Binding of *Griffonia simplicifolia* I-B₄ Lectin in the Substantia Gelatinosa of the Rat Spinal Cord

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Histochemical distribution of sugar chains in the substantia gelatinosa of the dorsal horn in the rat spinal cord, where there are mainly small-diameter sensory neurons, was studied with labeled lectins used as a probe. Cryostat sections were prepared from the pre-fixed thoracic spinal cord. For light microscopy, the sections were stained with the biotinylated lectins followed by avidin-Cy3, and observed under a confocal laser scanning microscope. For electron microscopy, the sections were treated with horseradish peroxidase (HRP)-labeled lectins. Among more than 20 different kinds of lectins tested, GS I-B₄, *Griffonia simplicifolia* I-B₄, was shown light-microscopically to selectively bind to sites in nerve fibers of the substantia gelatinosa, and also to those on the surface of unmyelinated nerve fibers and Schwann cells in the dorsal root. Electron microscopy revealed that GS I-B₄ bound to the axolemma of the unmyelinated nerve fibers and microglial cell membranes in the substantia gelatinosa, and also to the axo-

lemma of the unmyelinated nerve fiber and to the surface layer of the myelinated nerve fiber in the dorsal root, i.e., to the Schwann cell membrane. On the other hand, GS I-B₄ binding was negative in the oligodendroglia except for the trans side of the Golgi complex.

The present results indicate that the carbohydrate structure containing Gal α 1-3Gal sequence and related sugar structures, which has a strong affinity for GS I-B₄, is distributed in the axolemma of the unmyelinated nerve fibers, in the microglial cell membranes in the substantia gelatinosa, and in the Schwann cell membranes in the dorsal root. A marked difference between the oligodendroglial cell membrane and the Schwann cell membrane was found in terms of the distribution of Gal α 1-3Gal-type sugar structures. Differences in these myelin-forming cells may have some relevance to their functions as well as to demyelinating disease processes selectively confined to the central or the peripheral nervous system.

Key words: *Griffonia simplicifolia* I-B₄ lectin, Substantia gelatinosa, Nerve fiber, Schwann cell membrane

I. Introduction

Lectin histochemical techniques have been widely used for the cytochemical detection of glycoconjugates on the cell surface, and in the cytoplasmic organelles of various tissues and organs including the central nervous system (CNS) [1, 4, 7–14, 16–23]. However, information about the distribution of glycoconjugates in neural tissue is not sufficient, especially in terms of its function and

relation to diseases.

Although more than 70 different kinds of lectins are now available, lectins successfully applied to the study of the nervous system are somewhat restricted in number [12]. Among them, isolectin I-B₄ from the seeds of *Griffonia simplicifolia* (GS I-B₄) has been reported to bind to small-diameter neurons in the somatic sensory ganglia [16]. From this characteristic of GS I-B₄, we decided to examine the substantia gelatinosa where there are mainly small-diameter sensory neurons. In addition, we also examined the dorsal root for comparison between the central and peripheral nervous tissues. Confocal laser scanning microscopy, as well as electron microscopy, was employed

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to elucidate the precise distribution of GS I-B₄ binding sites.

II. Materials and Methods

Animals and tissue preparation

Five female and six male Wistar rats weighing 250–400 g were used in this study. Under diethylether anesthesia, the rats were perfused transcardially with about 100 ml of 10 mM phosphate-buffered saline (PBS, pH 7.4) and then with 200 ml of 4% formaldehyde-PBS per rat at room temperature. The thoracic spinal cord was immediately dissected out, cut into pieces of 2 corpora vertebrae in length, and placed in 2.5% glutaraldehyde for 2 hr at 0°C. After having been washed with PBS, the thoracic spinal cords were soaked in 10% sucrose-10 mM PBS for 24 hr at 4°C and then in 20% sucrose-10 mM PBS for 48 hr at 4°C. Frozen sections (10 μ m thick) were cut with a cryostat and mounted on glass slides that had been pre-coated with neoprene and incubated at 37°C for at least 24 hr to secure their attachment to the slides.

