Regulation of Chloroplast Differentiation

Proceedings of an International Meeting on the Regulation of Chloroplast Differentiation, held in Rhodes, Greece July 14–20, 1985

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ISOLATION OF A FRACTION ENRICHED IN ENVELOPE MEMBRANES FROM PURIFIED PEA (PISUM SATIVUM) ETIOPLASTS

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INTRODUCTION

The envelope membrane is a feature common to all members of the plastid family. It is conservatively retained as a two membrane barrier, consisting of inner and outer envelope, at all levels of plastid development, e.g. proplastids, etioplasts, chromoplasts, chloroplasts (Douce et al., 1984). Studies on chloroplast envelopes from pea and spinach have revealed its essential role in the proper function of the organell (Douce et al., 1984, Soll et al., 1980, Cline et al., 1982, Pfisterer et al., 1982). No data are available so far on the function of the plastid envelope in chloroplast development, though some initial reports have been published from etioplasts envelope of cereals (Sandelius and Selstam, 1984, Hönighaus and Feierabend, 1985). These results are difficult to compare with data obtained from dicotyledons. We have therefore developed a method to purify etioplasts from etiolated pea plants and to isolate envelope membranes of these plastids thus enabling us to compare directly data from different developmental stages.

MATERIAL AND METHODS

Pea plants (Pisum sativum, var. Miranda) were soaked for 12 hours in running tap water and grown in vermiculith in the dark for 11 days at 23°C. About 200 gr. of primary leaves were normally used for the etioplast preparation. Leaves were homogenized in a blendor equipped with razor blades (Kanangara et al., 1977) using the following buffer;

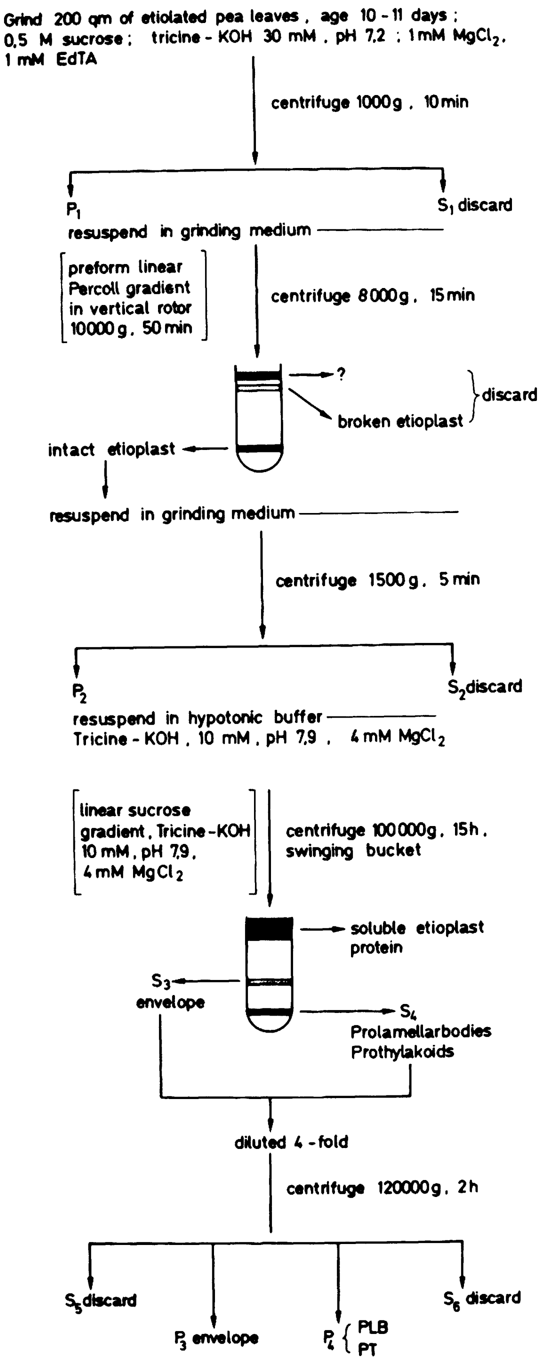

Figure 1. Purification scheme of pea etioplast subfractions

0.5 M sucrose, 1 mM $MgCl_2$, 1 mM EdTA, 30 mM N-(tris-(hydroxymethyl) methyl) glycine-KOH, pH 7.2. The slurry was filtered through a nylon-net (30 µm aperture) and a crude etioplast pellet was obtained after centrifugation at 1000 g for 10 min. The plastids were further purified on a linear siliciasol gradient. The gradient was made of 5 ml 80% percoll cushion and 20 ml 30% percoll solution. A linear gradient was formed by centrifugation at 10.000 g for 50 min. in a vertical rotor (see also Fig. 1). The purified, intact, etioplasts were recovered from the interface 80% percoll/linear gradient after centrifugation for 15 min. at 8000 g. The etioplast suspension was diluted in excess isolation medium and washed free of percoll. They were then lysed in hypotonic buffer (see Fig. 1) and the plastid components separated on a linear sucrose gradient

