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Glycoproteins in the whorls of membrane produced by oligodendroglia in culture

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Two glycoproteins of 99 kDa and 77 kDa which exhibit intense binding to wheat germ agglutinin have been purified from the whorls of membrane produced by oligodendroglia in culture. The whorls of membrane were isolated by gradient centrifugation from purified bovine oligodendroglia maintained in culture. The two glycoproteins were solubilized from the membranes using a non-ionic detergent and purified by Sephadex LH-60 chromatography, wheat germ agglutinin affinity chromatography, and SDS-polyacrylamide pore gradient gel electrophoresis. HPLC peptide mapping of the 99-kDa and 77-kDa glycoproteins revealed structural differences between the two proteins. Peptide mapping suggested that the 99-kDa glycoprotein from the whorls of membrane may be homologous to that from the plasma membranes. The 77-kDa glycoproteins from both sets of membrane may also be structurally related. Lectin binding studies showed that both glycoproteins from the whorls of membrane bound to wheat germ agglutinin, succinylated wheat germ agglutinin, concanavalin A, and lentil lectin, indicating the presence of high mannose and hybrid type oligosaccharide side-chains.

Introduction

In the central nervous system oligodendroglia produce massive amounts of membranes which form the highly compacted multilamellar myelin membranes. These membranes surround axons, enabling saltatory conduction of nerve impulses to occur. In culture, oligodendroglia produce whorls of membrane which have a similar density, enzyme, and lipid compositions as mature myelin, but have a more complicated protein pattern [1,2]. We believe that these whorls of membrane may be an early form of myelin which is further modeled to become myelin. These worls of membrane have two major glycoproteins that bind intensely to the lectin, wheat germ agglutinin. The two glycoproteins are not detected in mature compacted myelin; however, glycoproteins of similar molecular weights are found in the plasma membranes of oligodendroglia [2]. The glycoproteins from the plasma membranes have been purified and studied in earlier studies [3]. To determine whether the glycoproteins in the whorls of membrane are the same as those in the plasma membranes, they were

Abbreviations: HPLC, high-performance liquid chromatography; P-9-L, polyoxyethylene-9-lauryl ether; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; TLCK, N^{α}-*p*-tosyl-L-lysine chloromethyl ketone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; %T, polyacrylamide concentration defined as percentage of total monomers; $\%C_{Bis}$, percentage *N*,*N*′-methylenebisacrylamide crosslinker.

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purified from the whorls of membrane using affinity chromatography. Sensitive techniques useful for characterizing limited quantities of purified glycoproteins were necessary for these studies.

Experimental procedures

Materials

Acrylamide, *N,N'*-methylene bisacrylamide, high molecular weight SDS polyacrylamide gel electrophoresis standards, SDS, Affi-gel 10, 2-mercaptoethanol were purchased from Bio-Rad Laboratories. Na ^{125}I (Cat. No. IMS. 300) was obtained from Amersham. Wheat germ agglutinin, Trizma-Base, chloramine T, sodium bisulfite, TLCK-treated α-chymotrypsin (type VII), papain (type III), Sephadex LH-60-120, GlcNAc, PMSF, polyoxyethylene-9-lauryl ether, Triton X-100, neuraminidase (type V), Concanavalin A-Sepharose 4B (C-9017), *Tetragonolobus purpureas*-agarose (L-3257), *Dolichos biflorus* agarose (L-2007), *Lens culinaris* agarose (lentil) (L-2132), *Ricinus communis* agglutinin-120 agarose (L-2757) and *glycine max* agglutinin (soybean) agarose (L-1757) were from Sigma. Trifluoroacetic acid was purchased from Aldrich; *Staphylococcus aureus* V8 protease from Miles Scientific; low molecular weight electrophoresis standards from Pharmacia; ultrapure urea from Schwarz-Mann; XAR-5 X-ray film from Kodak; Lighting plus intensifying screens from Dupont; Iodobeads from Pierce; Dulbecco's phosphate-buffered saline (Cat. Nos. 310-4200 and 310-4080), from Gibco Laboratories; Centricon 30 miniconcentrators from Amicon; HPLC-grade acetonitrile from Burdick and Jackson. The HPLC column, HS-5 HCODS (Cat. No. 0258-0152) was purchased from Perkin-Elmer. Bovine brains were obtained from C.J. Schmidt and Co., Baltimore, MD.

