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Immunocytochemical Localization of Storage Protein in Pea (*Pisum sativum*) Cotyledon

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The pattern of seed storage protein, vicilin, deposition and site of intracellular localization was examined in cotyledon cells of pea (*Pisum sativum*) seed using the immunocytochemical methods. The vicilin was confined to the cisternae of the rough endoplasmic reticulum and dictyosome as well as protein granules newly formed in rough endoplasmic reticulum. Vacuolar protein deposits and protein bodies were also labelled by gold particles. After small protein bodies were formed in the rough endoplasmic reticulum, they were transported to large protein bodies and then fused together. Electron dense protein granule, elaborated in the dictyosome, appears to be transported from dictyosome to protein body. A few unlabelled protein granules seem to be accumulated in other type of proteins than vicilin.

Keywords: pea, cotyledon cell, storage protein, immunocytochemistry

Leguminous seeds have been considered as an important protein resource. These proteins in mature seeds were accumulated within the cotyledon parenchyma cells in small, membrane-bounded organelles known as protein bodies (Graham and Gunning, 1970; Craig *et al.*, 1980a, b).

The major storage proteins of pea were legumin and vicilin which together made up-70% of the total protein of the mature seed (Varner and Schidlovsky, 1963; Thomsons *et al.*, 1978).

The physiochemical nature of vicilin of legumes and the patterns of synthesis during seed development have been studied (Varner and Schidlovsky, 1963; Graham and Gunning, 1970; Thomsons *et al.*, 1978; Craig *et al.*, 1980a, b; Craig and Millerd, 1981). However, little further information was available about the involvement of the endoplasmic reticulum in the synthesis of storage proteins and in the sequence of events before their accumulation in the protein bodies. To study the location of biosynthetic pathway at the cellular and subcellular level, immunocytochemical technique were useful approach (Bollini and Chrispeels, 1978; Craig and Millerd, 1981; Craig and Miller, 1984).

Therefore, in this study, we examined how the vicilin was formed, accumulated and stored within cytoplasm using the anti-vicilin antibody with electron microscopic immunocytochemistry.

MATERIALS and METHODS

Plant meterials

Seeds of pea (*Pisum sativum*) were obtained from Kim Po cultivate land and stored at 4°C.

Preparation for electron microscopy

Cotyledon tissues were fixed in 2% glutaraldehyde in 25 mM sodium phosphate buffer (pH 7.2, 2 hr) and rinsed in 25 mM sodium phosphate buffer. Then the tissues were dehydrated in ethanol-acetone series and embedded in Spurr resin. Sections were collected on 200 mesh copper grids, counterstained with uranyl acetate and lead citrate and viewed in a JEM 100 CX-II electron microscope at 100 kV.

Immunogold labelling

The sections were collected on collodion coated nickel grids, and floated for 20 min on 20 μL of

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PBSTween (25 mM, pH 7.2, 0.2% Tween-20) containing 1% bovine serum albumin (BSA). The grids were incubated for 30 min on 20 ul of anti-vicilin (Lee *et al.*, 1992) diluted 1 : 250 (v/v) with PBST-BSA. Non-specifically binding antibodies were removed by washing the sections 7 times on a drop of PBSTween. The sections were then incubated for 20 min in goat anti-rabbit IgG conjugated with 30 nm gold particles (GAR-G30; Janssen pharmaceuticals) diluted 1 : 20 (v/v) in PBST-BSA. After washing in PBSTween and distilled water, they were stained with 2% aqueous uranyl acetate. Control sections were treated same as above, except that anti-vicilin was replaced with PBSTween-BSA. Stained grids were examined in a JEM 100 CX-II electron microscope at 100 kV.

RESULTS

The cotyledon cell contained numerous vacuoles in the peripheral cytoplasm. In some vacuoles an almost continuous protein layer lined the tonoplast with islands of protein 'free' vacuole, while in others there was less protein, forming isolated aggregates along the tonoplast. Electron dense peripheral deposits lined the vacuoles, with some clumped deposit within the vacuole. Numerous small, electron dense vesicles were also present in the cytoplasm (Fig. 1).

