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Purification and characterization of two glycoproteins from oligodendroglial plasma membranes

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Two major glycoproteins of 99 kDa and 77 kDa have been purified from oligodendroglial plasma membranes. These two glycoproteins exhibit intense binding to the lectin, wheat germ agglutinin. The 99-kDa and 77-kDa glycoproteins were purified by Sephadex LH-60 chromatography, wheat germ agglutinin affinity chromatography and SDS-polyacrylamide pore gradient gel electrophoresis. Re-electrophoresis of excised gel slices containing the two glycoproteins demonstrated their apparent homogeneity. The isoelectric points of the 99-kDa and 77-kDa glycoproteins were 6.15 and 6.00, respectively. Peptide mapping revealed structural differences between the two glycoproteins. Lectin binding studies with radiolabeled succinylated wheat germ agglutinin demonstrated that the binding of the 99-kDa and 77-kDa glycoproteins to wheat germ agglutinin was due to *N*-acetyl-D-glucosamine residues in the oligosaccharide side-chains.

Introduction

Only a handful of the major glycoproteins in brain have been purified and characterized thus far, due to their limited abundance. Brain membranes are as enriched in glycoproteins as are membranes from other tissues. These glycoproteins have been implicated in various reactions involving the cell surface such as neuronal-glial interactions and neuronal recognition sites, as well as serving as receptors for neurotransmitters, drugs, hormones, viruses, and possibly even for immune surveillance. A glycoprotein enriched on the surface of one cell type in brain may never be visualized by our sensitive electrophoretic techniques if whole brain is being studied, due to the complexity of brain as a tissue. Brain is composed of several different cell types (neurons, astrocytes and oligodendroglia), all with long branching processes and intimate interconnections. In addition, the multilamellar compacted myelin membranes, which surround axons, enabling saltatory conduction of nerve impulses to occur, comprise about 50% of the mass of white matter. All of these cell structures and membranes add to the biochemical challenge of purification of specific components from brain. Nonetheless, several major glycoproteins from brain have been purified and characterized; these include those from the synaptic junctional complex [1], Thy-1 [2], N-Cam [3], NILE [4] and the myelin-associated glycopro-

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

tein [5]. Most of these glycoproteins constitute less than 1% of the total protein found in brain.

Recently we have identified two major glycoproteins present on the plasma membranes of oligodendroglia, the cell type that produces and maintains the myelin membrane during its lifetime [6]. These glycoproteins are not evident upon inspection of total white matter or gray matter glycoproteins; they are also not apparent in myelin or in neuronal plasma membrane glycoproteins (S.E. Poduslo, M.T. Filbin and D. Hampson, unpublished data). That these glycoproteins may be functionally significant is indicated by their presence in myelin and membranes purified from a child with a demyelinating disease, metachromatic leukodystrophy [7]. The presence of these two glycoproteins during the diseased state suggests that an alteration in the gene expression of oligodendroglia during active myelin synthesis has occurred. To determine whether the glycoproteins present in the diseased tissue are similar to those in oligodendroglial plasma membranes and whether they may play a role in demyelination necessitates their purification and characterization. Oligodendroglial plasma membranes were used as the starting material for the isolation of the glycoproteins, due to their enrichment in these membranes. Techniques suitable for characterizing limited quantities of purified glycoproteins have been employed in these studies.

Experimental procedures

Materials

Bovine brains were obtained from C.J. Schmidt and Co., Baltimore, MD. Polyacrylamide, *N,N'*-methylenebisacrylamide, high molecular weight SDS-polyacrylamide gel electrophoresis standards, Bio-Lyte ampholytes 3/10, and 2-mercaptoethanol were purchased from Bio-Rad Laboratories. $Na^{125}I$ was obtained from Amersham. Wheat germ agglutinin, Trizma Base, chloramine T, sodium bisulfate, TLCK-treated α-chymotrypsin (type VII), papain (type III), Sephadex LH-60-120, GlcNAc, PMSF, isoelectric focusing standards, polyoxyethylene-9-lauryl ether, Triton X-100, and neuraminidase (type V) were from Sigma. Trifluoroacetic acid was purchased from Aldrich; *Staphylococcus aureus* V8 proteinase from Miles Scientific; low molecular weight electrophoresis standards and isoelectric focusing standards (broad p*I* kit: 3–10) from Pharmacia; ultrapure urea from Schwarz-Mann; XAR-5 X-ray film from Kodak; Cronex intensifying screens from Dupont; Iodobeads from Pierce; Dulbecco's phosphate-buffered saline, cat. No. 310–4200, 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.

Methods

Iodination and solubilization of oligodendroglial plasma membranes. Oligodendroglia were purified from bovine white matter and maintained in culture overnight as previously reported [8]. The plasma membrane fraction was purified by sucrose gradient centrifugation [9], and was concentrated by centrifugation at about $100\,000 \times g$ for 60 min at 4°C in a Beckman Airfuge. The pellets were radiolabeled by incubation in 200 μl of 0.1 M phosphate buffered saline (pH 7.4), 50 μl of 0.50 M sodium phosphate (pH 7.4), 1 mCi of carrier-free $Na^{125}I$ (300–600 mCi/ml) and 3 Iodobeads [10,11] for 15–20 min at 25°C. The reaction was terminated by dilution 100-fold in 0.1 M phosphate buffered saline (pH 7.4), and centrifuged at $75\,000 \times g$ for 210 min.

The radiolabeled plasma membrane pellet was solubilized in 0.1 M phosphate buffered saline (pH 7.4) containing 1% P-9-L and 1 mM PMSF, incubated overnight at 4°C, and then centrifuged at about $100\,000 \times g$ for 60 min at 4°C. The supernatant fraction was collected and assessed for radioactivity in a Searle model 1197 automatic gamma counter. For some experiments, the purified plasma membranes were solubilized and the proteins radiolabeled as described. The reaction was terminated by removal from the Iodobeads.

