**31P and 1H NMR investigation of liquid crystal phase temperature dependence in rehydrated myelin**

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**Introduction**

In vivo assessment of myelin would offer invaluable information about numerous neurodegenerative disorders. MRI has great potential for non-destructive assessment through indirect (e.g. MT\(^1\) and T\(_2\) relaxometry\(^2\)) and direct detection methods (e.g. UTE MRI\(^3\)). Understanding the MR properties of the myelin signal would provide crucial insight into these methods. Myelin forms a lamellar liquid crystalline lipid phase whose structure gives rise to its distinctive super-Lorentzian (SL) lineshape in 1H NMR\(^4\). However, the polymorphic phase behavior of lipids is influenced by water content and temperature and may exist in lamellar liquid crystal, lamellar gel, cubic, and hexagonal phases, which would have profound effects on relaxation properties. 1H NMR spectra of anhydrous myelin extract suggest that it exists in a lamellar phase at physiologic temperatures\(^5\). Recent work has studied the MR properties of rehydrated myelin extract at room temperature\(^6\). While there have been previous temperature studies on model membrane systems\(^7,8\), the applicability of rehydrated myelin extract studies at physiologic temperatures is not well understood. Here, we studied the liquid crystal phase of rehydrated bovine myelin extract with 31P and 1H NMR over the temperature range from 10 to 50°C.

**Methods**

Following our previous protocol\(^3\), myelin lipids were extracted from bovine spinal cord tissue with a sucrose gradient method\(^6\) followed by lyophilization. Myelin lipid extract was suspended in excess 99.9% D\(_2\)O (Sigma-Aldrich) to regenerate a bilayer structure (88% w/w D\(_2\)O in lipid). 1H and proton-decoupled 31P NMR spectra were obtained at 9.4T (DMX-400, Bruker Instruments) for rehydrated myelin extract. The temperature was varied using the Bruker variable temperature control system from 10 – 50°C.

1H spectra exhibited a sharp HDO peak that would have confounded quantitative analysis. The HDO peak was removed through a previously reported spectral fitting procedure implemented in Matlab (Mathworks)\(^9\). Briefly, the HDO peak was modeled as a Lorentzian peak, while the myelin lipid resonances were modeled as a sum of four SL peaks representing general alkyl chain methylenes, cholesterol alkyl chain methylenes, terminal methyls, and choline. The 1H spectrum at 10°C was fit first and the computed chemical shifts, linewidths, and relative intensities were used as the initial conditions for the next higher temperature. Once the myelin lipid resonances were isolated, the 2\(^1^H\) and proton-decoupled 31P NMR spectra were calculated at each temperature with the nth moment being defined as 

\[
M_n = \int S(\omega)\omega^n d\omega / \int S(\omega)d\omega
\]

where \(\omega\) is the spectral frequency. Sharp transitions in the temperature dependence of \(M_n\) and the ratio \(M_2/M_1^2\) are known to indicate phase transitions\(^7\).

31P spectra were normalized to maximum peak intensity at each temperature to allow comparison of peak shape, which is known to distinguish between liquid crystal lamellar, cubic, and hexagonal phases\(^9\).

**Results and Discussion**

Fig. 1. 1H (A and B) and 31P (C) spectra of rehydrated bovine myelin extract at various temperatures. The spectrum at 50°C shows the greatest deviation in lineshape and may indicate an impending phase change, although further investigation is needed. The narrow peak in the 31P spectrum may arise from lipid hydrolysis or small vesicle formation.

In the 1H spectrum, the linewidth of the broad myelin resonances clearly narrows with increasing temperature (Fig. 1A). From the spectral fit, the relative fractions of the myelin lipid model components are constant with temperature as expected. The linewidths of each SL peak decrease with increasing temperature. As it makes up ~70% of the myelin lipid signal and its linewidth decreases with the fastest rate, the general alkyl chain methylenes dominate the temperature behavior of the myelin lipid resonances. It is also evident that the chemical shift differences between the HDO and myelin lipid resonances decreases with higher temperature.

Fig. 2 shows the moment analysis of the myelin lipid resonances in the 1H spectrum. \(M_2\) and the ratio \(M_2/M_1^2\) have a smooth linear dependence on temperature. The absence of sharp discontinuities indicates that there are no phase transitions in this temperature range in agreement with the 31P NMR data. The values of the moments are consistent in qualitative agreement with values reported for other liquid crystal systems\(^5\). In particular, decreasing \(M_2\) values with rising temperature is consistent with increased rotational and lateral diffusion of lipids leading to motional narrowing of the lineshape.

**Conclusion**

This work shows 1H and 31P NMR data of rehydrated bovine myelin extract, which indicates that myelin lipids exhibit a lamellar liquid crystalline phase over a temperature range from 10 to 50°C. The smooth behavior of the spectra with respect to temperature indicates that studies of rehydrated myelin extracts should be applicable to physiologic temperatures.

**References**


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