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BILAYERS OF DIPALMITOYL-3-*sn*-PHOSPHATIDYLCHOLINE

CONFORMATIONAL DIFFERENCES BETWEEN THE FATTY ACYL CHAINS

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SUMMARY

Dipalmitoyl-3-*sn*-phosphatidylcholine is specifically deuterated at the C-2 position of the fatty acyl chains. Using deuterium magnetic resonance it is then possible to probe the chain conformation in the vicinity of the polar head groups. Three separate quadrupole splittings are observed for bilayers of 1,2[2'-$^2H_2$] palmitoyl-3-*sn*-phosphatidylcholine, indicating that the two chains behave differently. The synthesis of phosphatidylcholines each deuterated in only one chain allows the assignment of the three resonances. It is concluded that the beginnings of the two chains have orientations parallel and perpendicular to the bilayer normal. The data further suggest the possibility of two long-lived conformations of the glycerol constituent.

INTRODUCTION

Deuterium magnetic resonance has proved to be a valuable tool for the investigation of lipid membranes. From the quadrupole splitting of partially deuterated lipids detailed information on the chain ordering and polar group conformation has been obtained. The present work is directed towards an analysis of the hydrocarbon chain conformation in the immediate neighbourhood of the glycerol backbone of the phospholipid bilayers.

We have shown previously that the two fatty acyl chains of dipalmitoyl-3-*sn*-phosphatidylcholine do not have physically identical behaviour in the liquid crystalline state [1]. Two slightly different quadrupole splittings were observed in some parts of the bilayer even though the two palmitic acyl chains were labelled at equivalent carbon atoms. The deuterium resonance spectra are even more surprising if the palmitic acyl chains are deuterated adjacent to the carbonyl groups. As can be seen from Fig. 1 deuteration of both fatty acyl chains at the C-2 position gives rise to spectra with three quadrupole splittings. In this work the origin of the three resonances is determined by synthesis of dipalmitoyl-3-*sn*-phosphatidylcholines deuterated in one fatty acyl chain only.

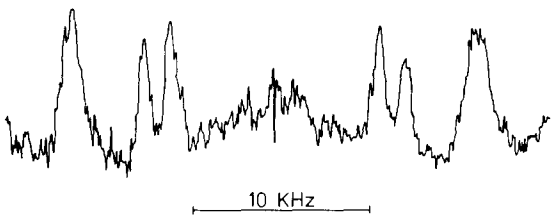
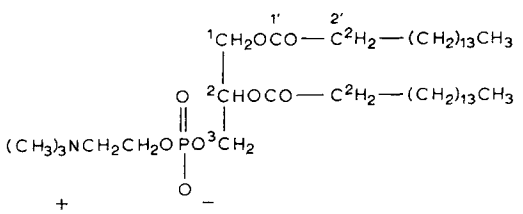


Fig. 1. An exceptionally good deuterium resonance spectrum at 13.8 MHz. Liquid crystalline bilayers of 1,2[2'-$^2H_2$]palmitoyl-3-*sn*-phosphatidylcholine at 70 °C. 90 000 free induction decays (2 h).

MATERIALS AND METHODS

1,2[2'-$^2H_2$]palmitoyl-3-*sn*-phosphatidylcholine (1) was synthesized from glycerophosphoryl choline and [2-$^2H_2$]palmitic acid anhydride as described previously [2].



(1)

1[2'-$^2H_2$]palmitoyl-2-palmitoyl-3-*sn*-phosphatidylcholine was prepared as follows: 1,2[2'-$^2H_2$]palmitoyl-3-*sn*-phosphatidylcholine was treated with phospholipase A (*Crotalus atrox*) according to a modified version of ref 3. To improve the solubility of dipalmitoyl-phosphatidylcholine in the ether phase, 10 % of pyridine was added. The lysophosphatidylcholine was purified by column chromatography on silica gel 60 (Merck), using chloroform/methanol/water (65 : 25 : 4, by vol.) followed by pure methanol as eluents. The lysophosphatidylcholine was reacylated with non-deuterated palmitic acid anhydride by the procedure of Robles and van den Berg [3].

1-Palmitoyl-2[2'-$^2H_2$]palmitoyl-3-*sn*-phosphatidylcholine was synthesized by preparing lysolecithin from non-deuterated 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (Fluka, Switzerland) followed by reacylating with [2-$^2H_2$]palmitic acid anhydride.

The purity of the specific lipids was established by thin-layer chromatography (chloroform/methanol/water; 65 : 25 : 4, by vol.), proton NMR, and optical rotation.

The bilayer phases were prepared by thoroughly mixing dipalmitoyl-3-*sn*-phosphatidylcholine (48.5 wt %) and water (51.5 wt %) in a sealed ampoule. For the powder type spectra 500 mg of liquid crystalline phase was used.

The ^2H NMR measurements were performed at 41.4 and 13.8 MHz with a Bruker HX-270-FT and a HX-90-FT spectrometer, respectively, both equipped with a variable temperature unit. The temperature unit was calibrated with a standard thermometer. The ampoule containing the bilayer phase was placed in a conventional 10 mm NMR tube. The Bruker HX-270-FT has a 3-fold higher sensitivity for deuterium resonance than the HX-90-FT instrument and this considerably simplifies the measurements.

