SHORT COMMUNICATIONS

THE LOCALIZATION OF ACID PHOSPHATASE IN THE SIEVE ELEMENT OF *PISUM**

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Acid phosphatase activity has been detected within the sieve-tube members of plants by many workers using the Gomori technique and light microscopy (Lester and Evert 1965; Flinn and Smith 1967). Unfortunately the limited resolution makes it difficult to determine the specific sites of activity of the reaction product of the enzyme; recent advances in histochemical techniques for electron microscopy have made it possible to investigate more specifically the sites of localization of the acid phosphatase reaction product by using the Gomori lead nitrate technique (Goldfischer, Essner, and Novikoff 1964; Catesson and Czanenski 1967; Bowen 1968; Figier 1968; Wardrop 1968).

Experimental

The region of the tissue studied was about 5 mm from the tip of the epicotyl. The epicotyl was obtained from pea seedlings germinated on filter papers in Petri dishes under continuous light for 5 days. For fixation purposes, the whole epicotyl still attached to the seed was immersed in a 4% solution of glutaraldehyde buffered with cacodylate buffer (pH 6·7) for 2 hr at room temperature. The required region was then sliced into very thin sections, and fixed again in the same fixative for another 2 hr. For localization of acid phosphatase activity, tissue slices were transferred from the cacodylate buffer washing after glutaraldehyde fixation to another washing in a 0·05M acetate buffer (pH 5) for 30 min. These slices were then incubated for 60 min at room temperature in a medium prepared according to Gomori (1952). They were then washed in the same acetate buffer for another 30 min; postfixation and embedding followed. Slices of tissue to be used as controls were either incubated in a medium without the substrate or were inactivated by exposure for 5 min to hot water (100°C) prior to incubation. Some tissues were incubated in a substrate medium containing 0·001M sodium fluoride; these tissues also acted as controls. Sections were observed in a Siemens Elmiskop I electron microscope at 80 kV.

Results and Discussion

In the young sieve elements acid phosphatase activity was localized within the cisternae of the endoplasmic reticulum (ER) and the saccules of dictyosomes (Figs. 2 and 3). The plasmalemma which lines the sieve element wall showed high levels of acid phosphatase reaction products (Fig. 2). Similar deposits of acid phosphatase–lead reaction product were also present in the ER-derived vacuoles (Figs. 5 and 7) [these vacuoles, which originate from the ER, are surrounded by single membranes (Figs. 5, 6, and 7)]. In the morphologically mature sieve element acid phosphatase–

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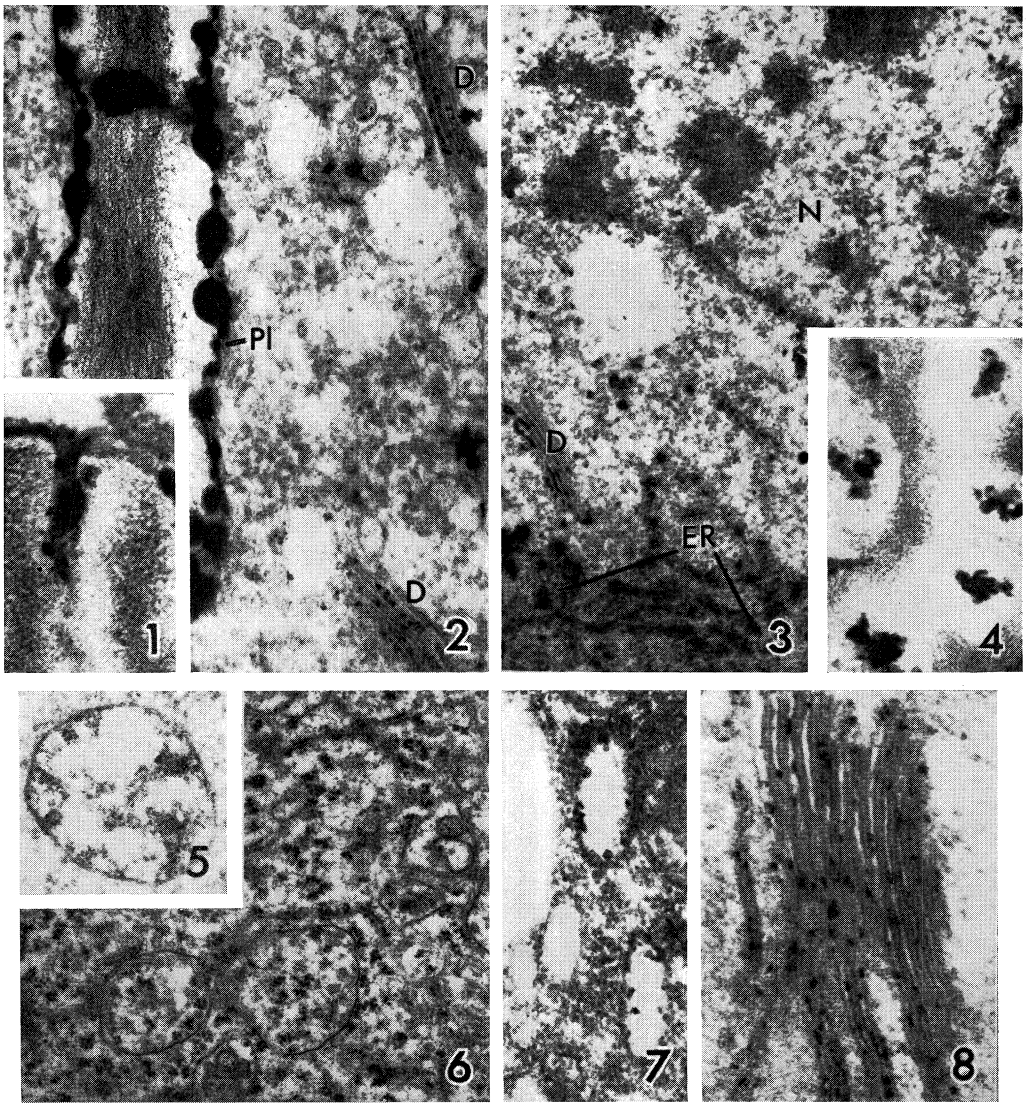

