# Loss of electrostatic cell-surface repulsion mediates myelin membrane adhesion and compaction in the central nervous system

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**During the development of the central nervous system (CNS), oligodendrocytes wrap their plasma membrane around axons to form a multilayered stack of tightly attached membranes. Although intracellular myelin compaction and the role of myelin basic protein has been investigated, the forces that mediate the close interaction of myelin membranes at their external surfaces are poorly understood. Such extensive bilayer–bilayer interactions are usually prevented by repulsive forces generated by the glycocalyx, a dense and confluent layer of large and negatively charged oligosaccharides. Here we investigate the molecular mechanisms underlying myelin adhesion and compaction in the CNS. We revisit the role of the proteolipid protein and analyze the contribution of oligosaccharides using cellular assays, biophysical tools, and transgenic mice. We observe that differentiation of oligodendrocytes is accompanied by a striking down-regulation of components of their glycocalyx. Both in vitro and in vivo experiments indicate that the adhesive properties of the proteolipid protein, along with the reduction of sialic acid residues from the cell surface, orchestrate myelin membrane adhesion and compaction in the CNS. We suggest that loss of electrostatic cell-surface repulsion uncovers weak and unspecific attractive forces in the bilayer that bring the extracellular surfaces of a membrane into close contact over long distances.**

myelination | PLP | adhesiveness

During the development of the central nervous system (CNS), oligodendrocytes generate a multilayered stack of membranes tightly attached at their cytosolic and external surfaces (1, 2). In electron micrographs of the cross-section of nerves, the typical repeating units of the myelin sheath with alternating electron-dense and light layers are seen. The major dense line is formed by myelin basic protein (MBP)-mediated compaction of the cytoplasmic leaflets of the myelin membrane (scheme in Fig. S1*A*). The less dense line, called the intraperiod line (IPL), represents the area at which the external surfaces of adjacent myelin membranes are brought together at a distance of 2–3 nm (3–5). Such close interactions of the external surfaces of a membrane over long distances are high unusual (6). One reason why bilayer-to-bilayer interactions do not occur is the repulsive forces generated by thermal undulations and by a polysaccharide-rich coat called the glycocalyx (7, 8). The glycocalyx, which covers the surface of most mammalian cells, can counteract adhesion by steric and electrostatic repulsion of its large and negatively charged oligosaccharide polymers (9–11). In most cases, the interaction of external cell surfaces is mediated by a specific lock-and-key type of adhesion molecules that are strong enough to overcome the repellent forces of the cell surface (12).

How the external surface of the myelin membrane is able to interact over very large areas is not understood. This question is of clinical interest, because knowledge of the forces that govern myelin membrane compaction is required to understand the mechanisms of myelin membrane disassembly seen in diseases such as multiple sclerosis and stroke (13).

One protein that has been implicated in myelin membrane adhesion is the proteolipid protein (PLP), a 30-kDa hydrophobic, four-span transmembrane protein forming two relatively small loops protruding into the extracellular space (14). Analysis of mice lacking both PLP and its splice isoform DM20 revealed that CNS myelin was physically unstable, for example when processed for electron microscopy (15, 16). However, PLP/DM20-deficient myelin is compacted in vivo with an abnormally condensed intraperiod line (16). It remains an open question of how myelin acquires its adhesive nature, as extracellular adhesion molecules have not yet been identified.

In this study, we hypothesized that the adhesion property of myelin might not require specific lock-and-key forces by adhesion molecules but could depend on weak unspecific forces of the lipid bilayer. We reinvestigated the role of PLP in myelin adhesion and that PLP itself is an adhesive strut (16, 17), testing the hypothesis that loss of repulsive forces from the surface of oligodendrocytes uncovers weaker generic interfacial forces of the membrane bilayer.

## Results

**PLP Increases Myelin Membrane Adhesiveness and Stability.** We set up a cellular assay to study the mechanisms that mediate the adhesion of the extracellular surfaces of myelin. In this assay, we determined the binding of purified myelin particles to myelin membrane sheets of cultured oligodendrocytes (Fig. S1*B*). Myelin was isolated from adult mouse brains by density centrifugation, sonicated, and then further purified to enrich for particles with the composition of myelin with molecules of the extracytoplasmic surfaces facing outward (Fig. S1*C*). By Western blotting and immunostaining, we confirmed the efficiency of the purification procedure for membranes of the IPL (Fig. S1 *D* and *E*). To determine whether these purified myelin particles specifically bind to the surface of mature oligodendrocytes (mimicking the formation of an IPL), a binding assay was performed with different glial cells. We found that myelin particles preferentially bind to oligodendrocytes when compared with other glia. Binding to oligodendrocyte precursor cells was also much less pronounced (Fig. S1*F*). By confocal microscopy, we confirmed the localization of the particles to the surface of oligodendrocytes (Fig. S1*G*).

To analyze the role of PLP in adhesion, myelin was prepared from wild-type and $Plp1^{null}$ mice (16) and the binding of particles to

