Characterization of a cytoskeletal matrix associated with myelin from rat brain

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Extraction of rat brain myelin in a buffer containing Triton X-100 yielded a soluble fraction and an insoluble residue that was enriched in cytoskeletal elements. Immunoblot analysis of the detergent-soluble fraction and the insoluble cytoskeletal residue showed that all of the tubulin and more than half of the actin were found within the cytoskeletal fraction. The distribution of myelin-specific proteins was also examined, and revealed that 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNPase) I and most of the myelin basic proteins (MBPs) were equally distributed between both fractions. By contrast, the large MBP (21.5 kDa) and CNPase II (50 kDa) were observed to partition almost entirely with the cytoskeletal fraction. Proteolipid protein was found predominantly in the detergent-soluble fraction, as was DM-20 protein. Analysis of the cytoskeletal fraction by sucrose-density-gradient centrifugation demonstrated that a distinct subset of lipids was tightly bound to the cytoskeletal protein residue. The cytoskeleton-associated lipid was considerably enriched in cerebroside and sphingomyelin by comparison with total myelin lipids. These results indicate that a cytoskeletal matrix is associated with multilamellar myelin, and suggest that this structure may play a fundamental role in myelinogenesis.

INTRODUCTION

Myelination in the central nervous system requires the synthesis of large amounts of a specialized plasma membrane by oligodendrocyte cells. Myelin extends from the oligodendrocyte and wraps round axons, where it functions as an insulator (Raine, 1984). Myelinated axons have high resistance and low capacitance, which allows the nerve impulse to be propagated along the fibre with a greater velocity and efficiency than is the case in unmyelinated axons (Waxman & Ritchie, 1985).

Most nucleated cells have an extensive cytoskeletal system that includes microtubules, microfilaments and intermediate filaments (Timasheff & Grisham, 1980; Lazarides, 1980). The extension of myelin processes from an oligodendrocyte requires a drastic change in the shape of the cell; therefore it seems reasonable to suppose that the cytoskeleton of the oligodendrocyte has a role in myelination, since plasma-membrane-cytoskeleton interactions have been implicated both in the control of cell shape and movement (Stossel, 1982; Niggli & Burger, 1987) and in the segregation of proteins into membrane domains (Carraway *et al.*, 1983; Gumbiner & Louvard, 1985).

Despite the wealth of biochemical knowledge that has accumulated over the years (reviewed by Lees & Brostoff, 1984), no single function has been attributed unequivocally to any of the myelin proteins, and their roles in myelin formation and stabilization remain obscure. Nevertheless, tubulin and actin co-purify with mouse brain myelin (de Néchaud *et al.*, 1983), and two reports have shown that MBP interacts with both actin

and tubulin. Interaction between MBP and F-actin results in the precipitation of a complex composed of both proteins with a morphology similar to the bundles of filaments observed when F-actin and troponin 1 interact (Barylko & Dobrowolski, 1984). Tubulin carboxypeptidase is inhibited by MBP, and this is due to the binding of MBP to the tubulin substrate (Modesti & Barra, 1986).

Non-ionic-detergent extraction has been used successfully to demonstrate associations between a cytoskeletal matrix and the plasma membrane in lymphocytes (Davies *et al.*, 1984; Apgar & Mescher, 1986), erythrocytes (Bennett, 1985) and platelets (Fox *et al.*, 1988). We have used a similar approach to identify interactions between cytoskeletal elements and proteins in myelin purified from rat brain. Furthermore, like Davies *et al.* (1984) and Apgar & Mescher (1986), we show here that this detergent-insoluble cytoskeletal residue is bound to a distinct subset of myelin lipids.

