Experimental Report

05/07/2014

Proposal: **8-02-644** **Council:** 4/2012

Title: Mechanical properties of myelin multilayers

This proposal is a new proposal

Researh Area: Biology

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Samples: Natural myelin and myelin lipids (on SiSiO2)

Instrument	Req. Days	All. Days	From	To
D16	7	6	01/07/2013	07/07/2013

Abstract:

The structure of natural myelin has been extensively studied by diffraction. Nevertheless, none of these studies has been concerned with the mechanical properties of interacting myelin membranes. Here we propose the use of specular and off-specular neutron scattering to study the mechanical properties of membrane multilayers prepared either from whole myelin or from myelin lipids. This will shed light on the role of membrane mechanics in myelin stability, lipid/protein phase separation and demyelination as it occurs in pathological disorders like multiple sclerosis.

Exp:8-02-644

MECHANICAL PROPERTIES OF MYELIN MULTILAYERS

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BACKGROUND:

The works on natural myelin diffraction are numerous, mainly concerned with myelin in nerve and/or isolated membranes. Nevertheless, none of those works has been concerned with splitting specular and off-specular scattering. This would allow studying the myelin membrane mechanical properties. In order to achieve the scattering vector splitting we formed planar layers from whole myelin (as well its total lipid fraction for comparisons). Myelin structure is sensitive to environmental conditions, for instance temperature, commonly used to isolate lipids rafts. Here we study mechanical properties on different conditions (including temperature and concentrations of NaCl and $CaCl_2$). Proper stabilization of the stack of the myelin membrane is a prerequisite for its proper function. Altered states of myelin membranes leads to pathological disorders, like multiple sclerosis. In this sense, understanding mechanical properties could help to understand myelin stability, lipid/protein domains and demyelination.

RESULTS:

Fig 1 shows one of the samples with higher diffraction signal. It is a lipid fraction isolated from myelin, measured at 37º C and nearly 100 % humidity (that means keeping sample and reservoir temperatures at the same value). Typically, up to third or fourth order peaks can be observed in lipid fraction samples. A single phase with a lamellar spacing of 5.7-5.8 nm is observed. A small peak at half the q value of the first diffraction peak denotes some asymmetry in the bilayers, which could arise from the many components nature of the system and the preferential partitioning of the lipids

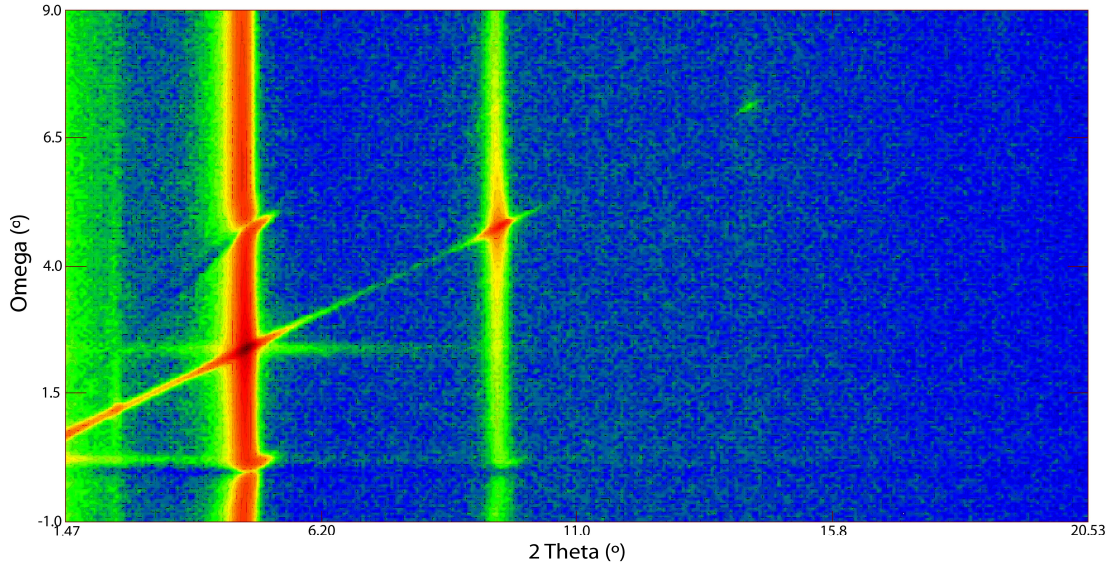

Figure 1: Diffractogram from a total lipid extract from myelin at 37º C and near full hydration.