Lectin staining for light microscopy

Cryostat sections mounted on glass slides were washed three times (10 min each) with PBS and treated with 1% bovine serum albumin (BSA)-PBS for 10 min; and they were then stained with biotinylated GS I-B₄ (Vector Lab. Inc., USA, 25 μ g/ml) for 1 hr at room temperature, washed three times (5 min each) with PBS, and incubated with avidin-Cy3 (Jackson Immunoresearch Lab. Inc., USA, 0.7 mg/ml) in 0.1% BSA-PBS for 30 min at room temperature. Nuclei were stained with SYBR Green I (Molecular Probes Inc., USA, 0.05 μ g/ml). After having been washed with PBS, the sections were observed under a laser scanning confocal microscope equipped with Ar/Kr ion lasers (MRC-1024, Bio Rad, UK).

Lectin staining for electron microscopy

Mounted cryostat sections were used as in light microscopy. Sections were washed three times (10 min each) with PBS and treated with 1% bovine serum albumin-PBS for 10 min, and then stained with horseradish peroxidase (HRP)-labeled GS I-B₄ (EY Lab. Inc., USA, 100 μ g/ml) overnight at 4°C. After having been washed three times (10 min each) with PBS, the specimens were immersed in 3,3'-diaminobenzidine solution (0.5 mg/ml in PBS) containing 0.005% H₂O₂ for 5 min at room temperature, and then washed with distilled water. Labeled specimens were post-fixed with 1% OsO₄ in 0.1 M phosphate buffer, dehydrated through a graded series of ethanol and propylene oxide, and then embedded in Epon. The ultrathin sections were observed under a transmission electron microscope (JEM-1010, JEOL, Tokyo, Japan) without post-staining.

III. Results

Light microscopic findings

GS I-B₄ was shown to bind exclusively to the substantia gelatinosa of the dorsal horn of the thoracic spinal cord and the dorsal root, whereas the white matter and the nucleus proprius cornu posterioris were negative (Fig. 1A). Under a magnified view of a part of the substantia gelatinosa, nerve fibers displayed GS I-B₄ sites having a dot-like appearance (Fig. 1B). On the other hand, in the dorsal root, the surface of the myelinated nerve fibers and the unmyelinated nerve fiber bundle were positive (Fig. 2A, B), but the axolemma of the myelinated nerve fibers was negative.

Electron microscopic findings

In accordance with the light microscopic findings, the axolemma of the unmyelinated nerve fiber in the substantia gelatinosa was positive for GS I-B₄ binding, whereas that of the myelinated nerve fibers was negative (Fig. 3). In addition, the microglial cell membrane was positive for GS I-B₄ sites (Fig. 4). In the oligodendroglia, the trans side of the Golgi complex was positive for GS I-B₄ binding, but the cell membrane was negative (Fig. 5). In the dorsal root, the axolemma of unmyelinated nerve fibers was positive for GS I-B₄ sites, but the axolemma of the myelinated ones was negative (Fig. 6). The Schwann cell membranes, including the nodal region of Ranvier were positively stained.

IV. Discussion

It has been known that GS I-B₄ specifically stained the sensory neurons [15, 16] and microglial cells [1, 14, 19] in some nervous tissues including the spinal cord. In the present study, we investigated detailed localization of GS I-B₄-positive sites in rat spinal cord by employing confocal laser scanning microscopy and transmission electron microscopy. GS I-B₄ specifically stained the axolemma of the unmyelinated nerve fibers in the substantia gelatinosa of the dorsal horn, and also the axolemma of the unmyelinated nerve fibers and the surface layer of the myelin sheath, i.e., the Schwann cell membrane, in the dorsal root. GS I-B₄ binding was also positive in the microglial cell membrane in accordance with previous reports [1, 14, 19]. The present results indicate that the Gal α 1-3Gal sequence and related sugar structures, possibly of glycoproteins having a strong affinity for GS I-B₄, are distributed on the axolemma of the unmyelinated sensory nerve fiber in the substantia gelatinosa of the spinal cord, on the Schwann cell membrane, and on the microglial cell membrane.

