Chain order profile in lipid H_{II} phases

Dear Sir:

In an interesting recent paper, Sternin et al. (1988) presented data on the orientational acyl chain order in the hexagonal H_{II} phase. Deuterium nuclear magnetic resonance (^2H NMR) measurements were made on perdeuterated tetradecanol in a mixture with 1-palmitoyl-2-oleoyl phosphatidylethanolamine in water. The data were analyzed by a novel method which involved first obtaining the oriented spectra from the ^2H NMR powder pattern (dePaking) and, second, assigning the resonances from the perdeuterated chain by uniformly dividing the intensity under the dePaked spectrum and then monotonically attributing the corresponding mean quadrupole splittings of these spectral segments to the methylene groups of the chain.

Relatively few order parameter measurements on hexagonal lipid phases are available, and therefore it was not possible to assess the fidelity of the new method of spectral analysis by comparison with other data, for example from measurements on specifically deuterated systems. In view of the importance of the results to the dynamic properties of inverted lipid phases, it is particularly useful to know to what extent the order parameters obtained are influenced by the method of spectral analysis.

In our continuing studies on the lamellar, L_α, to inverted hexagonal, H_{II}, phase transition in distearoyl phosphatidylethanolamine (DSPE) (Seddon et al., 1983, 1984) we have recently performed ^2H NMR measurements on both perdeuterated and specifically deuterated DSPE. Many of the single methylene resonances were resolved in the dePaked spectra from DSPE perdeuterated in the *sn*-2 chain in the H_{II} phase, and comparison with the results from specifically deuterated samples has permitted assignment of these resonances. This work therefore allows a direct comparison of the order parameters obtained by conventional means with the new method of analysis developed by Sternin et al. (1988).

The dePaked spectrum of DSPE perdeuterated in the *sn*-2 chain, dispersed in 2.4 M NaCl at 97°C is given in Fig. 1. At this temperature, the lipid is in the H_{II} phase (Marsh and Seddon, 1972; Seddon et al., 1983), as also evidenced by the low values of the outer quadrupole splittings. Many of the resonances are resolved as separate peaks, arising most probably from single methylene groups. The spectrum has been analyzed by the method of Sternin et al. (1988), as is indicated by the stick diagram given below the dePaked spectrum. An exact correspondence between the resolved peaks and the stick diagram is not obtained, although near coincidence is obtained in several cases. The resolved resonances in the dePaked spectrum have been assigned by comparison with the spectra from DSPE specifically deuterated in the *sn*-2 chain (Sankaram, M. B., and D. Marsh, manuscript in preparation). Plots of the quadrupole splitting versus chain segment position are compared with those given by the stick plot in Fig. 2. The individual quadrupole splittings from the specifically deuterated samples are also included for comparison. These do not fit exactly with those from the perdeuterated sample because the hexagonal transition temperature is somewhat different for the perdeuterated sample, but the fit suffices fully for the purposes of assignment.

Although the overall values of the quadrupole splittings given by the two methods in Fig. 2 are of rather similar magnitude, there are significant differences in the shape of the segmental profile. In particular, the method of Sternin et al. (1988) removes the order parameter plateau between the C-3 and C-6 positions and replaces it by a profile of steadily decreasing quadrupole splittings. On the other hand, it reproduces quite well the quadrupole splittings of the segments closer to the terminal methyl end of the chain. The large differences in the two assignments for the quadrupole splittings at the C-2 position arise from the special conformation of this group in the *sn*-2 chain, which is well known for the L_α-phase (Seelig and Seelig, 1975) and is preserved in the H_{II} phase. Such effects are difficult to allow for *a priori* in the method of analysis used by Sternin et al. (1988). If this is done on the basis of the specifically deuterated assignments, the fit between the two methods is improved somewhat, but the quadrupole splittings for the C-6

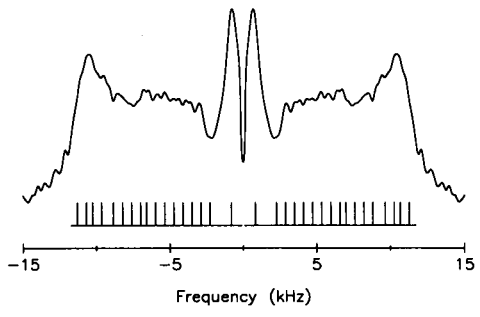

FIGURE 1 Depaked 46.1 MHz ^2H NMR spectrum of 1-stearoyl-2-[$^2H_{35}$]stearoyl-*sn*-glycero-3-phosphoethanolamine dispersed in 2.4 M NaCl and recorded at 97°C. The dePaked display corresponds to resonances for the magnetic field oriented parallel to the ordering axis. The stick plot represents contiguous sections of the spectrum whose intensities correspond to a single CD_2 groups, and is defined according to the convention of Sternin et al. (1988).

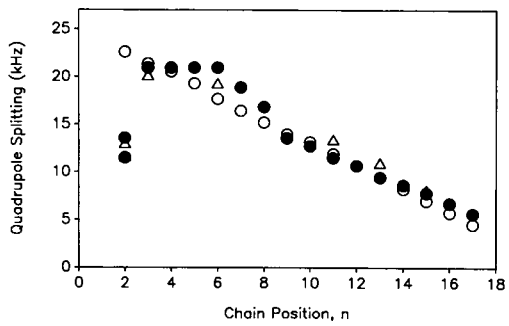

FIGURE 2 Profile of quadrupole splittings with chain segment position, C-*n*, for *sn*-2 chain perdeuterated DSPE dispersed in 2.4 M NaCl at 97°C in the H_{II} phase. (●) Based on direct assignment of the resonances by comparison with specifically deuterated derivatives. (△) Data from DSPE derivatives specifically deuterated at different positions in the *sn*-2 chain. (○) Based on the method of Sternin et al. (1988).

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and C-7 segments obtained by direct assignment still lie above the corrected data from the other method and the reverse becomes true for the C-3 segment. Although the C-2 feature is unlikely to be present for perdeuterated tetradecanol, this points to the general difficulty involved with perdeuterated chains that have specific conformations (e.g., *cis* double bonds) which violate the monotonic ordering of the quadrupole splittings.

In summary, as regards the chain order profile in the H_{II} phase, the method of Sternin et al. (1988) quantitatively reproduces the inherent reduction in molecular order parameter relative to the L_α phase for segments towards the terminal methyl end of the chain. For segments in the upper part of the chain, it only gives a qualitative impression of the reduction in the length of the order parameter plateau. With specifically deuterated DSPEs it is found that the plateau region extends from C-3 to C-11 in the L_α phase, but extends to no further than C-8, immediately on transition to the H_{II} phase (Sankaram, M. B., and D. Marsh, manuscript in preparation). The suppression of the plateau region is a general tendency of the method used by Sternin et al. (1988), arising from the uniform segmentation of the spectral intensity. Thus the broad conclusions concerning the comparison of the chain order profiles in the L_α and H_{II} phases are undoubtedly correct, but a detailed analysis of this important problem, such as would be necessary for a statistical mechanical treatment of the chain conformations in H_{II} lipid phases, requires direct assignment of the resonances.

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