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Behaviour of a glycosphingolipid with unsaturated fatty acid in phosphatidylcholine bilayers

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N-(Oleoyl)galactosylceramide with perdeuterated acyl chain was prepared by partial synthesis, and studied by wide line ^{2}H-NMR in phospholipid liposomes. Spectra were obtained for low glycolipid concentrations in bilayers of dimyristoyl-, distearoyl-, and 1-palmitoyl-2-oleoylphosphatidylcholines. In an attempt to isolate the effects of glycosphingolipid fatty acid *cis* unsaturation on glycolipid behaviour in membranes, spectral findings related to the above species were compared to literature NMR data for pure 1-palmitoyl-2-oleoylphosphatidylcholine bilayers in which the oleoyl chain of the phospholipid had been deuterated, and to analogously deuterated glycerol based lipids in *Acholeplasma laidlawii* membranes. The results for *N*-(oleoyl-d_{33})galactosylceramide proved to be qualitatively and quantitatively very similar to published data dealing with glycerol based lipids at comparable temperatures. In addition, the results were strikingly similar for glycolipids dispersed in saturated and unsaturated phospholipid host matrices. It would appear that the primary effects of *cis* 9,10 fatty acid unsaturation in glycosphingolipids (at low concentration in fluid phospholipid membranes) are the same as those of fatty acid *cis* unsaturation in glycerolipids. It further appears that the overall dynamic behaviour of *N*-(oleoyl)galactosylceramide in fluid phospholipid membranes is very similar to that of glycerolipids with comparable acyl chains.

Introduction

Glycosphingolipids (GSLs), the carbohydrate bearing lipids found at outer surfaces of mammalian cells, are thought to serve as structural elements of the plasma membrane and as specific recognition sites that may participate in a wide range of processes [1,2]. Available data suggest that both functions of this lipid family can be strongly influenced by their arrangement and behaviour in the membrane [1–4]. That this should be true in the case of GSL contribution to membrane structure seems straightforward. The relationship to their role as receptors may be less so: sensitivity to arrangement and behaviour in glycolipid function as recognition sites has been rationalized on the basis that their carbohydrate headgroups are in close, spatially restrictive, proximity to the membrane surface. The term, 'crypticity', was coined to describe one aspect of this phenomenon [5].

GSLs possess a single fatty acid as part of their ceramide backbone, and the nature of this fatty acid is widely considered to be a major potential modulator of their characteristics in membranes. In particular, length of the glycolipid fatty acid and its degree of hydroxylation have been considered for their ability to alter membrane structural features and specific binding events via their influence on GSL arrangement and behaviour (e.g. Refs. 6–11). However, as was recently pointed out by Reed and Shipley [12], although a great amount of work has been done on phospholipids with unsaturated fatty acids, very little is known about the effect of chain unsaturation on carbohydrate-bearing lipids. These workers compared the physical behaviour of the pure naturally occurring species, *N*-(oleoyl)galactosylceramide (*N*-(oleoyl)GalCer), in hydrated form, with that of its 18-carbon saturated fatty acid analogue, *N*-(stearoyl)GalCer, by differential scanning calorimetry and X-ray diffraction. Both

Abbreviations: GSL, glycosphingolipid; GalCer, galactosylceramide; DMPC, 1-α-dimyristoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; DSPC, 1-α-distearoylphosphatidylcholine.

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formed lipid bilayer structures over the temperature range examined. The derivative with saturated fatty acid displayed a main transition at 85°C from gel to liquid crystalline phase (see also Ref. 13). *N*-(Oleoyl)GalCer on the other hand, upon warming to 44.8°C, underwent a transition to a fluid metastable phase which converted over a period of some 24 h to a new gel phase with main transition of 55.5°C. The observation that *cis* unsaturation at the 9,10 position leads to a lowering of the main transition temperature is analogous to the situation that is well known for phospholipids: 1-palmitoyl-2-stearoylphosphatidylcholine has a main transition of 49°C, while its 2-oleoyl analogue exhibits melting at −3°C [13,14]. Quantitatively GSLs show much higher main transition temperatures than do their phospholipid counterparts of similar acyl chain composition, less effect of fatty acid nature on the main transition, and greater effect of headgroup nature on the main transition [1,15].

