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A CHLOROPLAST MEMBRANE LACKING PHOTOSYSTEM I

CHANGES IN UNSTACKED MEMBRANE REGIONS

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Summary

The structure and polypeptide composition of the photosynthetic membrane of a mutant of maize has been investigated. The thylakoid membranes of the mutant plants are deficient in Photosystem I activity, although Photosystem II is at near normal levels. SDS polyacrylamide gel electrophoresis of thylakoid membranes from the mutant shows them to be deficient in two polypeptide bands which have been associated with Photosystem I. Freeze-fracture studies of the membrane show that the absence of these polypeptides is associated with a measurable reduction in particle diameter on the unstacked protoplasmic fracture face. This fracture face is derived from the splitting of membranes in unstacked regions of the thylakoid membrane system. It is suggested that in membranes stacked by salts in vitro, Photosystem I activity may be confined to this region.

Introduction

The structural organization of the photosynthetic membrane has been dealt with in a number of recent reviews [1—4]. In most of these analyses, a class of small particles revealed by membrane splitting during the freeze-fracture process has been associated with the Photosystem I reaction complex. In a similar fashion, larger particles revealed in the membrane during freeze-fracturing have been associated with Photosystem II activity. In support of these suggestions, a recent report from this laboratory indicated that significant structural alterations in the photosynthetic membrane were found to be associated with the loss of Photosystem II activity in a mutant of tobacco [5]. In an

effort to learn more about the structural organization of the photosynthetic membrane, we have also undertaken an investigation of mutants of other species deficient in Photosystem I. I report here on studies of maize mutant *hcf**E1481, kindly provided by Dr. Donald Miles of the University of Missouri.

Materials and Methods

Seeds of the lethal mutant 1481 were kindly provided by Dr. Donald Miles of the University of Missouri. Ten-day-old seedlings, which had been grown in vermiculite in a controlled environment, were used for experiments. The seedlings received 15 000 lux for 14 h, and were 10 h in darkness during each 24-h period. Temperature was maintained at 25° C. Wild-type seedlings were grown under identical conditions.

Isolated chloroplasts were used for freeze-etch and gel electrophoresis studies. 10-day-old leaves were cut into small strips which were then ground in isolation buffer (300 mM NaCl, 2 mM $MgCl_2$, 50 mM Tricine/NaOH, pH 7.5) at high speed in a blender for 15 s, or with a mortar and pestle for 30 s. After filtering through eight layers of cheesecloth, the solution was centrifuged at 300 × *g* for 3 min, and the supernatant centrifuged at 3000 × *g* for 10 min. The resulting pellet was resuspended in the isolation buffer and pelleted again at 3000 × *g*. The pellet was then resuspended in a low-salt buffer (2 mM $MgCl_2$, 5 mM Tricine/NaOH, pH 7.5) to remove chloroplast outer membranes, and centrifuged at 6000 × *g* for 10 min to pellet the thylakoid membranes.

SDS-polyacrylamide gel electrophoresis was performed according to the methods of Laemmli [6]. Samples were prepared by the method of Apel et al. [7]. The solubilized membrane protein samples were either used fresh or stored frozen at −80° C; storage seemed to have no deleterious effect on the samples. The 8.5-cm long separating gel was 10% acrylamide, and the gels were run at 20 mA constant current at 25° C. The polypeptides were visualized by Coomassie blue staining.

Intact leaf fragments from wild-type and mutant leaves were examined in thin section by electron microscopy. Leaf fragments were cut into small pieces before fixation in 3% glutaraldehyde in 100 mM sodium phosphate buffer (pH 7.5) for 1 h. Following several rinses with the phosphate buffer, the leaves were post-fixed in 2% osmium textroxide for 30 min, rinsed and dehydrated through a graded series of acetone concentrations. The dehydrated material was embedded in Spurr's resin, cured overnight, thin sectioned, and stained with lead citrate and uranyl acetate.

The isolated thyalkoid membranes were also prepared for freeze-fracturing and deep-etching. Samples to be deep-etched were suspended in the low-salt buffer and quick-frozen in liquid freon before transfer to liquid nitrogen for storage. Samples to be freeze-fractured were infiltrated over 1 h with glycerol as a cryoprotectant to a final concentration of 25% (v/v) in the low-salt buffer; the membranes were recovered by centrifuging at 12 000 × *g* for 10 min, and were frozen as indicated above. The freeze-fracturing was performed as described by Moor and Mühlethaler [8]; freeze-fracturing was performed at −110° C, while deep-etching was done for 1 min following the final cut at −100° C. Replicas were cleaned with commercial laundry chlorine bleach, and

mounted on copper grids for electron microscopy.

