Bark and Leaf Lectins of *Sophora japonica* Are Sequestered in Protein-Storage Vacuoles

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ABSTRACT

The leguminous tree *Sophora japonica* contains a family of closely related, but distinct, lectins. Different members of this family are independently expressed in seeds, leaves, and bark (CN Hanksins, J Kindinger, LM Shannon 1987 Plant Physiol 83: 825–829; 1988 Plant Physiol 86: 67–10). The inter- and intracellular distribution of the bark and leaf lectins was studied by indirect postembedding immunogold electron microscopy. Aldehyde fixed bark and leaves postfixed with OsO₄ and embedded in LR White resin permitted sensitive and specific immunogold labeling while maintaining cellular ultrastructure. The leaf and bark tissue cells contain protein-filled storage vacuoles which occupy most of the cell's interior volume. The leaf and bark vacuoles closely resemble the protein bodies, or protein storage vacuoles, of seed cotyledons. The leaf and bark lectins were found to be exclusively sequestered in the protein-storage vacuoles of these tissues.

The leaf, bark, root, and stem tissue of several different legumes have been described as containing lectins or other immunologically CRM² (8, 9, 14, 18, 20, 22; see Ref. 3 for review). Partial amino acid sequences of these nonseed lectins or CRM indicate that each of these proteins is closely related to, but not identical to the seed lectin of the same plant (8, 9, 14). The lectins of legume seeds have been documented to be localized in storage vacuoles or protein bodies (see Ref. 3 for review). In contrast, immunologically related nonseed legume lectins have been localized by light microscopic immunocytochemistry in the vacuoles of soybean root tips (22), the cell wall in *Dolichos* stems and leaves (4), and the cytoplasm of *Dolichos* tissue culture and leaf cells (4, 13). The apparent variability of the localization of nonseed legume lectins contrasts with the electron microscopic localization of lectin in the vacuoles of adult wheat roots (17) and the light microscopic localization of lectin in protein-storage vacuoles of the Elder tree (*Sambucus*) bark (7).

Recent amino-terminal protein sequence and immunological cross-reaction data have shown that the seed, leaf, and bark lectins of the legume tree *Sophora japonica* are distinct but closely related gene products (8, 9). We have used high resolution electron microscopic immunocytochemistry to localize leaf and bark lectins. These observations demonstrate that the leaf and bark lectins are sequestered in protein-storage vacuoles.

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2 Abbreviations: CRM, cross-reacting materials; TBST, tris-buffered saline containing Tween-20.

3 Mention of trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

MATERIALS AND METHODS

*Sophora japonica* leaf samples were obtained from a tree growing on the U.C. Riverside campus (8) previously used for biochemical studies. Bark samples were obtained from a greenhouse-grown tree. Green, living bark samples were obtained as cross-sections of small lateral branches. All samples were fixed in 4% glutaraldehyde (Polysciences), 2% formaldehyde (EM grade, Polysciences) in 0.1 M sodium phosphate buffer (pH 7.4) at room temperature for several hours. The bark samples were further hardened at 7°C for at least 48 h. The leaf samples in fixative were transported from Riverside to Beltsville by overnight air express at ambient temperature and further processed on arrival. The samples were then postfixed in 1% aqueous OsO₄ (Polysciences) and dehydrated in a graded ethanol series. The samples were then transferred to LR White resin (Polysciences). Resin was changed several times over a 3-d period after which the blocks were polymerized for 48 h at 60°C. Silver colored sections were obtained with a Saphatone knife (LKB) and mounted on nickel grids. Indirect immunogold labeling was accomplished with 1:20 (for leaf sections) or 1:500 (for bark sections) dilutions of the primary anti-*Sophora* seed lectin serum (8) in TBST (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% v/v Tween-20). The sections were labeled for 30 min at 20°C, then washed in TBST, and then indirectly labeled with 7 nm polystyrene A-collloidal gold for 5 min at 20°C. The protein A-collloidal gold conjugate was prepared by the method of Slot and Geuze (19). After labeling, the grids were washed with TBST, then with double distilled water, and then stained with 5% aqueous uranyl acetate for 20 min followed by alkaline lead citrate (33 mg ml⁻¹) for 5 min. The grids were examined and photographed with a Hitachi H500 electron microscope.