MATERIALS AND METHODS

Materials

TLCK, Pipes and Hepes were from Sigma Chemical Co., Poole, Dorset, U.K. Triton X-100, acrylamide and bisacrylamide were supplied by Bio-Rad Laboratories, Watford, Herts., U.K. The proteinase inhibitors leupeptin and antipain were obtained from Peptide Institute, Osaka, Japan. LR White Medium Grade Resin was supplied by the London Resin Co., Woking, Surrey, U.K. All other chemicals were from BDH, Poole, Dorset, U.K., and of the highest purity available.

Abbreviations used: PAGE, polyacrylamide-gel electrophoresis; PBS, phosphate-buffered saline; TLCK, *N*^ε-*p*-tosyl-L-lysyl-chloromethane; MBP, myelin basic protein; CNPase, 2',3'-cyclic nucleotide 3'-phosphohydrolase; PLP, proteolipid protein.

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Mouse monoclonal antibodies versus tubulin were from Serotec, Oxford, U.K. Rabbit polyclonal antibodies against the following proteins were generously given: actin, from Dr. C. Bulinski, Columbia University, New York, NY, U.S.A.; CNPase and MBP from Dr. D. Colman, Columbia University, U.S.A.; PLP from Dr. E. Carey, University of Sheffield, U.K. Peroxidase-coupled antibodies were from North-East Biomedical Laboratories, Uxbridge, Middx., U.K., or the Scottish Antibody Production Unit, Carlisle, Scotland, U.K.; gold-coupled antibodies were from Janssen Life Sciences, Olen, Belgium.

Isolation of myelin

Myelin was isolated from the brains of 4–6-week-old Wistar rats of both sexes by a modification of the procedure of Norton & Poduslo (1973). Brains were removed from the animals and either frozen in liquid N₂ or homogenized (5%, w/v) immediately in ice-cold 0.85 M-sucrose in 10 mM-Hepes (pH 7.4)/3 mM-dithiothreitol/1 mM-TLCK with a Potter-Elvehjem glass homogenizer (Orme Scientific, Manchester, U.K.). The homogenate (40 ml) was overlaid with 2.5 ml of 0.25 M-sucrose/10 mM-Hepes (pH 7.4)/3 mM-dithiothreitol/1 mM-TLCK in polycarbonate centrifuge tubes and centrifuged at 70 000 g for 90 min at 4 °C in an MSE 3 × 70 ml swing-out rotor. Myelin was removed from the 0.25 M/0.85 M-sucrose interface and subjected to hypo-osmotic shock by resuspension in 10 vol. of ice-cold water with 1 mM-TLCK. The shocked membrane was recovered by centrifugation at 23 000 g for 30 min at 4 °C in a Sorvall SA600 rotor. The shock procedure was repeated twice. Purified myelin was resuspended in a small volume of water and used immediately or stored at –20 °C.

Extraction of myelin with non-ionic detergent

Rat brain myelin was resuspended in 0.5% (v/v) Triton X-100 in CSK (cytoskeleton) buffer [10 mM-Pipes (pH 6.9)/100 mM-KCl/2 mM-EGTA/1 mM-MgCl₂/20% (v/v) glycerol/leupeptin (10 µg/ml)/antipain (10 µg/ml)] at a concentration of 1 mg of protein/ml and incubated for 3 min at room temperature. After centrifugation (13 400 g for 5 min at room temperature; MSE Microcentaur) the supernatant was removed (soluble fraction) and the pellet was extracted again. The extracts were combined and the final pellet was resuspended in CSK buffer to a volume equivalent to that of the soluble fraction.

Immuno-electron microscopy

The detergent-insoluble pellet was broken up by extensive trituration, then incubated with 5% (v/v) goat serum for 1 h, followed by primary antibody in PBS/0.5% (w/v) fish gelatin (Sigma) at room temperature. After washing for 6 × 5 min in PBS/0.5% fish gelatin, gold-antibody conjugate in 5% goat serum was added and incubated for 1 h at room temperature. The sample was washed extensively in water and fixed with 2.5% (v/v) glutaraldehyde/0.1 M-sodium cacodylate overnight, then dehydrated with ethanol. After infiltration with LR White resin the sample was embedded in BEEM capsules and polymerized at 55 °C overnight. Ultra-thin sections were mounted on 200-mesh copper grids and stained with 8% (w/v) uranyl

acetate and 0.2% (w/v) lead citrate. Electron micrographs were obtained with a JEOL 100C electron microscope.

