JBC EDITORS' PICK HIGHLIGHT

A myelin sheath protein forming its lattice

DOI 10.1074/jbc.H120.014273

Hideaki Tsuge*
From the Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Japan

Edited by Paul E. Fraser

The formation of a mature, multilayered myelin sheath requires the compaction of lipid bilayers, but the molecular mechanism by which these bilayers condense is an open question. In this issue, Ruskamo *et al.* find that peripheral myelin protein P2 forms an ordered three-dimensional lattice within model membranes using *Escherichia coli* polar lipid liposomes. These data will help to understand the assembly, function, and structure of the myelin sheath.

Myelin is a lipid substance that is formed around nerve cell axons to insulate them and increase the rate of electrical impulses. The myelin sheath is formed in the central nervous system from oligodendrocytes and in the peripheral nervous system (PNS), from Schwann cells (1), in each case with an extension of the cell membrane wrapping around the axon. Transmission electron microscope observations have shown that the final structure consists of many membranes stacked together, with the distance between the two layers of a cell membrane in the double-bilayer becoming shorter and the adjacent double-bilayers tightly closing up (2). Despite this functional importance and structural complexity, structural types of protein in the myelin sheath—consisting of 20–30% protein and 70–80% lipid—are relatively few. The PNS proteins include the intrinsically disordered myelin basic protein (MBP), peripheral myelin protein zero (P0) and 22 (PMP22), and another peripheral membrane protein, P2. How do these lipids and proteins come together to organize the sheath structure?

Previous work from these authors has explored the formation of the myelin sheath by studying each of the individual proteins. They determined that MBP, found between two membrane bilayers, drives the formation of one structural feature of the sheath, the major dense line (2). P0 has an Ig-like extracellular domain and potentially involves the arrangement of the Ig-like domains, thus consequently stabilizing the intraperiod line (3). It is known that P2 can cause bilayer stacking (4), but the specific mechanism by which it mediates this process was unclear.

P2 is mainly expressed by Schwann cells in the PNS and is a typical fatty acid–binding protein (FABP) with a barrel formed of 10 antiparallel β strands and a helical lid capping the fatty acid–binding site (5). Generally, FABPs are thought to be involved in the intracellular transport of lipids, and indeed, P2 is located on the cytoplasmic side of compact myelin membranes (6). Myelin contains high levels of cholesterol (more than 25% of the total lipid content); thus, it has been postulated that P2 might function as a cholesterol transporter. Questions are whether and, if so, how P2 transports the cholesterol into the myelin membrane, how P2 organizes the myelin sheath, and whether these two functions are related to each other.

In their new study, Ruskamo *et al.* explore these questions in an investigation of the features and behavior of P2. The authors first show that mixing human P2 with *Escherichia coli* polar lipids makes myelin-like multilayered stacks as followed by cryo-EM (7). P2 is located in the 3.0-nm space between two membranes, although the exact orientation is unknown. Interestingly, the individual molecules of P2 formed an ordered 3D lattice, displaying consistent lateral spacing within the intermembrane space and across consecutive layers (Fig. 1). To gain further insight into this behavior, the authors next used crystallography, molecular dynamics, and membrane- and lipid-binding assays to investigate 12 P2 mutants distributed among two positively charged faces, a hydrophobic patch, and the hinge for the helical lid. P38G, a known "hyperactive" (8) mutation located at the hinge between the β barrel and the α-helical lid, had a crystallographic structure not different from WT P2 protein, but the fatty acid ligand was unusually absent, and thermal unfolding analysis indicated that it is more dynamic/less stable than the WT. P38G formed myelin-like multilayered stacks as well as WT and showed strong membrane, fatty acid, and cholesterol binding (7).

Mutations that removed positive charges also did not show changes in the crystallographic data, but did generally show decreased lipid binding and higher levels of fatty acid exchange. An assay with a cholesterol analog confirmed that WT and P38G do bind cholesterol. Finally, molecular dynamics calculations suggested that P2 membrane binding could be initiated in part by an interaction with Arg-88, a residue unique to P2 compared with other FABP members. These data point to a model in which P2 membrane binding, initiated by positively charged and hydrophobic contacts, leads to conformational dynamics linked to exposure of the fatty acid–binding site.