LH-60 chromatography of radioiodinated plasma membranes. Aliquots of 200–300 μl of radiolabeled solubilized plasma membrane proteins were applied onto an LH-60 column (1.0×50 cm), previously equilibrated in 0.1 M phosphate buffered saline (pH 7.4) containing 1% P-9-L. The solubilized proteins and glycoproteins were eluted by gravity, collected in 1-ml fractions, and the

fractions were assessed for radioactivity as described.

Lectin affinity chromatography. Wheat germ agglutinin was coupled to Affi-Gel 10 in 0.1 M Hepes (pH 7.5) for 24 h at 4°C, according to the Bio-Rad instructions. The immobilized gel contained 5–10 mg of wheat germ agglutinin per ml of packed gel and was stable for at least 3 months.

The void volume fractions from the LH-60 column, as determined by radioactivity measurements, were pooled and applied onto the wheat germ agglutinin Affi-Gel 10 column (0.5 × 3 cm), previously equilibrated in 0.1 M phosphate buffered saline (pH 7.4) containing 1% P-9-L. Several passes of the LH-60 void volume fraction through the wheat germ agglutinin affinity column were performed during a 60–90 min period at 25°C.

Non-binding material was removed from the affinity column by washing first in 0.1 M phosphate buffered saline containing 1% P-9-L, and then with 0.1 M phosphate buffered saline (pH 7.4). When the radioactivity of the eluted fractions reached background levels, bound glycoproteins were eluted with 1 M GlcNAc in 0.1 M phosphate buffered saline (pH 7.4) containing 1 mM PMSF. Glycoproteins not eluted with 1 M GlcNAc were eluted with 1% SDS.

The 1 M GlcNAc-eluted fractions were ultrafiltered and concentrated to 40–100 μl by use of a Centricon 30 miniconcentrator. The 1% SDS fractions were pooled, lyophilized, resuspended in a minimal volume of water (with occasional heating), and treated with 5 vol. of a cold acetone/ethanol mixture (1 : 1, v/v). After overnight incubation at 4°C, the precipitated material (containing the glycoproteins) was centrifuged in a Beckman microfuge for 3 min and dissolved in a minimal amount of water in preparation for electrophoresis.

SDS-polyacrylamide pore gradient gel electrophoresis. Oligodendroglial plasma membranes, solubilized in either 1% P-9-L or 10% SDS, were subjected to SDS-polyacrylamide pore gradient gel electrophoresis with use of either a 7–25% or 5–15% linear gradient gel of 0.75 mm thickness [12]. After electrophoresis, the proteins were fixed and were either stained with Coomassie blue or silver, or used in lectin binding studies [6,13].

Radiolabeled glycoproteins were visualized by autoradiography of the dried gels, using XAR-5 X-ray film and Lighting Plus intensifying screens [6,7]. Exposure time varied between 2 h and several days at −40°C.

Electrophoresis on a second SDS-polyacrylamide gel. The 99-kDa and 77-kDa glycoproteins were identified on 5–15% SDS-polyacrylamide pore gradient gels by autoradiography, and incubated in 0.125 M Tris-HCl (pH 6.8) containing 0.1% SDS and 1% 2-mercaptoethanol for 30 min. The gel slices and bromophenol blue marking dye were placed onto the stacking gel of a 1.5 mm 7.5% polyacrylamide vertical slab gel. Molecular weight markers were added to adjacent wells, and electrophoresis was allowed to proceed until the dye reached the bottom of the gel. The gel was then fixed, stained, destained, dried and subjected to autoradiography, as described.

Isoelectric focusing. The procedure for isoelectric focusing of the glycoproteins previously separated by electrophoresis was essentially that of Guilian et al. [13,14] with several modifications. The IEF gel (1.5 mm) consisted of 5.5% T, 2.6% C_{Bis}, 2% Triton X-100, 7 M urea, 10% glycerol and 2% ampholytes, Bio-Lytes 3/10. The gel slices were incubated for 30–60 min in IEF buffer (2% Triton X-100, 4 M urea, 2% Bio-Lytes 3/10 and 10% glycerol) and then placed into wells of the IEF gel (10-comb well). Pharmacia IEF standards (pH 3–10) and Sigma standards (lactate dehydrogenase and myoglobin) were used to determine the pH gradient of the gel. Electrophoresis was initiated at 150 V, constant voltage for 30 min, after which the voltage was increased to 800 V. Electrophoresis was allowed to proceed until 3000–4000 V/h were reached.

Peptide mapping by limited proteolysis. The 99-kDa and 77-kDa radiolabeled glycoproteins were separated by electrophoresis and reiodinated in the gel slices before peptide mapping. Unbound radiolabel was removed according to established procedures [15,16]. For peptide mapping, the gel slices were washed for 15 min with 0.125 M Tris-HCl (pH 6.8) containing 0.1% SDS, and were placed on the bottom of the stacking gel well with the appropriate digestive enzyme [17,18]. When the bromophenol blue dye front reached the end of the stacking gel (5% T and 1% C_{Bis}), the power was interrupted, but the temperature was main-

tained at 20°C. After 1 h, the power was turned on and electrophoresis proceeded, but the temperature was lowered to 4°C. After electrophoresis, the gel (15% T and 2.6% C_{Bis}) was stained with Coomassie blue, destained and dried. Each gel lane was cut into either 1-mm or 2-mm slices which were assessed for radioactivity.

