Short Communication

Increased Electron Density of Tonoplast Membranes in Washed Corn Root Tissue

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ABSTRACT

Washing corn (Zea mays L.) root segments for 4 hours results in increased tonoplast electron density in fixed and stained tissue. It suggested that previously reported increases in solute absorption rate with root tissue washing may lie with changes in the properties of the tonoplast.

In a study of the increased solute absorption which accompanies the washing of corn root tissue, Leonard and Hanson (4) reported that electron micrographs showed no detectable changes in subcellular structure due to washing, and thus the micrographs were not published.

More recent investigations (6) have shown that washing is accompanied by a small increase in the proportion of unsaturated fatty acids in the microsome fraction. Furthermore, washing root tissue in 1 μM linoleic acid for 4 hr will enhance the increase in phosphate absorption rates about 50%. Osmium tetraoxide reacts with unsaturated bonds (7), suggesting that the electron micrographs might show increased density of some cell membranes. Laties' (3) hypothesis that the dual mechanisms of salt uptake involve serial transport in plasmalemma and tonoplast was recalled. The electron micrographs were reexamined comparing plasmalemma and tonoplast in fresh and washed tissue. On this basis, unmistakable differences in the electron density of the tonoplast as a result of washing were found. Previously, we had been looking for something more dramatic in membrane proliferation, plasmalemma changes, or subcellular organization and had missed making comparisons of the tonoplast. This paper is to correct the deficiency in our earlier report.

METHODS AND MATERIALS

Segments of primary roots, 0.5 to 2.5 cm from the tip, were cut from corn seedlings (Zea mays L. WF9-Tms × M14), grown on paper toweling, and used fresh or after washing in 0.1 mM CaCl₂ for 4 hrs, as previously described (4). Phosphate absorption rates were determined on a portion of the segments: 0.17 and 0.62 μmole Pi/ hr·g fresh weight for fresh and washed sections, respectively.

For electron microscopy, fresh and washed segments were sliced longitudinally and fixed with 2.5% glutaraldehyde in 50 mM potassium phosphate buffer, pH 7.4. After 15 min at room temperature, fixation was continued for 75 min at ice temperature. After five washings in 50 mM phosphate buffer (pH 7.4), the segments were postfixed for 15 hrs in the cold in 1% OsO₄ in the same buffer. Following thorough rinsing in buffer, the sections were dehydrated in ethanol (50, 75, 80, 95, 100%) and embedded in Epon 812 resin essentially according to Luft (5). Microrme sections were stained in 2% uranyl acetate and Reynold's lead citrate. A Phillips EM-200 microscope was used. Comparisons were made on cortical tissues from three washed and three fresh segments.

RESULTS AND DISCUSSION

Figure 1 shows representative micrographs from fresh root tissue and tissue washed for 4 hrs at 30 C. Although there is some variation in the absolute density of the tonoplast, possibly due to variations in fixation and staining, comparisons using adjacent plasmalemma as a standard uniformly show the tonoplast to increase in electron density with washing.

Enlargements were made from areas where the cell membranes were distinct, and the negatives were scanned for plasmalemma and tonoplast density on a Joyce-Loebel MK IIIc double beam microdensitometer. Figure 2 gives representative scans from fresh and washed tissue. Table I gives the relative tonoplast densities from fresh and washed tissue compared to adjacent plasmalemma. No changes in plasmalemma density with washing were found, but tonoplast density increased to equal that of the plasmalemma.

This result of increased tonoplast electron density with washing has been confirmed in two repetitions. At this time, we have no explanation for the increase. Preliminary work indicates that differential staining is responsible (H. H. Mollenhauer, unpublished). A comprehensive study of this phenomenon is being undertaken.

Torii and Laties (8) found vacuolation of corn root cells to be accompanied by introduction of the low affinity system II ion accumulation mechanism, and they ascribe system II to the tonoplast. Hence, changes in the tonoplast during cell elongation might be expected. However, the corn root tissue used here was taken 0.5 to 2.5 cm back of the tip where vacuolation...
FIG. 1. Electron micrographs from fresh (A and B) and washed (C and D) corn root segments. T: tonoplast; P: plasmalemma. Magnifications: 29,000 (A), 63,000 (B), 38,000 (C), 48,000 (D).
is largely complete. A possible explanation is that aspects of tonoplast development may proceed independently of vacuolar enlargement, and perhaps some form of belated tonoplast maturation is being observed here. Earlier work in our laboratory (2) showed progressively changing kinetic constants for K⁺ uptake extending 35 mm back from corn root tips, suggesting changes in the carrier complex beyond the region of cell expansion. Eshel and Waisel (1) have recently studied Na⁺ uptake along the primary roots of corn seedlings, and they too found evidence for progressive changes in parameters of uptake well beyond the zone of cell elongation. Hence, the increased ion uptake rates with washing may largely reflect augmented transport rates in the tonoplast, with the structural and metabolic parameters of transport being modified independently of increases in tonoplast area.

**LITERATURE CITED**


**Table I. Relative Densities of Tonoplast and Plasmalemma**

Densitometer tracings (Fig. 2) were scaled in centimeters, and relative densities of adjacent tonoplast and plasmalemma were recorded. Means and standard error given for 24 comparisons each.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tonoplast</th>
<th>Plasmalemma</th>
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<tbody>
<tr>
<td>Fresh</td>
<td>7.3 ± 1.4</td>
<td>15.6 ± 2.4</td>
</tr>
<tr>
<td>Washed</td>
<td>15.8 ± 3.8</td>
<td>15.4 ± 2.4</td>
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**Fig. 2. Densitometer tracings across the tonoplast and plasmalemma from fresh and washed corn root segments. T: tonoplast; P: plasmalemma.**