RESULTS

**LR White Resin Embedding.** *S. japonica* leaf and bark tissues fixed with glutaraldehyde and formaldehyde, postfixed with OsO₄, and embedded in LR White retained structural preservation comparable to that normally results with epoxy resin embedding protocols (2). The comparative advantages and disadvantages of various tissue processing protocols for electron microscopic immunocytochemical assay of plant tissues has been reviewed (11). LR White-embedded tissue does differ from epoxy resin-embedded tissue in the staining characteristics of the chloroplasts. With this method the chloroplast stroma is intensely electron dense after staining with uranyl acetate and lead citrate (Figs. 1, 2, and...
4). The thylakoids are observed as negatively stained membranes against the dense stroma (Figs. 1, 2, and 4). Other organelles including the nucleus (Fig. 2), mitochondria (Fig. 2), endoplasmic reticulum (Fig. 2), and microbodies (data not shown) appeared to differ little in morphology and staining characteristics from material embedded in epoxy resin.

**Immunocytochemical Localization of Leaf Lectin.** At low magnification the leaf cells were observed to contain central vacuoles which occupy most of the cellular space. Unlike most leaf vacuoles, the vacuoles of *Sophora* leaves contain dense deposits of electron dense material. The leaf vacuole deposits in some cells are densely packed completely filling the vacuole, while in other leaf cells the vacuolar deposits are much more sparsely distributed (Fig. 1). The protein-filled vacuoles appear to be quite similar to the protein-storage vacuoles, or protein bodies of legume seed cotyledons. But unlike seed protein bodies, the protein filled leaf vacuoles are not subdivided into numerous smaller vacuoles.

The anti-*Sophora*-lectin serum has been previously shown to specifically cross-react with the *Sophora* leaf I and II lectins (8) as well as the bark lectin (9). Indirect immunogold labeling of LR White sections resulted in the specific labeling of the leaf protein deposits with the 7 nm colloidal gold particles (Figs. 2 and 4) indicating retention of antigenic determinants. All other subcellular organelles were observed to be free of gold particles except for a very sparse nonspecific and apparently random label. Controls in which nonimmune serum was substituted for the specific serum in a parallel labeling reaction resulted in the absence of gold particles on the leaf vacuole protein deposits (Fig. 3). Figure 4 shows a portion of a leaf vacuole which contains sparse protein deposits. The immunogold labeling is restricted to the protein deposits and is absent on the vacuolar sap.

**Immunocytochemical Localization of Bark Lectin.** *Sophora* bark was sectioned perpendicular to the surface to provide a transect through the tissue. The exterior surface and the outermost two cells of the bark are shown in Figure 5. These cells contain either very few vacuoles or a single pleomorphic vacuole which contains sparsely distributed protein deposits. The density of the vacuolar deposits was observed to increase toward the interior of the 10 to 12 cell thick bark. Cells in the middle of the bark contain a single pleomorphic vacuole which is completely filled with protein deposits. Figure 6 shows one such cell located six cells from the exterior surface of the bark. Immunogold labeling of plastic sections of the bark showed that the lectin was exclusively sequestered in the protein deposits (Fig. 7). The interior cells which contain completely filled vacuoles were observed to be more densely labeled than more exterior cells with more sparse vacuolar protein deposits (data not shown). The cell wall of the bark cells was devoid of gold particles although adjacent protein-storage vacuoles were labeled (Fig. 8).

**DISCUSSION**

The electron microscopic observations shown here demonstrate that protein-filled vacuoles are widely distributed among the cells of the leaf and bark of the tree legume *S. japonica*. Nonseed tissues in other plants have been shown to contain protein-filled vacuoles. For example, the leaves of soybean (*Glycine max*) contain a single cell layer referred to as the paraveinal mesophyll. The paraveinal mesophyll cells contain a large central vacuole filled with a storage glycoprotein (5, 23). Apparently
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Fig. 2. A portion of a Sophora leaf cell which contains completely filled vacuole(s) (V) is shown. The leaf lectin is localized in the protein matrix of the vacuole. The nucleus (N), mitochondria (M), and chloroplast (C) are unlabeled except for very few random nonspecific gold particles. Note that the chloroplast thylakoids are shown as negatively stained membranes against an electron dense stroma as a consequence of the preparative procedure (×40,000).

