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Electron Microscopical Studies on the Deposition Route of the Bark Lectin of *Sophora japonica*

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Abstract——Immuno-gold electron microscopical observation indicated that the bark lectin of *Sophora japonica* is localized in the Golgi apparatus and the intravacuolar electron-dense materials, suggesting that the lectin is transported through the Golgi apparatus on the way from the ER to the vacuole. On the other hand, it was sometimes observed that ER-like endomembrane contains electron-dense materials by conventional electron microscopy. Moreover, such endomembrane appeared to fuse the tonoplast. It is possible that some of the lectin molecules or subunits may be transported through the Golgi apparatus and the others of them may be transported directly from ER to vacuole in the parenchyma cells of the bark of *Sophora japonica*.

Key words: bark (phloem). immuno-gold electron microscopy, Golgi apparatus, lectin, *Sophora japonica*

1. Introduction

In our previous study it was found that the bark lectin of *Sophora japonica* increased in autumn, from September to December, and was located in ER lumen and vesicles, suggesting that the bark lectin is synthesized on ER-attached ribosomes[4]. This lectin is finally sequestered in vacuole[4,5]. There is, however, no information how the bark lectin is transported from ER to vacuole. The storage proteins in legume seeds are also synthesized on the ER-attached ribosomes, and finally packed in protein bodies. In the case of these proteins, their deposition has been revealed as follows. The seed storage proteins are synthesized on ER -attached ribosomes, sorted into ER lumen, and transported through Golgi apparatus into vacuole[6~13]. The vacuole which are filled with the proteins divided into smaller globules termed "protein body" on the last stage of seed maturation[19].

Recently, in the bark of woody plants several polypeptides which would be regarded as storage proteins were assessed[1]. Bark lectins are also thought as one of them[2~4]. However, it would be premature to regard their deposition as being

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analogous to that of the seed storage proteins. Because the initiation of the protein deposition in the seeds is different from the bark on the schedule of their tissue development, it is possible that the deposition of the bark lectin differ from the seed storage proteins. In the case of seeds, generally, the storage proteins are accumulated even in the early stage of their development, that is, the synthesis and deposition of the storage proteins occur simultaneously with the mitosis and cell expansion. On the other hand, the parenchyma cells in the bark have already differentiated before the end of summer, and the bark lectin is accumulated in autumn when the tissue development of the year has been completed[4]. There is no reports on the transportation of vacuolar proteins other than seeds or grains. In this paper, we localized lectin in the phloem parenchyma cells of *Sophora japonia* by electron microscopy and immuno-gold electron microscopy, and discussed the pathway of the bark lectin to vacuole.

2. Material and Methods

2.1 Plant material, fixation and embedding

An individual *Sophora japonica* growing on the campus of the Wood Research Institute, Kyoto University was used. Tissue block (approx. 3•3•2 cm^3) was harvested in autumn from the stem by using a chisel and cut into smaller pieces (approx. 0.5•1•2 mm^3) in the fixative of 4% paraformaldehyde, 0.2% glutaraldehyde, 0.5 mM $CaCl_2$ in 20 mM piperidine-N,N-bis(2-ethanesulfonic acid) (Pipes purchased from Dojin) (pH 7.2). Some pieces were fixed with the fixative described above at 4°C for 4 h. After washing with 20 mM 2-amino-(hydroxymethyl)-1,3-propanediol (Tris) buffer (pH 7.4) three times, they were dehydrated at 4°C with ethanol series (50, 75, 90, 99%) and embedded in hydrophilic methacrylate resin consisting of methyl methacrylate (Nacalai Tesque Co. Ltd.), glycol methacrylate (Nacalai Tesque Co. Ltd.) and Quetol 523M (Nisshin EM Co. Ltd.) (3:6:1 by vol.). The resin was cured by heating at 60°C catalyzed by 0.05% 2,2,-azo-bis-*iso*-butyronitrile, or by irradiation of ultraviolet at −10°C to −15°C catalyzed by 1% benzoyl peroxide in gelatin capsules. Other pieces were fixed in 1.5% paraformaldehyde, 2.5% glutaraldehyde in Pipes buffer at 4°C for 8 h, washed with 20 mM sodium cacodylate buffer (pH 7.4) three times, and postfixed with 1% OsO_4 at 4°C overnight. After being washed with cacodylate buffer and dehydrated in an ethanol series (50, 70, 90, 95, 99%) and acetone, they were embedded in Spurr's low-viscosity resin (Polysciences Inc.).

