Lectin Electron Microscopic Histochemistry of the Pseudoexfoliative Material in the Skin
Fujio Amari,* Seiji Nagata,* Junichi Umihira,* Masahiko Nohara,* Nobuteru Usuda,† and Katsuzo Segawa*

Purpose. To investigate the hypothesis that pseudoexfoliation (PSX) syndrome is a systemic disorder, the authors composed the composition of glycoconjugates in the intraocular and extraocular PSX material at the electron microscopic level, using a panel of lectins as cytochemical probes.

Methods. The authors examined 8 lid skins, 11 trabecular tissues, and 3 cataractous lenses from human eyes with PSX syndrome. Tissues were processed for electron microscopical histochemistry and stained with PNA, RCA120, DBA, SBA, ConA, WGA, UEA-I, and Lotus, with an indirect lectin-colloidal gold technique.

Results. Both the intraocular and extraocular PSX materials manifested almost identical reactivity to lectins, which indicated that glycoconjugates in the PSX material contained with sugar residues of galactose (PNA, RCA120), α-mannose (ConA), and N-acetyl-D-glucosamine (WGA). On the other hand, it was indicated that sugar residues of N-acetyl-D-galactosamine (DBA, SBA) and fucose (UEA-I, Lotus) were absent. Granular inclusions and microfibrils in the capsule and ocular zonules were stained similarly and weakly.

Conclusions. The intraocular and extraocular PSX materials contained the same sugar residues of glycoconjugates, which suggested that those materials had the same nature. This study, the first documentation of lectin-binding sites on the extraocular PSX material, supported the hypothesis of PSX syndrome as a systemic disorder. Invest Ophthalmol Vis Sci. 1994;35:3962-3966.

Lectins, carbohydrate-binding proteins or glycoproteins of plant or animal origin, have an affinity for specific terminal sugars and some internal sugars, sugar linkages, or oligomers in complex carbohydrates. This specific property makes lectins valuable probes for studying glycoconjugates, and many kinds of lectin have been employed to provide insight into the biochemical composition of membranes and to identify cell types in cytochemical studies of tissue slices and cultured cells. The specific lectin-binding patterns have been determined in many kinds of tissues from various organs, under normal or pathologic conditions.

Pseudoexfoliation (PSX) syndrome has been considered to be a disorder with the PSX material in the anterior segment of the eye. Findings of the PSX material in the conjunctiva and around posterior ciliary vessels raised the possibility that the production and deposition of the PSX material occurred in the whole body. The importance of this suggestion was emphasized by the discovery that similar PSX material was demonstrated in lid skins, various peribulbar tissues, and visceral organs.

We used lectins as cytochemical probes for studying molecular details of the extraocular PSX material. The electron microscopic histochemical study using biotin-labeled lectin and colloidal gold streptavidin as an electron-dense marker was used in a postembedding labeling technique to reveal lectin-binding sites with thin sections of Lowicryl K 4M embedded material. In this report, we describe the distribution of binding sites for peanut agglutinin (PNA), ricinus communis agglutinin (RCA120), dolichos biflorus agglutinin (DBA), soybean agglutinin (SBA), concanavalin A (ConA), wheat germ agglutinin (WGA), ulex europaeus agglutinin (UEA-I), and lotus tetragonolobus (Lotus) in the PSX material of the lens capsule, trabecular tissue, and skin.

MATERIALS AND METHODS. Materials. Eight lid skins, 11 trabecular tissues, and 3 cataractous lenses were obtained surgically from patients with the clinical diagnosis of capsular glaucoma. Comparisons were made with similar surgical tissues; seven lid skins, six trabecular tissues, and three lenses, all without the PSX material clinically. The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from each patient before biopsy specimens were taken.

Tissue Preparation for Electron Microscopic Histochemistry. Tissues were processed as reported previously with some modifications. The specimens were fixed in freshly prepared fixative of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M sodium phosphate, pH 7.4, with the addition of 0.15 M NaCl for 24 to 48 hours at 4°C, and washed with 0.1 M lysine in phosphate-buffered isotonic saline (pH 7.4) three times for 60 minutes. The specimens were then dehydrated in a graded series of cold ethanol and embedded in the Lowicryl K4M resin for 50 hours at −20°C. Ultrathin sections were cut with a diamond knife and picked up on formvar membrane-coated nickel grids.

