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BRAIN RESEARCH

Research Report

Effects of osmolality on PLP-null myelin structure: Implications re axon damage

Jack Rosenbluth, Rolf Schiff, Pokman Lam*

Department of Physiology and Neuroscience, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA
Department of Rehab. Medicine, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA

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ABSTRACT

In order to test the adhesiveness of PLP-null compact myelin lamellae we soaked aldehyde-fixed CNS specimens from PLP-null and control mice overnight in distilled water, in Ringer's solution or in Ringer's solution with added 1 M sucrose. Subsequent examination of the tissue by EM showed that both PLP-null and control white matter soaked in Ringer remained largely compact. After the distilled water soak, control myelin was virtually unchanged, but PLP-null myelin showed some decompaction, i.e., separation of myelin lamellae from one another. After the sucrose/Ringer soak, normal myelin developed foci of decompaction, but the great majority of lamellae remained compact. In the PLP-null specimens, in contrast, many of the myelin sheaths became almost completely decompacted. Such sheaths became thicker overall and were comprised of lamellae widely separated from one another by irregular spaces. Thus, in normal animals, fixed CNS myelin lamellae are firmly adherent and resist separation; PLP-null myelin lamellae, in contrast, are poorly adherent and more readily separated. Mechanisms by which impaired adhesiveness of PLP-null myelin lamellae and fluctuations in osmolality in vivo might underlie slowing of conduction and axon damage are discussed.

1. Introduction

Inactivation of the proteolipid protein (PLP) gene results in the formation of CNS myelin of near normal structure and abundance despite the complete lack of PLP and its smaller isoform, DM20 (Klugmann et al., 1997). These animals survive well, but develop progressive degeneration of CNS axons beginning at ~6–8 wk (Griffiths et al., 1998), reminiscent of the axon loss thought to underlie secondary progression in the relapsing/remitting form of multiple sclerosis (Trapp et al., 1998).

Despite its relatively normal appearance, previous studies have noted inconspicuous defects in PLP-null myelin consisting primarily of widening and irregularity of the interlamellar spaces (Rosenbluth et al., 2006). This subtle decompaction, visible in electron micrographs of fixed tissue, corresponds closely to the greater disorder and wider extracellular spaces between lamellae found in X-ray diffraction studies of unfixed PLP-null myelin (Yin et al., 2006) and is consistent with defective adhesion of myelin lamellae along their external surfaces due to loss of homophilic bonding of PLP molecules between adjacent myelin lamellae. Chemical modification of PLP, thought to interfere with homophilic bonding, also results in decompaction (Bizzozero et al., 2001, 2004).

We have suggested previously that defective compaction of PLP-null myelin could result in axon damage from leakage of

* *Corresponding author*. Department of Physiology and Neuroscience, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA. Fax: +1 212 263 8007.
E-mail address: rosenj03@med.nyu.edu (J. Rosenbluth).

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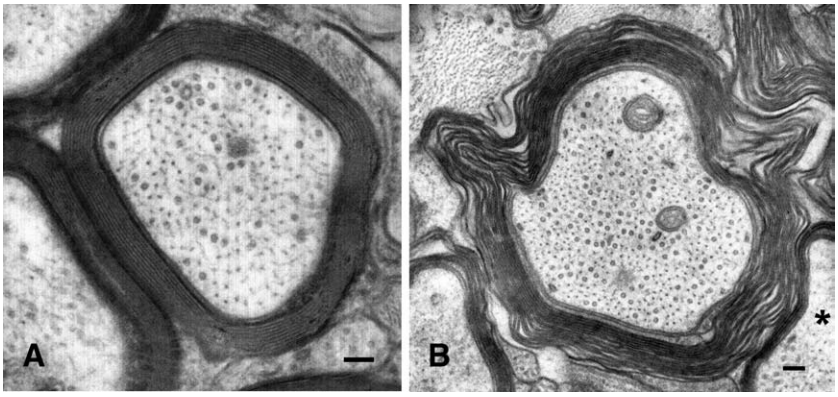