HPLC peptide mapping. The radiolabeled reiodinated gel slices of the 99-kDa and 77-kDa glycoproteins were washed extensively in 10% methanol, lyophilized, and suspended in 450 μl of 0.05 M NH_4HCO_3 (pH 7.8). TLCK-treated α-chymotrypsin (50 μg in 50 μl of 0.05 M NH_4HCO_3, pH 7.8) was added and the samples were incubated for 8 h at 37°C; an additional 50 μg of α-chymotrypsin were added, and the incubation was allowed to proceed for 16 h. The supernatant fraction was removed, and the gel was washed for 2 h with 0.50 ml of 0.05 M NH_4HCO_3 (pH 7.8). The supernatant fractions were pooled and lyophilized. The lyophilized material was suspended in 200 μl of 0.5% trifluoroacetic acid in H_2O (v/v) and centrifuged for 2 min in a Beckman microfuge; 175 μl of the clarified supernatant fraction were injected onto a Perkin Elmer HS-5 HCODS/PAH C_{18} column and eluted at 0.5 ml/min. The following conditions were employed for reversed phase HPLC analysis: solvent A: 0.1% trifluoroacetic acid; solvent B: 0.1% trifluoroacetic acid in acetonitrile. The program was 25 min of solvent A; 35 min of a linear gradient of solvents A and B with solvent B (0–20%); 20 min of 20% solvent B; 40 min of a linear gradient with solvent B (20–80%); 10 min of solvent B; followed by re-equilibration using solvent A for 20 min. Fractions (1 min) were collected and analyzed for radioactivity.

Lectin binding. Wheat germ agglutinin and succinylated wheat germ agglutinin were radioiodinated by the procedure using Iodobeads and were extensively dialyzed. Aliquots of radiolabeled wheat germ agglutinin (spec. act. 70000–100000 cpm per μg lectin) were stored at −40°C. Alternatively, radiolabeled wheat germ agglutinin was affinity-purified with the use of ovomucoid-Sepharose 4B [19]. Radiolabeled lectin binding was performed on the slab gels of the separated glycoproteins [5].

Lectin binding on transfers. After electrophoresis, the proteins were electrophoretically transferred onto nitrocellulose paper using 50 mM Na_2HPO_4 (pH 5.5), 2 mM EDTA and 0.05% SDS as the transfer buffer (unpublished data), at 1.6 amp for 3 h at 4°C for two gels. After transfer, the nitrocellulose paper was cut into strips and soaked in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8.1 mM KH_2PO_4, 1.47 mM Na_2HPO_4), containing 2% polyvinylpyrrolidone (Ref. 19 and unpublished data). Aliquots containing the radiolabeled lectins (wheat germ agglutinin or succinylated wheat germ agglutinin) were filtered through a Millipore GS 0.22 μm filter into 50–100 ml of 2% polyvinylpyrrolidone in phosphate buffer. The nitrocellulose strips were added and incubated for 4–6 h at room temperature, dried and subjected to autoradiography. Sialic acid was removed from glycoproteins immobilized on nitrocellulose by incubating the strips with 20 mU of *Clostridium perfringens* neuraminidase (type V) for 16 h at 25°C in 50 mM sodium acetate (pH 5.0) (Worthington). Alternatively, sialic residues were removed from the glycoproteins by incubating the nitrocellulose strips in 0.05 N H_2SO_4 at 80°C for 1 h [20].

Other methods. Myelin was purified from bovine brain subcortical white matter, according to our established procedures [21]. Proteins were determined by the method of Lowry et al. [22].

The molecular weights of the glycoproteins were determined by comparison of their electrophoretic mobility with known standards. The glycoproteins on the slab gels or X-rays were scanned on a Shimadzu CS 930 densitometer, and the molecular weights determined by evaluating the relationship of the log (M_r) versus log (%T) for the molecular weight standards, using a least-squares linear regression analysis [12].

Results

Solubilization and identifiation of the 99-kDa and 77-kDa glycoproteins

Many detergents were screened for their efficiency in solubilizing the oligodendroglial plasma membranes. Besides SDS, the only effective nonionic detergent was P-9-L (critical micelle concentration, 100 μM [23]). Over 90% of the radiolabeled plasma membrane components were

TABLE I

SOLUBILIZATION OF RADIOIODINATED OLIGODENDROGLIAL PLASMA MEMBRANES

Radiolabeled plasma membranes were solubilized in 1% P-9-L in 0.1 M phosphate buffered saline (pH 7.4) containing 1 mM PMSF. After 2 h at 9°C, the mixture was centrifuged at $100000 \times g$ for 1 h in a Beckman Airfuge as mentioned in Experimental procedures. The supernatant and pelleted fractions were evaluated for radioactivity.

Expt.	Soluble	Pellet	Solubilized (%)
1	$2.37 \cdot 10^8$	$2.43 \cdot 10^7$	91.7
2	$1.07 \cdot 10^9$	$7.83 \cdot 10^7$	93.2
3	$1.95 \cdot 10^8$	$2.29 \cdot 10^6$	98.8
4	$7.82 \cdot 10^8$	$8.75 \cdot 10^7$	89.9