Methods

Isolation and solubilization of oligodendroglial whorls of membrane lamellae. Oligodendroglia were purified from bovine white matter using bulk-isolation methods and maintained in culture 16–20 h as previously reported [1,4]. During this time oligodendroglia produced whorls of membrane lamellae, adjacent to the cell soma. These whorls of membrane were purified by sucrose gradient centrifugation [1]; two membrane fractions at 0.32 M sucrose and 0.5–0.6 M sucrose were combined, diluted with water, and concentrated by centrifugation at $100\,000 \times g$ for 45–60 min at 4°C in a Beckman Airfuge. The plasma membranes were found at 1.0 M sucrose [1]. The whorls of membrane pellets were solubilized in phosphate-buffered saline, containing 1% P-9-L and 1.0 mM PMSF, by sonicating the mixture in a Branson ultrasonic cleaning bath. The mixture was incubated 60 min at 4°C, prior to centrifuging at $100\,000 \times g$ for 45 min. The supernatant fraction contained the solubilized membrane glycoproteins.

Radioiodination of solubilized membrane glycoproteins. The solubilized glycoproteins were radioiodinated in the presence of Iodobeads, as previously described [3,5].

Purification of the two major glycoproteins. The solubilized radioiodinated glycoproteins from the whorls of membrane were subjected to Sephadex LH-60 chromatography, as described previously [3]. The void volume fractions containing the solubilized glycoproteins were recirculated 3–6 times through a wheat-germ agglutinin Affi-gel 10 column (0.5 × 3 cm). The two glycoproteins were eluted using either 1 M *N*-acetyl-D-glucosamine in phosphate-buffered saline (pH 7.4) or 1% SDS. The glycoproteins in the 1 M *N*-acetyl-D-glucosamine fraction were concentrated in a centricon 30 miniconcentrator. To concentrate the glycoproteins in the 1% SDS fraction, the fraction was lyophilized, resuspended in a minimal volume of water (with occasional heating), and mixed with 5–10 vol. of a −20°C acetone/ethanol mixture (1 : 1, v/v). The glycoproteins which precipitated in 1–2 h at −20°C were then centrifuged and prepared for SDS-gel electrophoresis [3].

SDS-polyacrylamide pore gradient gel electrophoresis. The glycoproteins were subjected to 5–12% SDS-polyacrylamide pore gradient gel electrophoresis as described previously [2,3,6]. Radiolabeled glycoproteins were visualized by autoradiography of the dried gels, using XAR-5 X-ray film and Lighting plus intensifying screens [2].

Electrophoresis on a second SDS-polyacrylamide gel. The 99 kDa and 77 kDa glycoproteins were identified on a 5–12% SDS-polyacrylamide pore

gradient gel by autoradiography, excised from the gel and prepared for a second electrophoresis step on an 8% SDS-polyacrylamide gel electrophoresis system [3]. The glycoproteins were identified by autoradiography.

Peptide mapping by limited proteolysis. The 99 kDa and 77 kDa glycoproteins were digested with either papain or α-chymotrypsin placed in the stacking gel (5% T and 1%C_{Bis}) before electrophoresis [7]. The peptides were then separated on a 17% T and 1%C_{Bis} SDS-polyacrylamide gel, as described previously [3].

HPLC peptide mapping. The radioiodinated whorls of membrane 99 kDa and 77 kDa glycoproteins were digested with 0.1% (50 μg/50 μl) TLCK-heated α-chymotrypsin for 8 h at 37°C. An additional 50 μg of enzyme was added and the digestion proceeded for 16 h [3]. The radioiodinated peptides were analyzed by reversed-phase HPLC as described [3]. Separation of the α-chymotrypsin peptides occurred, using a Perkin-Elmer HS-5 HCODS/PAH C_{18} column; fractions were eluted at 0.5 ml/min. The following conditions were used for the analysis: solvent A, 0.1% trifluoroacetic acid; solvent B, 0.1% trifluoroacetic acid in acetonitrile. The program was 15 min of solvent A; 60 min of a linear gradient of solvents A and B with solvent B (0–20%); 30 min of 20% solvent B; 60 min of a linear gradient with solvent B (20–100%); followed by re-equilibration using solvent A for 20 min. Fractions (1 min) were collected and analyzed for radioactivity.