Electrophoresis and immunoblotting

Proteins were analysed by SDS/PAGE on 5–15% acrylamide gradient slab gels in the discontinuous system of Maizel (1971) as modified by Kreibich & Sabatini (1974). Samples were boiled for 2.5 min in a sample buffer containing 2.5% (w/v) SDS and 125 mM-dithiothreitol. Protein bands were made visible by staining for at least 30 min in 0.2% (w/v) Coomassie Blue in 50% (v/v) methanol and were destained in 25% (v/v) methanol/10% (v/v) acetic acid.

For immunoblotting, the proteins were separated by SDS/PAGE and then transferred to nitrocellulose paper (0.45 µm pore) (Schleicher and Schuell; Anderman, Kingston upon Thames, U.K.) at 0.25 A for 6 h by the method of Towbin *et al.* (1979). After transfer, a strip was cut and stained in 0.1% (w/v) Amido Black, destained in 25% methanol/10% acetic acid and finally immersed in water. For immunostaining, strips were incubated overnight in PBS/0.2% (w/v) gelatin/0.1% (v/v) Triton X-100 (Buffer I), then incubated in fresh Buffer I containing the appropriate dilution of primary antibody for 1 h at room temperature. Blots were washed for 4 × 5 min in Buffer I, then incubated in Buffer I with either goat anti-rabbit IgG (1:1000 dilution) or sheep anti-mouse IgG (1:500), both conjugated to horseradish peroxidase. After incubation for 1 h at room temperature in the dark, the blots were washed four times in Buffer I and finally in PBS. Immunostained proteins were detected by diaminobenzidine (Sigma) (50 mg/100 ml) in 50 mM-Tris/HCl (pH 7.4) followed by 30% (v/v) H₂O₂ (10 µl/10 ml); the reaction was stopped by rinsing the blots in 2% SDS.

Sucrose-density-gradient centrifugation

Rat brain myelin (2 mg of protein) and the cytoskeletal pellet from 10 mg of rat brain myelin protein were made to 1 ml volume in 10 mM-Hepes, pH 7.3, and centrifuged (50 000 g for 20 h at 4 °C; MSE 6 × 16.5 ml swing-out rotor) through a continuous 10–40% (w/v) sucrose gradient in 20 mM-Hepes, pH 7.3. Fractions (0.5 ml) were collected, and 50 µl of each was solubilized in 2% SDS at 50 °C for 15 min and the A₂₈₀ measured. A further 50 µl from each fraction was analysed by SDS/PAGE. Lipid was extracted from the remainder of each fraction. Sucrose concentrations of solutions were determined from their refractive index.

Lipid analysis

Lipid was extracted by the method of Bligh & Dyer (1959). Dried samples were dissolved in 20 µl of chloroform/methanol (1:1, v/v) and spotted on activated (100 °C for 1 h) Kieselgel 60 t.l.c. plates (Merck). After resolution of the lipids in methyl acetate/propan-2-ol/chloroform/methanol/0.25% (w/v) KCl (25:25:25:10:9, by vol.; Bürgisser *et al.*, 1986), the plates were dried in air and the lipids were stained lightly with iodine vapour.

Lipid extracts from either whole myelin or the cytoskeletal pellet were analysed quantitatively for phospholipid (Eng & Noble, 1968), sulphatides (Kean, 1968), cerebroside (Macala *et al.*, 1983) and cholesterol (Sperry & Brand, 1943). Individual phospholipids were

quantified by scraping the relevant spots off the t.l.c. plate and subjecting them to phosphate analysis.