Fig. 1 – Myelin after overnight soak in distilled water. (A) Control optic nerve. The fibers appear as rounded polygons surrounded by compact myelin. Bar=0.1 μm. (B) PLP-null optic nerve. The outer contour of the myelin shows the protrusions characteristic of this mutant. A moderate degree of lamellar separation is visible in the sheaths at center and upper right. The thinner sheath around the axon at the lower right (*) appears compact. Bar=0.1 μm.

ions and other solutes through extracellular pathways in the internodal myelin (Rosenbluth et al., 1996b). In the present paper we present further evidence that PLP-null myelin lamellae are poorly adherent in that they are more readily separable than normal in non-isosmotic solutions.

Our results provide an explanation for the marked differences in PLP-null myelin structure reported in studies that have used different methods for preparing the tissues. In addition, they introduce the possibility that variations in osmolality could increase the leakiness of PLP-null myelin sheaths in vivo as a potential basis for conduction defects and axon damage.

2. Results

2.1. *Compact myelin*

2.1.1. *Ringer's soak*

An overnight soak in Ringer's solution (R) following aldehyde fixation corresponds to the conditions used in preparing the specimens in our previous study of PLP-null myelin compared with control (Rosenbluth et al., 2006). Data obtained from the current Ringer's soaked group confirmed the earlier observations, which will be reviewed briefly here but not illustrated.

Cross sections through control specimens showed myelinated nerve fiber profiles whose outlines were primarily in the form of rounded polygons. 'Splits' in myelin sheaths occurred infrequently. Ferricyanide, which acts as an extracellular tracer (Aguas, 1982), was found between lamellae in some of the thin sheaths, but in thicker sheaths it was confined to the inner and outer regions and did not extend between lamellae in the middle. Myelin generally appeared compact with a regular periodicity.

PLP-null specimens differed in that the outlines of the sheaths were more varied in shape, with frequent redundant folds involving all or some of the lamellae extending away from the axon. As a result the external contour of the fibers often looked scalloped or irregular, as if the sheath was too large for the enclosed axon. Where ferricyanide density could be seen, it more often infiltrated between layers throughout even thicker sheaths in an irregular manner (Fig. 3b in Rosenbluth et al., 2006). The width of the ferricyanide-labeled spaces between layers was variable, resulting in corresponding variation in the repeat period of the compact lamellae. Nevertheless, at low magnification, PLP-null myelin sheaths were close to normal in appearance and compaction at all ages examined.

Table 1 – Extent of decompaction in fixed PLP-null and control myelin under different osmotic conditions

	R		1MS		DW	
	PLP-null (*n*)	Control (*n*)	PLP-null (*n*)	Control (*n*)	PLP-null (*n*)	Control (*n*)
0–1/4	44.5% (89)	80% (223)	10% (28)	63% (94)	45% (209)	90% (311)
>1/4–1/2	24.5% (43)	15% (43)	5% (13)	19% (29)	10% (47)	10% (31)
>1/2–3/4	5.5% (11)	1% (3)	3% (8)	11% (16)	4% (18)	0% (0)
>3/4	25.5% (51)	4% (11)	82% (226)	7% (10)	41% (188)	0% (0)

After an isosmotic soak (R), 4% of control myelin sheaths and ~25% of PLP-null myelin sheaths showed decompaction around >3/4 of their circumference. However, the *p* value for this apparent difference was 0.19 (n.s.). In contrast, comparison of the >3/4 bin for PLP-null specimens soaked in R (~25%) with PLP-null specimens soaked in 1MS (82%) showed a highly significant difference ($p<0.00001$), as did comparison of the >3/4 bin for PLP-null specimens in R (~25%) with PLP-null specimens soaked in DW (41%) ($p<0.0001$). Under all three conditions, control myelin showed relatively little decompaction.