2.2 Electron microscopy

Ultrathin sections were prepared from Spurr's resin-embedded sample. They were mounted on 150 mesh copper grids supported by folmvar film, and stained

with uranyl acetate and lead citrate.

2.3 Immuno-gold electron microscopy

Ultrathin sections were prepared from the Spurr's epoxy resin-embedded-samples and the hydrophilic methacrylate-embedded-samples (Methacrylate). The former were etched with 30% H_2O_2 and then mounted on 150 mesh copper grids supported by folmvar film. The latter were directly mounted on the grids supported

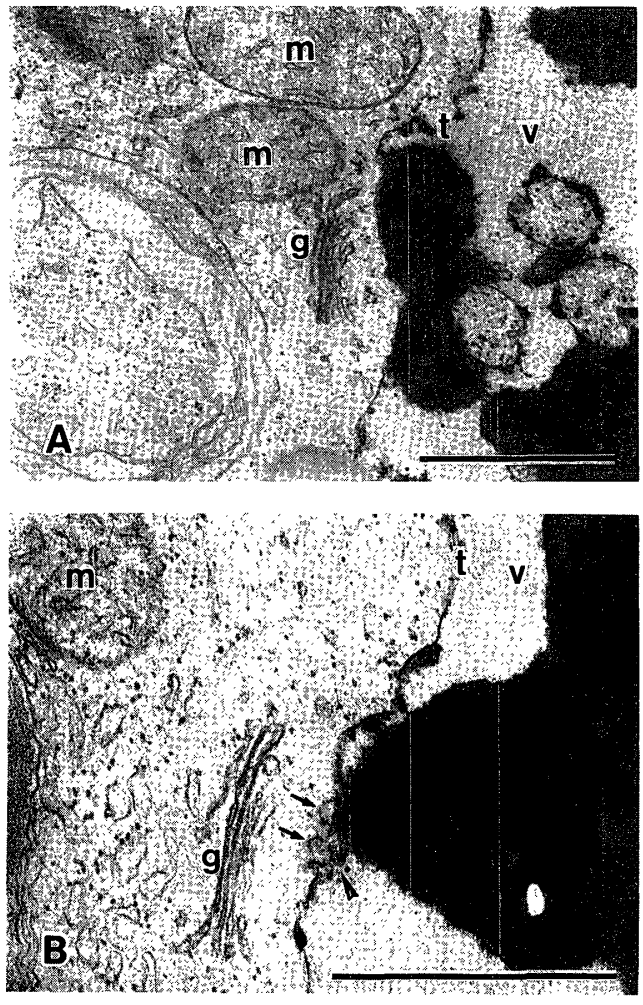

Fig. 1. Immuno-gold electron micrographs on the sections etched by H_2O_2 after embedding into Spurr's epoxy resin.
A, Gold particles are observed on the electron-dense materials in the vacuole, and the Golgi apparatus was observed at just back of the electron-dense clump. **B**, The tonoplast between the electron-dense clumps and the Golgi apparatus forms invagination (*arrows*), and electron-dense materials were observed between the invagination and the clumps (*arrowhead*) g, Golgi apparatus; m, mitochondrion; t, tonoplast; v, vacuole. Marker bars=1 μm.

by folmvar film. Both H_2O_2 etched and the Methacrylate sample were treated with the immuno-gold detecting method. Sections on grids were blocked with 1% BSA in 10 mM PBS (pH 7.2) for 30 min, followed directly by incubation for 1 h with anti-SJA antibody (E.Y. Laboratories Inc.) diluted 20-fold with 0.1% BSA in 10 mM PBS. After washing with PBS six times, they were left at room temperature for 30 min with protein A-colloidal gold (Funakoshi Yakuhin Co. Ltd.) diluted 10-fold with 0.1% BSA in 10 mM PBS. They were then washed with 10 mM PBS twice, followed by distilled water six times. Lastly, they were air-dried and stained with uranyl acetate for 20 min, and observed under an electron microscope (H-700; Hitachi).

