Super-Lorentzian framework for investigation of T_2^* distribution in myelin

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Introduction

Deficiencies of myelin, a lipid bilayer sheath critical for normal function of white matter (WM), lay at the core of numerous neurodegenerative disorders such as multiple sclerosis and schizophrenia (1). At present, there are few alternatives to destructive histologic methods to directly assess myelin. The short T_2^* of myelin protons make ultra-short echo time (UTE) MRI a potential imaging modality to directly detect myelin (2). In contrast, indirect MRI methods such as magnetization transfer and T_2 relaxometry are based on complex interactions between water and myelin, which can lead to ambiguities in data analysis. Characterizing the T_2^* distribution of myelin is key to developing optimal UTE methods for myelin imaging. Previous attempts have used multi-exponential fitting of the FID (3), which is not only an ill-posed problem (4), but also theoretically incorrect. Myelin is a liquid crystalline lipid system that is described by a sum of super-Lorentzians (SL) rather than a multi-Lorentzian lineshape (5, 6). Here, we use this SL framework to calculate T_2^* distributions by fitting ^{1}H NMR spectra of myelin lipid extract and intact rat spinal cord (SC).

Theory and Methods

According to Wennerström (6), due to averaging effects from translational and rotational diffusion, for a given orientation of a lipid bilayer, the lineshape can be expressed as: $L(\delta\text{-}\delta_0, \theta) = |3\cos^2\theta - 1|^{-1} f[(\delta\text{-}\delta_0)/|3\cos^2\theta - 1|]$ (Eq.1), where δ is the chemical shift centered at δ_0, θ is the angle of the lipid bilayer surface normal with B_0, and $f(x)$ is any highly peaked lineshape such as a Gaussian or Lorentzian. The SL lineshape, $L_{SL}(\delta\text{-}\delta_0)$, results from a uniform sampling of θ from 0 to $\pi/2$: $L_{SL}(\delta\text{-}\delta_0) = \int L(\delta\text{-}\delta_0, \theta)\sin\theta\, d\theta$ (Eq.2). By assuming $f(x)$ to be a Lorentzian, it can be seen from Eqs. 1 and 2 that a SL is composed of a series of scaled Lorentzians. From the widths and intensities of these Lorentzians, the T_2^* distribution of a single SL can be calculated. Multiple SLs have been used to fit NMR spectra of model membrane systems in which the SLs arise from protons at different chemical shifts, e.g. alkyl methylenes, terminal methyls, and choline (7). Therefore, it is possible in theory to perform a multi-SL fit of a ^{1}H NMR spectrum of myelin and calculate a T_2^* distribution.

Rat and bovine SC samples were harvested from Sprague-Dawley rats (Charles River Labs) and a local butcher. Myelin lipids were extracted from bovine SC tissue with a sucrose gradient method (8), dissolution in a ternary mixture (chloroform/methanol/water), and lyophilization. Previous work has shown that this protocol extracts myelin lipids with little to no protein (2). Dehydrated myelin lipid extract was then re-suspended in 99.9% D_2O (Sigma-Aldrich) to regenerate a bilayer structure. ^{1}H NMR spectra at 9.4T (DMX-400, Bruker Instruments) were obtained for a freshly excised rat thoracic SC immersed in Fomblin (Sigma-Aldrich), as well as the myelin lipid extract. Rat SC was immersed in D_2O for 24 hrs prior to experiments to reduce the dominant tissue water peak.

Spectral fitting was performed in Matlab (Mathworks). Four SLs were used to represent general alkyl chain methylenes, cholesterol alkyl chain methylenes (as they have shorter chain lengths), terminal methyls, and choline, while a single Lorentzian was used to model residual HDO. The chemical shifts of each SL were set to the known isotropic shift of the various moieties, and the width and relative intensities were free parameters.