Size measurements of the freeze-fracture face particles were made by enlarging the electron micrographs to 200 000 × and using a 7 × ocular micrometer to measure the diameters of the particles perpendicular to the direction of shadowing.

Statistical analyses of the particle size distributions were done using the Student's two-tailed *t*-test.

Results

The photosynthetic characteristics of mutant 1481 have been described elsewhere [9]. *P*-700 content is reduced to a level 10% that of wild-type membranes, Photosystem I activity is severely reduced, while Photosystem II

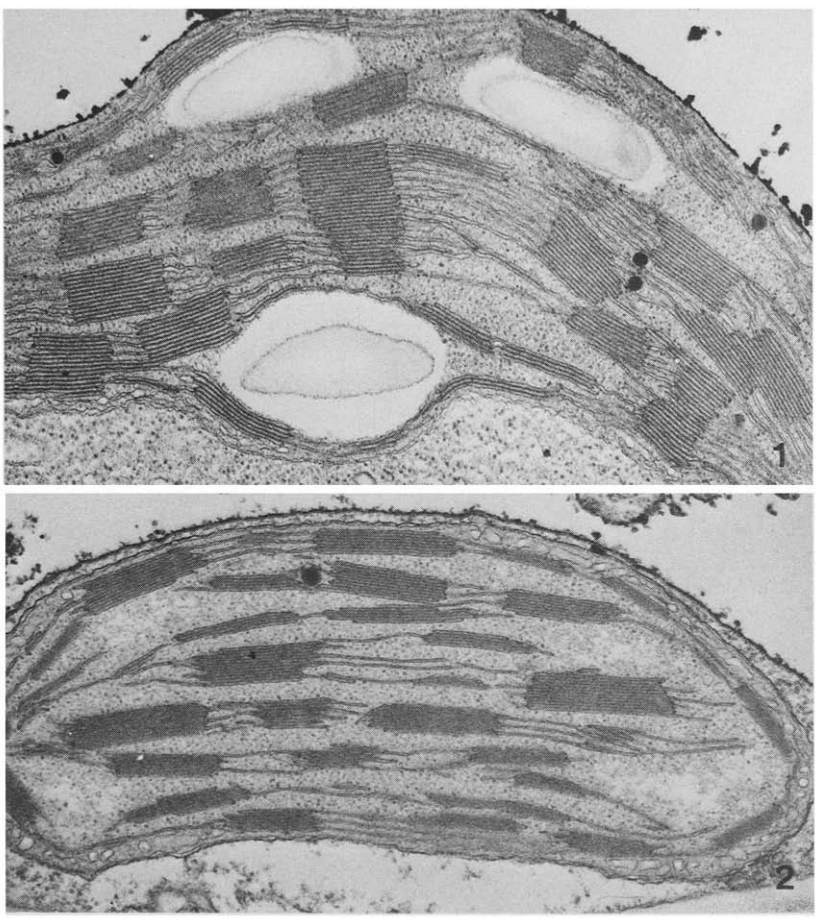


Fig. 1. Thin section of a wild-type maize chloroplast. The usual organization of photosynthetic membrane is apparent. Three large starch granules are also present in the plastid. Magnification: ×29 000.

Fig. 2. Thin section of a chloroplast from the lethal 1481 mutant. Membrane organization is very similar to that of the wild-type (WT) organelle, but starch grains are not present. Magnification: ×29 000.

activity is near normal. The appearance of mutant and wild-type chloroplasts is illustrated in Figs. 1 and 2.

Polyacrylamide gel electrophoresis of thylakoid membranes solubilized in sodium dodecyl sulfate was carred out to analyze the polypeptide composition of mutant and wild-type membranes. Representative gels are shown in Fig. 3. Although both samples are quite similar, there are several distinct changes associated with the mutation. A polypeptide band at an apparent molecular weight of 68 000 is severely reduced or eliminated in the mutant, and a band at 18 000 is weakened. In addition, gels run under non-denaturing conditions showed no high molecular weight chlorophyll-protein bands in the region where 'chlorophyll-protein complex I' was observed in wild-type membranes.