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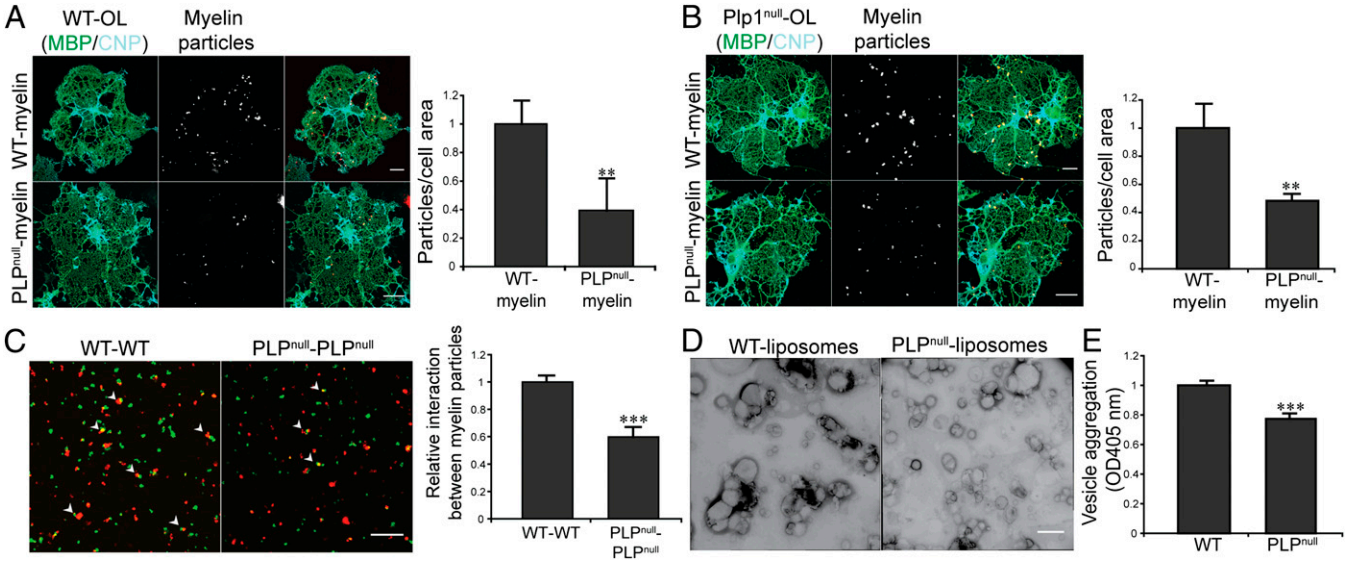


**Fig. 1.** PLP promotes membrane-to-membrane interaction. (*A*) Wild-type and PLP$^{null}$ myelin particles were added to wild-type oligodendrocytes (OL) and the number of particles per cell area was quantified. Data were normalized relative to wild-type ($n = 3$ different experiments, $^{**}P < 0.01$; *t* test). (Scale bars, 20 μm.) MBP, myelin basic protein; CNP, 2′,3′-cyclic-nucleotide 3′-phosphodiesterase. (*B*) A higher number of WT particles compared with PLP$^{null}$ particles bound to Plp1$^{null}$ cells ($n = 3$, $^{**}P < 0.01$; *t* test). (Scale bars, 20 μm.) (*C*) Myelin particles were stained with two different lipophilic dyes and incubated at room temperature. The fraction of interacting particles (arrowheads) was determined. The data were normalized relative to wild-type ($n = 3$, $^{***}P < 0.001$; *t* test). (Scale bar, 20 μm.) (*D*) Proteoliposomes were prepared from myelin derived from wild-type and Plp1$^{null}$ mice. Electron microscopic negative staining showed a higher fraction of aggregating wild-type vesicles compared with PLP$^{null}$. (Scale bar, 2 μm.) (*E*) Liposome aggregation was carried out by measuring the optical density of samples at 405 nm. Increased aggregation was observed with wild-type proteoliposomes compared with PLP$^{null}$ myelin ($n = 6$, $^{***}P < 0.001$; *t* test).

wild-type oligodendrocytes was evaluated. Quantification of particle attachment per cell area revealed binding of a higher number of particles from wild-type compared with PLP$^{null}$ myelin (Fig. 1*A*). When the reverse experiment was performed, that is, comparing the binding of purified wild-type myelin particles with oligodendrocytes from wild-type or Plp1$^{null}$ mice, particle binding was preferentially observed to wild-type oligodendrocytes (Fig. S2*A*).

Next, we determined whether the presence of PLP both in myelin particles and on the surface of oligodendrocytes enhances binding efficiency. To address this issue, we performed experiments in which PLP was absent in both the myelin particles and the oligodendrocytes or in only one of them. These experiments revealed that PLP was able to promote myelin particle attachment to oligodendrocytes when present even on only one of the interacting surfaces, suggesting that PLP might act at least in part by nonhomophilic interactions (Fig. 1*B* and Fig. S2*B*).

To obtain further evidence for the adhesive role of PLP, we set up a myelin particle binding assay. In this assay, particles were labeled with lipophilic dyes (PKH26, red; PKH67, green) and incubated together, and the percentage of interacting particles was determined by image analysis. The results from this assay confirmed that particles from wild-type myelin were more adhesive than particles prepared from PLP$^{null}$ myelin (Fig. 1*C*). Interestingly, the size of myelin particles prepared from Plp1$^{null}$ mice was significantly smaller than particles from wild-type mice (Fig. S2*C*). This fragmentation of particles during the isolation process is another indirect indication of lower adhesive forces of myelin lacking PLP.

To obtain direct evidence for an adhesive role of PLP, we performed proteoliposome aggregation assays using liposomes prepared from lipid extracts of wild-type and PLP-deficient myelin. Immunoblotting experiments confirmed the incorporation of PLP into the proteoliposomes prepared from wild-type myelin (Fig. S2*D*). Negative-staining electron microscopy and optical density measurements revealed higher aggregation for liposomes prepared from wild-type compared with the vesicles prepared from PLP knockout myelin lipids (Fig. 1 *D* and *E*), confirming the adhesive nature of PLP (18, 19).