Fig. 3. An immunological control is shown in which protein-filled leaf vacuoles are devoid of gold particles which demonstrates the specificity of the immunocytochemical labeling. ×37,500.

Fig. 4. Immunogold labeling of a partially filled leaf vacuole (V) is shown. Note that the immunogold labeling is restricted to the aggregated protein (arrowheads) precipitated against the vacuolar membrane while the vacuolar sap is unlabeled. Also shown is a portion of chloroplast (C) (×45,800).
Fig. 5. A low magnification survey micrograph of the outer two cell layers of the Sophora bark is shown. The outermost cells along the exterior surface (ES) are elongated, while the other cells from the second cell layer inward are ovoid in shape. The vacuoles (V) of the outer two cell layers contain diffusely distributed protein deposits (×4,000).

Fig. 6. A low magnification micrograph of a bark cell six cell layers from the exterior surface is shown. Cells in the interior of the bark contain single pleomorphic vacuoles (V) which are completely filled with protein. Other prominent organelles shown include nucleus (N), chloroplast (C), mitochondria (M), and microbodies (B) (×5,400).

Fig. 7. A high magnification micrograph of a portion of an interior bark cell which has been labeled by the immunogold procedure. Note that the gold particles specifically label the vacuole (V) matrix while adjacent chloroplast (C), mitochondria (M), and nucleus (N) are unlabeled (×36,000).

Fig. 8. An adjacent protein-filled vacuole (V) and cell wall (CW) of an interior bark cell is shown. The vacuole is specifically labeled by immunogold particles while the cell wall is devoid of label (×36,000).
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this material functions as a nitrogen reserve for seed fill (6). The bark of Elder trees (Sambucus nigra) contain a phloem storage parenchyma which contains cells with a lectin localized in a protein-storage vacuole (7) which is thought to serve a seasonal storage function (15). Similarly, the bark of apple trees contain storage proteins which provide seasonally available nitrogen reserves (10, 16). Whether the abundance of the Sophora bark or leaf vacuolar proteins and in particular the lectin varies on a seasonal basis must still be determined.

The protein-filled vacuoles of legume seed cotyledons are usually termed protein bodies. The protein bodies contain the same spectrum of acid hydrolases as vacuoles (21), and are derived from the subdivision of preexisting vacuoles during the course of seed maturation (1). The presence of protein-filled vacuoles in nonseed tissues of S. japonica raises a question of terminology. Should the protein-filled vacuoles of seed tissues be differentiated from those of nonseed tissues? The term ‘protein-storage vacuole’ is descriptive of the morphology and the apparent function of the protein-filled seed, leaf, and bark vacuoles.

Previous light microscopic localizations of nonseed legume lectins in soybean (22) and Dolichos (4, 13) have resulted in an uncertainty of whether these proteins are localized in vacuole, cell wall, or cytoplasm. The much higher resolution afforded by electron microscopic immunocytochemistry has allowed us to determine that bark and leaf lectins are localized in the protein-storage vacuoles. We have also been able to demonstrate that lectins are absent from many organelles or portions of organelles below the resolution capability of light microscopy.

It is interesting that the bark, leaf, and seed tissues have each evolved cells containing protein-storage vacuoles with a distinct tissue-specific lectin gene product (8, 9), even though each lectin must have evolved from a common vacuolar protein. In contrast, although protein-storage vacuoles are found in different cell types of jackbean (Canavalia ensiformis) cotyledons, only the protein-storage vacuoles of fully differentiated storage parenchyma cells contain the lectin concanavalin A (12). The physiological function of the carbohydrate binding property of legume lectins, if indeed any function exists, remains unknown. The presence of multiple tissue-specific lectins in storage vacuoles argues that lectins may have an important, if yet undefined role in the physiology of protein-storage vacuoles beyond a possible function as stored nitrogen reserves.

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