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DEUTERIUM NMR STUDIES OF CEREBROSIDE-PHOSPHOLIPID BILAYERS

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Summary

^{2}H-NMR was used to probe the interaction of non-hydroxy fatty acid cerebroside and 2-hydroxy fatty acid cerebroside with the polar head group and with the acyl chains of dipalmitoylphosphatidylcholine in unsonicated bilayers. It is shown that the interior of the bilayer exhibits uniformly increasing orientational order as the concentration of both types of cerebroside increases, whereas the surface of the bilayer, as reflected by the head group motion, becomes disordered. The extent of the disorder at the surface is dependent upon the type and concentration of the cerebroside. These results are discussed in terms of hydrogen-bonding interactions.

Glycolipids are present in many biological membranes. However, only in specialized membranes such as the microvilli of the intestine [1, 2] and the myelin membrane [3] are they found in high concentrations; for example, glycolipids in the form of cerebrosides occur in the myelin membrane at a level of 20—25% of the total lipids [3]. Furthermore, during the development of the brain, the fatty acid composition of the cerebrosides is altered. In young animals, the non-hydroxy fatty acid-containing cerebrosides comprise 60% of the total cerebrosides; while in the adult brain, hydroxy fatty acid-containing cerebrosides account for 70% of the total cerebrosides [4, 5]. This change in their fatty acid composition results in altered physical properties of the cerebrosides, as determined by X-ray diffraction [6—8],

Abbreviation: DPPC, dipalmitoylphosphatidylcholine.

infrared spectroscopy [7], and monolayer studies [6—9]. Because the above mentioned membranes have special functions, the question arises as to whether a high concentration of glycolipids, especially cerebrosides, imparts any unique physical properties to phospholipid bilayers. In this communication, we report on the use of deuterium nuclear magnetic resonance (NMR) [10] to probe the interaction of non-hydroxy fatty acid and hydroxy fatty acid cerebroside with the polar head group and acyl chains of dipalmitoylphosphatidylcholine (DPPC) in unsonicated bilayers.

The effect of incorporating an increasing concentration of non-hydroxy fatty acid-containing cerebrosides into the DPPC bilayer at 53° C is shown in Fig. 1A. A striking decrease in the intensity of the quadrupolar pattern is observed upon addition of 10 mol% of non-hydroxy fatty acid cerebroside, i.e., the ratio of the amplitude of the zero frequency resonance to the amplitude of the residual quadrupole doublet increases. At a cerebroside concentration of 33 mol%, the quadrupole doublet begins to re-emerge and it becomes the prominent feature at 50 mol%. The effect of hydroxy fatty acid-containing cerebrosides on the head group labeled DPPC is quite different as shown in Fig. 1B. First, there is no sharp decrease of the quadrupole doublet intensity at 10 mol% cerebroside and the doublet remains evident at all concentrations. Second, not until a concentration of 20 mol% hydroxy fatty acid cerebroside is reached does there occur an appreciable decrease in the quadrupole doublet intensity. At the highest concentrations (50 mol%) the spectra of both types of cerebroside-DPPC bilayers become similar. The resonance at zero frequency is not due to residual ^2H_2O in the sample because deuterium depleted H_2O was used as the solvent. Furthermore, when equivalent samples were prepared using unlabeled DPPC, no resonance was observed at zero frequency.

The effect of incorporating an increasing concentration of non-hydroxy fatty acid- or hydroxy fatty acid-containing cerebrosides into bilayers of DPPC labeled with ^2H in the acyl chains at the 10-position is shown in Table I. The residual quadrupole splitting, $\Delta\nu_Q$, of the deuterated acyl chains of DPPC increases as the amount of each cerebroside increases. Only a single set of doublets is observed at all compositions. The magnitude of $\Delta\nu_Q$ at $T = 53°$ C is essentially the same for each type of cerebroside at every mol percentage and it increases uniformly with increasing cerebroside concentration. Thus, within the bilayer both non-hydroxy fatty acid and hydroxy fatty acid cerebroside act to increase the orientational order to the same extent.