Finally, the interaction of myelin particles with oligodendrocytes was measured quantitatively via an atomic force microscope (20, 21). Myelin particles were captured by a poly-D-lysine–coated cantilever, brought into contact with the surface of oligodendrocytes for a given time of 5 s, and then withdrawn at a constant speed (Fig. 2*A*). During this separation process, the cantilever deflection is recorded as a function of the vertical displacement of the z-piezo, providing information about the adhesive strength between the particles and the cells. The obtained force–distance curves revealed significant differences in maximum adhesion strengths during the binding of wild-type particles to wild-type cells compared with the interaction between particles and cells prepared from PLP knockout mice (Fig. 2 *B* and *C*). Medians of the most probable unbinding forces were 219 and 191 pN, respectively (Fig. 2*D*). Interestingly, force–distance curves of measurements with particles from PLP$^{null}$ myelin showed significantly higher perturbations than those for measurements with wild-type particles (Fig. 2 *B* and *C*). We attributed these increased fluctuations to the instability of particles lacking PLP leading to thermally excited oscillations of the cantilever during movement due to undulations of the liquid particles.

To investigate the effect of PLP on myelin adhesion in vivo, we reanalyzed the myelin ultrastructure of Plp1$^{null}$ mice by performing electron microscopy of optic nerves at age postnatal day 21. As seen previously (16), myelin integrity was highly dependent on the fixation protocol. To obtain a higher degree of tissue preservation, we applied high-pressure freezing followed by freeze substitution. As observed previously (22), in an area with optimal tissue preservation, myelin from Plp1$^{null}$ mice was compacted similarly to wild-type myelin. Only in an area with suboptimal tissue preservation were split myelin lamellae observed in the optic nerves of Plp1$^{null}$ mice (Fig. S3*A*). When conventional embedding was performed on fixed optic nerves, prepared by aldehyde immersion fixation, more consistent and reproducible splitting of the myelin sheaths was seen in the myelin lacking PLP (Fig. S3*B*). These data confirm that myelin is of reduced stability in Plp1$^{null}$ mice.

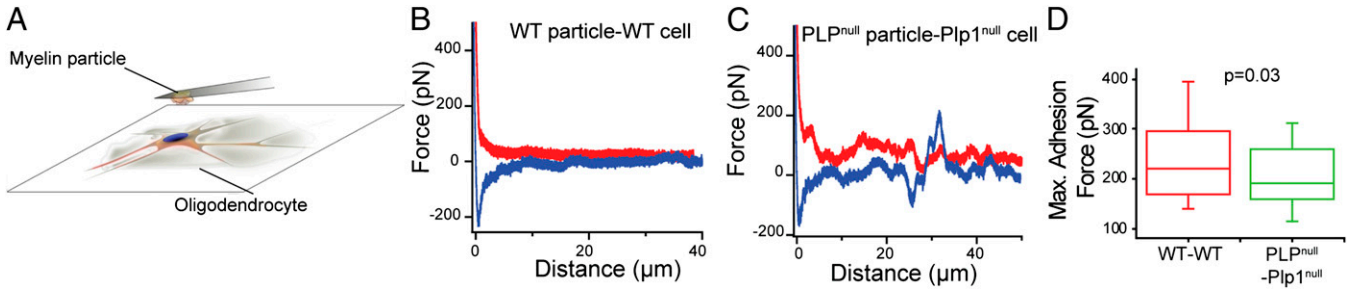


**Fig. 2.** Adhesive strength of myelin particles lacking PLP. (*A*) Scheme illustrating the used setup for force spectroscopy measurements. The myelin particles were bound electrostatically to the poly-D-lysine–coated cantilever and brought into contact with a spread oligodendrocyte. Upon cantilever retraction, the interaction between particle and cell is mirrored in the recorded force–distance curve. (*B* and *C*) Exemplary force–distance curves of wild-type (*B*) or mutant (*C*) myelin particles interacting with the corresponding oligodendrocytes obtained by force spectroscopy; red, approach curve; blue, retraction curve. (*D*) Boxplots for the maximum adhesion force of myelin particles interacting with oligodendrocytes of WT samples ($n = 114$) as compared with PLP$^{null}$ ($n = 70$, $P = 0.03$; Wilcoxon rank-sum test).

**Reduction in Sialic Acid Content During Oligodendrocyte Maturation Enhances Their Membrane Adhesiveness.** Despite the stabilizing function of PLP, myelin compaction in the null mutant suggests that PLP itself is unlikely to play a prevailing role in membrane compaction. We therefore asked which other mechanisms contribute to the attachment of the extracytoplasmic leaflets of the myelin membrane bilayer. In general, the interaction of plasma membranes over long distances is prevented by a number of repulsive forces such as the steric and electrostatic repulsion generated by the large and charged polymers of the glycocalyx (12, 23).

We used the lectins concavalin A, peanut agglutinin, and wheat germ agglutinin (WGA) (24) to systematically analyze the changes in sugar residues during oligodendrocyte differentiation in culture. Strikingly, whereas all lectins stained oligodendrocytes at day 2 in culture, we observed only weak staining at day 5 in culture (Fig. S4*A*). One main component of the repulsive glycocalyx is negatively charged sialic acid (25). We therefore used the lectin MAL II (*Maackia amurensis* lectin) to specifically evaluate the sialic acid content of differentiated oligodendrocytes. Again, a much weaker binding of the lectin was observed in mature oligodendrocytes compared with immature oligodendrocytes, microglia, or astrocytes (Fig. S4*B*). Lectin stainings were also performed on brain slices from 2-mo-old mice. Consistent with a low content of sialic acids in myelin, on vibratome sections both WGA and MAL II showed very weak labeling of the white matter compared with the gray matter (Fig. S4*C*).