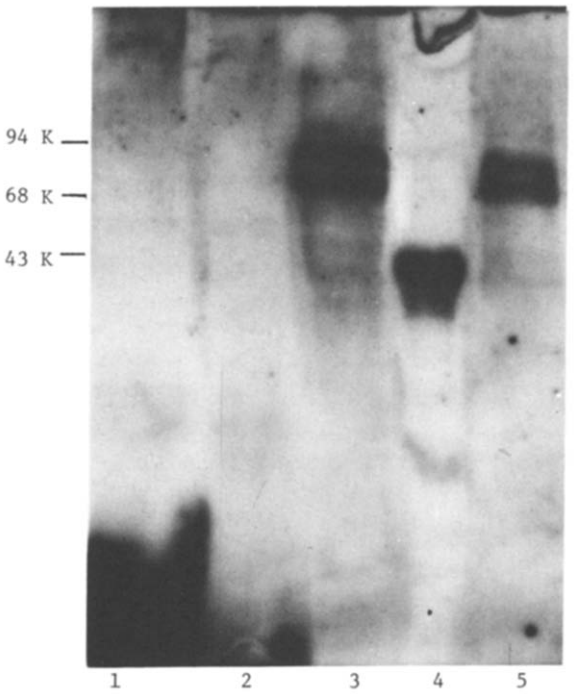

Fig. 1. Radiolabeled wheat-germ agglutinin binding. Myelin was solubilized with 10% SDS and the oligodendroglial plasma membranes were solubilized with 1% P-9-L. Both samples were treated with SDS-sample buffer, heated at 100°C for 3 min, and subjected to electrophoresis on 7–25% SDS-polyacrylamide gel slabs as described in Experimental procedures. The glycoproteins were identified by binding to radioiodinated wheat germ agglutinin. Lane 1, 100 μg bovine light myelin fraction; lane 2, 100 μg bovine heavy myelin fraction; lane 3, 25 μg oligodendroglial plasma membranes; lane 4, Pharmacia low molecular weight standards (ovalbumin); lane 5, 20 μg oligodendroglial membrane whorls.

solubilized in 1% P-9-L after sonication and centrifugation (Table I). If nonradiolabeled plasma membrane components were solubilized in 1% P-9-L, separated on 7–25% SDS-polyacrylamide gradient slab gels, and assessed for binding to radiolabeled wheat germ agglutinin (Fig. 1), both the 99-kDa and the 77-kDa glycoproteins had been solubilized as evidenced by their intense binding to this lectin. (A preliminary study [6] identified the two major wheat germ binding glycoproteins as having molecular masses of 95 kDa and 78 kDa, respectively. In this report, these two glycoproteins, after being purified, were found to have molecular masses of 99 kDa and 77 kDa, as separated on 5–15% SDS-polyacrylamide pore gradient slab gels.) Adjacent lanes of purified myelin glycoproteins showed little or no binding to wheat germ agglutinin, even though greater quantities of protein were applied. The intense binding at the front of the myelin samples is due

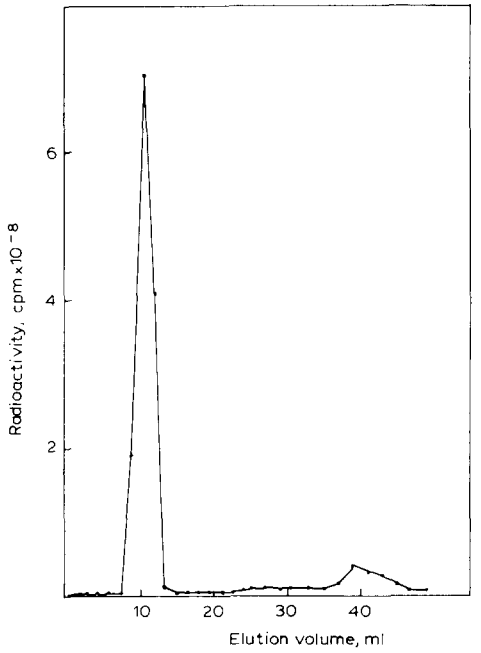

Fig. 2. Elution profile of solubilized radioiodinated oligodendroglial plasma membranes on a Sephadex LH-60 column. Solubilized radioiodinated plasma membranes (200–300 μl) were applied onto an LH-60 column (1.0×50 cm), previously equilibrated in 1% P-9-L and 0.1 M phosphate buffered saline (pH 7.4) with descending elution. The effluent was collected in 1.5-ml fractions and assayed for radioactivity using a Searle Model 1197 automatic gamma system.

to nonspecific binding to lipids. In other experiments using other lectins (concanavalin A or *Ulex europaeus*), there was considerable binding to myelin glycoproteins under these conditions [6].

Purification of the 99-kDa and 77-kDa glycoproteins

If the P-9-L solubilized, radiolabeled plasma membrane fraction was applied onto a Sephadex LH-60 column (removes both free ^{125}I and lipids), the radiolabeled proteins were contained in the void volume. These fractions were pooled and applied to a wheat germ agglutinin affinity column. The bound material was eluted from the column in sequential order by 1.0 M GlcNAc and 1% SDS (Fig. 3). Approx. 1% of the Sephadex LH-60 void volume fractions bound to the wheat germ column (Table II); greater then 70% of the bound material could be eluted by 1.0 M GlcNAc and 1% SDS.

To determine the composition of these fractions, the materials eluting with 1 M GlcNAc and the SDS fractions were concentrated, separated on an SDS 5–15% polyacrylamide gradient slab gel, and visualized by autoradiography. Both fractions contained the 99-kDa and the 77-kDa glycoproteins and several minor glycoproteins (data not shown). To assess the homogeneity of the 99-kDa and 77-kDa glycoproteins, after purification and separation on the 5–15% polyacrylamide gradient slab gel electrophoresis, the two glycoproteins were subjected to further electrophoresis on 7.5% polyacrylamide gels. Since the aspartyl–prolyl peptide bond has been shown to be susceptible to hydrolysis with mild acid [24], the 5–15% polyacrylamide gradient gels were exposed to the fixative and staining solutions for only short times (approx. 1 h) before autoradiography. Fig. 4A and B shows the densitometer tracings of the autoradiogram of the 99-kDa and the 77-kDa glycoproteins after electrophoresis on the second polyacrylamide gel obtained after a 48 h exposure with the 7.5% gel. These results demonstrate that the two glycoproteins isolated by 5–15% SDS polyacrylamide pore