It appears that non-hydroxy fatty acid and hydroxy fatty acid cerebroside exert a differential effect on the DPPC bilayer as monitored by ^2H-NMR. The interior of the DPPC bilayer becomes more ordered as cerebroside content increases, whereas the surface of the bilayer, as reflected by the head group motion, becomes disordered. However, the extent of the disorder at the surface of the bilayer is dependent upon the type and concentration of cerebroside. One possible hypothesis to explain the results obtained with two different types of cerebrosides is the occurrence of self association of the cerebroside molecules caused by hydrogen bond formation. The presence of the hydroxyl group might facilitate this self-association for the hydroxy

fatty acid-containing cerebroside so that at low concentration (10 mol%) it could form dimers and perturb a smaller fraction of the DPPC headgroups. On the other hand, the non-hydroxy fatty acid-containing cerebroside, lacking the hydroxyl group at the 2-position in the acyl moiety, would be more uniformly dispersed and perturb a larger fraction of the DPPC headgroups in the bilayer at low concentrations. This would account for the marked decrease in the quadrupole doublet intensity of the head group at low concentration (10 mol%) of non-hydroxy fatty acid cerebroside. At cerebroside

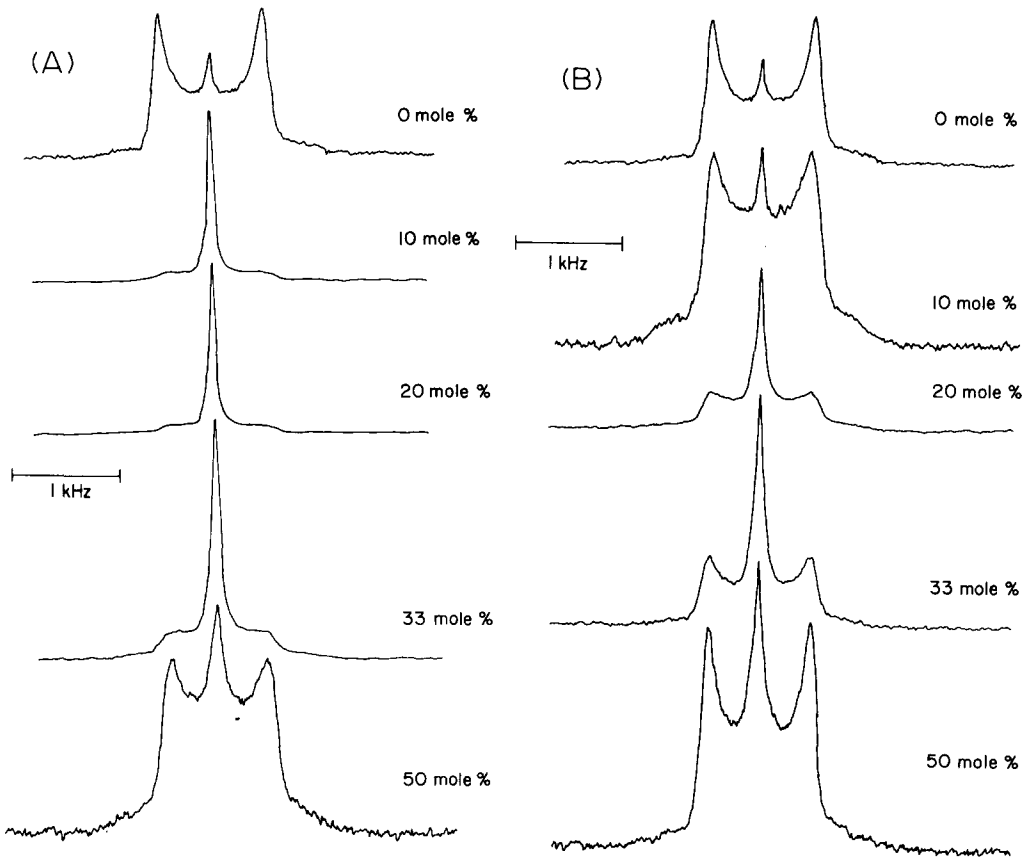

Fig. 1. ^{2}H-NMR spectra at $T = 53^\circ$C of unsonicated bilayers composed of dipalmitoylphosphatidylcholine (DPPC) and various mol% (A) non-hydroxy fatty acid cerebroside and (B) 2-hydroxy fatty acid cerebroside. The DPPC is labeled with ^{2}H at the *N*-methyl carbons of the choline moiety. The total cerebroside fraction was isolated from bovine brain [4]. Hydroxy fatty acid- and non-hydroxy fatty acid-containing cerebrosides were separated by silicic acid chromatography and were approx. 98% pure as judged by HPLC [4]. [*Me*-^{2}H]Choline was prepared using ethanolamine and C^2H_3I (Kor Isotopes, Cambridge, MA) as described by Brulet and McConnell [12]. The [*Me*-^{2}H]-labeled DPPC was prepared by coupling [*Me*-^{2}H]choline to dipalmitoylphosphatidic acid according to Sears et al. [13] Deuterium depleted H_2O (Aldrich) was used to prepare all samples. Each sample was taken to dryness from a $CHCl_3/CH_3OH$ solution. The sample was hydrated with 1 ml of deuterium-depleted H_2O, vortexed at 80°C for several min and then allowed to stand at room temperature for 30 min. This temperature was chosen to insure that the cerebrosides would be in the liquid crystalline state. The sample was then frozen at —78°C and lyophilized overnight. The lyophilization procedure was repeated. The sample for study was prepared by adding 1.7 ml of unbuffered deuterium-depleted H_2O and vortexing at 80°C for several min. All experiments were carried out at 41.4 MHz with no proton decoupling. The temperature was maintained at 53° ± 0.5°C. 35 μmol of phospholipid were present in all samples.