Finally, we performed metabolic labeling experiments to substantiate our conclusion of lower sialic acid content in differentiated

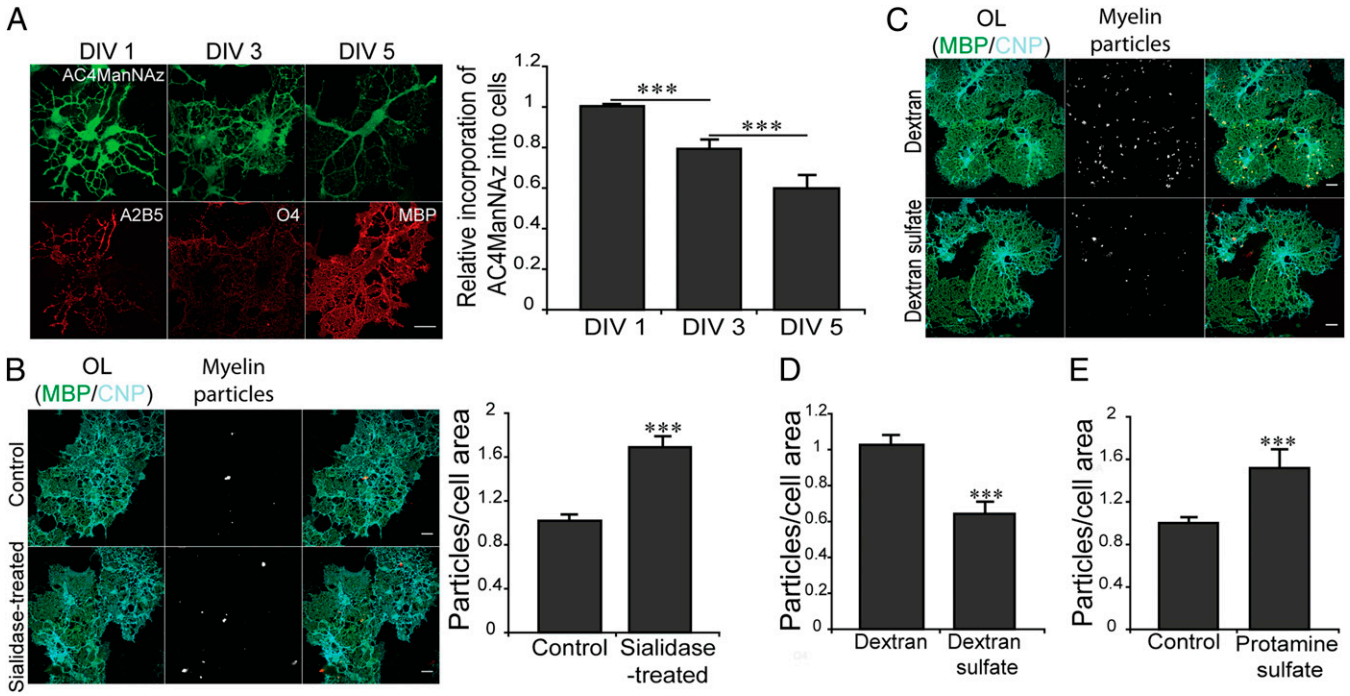


**Fig. 3.** Reduction in sialic acid content enhances myelin adhesiveness. (*A*) Oligodendrocytes at different stages of differentiation were metabolically labeled with 50 μM Ac4ManNAz for 24 h. The fluorescent alkyne was used to detect the incorporated sialic acids ($n = 4$, $^{***}P < 0.001$; one-way ANOVA). (*B*) Cells at 3 d in culture (DIV) were treated with 5 U/mL sialidase for 1 h at 37 °C and the binding of particles was evaluated. Data were normalized relative to control ($n = 4$, $^{***}P < 0.001$; *t* test). (*C* and *D*) Fully differentiated oligodendrocytes (DIV 5) were treated with 5 mg/mL dextran or dextran sulfate for 30 min at 37 °C and the binding of particles was determined ($n = 4$, $^{***}P < 0.001$; *t* test). (*E*) Oligodendrocytes at DIV 3 were incubated with 50 μg/mL protamine sulfate for 15 min at 37 °C and the binding assay was performed ($n = 5$, $^{***}P < 0.001$; *t* test). (Scale bars, 20 μm.)

oligodendrocytes. A sugar derivative, tetraacetylated *N*-azidoacetyl-D-mannosamine (Ac4ManNAz), was used to chemically modify and label sialic acids in cultured oligodendrocytes. Metabolic labeling was performed with Ac4ManNAz for 24 h, and the synthesized azido-sugars were visualized with a fluorophore-conjugated alkyne. We observed a significant reduction in sialic acid biosynthesis with the differentiation of oligodendrocytes (Fig. 3*A*). In comparison, astrocytes did not change their sialic acid metabolism during the same time span in culture (Fig. S5*A*).

One possible mechanism by which oligodendrocytes increase their adhesiveness is the removal of sialic acid molecules from their cell surface. Because myelin particles attached poorly to the cell surface of immature oligodendrocytes (Fig. S5 *B* and *C*), we asked whether binding could be increased by removing sialic acids from their plasma membrane. Indeed, the treatment with neuraminidase (sialidase) resulted in a pronounced increase in the number of binding myelin particles (Fig. 3*B* and Fig. S5*D*).

Because sialic acids contribute to the negative charges of the plasma membrane, we asked whether manipulating the charge of the cell surfaces has any influence on myelin particle attachment. We applied the negatively charged dextran sulfate and the positively charged protamine sulfate to change cell-surface charge, as previously established for endothelial cells (26). Treatment of differentiated oligodendrocytes with dextran sulfate reduced particle binding (Fig. 3 *C* and *D*), whereas charge neutralization with protamine sulfate increased myelin particle attachment to oligodendrocytes (Fig. 3*E*). Thus, cell-surface charge regulates the adhesiveness of the oligodendrocyte cell surface.

