Vacuolar Protein in Apical and Flower-Petal Cells*

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Summary. Vegetative apices, floral apices and flower petals of five Solanaceae (potato, tomato, tobacco, petunia and nightshade) and of corn and Nigella were examined with an electron microscope for the presence of protein bodies in the cell vacuoles. Electron-dense bodies were found in vacuoles of all plants investigated but not in every tissue examined. The bodies observed in the apices are similar to the protein bodies previously found in tomato leaves where they appear to be related to the presence of chymotrypsin inhibitor I protein (Shumway et al., 1970). The bodies appeared in very young cells in small vacuoles, disappearing as the cell matured. They are apparently related to the growth and development of the new cells. The results suggest that plants may regulate specific proteins within the apical region through selective synthesis and degradation of proteins accompanied by compartmentalization in the vacuole.

Introduction

Biochemical investigations involving cell fractionation have provided insights into the compartmentation of metabolic functions within cells. When coupled with ultrastructural information, such studies provide an increased understanding of the role of intracellular compartmentation, particularly during developmental processes. The present work provides ultrastructural evidence that one compartment, the vacuole, plays a role in protein storage and utilization in the cells of the shoot apices of some plants. This ultrastructural evidence is compatible with a number of previous biochemical observations.

Ryan and Balls (1962) found that a powerful inhibitor of chymotrypsin, called chymotrypsin inhibitor I, was present in potato tubers. This protein is unusually resistant to heat, acid and alkali. The inhibitor could be detected at various growth stages in nearly all tissues of the

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potato plant and its presence coincided with establishment and maintenance of meristematic tissues (Ryan, 1967).

Shumway et al. (1970) have shown that tomato leaf tissue containing chymotrypsin inhibitor I protein also contains protein in the form of electron-dense material in the cell vacuoles. Mature tomato leaf cells are usually devoid of inhibitor I protein and the vacuoles lack electron-dense material. But under conditions resulting in production of inhibitor I there was a corresponding increase in vacuolar protein. The authors suggested that the vacuole is a temporary storage site for protein which may play an important role in plant growth and development. Under this concept, the plant-cell vacuole is something more than a terminal site for deposition of waste products. The work by Matile (1966) and Matile and Moore (1968) on corn root tips, showing the presence of several hydrolytic enzymes in the vacuoles, forms an independent support of this viewpoint.

In terms of cell division, the apical meristem is a highly active area in the plant. It seems to receive priority on some metabolites. This region, or that just behind it, is a possible site for protein synthesis and utilization during development and maturation of the new cells. This paper shows that protein bodies exist as transient components of apical cells of many plants, and further strengthens the hypothesis that the protein of the apical meristem vacuoles is utilized during growth and development.

Materials and Methods

Potato (Solanum tuberosum L.), tomato (Lycopersicum esculentum Mill.), tobacco (Nicotiana tabacum L.), corn (Zea mays L.), and Nigella damascena L. were grown from seed. Nightshade (Solanum nigrum L.) and petunia (Petunia hybrida Hort.) were obtained as young plants. All plants were grown during the summer in a greenhouse with no supplemental lighting. The soil in all cases was treated with fertilizer (16% nitrogen, 8% phosphate, 4% potassium) at a rate of about 1 g in 100 g soil.

After the plants were about 30 cm high, the apex, floral buds, and flowers were dissected. For electron microscopy, tissues were fixed in 6% glutaraldehyde in 0.2 M phosphate buffer, pH 7. The solution containing the specimens was evacuated for 5-10 min, then left on ice for 90 min. The specimens were washed in buffer for 5 min and left in 1% OsO₄ buffered at pH 7 for 90 min. Following dehydration in ethanol and propylene oxide, the tissues were embedded in Araldite 6005. Polymerization was at 70°C for 30-36 h. Sections 60-90 nm thick were obtained using a diamond knife on a Porter-Blum ultramicrotome MT-2 or MT-2B. The sections were stained with 2% aqueous Ba(MnO₄)₂ for 5 min and viewed in a Zeiss EM 9A or 9S electron microscope.