Freeze-fractured thylakoid membranes from all higher plants studied show four distinct fracture faces, two associated with stacked regions of the membrane system, and two associated with unstacked regions [10]. The same four fracture faces are visible in both wild-type (Figs. 4 and 5) and mutant membranes (Figs. 6 and 7). In order to carefully compare the organization of each

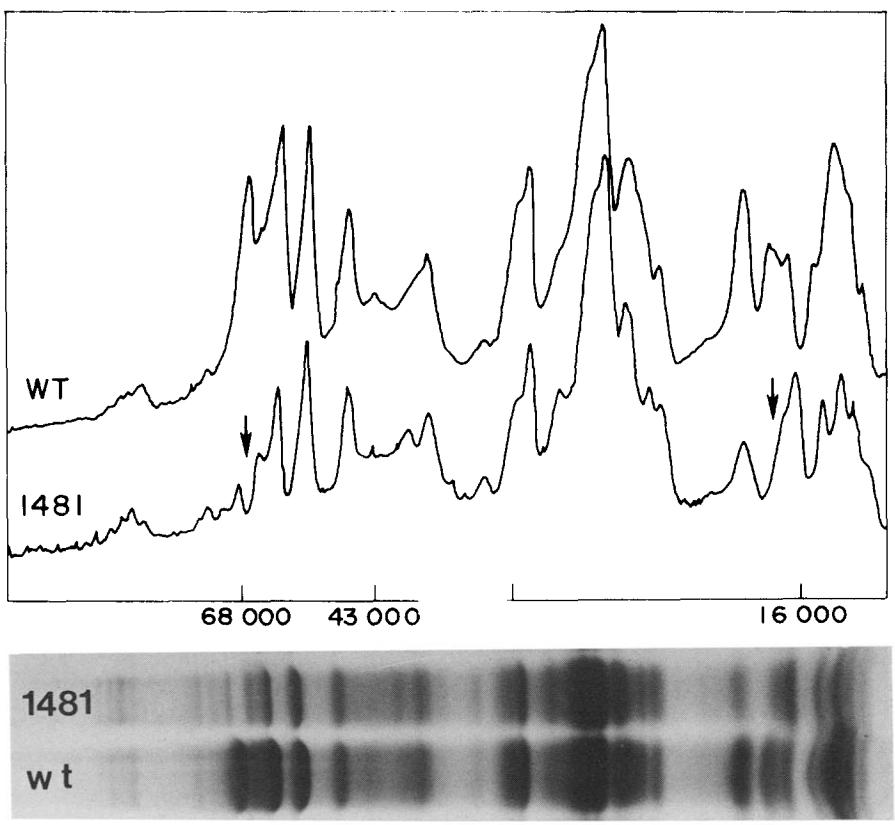


Fig. 3. SDS-polyacrylamide gel electrophoresis of thylakoid membrane polypeptides from wild-type and mutant plants. The absence of a major polypeptide at 68 000 daltons and reduction of a component at 18 000 daltons are indicated by arrows in the densitometer tracing. Samples were stained with Coomassie brilliant blue. The polypeptides at 68 000 daltons and 18 000 daltons are also observed in preparations enriched for Photosystem I activity [12].