Next, we analyzed whether it is possible to reduce the adhesiveness of oligodendrocytes by reintroducing sialic acids to the cell surface of mature oligodendrocytes. One component that is lost from the cell surface during oligodendrocyte differentiation is the gangliosides, which are glycolipids with a complex oligosaccharide tree containing terminal sialic acids (27). We thus asked whether the reincorporation of gangliosides into the membrane of oligodendrocytes influences their adhesiveness toward myelin particles. Indeed, when total brain gangliosides were loaded onto oligodendrocytes, a notable decrease of particle binding was observed (Fig. 4*A*). In line with these experiments, incorporation of gangliosides into myelin liposomes resulted in a dramatic reduction in their aggregation (Fig. 4*B*). Because these data demonstrate a repulsive effect of negatively charged sialic acid-containing molecules in myelin membrane adhesion, we asked whether increasing the sialic acid content in oligodendrocytes affects myelin structure in vivo.

Levels of polysialic acid are dramatically decreasing during oligodendrocyte differentiation, and one reason for this is the down-regulation of the polysialyltransferase ST8SiaIV (28, 29). Transgenic mice expressing ST8SiaIV under the Plp1 promoter have been generated (PLP-ST8SiaIV) (30). One conclusion from this study was that the down-regulation of polysialic acid is a prerequisite for normal myelination, because these mice had fewer mature oligodendrocytes and formed less myelin (30). We used conventional embedding fixation of optic nerve to analyze the ultrastructure of myelin in these mice. We found that axons in the optic nerves were myelinated. However, the ultrastructure of myelin from 20-wk-old mice appeared to be altered, as splitting of the myelin lamellae was frequently seen. Quantification revealed a significant increase in the average distance of the myelin membrane bilayers within a sheath compared with control (Fig. 4*C*). To analyze possible changes in myelin adhesiveness, we performed myelin particle interaction assays using purified myelin isolated from PLP-ST8SiaIV mice. These experiments revealed a significant reduction in the number of interacting particles isolated from PLP-ST8SiaIV mice compared with controls (Fig. 4*D*), supporting our conclusion that sialic acids convey repulsive forces to a membrane.

If loss of sialic acids were a prerequisite for myelin membrane adhesion, it should be possible to increase the attachment of myelin particles to nonmyelinating cells, such as the epithelial cell line potoroo kidney (PtK2), by removing sialic acids from its surface. First, we tested whether the expression of PLP in transfected PtK2 cells resulted in an increase in myelin particle

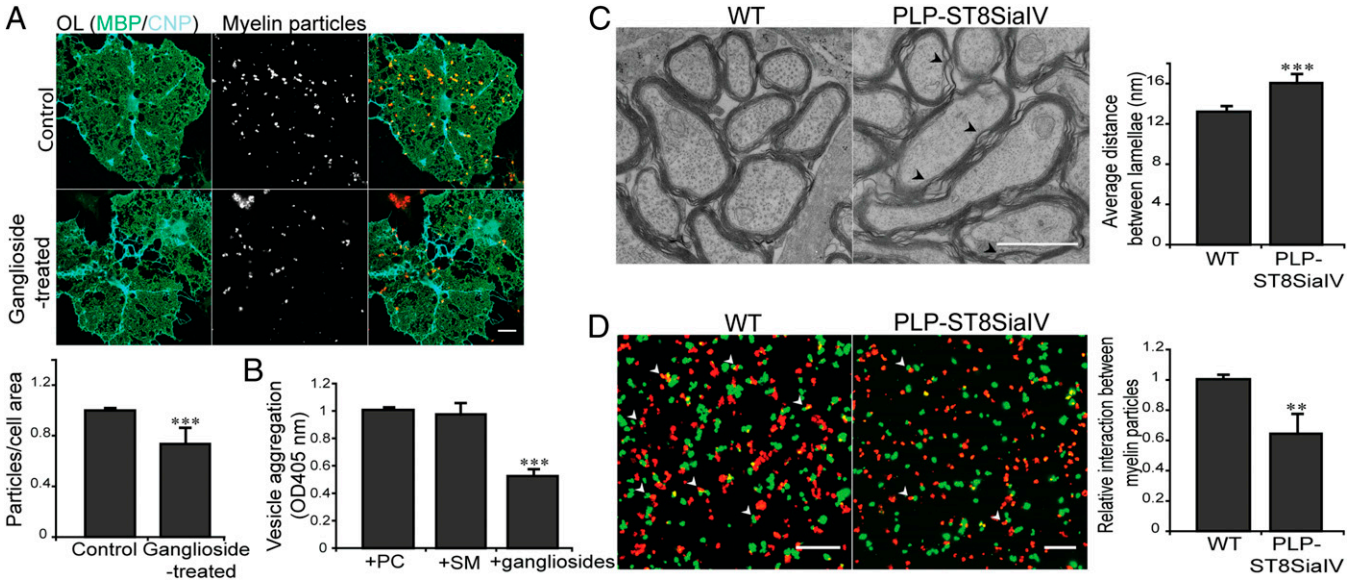


**Fig. 4.** Increase in sialic acid content reduces membrane adhesiveness. (*A*) Total brain gangliosides (80 μM) coupled to defatted BSA were added to differentiated cells for 1 h at 37 °C. After extensive washing with PBS, a binding assay was performed. Data were normalized relative to control ($n = 6$, $^{***}P < 0.001$; *t* test). (Scale bar, 20 μm.) (*B*) Exogenous lipids were incorporated into myelin liposomes to obtain a concentration of 10% total lipids. A liposome aggregation assay revealed that gangliosides reduce aggregation compared with sphingomyelin (SM) and phosphatidylcholine (PC) ($n = 4$, $^{***}P < 0.001$; one-way ANOVA). (*C*) Conventional embedding fixation of optic nerves from 20-wk-old mice showed an increase in myelin lamella splitting (arrowheads) for PLP-ST8SiaIV mice compared with wild-type ($n = 15$ multiple image alignments from three animals, $^{***}P < 0.001$; *t* test). (Scale bar, 1 μm.) (*D*) A decrease in particle-to-particle interaction (arrowheads) was observed between WT particles and particles prepared from PLP-ST8SiaIV mice ($n = 3$, $^{**}P < 0.01$; *t* test). (Scale bars, 20 μm.)

attachment. However, only subtle changes were observed. In contrast, treatment with sialidase resulted in a dramatic increase in particle binding to PLP-expressing cells (Fig. S6 *A* and *B*), suggesting that loss of repulsive forces also uncovers attractive forces by PLP.

