ISOLATION OF TONOPLAST VESICLES FROM KIWI FRUIT CELLS:
ELECTROCHEMICAL INVESTIGATION OF K\(^+\) AND H\(^+\) MEMBRANE TRANSPORT
LINKED TO A MAGNESIUM-ATP HYDROLYTIC ACTIVITY

Jean-Pierre Rona, F. Chedhomme, M. Convert
and Michèle Monestiez

Laboratoire d'Electrophysiologie des Membranes
Unité Associée C.N.R.S. No. 1180
Université de Paris VII, 2, Place Jussieu
75251-Paris-Cedex 05, France

INTRODUCTION

Mechanical breaking of giant cells of the peripheral layer of Actinidia
chinensis fruits produces in the extraction juice, many large vesicles that appear
like free vacuoles or protoplasts (Chedhomme and Rona, 1984). The latter are of
a much smaller size than the initial cells; these pseudoprotoplasts are always devoid
of nuclei as in the case with "vacuoplasts" of Poterioochromonas malhamensis
(Jochem et al., 1983).

Several findings (Chedhomme and Rona, 1984 and 1986) suggest that these
membrane structures are due to fragmentation followed by revesculation of the
tonoplast surrounding the cytoplasmic strands of giant cells of this zone.

The study of these structures with Nomarski's interference phase contrast
microscopy, shows that revesculation occurs either directly in the endovacuolar
medium through fragmentation of the central vacuole (type A vesicles), or on the
layer of cytoplasmic strands which surrounds the largest vacuoles (type B and C
vesicles). During the resealing of the tonoplast around the cytoplasm of the strands,
small secondary vacuoles may remain trapped, giving the vesicle its protoplast
aspect (type B vesicle, Chedhomme and Rona, 1986).

The diversity of formation patterns of vesicles implies a change in the
tonoplast interface orientation with respect to their usual contact medium:
depending on whether the membrane reforms around the vacuolar juice or around
the cytoplasmic strands, the tonoplast surface initially in contact with the
endovacuolar medium will either remain in contact with this medium, as for intact
isolated vacuoles (Rona et al., 1980), or, by contrast, be in contact with the outer
environment, when the cytoplasm is trapped within the vesicles.

The purpose of the present investigation is to confirm our previous results
pointing to the tonoplast as the membrane limiting the different types of vesicles
present in Kiwi juice. This study uses electrophysiological data pertaining to the
structures involved and the specific ability (anion sensitive vacuolar ATPase) of
the vacuolar membrane to hydrolyse MgATP (Spanswick et al., 1984).
MATERIAL AND METHODS

Vesicles were collected from the cellular juice that appeared after excision of the peripheral layer of the fruit of *Actinidia chinensis* var. Hayward. Fruit conservation and juice collection methods have been described previously (Chedhomme and Rona, 1986).

Preparation of the Biological Material

After collection of Kiwi juice, the suspension was divided into two batches. In the first batch, the vesicles were studied in the juice directly, about 15 minutes after extraction from the fruit (measurement of PD and K⁺, H⁺ internal ionic activity), the vesicles of the second batch were purified by centrifugation in discontinuous gradient (Ficoll 5/10/20%) and resuspended in an artificial medium with pre-controlled ionic status and osmotic potential (Fig. 1); the purified vesicle suspension was used for electrophysiological measurements in the artificial medium at pH 6.5 or at pH 8 (Hepes-Tris, 25 mM); 0.7 M mannitol was added to maintain the osmotic potential around - 2.2 MPa, close to the cellular juice potential (Chedhomme and Rona, 1986).

Approximately 60% of type A vesicles settled at the 5% - 10% Ficoll interface, and 50% of type B and C vesicles settled at the 10% - 20% Ficoll interface.

![Procedure for purification of tonoplastic vesicles in buffered medium](image)

**Figure 1**

Procedure for purification of tonoplastic vesicles in buffered medium

(Hepes-Tris 25 mM, mannitol 0.7 M, pH 6.5)

ATP-Hydrolysis

For measurements of enzymatic activity, fruits were peeled and the outer pericarp cut into small pieces, was homogenized in a laminar grinder with saccharose (0.25 M), Hepes-Tris 25 mM (pH 7.4) and 0.5% β-mercaptoethanol. The homogenate was strained through cheesecloth and centrifuged at 8000 g/10 min, 13000 g/15 min and 30,000 g/90 min, consequently.

1 ml of membrane preparation was layered onto a 10 ml discontinuous saccharose gradient and the fractions were collected at the interfaces d = 1.10 - 1.15 and 1.15 - 1.20 after centrifugation at 110,000 g for 60 min.

The release of Pᵢ (Peterson, 1978) in the membrane fraction as a result of ATP hydrolysis was measured in 100 µM sodium orthovanadate, 100 µM ammonium molybdate, and 0.2 mM azide with a Perkin Elmer, Lambda 5 spectrophotometer.