Although this sample renders very clear Bragg peaks, the roughness might be not only due to membrane fluctuations but also to static roughness, which difficult the analysis.
In previous work our group has arranged a setup for the study of membranes in bulk buffer. Thus, the same lipid sample in bulk buffer (100 mM NaCl plus 20 mM Ca^{2+}) was tested in conditions closer to the physiological ones. Here the introduction of bulk water increases the period to 6.2-6.3 nm and helps to eliminate static scattering. Fig 2 shows a preliminary analysis of the data; the bending rigidity, kappa, appears to be in the typical range for lipids (about 8 kT). The compression modulus is 3.7 MPa, also in the range of what we have observed in previous studies (Schneck et al., 2009; 2011). This data was obtained for the first time for myelin systems.

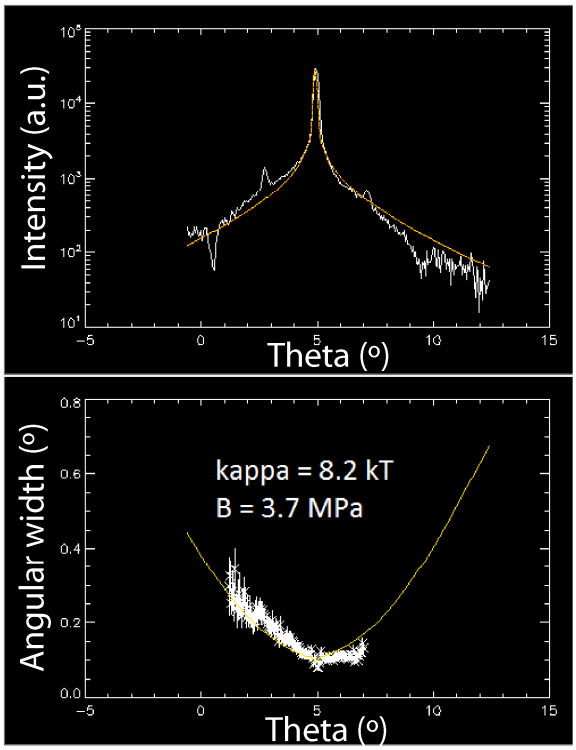

Figure 2: Measured (white) and simulated (color) second Bragg sheets of myelin lipids measured at 37° C in bulk buffer (see text). Upper graph: intensity integrated along omega as a function of theta (angle between the incident beam and the sample plane). Lower graph: angular width (in degrees) of the second Bragg sheet along omega as a function of theta. From these fittings the bending rigidity (Kappa) and the compressional modulus (B) are obtained, which accounts for the mechanical properties of the stack of membranes (Schneck et al., 2009).

For whole myelin we succeeded in registering good first order diffraction peaks which already renders a semi-quantitative view of the mechanical properties of the different membrane domains (phases with different periodicities). The most compact phase of whole myelin has a spacing of 5.8 nm in D_2O vapor and 6.3 nm under buffer, exactly the same behavior as for pure lipids. This allows us to conclude that are lipid rich domains (Oliveira et al., 2010). We were also able to measure a phase with the native spacing for central nervous system myelin (~7.8 nm) showing a clear first order diffraction peak. We do not detect higher order peaks needed to quantitatively analyze the data in the framework of the kinematic approximation but we conclude that the native (protein rich) phase has a higher relation of specular/off specular scattering than the lipid rich compacted phase. We are currently obtaining more quantitative analysis of the data.

References:
Schneck E, Oliveira RG, Rehfeldt F, Demé B, Brandenburg K, Seydel U, Tanaka M. Phys Rev E 80, 041929 (2009).
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Oliveira RG, Schneck E, Funari S, Tanaka M, Maggio B. Biophys J 99:1500-1509 (2010).