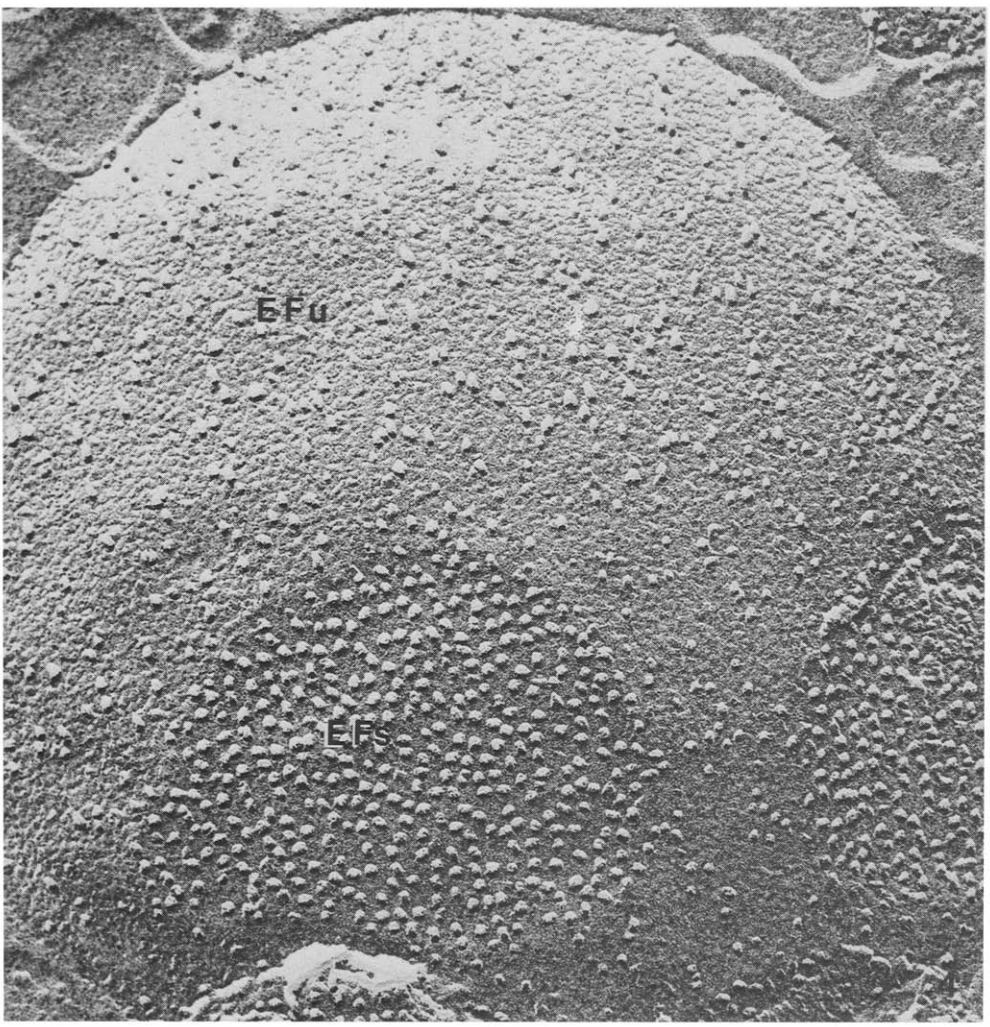


Fig. 4. Freeze-fractured wild-type thylakoids. Only the fracture face is observed in this micrograph, and one of the two stacked areas at the lower part of the membrane has been marked with the appropriate nomenclature (EFs). The large ectoplasmic fracture face particles are concentrated in the stacked regions, and the unstacked region (EFu) shows a pockmarked background compared to the stacked areas. Magnification: ×100 000.

membrane type, I have carried out a systematic analysis of particle sized on each of the four fracture faces of both mutant and wild-type membrane. The results of these measurements are shown in Fig. 8 for the protoplasmic fracture face and Fig. 9 for the ectoplasmic fracture face. Significant differences between mutant and wild-type fracture faces are observed only on the unstacked protoplasmic fracture face.

The differences shown in particle-size histograms can also be observed directly from electron micrographs. Fig. 5 shows a extensive protoplasmic fracture face, containing both stacked and unstacked membrane regions from a wild-type thylakoid membrane. Note the apparent difference in average particle