Protein determination

Protein content was routinely measured by the method of Lowry *et al.* (1951), with the inclusion of SDS to solubilize membrane proteins.

RESULTS

Immuno-electron microscopy

The electron micrographs (Fig. 1) show that CNPase and actin are located on the periphery of the detergent-insoluble pellet, whereas tubulin and MBP are distributed throughout the residue. A regular structural organization in the residue was not apparent, which suggests that tubulin and actin are not organized into long microtubules and microfilaments respectively in the cytoskeletal matrix. In mammalian erythrocytes it is known that the plasma-membrane-associated microfilaments are also very short (Bennett, 1985).

Protein distribution in the myelin-associated cytoskeleton

Triton X-100-soluble and -insoluble (cytoskeletal) fractions were prepared from purified rat brain myelin and analysed by immunoblotting as described in the Materials and methods section. The protein content of the insoluble residue represented 25% of total myelin protein. Fig. 2(a) shows that only a small number of proteins remain unextracted in the insoluble pellet. Figs. 2(b) and 2(c) show that all of the tubulin and more than half of the actin are found in this cytoskeletal fraction. The retention of tubulin and actin in the cytoskeletal residue implies that these proteins are present in macromolecular structures. It seems most likely that these would involve other proteins, since electron microscopy had not revealed any evidence for the presence of large microtubules or microfilaments.

CNPase in rat brain myelin comprises two polypeptides, of 48 kDa (CNPase I) and 50 kDa (CNPase II) respectively (Lees & Brostoff, 1984). The CNPase I (lower band) is distributed equally between the detergent-soluble fraction and the insoluble residue; however, there is a considerable enrichment of CNPase II (upper band) in the cytoskeleton (Fig. 2d). PLP, by contrast, is almost completely extracted by the detergent, as would be expected of an integral membrane protein (Fig. 2f). Most of the MBPs are distributed equally between the soluble and cytoskeletal fractions (Fig. 2e); however, of considerable interest is the finding that the largest MBP (21.5 kDa) is found almost exclusively with the cytoskeleton. The other MBP species do not show this preferential association with the cytoskeleton.

Lipid analysis of the myelin-associated cytoskeleton

Since others have found residual lipid in cytoskeletal preparations from purified plasma membranes (Davies *et al.*, 1984; Apgar & Mescher, 1986), we wished to know if there were any lipids in the myelin-associated cytoskeleton. The lipid compositions of rat brain myelin and the Triton X-100-insoluble pellet were analysed, and the results are shown in Table 1. The results shown for rat brain myelin are consistent with values published previously by other workers (for a review see Norton &

Cammer, 1984). We were also able to demonstrate the presence of lipid in the insoluble cytoskeletal residue. This lipid persisted in spite of repeated extractions of the pellet.

The lipids associated with the cytoskeletal fraction represent a subset of the total myelin membrane lipid. There is a considerable depletion of the phospholipid content of the cytoskeletal residue by comparison with the intact membrane. Most of the phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol are removed by the detergent, whereas there is a considerable enrichment of sphingomyelin in the detergent-insoluble pellet. The phosphatidylcholine and cholesterol contents of the pellet are similar to those of the native membrane. These results are very similar to those of Davies *et al.* (1984) and Apgar & Mescher (1986) in their studies on lymphocyte membranes. A striking feature of the lipid analysis is the considerable enrichment of cerebrosides (other than sulphatide) found associated with the detergent-insoluble pellet. The hydroxy- and non-hydroxy-cerebrosides appear to be equally enriched in the cytoskeletal residue.

