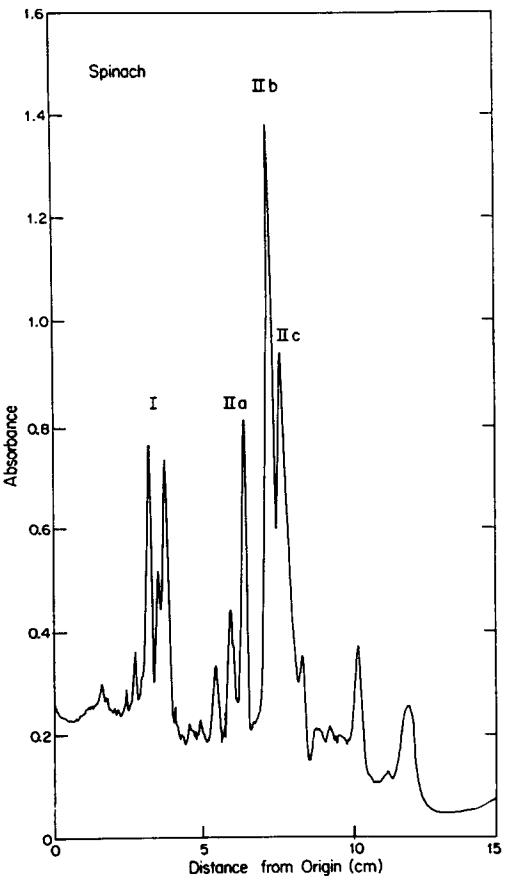

Fig. 2. Electrophoretic separation of the chloroplast membrane polypeptides from spinach.

shows close similarity. It has previously been shown for spinach and *C. reinhardi* that the group of polypeptides designated I are associated with a membrane fraction rich in Photosystem I activity and the group of polypeptides labelled IIa, b and c are found to predominate in a fraction enriched in Photosystem II activity [18]. In contrast to the wild-type barley chloroplasts, the membrane polypeptides obtained from the mutant strain of barley (Fig. 1B) have markedly reduced amounts of polypeptides in the region of Group II polypeptides b and c, while polypeptide IIa is increased compared to the wild-type profile. The two small peptide peaks in the Group II region of the mutant strain do not appear to be coincident with the Group II polypeptides b and c of the wild-type strain.

Treatment of wild-type barley chloroplasts with digitonin yields two subchloroplast fractions, the smaller one (D-144) being a Photosystem I fraction and the larger one (D-10) being enriched in Photosystem II analogous to the fractions obtained from the digitonin treatment of spinach chloroplasts [13]. As shown in Fig. 3A, the polypeptide profile of the wild-type barley D-10 fraction is enriched in Group II polypeptides as compared to the untreated chloroplast membranes (Fig. 1A) whereas

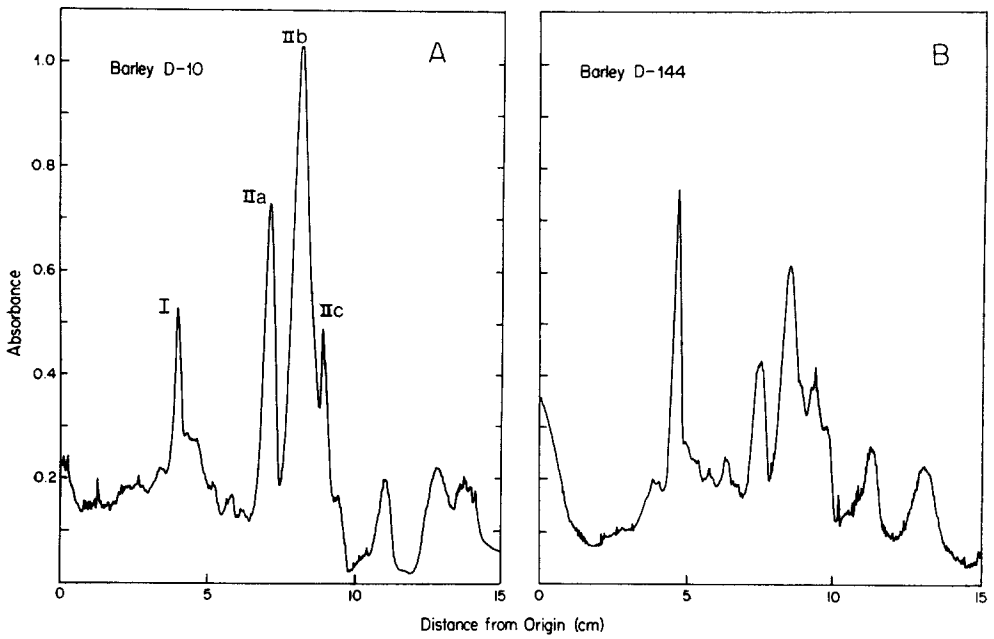

Fig. 3. Electrophoretic separation of chloroplast membrane polypeptides obtained from the digitonin fractionation of wild-type barley chloroplasts. A. D-10 fraction enriched in Photosystem II. B. D-144 fraction enriched in Photosystem I.