3. Results and Discussion

On the sections prepared from H_2O_2-etched-sample gold particles, indicating lectin localization, were observed only in the electron-dense clumps of the vacuole (Fig. 1). the gold particles were not observed on any organelle. However, Golgi apparatus was often seen at just back of the electron-dense clumps (Fig. 1). Moreover, the tonoplast between the Golgi apparatus and the clump formed several

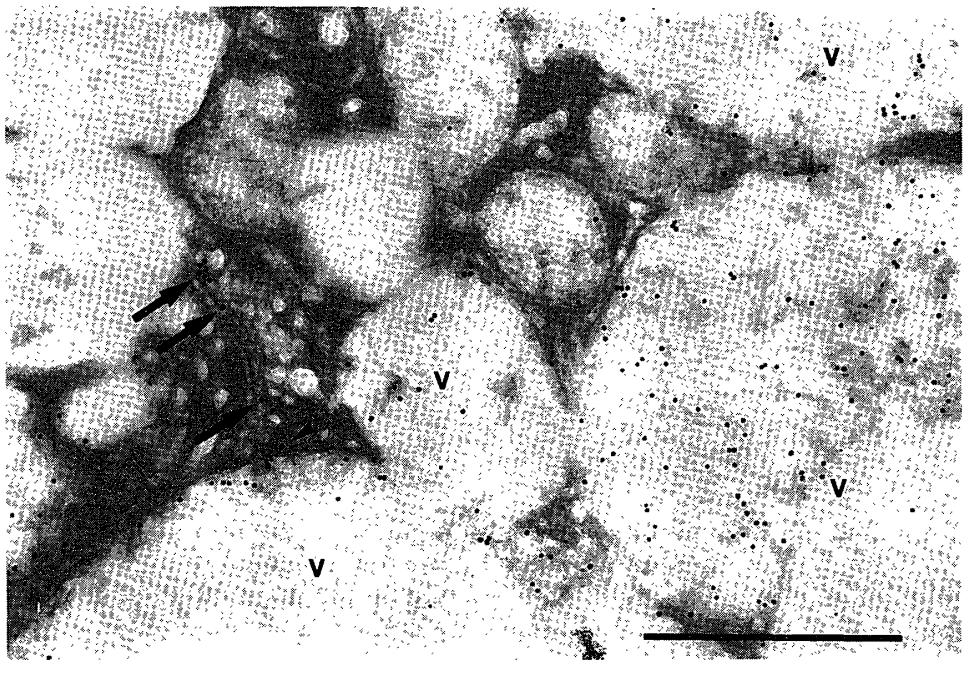

Fig. 2. Immuno-gold electron micrographs on the sections embedded in hydrophilic methacrylate resin.
Gold particles are seen on the materials in vacuole, Golgi apparatus (*arrows*) and vesicles (*arrowhead*). v, vacuole. Marker bars=1 μm.

small invagination (arrows in Fig. 1B), and the electron-dense material was observed between the invagination andt he clumps (arrowhead in Fig. 1B). On the sections prepared from Methacrylate sample gold particles were observed in the lumen of the Golgi apparatus (arrows in Fig. 2) and its vesicles (arrowheads in Fig. 2), and of course in the vacuoles. These results are consistent with the immunocytochemical observations on seed lectins in developing cotyledon, such as Con A, the seed lectin of *Canavalia ensformis*[14], PHA, the seed lectin of *Phaseolus vulgaris*[15], and the seed lectin of *Bauhinia purpurea*[16]. The synthetic pathway of these lectins was also studied biochemically and demonstrated that they are synthesized on ER