RESULTS AND DISCUSSION

Fig. 2 shows a comparison of three deuterium resonance spectra of dipalmitoyl-3-*sn*-phosphatidylcholine with the deuterium label at the C-2 position of the palmitic acyl chains. Fig. 2A arises from phosphatidylcholine with both fatty acyl chains labelled, Figs 2B and 2C correspond to phosphatidylcholines with one deuterated chain only. The intensity ratio of the three signals in Fig. 2A is approx. 2 : 1 : 1. Spectrum 2A is simply the sum of the spectra 2B and 2C and the assignment of the resonances is thus readily made.

The largest quadrupole splitting is due to the C^2H_2 group in the chain attached at position 1 of the glycerol moiety (chain 1). The magnitude of this splitting and its variation with temperature are identical with the results obtained for the consecutive eight segments of the chain [1]. The two smaller splittings arise from the palmitic acyl chain attached at position 2 of the glycerol moiety (chain 2). This spectrum is unusual in two respects: Firstly, there are two doublets arising from one C^2H_2 group. Secondly, both splittings are distinctly smaller than the splittings found for chain 1 or the other segments in chain 2. The occurrence of two splittings for the same C^2H_2 group can be accounted for by at least two different models. One possibility would be that the two deuterium atoms of the CD_2 group are not motionally equivalent and thus produce two different signals. However, this interpretation seems not very probable since only one splitting shows a variation with temperature, whereas the other is temperature independent (Fig. 3).

An alternative explanation would be the assumption of two long lived conformations of the lipid molecule with two different orientations of chain 2. The two conformations must be almost equivalent energetically and must be separated by a large energy barrier. Molecular models indicate that two conformations can indeed be constructed for the glycerol constituent of phosphatidyl choline, the transition

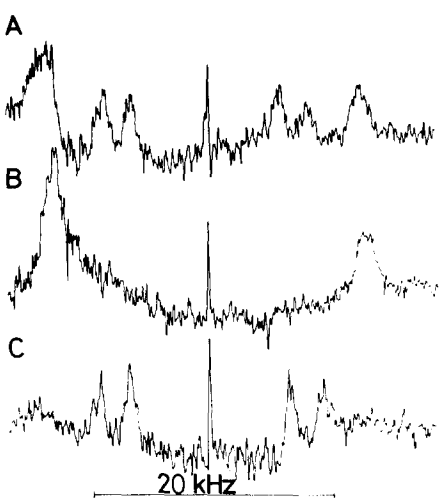


Fig. 2. Deuterium resonance spectra measured with a superconducting magnet (41.4 MHz) at 50 °C; 200 000 free induction decays [1 h]. Liquid crystalline bilayers of (A) 1,2[2′-$^2H_2$]palmitoyl-3-*sn*-phosphatidylcholine; (B) 1[2′-$^2H_2$]palmitoyl-2-palmitoyl-3-*sn*-phosphatidylcholine; (C) 1-palmitoyl-2[2′-$^2H_2$]palmitoyl-3-*sn*-phosphatidylcholine.

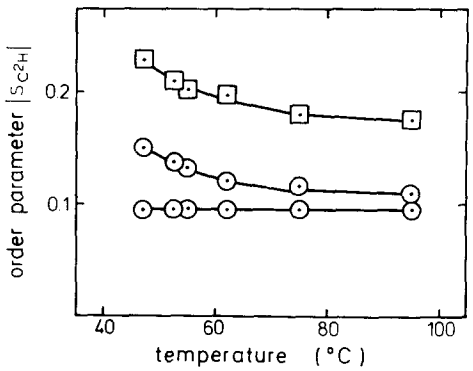


Fig. 3. Temperature dependence of the order parameter $|S_{C-^2H}|$ of 1,2[2'-$^2H_2$]palmitoyl-3-*sn*-phosphatidylcholine bilayers. ⊡, chain 1; ○, chain 2.

between the two conformations being strongly hindered for steric reasons. The existence of a conformational equilibrium of the glycerol constituent is further suggested by the fact that deuteration of dipalmitoyl-3-*sn*-phosphatidylcholine at carbon atom 3 of the glycerol backbone also produces two doublets [4]. However, further experiments are required before a definite conclusion can be reached.

The quadrupole splittings of the C-2 segment of chain 2 are much smaller than the corresponding splitting of chain 1. This result is indicative of different initial orientations of the two chains with respect to the bilayer surface. A statistical mechanical analysis of the deuterium data shows that in most parts of the bilayer both fatty acyl chains are extended perpendicular to the bilayer surface except for the beginning of chain 2 which is oriented essentially parallel to the bilayer surface [5].

Additional evidence for a bent configuration of chain 2 is provided by recent X-ray studies of single crystals of phosphatidylethanolamine bilayers [6]. These investigations clearly demonstrate that in the bilayer crystal the first segments of chain 2 are extended parallel to the bilayer surface. In the liquid crystalline state the influence of the initial orientation on the following chain segments is rapidly lost, since the quadrupole splittings of all other segments in chain 2 are found to be similar to those of chain 1 segments. Nevertheless small differences between quadrupole splittings of corresponding segments of the two chains are also observed in other parts of the bilayer as has been pointed out earlier. This effect can now be understood as resulting from the different initial orientations of the two chains. Due to the conformational twisting of chain 2 chemically equivalent segments of both chains are positioned at different distances from the bilayer surface and thus they experience slightly different fluctuations.

ACKNOWLEDGEMENT

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