## Discussion

The interaction of cell membranes is critical in many biological processes. A particularly fascinating process is the association of the extracellular leaflets of the myelin membrane bilayer in oligodendrocytes and Schwann cells (4, 31). This interaction is unusual, because the two opposing surfaces of the plasma membrane have to associate over very large areas. Such extensive bilayer–bilayer interactions are, in most cells, prevented by a dense and confluent layer of large and negatively charged oligosaccharides that protects cells from unspecific adhesion with molecules and other cells (32). Adhesion occurs in many cases by lock-and-key forces of adhesion molecules, which override the repulsive forces of the membrane bilayer (12). These interactions result in the formation of specific membrane attachment sites, in which the size of the domains is limited by the osmotic pressure produced by the repelling molecules at the edges of the adhesive sites (12, 23). Here we propose a model that the interaction of the extracellular leaflets of the myelin membrane does not require strong lock-and-key forces but is caused by the loss of the repulsive molecules from the plasma membrane. We provide evidence that oligodendrocytes lose major components of their glycocalyx with the differentiation of the cells. Using a number of different lectins to stain various carbohydrates, we show that sugar residues, including the negatively charged sialic acids, are virtually absent from the surface of mature oligodendrocytes.

Results from several different experiments show that loss of electrostatic cell-surface repulsion is a requirement for bilayer–bilayer interaction. First, removal of sialic acids by neuraminidase promotes myelin particle attachment. Second, charge neutralization by cationic protamine sulfate increases particle binding. Third, substituting sialic acids with dextran sulfate restores the repulsive effects of the cell surface toward myelin membrane particles. Finally, increased polysialic acid content in myelin from transgenic mice expressing sialyltransferase results in splitting of myelin lamellae. Thus, electrostatic forces are likely to be a key mechanism governing the adhesion of the extracellular leaflets of individual myelin layers. However, loss of steric repulsion is another possible factor. Indeed, several proteins with large extracellular domains such as chondroitin sulfate proteoglycan 4 (NG2) and CD44 are down-regulated during oligodendrocyte differentiation (33, 34). Interestingly, reexpression of CD44 into mature oligodendrocytes causes dysmyelination in mice (35).

There are several ways oligodendrocytes might restructure their plasma membrane to promote its adhesiveness. Down-regulation of enzymes involved in the synthesis of sialic acids is one possibility. For example, a reduction of ST8SiaIV mRNA levels has been observed during oligodendrocyte differentiation (36, 37). Pathway analysis of published transcriptome data reveals that several other enzymes involved in glycosylation pathways, such as enzymes involved in glycosphingolipid and *N*- and *O*-glycan biosynthesis, are underrepresented in myelinating oligodendrocytes (38). We also find a reduction in sialic acid synthesis by metabolically labeling maturating oligodendrocytes. Another possibility for reducing the content of glycoproteins in myelin is provided by MBP as a diffusion barrier for proteins with large cytoplasmic domains (39).

Thus, by reduced synthesis and by preventing targeting, many large and charged glycoproteins may be restricted from being incorporated into myelin. Similar mechanisms may also hold for lipids. During the differentiation of oligodendrocytes, major changes in lipid synthesis occur. Whereas oligodendrocyte precursor cells synthesize gangliosides with negatively charged and branched sugar residues attached to their head groups, differentiated oligodendrocytes produce galactosylceramide as their major glycolipid (40, 41). Interestingly, studies on artificial bilayers reveal relatively strong adhesive forces between galactosylceramides, even a much stronger value than measured for phosphatidylcholine (42). Our study provides evidence that the shift in the synthesis of specific glycolipid species is functionally relevant for the adhesiveness of the oligodendrocyte plasma membrane. When gangliosides are reintroduced into differentiated oligodendrocytes, attachment of myelin particles is reduced.

The co-ordinated change in the expression of adhesion molecules along with a reduction in repellers mediates cell–cell interaction. For instance, up-regulation of integrins together with the down-regulation of the repulsive molecule CD43 is a prerequisite for the penetration of leukocytes through the endothelium of blood vessels (43, 44). Therefore, one important question is whether the loss of a repulsive glycocalyx from the extracellular region of the myelin membrane is sufficient to promote a close bilayer–bilayer interaction over large areas or whether specific adhesion molecules are required for the compaction of the extracellular leaflets of the myelin membrane. The adhesive role of PLP has been controversial. The controversy stems from different results from mice lacking PLP. Analysis of one mouse line, termed $plp^{neo}$, revealed a disrupted myelin ultrastructure with missing intraperiod lines and severely splitted extracytoplasmic surfaces (15). The complete $Plp1^{null}$ mouse did not confirm this severe phenotype and showed instead the presence of compacted myelin, even more tightly condensed with a reduced periodicity (16, 45). In these mice, delaminated myelin was only found in regions in which the fixation of the tissue was poor (22, 46). Subsequent studies confirmed that myelin from $Plp1^{null}$ mice defragments more easily (47, 48). Thus, changes in fixation conditions are most likely to explain the reported differences in myelin ultrastructure between these two Plp1 mutant mouse lines. Using a number of different assays, we now find that PLP indeed possesses adhesive forces; however, even without these forces, the extracytoplasmic surfaces of myelin membranes stick together. Thus, not the expression of PLP but rather the loss of repulsive forces between adjacent membrane bilayers might drive myelin compaction at the IPL. Other, yet to be identified interactions cannot be excluded, but we believe that strong lock-and-key forces by specific adhesion molecules are unlikely. One reason why oligodendrocytes may use weak forces for the alignment of extracellular membrane surfaces might lie in the way myelin is generated. Newly synthesized membrane bilayers have to glide along each other within the developing myelin sheath, which requires dynamic and weak connections.