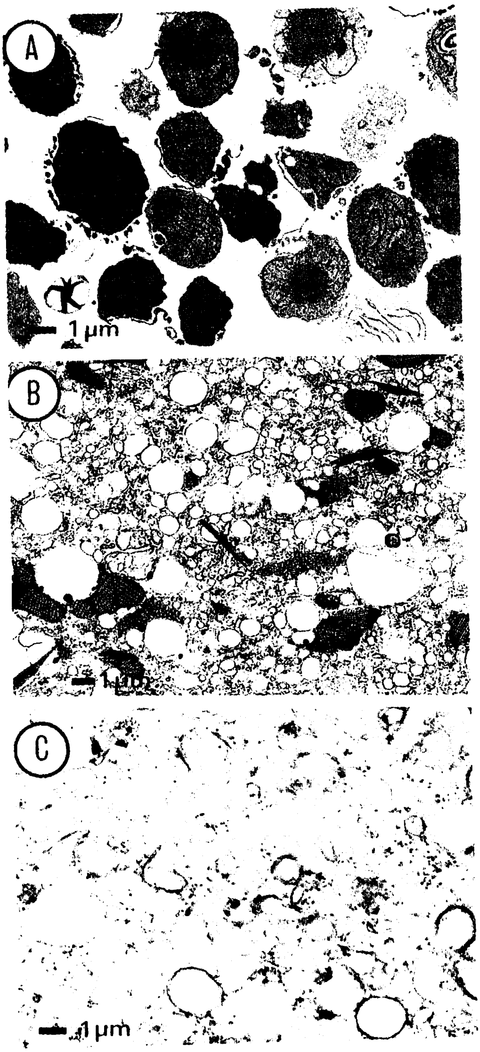

Figure 2. Electronmicroscopic studies; A) isolated pea etioplasts after purification on a linear percoll gradient; B) mixture of prolamellar bodies and prothylakoids, obtained from fraction 10 of the linear sucrose gradient (see Fig. 1, Fig. 5); C) envelope membranes, fraction 6 (Fig. 5)

(0.6-1.2 M sucrose, underlayed with a 55% sucrose cushion) buffered as in Fig. 1. Envelope membranes and a mixture of prolamellar bodies and prothylakoids were recovered from the gradient and pelleted by centrifugation (Fig. 5).

Contamination of the etioplast fraction by mitochrondria and peroxisomes were tested using cytochrome-c-oxidase and OH-pyruvate-reductase, respectively, as marker enzymes (Jackson and Moore, 1979) (Table 1).

Lipid analysis was done by thin layer chromatography on HPTLC-plates (silica-gel 60, Merck) using acetone benzene/water (91/30/8) as a solvent system. Lipids were visualized with the following stain (made from 2 gr $FeSO_4 \times 7H_2O$, 180 mg $KMnO_4$, 6 ml H_2SO_4, 200 ml H_2O. The plates were heated for 10 min. at 120°C and then scanned at 583 nm. Calibration experiments had shown, that the different lipid species stain with the same intensity ($\pm$ 5%).

Function	Volume (ml)	cytochrome-c-oxidase total activity (mol/min)	mol/mg·min.	HO-Pyruvat-reductase total activity (nmol/min)	µmol/mg·min.
crude pellet, P_1	10	2745	25.3	2.42	0.022
top layer Percoll gradient, 0_1	2	1220	36	0.66	0,0197
broken etioplasts 0_2	4	1028	33.2	0.51	0,0166
purified, intact etioplast	1	6.4	0.74	0.005	0,0006

TABLE 1. Contaminations of percoll purified, intact etioplasts were determined as in Jackson and Moore 1979.

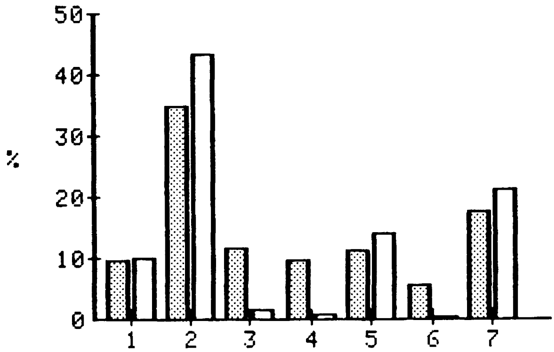

Figure 3. Polar lipid composition of envelope membranes from chloroplasts and etioplasts; 1,MGDG; 2,DGDG; 3,SL; 4,PG; 5,PE; 6,PI; 7,PC: ▩ , chloroplasts ☐ , etioplasts

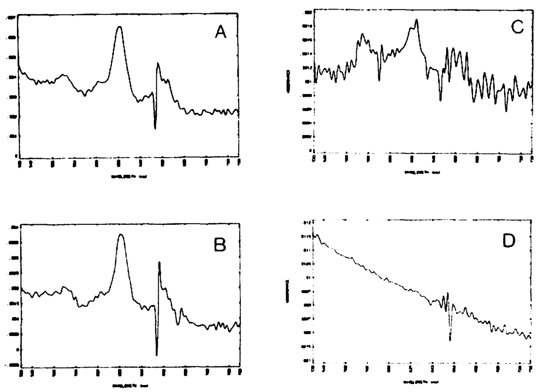

Figure 4. Distribution of protochlorophyllide (λmax 620 nm) in different fractions. Similar amounts of protein were extracted and analysed spectrophotometrically. A) intact etioplasts, B,C,D) fraction 10, 8,6 respectively of linear sucrose gradient.

