DEUTERIUM MAGNETIC RESONANCE STUDIES OF PHOSPHOLIPID BILAYERS

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Summary

L-α-dipalmitoyl lecithin is selectively deuterated at two different chain positions. The residual quadrupole splittings of the corresponding phospholipid bilayers are measured by means of deuterium magnetic resonance and evaluated in terms of the segmental order parameters. The results are briefly compared with other esr and nmr investigations of lipid bilayers.

An attractive possibility for studying the ordering, conformation and dynamics of fatty acyl chains in lipid bilayers is the use of deuterium magnetic resonance (DMR). If a chain proton is replaced by a deuteron, the DMR spectrum should correspond to a doublet, the separation of the two lines being directly related to the order parameter of the labeled chain segment (1). Such an information is difficult to obtain from conventional proton-nmr or carbon-13 nmr spectroscopy. Compared to the nitroxide spin label method, which also allows a determination of order parameters, the deuterium label method has the advantage of not perturbing the bilayer structure.

Selective deuteration combined with DMR spectroscopy has been applied to thermotropic liquid crystals as early as 1965 (2), but the widespread use of this method has been hampered by the low sensitivity associated with DMR. To circumvent the sensitivity problem Oldfield et al. synthesized perdeuterated dimyristoyl lecithin (3). Using broad line nmr they were able to detect a quadrupole splitting of 27 KHz (at a temperature of 30° C; that is 7° C above the gel ⟶ liquid crystalline phase transition), but the assignment and the molecular interpretation of the signal were difficult. A better resolution was obtained by Charvolin et al. using oriented samples of perdeuterated potassium laurate bilayers (4). Again the assignment of the lines

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was not unambiguous. Only recently has the development of appropriate Fourier-Transform instrumentation made it possible to detect DMR signals even at low concentrations of deuterium (1, 5, 6). Saito et al. intercalated selectively deuterated lauric acid into sonicated lecithin bilayers (5). Only one DMR signal was observed, from which information about the correlation time was deduced. In our laboratory, we have investigated selectively deuterated bilayers composed of decanoic acid and decanol (1, 6). The quadrupole splitting was measured for each chain segment. This allowed a determination of the order parameter, which was compared with the corresponding nitroxide spin label results. The two methods differed from each other, leading to two different pictures for the ordering of the hydrocarbon chains. Hence it seemed appropriate to investigate the situation in phospholipid bilayers, since they are more closely related to biological membranes. Here we show that applying the deuterium label method to phospholipid systems is experimentally feasible and yields the order parameter of the chain segments.

Experimental

L-α-dipalmitoyl lecithins, deuterated in both palmitic acyl chains either at carbon atom 2 or at carbon atom 5, were synthesized as follows: 2, 2-dideuteropalmitic acid was obtained from palmitic acid by deuterium exchange (7). 5, 5-dideuteropalmitic acid was prepared by $LiAlD_4$ reduction of methyl laurate, followed by chain elongation via two malonic ester condensations. The acids were characterized by their melting points and their proton-nmr and DMR spectra. L-α-dipalmitoyl lecithin (DPL) was synthesized according to reference (8). The specifically deuterated lipids were characterized as follows: TLC in chloroform - methanol - water (65:25:4 by Vol.) gave a single spot. The proton-nmr spectra were identical with non-deuterated reference material with two exceptions. For the C-2 deuterated DPL the four protons at 2.3 ppm were missing. For the C-5 deuterated DPL the integrated intensity at 1.2 ppm was reduced by four protons. The DMR spectra showed a single line for each deuterated DPL. The chemical shift corresponded to the chemical shift of the replaced protons. The measurement of the optical rotation in methanol - chloroform (1:1 by Vol.) yielded the following results: non-deuterated L-α-DPL, purchased

from Fluka, Switzerland, $[\alpha]_D^{29} = +6.9^o$; C-2 deuterated L-α-DPL $[\alpha]_D^{29} = +7.0^o$; C-5 deuterated L-α-DPL $[\alpha]_D^{29} = 6.9^o$.

DPL bilayers were prepared by thoroughly mixing DPL (60 wt. %) and water (40 wt. %) and heating the mixture in a sealed ampoule to 60° C. About 400 mg material were used for measuring powder type spectra in a 10 mm nmr tube. Oriented DPL bilayers were prepared by pressing the same phase between a stack of about 30 thin glass plates.

The DMR measurements were performed at 13.78 MHz with a Bruker HX-90-FT spectrometer equipped with a variable temperature unit.