Studies of pure lipid species are crucial to an understanding of the behaviour of cell membranes. However, in most cases GSLs are minor membrane components, comprising as little as a few % of total plasma membrane lipid. Hence it has been pointed out that one must also consider systems of greater complexity, designed to mimic specific aspects of glycolipid-related phenomena relevant to cells [1,2]. In the present article we describe for the first time an examination by ^{2}H-NMR of the behaviour of a GSL with an unsaturated fatty acid in phospholipid membranes. The sensitivity of deuterium wide line NMR to molecular dynamics and orientation in membranes, and the non-perturbing nature of the deuterium probe, make it a potentially very useful technique for addressing the question of lipid behaviour in membranes [16–18]. The major question addressed in this work was, how the presence of a *cis* double bond in a GSL fatty acid might be expected to influence the behaviour of this lipid family at low concentrations in phospholipid-rich membranes. The problem was approached by assembling *N*-(oleoyl-d_{33})-GalCer into bilayers of several typical saturated and unsaturated phosphatidylcholines, and quantitatively comparing the resultant ^{2}H-NMR spectra to those of well characterised glycerolipid systems (particularly POPC) bearing deuterated oleic acid at the *sn*-2 position.

Materials and Methods

1,2-Dimyristoyl-, 1,2-distearoyl- and 1-palmitoyl-2-oleoylphosphatidylcholines (DMPC, DSPC and POPC, respectively) were obtained from Avanti Polar Lipids, Birmingham, AL, and were used without further purification. Galactosylceramide (GalCer) (Type II from beef brain) was from the same commercial source. Perdeuterated oleic acid (Cambridge Isotope Laboratories) was attached to GalCer in place of the natural fatty acid mixture to produce *N*-(oleoyl-d_{33})GalCer according to the partial synthetic route described earlier for the preparation and purification of probe labelled simple glycosphingolipids [19]. Reactions and purification were followed by thin layer chromatography on Merck silica gel 60 plates, eluted with 65 : 15 (v/v) $CHCl_3/CH_3OH$, and developed with ninhydrin or sulphuric acid/ethanol spray. Organic solvents were reagent or spectral grade.

Lipid samples were prepared for spectroscopy by dissolving all components at the desired final ratio in 2 : 1 (by volume) $CHCl_3/CH_3OH$ and removing solvent by evaporation under a stream of nitrogen. They were then maintained under vacuum (rotary pump) for at least 3 h to remove traces of solvent. The resultant lipid films were successively hydrated with 150 μl of deuterium depleted water (MSD Isotopes) and lyophilized three times, prior to final rehydration with deuterium depleted water (total volume 150–200 μl). They were then subjected to eight freeze-thaw cycles during which the temperature was raised above the main transition of the lipid matrix involved, with vortexing.

^{2}H-NMR spectra in Figs. 5 and 3A were acquired at 30.7 MHz on a 'home-built' spectrometer. Spectra were recorded using a quadrupolar pulse sequence [20] with full phase cycling and quadrature detection [21]. Moment analysis was performed on an Intel Series II microcomputer development system which was interfaced to a slave microcomputer used for data acquisition. All other ^{2}H-NMR spectra were acquired at 55.26 MHz on a 'home-built' spectrometer, and were recorded using the quadrupolar echo sequence [20] with full phase cycling and quadrature detection [18,22]. The 90° pulse lengths were 1.9 μs and the separation between the two pulses in the quadrupolar echo was 30 μs. The time domain quadrupolar echo signal was filtered and symmetrized about the top of the echo using a procedure described elsewhere [18,22a]. Spectral simulations and moment analyses were performed on a μVAX II computer interfaced to a 'home-built' data acquisition computer.

Results and Discussion

^{2}H-NMR spectra of pure *N*-(oleoyl-d_{33})GalCer in hydrated form at 15°C and 52°C are shown in Fig. 1. ^{2}H-NMR spectra are strongly influenced by factors that include orientation of the labelled molecules and the nature of their reorientation with time [16–18]. Anisotropic molecular motion typical of lipids within a fluid membrane leads to incomplete averaging of quadrupolar interactions, resulting in spectra characterised by residual splittings (quadrupole couplings), $\Delta\nu_Q$, of spectral lines. The quadrupole coupling for the

90° orientation of the molecular axis of rotation with respect to the magnetic field is directly related to the C-^{2}H bond order parameter, S_{CD}, by:

$$\Delta\nu_Q = (3/4)(e^2qQ/h)S_{CD}$$

where (e^2qQ/h) is the quadrupolar coupling constant (170 kHz for the aliphatic C^2H_2 residue [16]). Thus S_{CD} provides a measure of time averaged angular fluctuations of the C-^{2}H bond with respect to the director axis of motion (generally perpendicular to the plane of the bilayer for membrane lipids). Since the fatty acyl chain of *N*-(oleoyl)GalCer was perdeuterated in the experiments described here (inset to Fig. 1), the spectra derived are superpositions of spectra from deuterons attached to each of the fatty acid chain carbons.