Sucrose-density-gradient analysis

In order to ensure that the lipid extracted from the cytoskeletal fraction was genuinely associated with the cytoskeletal residue, purified rat brain myelin and the detergent-insoluble pellet were subjected to continuous sucrose-density-gradient centrifugation. Measurements of the protein content of the myelin and cytoskeletal fractions revealed two distinct peaks (Fig. 3) at approx. 23% and 36% (w/v) sucrose respectively. Myelin has been found to band at around 23% sucrose in previous analyses of this type (Waehnel, 1978). The differences in density between myelin and the cytoskeletal residue must reflect their very different lipid/protein ratios (see Table 1). The results from this experiment also indicate that both the myelin and myelin-associated cytoskeleton fractions are quite homogenous, since most of each sample bands in a relatively narrow density range.

The protein components of each fraction were characterized by SDS/PAGE. Fig. 4 shows that most of the myelin proteins are present in fractions 9–11, which correspond to the peak of protein seen in Fig. 3. There is, however, a small amount of CNPase I and II in fraction 3. This fraction must contain considerable amounts of lipid, since it is much less dense than the bulk of the myelin. This fraction is located at the top of the sucrose gradient (approx. 10% sucrose), and is of interest, since it has been detected when peripheral-nervous-system myelin is subfractionated on continuous sucrose gradients (Matthieu *et al.*, 1980). It has been suggested by these workers that the floating 'pad' membrane of the peripheral nervous system may be derived from the Schwann-cell plasma membrane. If this is so, it suggests that a considerable fraction of the CNPases may be localized in the oligodendrocyte plasma membrane or in membranes which are morphologically and biochemically different from that of myelin. No proteins were detected in fractions 15–30 in this gradient.

Consistent with the protein profile shown in Fig. 3, Fig. 4 shows that most of the proteins of the cytoskeletal residue were present in fractions 20–26. The protein composition of these fractions is very like that shown in Fig. 2(a), where the cytoskeleton-associated proteins are stained with Amido Black after transfer to nitrocellulose.

Fig. 1. Immuno-electron microscopy of detergent-insoluble residues of rat brain myelin

Samples were processed for immuno-gold-electron microscopy as described in the Materials and methods section. They were then probed with specific antibodies against the following proteins, followed by gold-labelled second antibodies: tubulin (*a*); actin (*b*); CNPase (*c*); MBP (*d*). The scale bar represents 100 nm in each case.

Fig. 2. Protein distribution between the detergent-soluble and -insoluble fractions derived from rat brain myelin

Rat brain myelin was extracted with Triton X-100 as described in the Materials and methods section. Equal volumes of the soluble (S) and insoluble (cytoskeletal; C) fractions were electrophoresed on 5–15% gradient gels, and the proteins were electrotransferred to nitrocellulose paper. Strip (a) was stained with Amido Black; stripes (b)–(f) were immunoblotted with antibodies versus tubulin (b), actin (c), CNPase (d), MBP (e) and PLP (f). M_r markers are shown at the left.

Once again the detergent-insoluble residue is found to be highly enriched in tubulin and the 21.5 kDa MBP. The two CNPases, actin, and the other MBPs, together with a small amount of PLP, are also clearly visible in lanes 23–25. It is noteworthy that the relative amounts of these proteins do not appear to vary over the peak, which suggests that the cytoskeletal residue is a homogeneous macromolecular complex. No proteins were detected in fractions 1–18 for this sample.

T.l.c. analysis of the sucrose-density-gradient fractions shown in Fig. 5 reveals that myelin membrane lipids do

remain associated with the detergent-insoluble proteins. Comparison of the lipids from intact myelin (Fig. 5a, fraction 10) with the lipids associated with the cytoskeleton (Fig. 5b, fraction 24) shows that their compositions are different and that an enrichment of specific lipids with the cytoskeleton exists. The co-migration of a discrete set of proteins and their associated lipids in the detergent-insoluble fraction suggests that this material may represent a sub-membranous protein matrix that persists even when most of the myelin proteins and lipids have been removed.