Results and Discussion

The observed quadrupole splitting $\Delta\nu$ is related to the order parameter S_{CD} of the CD bond according to

$$|\Delta\nu| = (3/4)\,(e^2qQ/h)\,|S_{CD}|.$$

The deuteron quadrupole splitting constant (e^2qQ/h) has been found to be 170 KHz for paraffinic chains (9). The average orientation of the CD bond is more or less perpendicular to the bilayer normal; hence it is safe to assume that S_{CD} is negative. S_{CD} is related to the chain segment order parameter S_{Mol} according to (1):

$$S_{Mol} = -2\,S_{CD}$$

S_{Mol} describes the average orientation of the carbon chain at the position of the deuterium label. S_{Mol} can be compared directly with the spin label order parameter S_3.

Figure 1 shows a DMR spectrum typical for a random distribution of C-5 deuterated DPL bilayers. The experimental quadrupole splittings and the corresponding order parameters are summarized in the table. The quadrupole splittings increase with decreasing temperature, but below the liquid crystalline ⟶ gel phase transition (~40° C) no signal could be detected. The DMR measurements thus clearly reflect the phase transition, but quantitative structural information is as yet limited to the liquid crystalline state of the bilayers. The C-5 deuterated bilayers have been oriented also between glass plates. The signal-to-noise ratio of these measurements was generally poor, due to the small amount of material which could be oriented.

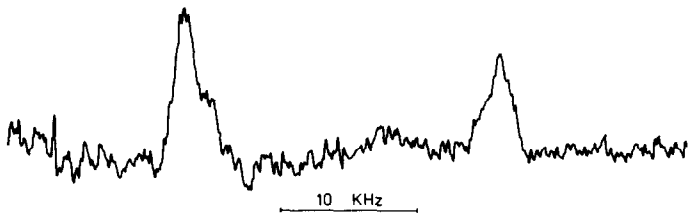

Figure 1 Deuterium magnetic resonance spectrum of bilayers of L-α-dipalmitoyl lecithin deuterated at carbon atom 5 of both chains. Temperature 60° C. Pulse width 18 μs. 40 000 scans.

Table

Quadrupole splittings and order parameters of L-α-dipalmitoyl lecithin deuterated at the C-5 position

Temp. [° C]	$\Delta\nu$ [KHz]	S_{CD}	S_{Mol}
40	$28.1_6 \pm 0.15$	-0.22_1	0.44_2
45	$25.7_5 \pm 0.15$	-0.20_2	0.40_4
50	$24.5_8 \pm 0.15$	-0.19_3	0.38_6
60	$23.8_3 \pm 0.15$	-0.18_7	0.37_4

Two sharp lines were obtained if the glass plates were inclined around the magic angle. The quadrupole splitting was small (5 to 10 KHz) and collapsed at the magic angle ($\pm$ 10°). The result is important because it indicates that on the time scale of this DMR experiment the axis of motional averaging is identical with the normal on the bilayer surface.

The results obtained with C-2 deuterated DPL bilayers are more difficult to understand. At 60° C three pairs of signals are seen in the powder-like spectrum. The intensity ratio of the three signals is approximately 2:1:1. The corresponding quadrupole splittings are 23.1 KHz, 14.9 KHz and 12.0 KHz, respectively. Lowering the temperature increases the separation of the lines. Again the signals vanish below the liquid crystalline $\longrightarrow$ gel tran-

sition point. The quadrupole splitting associated with the most intense peak is practically the same as that observed for C-5 deuterated bilayers under the same conditions. The appearance of two smaller peaks indicates however that the conformational situation is rather complicated close to the polar head. At present, we have no satisfying explanation for these resonances.

Let us briefly discuss our results in the light of other esr and nmr investigations. Spin label studies of phospholipid bilayers yield order parameters $S_3 \approx 0.6$ at the C-5 position (10, 11). Furthermore they seem to indicate a collective tilt of the fatty acyl chains by about 30° (11). Both results are at variance with the DMR experiments. A possible explanation is the different time scale of the two methods. Assuming a lifetime of the collective tilt of about 10^{-5} sec, this would be too long to affect the spin label order parameter, but would be short enough to be included in the deuterium order parameter.* The ratio S_{Mol}/S_3 should then correspond to approximately $(1/2)(3\cos^2 30^{\circ} - 1) \approx 0.63$. Although this compares favorably with the experimental results, other explanations cannot be ruled out. A second aspect is the dependence of S_{Mol} on the distance from the polar head. The constant quadrupole splitting of about 25 KHz observed for the C-2 and C-5 position of DPL and also for perdeuterated dimyristoyl lecithin (3) suggests a rather constant order parameter for most chain segments. This is supported by proton-nmr measurements (12, 13) and also by the deuterium results for the above mentioned simpler bilayers (4, 6). However, before a definite conclusion can be reached, some more deuterated DPL bilayers must be investigated.

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