Interestingly, removal of repulsive molecules has also been implicated in axon–oligodendrocyte interactions. For example, loss of polysialic acid from neural cell adhesion molecule (NCAM) in neurons initiates myelination (49, 50). Moreover, a recent study provides evidence that oligodendrocytes are able to wrap their membrane around synthetic nanofibers, suggesting that unspecific interactions of axons and oligodendrocytes are sufficient to trigger myelination (51). The transgenic animals used in this study express ST8SiaIV under the Plp1 promoter, which increases the sialic acid content of NCAM in mature oligodendrocytes (30). Because myelin formation per se is unaffected, we do not attribute the delamination phenotype to impaired oligodendrocyte–axon interactions. We rather suggest that the splitting of the myelin lamellae is due to the altered composition of myelin and its reduced stability.

In summary, we suggest a model in which the adhesion of the extracellular surfaces of myelin requires the down-regulation of repulsive components of the glycocalyx, which uncovers weak and unspecific attractive forces in the bilayer that bring the extracellular surfaces into close contact over large areas (Fig. S6*C*). Therefore, changes in pH or ion concentration are likely to influence the

apposition of myelin bilayers and might contribute to myelin destabilization in diseases such as multiple sclerosis or stroke.

## Materials and Methods

Primary culture of oligodendrocytes, myelin purification, and myelin particle preparation and labeling as well as the particle–cell binding assay are discussed in *SI Materials and Methods*. Details of immunoblotting, immunostaining, and microscopic analysis are also provided in *SI Materials and Methods*. All animal experiments were performed under approval of the responsible local organization (LAVES, Oldenburg).

