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Effects of fatty acid alpha-hydroxylation on glycosphingolipid properties in phosphatidylcholine bilayers

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The role of glycosphingolipid fatty acid α-hydroxylation as a modulator of glycolipid organization and dynamics was considered by ^{2}H-NMR in bilayer membranes. For these experiments, galactosylceramides were prepared in which the natural fatty acid mixture was replaced with perdeuterated 18-carbon hydroxylated or non-hydroxylated stearic acid. The L-stereoisomer of *N*-(α-OH-stearoyl-d_{34})galactosylceramide and its naturally-occurring D-α-OH analogue, were isolated for independent study. Bilayers were formed using 10 mol% galactosylceramide in a shorter chain phospholipid, dimyristoylphosphatidylcholine, in an attempt to reproduce several features of glycolipid–phospholipid interactions typical of cell membranes. Spectra of deuterated galactosylceramide in gel phase phospholipid membranes indicated that α-hydroxylation led to greater motional freedom and/or conformational disorder, with no measurable difference between D- and L-α-OH fatty acid derivatives. In fluid phosphatidylcholine bilayers the effects were modest. Glycolipid fatty acid hydroxylation led to broadening of the range of order parameters associated with methylene groups near the membrane surface (frequently referred to as the 'plateau region') – this effect being more marked for the naturally-occurring (D) stereoisomer. The degree of overall molecular order sensed by the glycolipid fatty acid chain in a fluid host matrix was minimally affected by α-hydroxylation; although the plateau region of the D isomer was slightly more ordered than that of the L isomer and the non-hydroxylated species. These results suggest that a significant aspect of the α-hydroxy group effect on glycosphingolipid behaviour in bilayer membranes with low glycolipid content was interference with glycolipid packing amongst host phospholipids in the upper portion of the acyl chains. For the D stereoisomer, there was some evidence that the hydroxy group led to strengthening of interlipid interaction near the membrane surface.

Introduction

Glycosphingolipids (GSLs) are the carbohydrate-bearing lipids of animal cells. Generally they are minor membrane constituents, although restriction to the outer bilayer leaflet raises their effective concentrations. In a few tissues they may comprise up to 30% of total membrane lipid. Hence, this family of sphingolipids is considered to have the potential to contribute to membrane physical properties, in addition to having an established role as recognition sites that may mediate the interaction of the cell with its surroundings [1,2]. Subtle details of GSL physical behaviour and arrangement in membranes have been demonstrated to be involved with both their structural and receptor roles [1–4]. Moreover they have structural features that set them distinctly apart from phospholipids, and which influence GSL physical behaviour and arrangement; these include donor H-bonding groups in the carbohydrate headgroup and sphingosine backbone, a disproportionately long (single) fatty acid, and often α-hydroxylation of the fatty acid. Fatty acid hydroxylation, at C-2, is a very common feature of naturally occurring GSLs. There has been considerable speculation with

Abbreviations: GSL, glycosphingotipid; GalCer, galactosylceramide; DMPC, L-α-dimyristoylphosphatidylcholine.

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regard to the possible influence of this phenomenon on the carbohydrate-bearing lipids of cell membranes. Thus Ki et al. noted that nerve conduction velocity in *Caudata* species having no α-hydroxy fatty acids in their myelin cerebrosides was significantly reduced [5], while Gahmberg and Hakomori have suggested that α-hydroxylation of the fatty acid should be considered for its potential to affect phenotypic expression (i.e. receptor function) of a given GSL via control of 'crypticity' [6,7]. There exists the concept that intermolecular hydrogen bonding amongst glycolipids in membranes may be a very significant force controlling their functions [1,2].

The bases of effects associated with fatty acid hydroxylation are unknown. Bunow and Levin have pointed out that the hydroxylated fraction of pure galactosylceramide (GalCer) from beef brain forms a more disordered gel phase than does the non-hydroxy fraction, and suggest that this may be due to a spatially perturbing effect of the substituent group [8]. Reduction of both the temperature and enthalpy of the gel-to-liquid crystalline phase transition in pure GalCer by the presence of an α-hydroxyl group [9,10] has been related to its causing disruption of the tight acyl chain packing characteristic of a gel phase [9]. Hydroxylation of the fatty acid in GalCer was observed to raise the kinetic energy barrier to reaching a stable bilayer gel state [9]. In a study of pure sulfated GalCer (cerebroside sulfate), it was noted that, in this system too, hydroxylation of the fatty acid inhibited certain organizational changes necessary to give rise to the most stable, ordered phase [11]. However, these authors recorded a higher phase transition temperature and enthalpy for the gel-to-liquid crystalline transition of the α-hydroxylated material than the non-hydroxylated. In this case there was the added influence of repulsive effects from the charged sulfate group; and the authors suggested that the α-hydroxyl function might contribute to a hydrogen-bonding network, overcoming this repulsion. The possibility of intermolecular hydrogen bonding between beef brain GalCer molecules with hydroxy fatty acids in fluid phospholipid bilayers has also been raised [12]. However, Johnston and Chapman found DSC evidence of greater miscibility of α-hydroxy cerebrosides with phospholipids, and considered this to be the result of reduced GSL-GSL attractive forces when dispersed in a phospholipid membrane [13].