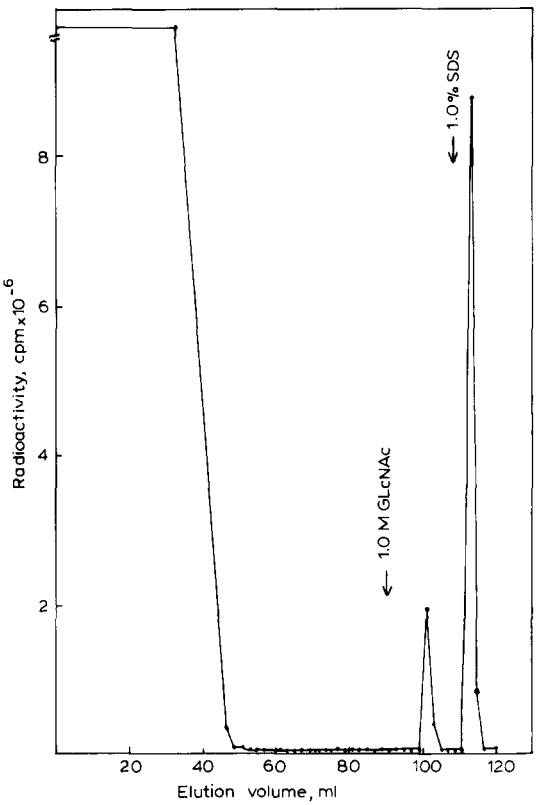

Fig. 3. Affinity chromatography of the Sephadex LH-60 void volume fraction on a wheat germ agglutinin Affi-Gel 10 column. The Sephadex LH-60 void volume fraction was applied onto a wheat germ agglutinin column, previously equilibrated in 1% P-9-L and 0.1 M phosphate buffered saline (pH 7.4). After recycling the Sephadex LH-60 void volume fraction through the wheat germ column several times over a 60–90 min period, non-binding material was removed by washing with 1% P-9-L in 0.1 M phosphate buffered saline (pH 7.4) and finally with 0.1 M phosphate buffered saline (pH 7.4) as mentioned in Experimental procedures. Bound glycoproteins were eluted from the wheat germ column with 1 M GlcNAc, followed by 1% SDS. The collected fractions were assayed for radioactivity.

TABLE II

FRACTIONATION ON WHEAT GERM AGGLUTININ AFFI-GEL 10 AFFINITY COLUMN

Sephadex LH-60 void volume fraction (Fig. 2) was applied onto the top of a wheat-germ agglutinin affinity column (Fig. 3), washed with P-9-L in 0.1 M phosphate buffered saline and 0.1 M phosphate buffered saline (pH 7.4). Bound material was eluted with 1 M GlcNAc and 1% SDS as described in Fig. 3.

Fraction(s)	cpm ^{125}I	Recovered cpm (%)
1. Non-binding material	$1.424 \cdot 10^9$	98.97
2. 1 M GlcNAc	$2.39 \cdot 10^6$	0.17
3. 1% SDS	$9.672 \cdot 10^6$	0.67
4. Remaining on gel	$2.86 \cdot 10^6$	0.19

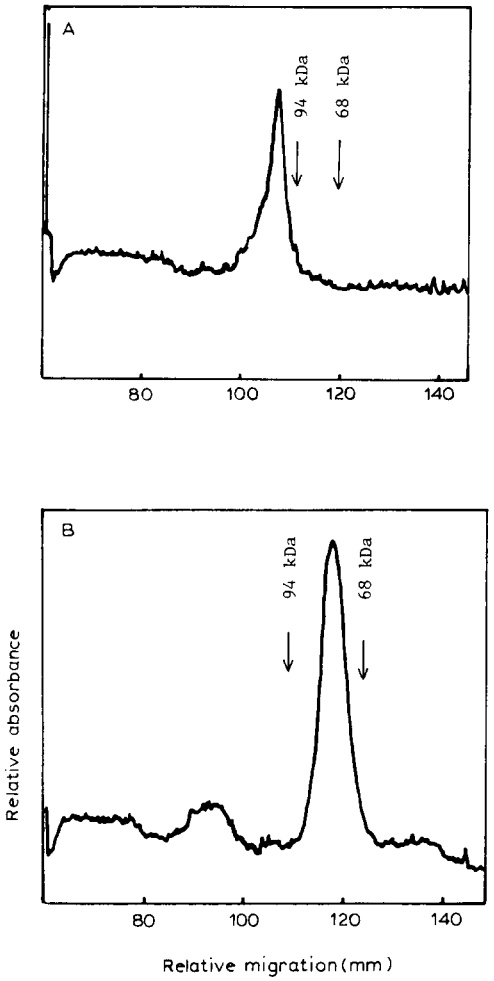

Fig. 4. Electrophoresis on a second slab gel to determine the apparent homogeneity of the 99-kDa and 77-kDa oligodendroglial plasma membrane glycoproteins. The glycoproteins were identified on a 5–15% polyacrylamide pore gradient gel and excised from the gel. The gel slices were subjected to 7.5% SDS-polyacrylamide gel electrophoresis as described in Experimental procedures. The figure shows the densitometer tracing of the autoradiogram: A, 99-kDa glycoprotein; B, 77-kDa glycoprotein.

gradient gel electrophoresis are indeed sufficiently homogeneous for any type of structural studies, such as peptide mapping.

Characterization of the glycoproteins

To determine the isoelectric points, the areas of the 5–15% SDS-polyacrylamide pore gradient gels containing the two purified radiolabeled glycoproteins were excised and analyzed by isoelectric focusing. The isoelectric point of the 99-kDa glycoprotein was found to be 6.15 and that of the 77-kDa glycoprotein to be 6.0.