Staining Protocol. The eight lectins were pur-
chased from Seikagaku Kougyou (Tokyo, Japan). The specificity of these lectins was reported previously (Table 1).1 The grids were first placed on a drop of 0.05 M trometamol (Tris)-HCl buffer, pH 7.5, with the addition of 0.15 M NaCl (TBS), containing 5 mg/ml bovine serum albumin for 30 minutes at room temperature, followed by incubation with the biotin-labeled lectins in a moist chamber overnight at 4°C. The lectin grids were rinsed again and counterstained with uranyl acetate and lead citrate before examination under a Hitachi (Tokyo, Japan) HS-9 transmission electron microscope at 75 kV.

**Control Staining.** The control procedures included the use of inhibitory and noninhibitory sugars before incubation with the lectins; omission of the lectins as a negative control; specimens of kidneys and intestines employed as a positive control; and fibroblasts were often seen around the PSX fibers. Elastic fibers (Fig. 1B). The particles were not clearly arranged at regular intervals. The lectin dilutions in TBS plus 5 mg/ml bovine serum albumin, as determined by previous experimental work, were 1:20. After six rinses with TBS, the grids were further incubated with 15 nm colloidal gold streptavidin (Amersham, Chalfont, UK) for 3 hours at room temperature. Those dilutions were determined to be 1:50. The grids were rinsed again and counterstained with uranyl acetate and lead citrate before examination under a Hitachi (Tokyo, Japan) HS-9 transmission electron microscope at 75 kV.

**Control Staining.** The control procedures included the use of inhibitory and noninhibitory sugars before incubation with the lectins; omission of the lectins as a negative control; specimens of kidneys and intestines employed as a positive control for the lectins that did not bind to the PSX material and neighboring tissues.

**RESULTS.** The PSX material was found in all specimens of capsular glaucomas. Most PSX fibers were from 20 to 40 nm in diameter and banded at 20 to 50 nm. On the lens capsule, a few microfibrils were seen as unbranched rods 10 nm in diameter. Granular inclusions were rich in the anterior to equatorial region of the capsule and distributed throughout the full thickness of the capsule. Vertically or radially oriented microfibrils of 10 nm diameter from the grooves of the lens epithelium were also prominent in the anterior to equatorial region. The PSX fibers among the trabecular meshwork were shown almost the same ultrastructure. In the lid skin, the similar fibers were also observed mainly in the papillary dermis. Compared with the intraocular PSX fibers, the extraocular fibers had obscure bands and outlines with dark granular matrices around them. Elastic fibers, collagen fibers, and fibroblasts were often seen around the PSX fibers.

The lectin-binding patterns of PNA, RCA120, DBA, SBA, ConA, WGA, UEA-I, and Lotus, are summarized in Table 2. The PSX fibers in the 11 eyes stained for 4 kinds of lectin, with some differences in binding intensity (Fig. 1A). The reactivity of the PSX fibers for lectins was similar among all specimens obtained from the eyes. The positive staining of lectins indicated that glycoconjugates in the PSX fibers contained sugar residues of galactose (PNA, RCA120), α-mannose (ConA) and N-acetyl-D-glucosamine (WGA). Binding of lectins for N-acetyl-D-galactosamine (DBA, SBA) and fucose (UEA-I, Lotus) were negative. The gold particles for each lectin preferentially distributed at the margin of the PSX fibers (Fig. 1B). The particles were not clearly arranged at regular intervals. The capsule itself was not labeled, but granular inclusions and microfibrils were labeled similarly to the PSX fibers (Fig. 2A). The lens epithelium stained for all lectins except UEA-I and Lotus. Ocular zonules and zonular lamella exhibited similar labeling (Fig. 2B). In the lid skin, positive reactivities of the PSX fibers with those four lectins were similar (Figs. 3A, 3B). Elastic fibers in the papillary dermis showed some elastic change, irregular in outline with loosely aggregated elastic skeleton fibers, and sometimes they appeared to undergo granular and fibrillar degenera-