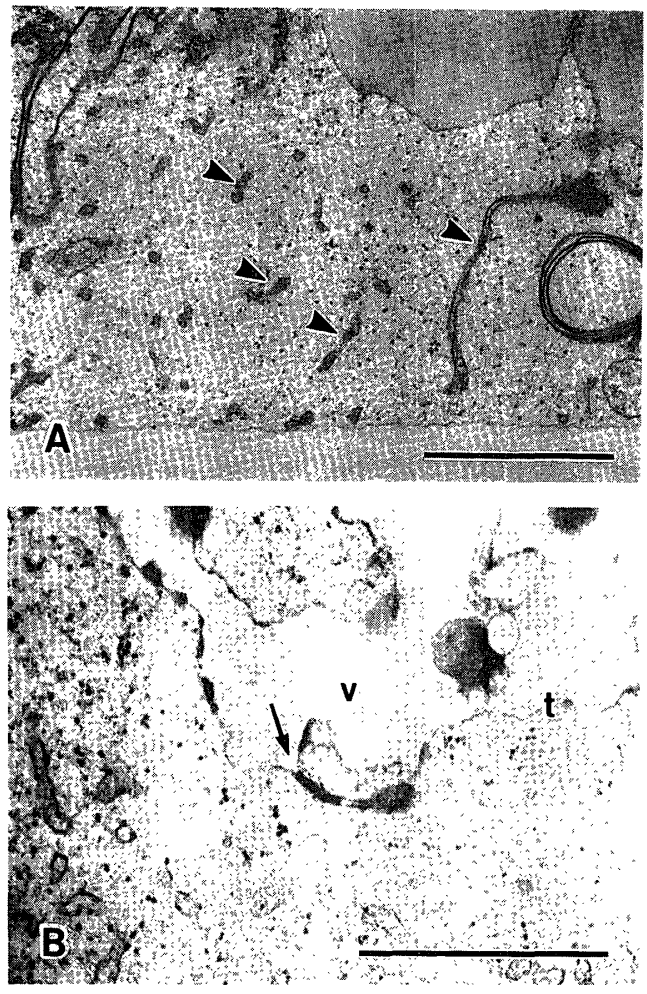

Fig. 3. Conventional electron micrographs of the phloem ray parenchyma cells of *Sophora japonica*.
A, The ER-like endomembrane was filled with the electron-dense materials (*arrowheads*). **B**, The ER-like endomembrane containing the electron-dense materials appeared to fuse to tonoplast (*arrow*). t, tonoplast; v, vacuole; Marker bars=1 μm.

-attached ribosomes, sorted into ER lumen, and after passing through the Golgi apparatus, accumulated in vacuole (protein body)[6,9,12,13]. Therefore, our result suggests that the bark lectin may also pass through Golgi apparatus. This deposition route is also demonstrated in the other storage proteins in seeds of dicotyledons[6~8,10,11].

Originally, the idea that the vacuolar proteins are passed through the Golgi apparatus on the way to the vacuole was raised by the observation that the Golgi -derived vesicles were filled with the electron-dense materials similar to the deposits in the vacuoles of developing cotyledons[20]. In the parenchyma cells of the bark, however, the dense-vesicles were never observed. On the other hand, electron-dense materials were observed in the lumen of the ER-like endomembrane system (Fig. 3A), and moreover, it was sometimes observed that the ER-like endomembrane containing the electron-dense materials appeared to fuse the tonoplast directly (Fig. 3B). These results suggest that ER may directly be involved in the secretion of electron-dense materials to vacuole. It is possible that all of the lectin molecule may not be transported through the Golgi apparatus. The bark lectin of *Sophora japonica* is tetrameric molecules constructed with three or more types of subunits[17,18], and is mixture of the five molecular species which are able to be separated with ion-exchange chromatography[18]. It would be possible that only some type(s) of lectin molecules or some subunit(s) may be transported through the Golgi and the others directly from the ER to the vacuole. The two-route model, that some type is transported via Golgi apparatus and the other type bypassing the Golgi within the certain protein species, was also proposed on the secretion of α-amylase in the barley germination[21].

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