The spectra in Fig. 1 are consistent with a recent report on the phase behaviour of pure hydrated *N*-(oleoyl)GalCer as examined by DSC and X-ray diffraction [11]. A transition was recorded from gel to metastable liquid crystal phase at 44.8°C: cycling the sample temperature between 49°C and 0°C over a period of 24 h led to the appearance of a new gel phase with a main transition temperature of 55.5°C. Throughout the temperature range studied, the structures formed were reported to be lamellar. The appearance of the 15°C spectrum is typical of deuterated lipids in the gel phase. A first moment, M_1, of $125 \cdot 10^3$ s^{-1} was calculated, which is indicative of very slow molecular reorientation on the NMR timescale (M_1 is obtained as a weighted average of spectral splittings, and, as such, is directly related to the degree of probe motional order [20–22]). The 52°C spectrum was subsequently obtained within 5 h of raising the sample temperature. It displays a number of sharp quadrupole-split peaks characteristic of deuterated lipids undergoing rapid axially symmetric motion in fluid bilayers. The first moment at this higher temperature was $34.5 \cdot 10^3$ s^{-1}, consistent with much greater motional freedom of the GSL acyl chain. The unsplit central peak is prominent, and would be adequately accounted for by the presence of a population of highly curved liposomes (diameter some 100 nm) that permit rapid reorientation of glycolipid molecules relative to the spectrometer magnetic field, via lateral diffusion within the membrane and via tumbling of the entire liposome (with resultant collapse of quadrupole splittings) [9]. Indeed it is known that pure GalCer forms structures in the size range 100–500 nm, which would be consistent with such an observation [9,23]. The spectrum of the same sample raised to 65°C shows collapse of essentially all resolved quadrupolar couplings to result in a single intense peak with a first moment of $20.6 \cdot 10^3$ s^{-1}. Neither this latter phenomenon, nor the relatively intense unsplit central resonance referred to above in the 52°C spectrum, were noted previously in studies of deuterated oleic acid attached to the glycerolipid, 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) [24]. However, POPC forms liposomes much larger than 100 nm in diameter (Ref. 9 and our unpublished observation by freeze-fracture electron microscopy), and collapse of quadrupolar splittings for large liposomes is not expected due to their greater radius of curvature and slow tumbling.

Fig. 2 shows ^{2}H-NMR spectra of *N*-(oleoyl-d_{33})GalCer at 10 mol% in gel and liquid crystal host

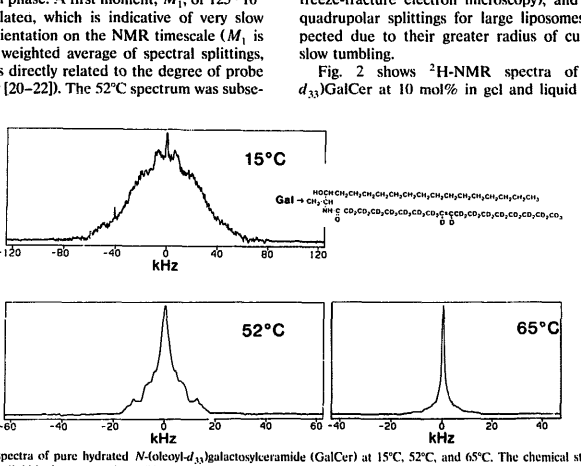

Fig. 1. ^{2}H-NMR spectra of pure hydrated *N*-(oleoyl-d_{33})galactosylceramide (GalCer) at 15°C, 52°C, and 65°C. The chemical structure of the probe-labelled glycolipid is shown as an inset. The sample comprised 5 mg (5.8 μmol) of deuterated GSL and was run at 15°C, prior to warming to the higher temperatures with an equilibration time of 30 min. Note: ν_0 = 55.26 MHz.