Lectin affinity chromatography. Wheat germ agglutinin-agarose: Solubilized whorls of membrane glycoproteins were subjected to chromatography on wheat germ agglutinin, immobilized to Affi-gel 10 as described previously [3]. Concanavalin A-Sepharose 4B: Whorls of membrane glycoproteins were solubilized in 10 mM Tris-HCl (pH 7.5), 1% P-9-L, 1 mM $MnCl_2$ and then applied to a Con A-Sepharose 4B column, pre-equilibrated with sample buffer. After washing the column extensively with 10 mM Tris-HCl (pH 7.5) containing 1% P-9-L, and then with 10 mM Tris-HCl (pH 7.5), bound glycoproteins were eluted with 400 mM α-methyl glucopyranoside. Any glycoproteins not eluted with the specific sugar, were eluted in 2% SDS, 1% 2-mercaptoethanol, 10% sucrose, 0.125 M Tris-HCl (pH 6.8) at 100°C for 2 min. *Lens culinaris* lectin (lentil)-agarose: The whorls of membranes glycoproteins were solubilized in 50 mM Hepes (pH 7.5) containing 1% P-9-L, 0.15 M NaCl, 1 mM $MnCl_2$ and 0.25 mM PMSF [3,8,9]. After applying the solubilized glycoproteins to the lentil-lectin agarose column (pre-equilibrated with sample buffer), the column was first washed with 50 mM Hepes (pH 7.5) containing 1% P-9-L, and then with 50 mM Hepes (pH 7.5). Glycoproteins were eluted with either 400 mM α-methylmannoside or by incubating the gel with 0.05 M Tris-HCl (pH 6.8), 1% SDS, 3% 2-mercaptoethanol and 4% sucrose at 100°C for 2 min.

Binding to other lectins. The whorls of membrane glycoproteins were solubilized in phosphate-buffered saline (pH 7.4) containing 0.9 mM $CaCl_2$, and 0.5 mM PMSF. The solubilized mixtures were subjected to lectin affinity chromotography on either *Dolichos biflorus* agarose, soybean agglutinin-agarose, *lotus tetragonolobus* agarose, or *Ricinus communis* agglutinin-120 agarose columns [10,11]. After extensively washing the columns with solubilization buffer, followed by phosphate-buffered saline (pH 7.4) the glycoproteins were eluted using 0.2 M *N*-acetyl-D-galactosamine (*Dolichos biflorus* and soybean agglutinin), 0.2 M α-L-fucose (*Lotus tetragonolobus*), or 0.2 M D-galactose (*Ricinus communis* agglutinin-120). Any glycoproteins not eluted by the specific monosaccharide were eluted by heating the gels at 100°C for 2 min in SDS-sample buffer (0.05 M Tris-HCl (pH 6.8), 1% SDS, 3% 2-mercaptoethanol and 4% sucrose).

Lectin binding on nitrocellulose paper. Wheat germ agglutinin and succinylated wheat germ agglutinin were radioiodinated in the presence of *N*-acetyl-D-glucosamine by the Iodobead procedure, as described previously [3,5]. Peanut agglutinin was radioiodinated in the presence of D-galactose, and *Ulex europaeus* I was radioiodinated in the presence of α-L-fucose. The whorls of membrane glycoprotein were solubilized either in phosphate-buffered saline (pH 7.4) containing 1% P-9-L, or 1% SDS and subjected to separation by 8% SDS-polyacrylamide gel electrophoresis. Following electrophoresis, the glycoproteins were electrophoretically transferred onto nitrocellulose paper as described [3,12]. After transfer, the nitrocellulose paper was cut into strips and in-

cubated with ^{125}I-labeled lectins [3,13].

Neuraminidase treatment. The whorls of membrane lamellae were solubilized in 50 mM sodium acetate, pH 5.0, containing 1% P-9-L. After solubilization, 30 mU of *Clostridium perfringens* neuraminidase (type V) were added to the solubilized whorls of membrane glycoproteins which were incubated for 16 h at 25 °C [3].