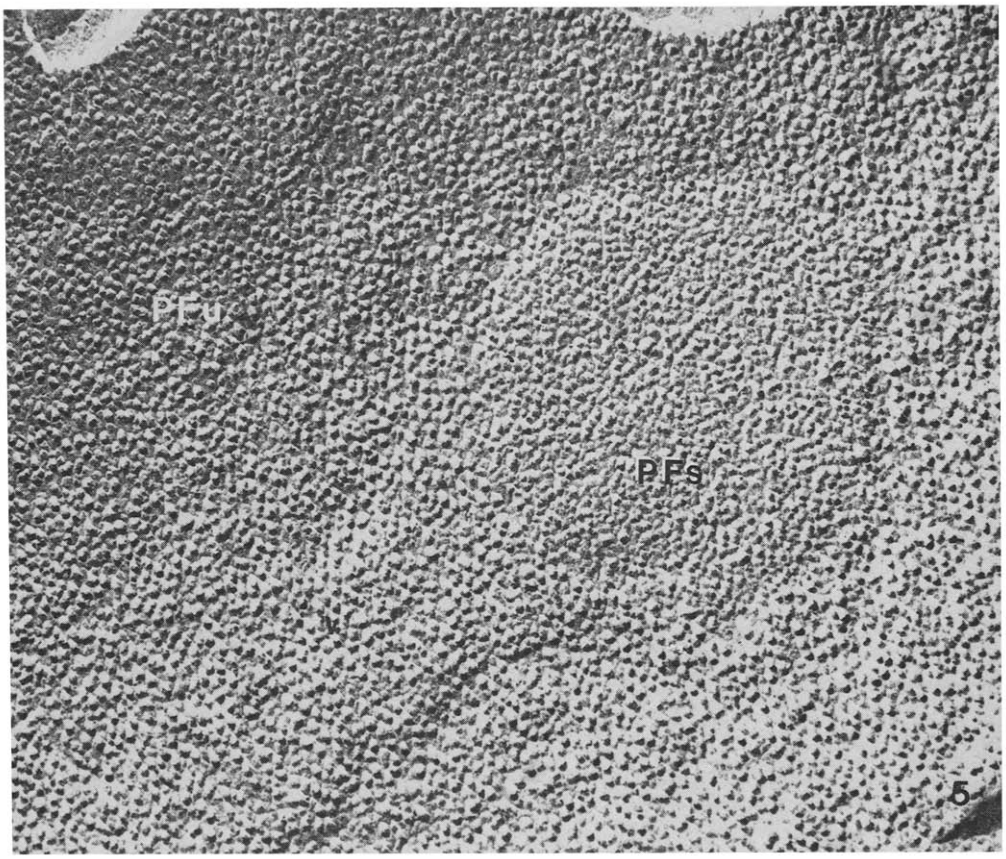


Fig. 5. Freeze-fractured wild-type thylakoids, showin protoplasmic fracture faces. (Stacked (PFs) and unstacked (PFu) regions are indicated. Note that the stacked region can be recognized in part because the intramembrane particles seen in stacked regions are somewhat smaller than those present on the adjacent unstacked fracture face. Magnification: ×100 000.

size between the stacked and unstacked region: particles are clearly larger on the unstacked face. A similar micrograph prepared from a sample of mutant thylakoid does not show this difference. In Fig. 7, although stacked and unstacked regions can be distinguished, the particles found on each seem to be virtually indistinguishable on the basis of size. A normal feature of wild-type thylakoids, namely, the presence of larger particles on the unstacked protoplasmic fracture face when compared with the protoplasmic fracture face, is therefore not observed in the mutant photosynthetic membrane.

Discussion

The polypeptide differences apparent between wild-type and mutant 1481 thylakoid membranes seem consistent with bioenergetic studies [9] indicating a deficiency in Photosystem I activity. Purified Photosystem I preparations have been reported to contain a major polypeptide in the vicinity of 68 000,