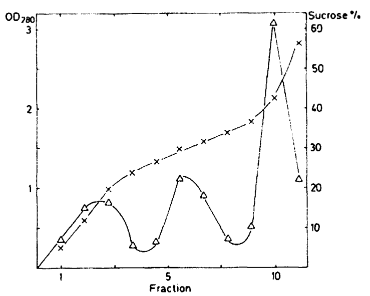

Figure 5. Separation of etioplasts components on a linear sucrose gradient. Envelope fractions 6,7; Prothylakoids and prolamellar bodies, fraction 10.

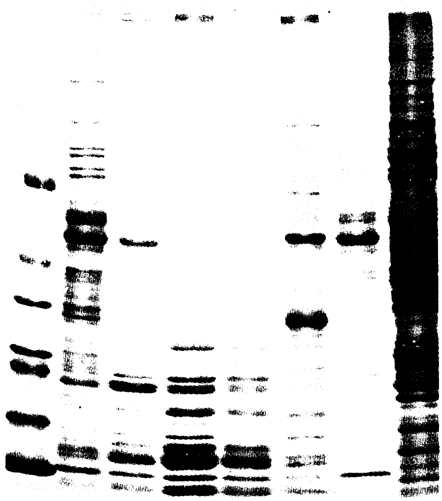

Figure 6. SDS-Polyacrylamide gelelectrophoresis of etioplasts subfractions obtained from the linear sucrose gradient from left to right lane;
1, Molecular weight standards 12,20,24,29,36,45,68 kDa;
2, etioplasts; 3, fract. 6;
4, fract. 7; 5, fract. 8;
6, fract. 10; 7, fract. 3;
8, fraction 2.

CONCLUSIONS

We have developed a large scale method for the purification of etioplasts from dark grown pea. As outlined in Fig. 1 and shown in Table 1 for the marker enzyme distribution a very pure etioplast fraction is obtained in respect of mitochondrial and peroxisomal contaminations. Electronmicroscopy excludes also the contamination by membranes of other cell compartments (Fig. 2A). To have highly purified plastids is a prerequisite for the isolation of envelope membranes (Douce et al., 1984). Our separation procedure, as summarized in Fig. 1 has yielded a discret membran fraction different from the mixture of prothylakoid and prolamellar bodies. Initial attempts to characterize this fraction are outlined in Fig. 3-6 and support our view that we have obtained envelope membranes from pea etioplasts.

Our data on the polar lipid composition of envelope membranes show major differences in the content of sulfur lipid (SL), phosphatidylglycerol (PG) and phosphatidylinositol (PI) which are much less in envelope membranes from etioplasts. The content of phosphoethanolamine (PE), a lipid which is normally not found in envelope (Douce et al., 1984) is probably due to contamination with rough endoplasmatic reticulum and currently under further investigation. No differences are observed in the galactolipid content (MGDG, DGDG) As. shown in Fig. 4, no protochlorophyllide is detectable in the envelope membrane fraction, indicating that this fraction is devoid of prolamellar bodies. These results are further supported by polyacrylamidegel electrophoresis with subsequent Western-blot analysis stained for coupling-factor using specific antibodies. In fraction 6 (envelope fraction) coupling factor was at the limit of detection while fraction 9 and 10 (prolamellarbodies, prothylakoids) showed high coupling factor content (compare also Fig. 6, data not shown).

REFERENCES

Douce R, Block MA, Dorne AJ, Joyard J (1984). The plastid envelope membranes. Subcell Biochemistry 10, 1-84.

Soll J, Kemmerling M, Schultz G, (1980). Tocopherol and plastoquinone synthesis in chloroplast subfractions. Arch Biochem Biophys 204, 544-550.

Cline K, Werner-Washburne M, Andrews J, Keegstra K (1981). Separation and characterization of inner and outer envelope membranes of pea chloroplast. Proc Natl Acad Sci USA 78, 3595-3599

Pfisterer J, Lachmann P, Kloppstech K (1982). Transport of proteins into chloroplasts. Eur J Biochem 126, 143-148.

Sandelius AS, Selstam E (1984). Localization of galactolipid biosynthesis in etioplasts isolated from dark grown wheat. Plant Physiol 76, 1041-1046.

Hönighaus R, Feierabend J (1985). Origin and developmental changes of plastid envelope proteins and translocator activities. Planta in press.

Kannangara CG, Gough SP, Hansen B, Rasmussen JN, Simpson DJ (1977). A homogenizer with replacable razor blades for bulk isolation of active barley plastids. Carlsberg Res Commun 42, 431-439.

Jackson C, Moore AL (1979). Isolation of higher plant mitochondria. In Plant Organelles, Reid E (ed) Ellis Horwood publisher, pp 1-13.

This work was supported in part by the Deutsche Forschungsgemeinschaft and Freunden und Förderern der Universität München.