Table 1. Lipid composition of the Triton X-100-insoluble cytoskeleton residue compared with that of myelin

Lipids were extracted from whole myelin and from the detergent-insoluble residue and analysed as described in the Materials and methods section. All values are the means of measurements made on at least three different preparations, and the standard deviations were, in all cases, less than 15% of each mean value: ND, not detected.

Component	Composition	
	Myelin	Triton X-100-insoluble residue
Total lipid ($\mu\text{mol}/\text{mg}$ of protein)	2.46	0.73
Cholesterol (mol% total lipid)	39.8	38.4
Cerebroside (mol% total lipid)	11.4	35.6
Non-hydroxy-cerebroside (mol% cerebroside)	32	23
Hydroxy-cerebroside (mol% cerebroside)	68	77
Sulphatide (mol% total lipid)	5.3	1.4
Phospholipid (mol% total lipid)	43.5	19.5
Phosphatidylethanolamine (mol% phospholipid)	45	13
Phosphatidylcholine (mol% phospholipid)	32	39
Phosphatidylserine (mol% phospholipid)	13	ND
Phosphatidylinositol (mol% phospholipid)	4	ND
Sphingomyelin (mol% phospholipid)	6	48

Fig. 3. Sucrose-density-gradient profile of rat brain myelin and the detergent-insoluble fraction derived from it

Rat brain myelin (2 mg of protein) and the Triton X-100-insoluble pellet (3 mg of protein isolated from 10 mg of myelin protein) were layered on top of 10–40% (w/v) sucrose gradients and centrifuged for 16 h at 50000 *g*. Fractions (0.5 ml) were collected, and 50 μ l portions of each were analysed for protein content (A_{280}). $\circ$, Rat brain myelin; $\bullet$, detergent-insoluble pellet; $\square$, sucrose concentration.

DISCUSSION

Many nucleated cells have been shown to have extensive cytoskeletal systems, and non-ionic-detergent extraction of both cells and isolated membranes from several mammalian sources has demonstrated the occurrence of detergent-insoluble components associated with the plasma membrane (Mescher *et al.*, 1981; Davies *et al.*, 1984; Apgar *et al.*, 1985; Bennett, 1985; Apgar & Mescher, 1986; Fox *et al.*, 1988). Partial characterization of the components of these detergent-insoluble fractions has led to suggestions that they represent sub-membranous cytoskeletal complexes associated with the membrane. Furthermore, studies conducted on lymphoid and murine tumour cells have led to the proposal that the detergent-insoluble material represents a distinct membrane skeletal structure. The results presented here on the characterization of myelin-associated cytoskeleton show that some of the major central-nervous-system myelin proteins are associated with a detergent-insoluble residue. The fact that the unique protein and lipid distribution of the insoluble residue remains unaltered even after prolonged extraction in the presence of Triton X-100 suggests that it represents a distinct macromolecular complex. This view is supported by the co-migration of the component lipids and proteins through a continuous sucrose-density gradient.

Our ability to extract the major intrinsic membrane

protein of central-nervous-system myelin, PLP, suggests that, unlike other proteins of this class thought to participate in both cytoskeletal and extracellular-matrix interactions (Ali & Hynes, 1977; Hay, 1985), this protein either has no direct association with cytoskeletal elements, or that, if an interaction exists, it is through extrinsic-type 'linker' proteins, as is the case for Band III in the erythrocyte cytoskeleton (Gratzer, 1981; Nelson & Lazarides, 1984).

In contrast with PLP, the two major groups of extrinsic proteins of myelin, the MBPs and the CNPases, did associate with the cytoskeletal residue. For the MBPs, the 21.5 kDa species was considerably enriched in the cytoskeletal fraction, which may reflect a distinct role that this protein plays in the formation and/or stabilization of myelin.