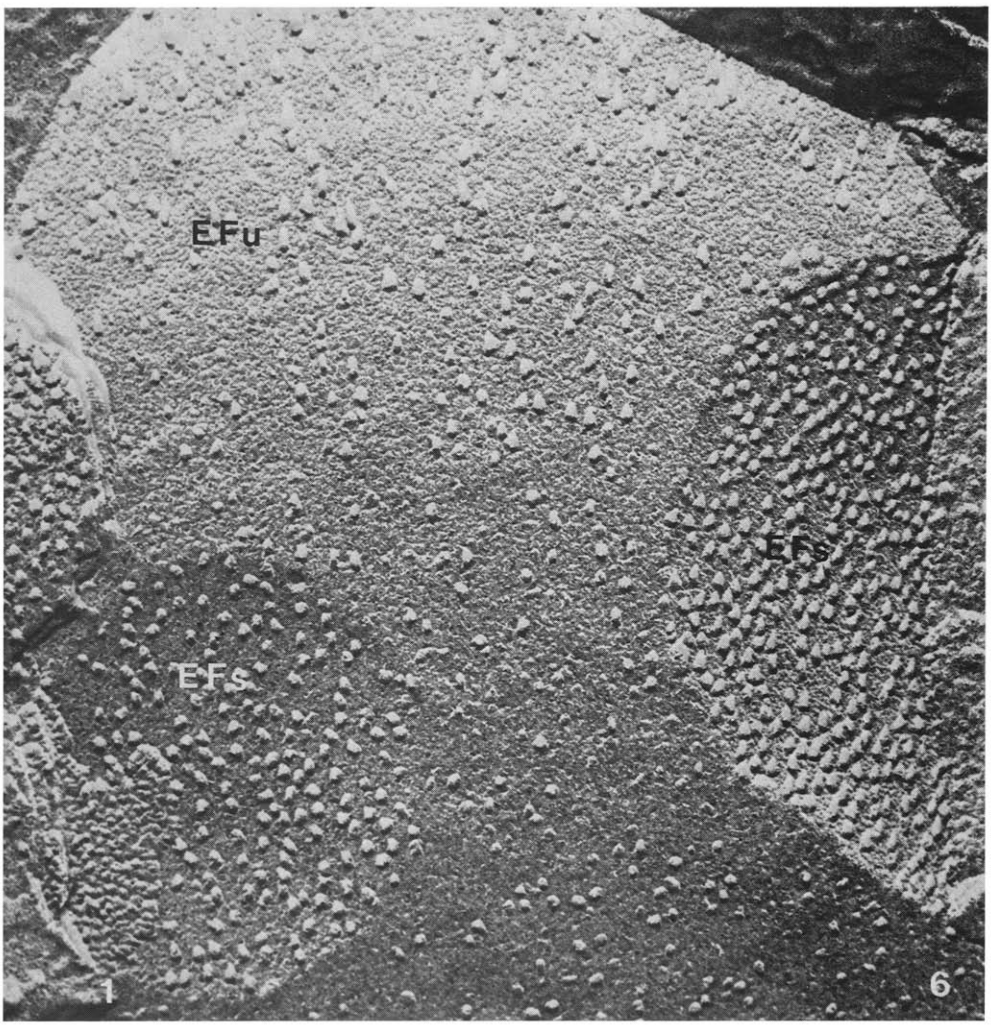


Fig. 6. Freeze-fracture thylakoids, ectoplasmic fracture faces, from the Photosystem I-deficient 1481 mutant. Both stacked (EFs) and unstacked (EFu) fracture faces seem to resemble those of wild-type thylakoids (Fig. 4), although the small pits or depressions visible on the EFu face do not seem as pronounced as those on the wild type. Magnification: ×100 000.

and several components of lower molecular weight (Bengis and Nelson [8] report polypeptides of 70 000, 25 000, 20 000, 18 000, 16 000, and 8000). Although the biochemical nature of the defect in mutant 1481 cannot be specified at this time, the reduction in bands associated with Photosystem I supports the notion that several integral components of the Photosystem I reaction complex are missing or present in smaller amounts in the thylakoid membranes of the mutant.

Although the general pattern of structure in the membranes as revealed by freeze-fracturing is not altered as a result of this mutation, specific changes are observed on both fracture faces of thylakoid membranes in unstacked regions. These changes are consistent with a reduction in size of an internal membrane