Since both the 99-kDa and the 77-kDa glycoproteins were intensely labeled with ^{125}I-labeled wheat germ agglutinin, it was of interest to compare their primary structures by peptide mapping to determine whether they had completely different structures, or whether there was a possible precursor/product relationship between them. The glycoproteins on the gel slices were reiodinated and subjected to limited proteolysis by using *S. aureus* V8 proteinase or papain.

Whereas the peptide maps generated by papain (Fig. 5A and B) were similar, those generated by *S. aureus* (Fig. 5C and D) were clearly different for the two glycoproteins. Papain cleavage generated a common peptide 44, but peptides 48 and 54 were also prominent in the 77-kDa glycoprotein. There were several more peptides (especially peptides 40 and 46) generated by *S. aureus* V8 proteinase action from the 77-kDa glycoprotein than from the 99-kDa glycoprotein.

The structural relatedness of the two glycoproteins was also examined by HPLC mapping of their α-chymotryptic digests (Fig. 6). Peptides common to both glycoproteins were peptides I, II, VI and X. However, the 99-kDa glycoprotein also had distinctly different peptides (peptides IV, VII, VIII, IX and XI). The 77-kDa glycoprotein had a unique peptide in peptide XII.

Lectin binding studies

Lectin binding studies were performed to determine which monosaccharide was involved in wheat germ agglutinin binding to the two glycoproteins. After transfer of the solubilized oligodendroglial plasma membrane glycoproteins separated on 7–25% SDS-polyacrylamide pore gradient gel slabs, the nitrocellulose strips were treated with neuraminidase, 0.05 N H_2SO_4, or were untreated. As shown in Fig. 7, neither acid nor neuraminidase treatment had any effect on wheat germ agglutinin binding. Incubation of the glycoproteins in the presence of the competing sugar, 0.2 M GlcNAc, eliminated all binding to this lectin. Furthermore, both the 99-kDa and the 77-kDa glycoproteins bound to ^{125}I-labeled succinylated wheat germ agglutinin, whereas a standard

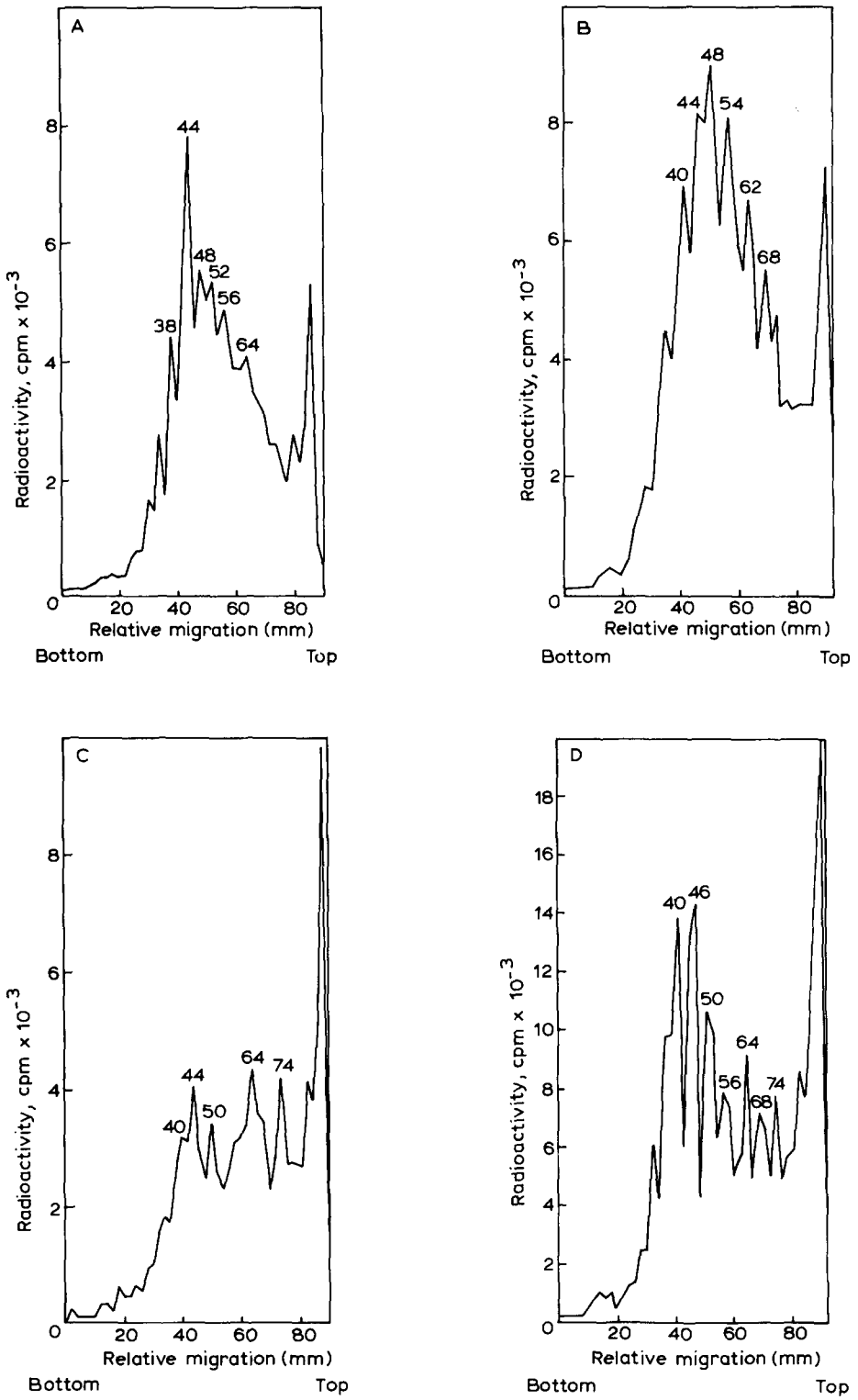

Fig. 5. Peptide mapping of the 99-kDa and 77-kDa glycoproteins by limited proteolysis. Details are described under Experimental procedures. Peptide maps generated by papain: A, 99-kDa glycoprotein; B, 77-kDa glycoprotein. Peptide maps generated by *S. aureus* V8 proteinase: C, 99-kDa glycoprotein: D, 77-kDa glycoprotein.

