LECTIN BINDING SITES IN AXON-MYELIN-SCHWANN CELL COMPLEX

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The distribution of α -D-N-acetylgalactosamine residues on the membranes of axon-myelin-Schwann cell complexes of rabbit and rat sciatic nerves was visualized by means of a one-step method with HRP labelled soybean agglutinin. The compact myelin was found to be very rich in lectin binding sites whereas mesaxons and split myelin lamellae, as well as the Schwann cell plasma membrane were almost negative. The labelling of the axolemma was most pronounced in the nodal and paranodal regions. The smooth endoplasmic reticulum and mitochondria in axons and glial cells were also labelled.

The lectins are extensively used as histochemical markers because of their selective and direct binding to the monosaccharide residues of glycoproteins and glycolipids. It is well known that most proteins in the peripheral myelin are glycosylated (11). A visualization of Con-A binding sites in rat sciatic nerve myelin has been carried out (18). The soybean agglutinin (SBA) specifically recognizes the α -D-N-acetylgalactosamine (α -D-GalNAc) and to a lesser extent α -D-galactose (α -D-Gal) in the carbohydrate moieties of glycoconjugates (1). The aim of the present study was to establish and to compare the distribution of SBA binding sites in the different membranes of the axon-myelin-Schwann cell complex.

MATERIAL AND METHODS

The sciatic nerves of adult rabbits, and of 10 day-old rabbits and rats were studied. The nerves were removed under thiopental narcosis, cut into pieces 1–2 mm small and fixed in 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2, for 1 hr at 4°C. The samples were then cut with a razor blade into pieces under 0.5 mm each, washed in PBS and immersed in peroxidase-lectin solution in PBS (40 μ g/ml) for 45 min at room temperature with constant gentle agitation (8). Peroxidase-labelled lectin from Glycine max (Soybean) was obtained from Sigma (St. Louis, U.S.A.). After three changes in PBS, 5 min each, the samples were incubated in a solution of 0.06% 3,3'-diaminobenzidine and 0.01% hydrogen peroxide in 0.05 M Tris buffer, pH 7.6, for 20 min at room temperature in the dark (10). They were next washed several times in Tris buffer, postfixed in 1% OsO₄ in 0.1 M cacodylate buffer, pH 7.2, for 1 hr at 4°C, briefly dehydrated and embedded

in Durcupan ACM (Fluka, Switzerland). Ultrathin sections were cut with an LKB ultramicrotome and were examined unstained or slightly stained with lead citrate in a Hitachi H500 electron microscope. The controls included samples a) treated with peroxidase, type VI (Sigma, St. Louis, U.S.A.), 40 $\mu\text{g/ml}$ in PBS, for 45 min at room temperature without lectin and b) incubated only in the solution for demonstration of peroxidase according to Graham and Karnovsky (10) for exclusion of endogenous peroxidase.

RESULTS

A large amount of SBA was found associated with the compact myelin (Fig. 1). The major dense lines were labelled better than the intraperiod lines. In adult rabbits the membranes, split in the paranodal loops, as well as in the Schmidt-Lanterman clefts, were negative or showed only scant lectin binding sites (Figs. 2, 3, 4). In 10 day-old rabbits and rats these membranes were often markedly labelled (Fig. 5). The major dense lines were most heavily labelled in their terminal parts immediately before they split. The regions of the axo-glial contacts opposite the paranodal loops were also labelled (Fig. 2). A large amount of reaction deposits were found in the glial cytoplasm, evaginated in the paranodal myelin sheath (Fig. 3). As a rule no reaction deposits were found along the mesaxons and the peripheral and inner internodal lamellae, split off from the compact myelin (Figs. 6, 8). Single reaction deposits on these membranes were occasionally observed in 10 day-old rabbits and rats.

The lectin binding reaction was strongly manifested on the smooth reticulum of the axoplasm, especially in its nodal and paranodal regions. The axoplasmic cisternae were outlined or filled with reaction product. Mitochondria and lysosomes were also labelled. An intensive reaction was often seen on the sublemmal cisternae. Single reaction granules were scattered on neurofilaments and microtubules (Figs. 1, 7).

The axolemma of the myelin nerve fibers was slightly labelled by fine reaction granules (Fig. 9). In 10 day-old animals an accumulation of granules was associated with the axolemma at the site where the inner mesaxon begins (Fig. 8). The nodal and paranodal axolemma was better labelled than its internodal part (Fig. 10).

The Schwann cell cytoplasm exhibited a few lectin binding sites, associated with the membranes of the mitochondria and endoplasmic reticulum (Fig. 11). The paranodal cytoplasm was better labelled than the outer, perikaryonic cytoplasm. The Golgi apparatus was negative. The nuclear envelope of the Schwann cell was covered by reaction product (Fig. 12).

FIG. 1. The compact myelin (M) is strongly SBA labelled. (A)-axon. Reaction deposits on the axoplasmic reticulum profiles (large arrow), neurofilaments (small arrow) and axolemma (arrowhead). Adult rabbit. $\times 78,000$

FIG. 2. Paranodal region of myelinated axon. SBA binding sites on the major dense lines (large arrowhead) and the intraperiod lines (small arrowhead). An accumulation of reaction deposits in the terminal parts of the major dense lines (arrow) before they split in paranodal loops. The axo-glial contacts are also labelled (double arrow). Adult rabbit. $\times 170,000$

FIG. 3. Paranodal region of myelinated axon. An accumulation of reaction deposits (arrow) in the Schwannian cytoplasm, evaginated in the paranodal myelin sheath. Adult rabbit. $\times 170,000$

The axolemma and axoplasmic organelles of the nonmyelinated fibers were also well labelled (Fig. 12). Their mesaxons were usually negative.