the D-144 membrane fraction is somewhat enriched in the Group I polypeptides. This separation is similar to that reported previously for detergent fractions obtained from spinach and *C. reinhardi* [18].

When the profile of the chloroplast membrane polypeptides from the chlorophyll-deficient mutant strain of pea is compared with that of the wild-type strain, the effect of the mutation is also found to be on the Group II polypeptides. The membrane polypeptides of the wild-type pea chloroplasts are resolved into two groups (Fig. 4A) that are similar to those seen in spinach, wild-type *C. reinhardi* [18], and the wild-type barley described above except that the Group II polypeptide *c* is not as distinct in the pea chloroplast profile as it is in that of spinach. Comparison of the membrane polypeptide profile of the mutant strain pea chloroplasts with that of the wild-type pea chloroplasts (Fig. 4) shows that the mutant pea is deficient in Group II polypeptides b and c with IIb being more reduced than IIc. However, the reduction in Group II polypeptides b and c is less marked than is the case with the mutant strain of barley.

Digitonin fractionation of wild-type pea chloroplast leads to a very good separation of the two groups of polypeptides associated with the two detergent fractions, D-10 and D-144 (Fig. 5), supporting the assumption that the two groups of polypeptides are associated with distinct membrane fractions [18].

The picture presented for the agranal bundle sheath cell chloroplasts of maize is similar to the mutants being discussed here: there is a reduction in the amount of Group II polypeptides. The profiles of the maize mesophyll cell and bundle sheath

cell chloroplasts membrane polypeptides are shown in Fig. 6, and once again the two groups of polypeptides can be distinguished. The twin band seen in the Group I region (Fig. 6A) appears to be characteristic for maize mesophyll cell chloroplasts and it is seen also in membranes which have not been extensively purified by washing and EDTA extraction. This twin band is absent from the profile of membrane polypeptides from bundle sheath cell chloroplasts. When the polypeptides of the bundle sheath cell chloroplasts membranes are compared with those from mesophyll cell chloroplasts, it is seen that there is a marked reduction in the Group II polypeptides b and c.

Maize mesophyll chloroplasts are fractionated with digitonin to yield a D-10 fraction enriched in Photosystem II and a small D-144 fraction enriched in Photosystem I. As seen in Fig. 7, the D-10 fraction is enriched in the Group II polypeptides and the D-144 fraction is enriched in Group I polypeptides.

