^{31}P and ^{1}H 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 ^{1}H 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. ^{1}H 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[3,6]. While there have been previous temperature studies on model membrane systems[7], 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 ^{31}P and ^{1}H NMR over the temperature range from 10 to 50ºC.

Methods

Following our previous protocol[3], myelin lipids were extracted from bovine spinal cord tissue with a sucrose gradient method[8] followed by lyophilization. Myelin lipid extract was suspended in excess 99.9% D_2O (Sigma-Aldrich) to regenerate a bilayer structure (88% w/w D_2O in lipid). ^{1}H and proton-decoupled ^{31}P 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.

^{1}H 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)[3]. 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 ^{1}H 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 2nd (M_2) and 4th (M_4) moments 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 ω is the spectral frequency. Sharp transitions in the temperature dependence of M_2 and the ratio M_4/M_2^2 are known to indicate phase transitions[7].

^{31}P 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].

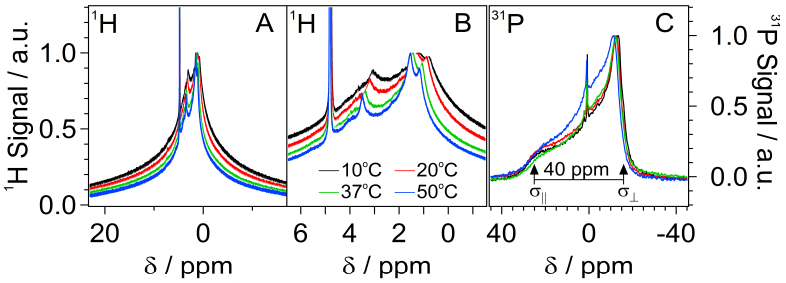

Fig. 1. ^{1}H (A and B) and ^{31}P (C) spectra of rehydrated bovine myelin extract at various temperatures. B is an expanded spectrum of A to show the details of the myelin lipid resonances. The sharp resonance in the ^{1}H spectra is HDO. The chemical shifts of the parallel ($\sigma_{\parallel}$) and perpendicular ($\sigma_{\perp}$) orientations are labeled in the ^{31}P spectrum.

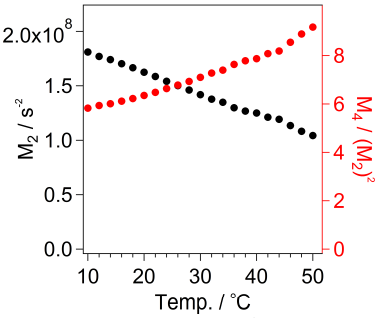

Fig. 2. Moment analysis of myelin lipid resonances from ^{1}H spectra. Black: 2nd moment (M_2) vs temperature. Red: 4th and 2nd moment ratio $(M_4/(M_2)^2$ vs temperature.

Results and Discussion

Fig. 1 shows ^{1}H and ^{31}P spectra of rehydrated bovine myelin extract as a function of temperature. The asymmetric lineshape of the ^{31}P spectrum is consistent with an axially symmetric lamellar-phase liquid crystal (Fig. 1C). In addition, the chemical shift difference between the parallel ($\sigma_{\parallel}$) and perpendicular ($\sigma_{\perp}$) orientations (~40 ppm) is similar to that of a common model membrane system (1,2 dipalmitoyl-3-*sn*-phosphatidylcholine)[10]. The consistency of the lineshape suggests that the lamellar phase is stable in this temperature range. 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 ^{31}P spectrum may arise from lipid hydrolysis or small vesicle formation.

In the ^{1}H 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 decreases 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 ^{1}H spectrum. M_2 and the ratio M_4/M_2^2 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 ^{31}P NMR data. The values of the moments are in qualitative agreement with values reported for other liquid crystal systems[7]. 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 ^{1}H and ^{31}P 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 50ºC. The smooth behavior of the spectra with respect to temperature indicates that studies of rehydrated myelin extracts should be applicable to physiologic temperatures.

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