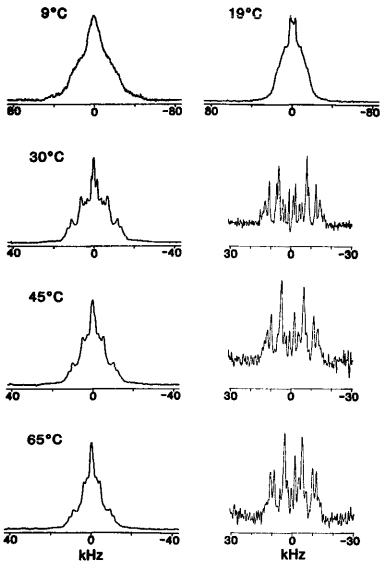

Fig. 2. ^{2}H-NMR spectra of *N*-(oleoyl-d_{33})GalCer incorporated at 10 mol% into fully hydrated bilayers of the phospholipid, dimyristoylphosphatidylcholine (DMPC) at selected temperatures. Depaked spectra are shown to the right for samples run at 30°C, 45°C, and 65°C. Each sample contained 5 mg (5.8 μmol) of deuterated GSL.

bilayer matrices of DMPC (the main transition temperature of pure DMPC is 23°C, with a pretransition at 10–14°C [25,26]). At 9°C the spectrum is typical of deuterated lipids in gel phase bilayers [18]. Upon warming the sample to 19°C the spectrum shows some evidence of glycolipid axial motion, but retains features of gel phase spectra. The measured first moments were $84.4 \cdot 10^3$ s^{-1} and $60.0 \cdot 10^3$ s^{-1}, respectively. Spectra run at 30°C (Fig. 2) show no features suggestive of the presence of significant gel phase, in spite of the fact that the temperature involved is well below the pure glycolipid main transition. This indicates that under such conditions *N*-(oleoyl)GalCer is dispersed into the fluid host matrix. A phase diagram has not been published for DMPC/GalCer; however, in our previous experiments involving low concentrations of deuterium-labelled and spin-labelled GalCer with saturated fatty acids in DMPC, apparent dispersion or the glycolipid into a fluid host matrix was also found at 30°C [11,27]. Skarjune and Oldfield first remarked on this resul: ɔr 17% glucosylceramide in DMPC [28]. ^{2}H-NMR spectra of 10 mol% *N*-(oleoyl-d_{33})GalCer in DMPC at 45°C and 65°C are included in Fig. 2, with their depaked counterparts.

The 30°C, 45°C, and 65°C spectra in Fig. 2 are superpositions of powder spectra having lineshapes characteristic of rapid axially symmetric motion in fluid bilayers. First moments at these temperatures above the host matrix main transition were reduced to $40.3 \cdot 10^3$, $35.2 \cdot 10^3$ and $32.1 \cdot 10^3$ s^{-1}, respectively. The existence of features that are potentially assignable to specific fatty acid methylene groups is most obvious in the depaked spectra, as individually resolved pairs (and overlapping pairs) of peaks related to specific deuterated carbon atoms (and groups of carbon atoms). GSLs with unsaturated deuterated fatty acid chains have not been previously reported, hence prior assignments of their spectra do not exist. However, extensive wide line ^{2}H-NMR investigations have been made of bilayer membranes containing glycerol based lipids with selectively deuterated oleic acid chains. This previous work comes from studies of three systems: unsonicated bilayers of pure POPC bearing deuterated oleic acid at the *sn*-2 position [24,29]; *Escherichia coli* membranes with biosynthetically incorporated deuterated oleic acid [30]; and *Acholeplasma laidlawii* membranes with biosynthetically incorporated deuterated oleic acid [31]. The striking observation was made by these workers that the ^{2}H quadrupolar splittings for deuterated oleic acid which had been incorporated into *Acholeplasma laidlawii* (predominantlv as simple neutral glycosyl diacylglycerols with some phosphoglycerides) and into *Escherichia coli* (predominantly as phosphatidylglycerol and phosphatidylethanolamine), corresponded very closely to those seen by Seelig's group [24,29] for deuterated oleic acid attached to the glycerol backbone of POPC in single-component (fluid) liposomes. The only exception was in the C_2 methylene group (i.e. at the membrane surface).