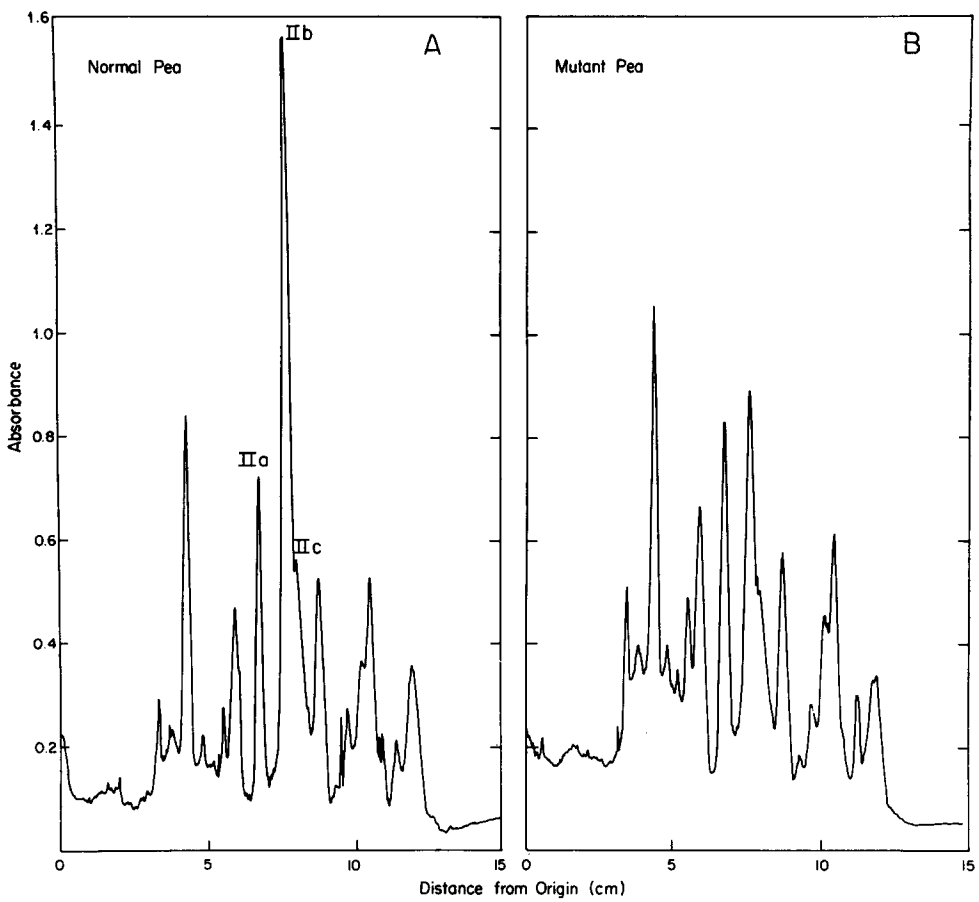

Fig. 4. Electrophoretic separation of the chloroplast membrane polypeptides isolated from pea. A. Wild-type. B. Chlorophyll-deficient mutant strain.

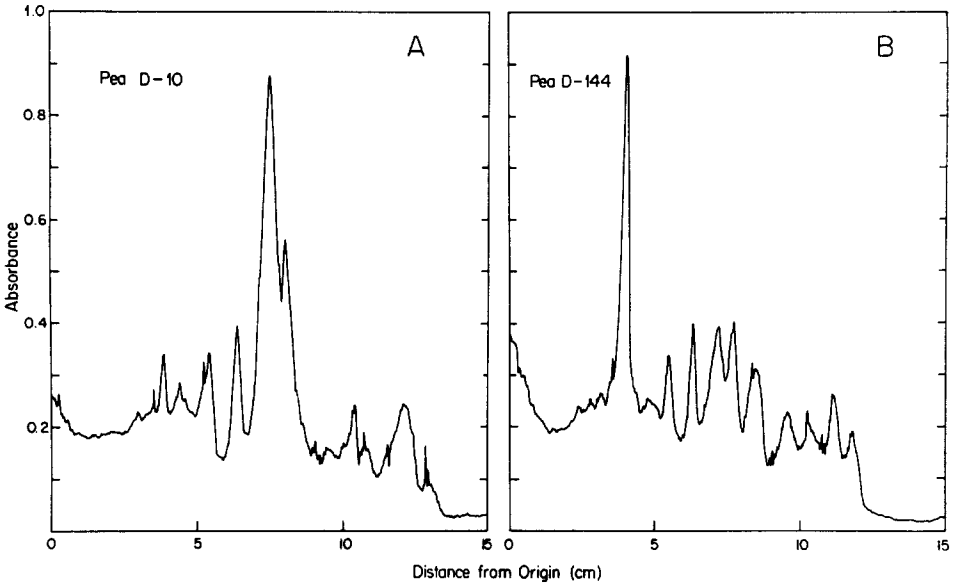

Fig. 5. Electrophoretic separation of chloroplast membrane polypeptides obtained from the digitonin fractionation of wild-type pea chloroplasts. A. D-10 fraction enriched in Photosystem II. B. D-144 fraction enriched in Photosystem I.

DISCUSSION

The polypeptides of the chloroplast membranes from three higher plants have been analyzed. Both stacked and unstacked membranes exist in each species. The chloroplast membranes of the wild-type strains of barley and pea and of the mesophyll cells of maize are stacked, and they possess strikingly similar profiles of membrane polypeptides following sodium dodecylsulphate–polyacrylamide electrophoresis. These profiles resemble the ones presented earlier for spinach and wild-type *C. reinhardi* [18]. As shown by Remy [19], essentially similar results are obtained when the chloroplast membrane polypeptides of spinach, wheat, and barley are examined by rather different solubilization and electrophoresis procedures. The chloroplast membrane polypeptides fall into two principal groups on the basis of digitonin or Triton X-100 fragmentation of the chloroplasts [18, 19]: one group predominates in the membrane fraction enriched for Photosystem I and a second group is associated with the membrane fraction enriched for Photosystem II activity. In the higher plant chloroplasts studied here, Polypeptide IIb is the major polypeptide of Group II with Polypeptide IIc being the minor constituent, whereas the reverse is true for *C. reinhardi* [18] where IIc is always the dominant polypeptide of Group II [11, 18]. The similar results obtained upon analysis of four rather disparate higher plants and a unicellular green alga suggest that there is an underlying similarity in the genetic specification of these membrane proteins.