Figures 2-8 were electron micrographs of immunogold localization in cotyledon tissue, which was exposed to anti-vicilin and goat anti-rabbit conjugated gold particles (30 nm). The goat anti-rabbit conjugated gold particles were labelled within the cisternae of the rough endoplasmic reticulum (rER) and dictyosome as well as protein granules which were newly formed in rER. The electron dense vesicles which were associated with rER and dictyosome in the cytoplasm were labelled (Fig. 2). Gold particles were labelled within the protein body and formed linear patterns paralleling the rER cisternae (Fig. 3).

Protein accumulation in vacuoles occurred near the rER and dictyosome (Figs. 4, 5). The electron dense granules were also labelled and they were formed throughout the cytoplasm. Small protein granules (Fig. 4) formed in rER were transported to large protein body. The gold particles were labelled on the protein body which were dispersed throughout the cytoplasm. Cisternae of dictyosome filled with elelctron dense materials that were examined in the vacuolar protein deposits. Electron dense protein granules appeared to be elaborated in the dictyosome, were finally incorporated into the protein body (Fig. 5).

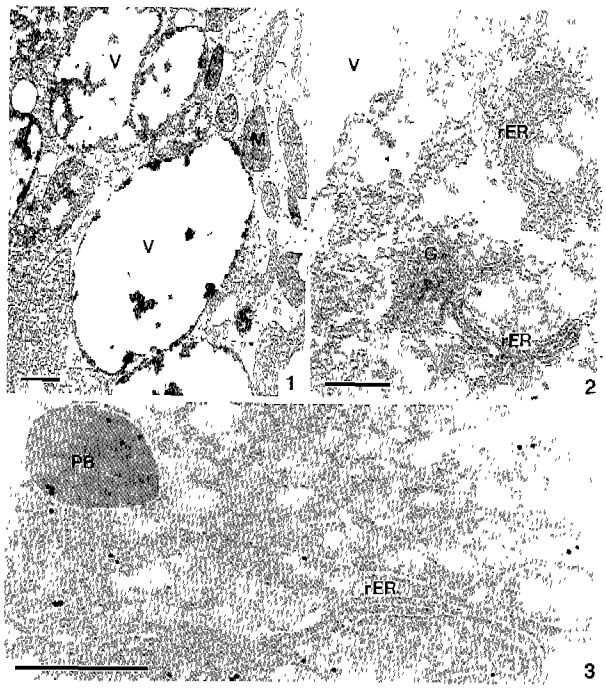

Fig. 1. Electron micrograph of pea cotyledon tissue. Electron dense peripheral deposits lined the vacuoles (V), with some clumped deposits in the vacuole. Numerous small, electron dense vesicles occurred in the cytoplasm. Figs. 2-9. Electron micrographs of immunogold localization in pea cotyledon tissue exposed to anti-vicilin and goat anti-rabbit conjugated gold particles.

Fig. 2. The vicilin was localized within the cisternae of the rough endoplasmic reticulum (rER) and dictyosome (D).

Fig. 3. Gold particles were labelled on the protein body (PB) near the rER. M, mitochondria. Bars indicated 1 μm.

The cytoplasmic and vacuolar protein granules thought to be formed in rER, accumulated in vacuoles to form protein body and were labelled by gold particles (Fig. 6). Peripheral vacuolar deposits and free protein bodies were heavely labelled (Figs. 6-8). The protein bodies were small and their matrix had more electron density. Highly dilated rER was associated with the vacuole. Vacuolar protein deposits and protein bodies are densely labelled. Very few gold particles were labelled the cytoplasm, except where rER was visible (Figs. 7, 8). After small protein bodies were formed in the rER, they were transported to large protein bodies and then fused together (Fig. 7).

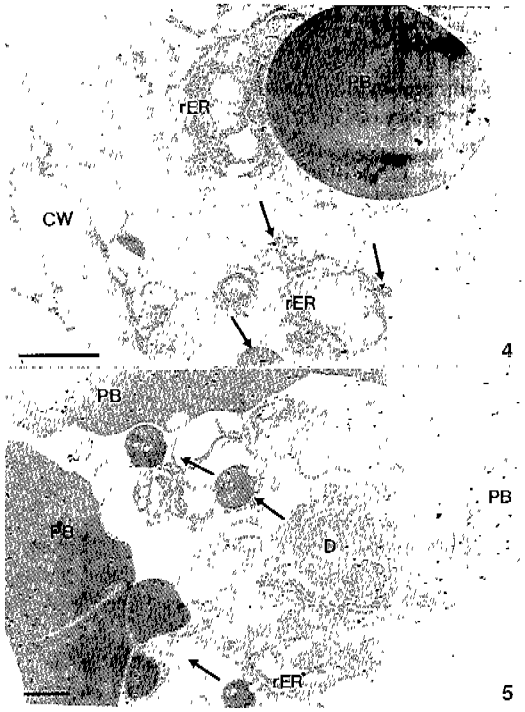

Fig. 4. Gold particles were labelled on the protein body (PB) which dispersed throughout the cytoplasm. Small protein granules (arrows) newly formed in rER appeared to be transported to large protein body.