Given the uniformity of published data for selectively deuterated oleic acid attached in *sn*-2 linkage to glycerolipids, it is possible to use the spectral $\Delta\nu_Q$ values from published experiments with POPC [24,29], supplemented with data for *Acholeplasma laidlawii*, which is more encyclopedic, (Table I) to generate a computer simulated spectrum for perdeuterated oleic acid attached in *sn*-2 linkage to a glycerol backbone in the above membranes at 25–27°C. The result is presented in Fig. 3A, with the experimental 30°C spectrum for 10 mol% *N*-(oleoyl-d_{33})GalCer in DMPC for comparison (3B). Figs. 3A and 3B are virtually identical. This observation is instructive since ^{2}H-NMR spectra are very sensitive to subtleties of orientation and dynamics of the molecules to which they are attached. Also shown is the spectrum of the saturated fatty acid

TABLE I

Literature ^2H-NMR spectral data for deuterated oleic acid attached at the sn-2 position of glycerol based lipids in fluid membranes, tabulated for comparison with exoerimental data from samples of N-(oleoyl-d_{33})GalCer in various ,.uid phosphatidylcholine bilayers

Quadrupole splittings for pure POPC, and for *Acholeplasma laidlawii* were taken from Refs. 24, 29 and 31, respectively. Literature data are presented as a function of ^2H location along the fatty acid chain ('Carbon No.') with the corresponding value of $\Delta\nu_Q$ (values for carbons No. 13 and No. 15 were interpolated by drawing a smooth curve through *Acholepiasma* data points [31]). Experimental data are presented as a function of resolvable quadrupolar splitting, $\Delta\nu_Q$ from high to low, with the corresponding suggested ^2H location(s) assigned by comparison with the simulated spectrum, Fig. 3A.

Literature $\Delta\nu_Q$ values (kHz) for ^2H in the oleate chain		
Carbon No.	*A. laidlawii* (25°C)	POPC (27°C)
2	20.0	10.8
	25.5	16.4
3	23.8	
4	24.0	
5	25.8	
6	22.0	
7	22.0	
8	15.6	15.6
9	14.8	13.4
10	4.3	2.5
11	7.8	6.2
12	13.5	13.0
13	13.3	
14	13.0	
15	12.0	
16	11.0	
17	8.0	
18	3.0	

Experimental $\Delta\nu_Q$ values (kHz) for ^2H in the oleate chain on GalCer				
Carbon No.	DMPC (30°C)	POPC (27°C)	DMPC (65°C)	DSPC (65°C)
5	31.6	28.8	27.0 (?)	27.3
3&4	27.2	26.4	22.6	23.4
6&7	23.2	22.6	18.8	19.9
8&2 (?)	15.8	15.0	12.7	
2 (?), 9, 12, 12, 13, 14	14.0	13.2	8.7	12.9
15, 16				
17	9.6	9.4		9.3
11	7.2	7.4		7.0
18&10	3.2	3.0	1.8	3.1

GSL, *N*-(stearoyl-d_{35})GalCer at 30°C in the same host matrix (Fig. 3C).

Peak assignments are illustrated in the simulated spectrum 3A based on the published data (for deuterated oleic acyl chains attached in *sn*-2 linkage to a glycerol backbone) which were used in generating it (Table I). Its close resemblance to 3B permitted likely assignment of the GalCer spectrum with little difficulty (Table I). At 45°C and 65°C (Fig. 2) *N*-(oleoyl-d_{33})GalCer spectra display some qualitative and quantitative differences from the spectrum at 30°C and from the simulated spectrum in Fig. 3A – consistent with the expectation that ^2H-NMR spectra are sensitive to lipid dynamics: temperature-induced disordering of the fatty acyl chain has occurred accompanied by changes in the relative peak positions.