Both the chlorophyll-deficient mutant strains of barley and pea possess fewer stacked membranes than do their respective wild-types [2, 3]. Comparison of the amount of stacked membranes in the wild-type and mutant strains showed that the

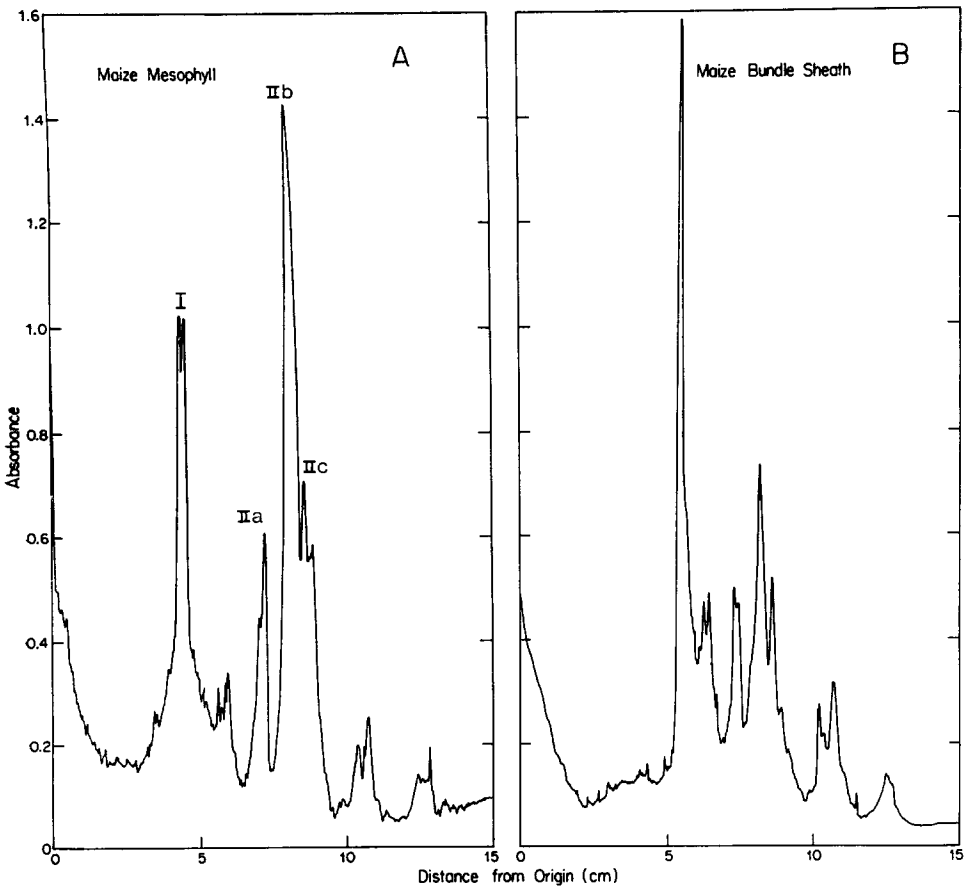

Fig. 6. Electrophoretic separation of the chloroplast membrane polypeptides isolated from the two types of maize cells. A. Mesophyll cells. B. Bundle sheath cells.

reduction in membrane stacking was more marked for the barley mutant than for the pea mutant strain [3]. The bundle sheath cell chloroplasts of maize are virtually without stacked membranes [10]. The Group II polypeptides are affected in all three strains: Polypeptides IIb and IIc are probably absent from the barley mutant membranes and are almost absent from the maize bundle sheath cell membranes and they are markedly reduced in the pea mutant membranes. In contrast, the Group II polypeptide a is not affected in these three strains. All three Group II polypeptides are absent from the unstacked membranes [20] of mixotrophically grown *ac-5* mutant strain of *C. reinhardi* [21].The results presented here suggest that the Group II polypeptides b and c are associated with membrane stacking in higher plant chloroplasts. The group II polypeptides b and c are not required for Photosystem II activity, however, for both the barley [6] and pea [3] mutants have good Photosystem II activities. Maize agranal bundle sheath cell chloroplasts also possess Photosystem II activity at high light intensities [12].