### TABLE 1. Binding Specificities of Lectins Employed

<table>
<thead>
<tr>
<th>Lectin Acronym (Latin name)</th>
<th>Binding Preference</th>
<th>Inhibitory Sugars Used in Negative Control</th>
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<tbody>
<tr>
<td>PNA (Arachis hypogaea)</td>
<td>Terminal Galβ1,3GalNAc</td>
<td>Gal, lactose</td>
</tr>
<tr>
<td>RCA120 (Ricinus communis agglutinin)</td>
<td>Galβ1-4GalNAc &gt; βGal &gt; αGal</td>
<td>Gal, lactose</td>
</tr>
<tr>
<td>DBA (Dolichos biflorus)</td>
<td>Terminal FP &gt; GalNAcα1, 3GalNAc &gt; GalNAcα1, 3Gal</td>
<td>GalNAc</td>
</tr>
<tr>
<td>SBA (Glycine max)</td>
<td>TerminalαβGalNAc &gt; αβGal</td>
<td>GalNAc</td>
</tr>
<tr>
<td>ConA (Canavalia ensiformis)</td>
<td>αMan &gt; αGlc &gt; GlcNAc</td>
<td>Man</td>
</tr>
<tr>
<td>WGA (Triticum vulgaris)</td>
<td>GlcNAc(β1,4GlcNAc)1,2 &gt; βGlcNAc</td>
<td>GlcNAc</td>
</tr>
<tr>
<td>UEA-I (Ulex europaeus)</td>
<td>αFuc</td>
<td>Fuc</td>
</tr>
<tr>
<td>Lotus (Lotus tetragonolobus)</td>
<td>αFuc</td>
<td>Fuc</td>
</tr>
</tbody>
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Gal = Galactose; GalNAc = N-acetyl-D-galactosamine; GlcNAc = N-acetyl-D-glucosamine; FP = forssman pentasaccharide; αFuc = fucose.

### TABLE 2. Lectin Binding Patterns

<table>
<thead>
<tr>
<th>PSX Fibers (Lid Skins)</th>
<th>PSX Fibers (Eyes)</th>
<th>Ocular Zonules</th>
<th>Microfibrils (Capsules)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNA</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td>RCA120</td>
<td>3+</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>DBA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SBA</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ConA</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>WGA</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
</tr>
<tr>
<td>UEA-I</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lotus</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

The intensity of staining was graded — (negative) to 3+ (intense).
FIGURE 1. Immunogold labeling of PNA on the pseudoexfoliative fibers (PSX) on the lens capsule. Note the moderate labeling of the PSX fibers (A). The label was found to be located at the margin of the PSX fibers (B). PNA = Peanut agglutinin. (A) Bar = 0.5 μm. (B) Bar = 0.2 μm.

FIGURE 2. Immunolocalization of ricinus communis agglutinin (RCA120) in the lens capsule. Note the labeling of granular inclusions (arrows) and vertically and radially oriented microfibrils (MF) from the grooves of the lens epithelium (EP) (A). Labeling of ConA. The gold label is located along zonules and zonular lamella (B). ConA = Concanavalin A. (A) Bar = 0.5 μm. (B) Bar = 0.2 μm.

ultrastructures similar to the extraocular PSX fibers in the lid skin, they also had the same carbohydrate residues revealed by lectin bindings. Our observations suggested the presence of glycoconjugates with galactose, α-mannose, and N-acetyl-D-glucosamine residues in the PSX fibers, which seemed to be present mainly at the margin of the fibers.

The presence of various molecular components in the PSX material, fibrillin, elastin, amyloid P, vitronectin, type IV collagen, heparan sulfate, chondroitin sulfate, laminin, entactin-nidogen, and fibronectin, have been revealed immunohistochemically. However, most of these studies were performed on the intraocular PSX material. Concerning the glycoconjugates of the PSX material, there have been a few light microscopic histochemical analyses concerning the intraocular PSX material, and none concerning the extraocular material.

Previous reports of light microscopic lectin binding in the PSX material are similar to our results except for a couple of lectins. Streiten et al. observed that DBA had a moderate reaction, whereas SBA and

DISCUSSION. The present study showed that the intraocular PSX fibers on and within the lens capsule and among the trabecular meshwork not only had
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PSX

CL

FIGURE 3. Staining of the pseudoexfoliative fibers (PSX) with WGA in the lid skin. Note the moderate labeling of the PSX fibers adjacent to collagen fibers (CL) (A). The label was located at the margin of the PSX fibers as seen with the intraocular PSX fibers (B). WGA = Wheat germ agglutinin. (A) Bar = 0.5 μm. (B) Bar = 0.2 μm.

UEA-I showed intense reaction with the PSX material. Hietanen et al5 reported that SBA gave a positive reaction. In this study, there was no binding with DBA, SBA, or UEA-I. We used lectin-gold complex as a cytochemical probe to localize glycoconjugates and performed low-temperature embedding with the hydrophilic resin Lowicryl K4M. In previous studies,4,5 lectin binding was studied by light microscopy on paraffin-embedded tissues. Paraffin embedding is known to cause the change of reactivities of tissues for lectin staining.6 Furthermore, it has been recognized that at low temperatures, tissue processing does not significantly alter the characteristics of molecules in the tissues, such as antigenicity.7 The difference in staining patterns observed in our study and others may be explained by the use of paraffin-embedded specimens or by different staining techniques.