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1. Sherman DL, Brophy PJ (2005) Mechanisms of axon ensheathment and myelin growth. *Nat Rev Neurosci* 6(9):683–690.
2. Min Y, et al. (2009) Interaction forces and adhesion of supported myelin lipid bilayers modulated by myelin basic protein. *Proc Natl Acad Sci USA* 106(9):3154–3159.
3. Baumann N, Pham-Dinh D (2001) Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol Rev* 81(2):871–927.
4. Aggarwal S, Yurlova L, Simons M (2011) Central nervous system myelin: Structure, synthesis and assembly. *Trends Cell Biol* 21(10):585–593.
5. Arroyo EJ, Scherer SS (2000) On the molecular architecture of myelinated fibers. *Histochem Cell Biol* 113(1):1–18.
6. Israelachvili JN (2010) *Intermolecular and Surface Forces* (Academic Press, London, San Diego), 3rd Ed.
7. Foa C, Soler M, Benoliel A-M, Bongrand P (1996) Steric stabilization and cell adhesion. *J Mater Sci Mater Med* 7(3):141–148.
8. Bell GI, Dembo M, Bongrand P (1984) Cell adhesion. Competition between nonspecific repulsion and specific bonding. *Biophys J* 45(6):1051–1064.
9. Sabri S, Pierres A, Benoliel AM, Bongrand P (1995) Influence of surface charges on cell adhesion: Difference between static and dynamic conditions. *Biochem Cell Biol* 73(7-8):411–420.
10. Sabri S, et al. (2000) Glycocalyx modulation is a physiological means of regulating cell adhesion. *J Cell Sci* 113(Pt 9):1589–1600.
11. Morris JE (1993) Proteoglycans and the modulation of cell adhesion by steric exclusion. *Dev Dyn* 196(4):246–251.
12. Sackmann E, Goennenwein S (2006) Cell adhesion as dynamic interplay of lock-and-key, generic and elastic forces. *Prog Theor Phys* (Suppl 165):78–99.
13. Sospedra M, Martin R (2005) Immunology of multiple sclerosis. *Annu Rev Immunol* 23:683–747.
14. Popot JL, Pham Dinh D, Dautigny A (1991) Major myelin proteolipid: The 4-alpha-helix topology. *J Membr Biol* 123(3):278.
15. Boison D, Büssow H, D'Urso D, Müller HW, Stoffel W (1995) Adhesive properties of proteolipid protein are responsible for the compaction of CNS myelin sheaths. *J Neurosci* 15(8):5502–5513.
16. Klugmann M, et al. (1997) Assembly of CNS myelin in the absence of proteolipid protein. *Neuron* 18(1):59–70.
17. Sinoway MP, Kitagawa K, Timsit S, Hashim GA, Colman DR (1994) Proteolipid protein interactions in transfectants: Implications for myelin assembly. *J Neurosci Res* 37(5):551–562.
18. Palaniyar N, Semotok JL, Wood DD, Moscarello MA, Harauz G (1998) Human proteolipid protein (PLP) mediates winding and adhesion of phospholipid membranes but prevents their fusion. *Biochim Biophys Acta* 1415(1):85–100.
19. Bizzozero OA, Howard TA (2002) Myelin proteolipid protein-induced aggregation of lipid vesicles: Efficacy of the various molecular species. *Neurochem Res* 27(11):1269–1277.
20. Müller DJ, Helenius J, Alsteens D, Dufrêne YF (2009) Force probing surfaces of living cells to molecular resolution. *Nat Chem Biol* 5(6):383–390.
21. Janshoff A, Neitzert M, Oberdörfer Y, Fuchs H (2000) Force spectroscopy of molecular systems—Single molecule spectroscopy of polymers and biomolecules. *Angew Chem Int Ed Engl* 39(18):3212–3237.
22. Möbius W, Patzig J, Nave K-A, Werner HB (2008) Phylogeny of proteolipid proteins: Divergence, constraints, and the evolution of novel functions in myelination and neuroprotection. *Neuron Glia Biol* 4(2):111–127.
23. Sackmann E, Bruinsma RF (2002) Cell adhesion as wetting transition? *ChemPhysChem* 3(3):262–269.
24. Niehaus A, Trotter J (1997) Lectin-binding properties of oligodendrocyte lineage cells aid in defining functionally important surface proteins. *Glia* 20(3):173–183.
25. Fujimoto I, Bruses JL, Rutishauser U (2001) Regulation of cell adhesion by polysialic acid. Effects on cadherin, immunoglobulin cell adhesion molecule, and integrin function and independence from neural cell adhesion molecule binding or signaling activity. *J Biol Chem* 276(34):31745–31751.
26. Strilić B, et al. (2010) Electrostatic cell-surface repulsion initiates lumen formation in developing blood vessels. *Curr Biol* 20(22):2003–2009.
27. Cochran FB, Jr., Yu RK, Ledeen RW (1982) Myelin gangliosides in vertebrates. *J Neurochem* 39(3):773–779.
28. Trotter J, Bitter-Suermann D, Schachner M (1989) Differentiation-regulated loss of the polysialylated embryonic form and expression of the different polypeptides of the neural cell adhesion molecule by cultured oligodendrocytes and myelin. *J Neurosci Res* 22(4):369–383.
29. Bartsch U, Kirchhoff F, Schachner M (1990) Highly sialylated N-CAM is expressed in adult mouse optic nerve and retina. *J Neurocytol* 19(4):550–565.
30. Fewou SN, Ramakrishnan H, Büssow H, Gieselmann V, Eckhardt M (2007) Down-regulation of polysialic acid is required for efficient myelin formation. *J Biol Chem* 282(22):16700–16711.
31. Garbay B, Heape AM, Sargueil F, Cassagne C (2000) Myelin synthesis in the peripheral nervous system. *Prog Neurobiol* 61(3):267–304.
32. Curry FE, Adamson RH (2012) Endothelial glycocalyx: Permeability barrier and mechanosensor. *Ann Biomed Eng* 40(4):828–839.
33. Nishiyama A, Komitova M, Suzuki R, Zhu X (2009) Polydendrocytes (NG2 cells): Multifunctional cells with lineage plasticity. *Nat Rev Neurosci* 10(1):9–22.
34. Bouvier-Labit C, Liprandi A, Monti G, Pellissier JF, Figarella-Branger D (2002) CD44H is expressed by cells of the oligodendrocyte lineage and by oligodendrogliomas in humans. *J Neurooncol* 60(2):127–134.
35. Tuohy TMF, et al. (2004) CD44 overexpression by oligodendrocytes: A novel mouse model of inflammation-independent demyelination and dysmyelination. *Glia* 47(4):335–345.
36. Nait Oumesmar B, et al. (1995) Expression of the highly polysialylated neural cell adhesion molecule during postnatal myelination and following chemically induced demyelination of the adult mouse spinal cord. *Eur J Neurosci* 7(3):480–491.
37. Stoykova LI, Beesley JS, Grinspan JB, Glick MC (2001) ST8Sia IV mRNA corresponds with the biosynthesis of alpha2,8sialyl polymers but not oligomers in rat oligodendrocytes. *J Neurosci Res* 66(3):497–505.
38. Dugas JC, Tai YC, Speed TP, Ngai J, Barres BA (2006) Functional genomic analysis of oligodendrocyte differentiation. *J Neurosci* 26(43):10967–10983.
39. Aggarwal S, et al. (2011) A size barrier limits protein diffusion at the cell surface to generate lipid-rich myelin-membrane sheets. *Dev Cell* 21(3):445–456.
40. Jackman N, Ishii A, Bansal R (2009) Oligodendrocyte development and myelin biogenesis: Parsing out the roles of glycosphingolipids. *Physiology (Bethesda)* 24(5):290–297.
41. Chrast R, Saher G, Nave K-A, Verheijen MHG (2011) Lipid metabolism in myelinating glial cells: Lessons from human inherited disorders and mouse models. *J Lipid Res* 52(3):419–434.
42. Kulkarni K, Snyder DS, McIntosh TJ (1999) Adhesion between cerebroside bilayers. *Biochemistry* 38(46):15264–15271.
43. Remold-O'Donnell E, Parent D (1994) Two proteolytic pathways for down-regulation of the barrier molecule CD43 of human neutrophils. *J Immunol* 152(7):3595–3605.
44. Gahmberg CG (1997) Leukocyte adhesion: CD11/CD18 integrins and intercellular adhesion molecules. *Curr Opin Cell Biol* 9(5):643–650.
45. Duncan ID, Hammang JP, Trapp BD (1987) Abnormal compact myelin in the myelin-deficient rat: Absence of proteolipid protein correlates with a defect in the intraperiod line. *Proc Natl Acad Sci USA* 84(17):6287–6291.
46. Rosenbluth J, Nave K-A, Mierzwa A, Schiff R (2006) Subtle myelin defects in PLP-null mice. *Glia* 54(3):172–182.
47. Rosenbluth J, Schiff R, Lam P (2009) Effects of osmolality on PLP-null myelin structure: Implications re axon damage. *Brain Res* 1253:191–197.
48. Jurevics H, et al. (2003) Normal metabolism but different physical properties of myelin from mice deficient in proteolipid protein. *J Neurosci Res* 71(6):826–834.
49. Charles P, et al. (2000) Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molecule. *Proc Natl Acad Sci USA* 97(13):7585–7590.
50. Jakovcevski I, Mo Z, Zecevic N (2007) Down-regulation of the axonal polysialic acid-neural cell adhesion molecule expression coincides with the onset of myelination in the human fetal forebrain. *Neuroscience* 149(2):328–337.
51. Lee S, et al. (2012) A culture system to study oligodendrocyte myelination processes using engineered nanofibers. *Nat Methods* 9(9):917–922.