Occasional very fine reaction granules were seen in contact with the glial plasma membrane and basal lamina. The reaction at this site was better manifested in 10 day-old rabbits and rats, especially in the nonmyelinated fibers and nodes of myelinated fibers. Fine reaction granules were seen on the periphery of the endoneurial collagen fibers (Figs. 11, 12).

The fibers in stages of promyelination and of initial myelination in 10 day-old rabbits and rats showed the same pattern of reaction: their mesaxons and loose myelin lamellae as a rule were negative, but the reaction appeared immediately after these membranes assembled to form compact myelin (Fig. 13).

There were no differences between the stained and unstained sections. Both controls were negative.

DISCUSSION

Lectins selectively bind to the carbohydrate residues of glycoproteins and glycolipids. It is well known that the most proteins in the PNS myelin are glycoproteins (11).

The strong labelling of the major dense lines, as well as the labelling of the intraperiod lines of the compact myelin may be due to the presence of α -D-GalNAc residues in some minor glycoproteins (11, 14) and glycolipids, including gangliosides (3, 13) available here.

Practically no α -D-GalNAc residues were detected in the mesaxons, split lamellae of the compact myelin, Schmidt-Lanterman clefts and paranodal loops in adult rabbits. All these membranes are considered to be myelin-forming ones (5, 6). It has been shown that the myelin-associated glycoprotein (MAG), immunocytochemically established in these membranes, prevents the fusion of the cytoplasmic leaflets of the glial membranes (16). Our data suggest that at least in adult rabbits, MAG does not possess α -D-GalNAc residues.

The positive reaction of the axolemma is related to its complex glycoprotein composition (9), including membrane bound enzymes of glycoprotein nature (4). The axoplasmic smooth reticulum and mitochondria are characterized by a very strong lectin binding capacity. These organelles are involved in the fast axoplasmic flow of numerous glycoproteins (12, 17). The SBA binding of neurofilaments and

FIG. 4. The compact myelin (M) is well labelled, whereas the Schmidt-Lanterman cleft (large arrow) is negative. An accumulation of reaction product adjacent the cleft (arrowhead). The axolemma (small arrow) is also labelled. Axon-(A). Adult rabbit. $\times 69,000$

FIG. 5. Single SBA binding sites on the membranes, split in the Schmidt-Lanterman cleft (arrowheads). An accumulation of reaction product on the terminal parts of the major dense lines (small arrow). Reaction deposits on the intraperiod lines (large arrow). 10 day-old rabbit. $\times 320,000$

FIG. 6. The inner mesaxon of the myelin sheath (M) is negative (arrow). Axon-(A). 10 day-old rabbit. $\times 85,000$

FIG. 7. Large amount of reaction product is associated with the membranes and partly the interior of the smooth axoplasmic reticulum (arrows). Single reaction deposits on neurofilaments and microtubules (arrowheads). Axon-(A), myelin sheath-(M). Adult rabbit. $\times 92,000$

FIG. 8. The outer (large arrow) and inner (small arrow) mesaxons are negative; an accumulation of reaction product on the axolemma opposite to the inner mesaxon. Single reaction on the split innermost and peripheral myelin lamellae (arrowheads). 10 day-old rat. $\times 78,000$

FIG. 9. Both leaflets of the internodal axolemma are slightly labelled by fine reaction deposits (arrow). Axon-(A). Adult rabbit. $\times 85,000$

FIG. 10. The nodal (arrow) and paranodal (double arrow) parts of the axolemma are well labelled. The myelin lamellae, split in paranodal loops, are negative (large arrow). Adult rabbit. $\times 92,000$

microtubules may be connected with glycoproteinous microvesicles transported by these cytoskeletal structures rather than with their own composition (7).

α -D-GalNAc dependent reaction was either absent or strongly reduced along the Schwannian plasmalemma. It can not be ruled out that these residues are inaccessible for the labelled SBA. In contrast to the axoplasmic organelles the SBA binding of the Schwannian organelles was rather scant.

In a cytochemical study of peripheral myelin Wood and McLaughlin (18) have observed Con-A binding sites only in the intraperiod lines of the compact myelin. Streit *et al.* (15) have not seen SBA reaction at the peripheral nodes of Ranvier using the light microscope.

The role of the glycoconjugates in cell-to-cell interactions and communication is well known (2). Taking into account the heavy labelling of the axoplasmic structures, the absent or strongly reduced reaction of the Schwannian plasmalemma and myelin-forming membranes, and its absence in the Golgi apparatus, one may speculate that glycoconjugates participate in the transfer of α -D-GalNAc from the axon to the myelin sheath.

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FIG. 11. The myelin sheath (M) exhibits many SBA binding sites. Single reaction deposits are connected with the mitochondria (large arrow) and endoplasmic reticulum profiles (small arrow) of the Schwann cell. Tiny reaction granules on the basal lamina (small arrowhead) and endoneurial collagen fibers (large arrowhead). 10 day-old rabbit. $\times 51,000$

FIG. 12. Nonmyelinated axons. Only the axolemmal part (arrowhead) of the simple axon-Schwann cell sheath of the axons is SBA labelled. The axoplasmic mitochondria (large arrows), reticulum profiles (small arrow) are also labelled. The outer membrane of the nuclear envelope is covered by reaction granules (double arrow). Nucleus of the Schwann cell-(N). Adult rabbit. $\times 34,500$

FIG. 13. Nerve fiber in an early stage of myelinization. Axon-(A). SBA binding sites on the compact myelin (M). The nonassembled membranes (arrow) of the myelin sheath are negative. 10 day-old rat. $\times 130,000$

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