For light microscopy, the tissues were fixed with 6% glutaraldehyde in 0.2 M phosphate buffer, pH 7, dehydrated to 50% ethanol, passed through a tertiary butanol series, and embedded in paraffin. The embedded specimens were sectioned at 10 μ. A specific protein stain, mercuric bromophenol blue (Mazia et al., 1953), was used to identify protein in the vacuolar bodies.
Results

Electron micrographs of vegetative apices, floral apices, and flowers of five species of the Solanaceae, of Nigella and of corn show that vacuoles in many of the cells contain electron-dense material. The vacuolar material was found as many small bodies, or as a few large bodies, or as unaggregated, dense strands. Light microscopy of apical meristems of tomato showed that the vacuolar material gave a positive reaction with mercuric bromophenol blue. Thus it appears that much of the electron-dense material in the vacuoles is protein. Because of the low resolution of light microscopy, and the small size of these bodies, we could not unambiguously detect them in apices of plants other than tomato.

Electron micrographs show vacuolar bodies in the cells of the vegetative apex, floral apex, and flower of tomato. Fig. 1 shows typical bodies in the vegetative apex cells of tomato. As the cells mature and differentiate the vacuolar protein seems to increase in amount and then decrease and disappear. Fig. 2 shows portions of one longitudinal section of a vegetative apex of tomato. Small protein bodies exist in the small vacuoles of the young cells near the tip (Fig. 2a). In the older cells further

Fig. 1. Vacuolar bodies in cells near the tip of a vegetative apex of tomato. ×3114. The bar in Figs. 1–7 represents 10 μ.
Fig. 2a—e. Adjacent micrographs from a longitudinal section of a tomato apex. The top of Fig. 2a is about 6 cells below the tip. ×3000
Fig. 2c and d
Fig. 3. Same apex as Fig. 2 but 2 mm from tip. $\times 3550$
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Table 1. The presence of protein bodies in cell vacuoles as determined by electron microscopy

<table>
<thead>
<tr>
<th>Plant</th>
<th>Leaf</th>
<th>Vegetative apex</th>
<th>Floral apex</th>
<th>Flower petal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato</td>
<td>+</td>
<td>+++</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Tomato</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Tobacco (white)</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tobacco (green)</td>
<td>NO</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Petunia</td>
<td>NO</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Nightshade</td>
<td>NO</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Nigella</td>
<td>NE</td>
<td>+</td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>NO</td>
<td>++</td>
<td>NO</td>
<td>NE</td>
</tr>
</tbody>
</table>

+= A few bodies present; ++ = several bodies present; +++ = many conspicuous bodies present; NO = bodies not present; NE = not examined.

down from the tip, vacuole size increases, but the number of vacuoles decreases, and the protein bodies become larger (Fig. 2b, c). In cells still further down from the tip, the proplastids have begun to differentiate into chloroplasts (Fig. 2d). The vacuoles are larger and vacuolar protein is less visible. Eventually (about 1.1 mm down in this tip) vacuolar protein bodies cannot be detected (Fig. 2e). At about 2 mm from the tip the chloroplasts are well developed and no protein bodies are found (Fig. 3).

Fig. 4 shows protein bodies in the vacuoles of cells of a tomato floral apex. It is our impression that there is less vacuolar protein in floral apices than in vegetative apices, but this has not been quantitated. The fate of the bodies in floral apices was not studied in the same detail as in the vegetative apex.

The protein bodies are not confined to the apex but are also found in the developed flower petals of tomato. Fig. 5 shows protein bodies in the cell vacuoles of tomato flower petal. In these cells the vacuolar protein bodies occupy a smaller percentage of the total vacuolar space than in the apical cells.

Protein bodies similar to those in the same tissues of tomato are also present in vacuoles of apical tissues and flowers of other members of the Solanaceae. Fig. 6, for example, shows many protein bodies in the vacuoles of a vegetative apex of tobacco. Fig. 7 shows that such bodies are also present in the vacuoles in tobacco flower cells. In vegetative apices of petunia and nightshade, protein bodies are present in the vacuoles, but they are small, less compact, and few in number in comparison to the bodies in vegetative and floral apices of tomato or tobacco.