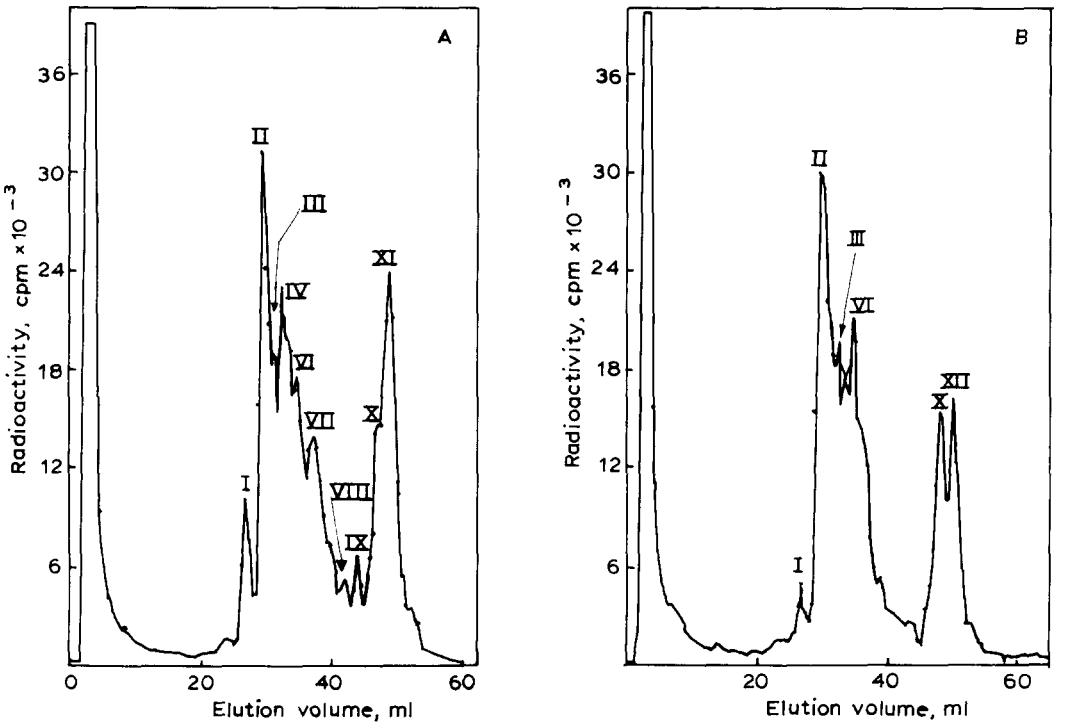

Fig. 6. HPLC peptide mapping of the 99-kDa and 77-kDa glycoproteins. The gel slices containing the 99-kDa and 77-kDa glycoproteins were incubated with TLCK-treated α-chymotrypsin in 50 mM NH_4HCO_3 (pH 7.8) for 28 h. After washing the gel with 50 mM NH_4HCO_3 (pH 7.8) the supernatant fractions were pooled and lyophilized as mentioned in Experimental procedures. 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.