Results

The radioiodinated solubilized whorls of membrane glycoproteins were applied onto a Sephadex LH-60 column to remove free ^{125}I and lipids. The column was washed with phosphate-buffered saline (pH 7.4) containing 1% P-9-L. The void volume fractions (data not shown) were applied onto a wheat germ agglutinin Affi-gel 10 column which had been previously equilibrated in phosphate-buffered saline (pH 7.4) containing 1% P-9-L. After washing the column to remove non-specifically bound material, the glycoproteins were eluted in sequential order with 1.0 M *N*-acetyl-D-glucosamine and 1% SDS [3]. Approx. 98% of the Sephadex LH-60 void volume fraction did not bind to the wheat germ agglutinin column (Table 1); 70% of the bound material was eluted with 1 M *N*-acetyl-D-glucosamine and 1% SDS.

The SDS fractions and 1 M *N*-acetyl-D-glucosamine fractions were separated on an 5–12% SDS polyacrylamide gradient slab gel. The two glycoproteins were visualized by autoradiography and subsequently applied to an 8% SDS-polyacrylamide slab gel. The gel was dried and the purified glycoproteins were visualized by autoradiography. Densitometric scans of the autoradiogram confirmed the presence of a single band for each glycoprotein (Fig. 1).

To determine whether the glycoproteins had a high content of sialic acid, the solubilized glycoproteins were treated with neuraminidase. The glycoproteins were separated by electrophoresis, transferred to nitrocellulose, and incubated with ^{125}I-labeled wheat germ agglutinin. Untreated glycoproteins were analyzed as a control. There were no differences in molecular weights between the untreated glycoproteins and the neuraminidase-treated glycoproteins (data not shown).

The structural relatedness between the 99-kDa glycoproteins in the whorls of membrane and plasma membranes were examined by limited proteolysis using α-chymotrypsin (Figs. 2A and 2B). The fragmentation patterns resulting from limited digestion of the 99-kDa glycoproteins by α-chymotrypsin were very similar, suggesting homology in their primary structures. Four to five major peptides [1–4] were present in each peptide map (Figs. 2A and 2B). Peptide maps generated by α-chymotrypsin digestion of the 77-kDa glycoproteins from the two sets of membranes were also remarkably similar, suggesting homology be-

TABLE I

FRACTIONATION OF THE WHORLS OF MEMBRANE RADIOLABELED COMPONENTS ON WHEAT GERM AGGLUTININ AFFI-GEL 10 AFFINITY COLUMN

The Sephadex LH-60 void volume fraction was subjected to wheat germ affinity chromatography, as described in Experimental Procedures. Bound material was eluted with 1 M *N*-acetyl-D-glucosamine (GlcNAc) and 1% SDS.

Fractions	% of recovered material
Non-binding material	98.2
1.0 M GlcNAc eluting material	0.06
1% SDS eluted fraction	1.27
Remaining on gel	0.55

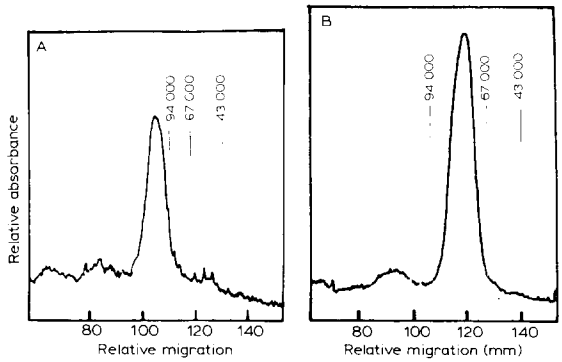

Fig. 1. Homogeneity of the purified glycoproteins. Electrophoresis on a second SDS-polyacrylamide slab gel to determine the apparent homogeneity of the 99-kDa and 77-kDa glycoproteins purified from the whorls of membrane. The glycoproteins were identified on a 5–12% polyacrylamide pore gradient slab gel and excised from the gel. The gel slices were subjected to 7% SDS-polyacrylamide gel electrophoresis as described in Experimental Procedures. The figure depicts the densitometer tracing of the autoradiogram: (A) 99-kDa glycoprotein; (B) 77-kDa glycoprotein.