Successful fractionation of the photosystems by digitonin requires that chloro-

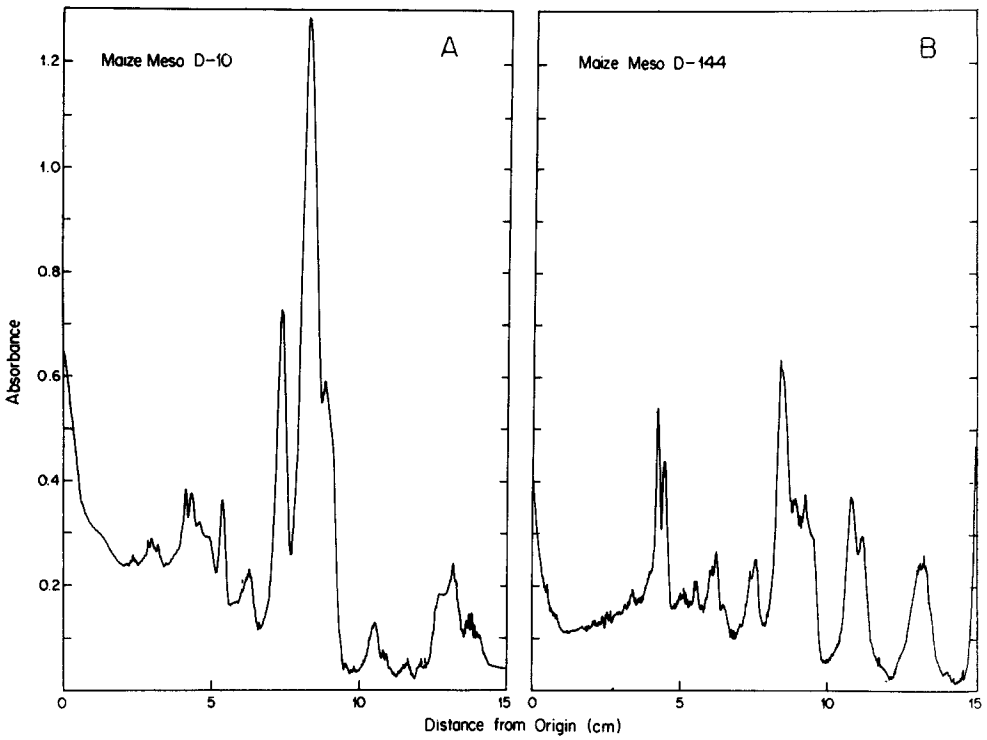

Fig. 7. Electrophoretic separation of chloroplast membrane polypeptides obtained from the digitonin fractionation of maize mesophyll cell chloroplasts. A. D-10 fraction enriched in Photosystem II. B. D-144 fraction enriched in Photosystem I.

plasts possess stacked membranes. Neither the barley mutant strain [21] nor the agranal bundle sheath chloroplasts of maize (Anderson, J. M., unpublished results) are fractionated with digitonin. This is true also for the unstacked membranes of mixotrophically grown *ac-5* cells of *C. reinhardi* [22]. It cannot be concluded, however, that the presence of Group II polypeptides is necessary for fractionation since the pea mutant which is relatively deficient in Group II polypeptides can be fractionated with digitonin (Boardman, N. K. and Thorne, S. W., unpublished results). Moreover, spinach chloroplasts whose membranes have been unstacked by suspension in low-salt tricine buffer [23] are not fractionated by digitonin [24], yet they retain their Group II polypeptides.

The Group II polypeptides have been affected in different ways in the organisms studied so far. In the case of *C. reinhardi*, the mutation of a single Mendelian gene has affected the synthesis of three polypeptides [11]. The various ways to interpret this observation within the framework of one gene–one polypeptide are discussed elsewhere [25]. In the case of both the barley and pea mutant strains, a single Mendelian gene has been affected by mutation and, it appears that two polypeptides have been affected.

The bundle sheath cell chloroplasts of maize and other C_4-plants having agranal bundle sheath cell chloroplasts present a complex picture for analysis, since it is

known that chloroplasts in the young bundle sheath cells have stacked membranes and the ability to form stacks is lost as maturation progresses [26, 27]. Perhaps the expression of nuclear or chloroplast genes in bundle sheath cells becomes altered as the cells or chloroplasts develop.

ACKNOWLEDGEMENTS

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