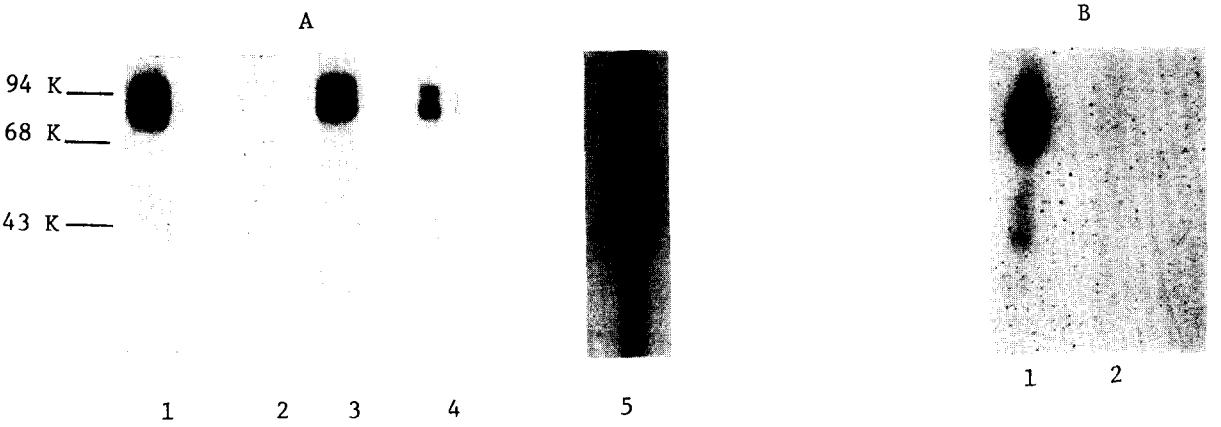

Fig. 7. Radioiodinated-lectin binding to electrophoretically separated oligodendroglial plasma membrane glycoproteins. Oligodendroglial plasma membranes and bovine fetuin were solubilized with 1% P-9-L, separated on a 7–25% polyacrylamide pore gradient gel slab, and electrophoretically transferred to nitrocellulose as described under Experimental procedures. The nitrocellulose paper was cut into 4-mm-wide strips and subjected to specific treatments as described below; the nonspecific binding sites were blocked with 2% polyvinylpyrrolidone and incubated with the appropriate ^{125}I-labeled lectin as mentioned in Experimental procedures. Lectin binding to the glycoproteins was visualized by autoradiography. A, incubation with radiolabeled wheat germ agglutinin: 1, oligodendroglial plasma membranes (10 μg protein); 2, oligodendroglial plasma membranes (10 μg) incubated with 0.2 M GlcNAc; 3, oligodendroglial plasma membranes (10 μg), prior treatment with neuraminidase; 4, oligodendroglial plasma membranes (5 μg), prior treatment with 0.05 N H_2SO_4; 5, bovine fetuin (15 μg). B, incubation with radiolabeled succinylated wheat germ agglutinin: 1, oligodendroglial plasma membranes (10 μg); 2, bovine fetuin (15 μg).

glycoprotein, fetuin, exhibited no binding. This latter result was expected since fetuin, which contains three *N*-linked oligosaccharide chains and three *O*-glycosidically linked chains [25], had been shown to bind to wheat germ agglutinin by its sialic acid residues. These binding studies indicated that the interaction of wheat germ agglutinin with the two glycoproteins appeared to be to internal GlcNAc residues on the glycoprotein's carbohydrate moieties.

Discussion

By silver staining, 70–100 bands are easily visualized when oligodendroglial plasma membrane proteins and glycoproteins are separated by SDS gradient slab gel electrophoresis. Using a combination of adsorption and lectin affinity chromatography and SDS gradient slab gel electrophoresis, the 99-kDa and 77-kDa glycoproteins have been purified from oligodendroglial plasma membranes. In these studies the solubilized plasma membrane fractions were radiolabeled with $Na^{125}I$ to increase the sensitivity of detection for both the glycoproteins and any contaminating components. It did not appear that introducing ^{125}I into the glycoprotein molecule had any major effects on the lectin-glycoprotein interactions due to the strong binding to the affinity column. This result was contrary to those reports using immobilized concanavalin A or lentil lectin [26].

Approx. 1% of the radiolabeled plasma membranes bound to the wheat germ agglutinin column, of which 17% of the material could be eluted with the specific inhibiting sugar (GlcNAc). However, even after elution with 1% SDS, 30% of the radiolabeled material was still retained on the column. Elution of glycoproteins bound to wheat germ agglutinin columns is variable; some reports state very low recoveries of glycoproteins with either SDS or GlcNAc elution [27–29], while another group reports a 100% recovery of glycoproteins [30]. These divergent results may be due to the nature of the glycoproteins under study.

That the binding of the 99-kDa and the 77-kDa glycoproteins to wheat germ agglutinin is specific is supported by the lack of binding in the presence of the inhibiting monosaccharide, GlcNAc. The apparent homogeneity of these purified glycoproteins was demonstrated by their migration as single peaks on a second type of electrophoresis gel. Further proof would require demonstrating a single N-terminus or immunoprecipitation with specific antibodies.

Peptide mapping by both limited proteolysis and HPLC were used to compare structurally the 99-kDa and the 77-kDa glycoproteins. The peptide maps for the two glycoproteins were different in that each map contained peptides unique to each of the two glycoproteins. In addition, HPLC mapping of TLCK-treated α-chymotrypsin digestions revealed different overall maps for the two glycoproteins. The number of peptides generated by α-chymotrypsin digestion was surprisingly low. This may be due either to our detection system (using radioiodinated peptides which must have tyrosine, histidine or phenylalanine residues) or to possible stearic hindrance due to either acquisition of the tertiary structure after removal of SDS or to nearby oligosaccharide chains [31,32].

Wheat germ agglutinin appears to bind primarily to internal *N*-acetylglucosamide residues on the two glycoproteins, since there was binding to both wheat germ agglutinin and succinylated wheat germ agglutinin. The glycoprotein used as control, fetuin, exhibited binding to only wheat germ agglutinin due to its sialic acid residues on the complex *N*-linked oligosaccharide chain structure.

Two glycoproteins with molecular weights of approx. 100 000 have been purified from brain by other investigators. The synaptic junctional complex has a glycoprotein of 110 kDa which is not present in microsomes, axolemma, myelin or synaptic vesicles [1]. The myelin-associated glycoprotein, purified from myelin, has a molecular weight of 100 000–110 000 and is found in the periaxonal region of the myelin sheath [5]. Both of these glycoproteins bind strongly to concanavalin A; this lectin coupled to Sepharose or agarose has been used for the purification of these glycoproteins. The glycoprotein 110 from synaptic junctions also binds to wheat germ agglutinin, but binding is considerably reduced after incubation with neuraminidase [1]. That the 99-kDa glycoprotein from oligodendroglial plasma membranes is different from these two glycoproteins is attested to by its strong affinity for wheat germ agglutinin, which is unaltered by neuraminidase treatment,

and by its lack of staining in purified myelin.

Thus two glycoproteins, 99-kDa and 77-kDa, have been purified from oligodendroglial plasma membranes by wheat germ agglutinin chromatography. When specific antibodies to these glycoproteins are prepared, we will be able to determine whether they may be cell-specific markers for oligodendroglia. Major wheat germ agglutinin-binding glycoproteins will now be isolated and analyzed from the whorls of membrane (an early form of myelin) produced by bovine oligodendroglia in culture [33] and from the myelin and membranes of diseased tissue from metachromatic leukodystrophy to try to determine the role of these glycoproteins during development and during demyelination.

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