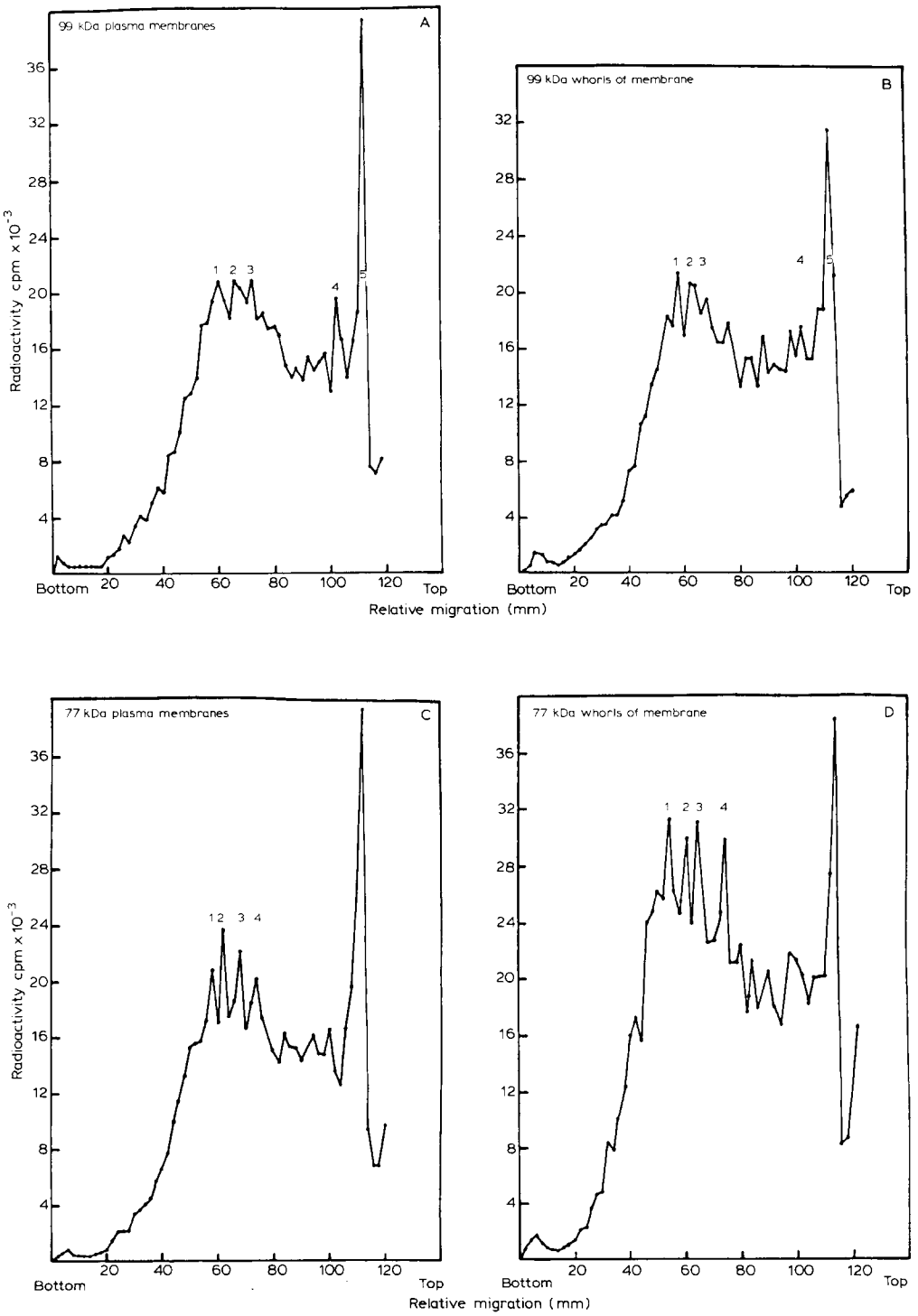

Fig. 2. Peptide mapping using limited digestion with α-chymotrypsin. The 99-kDa and 77-kDa glycoproteins from the whorls of membrane and the plasma membranes were subjected to α-chymotrypsin digestion and the peptide maps were generated. (A) Plasma membrane 99-kDa glycoprotein; (B) whorls of membrane 99-kDa glycoprotein; (C) plasma membrane 77-kDa glycoprotein; (D) whorls of membrane 77-kDa glycoprotein.

tween these two proteins (Figs. 2C–D). The peptide maps of the 77-kDa glycoproteins also contained four major peptides, along with several other peptides. If the four maps of peptides were compared (Figs. 2A–D), similarities in the fragmentation patterns were found, suggesting that the 99-kDa and 77-kDa glycoproteins may have similar α-chymotrypsin-sensitive cleavage sites in the primary structures.

