Ion permeability of maize root membrane vesicles: Studies with light scattering

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Abstract

A possible modulation of permeabilities of membrane vesicles to anions and cations was explored by light scattering techniques, evaluated by measuring the capacity of the vesicles to shrink and swell in response to changes of the osmolarity of the incubation medium. Membrane fractions were obtained by phase partition. Purity was evaluated by detection and quantification of membrane enzyme markers: vanadate-sensitive ATPase for the plasma membrane, nitrate-sensitive ATPase for the tonoplast and azide-sensitive ATPase for mitochondria. Membrane vesicles (250 μg protein) were exposed to hypertonic solutions of salts (0.6 osmolar). Kinetics of the changes in apparent absorbance at 546 nm were observed by the addition of potassium, nitrate and chloride salts. The diffusion of ions into vesicles was induced by an osmotic gradient across the membrane and brought about volume changes of vesicles. Upon addition of vesicles to the different solutions the following ion permselectivity sequences were observed: \( \text{PNO}_3^- > \text{PCl}^- > \text{PSO}_4^{2-} \) and \( \text{PK}^+ > \text{PNa}^+ > \text{PNH}_4^+ \).


Introduction

Preparations of isolated vesicles represent an ideal system for the study of transport processes across native membranes. It becomes possible to characterize single transport systems without the complications of transport in the intact cell. Solute transport can be studied in the absence of metabolic transformation or compartmentation (Briskin, 1990). A number of investigators have examined transport processes at the plasma membrane (Giannini et al., 1987a, b, 1988 b; De Michielis and Spanswick, 1986); tonoplast (Blumwald and Poole, 1985; Briskin et al., 1985; Poole et al., 1984; Schumaker and Sze, 1985), and endoplasmic reticulum (Buckhout 1983, 1984; Bush and Sze, 1986; Giannini et al., 1988a) vesicles. Major progress in plasma membrane-associated transport processes by using isolated vesicles has only been achieved within the last few years.

The purpose of the present study was to investigate the relative permeabilities of the plasma membrane vesicles to various ions by spectrophotometry of salt-induced osmotic swelling. For this reason, plasma membrane vesicles were purified by partitioning in aqueous two-phase systems. It has been shown that the plasma membrane has a high partition in phase systems where chloroplast, mitochondria and endoplasmic reticulum have a low partition (Albertsson et al., 1981). In aqueous two-phase systems the mixed population of membrane vesicles will partition between the two phases, partly according to their surface charge and partly according to hydrophilic/hydrophobic properties of their outer surface (Albertsson et al., 1981; Larsson and Andersson, 1979).

Materials and methods

Plant material. Maize seeds (LG 11) were germinated in rolls of filter paper soaked in 0.1 mM CaSO₄ solution in pots at 25°C in the dark. Root material was taken for experiments after three days; whole primary
roots were cut from the seedlings and collected in cold distilled water. All the following operations were performed at 4°C.

**Preparation of plasma membrane vesicle.** The primary roots were homogenized with a chilled mortar and pestle in 0.25 M sucrose, 3 mM EDTA, 2.5 mM DTT, and 25 mM Tris-Mes (pH 7.7). The homogenate was filtered through four layers of cheesecloth and centrifuged at 10,000 g for 15 min in the large swinging rotor TH-641 of a Sorvall centrifuge (OTD B). The supernatant was centrifuged at 55,000 g for 30 min and the pellet was suspended in suspension buffer (0.3 M sucrose, 1 mM DTT, 1mM Tris-Mes, pH 7.2), frozen in liquid N2 and stored at -70°C. Before use the suspension was diluted in 5 mM potassium phosphate buffer (pH 7.8) containing 0.25 M sucrose to a final weight of 6g and subjected to phase partition in an aqueous polymer two-phase system. The 36g phase mixture contained a final concentration of 6.3% (w/w) Dextran T500, 6.3% (w/w) PEG 4000, 0.25 M sucrose, 5 mM K-phosphate (pH 7.8) and the pellet. It was equally divided to six tubes, mixed by 20-30 inversions of the tube and centrifuged at 1,000 g for 5 min. 90% of the upper phase was removed with a Pasteur pipette, diluted with an equal volume of the suspension buffer and centrifuged at 100,000 g for 1 h to produce the plasma membrane vesicles. Similarly, 90% of the lower phase was removed, diluted with the suspension buffer and centrifuged at 100,000 g for 1h.

**Shrinking and swelling.** At zero time the fraction of upper phase (250 µg protein) was diluted with 1.0 mL of the specified solutions and the light scattering recorded every 10 sec. at 21°C as the absorbance at 546 nm (A_{546}) with a Beckman DU-70 spectrophotometer. A decrease in A_{546} reflects swelling of the membrane vesicles.

**Enzyme assay.** In order to identify the plasma membrane and assess contamination by other membrane components during the membrane isolation procedure, the following markers were evaluated: Vanadate-sensitive plasmalemma ATPase was assayed in 30 mM Tris-Mes, pH 6.5, containing 3 mM ATP, 3 mM MgSO4, 25 mM K2 SO4 and in the presence and absence of 5 mM Na3VO4. 0.02% (w/v) Triton X-100 was added to disrupt right-side-out membrane vesicles such that the vesicles became permeable to ATP without additional stimulatory or inhibitory effect on the ATPase activity. Nitrate-sensitive tonoplast ATPase was assayed in the incubation medium (3 mM ATP, 3mM MgSO4, 30 mM Tris-Mes, pH 8.0, and 0.02% (w/v) Triton X-100) in the presence of 50 mM KCl or KNO3. Azide-sensitive mitochondrial ATPase was measured in 30 mM Tris-Mes, pH 8.5, containing 3 mM ATP, 3 mM MgSO4, 50 mM KCl, and 0.02% (w/v) Triton X-100, in the presence or absence of 1 mM NaN3. ATPase activity was determined at 38°C for 30 min. in a reaction volume of 1.0 mL by a modified Peterson (1978) procedure. Proteins were determined by the method of Bradford (1976).

**Results and discussion**

**Characterization of the membrane vesicles**

Plasmalemma vesicles were purified by aqueous polymer two-phase partitioning. Table 1 shows the specific activities of the marker enzymes in the microsomal fraction and in the upper phase (U) and the lower phase (L) after phase partition. Low activities of the mitochondrial and tonoplast markers in the U phase were found, resulting in a higher purity of the plasma membrane fraction. These results are in agreement with Briskin et al. (1987) who have shown that plasma membrane markers are distributed almost exclusively in the upper phase while the markers for intracellular membranes are found mainly in the lower phase. Hallberg and Larsson (1981) have shown that intact protoplasts have a high affinity for the polyethylene glycol-rich upper phase. Our plasma membrane vesicles which were partitioned in the upper phase are expected to resemble in properties these protoplasts and represent therefore the right-side-out population, if true, the activity found in the lower phase might represent the inside-out vesicles which had to be excluded from upper phase because of their different surface properties. The presence of the inside-out vesicles population in the lower phase might be the result of thawing the vesicles after their storage at -70°C (Palmgren et al., 1990).

**Permeability properties of the membrane vesicles**

In this study we have examined the movement of cations and anions across the plasma membrane vesicles of maize roots by the use of light scattering techniques. The first requirement of a membrane fraction to be used for the study of transport is a high proportion of osmotically intact vesicles. This can be evaluated