P2 mutations in the myelin sheath lead to a variety of neurological diseases. Charcot-Marie-Tooth disease is a hereditary peripheral neuropathy caused by mutations in dozens of genes, including that coding for P2 (8, 9). Whereas the P2 Charcot-Marie-Tooth disease mutations (I43N, T51P, and I52T) have small effects on the crystal structure, they destabilize the P2 protein and affect its membrane-binding properties (10). These mutants with reduced stability may cause an abnormality in the myelin function and myelin stability. Specifically, the disease variants are more dynamic than WT P2, and the β-barrel structure of P2 opens more easily, which might be of relevance for ligand exchange. These mutations show membrane stacking, and it will be interesting to determine whether these mutants can form the P2 3D lattice.

* For correspondence: Hideaki Tsuge, tsuge@cc.kyoto-su.ac.jp.
This is an Open Access article under the CC BY license.

ASBMB
© 2020 Tsuge. Published under exclusive license by The American Society for Biochemistry and Molecular Biology, Inc.

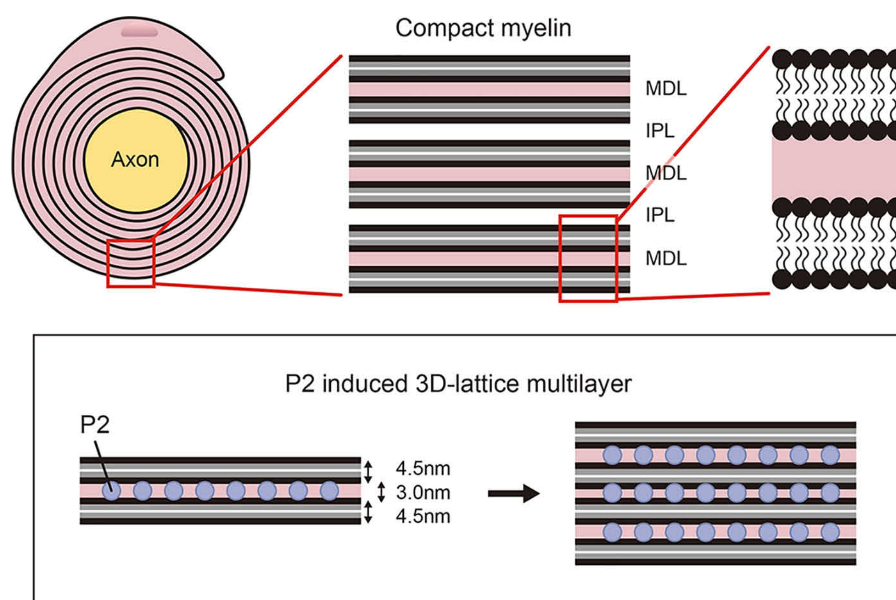

Figure 1. The myelin sheath structure and P2-induced 3D lattice multilayer. The multilayered myelin sheath is lapped around the axon. The compaction between two layers of the cell membrane forms the major dense line (*MDL*) and intraperiod line (*IPL*) (*top*). P2 induced a 3D-lattice multilayer, where P2 is located between two bilayers (*bottom*).

The myelin protein MBP, P0, and PMP22 in addition to P2 are essential players to form myelin-like membrane multilayers. The new insights from Ruskamo *et al.* provide important new details in our understanding of P2 function, but of course the final goal is to reveal how myelin proteins act together in forming the native myelin sheath. It will be exciting to learn more about how these dynamics are linked to lattice formation and membrane compaction, and we eagerly await more explorations by cryo-EM, which would bring more details of the myelin structure into focus at high-resolution.

Finally, are there ways we can take advantage of P2's unique abilities? In recent advances of cryo-EM single-particle analyses, the structures of many membrane proteins have been analyzed in detergent micelles and protein-reconstituted nanodiscs. In some cases, liposomes would be more advantageous because they have a different environment in the inner and outer sides of the membrane. However, in high-resolution cryo-EM data, achieving specimen thicknesses below ~100 nm is important. Perhaps membrane modulation proteins such as P2 may be applicable to make various artificial membranes such as a flat liposomes, advancing our understanding of this protein and many others at the same time.

