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HIGH RESOLUTION GEL ELECTROPHORESIS OF CHLOROPLAST MEMBRANE POLYPEPTIDES

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HIGH RESOLUTION GEL ELECTROPHORESIS OF CHLOROPLAST MEMBRANE POLYPEPTIDES*

*Work done under the auspices of the U. S. Atomic Energy Commission.

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ABSTRACT

In the present study we extend previous work from this laboratory on the polypeptide composition of photosynthetic lamellae. Using a high resolution SDS-slab[1] gel electrophoresis technique, we show that both grana and stroma lamellae have qualitatively very similar polypeptide compositions although some clear quantitative differences are demonstrated.

Higher plant chloroplasts contain a complex system of internal membranes, usually differentiated into appressed grana lamellae and unpaired stroma lamellae which connect the grana. During the past few years, there has been a great deal of interest in the comparative chemistry of grana and stroma lamellae, mainly as a result of reported differences in the functional capabilities of these two types of membranes (12). Though the lipids of photosynthetic membranes have been thoroughly investigated (1), little progress in the characterization of membrane proteins was achieved until recently. The introduction of SDS for solubilization of these proteins in conjunction with acrylamide gel electrophoresis of the resulting polypeptides provides a powerful analytical technique.

Previous reports from this laboratory on the characterization of chloroplast internal membrane polypeptides using SDS-acrylamide gel electrophoresis were limited in resolution (10,11). In this paper we have extended the earlier observations using a high resolution system and show that grana and stroma lamellae have qualitatively similar polypeptide composition, although various quantitative differences are apparent.

MATERIALS AND METHODS

Fresh market spinach, Spinacia oleracea L., plants were used in this work. The grana and stroma lamellae were prepared by mechanical disruption of Class II chloroplasts, as described by Sane et al (12). A portion of the French Press homogenate was pelleted at 160,000 xg for one hour and termed FP fraction; the remaining suspension was subjected to fractional centri-

fugation to separate the grana stacks from the unappressed stroma lamellae. The fractions were EDTA (1.0mM, pH 8.0) washed for 30 minutes.

The lipids were extracted by repeated washing with chloroform: methanol (1:2, v/v) and the protein pellet, after drying, was dissolved in 0.06M tris (pH 6.8), 5% w/v β mercaptoethanol, 2% w/v SDS and 5% w/v glycerol at a concentration of approximately 1 mg/ml (8).

The electrophoretic method employs a discontinuous SDS buffer system (8) in a thin slab gel apparatus (HOEFER SCIENTIFIC INSTRUMENTS, San Francisco). Two glass plates (30 cm long, 18 cm wide) sandwich a 0.75 mm thick slab gel between them. A 5% w/v stacking gel and a 9% w/v separating gel, as described by Studier (13) and Ames (2) were used. The separating gel was prerun for 8 hours at 300 volts and after that the stacking gel was poured. Sixty µl of sample solution were then carefully layered in the wells. The voltages applied were 50 V for 10 min., 300 V for 10 min., and 650 V for 4 hours.

Gels were stained for protein with Coomassie Brilliant Blue R and destained sequentially (4). Proteins with known molecular weights, run in wells parallel to the samples, provided molecular weight assignments (15).

RESULTS AND DISCUSSION

SDS-disc gel electrophoresis of chloroplast membrane proteins showed the heterogeneity of polypeptide composition of these membranes. Although variable degrees of resolution have been achieved, resulting in a few or up to 20 components, most studies show a rather consistent pattern for the chloroplast membrane peptides from different plant species (3,6,7,9,11).

Using high resolution SDS-slab gel electrophoresis we have shown a far more complicated image of the polypeptide composition of photosynthetic membranes. The original FP electrophoretic profile (Figure 1) reveals more than 50 clearly discrete bands, dispersed in a relatively large range of molecular weight though not all of them are apparent in the photograph in Figure 1. The additional bands seen in Figure 1 are partially due to resolution of peaks superimposed in disc gel but also to the solubilization procedure used here. In many cases the solubilization conditions produce only partial depolymerization of oligomeric proteins with the consequence that several polypeptide species may be present at the same band and fewer bands are seen. Despite the differences in resolution achieved, it is interesting that the same major bands reported in disc gel are also seen in our results, namely the components with the assigned molecular weights of 60, 56, 30 and 23 Kd.

The variety of low molecular weight components shown here is usually seen in a disc gel as one or a few broad bands. Also, the high molecular weight polypeptides (>60 Kd) resolved here are, in general, not visible in disc gel preparations.

There is a remarkable similarity of grana (G) and FP fractions, both qualitatively and quantitatively (see Figure 1). This is easily explained by the fact that in mature spinach chloroplasts the grana stacks constitute up to 80% of the internal membrane system of this organelle (5).

The polypeptide composition of grana lamellae, on the other hand, shows several differences from that of stroma membranes (see Figure 1). Quantitatively, the most prominent are the large decrease of the 23 Kd and 30 Kd peaks in the stroma lamellae which, in turn are enriched in the 60,

56, and 54 Kd components. These three polypeptides are most probably associated with proteins (coupling factor and Fraction I protein) attached to the outside surfaces of chloroplast membranes and are usually washed out to some extent by the EDTA treatment. The large amount of these components associated with the stroma fraction may result, in part, from their co-sedimentation with the single lamellae, pelleted at high centrifugal force or also from the comparatively large exposed area of the single membrane available to adsorb these proteins.