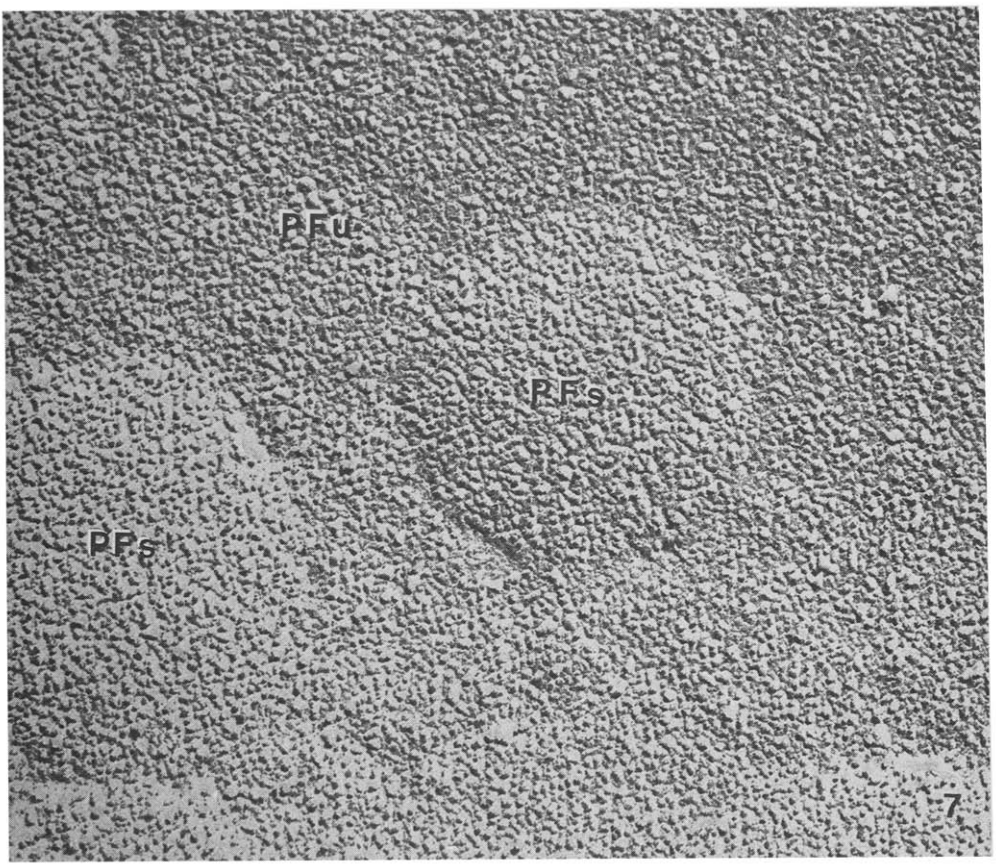


Fig. 7. Protoplasmic fracture faces from the Photosystem I-deficient 1481 mutant. Note that although stacked regions (PFs) can still be recognized, particle sizes in stacked and unstacked regions are now virtually the same, and it is difficult to distinguish stacked and unstacked regions. Compare with Fig. 5. Magnification: ×100 000.

structure which, after the membrane has been split in freeze-fracturing, appears on the unstacked protoplasmic fracture face. No other changes in membrane organization seem to be associated with the mutation.

These results are not sufficient to identify the unstacked protoplasmic fracture face particles visible in the wild-type thylakoid as representing the Photosystem I reaction complex. Nevertheless, they are certainly consistent with that idea. The large number of small particles found in unstacked regions on the protoplasmic fracture face might represent in part incomplete Photosystem I centers, missing crucial components as a result of the mutation. Such

Fig. 9. Histograms presenting data from measurements on the protoplasmic fracture faces (PFu, unstacked and PFs, stacked) of both thylakoid types. Although particle diameters for the PFs fracture face are similar, large differences are detected for the PFu fracture faces (109 Å average diameter for wild type vs. 93 Å for the mutant). A *t*-test comparing the significance of this difference showed that the probability of these two populations arising from a change sampling of one group was less than 0.01%. Therefore, a statistically significant difference is observed in particle diameters on the PFu fracture faces of thylakoids from wild-type and mutant plants.