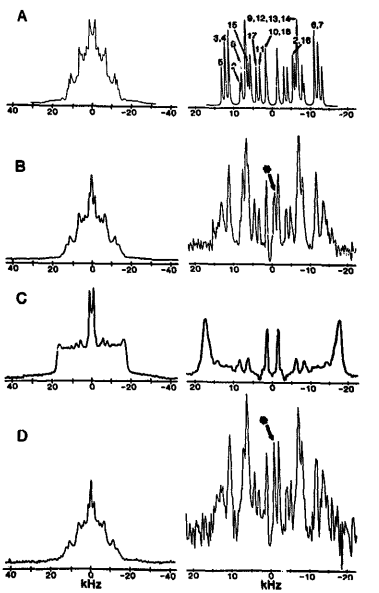

Fig. 3. Comparison of ^2H-NMR spectra for deuterated oleic acid attached to a substituted glycerol vs. a glycosyl sphingosine backbone, in fluid bilayer membranes. (A) Computer simulated spectrum for oleic acid-d_{33} attached in *sn*-2 linkage to glycerolipids (from literature data for 1-palmitoyl-2-oleoylphosphatidylcholine at 27°C [24,29], and *Acholeplasma laidlawii* membranes at 25°C [31] – see caption to Table I and Fig. 4). (B) Experimentally derived spectra for *N*-(oleoyl-d_{33})GalCer at 10 mol% in fluid DMPC at 30°C. (C) Experimentally derived spectra for *N*-(stearoyl-d_{35})GalCer at 10 mol% in fluid DMPC at 30°C. (D) Experimentally derived spectra for *N*-(oleoyl-d_{33})GalCer at 5 mol% in fluid POPC at 30°C. In each case the powder spectrum is shown on the left, and the depaked spectrum on the right. Arrows in (A) indicate accepted spectral assignments from the literature quoted, as listed in Table I. The single arrow in (B) and (D) indicates the position of the sharp, unsplit central peak which is not expected to be present in the simulated spectrum.

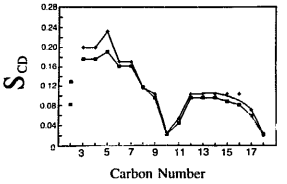

Fig. 4. Plot of the order parameters, S_{CD}, for deuterated oleic acid attached in *sn*-2 linkage to glycerolipids, taken from the literature [24,29,31] used to produce Fig. 3A above (■), superimposed upon tentative assignments based on correspondence for *N*-(oleoyl-d_{33})GalCer at 10 mol% in fluid DMPC at 30°C (+). Values for carbons No. 13 and No. 15 in the glycerolipid plot were interpolated by drawing a smooth curve through points on either side.

Order parameters, S_{CD}, corresponding to the unsaturated glycerolipid spectral splittings used to generate Fig. 3A are shown plotted in Fig. 4. It is well known that for deuterated oleic acid attached to the *sn*-2 position of a glycerol backbone, the shape of the S_{CD} profile as a function of position in the fatty acid chain is very different from the classic pattern seen for its saturated fatty acid analogue [16–18]. The major distinction is that peaks associated with deuterons at C-9 and C-10 (the double bond location) manifest much smaller quadrupolar splittings. This has been demonstrated to arise primarily from the fact that the double bond (with attached deuterons) makes an angle of 7–8 degrees to the motional director (the bilayer normal) [29]. The double bond also exerts a measurable effect on methylene carbons immediately above and below it in the chain (i.e. C-8 and C-11, respectively), as evidenced by their lower S_{CD} values than those seen for saturated analogues. When the tentative peak assignments for the 30°C spectrum obtained for *N*-(oleoyl-d_{33})-GalCer in DMPC are used to generate an analogous plot (Fig. 4), they suggest that very much the same phenomena operate in the case of this unsaturated fatty acid GSL dispersed in a fluid phosphatidylcholine matrix.

The literature concerning NMR of lipids having deuterated unsaturated fatty acids deals with membranes composed almost entirely of glycerol based species, and in which all, or a very large fraction, of the membrane lipids possessed unsaturated fatty acids – the simplest system reported being pure (deuterated) POPC [24,29–31]. A corresponding spectrum of *N*-(oleoyl-d_{33})GalCer in fluid POPC bilayers is presented in Fig. 3D. POPC mixtures with GalCer from beef brain have been reported to exhibit phase separation over a wide range of temperature and composition [13], presumably because of the great difference in their phase transition temperatures. Within this range there is a tendency for the glycosphingolipid to exist in rigid domains, in equilibrium with fluid regions enriched in POPC. For the spectrum in Fig. 3D, 5 mol% GalCer in POPC at 27°C was selected, since this corresponds to a point that lies substantially above the fluidus of the published POPC/glycolipid phase diagram [13]. Freeze-etch electron microscopy of this sample at 27°C showed the liposomes to be multilamellar structures ranging about 1000 nm in diameter, and to possess membrane features known to be associated [32,33] with liquid crystal phase only. The spectral appearance is the same as that seen for *N*-(oleoyl-d_{33})GalCer in fluid bilayers of DMPC at a comparable temperature (3B).

