Fractionation and characterization of cellular membranes from root tips of garden cress (*Lepidium sativum* L.)

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**Abstract.** The first step in the gravitropic reaction chain, i.e., perception, is known to occur in the statenchyma of the root cap. Because of the importance of the root tip in graviperception, a procedure has been developed to isolate root tips from garden cress (*Lepidium sativum* L.). The root tip fraction contains the tissues of the root cap plus the lower half of the meristem zone, but is clearly separated from the tissues of the elongation zone, the zone of gravitropic response. Membranes from the root tip and root base fractions have been centrifuged on sucrose density gradients and the marker enzyme profiles analyzed. These results show that the marker enzyme profiles for vacuoles, dictyosomes, mitochondria, and plasma membranes are similar in the root tip or root base fractions. The endoplasmic reticulum (ER) has a shoulder of cytochrome c reductase activity at a density of 1.16 g cm⁻³ which is distinct from the other enzyme activities and is only observed in root tip preparations. The specific enzyme activity for ER, cytochrome c reductase, was enriched in root tip membranes 1.7 fold. This latter increase is interpreted as at least in part an increased ER content in the root tip.

**Key words:** Endoplasmic reticulum – Gravitropism – *Lepidium* – Membrane fractionation – Root (membranes).

**Introduction**

In the root the cells responsible for sensing the gravity force (statocytes) are located in the root cap (Sievers and Volkmann 1972; Barlow 1974; Juniper 1976; Volkmann and Sievers 1979; Jackson and Barlow 1981). The structure of these cells has been extensively studied, especially in *Lepidium sativum* L. (Iversen 1969; Sievers and Volkmann 1972, 1977a, 1977b; Volkmann 1974; Sievers et al. 1976). The structural polarity of the statocytes is not gravity induced but genetically determined, indicating a structural-functional relationship (Sievers et al. 1976).

Although little is currently known concerning the mechanism of gravity perception and signal transmission, there are observations that might indicate important components in this process. For example, both gravitropic and agravitropic lateral roots contain sedimentable amyloplasts; however, only gravitropic lateral roots contain an underlying endoplasmic reticulum (ER) complex (Schmitz 1977). Or, when the starch in the amyloplasts is artificially depleted following hormone treatment, the roots no longer respond to gravity (Iversen 1969). Even short physical contact between the amyloplasts of the statenchyma and the distal ER has been suggested to be sufficient for graviperception (Hensel and Sievers 1981). In addition, the unique function of the statocyte ER may be related to the unique structure as indicated by the high capacity of the ER to bind Concanavalin A (Schneider and Sievers 1981).

More recent results involving electro-physiological measurements in *Lepidium* roots (Behrens et al. 1982) show a symmetrical pattern of external current flow with current leaving the elongation zone and entering the root cap and meristem zone. After 90° rotation to the horizontal an apparent reversal of current flow occurs. Current leaves the root cap on the upper surface of the root but enters the root cap on the lower surface. The authors interpret these results in terms of altered ion trans-
port across the plasma membrane, which may be involved in graviperception.

Thus, there are indications that membranes of the ER and the plasma membrane and the interaction of the amyloplasts with the ER may be important in the early reactions of the perception process. We have developed a procedure for isolation of root tips from *Lepidium* and report here an ultrastructural comparison of isolated and control root tips, the qualitative distribution of membranes on sucrose density gradients based on marker enzyme analysis and the quantitation of membrane content based on marker enzyme activity.

**Materials and methods**

*Plant material.* Seeds of *Lepidium sativum* L. cv Krause (ca. 400 ml dry volume) were soaked for 30 min in tap water and spread evenly on wire mesh racks one layer thick. The seeds were grown in the dark at 24±2°C for 34 h. The roots protruding through the wire mesh were harvested and the cut roots stored in ice-cold 50 mM sucrose solution until needed (maximum storage 2 h). Roots grown for 34 h had an average, in

**Isolation of root tips.** Roots were collected by filtration through nylon cloth and chopped in 50 mM sucrose with a mechanical chopper similar to that described by Cunningham et al. (1966). The root material was chopped for 3 min at approximately 240 cycles per min, washed in 50 mM sucrose and mixed with 200 ml 20% Percoll (Pharmacia Fine, Uppsala, Sweden) solution in 30 mM sucrose and stood on ice for 5 min. The tissue separated at unit gravity into an interface (root base fraction) and a pellet fraction. The underlying pellet fraction was recovered and subsequently mixed with 40% Percoll and stood on ice as before. The resulting pellet was recovered and designated the root tip fraction.