As with the MBPs, there is a selective enrichment of one of the CNPase species (CNPase II) with the detergent-insoluble fraction. CNPase II and MBPs are phosphorylated rapidly both *in vivo* and *in vitro* (Agrawal *et al.*, 1982; Bradbury & Thompson, 1984; Thompson *et al.*, 1986). Phosphorylation of tubulin enhances its interaction with membranes in a reversible fashion (Hargreaves *et al.*, 1986). A strong and stable association between the cytoskeleton and the myelin membrane, mediated by CNPase II (and perhaps by 21.5 kDa MBP), could therefore be envisaged, with a flexibility of movement between the structures allowed for by the revers-

Fig. 4. Protein profile of sucrose-density-gradient fractions obtained after sub-fractionation of rat brain myelin and the detergent-insoluble pellet

Portions (50 μ l) of the sucrose-density-gradient fractions (see Fig. 3) were analysed by SDS/PAGE in 5–15% gradient gels, and the proteins were stained with Coomassie Blue.

ible phosphorylation and dephosphorylation of these proteins. Enrichment of both CNPase II and the 21.5 kDa MBP in the Triton X-100-insoluble residue have also been observed by Pereyra *et al.* (1988) upon extraction of rabbit central-nervous-system myelin.

Lipid analysis showed that there is a considerable enrichment of sphingomyelin and cerebrosides (other than sulphatides) associated with the detergent-insoluble matrix of purified myelin. The properties of the lipid bilayer in the native membrane may therefore be

Fig. 5. Lipid content of fractions after sucrose-density-gradient centrifugation of rat brain myelin and the detergent-insoluble residue derived from it

Lipids were extracted from portions (400 μ l) of alternate fractions (see Fig. 3) and subjected to analysis by t.l.c. as described in the Materials and methods section. Lipids were detected by briefly exposing the t.l.c. plates to iodine vapour. Rat brain myelin (a); Triton X-100-insoluble pellets (b). Abbreviations: Chol, cholesterol; non-OH-Cer, non-hydroxy-cerebroside; OH-Cer, hydroxy-cerebroside; Su, sulphatide; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin.

influenced strongly by the presence of tightly bound cytoskeletal lipid. There is some evidence that the spectrin matrix has a role in maintaining lipid asymmetry in the membranes of erythrocytes (Williamson *et al.*, 1982) and in lymphocytes (Del Buono *et al.*, 1988). The myelin-associated cytoskeleton may exert a similar influence, since the binding of lipids directly to cytoskeletal elements could modulate the transbilayer distribution of sphingomyelin, cerebrosides and phosphatidylethanolamine. Cerebrosides are thought to be the lipid most characteristic of myelin. The observation that so much of this particular class of lipid is found associated with the detergent-insoluble fraction isolated from purified myelin suggests that it may have a dynamic rather than a purely structural role to play in myelinogenesis. Support for this view has come from the observations by Dyer & Benjamins (1988) on galactocerebroside patching

induced by anti-galactocerebroside antibodies. They observed that galactocerebroside segregated over MBP domains and, since patching has been shown to involve re-organization of the cytoskeleton in other systems (Ash *et al.*, 1977), they suggest that galactocerebroside patching involves a linkage between the oligodendrocyte cytoskeleton and galactocerebroside mediated by MBP.

Non-ionic-detergent extraction provides a method whereby an enrichment of cytoskeletal components can be obtained rapidly and, provided that precautions are taken to minimize depolymerization of the cytoskeletal elements, a biologically relevant fraction can be isolated in which protein-protein and protein-lipid associations can be studied. As pointed out by Niggli & Burger (1987), this approach has potential drawbacks. In particular, detergent insolubility might be a result of non-specific aggregation and precipitation. In the present study it was encouraging to observe that the protein and lipid contents of the detergent-insoluble pellet before and after resolution by sucrose-density-gradient centrifugation were identical.

Work on establishing a role for the cytoskeleton in myelinogenesis is at an early stage and, although these results are consistent with the idea that the myelin membrane associates with a cytoskeletal matrix, further work is needed to understand its role in myelin formation and/or stabilization.

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