We detected a difference in the stainability of GS I-B₄ between the axolemma of the myelinated and unmyelinated nerve fibers. It may be possible that the non-stainability of the axolemma of myelinated nerve fibers in the electron microscopic study was due to the

Figs. 1, 2. Laser scanning confocal microscopic view of frozen sections of rat thoracic spinal cord. Stained with biotinylated *Griffonia simplicifolia* I-B₄ (GS I-B₄) in combination with avidin-3. Red, GS I-B₄ positive reaction; green, nucleus stained with SYBR green I.

Fig. 1. **A:** Dorsal horn. Substantia gelatinosa (SG) is strongly stained. White matter (W) and nucleus proprius cornu posterioris (NPCP) are negative. DR, dorsal root. $\times 30$. **B:** Magnified view of a portion of **A** (X). Nerve fibers show positive reaction with a dot-like appearance (arrows). $\times 300$.

Fig. 2. **A:** Dorsal root (DR). The surface layer of myelinated nerve fibers (arrows) and the unmyelinated nerve fibers (arrowheads) are positive for GS I-B₄ binding sites. $\times 50$. **B:** Magnified view of a portion of **A** (X). The surface layer (arrows) of myelinated nerve fibers (M) and the unmyelinated nerve fibers (U) are positive. S, Schwann cell. $\times 370$.

Figs. 3–5. Electron micrographs of the thoracic spinal cord stained with HRP-labeled GS I-B₄.

Fig. 3. The axolemma is positive in unmyelinated nerve fibers (U), whereas it is negative in myelinated ones (M). $\times 60,000$.

Fig. 4. The microglial cell membrane (arrow) is positive. There are three peroxisomes visible in the cytoplasm. $\times 27,000$. N, nucleus.

Fig. 5. The trans side of the Golgi complex (G) of this oligodendroglia is positive for GS I-B₄ binding, but the cell membrane is negative. The axolemma in unmyelinated nerve fibers (arrows) is positive, but negative in myelinated ones (M). $\times 15,000$. N, nucleus.

Fig. 6. Dorsal root. The Schwann cell membrane, the node of Ranvier (R) and the axolemma of unmyelinated nerve fibers are positive, but the axolemma of myelinated nerve fibers (M) is negative. S, Schwann cell nucleus. $\times 25,000$.

Fig. 3

Fig. 4

Fig. 5

Fig. 6

incomplete penetration of the labeled ligand. However, this possibility can be ruled out because light microscopy, which permits assessment of the stainability of the surface of the section, also revealed negative staining of the axolemma in cross sections of the myelinated nerve fiber. The difference in the stainability of GS I-B₄ between the axolemma of the myelinated and the unmyelinated nerve fiber should be due to the difference in the sugar chains on the surface of each axolemma.

In contrast to the positive staining of the Schwann cell membrane, oligodendroglial cells were negative for GS I-B₄ except in their trans-Golgi membranes. The differences in the stainability of GS I-B₄ between the oligodendroglial cell membrane and the Schwann cell membrane should be emphasized, because this is a newly detected difference between the central and peripheral myelin-forming cells. Some differences of these myelin-forming cells are already known. Namely, oligodendroglial cells are derived embryologically from spongioblasts in the neural tube, whereas Schwann cells differentiate from the cells of the neural crests, which are located laterally to the neural tube; although both the myelin-forming cells have an ectodermal origin [2]. Morphologically a single oligodendroglial cell can myelinate a segment of several axons in its vicinity, whereas a single Schwann cell myelinates a segment of only one axon [3]. Biochemically, the Schwann cell is known to produce Protein zero (P₀), a glycoprotein that comprises about 50% of myelin proteins in the peripheral nervous system [6]; whereas the oligodendroglial cell produces a proteolipid protein that represents more than 50% of the myelin proteins in the CNS [5].

GS I-B₄-binding carbohydrate structures containing Gal α 1-3Gal-type sugar residues may be glycoproteins other than P₀ glycoprotein, because the myelin itself was negative for GS I-B₄ staining. The above stated differences between Schwann cells and oligodendroglial cells, including our results, possibly show some different functions of these myelin-forming cells and also may have some bearing on the central versus peripheral demyelinating disease processes.

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VI. References

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