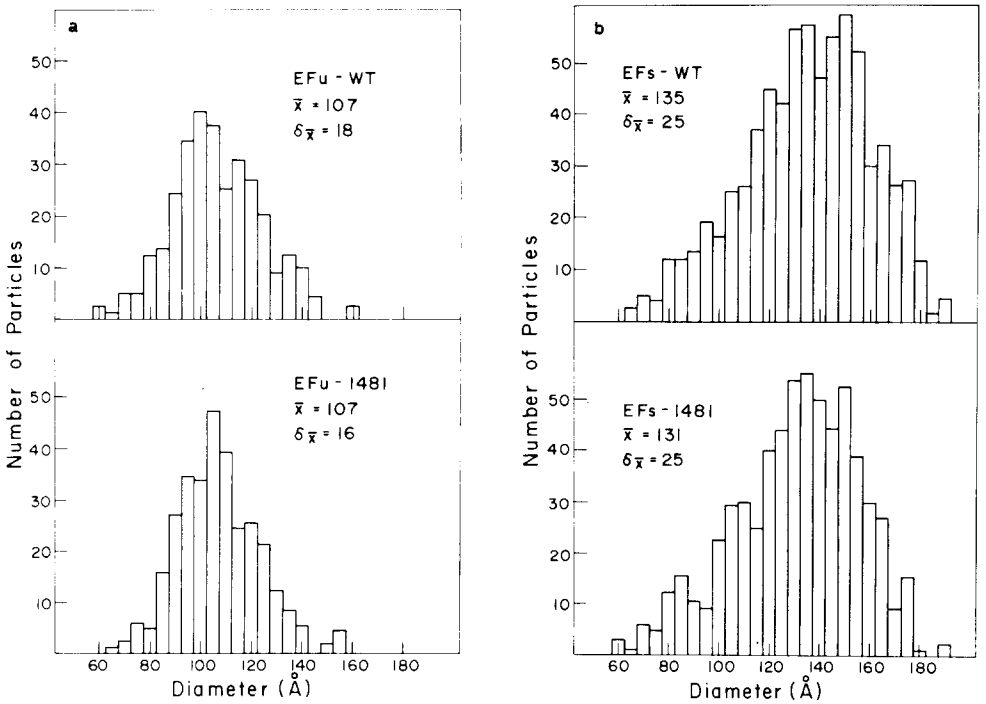
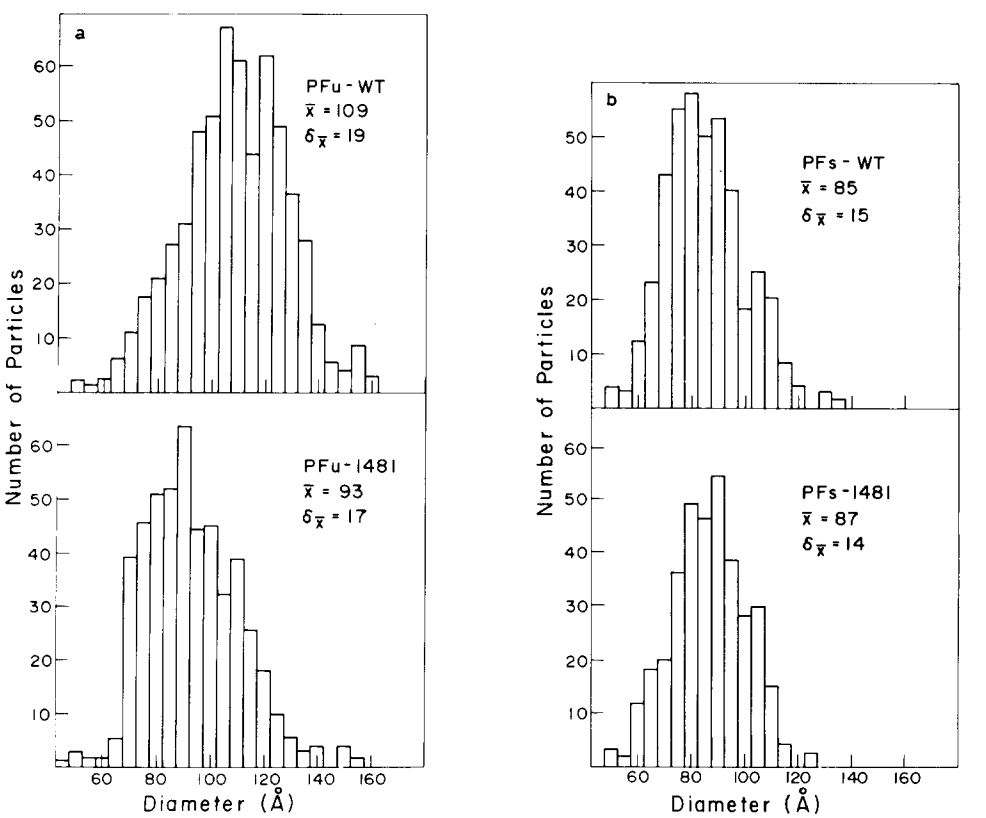


Fig. 8. Histograms presenting data from particle size measurements on ectoplasmic fracture faces (EFu, unstacked and EFs, stacked) of wild-type and mutant thylakoid membranes. No significant differences are detected between wild-type and mutant membranes.



centers might therefore lack Photosystem I activity, and appear much smaller in freeze-fracture due in part to the absence of a large polypeptide required for proper Photosystem I function. Equally interesting is the observation that no structural changes have been found in stacked regions of the mutant membrane system. This 'segregation would confine a majority of the Photosystem II centers to the stacked regions of the membrane system, while a majority of the Photosystem I centers would be found in unstacked regions. It is tempting to suggest that stacking (at least of the type which occurs in vitro) effects a spatial separation of the two photosystems reflected in the segregation of various particle size classes in stacked and unstacked regions of the membrane system. If this were indeed the case, one might suppose that an important physiological tool to adjust energy distribution and electron flow between the photosystems in vivo might be the stacking and unstacking of the chloroplast membrane system.

Recent studies by Simpson and his coworkers [13] which have suggested that the protoplasmic fracture face particles in stacked regions are related to chlorophyll protein complex II (a light-harvesting chlorophyll *a/b* protein) also support this idea. The deficiency in Photosystem I seems to cause no structural changes on the protoplasmic fracture face of mutant 1481, while the structural changes associated with the absence of chlorophyll-protein complex II have been observed only in stacked regions of the thylakoid membrane system [13,14].

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