**Homogenization and centrifugation.** Root material was homogenized with a mechanical razor blade chopper for 4 min at approximately 400 cycles per min in 50 mM Tris(hydroxymethyl)aminomethane-HCl (Tris), pH 8.0, at 20°C, 50 mM KCl, 14 mM mercaptoethanol, 1 mM (ethylene-his(oxyethylenenitrile))tetraacetic acid (0.1 mM in the presence of MgCl₂), ± 3 mM MgCl₂, and 0.4 mM sucrose. The ratio of tissue fresh weight to buffer volume was 1:2. The homogenate was filtered through a single layer of nylon cloth, centrifuged at 4,000 rpm for 15 min (HB 4 Sorvall rotor) and chromatographed through a Sepharose CL-2B gel filtration column (Pharmacia Fine, Uppsala, Sweden). The column (2-10 cm) was equilibrated and eluted with homogenization buffer at a flow rate of 85 ml h⁻¹. Fractions of 1 ml were collected and void-volume fractions pooled and used for subsequent membrane fractionation. Void-volume fractions were cleanly separated from soluble, retained fractions as judged by carotenoid measurements (data not shown).

The pooled void-volume fractions were layered onto a continuous, 1.5—0.4 M (1.6—0.4 M in high-Mg²⁺ gradients) sucrose gradient and centrifuged for 4 h at 25,000 rpm (Spinco SW 27 rotor). All sucrose solutions were prepared in homogenization buffer minus mercaptoethanol. Gradients were fractionated from the bottom into 0.5 ml fractions and either analyzed immediately or frozen at −20°C until needed with the maximum storage time being 48 h.

Enzyme assays. ATPase and IDPase activity was determined by the measurement of released inorganic phosphate, using the modified method of Scherer and Morré (1978). Fractions to be tested for ATPase activity were chromatographed through Sephadex G50 columns (bed volume 2 ml) equilibrated with 0.1 M 2-(N-morpholino)ethanesulfonic acid-Tris buffer, pH 5.5, at 20°C, plus 50 mM sucrose. Void-volume fractions were collected and 50 µl samples were taken for the ATPase assay. The assay was run in the above buffer plus 5 mM MgCl₂ plus or minus 50 mM KCl in a total volume of 100 µl. The assay was started by the addition of 10 µl 30 mM ATP and continued for 30 min at 30°C. The reaction was terminated with 400 µl 10% trichloroacetic acid (TCA) and inorganic phosphate was measured. Non-specific ATP-phosphatase activity was taken as the enzyme activity in the absence of MgCl₂. IDPase activity was assayed plus or minus 0.1% Triton X-100, according to Bowles and Kauss (1976), in a total volume of 100 µl containing 50 µl membrane fraction, according to Pennington (1961). The assay for α-mannosidase was performed by the method of Boller and Kende (1979) as modified by Scherer (1981).

Sterol-UDP-glucose transferase was assayed by a modification of the method of Hartmann-Bouillon et al. (1979). Membrane fractions (60 µl) were incubated for 3 min at 30°C with 20 µl 0.3 M Tris-HCl, pH 8.0, containing 0.8% Triton X-100 (w/v) and 1 mM mercaptoethanol and the reaction started with the addition of 20 µl 1.7 mM UDP-glucose containing 0.35 kBq UDP-[14C]glucose (Amersham Buchler, Braunschweig, FRG). The assay was continued for 30 min and stopped by boiling for 1 min. The isolation of steryl glucoside (SG) and 6-acyl-sterol glucoside (ASG) was conducted as described by Quantin et al. (1980). Glucan synthetases were assayed by the procedure of Jessaitis et al. (1977). Glucan synthetase I was assayed at 30°C for 30 min and glucan synthetase II at 30°C for 45 min. The glucan synthetase II activity was assayed plus or minus 1 h.

Fraction purity was determined by accurately weighing 100 µl samples, or refractometrically. Both methods gave identical results. Protein was determined following TCA precipitation by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

**Microscopy.** For electron microscopy, roots or root tips were fixed in KMnO₄ (2%) at room temperature and subsequent dehydration and embedding were performed as described by Sivers and Volkmann (1972). Root tip and root base fractions to be analyzed by light microscopy were stained with iodophenol to visualize the statenchyma in the calyptra. Fraction purity was determined by counting the number of intact root tips under low-power magnification. The data is expressed as percent root tip tissue and represents the results of three determinations from each of four separate preparations. Root length was determined by measuring photographically enlarged root tips to the nearest 0.1 mm.

**Results**

*The isolation and characterization of root tip fractions.* The root fractionation procedure as de-