The relatedness between the 99-kDa glycoproteins in both sets of membranes was also examined by limited proteolytic digestion using papain (Figs. 3A and 3B). Both peptide maps had five similar peptide fragments, suggesting again that the two 99-kDa glycoproteins were structurally related.

Reversed-phase HPLC was used to compare the structures of the 99-kDa and 77-kDa glycoproteins in the whorls of membrane. The glycoproteins were digested with α-chymotrypsin; the separation by HPLC is presented in Figs. 4A and 4B. Major differences were found in the peptides that eluted in the 35–45 ml area in the two HPLC peptide maps; e.g., peptides I, II, and III of the 77-kDa glycoprotein (Fig. 4) and peptides I, II and III of the 99-kDa glycoprotein in Fig. 4A were different. Peptide IV was missing from the HPLC peptide map of the 77-kDa glycoprotein.

TABLE II

INTERACTION OF LECTINS WITH THE 99-kDa AND 77-kDa GLYCOPROTEINS IN THE WHORLS OF MEMBRANE

(A) Binding to immobilized lectins

	Binding
Wheat germ agglutinin agarose	+
Concanavalin A-Sepharose	+
Lens culinaris agarose	+
Ricinus communis agglutinin agarose	–
Lotus tetragonolobus agarose	–
Dolichos biflorus agarose	–
Glycine max agglutinin agarose	–

(B) Binding to radiolabeled lectins

	Binding
Wheat germ agglutinin	+
Succinylated wheat germ agglutinin	+
Ulex Europaeus I	–
Arachis hypogae	–

A combination of lectin affinity chromatography and radiolabeled lectin binding was used to characterize the carbohydrate moieties on the glycoproteins. The two glycoproteins from the whorls of membrane bound to wheat germ agglutinin, concanavalin A, and lentil lectin columns (Fig. 5).

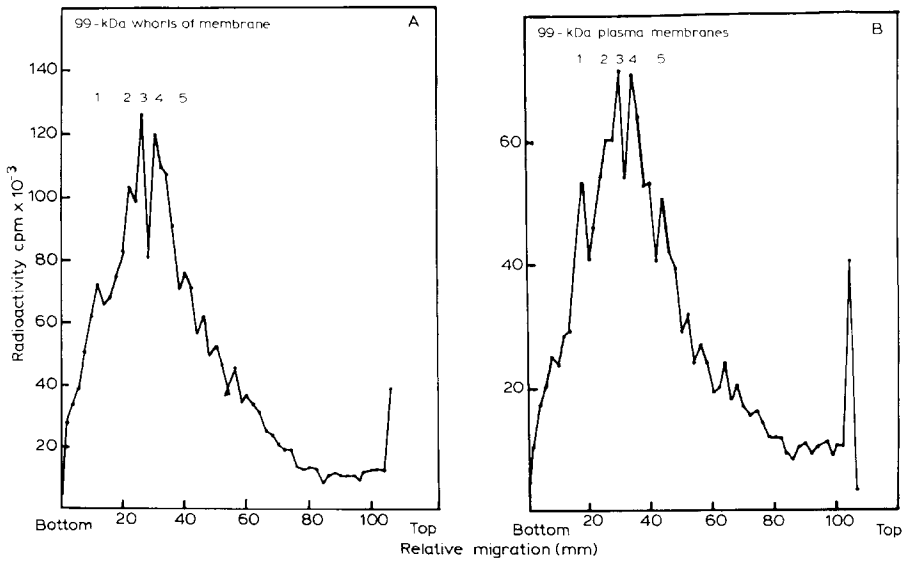

Fig. 3. Peptide mapping using limited digestion with papain. (A) Whorls of membrane, 99-kDa glycoprotein; (B) plasma membrane, 99-kDa glycoprotein.

