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Metabolically-incorporated deuterium in myelin from mice localized by neutron diffraction

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Myelin is a natural and dynamic multilamellar membrane structure that continues to be of significant biological and neurological interest, especially with respect to its biosynthesis and assembly during its normal formation, maintenance, and pathological breakdown. To explore the usefulness of neutron diffraction in the structural analysis of myelin, we investigated the use of in vivo labeling by metabolically incorporating non-toxic levels of D2O via drinking water into a pregnant dam (D-dam) and her developing embryos. All of the mice were sacrificed when the pups (D-pups) were 55 days old. Myelinated sciatic nerves were dissected, fixed in glutaraldehyde and examined by neutron diffraction. Parallel samples that were unfixed (trigeminal nerves) were frozen for mass spectrometry (MS).

The diffraction patterns of the nerves from deuterium-fed mice (D-mice) vs. the controls (H-mice) had major differences in the intensities of the Bragg peaks but no differences in myelin periodicity. Neutron scattering density profiles showed an appreciable increase in density at the center of the lipid-rich membrane bilayer. This increase was greater in D-pups than in D-dam, and its localization was consistent with deuteration of lipid hydrocarbon chains, which predominate over transmembrane proteins in myelin. MS analysis of the lipids isolated from the trigeminal nerves demonstrated that in the pups the percentage of lipids that had one or more deuterium atoms was uniformly high across lipid species (97.6% +/- 2.0%), whereas in the mother the lipids were substantially less deuterated (60.6% +/- 26.4%) with levels varying among lipid species and subspecies. The mass distribution pattern of deuterium-containing isotopologues indicated the fraction (in %) of each lipid (sub-)species having one or more deuteriums incorporated: in the D-pups, the pattern was always bell-shaped, and the average number of D atoms ranged from a low of ~4 in fatty acid to a high of ~9 in cerebroside. By contrast, in D-dam most lipids had more complex, overlapping distributions that were weighted toward a lower average number of deuteriums, which ranged from a low of ~3–4 in fatty acid and in one species of sulfatide to a high of 6–7 in cerebroside and sphingomyelin. The consistently high level of deuteration in D-pups can be attributed to their de novo lipogenesis during gestation and rapid, postnatal myelination. The widely varying levels of deuteration in D-dam, by contrast, likely depends on the relative metabolic stability of the particular lipid species during myelin maintenance. Our current findings demonstrate that stably-incorporated D label can be detected and localized using neutron diffraction in a complex tissue such as myelin; and moreover, that MS can be used to screen a broad range of deuterated lipid species to monitor differential rates of lipid turnover. In addition to helping to develop a comprehensive understanding of the de novo synthesis and turnover of specific lipids in normal and abnormal myelin, our results also suggest application to studies on myelin proteins (which constitute only 20–30% by dry mass of the myelin, vs. 70–80% for lipids), as well as more broadly to the molecular constituents of other biological tissues.

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