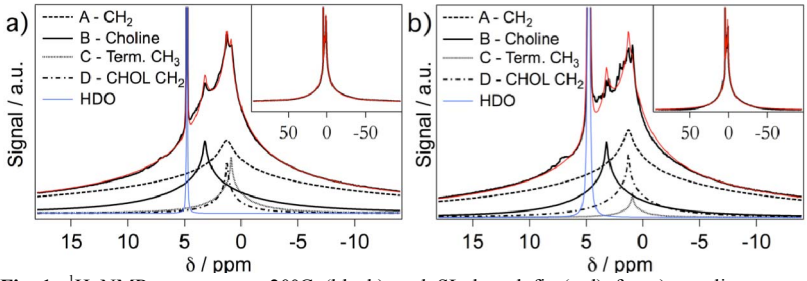

Fig 1. ^{1}H NMR spectra at ~20°C (black) and SL based fit (red) for a) myelin extract (R^2=0.999) and b) rat SC (R^2=0.997). The four SL and Lorentzian peaks of the fit are shown. Full spectral width is shown in inset with HDO peak truncated.

Results and Discussion

Fig. 1 shows myelin lipid extract and rat SC ^{1}H NMR spectra with the results of the four-component SL fits. Both spectra are comprised of a narrow peak from residual HDO and a broad resonance (linewidth ca. 1700Hz) from myelin. A large 4th to 2nd moment ratio (ca. 6.6), suggests that this broad resonance has a SL lineshape. The SC ^{1}H NMR spectrum had additional minor peaks from intracellular proteins and other non-myelin protons. Despite the additional complexity of the SC spectrum, both SL fits agree well with the ^{1}H NMR spectra ($R^2 > 0.99$). Fig. 2 shows the relative fractions (theoretical and fitted) of the four SL myelin components in myelin extract and intact rat SC. As expected, the signal is dominated (>70%) by alkyl methylene protons. Deviations from theoretical values may result from inaccuracies of the SL framework to describe non-chain alkyl protons, e.g. choline and terminal methyls.

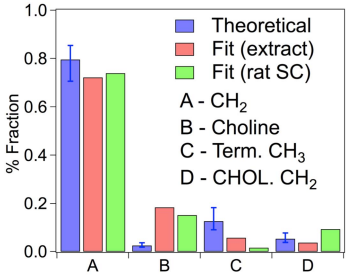

Fig 2. Relative fraction fitting results of the four SL components with expected theoretical fractions. Error bars account for variation in alkyl chain length.

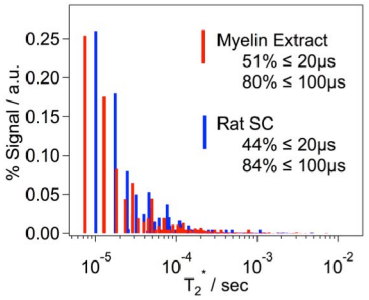

Fig 3. Calculated T_2* distributions for myelin lipid extract (red) and rat SC (blue). Signal fractions with T_2^* <20 and <100 μs are reported.

Fig. 3 shows the T_2^* distributions derived from the SL fits for the myelin lipid extract and SC. The T_2^* distributions are highly skewed with a wide range (10μs to 10ms). Despite this range, roughly 50% (80%) of the signal has a T_2^* less than 20μs (100μs). This result highlights the difficulty of direct myelin imaging even with UTE MRI. Further investigation is needed to study the system at body temperature as increased molecular motion is likely to result in longer effective T_2^*s.

Conclusion

This work uses a SL framework to characterize the T_2^* distribution of myelin, which would provide guidance toward developing UTE methods for myelin imaging. The results indicate that at ambient temperature ~50% of the myelin lipid proton signal has $T_2^* < 20$ μs.

References: 1. van der Knaap, MS et al, *Magnetic Resonance of Myelin, Myelination, and Myelin Disorders*, Springer-Verlag (1995). 2. Wilhelm, MJ et al, *Proc. of the ISMRM* (2011). 3. Horch et al, *Mag. Res. Med.*, **66**:24 (2011). 4. Epstein, CL et al, *SIAM Rev.*, **50**:504 (2008). 5. Bloom, M et al, *Chem. Phys. Lipids*, **4**:107 (1975). 6. Wennerstroem, H et al, *Chem. Phys. Lett.*, **18**:41 (1973). 7. Ulmius, J et al, *Biochim. Biophys Acta*, **389**:197 (1975). 8. Larocca, JN et al, *Curr. Protoc. Cell Biol.*, **3.25**:1 (2006). **Acknowledgements:** NIH T32 EB00814