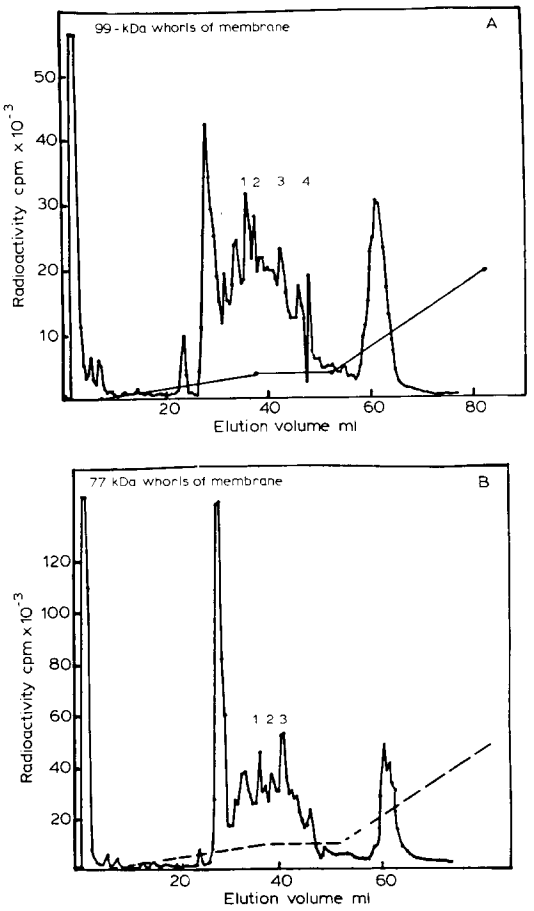

Fig. 4. HPLC peptide mapping of α-chymotrypsin digests of the whorls of membrane 99-kDa and 77-kDa glycoproteins. The gel slices containing the 99-kDa and 77-kDa glycoproteins were incubated with α-chymotrypsin as described in Methods. The peptides were solubilized in 0.5% trifluoroacetic acid and subjected to reversed-phase C_{18} HPLC. (A) HPLC profile of α-chymotryptic digest of the 99-kDa glycoprotein; (B) HPLC profile of α-chymotryptic digest of the 77-kDa glycoprotein.

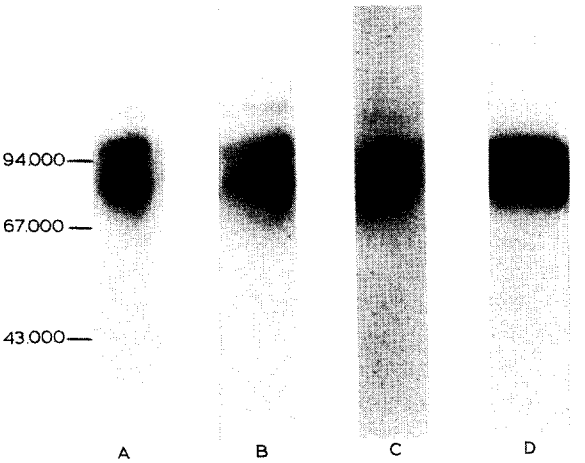

Fig. 5. Lectin binding to the 99-kDa and the 77-kDa glycoproteins from the whorls of membrane. The whorls of membrane were solubilized and subjected to lectin affinity chromatography (lanes 1, 2, 3) as described in Methods. The glycoproteins were eluted from the lectin columns, using both the specific monosaccharide and SDS. They were separated using 8% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose paper, and detected using ^{125}I-radiolabeled wheat germ agglutinin (lanes 1–3). Alternatively the glycoproteins were solubilized in SDS sample buffer, separated on an 8% polyacrylamide slab gel and transferred to nitrocellulose paper (lane 4). The nitrocellulose paper containing the separated glycoproteins was incubated with ^{125}I-labeled succinylated wheat-germ agglutinin as described previously. Lectin binding to the glycoproteins was visualized by autoradiography. (A) Wheat-germ agglutinin affinity chromatography; (B) concanavalin A affinity chromatography; (C) lentil lectin affinity chromatography; (D) binding of radiolabeled succinylated wheat germ agglutinin.

The two glycoproteins did not bind to *Lotus tetragonolobus* agarose, *Dolichos biflorus* agarose, *Ricinus communis* agglutinin 120-agarose, or soybean agglutinin agarose columns (data not shown). Lectin binding studies revealed that the two glycoproteins bound to ^{125}I-labeled wheat germ agglutinin and ^{125}I-labeled succinylated wheat germ agglutinin (Fig. 5). The two glycoproteins did not bind to radiolabeled *Ulex europaeus* or *Arachis hypogae* (peanut agglutinin) (Table II).