Fig. 5 illustrates wide line ^{2}H-NMR spectra of *N*-(oleoyl-d_{33})GalCer dispersed in hydrated bilayers of a longer chain saturated fatty acid phospholipid: the 18-carbon species, distearoylphosphatidylcholine (DSPC). DSPC in bilayer form is known to exhibit a pretransition at 46–49°C and a main transition to the fluid phase at 54–55°C [25,26]. Spectra are shown for 10 mol% *N*-(oleoyl-d_{33})GalCer in DSPC bilayers at 45°C, 52°C, and 65°C. The low-temperature spectra are similar to those already seen for this deuterated glycolipid in DMPC below the host matrix phase transition

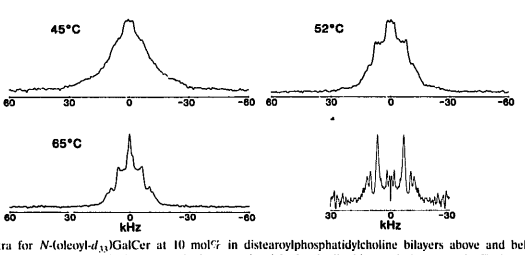

Fig. 5. ^{2}H-NMR spectra for *N*-(oleoyl-d_{33})GalCer at 10 mol% in distearoylphosphatidylcholine bilayers above and below the 54°C phase transition of the pure host matrix. The depaked spectrum is shown to the right for the liquid crystal phase sample. Each sample contained 5 mg (5.8 μmol) of deuterated GSL.

temperature (Fig. 2, 9°C and 19°C), exhibiting M_1 values of $61.8 \cdot 10^3$ and $46.9 \cdot 10^3$ s^{-1} for 45°C and 52°C, respectively. At 65°C in DSPC the spectral pattern seen is similar to that found in the DMPC matrix at 45°C and 65°C. The spectral first moment was calculated to be $36.2 \cdot 10^3$ s^{-1}.

Given the sensitivity of ^{2}H-NMR to probe orientation and dynamics, it is interesting that the spectral appearance of the glycosphingolipid studied was the same in both saturated and unsaturated host matrices at comparable temperatures.

Conclusions

While it is accepted that the cell membrane is based on the lipid bilayer, detailed knowledge of the relative arrangement of constituent molecules does not exist for any fluid membrane of mixed lipids with major structural differences; and the area remains one of intensive research [34]. Even simple mixtures of lipids in bilayer form pose challenging problems of analysis, and extrapolation from single-component lipid bilayers forms a major basis of existing theories. Hence, in our present study the most telling answer to the question of, how exchanging a GSL saturated fatty acid for its *cis* unsaturated analogue affects glycolipid behaviour in a fluid phosphatidylcholine bilayer matrix, is that, within the limitations of the probe, the result is nearly indistinguishable from the effect of the same change in a pure single component phosphatidylcholine and related glycerolipids. Insertion of a *cis* double bond at the 9,10 position has been shown to strikingly alter the properties and ^{2}H-NMR spectra of deuterated fatty acids attached at the *sn*-2 position of pure phospholipids in membranes. The alterations seen have been analyzed in detail [24,30,31] and are known to arise from the double bond making an angle of 7–8 degrees to the bilayer normal, as well as from local disordering effects. The experiments described here, in which deuterium probes were located within the fluid membrane, clearly demonstrate that the same mechanisms operate in the case of the (unsaturated) GSL.

Earlier workers found that ^{2}H-NMR spectra of oleic acid at the *sn*-2 position of glycerolipids in *Escherichia coli* and *Acholeplasma laidlawii* demonstrate striking similarities to those of comparably deuterated lipid model membranes [30,31]. Such similarities between model and cell membranes might not occur for GSLs since potential for behavioural differences between GSLs and phospholipids has been suggested, based on the carbohydrate headgroup vs. zwitterionic phospholipid headgroup, and on the existence of GSL donor H-bonding groups, [1,2]. However, X-ray crystallographic structures of phosphatidylcholine [35] and GalCer [36] have shown the *sn*-2 fatty acid to be in the same conformation as the single fatty acid of the GSL. Also, as was the case in the present study, basic similarities have been noted previously in spectroscopic characteristics of phospholipids and GSLs in fluid membranes [1,27,28,37]. Hence there is a basis for tentatively extrapolating our results with *N*-(oleoyl-d_{33})GalCer to glycosphingolipids in more complex systems.

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