The staining patterns of microfibrils on and within the lens capsule and capsular granular inclusions were similar to the PSX fibers with all the lectins used. Dark et al8 described a close association between these microfibrils and the PSX fibers. Our study confirmed that hypothesis in the view of the composition of glycoconjugates. Ocular zonules and zonular lamella had a similar but weak lectin binding added to their similarities, as Streeten et al reported.9 No binding was seen to the capsular granular inclusion in the control lens, which might indicate that the granular inclusions of capsular glaucoma have a different composition from granular inclusions from aging.

Because the lid skin is often exposed to sun, two types of elastosis—actinic and aging elastosis—were seen. In this study, PSX fibers and these elastoses were often intermingled. Because the staining patterns of the elastic fibers were not consistent, the elastotic process seemed to be accompanied by the change of glycoconjugates. Of interest were that some elastotic elastic fibers and atypical nondiagnostic fibers were stained like the PSX fibers, which prompted a certain relation between these fibers, as indicated previously.9

Changes in the content and distribution of glycoconjugates are often associated with pathologic processes. Lung cells do not stain with PNA unless treated with sialidase, whereas cells from patients with pneumonia stain abnormally with this lectin. Tumors of endothelial origin could be indicated by the intense staining of UEA-I, and invasiveness and metastatic capacity of melanoma may be represented by PNA reactivity.10 Therefore, some lectins are considered to be reliable markers for specific diseases. Our study suggested the possibility that the positive reaction of four kinds of lectin—PNA, RCA120, ConA, and WGA—is a diagnostic marker of PSX syndrome associated with the PSX fibers, both in the intraocular and extraocular sites. Consequently, it was indicated that lectins were useful probes in the identification of the PSX material and further detection of the extraocular PSX material in various organs.

FIGURE 4. Localization of WGA in the elastic fiber (E). The gold particles are scattered on the fiber and the surrounding microfibrils. WGA = Wheat germ agglutinin. Bar = 0.2 μm.
Key Words
skin, pseudoexfoliative material, lectin, electron microscopical histochemistry, glycoconjugates

References

Conjugacy of Spontaneous Blinks in Man: Eyelid Kinematics Exhibit Bilateral Symmetry
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Robert S. Baker,† Avrom D. Epstein,† and John D. Porter‡

Purpose. To provide a quantitative description of the conjugacy of human eyelid movements during spontaneous blinks.

Methods. Eyelid movements occurring during spontaneous blinks were recorded bilaterally using a modification of the electromagnetic search coil technique. In off-line analyses, covariation of amplitude, peak velocity, and duration of blink down phases were determined for the two eyelids. Interocular differences in the timing of blink onset and offset, and time to peak velocity, also were evaluated.

Results. Human blink motor control systems act to link tightly the spatial and temporal characteristics of movements of the two eyelids. Data show that human spontaneous blinks are conjugate. Analysis of interocular covariation of blink amplitude, peak velocity, and duration yielded linear functions with high correlation coefficients. Interocular comparison of eyelid movement durations during blinks showed a particularly high correlation. There were negligible interocular differences in blink down-phase onset time, termination time, and time to peak velocity. A small percentage of blinks exhibited interocular differences in amplitude and peak velocity of >20%; however, even in these cases, blink duration remained tightly linked.

Conclusion. Spatial and temporal properties of eyelid movements occurring during spontaneous blinks are conjugate. These data support the hypothesis that a bilateral gating mechanism regulates blink duration. Elements downstream from the gate may differentially and unilaterally alter blink amplitude and peak velocity, but the duration of blinks remains time-locked for the two eyelids. Invest Ophthalmol Vis Sci. 1994;35:3966–3971.

Human blinks are controlled by the reciprocal activity of two skeletal muscles, the levator palpebrae superiors and the orbicularis oculi (for review, see Schmidtke and Büttner-Ennever). To execute the smooth eyelid movements seen during blink, the activity in these antagonistic muscles is tightly coordinated. Blinking requires coordination between two brainstem cranial motor nuclei: levator palpebrae superioris motoneurons lie in the caudal central subnucleus of the oculomotor nerve, whereas orbicularis oculi motoneurons are in a subdivision of the facial nucleus. The central pathways responsible for the coordinated, reciprocal activity of levator palpebrae superiors and...