The peaks in the 23 Kd region have recently been associated with the PS II chlorophyll-protein complex (14). This complex, according to Thornber and co-workers, is the major location for chlorophyll b in higher plants and functions as a light-harvesting center for PS II. Thornber's hypothesis is strengthened by these data which show that the peaks in the 23 Kd region are enhanced in the 10 K fraction and depleted in the 160 K fraction. The 10 K fraction possesses both PS II and PS I and a low chl a:chl b ratio whereas the 160 K fraction contains only PS I and a much higher chl a:chl b ratio (12). Though the fractions differ greatly in pigment composition and electron transport reactions (12) the are qualitatively very similar in polypeptide composition. In this respect our results are at variance with previous studies (7,10,11) reporting differences; we attribute this disagreement to the much higher sensitivity of our technique which allows a finer analysis of polypeptide composition.

The complexity of polypeptide composition of the photosynthetic membranes shown here raises several interesting questions. One immediate challenge is the identification of some of these bands with electron carriers known to be present in these membranes. Tentative assignments have already been reported (5,7) but they are still largely speculative, and more detailed knowledge of the individual polypeptides is necessary before such identification can be done with certainty.

FOOTNOTES

[1]Abbreviations: SDS, sodium dodecylsulfate; Kd, kilodalton; PS II, photosystem II.

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LEGEND FOR FIGURE

Fig. 1. Slab gel electrophoretic pattern of subchloroplast fractions FP, grana and stroma lamellae. Membrane protein was solubilized with sodium dodecylsulfate and 60 μg of each sample were electrophoresed on 9% sodium dodecylsulfate-polyacrylamide as described under "Material and Methods."

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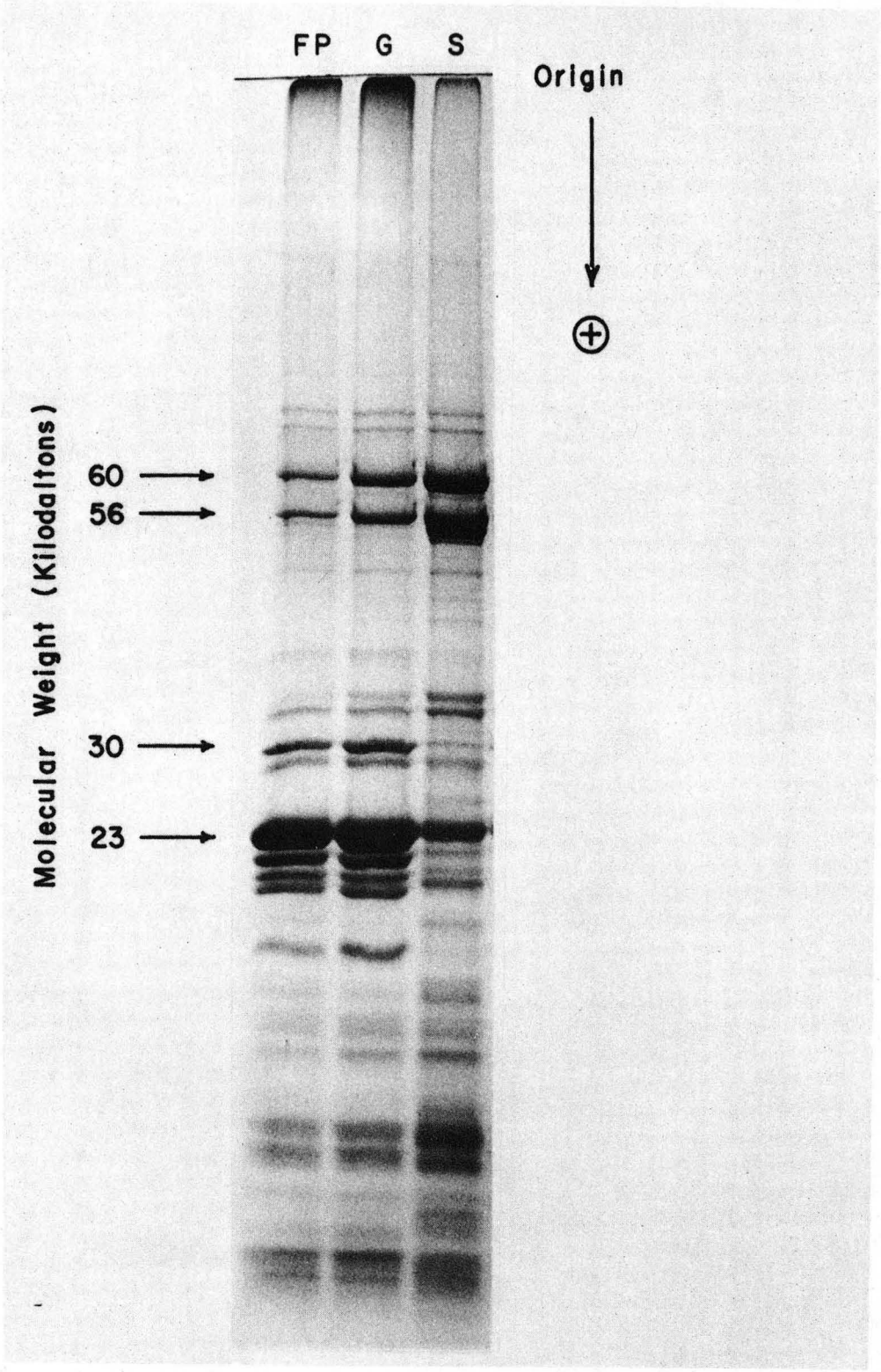

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Fig. 1.

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