Fig. 5. Electron dense protein granules (arrows), elaborated in the dictyosome, seemed to be transported from dictyosome to protein body. CW, cell wall. Bars indicated 1 μm.

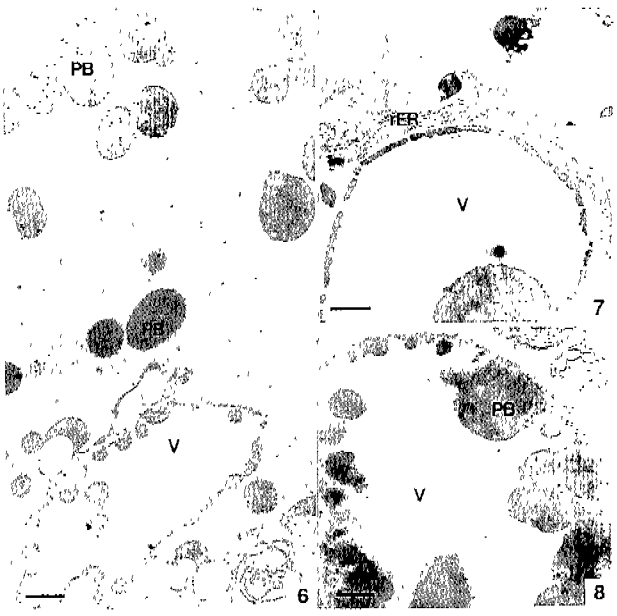

Fig. 6. Being labelled by gold particles, the cytoplasmic and vacuolar protein granules accumulated in vacuoles to form protein body.

Fig. 7. The rER was highly dilated and found in associated with the vacuole.

Fig. 8. Gold particles were densely labelled in vacuolar protein deposits and protein bodies. A few gold particles were labelled on the cytoplasm, except where rER is visible. Bars indicated 1 μm.

DISCUSSION

In developing seeds, reserve protein can be detected microscopically first as clumped deposits at the periphery of large vacuoles (Craig *et al.*, 1980b) which fragment give rise to the protein bodies as development processes (Craig *et al.*, 1980a). In this study, small protein bodies were deposited in the rER lumen and released into the cytoplasm as protein aggregates.

Bollini and Chrispeels (1978, 1979) and Baumgartner *et al.* (1980) observed that rER was the site of vicilin synthesis in developing bean cotyledons. According to Craig and Miller (1984) and Craig and Goodchild (1984a), vicilin was detected within the dictyosome cisternae and its vesicles. Also, Craig and Miller (1980) suggested that at least the glycosylated polypeptides of vicilin would pass through the dictyosome.

In our result, vicilin elaborated in the dictyosome was labelled with gold particles. It was suggested that vicilin was glycosylated and passes through the dictyosome.

Vicilin was also associated with the electron dense granules. The electron dense granules were distributed over the cytoplasm and also labelled with gold particles. The electron dense granule containing elaborated vicilin was formed at the dictyosome, and transported toward the protein bodies. The transported electron dense granules were finally incorporated into the protein body. In the legume seed, vicilin was associated with electron dense dictyosome vesicles which were suggested a vehicle by which newly formed protein was relocated into the vacuoles (Craig and Goodchild, 1984a).

Electron dense granules were labelled with gold particles and the protein bodies distributed over the cytoplasm were labelled uniformly with gold particles. But, very few gold particles were observed in the cytoplasm. Accordingly, we considered that vicilin was preserved in protein bodies. These results coinsided with the works of Baumgartner *et al.* (1980) and Craig *et al.* (1984a, b) who postulated that formed vicilin was initially sequestered within

the rER and then transported to the protein bodies.

In conclusion, from all these results, vicilin was formed in the rER and glycosylated in the dictyosome. Also, our observations indicated that the final site of vicilin deposition was the protein body, and that the rER and dictyosome play an essential role in the deposition process.

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