Fig. 1.—A slightly oblique longitudinal section through a sieve-plate pore. Heavy lead deposits can be recognized within the pore. ×45,600.

Fig. 2.—Longitudinal section of a young sieve element showing heavy lead reaction product along the plasmalemma (*Pl*), within the saccules of dictyosomes (*D*), and along the plasmodesma connection between the young sieve element on the right and the phloem parenchyma on the left. ×28,500.

Fig. 3.—Localization of acid phosphatase–lead reaction product within the endoplasmic reticulum (*ER*), the nuclear envelope, and the saccules of dictyosomes (*D*) in a relatively young sieve element. *N*, nucleus. ×25,600.

Fig. 4.—Association of acid phosphatase–lead reaction product with the sieve-plate pores. ×34,200.

lead reaction products have been observed in association with the sieve-tube reticulum (Fig. 8) and the sieve-plate pores (Figs. 1 and 4), but not with the nuclear ground substance, the mitochondria, the plastids, and the ground substances of the mictoplasm. No acid phosphatase–lead deposit was observed in tissue sections of control experiments.

Vacuoles derived from the ER have been studied by Poux (1962), who found that in the young leaves of some species of Gramineae provacuoles can originate from the ER. Recently Matile and Moor (1968) indicated further that the ER-derived vacuoles present in the meristematic cells of *Zea mays* also exhibit lysosome-like functions.

ER-derived lysosomal vacuoles similar to those described by Matile and Moor (1968) are also present in the young sieve element of the primary phloem of the epicotyl of *Pisum*. These vacuoles appear just before the disintegration of the tonoplast and they disappear rapidly soon after. The time at which these vacuoles disappear seems to coincide with the disintegration of the tonoplast, the disappearance of the chromatin materials within the nucleus, and the rapid disappearance of the ribosomes. Thus it is possible that during this period of sieve element cytoplasmic reorganization, acid phosphatase may be released from the ER and the ER-derived vacuoles into the cytoplasm, resulting in some kind of self digestion.

In the young sieve elements of the primary phloem of the epicotyl of *Pisum*, apart from the ER and the ER-derived vacuoles, both the nuclear envelope and the dictyosomes gave a very definite positive lead reaction product to the acid phosphatase test of Gomori. This is indeed not surprising since ontogenetically the ER, the nuclear envelope, and the dictyosomes are all closely related (Essner and Novikoff 1962). However, functionally the ER, the nuclear envelope, and the dictyosomes may be quite separate and the roles played by both the nuclear envelope and the dictyosomes during the development of the sieve elements are still not too clear.

In the morphologically mature sieve element acid phosphatase activity was confined to the sieve-tube reticulum and to the plasmalemma, indicating that both these structures still possess enzymatic activities.

The localization of acid phosphatase–lead reaction product at the sieve element plate pore sites was not very specific. It was not possible to determine whether it is the membrane structure (derived from either the ER or the sieve-tube reticulum) at the pore sites or the plasmalemma that lines the pore that contributes to the acid phosphatase activity. However, the "slime" materials present in the sieve element

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Fig. 5.—A vacuolar structure comparable to that seen in Figure 6. The section is unstained. Thin granules of the localized materials are present in association with the ground substance of the vacuole and the surrounding membrane. × 28,500.

Fig. 6.—At a later stage of development of the sieve element. Vesicular structures and vacuoles containing some ground substance and surrounded by single membranes are shown. × 19,000.

Fig. 7.—ER-derived vacuoles. Lead deposits from the Gomori reaction technique are mainly associated with the surrounding membranes. × 28,500.

Fig. 8.—Association of lead reaction products with the sieve-tube reticulum present in a morphologically mature sieve element. × 57,000.

of *Pisum* did not show any acid phosphatase activity. Therefore these observations are not in agreement with the light microscope observations made by Lester and Evert (1965) who, using Gomori's technique, showed that acid phosphatase activity is associated with the slime strands present in the sieve element of the secondary phloem of *Tilia americana*.

It is clear that further work is needed to clarify the roles played by the hydrolytic enzymes present in the sieve elements. There is, however, reasonable evidence that lysosomal activity does occur in the ER and the ER-derived vacuoles as the sieve element morphologically matures.

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