concentrations greater than 33 mol%, hydrogen bonds between the galactose moieties might occur and cause the formation of higher order clusters for both types of cerebrosides. If this were the case, then at the highest concentrations the non-hydroxy fatty acid- and hydroxy fatty acid-containing cerebrosides would produce similar degrees of perturbation of the DPPC bilayer surface and their spectra would be similar. This effect is observed at a concentration of 50 mol% cerebroside (Fig. 1). It should be noted that experimental results on gangliosides in phospholipid bilayers have also been explained in terms of hydrogen bonding interaction [11].

The results in Fig. 1 cannot be explained on the basis of incomplete mixing of the lipids since the quadrupole splitting observed for the deuterated acyl chains increases monotonically and only one doublet is observed at each concentration (see Table I). This serves as an internal standard and suggests that at all concentrations our samples are homogeneously mixed. The possibility of rapid exchange of the phospholipid molecule between distinct phases (i.e. gel and liquid crystalline) which might exist simultaneously in the bilayer at $T = 53°C$ should be considered. However, we have carried out similar experiments (Neuringer, L.J., Sears, B. and Jungalwala, F.B., unpublished data) at $T = 53°C$ wherein the cerebroside is labeled with deuterium at the 10-position. We find a uniform and monotonic increase in quadrupolar splittings. The magnitudes of $\Delta\nu_Q$ observed in the latter series of experiments are comparable to those listed in Table I. This result supports our view that the bilayer consists of a single phase (i.e. liquid crystalline) at 53°C.

The differential effect of non-hydroxy fatty acid- and hydroxy fatty acid-containing cerebrosides on the polar head group at the bilayer surface as opposed to their effects on acyl chains in the interior of the bilayer suggest that the changes observed in the fatty acid composition of cerebrosides during the development of the brain may serve as a subtle biochemical regulation of the physical surface properties of the myelin membrane.

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TABLE I

RESIDUAL QUADRUPOLE SPLITTING OF DI([10-$^2H_2$]PALMITOYL)PHOSPHATIDYLCHOLINE AS A FUNCTION OF CEREBROSIDE CONCENTRATION AT 53°C

Dependence of the residual quadrupole splitting $\Delta\nu_Q$ on concentration of non-hydroxy fatty acid- and hydroxy fatty acid-containing cerebrosides in unsonicated DPPC bilayers at $T = 53°C$. The DPPC is labeled at the C-10 position of the acyl chains. 10-Keto palmitic acid was prepared as described by Hubbell and MacConnell [14]. [10-$^2H_2$]Palmitic acid was synthesized as described by Tulloch [15]. [10-$^2H_2$]Dipalmitoyl phosphatidylcholine was prepared according to Robles and van den Berg [16]. Samples were taken to dryness and then hydrated with 0.5 ml of deuterium-depleted H_2O and vortexed for several min at 80°C as described in Fig. 1. 50 μmol of phospholipid were present in all samples.

Concentration of cerebroside	$\Delta\nu_Q$ (kHz)	
	Hydroxy fatty acid	Non-hydroxy fatty acid
0	20.8	20.8
10	22.5	22.7
20	24.1	23.7
33	24.3	25.4
50	28.0	27.0

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