Discussion

Previous investigations from this laboratory indicated that two glycoproteins in oligodendroglia exhibited intense binding to radiolabeled wheat germ agglutinin [2]. These glycoproteins were present in both the plasma membranes and in the whorls of membrane lamellae produced by oligodendroglia in culture. They are not present in purified compacted myelin. We wanted to explore whether these glycoproteins in the two different membrane fractions had any homology in their primary structures.

Results obtained by limited proteolytic digestion with α-chymotrypsin of the 99-kDa glycoprotein in the whorls of membrane and the

plasma membranes suggested that they are structurally similar glycoproteins (Fig. 2). Proteolytic digestion with papain further supported this observation. The 77-kDa glycoproteins in both sets of membranes appear to also be homologous.

HPLC peptide mapping revealed that the 99-kDa and the 77-kDa glycoproteins in the whorls of membrane lamellae are probably different proteins (Fig. 4), as the maps are quite different. This is similar to the finding that the two glycoproteins purified from plasma membranes were also different from each other [3].

As wheat germ agglutinin is specific for *N*-acetyl-D-glucosamine and sialic acid residues, while succinylated wheat germ agglutinin binds only to *N*-acetyl-D-glucosamine residues, the binding of the two glycoproteins is probably due to both the oligosaccharide and sialic acid moieties. Since wheat germ agglutinin has been shown to bind glycopeptides with hybrid-type structures and a bisecting *N*-acetylglucosamine residue [14], one may predict that the two glycoproteins in the whorls of membrane may contain hybrid-type oligosaccharides and/or an oligosaccharide with a bisected *N*-acetyl-D-glucosamine residue. The glycoproteins in the whorls of membrane also bind intensely to concanavalin A and to lentil lectin, suggesting glycopeptides with certain biantennary complex-type, hybrid-type, and high-mannose oligosaccharides.

The glycoproteins in the whorls of membrane did not bind to *Ricinus communis* agglutinin I (specific for terminal D-galactose and the galactosyl β1,4-*N*-acetylglucosamine sequences) or peanut agglutinin (specific for terminal D-galactose and the galactosyl β1,3-*N*-acetylgalactosamine sequences on O-linked sugars) [11,15]. Neither the 99-kDa or the 77-kDa glycoproteins appear to have these terminal sequences. The glycoproteins in the whorls of membrane also did not bind to soybean agglutinin or *Dolichus biflorus* indicating that there are probably no terminal *N*-acetylgalactosamine residues available [10,16–18]. Furthermore, they did not bind to the α-L-fucose binding lectins, *Lotus tetragonolobus* or *Ulex europaeus* I, although steric inaccessibility may be a factor in this lack of binding [11,18].

Other membrane glycoproteins purified from brain have included Thy-1 antigen which binds to lentil lectin [19,20]; rat brain acetylcholinesterase which binds to lentil lectin, wheat germ agglutinin, and *Ricinus communis* agglutinin [21]; and the nicotinic acetylcholine receptor which binds to concanavalin A, wheat germ agglutinin, and to a lesser extent to *Ricinus communis*, but not to the fucose-binding protein (*Lotus tetragonolobus*) [22]. These glycoproteins have in common with the whorls of membrane glycoproteins the high mannose type and possibly the bisecting hybrid type of oligosaccharides. Two brain glycoproteins of approx. 100 kDa have been purified from synaptic junctional complex (glycoprotein 110) and from myelin (the myelin-associated glycoprotein) [23, 24]. The former glycoprotein binds to wheat germ agglutinin, but binding is considerably reduced after neuraminidase treatment; this is different from our findings with the oligodendroglial glycoproteins. As there is no binding of wheat germ agglutinin to glycoproteins in purified myelin in the 100 kDa range, the glycoproteins in oligodendroglia appear to be different from those in myelin.

The results obtained by SDS-polyacrylamide gel electrophoresis, peptide mapping, and lectin-binding experiments with wheat germ agglutinin and succinylated wheat germ agglutinin indicate that the glycoproteins of 99-kDa and 77-kDa from the whorls of membrane lamellae and those from the plasma membranes have several biochemical properties in common. More definitive studies with larger quantities of these glycoproteins are necessary to unequivocally prove that the glycoproteins of similar molecular weights from the two sets of membranes are identical. The role that these two glycoproteins play during the course of myelination is unclear at this time.

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