Conflict of interest—The author declares that he has no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: PNS, peripheral nervous system; MBP, myelin basic protein; FABP, fatty acid–binding protein; 3D, three-dimensional.

References

1. Salzer, J. L. (2015) Schwann cell myelination. *Cold Spring Harb. Perspect. Biol.* **7,** a020529 CrossRef Medline
2. Raasakka, A., Ruskamo, S., Kowal, J., Barker, R., Baumann, A., Martel, A., Tuusa, J., Myllykoski, M., Bürck, J., Ulrich, A. S., Stahlberg, H., and Kursula, P. (2017) Membrane association landscape of myelin basic protein portrays formation of the myelin major dense line. *Sci. Rep.* **7,** 4974 CrossRef Medline
3. Raasakka, A., Ruskamo, S., Kowal, J., Han, H., Baumann, A., Myllykoski, M., Fasano, A., Rossano, R., Riccio, P., Bürck, J., Ulrich, A. S., Stahlberg, H., and Kursula, P. (2019) Molecular structure and function of myelin protein P0 in membrane stacking. *Sci. Rep.* **9,** 642 CrossRef Medline
4. Suresh, S., Wang, C., Nanekar, R., Kursula, P., and Edwardson, J. M. (2010) Myelin basic protein and myelin protein 2 act synergistically to cause stacking of lipid bilayers. *Biochemistry* **49,** 3456–3463 CrossRef Medline
5. Ruskamo, S., Yadav, R. P., Sharma, S., Lehtimaki, M., Laulumaa, S., Aggarwal, S., Simons, M., Bürck, J., Ulrich, A. S., Juffer, A. H., Kursula, I., and Kursula, P. (2014) Atomic resolution view into the structure-function relationships of the human myelin peripheral membrane protein P2. *Acta Crystallogr. D Biol. Crystallogr.* **70,** 165–176 CrossRef Medline
6. Trapp, B. D., Dubois-Dalcq, M., and Quarles, R. H. (1984) Ultrastructural localization of P2 protein in actively myelinating rat Schwann cells. *J. Neurochem.* **43,** 944–948 CrossRef Medline
7. Ruskamo, S., Krokengen, O. C., Kowal, J., Nieminen, T., Lehtimaki, M., Raasakka, A., Dandey, V. P., Vattulainen, I., Stahlberg, H., and Kursula, P. (2020) Cryo-EM, X-ray diffraction, and atomistic simulations reveal determinants for the formation of a supramolecular myelin-like proteolipid lattice. *J. Biol. Chem.* **295,** 8692–8705 CrossRef Medline
8. Hong, Y. B., Joo, J., Hyun, Y. S., Kwak, G., Choi, Y. R., Yeo, H. K., Jwa, D. H., Kim, E. J., Mo, W. M., Nam, S. H., Kim, S. M., Yoo, J. H., Koo, H., Park, H. T., Chung, K. W., *et al.* (2016) A mutation in PMP2 causes dominant demyelinating Charcot-Marie-Tooth neuropathy. *PLoS Genet.* **12,** e1005829 CrossRef Medline
9. Motley, W. W., Palaima, P., Yum, S. W., Gonzalez, M. A., Tao, F., Wanschitz, J. V., Strickland, A. V., Löscher, W. N., De Vriendt, E., Koppi, S., Medne, L., Janecke, A. R., Jordanova, A., Zuchner, S., and Scherer, S. S. (2016) *De novo* PMP2 mutations in families with type 1 Charcot-Marie-Tooth disease. *Brain* **139,** 1649–1656 CrossRef Medline
10. Ruskamo, S., Nieminen, T., Kristiansen, C. K., Vatne, G. H., Baumann, A., Hallin, E. I., Raasakka, A., Joensuu, P., Bergmann, U., Vattulainen, I., and Kursula, P. (2017) Molecular mechanisms of Charcot-Marie-Tooth neuropathy linked to mutations in human myelin protein P2. *Sci. Rep.* **7,** 6510 CrossRef Medline