Protein bodies are also present in vacuoles of vegetative and floral apex cells of Nigella, but in the flowers of Nigella, no bodies could be
Fig. 4. Vacuolar bodies in cells of a floral apex of tomato. ×3240

Fig. 5. Vacuolar bodies in cells of a mature flower petal of tomato. ×3276
Fig. 6. Vacuolar bodies in cells of a vegetative apex of tobacco. ×3600
Fig. 7. Vacuolar bodies in cells of a mature flower petal of tobacco. ×3570
found. Vegetative apices of corn do have bodies in cell vacuoles (Fig. 8), but none were found in floral spises of this plant.

The presence of protein bodies in cell vacuoles of different plants as determined by electron microscopy is summarized in Table 1. There are some bodies (+) present in the vegetative and floral apices of petunia, nightshade, and *Nigella*. More and larger bodies (++) are present in vegetative apex of potato, tabacco, and corn, and floral apices of tomato, tabacco, and flower of tomato, tabacco, petunia, and nightshade. Many conspicuous bodies (+++) are present in the vegetative apex of tomato.

**Discussion**

The presence of protein bodies in plant cell vacuoles, especially of seeds, has been reported before (Horner and Arnott, 1965), but not associated with aspects of growth and development of the apices. Matile
(1966) and Matile and Moore (1968) have shown that vacuoles of corn root tips contain several hydrolytic enzymes and they refer to these vacuoles as plant lysosomes.

Burr and West (1971) have reported, on the basis of ultrastructural work, that wound-healing and the development of branch septae in *Bryopsis* is marked by the involvement of vacuolar protein. This protein originates in matrices of the endoplasmic reticulum and migrates to the large central vacuole where the membrane around the protein body is lost. After changing structurally the protein leaves the vacuole to participate in cell wall formation.

Greyson and Mitchell (1969) have reported the presence of vacuolar protein bodies in flowering meristems but not in vegetative apices of *Nigella* and suggested that these bodies are important in metabolism at certain stages of plant development. Their published electron micrographs show vacuolar bodies that are very similar in appearance to some of the vacuolar protein bodies we have seen in vegetative and floral apices of tomato and tobacco. Our work on *Nigella* showed very little if any difference between vegetative and floral apices of *Nigella*, but our growth conditions were different from those used by Greyson and Mitchell.

Corn was investigated because it is not closely related to the *Solanaceae* and might be expected to give another indication of how widespread the presence of protein in vacuoles is in the plant kingdom. Vacuolar bodies were present in vegetative apices but, in contrast to the *Solanaceae* and *Nigella*, not in floral apices. This could be due to differences between corn and the Solanaceous plants in protein metabolism as well as nitrogen requirements during growth.

Ryan (1968) found a transitory existence of protein inhibitor I in all tissues of potato, except xylem and seeds where it was consistently not detectable immunologically. Shumway *et al.* (1970), using an immunological assay and electron microscopy, found that presence of inhibitor I is coincident with the presence of protein bodies in vacuoles of detached leaves of tomato. They suggested that the vacuole may be a storage site for transitory protein which may be important in cell metabolism. Evidence from both biochemical and ultrastructural studies suggested that inhibitor I protein and vacuolar protein bodies accumulate in leaflets in response to excision, light, and a high nitrogen concentration in the cell. These observations lead us to suggest that vacuolar protein may not be obligatory in nitrogen metabolism but may serve a useful purpose in regions of excess as a regulated reserve protein. Other factors of environment, such as insect damage, have recently been shown to effect large changes in inhibitor I concentration in tomato and potato leaves (Green and Ryan, 1972). Thus, the regulation of accumulation and utilization of inhibitor I in vegetative tissue is a complex process and depends upon the overall environment of individual plants.
We did not use the immunological method specific for inhibitor I to assay all tissues in which protein bodies were found. However, the location and general configuration of protein bodies in cell vacuoles of all plants examined is similar to the protein found in the detached tomato leaf where coincidence of inhibitor I and vacuolar protein is complete. Immunological assays of apex regions of tomato and tobacco have shown that inhibitor I is present.

Although we do not know that vacuolar protein bodies and protein inhibitor I are the same, our data suggest that the presence of protein bodies in the cell vacuoles is related to the presence of inhibitor I and that vacuolar protein bodies result from compartmentalization of this protein.

References