In the present article we have addressed by ^{2}H-NMR the effect of fatty acid hydroxylation on GSL molecular arrangement and dynamics when at low concentration in a phospholipid bilayer membrane. Three probe-labelled galactosylceramides were synthesized for this purpose, having perdeuterated 18-carbon fatty acids that were identical but for the presence in one case of a hydroxyl group at the L-α-position and in another a hydroxyl group at the D-α-position. These were assembled into lipid bilayers composed of the (shorter) 14-carbon fatty acid phosphatidylcholine, DMPC, since in general glycolipids have longer fatty acids than do their surrounding membrane phospholipids. The orientational ordering of the GSL stearic acid residue and its corresponding hydroxy analogue were compared in liquid crystalline and gel state bilayers. The presence of the spectroscopic probe on GalCer itself made it possible to focus selectively on phenomena related to the glycolipid even at the low concentrations reflective of natural membranes.

Materials and Methods

1,2-Dimyristoylphosphatidylcholine (DMPC) was obtained from Avanti Polar Lipids, Birmingham, AL; and was used without further purification. Galactosylceramide (GalCer) was isolated from beef brain, using the non-polar residue of a Folch extraction [14], or purchased from Avanti Polar Lipids, Birmingham, AL. The former material was applied to a column of Bio-Rad silicic acid 200–400 mesh, and eluted with a gradient of methanol in chloroform. Isolated GalCer co-chromatographed with known material on Merck silica gel 60 thin-layer chromatography plates eluted with 65:15 $CHCl_3/CH_3OH$ and developed with sulfuric acid/ethanol. Lyso-GalCer (i.e. with fatty acid removed) was produced from the above material by hydrolysis in stirred methanolic KOH at 97°C in a sealed glass culture tube [15] or in refluxing butanolic KOH [16] and was ninhydrin positive.

Preparation of α-acetoxy (perdeuterated) stearic acids was via the corresponding α-bromo intermediates. The α-bromo intermediates were generated by reacting octadecanoic acid (d_{35}) (MSD Isotopes Ltd.) with liquid bromine in the presence of PCl_3, followed by hydrolysis of the acid chloride product [17]. These were in turn converted to the corresponding α-acetoxy esters by refluxing with anhydrous sodium acetate in glacial acetic acid [18]. The mixture of stearic acid α-acetoxy esters was purified on a silicic acid column eluted with $CHCl_3$, and converted to the acid chloride form by refluxing with excess $SOCl_2$ [19]. These reactions were followed by TLC on silicic acid plates eluted with hexane/diethyl ether/formic acid (70:30:1, v/v). The α-acetoxy ester was identified by ^{1}H-NMR in C^2HCl_3. Probe-labelled D- and L-α-OH GalCer were synthesized by coupling the above α-acetoxy derivatives of the deuterated fatty acid chlorides with lyso-GalCer, followed by hydrolysis of the acetate group [20]. D- and L-α-OH stereoisomers of the GSL were separated using a silicic acid column eluted with a $CHCl_3/CH_3OH$ gradient, the L-isomer being significantly faster-running in the solvent system described [20]. Semi-synthetic non-hydroxy and D-α-hydroxy Gal-

Cer behaved similarly, but not identically, on TLC to natural beef brain GalCer non-hydroxy and D-α-hydroxy fractions, respectively: the semi-synthetic species running slightly slower than their corresponding natural mixtures, which included longer fatty acids, as reported previously [21].

Lipid bilayer membranes for these experiments were prepared by dissolving all components at the final desired ratio (10 mol% glycolipid) in 3:1 $CHCl_3$/CH_3OH, and removing the solvent under a N_2 atmosphere. Resultant films were further dried under vacuum (rotary pump) for 3 h at 22°C. Liposomes were generated by hydration of such films with deuterium depleted water (MSD Isotopes Ltd.). Samples were lyophilized three times from a volume of 200 μl of deuterium depleted water after which the hydrated samples were subjected to eight freeze-thaw cycles following the final hydration step. Total lipid used per sample was typically 60–90 mg, and the total volume was 200–300 μl. All samples were incubated 10°C above the transition temperature of the host matrix for 15 min to assure diffusional equilibrium within the bilayer.

^{2}H-NMR spectra were acquired at 30.7 MHz on a 'home-built' spectrometer operated by a Nicolet 1280 computer. The sample was enclosed in a glass dewar and the temperature was electronically regulated to within ±0.5 C°. Spectra were recorded using the quadrupolar echo pulse sequence [22] with full phase cycling [23] and quadrature detection. The π/2 pulse length varied from 2.2 to 2.5 μs with the 5 mm solenoid coil, and 5–6 μs with the 10 mm coil. Pulse spacing was 60 μs, and recycle time was 800 ms. Spectra were not folded about the Larmor frequency. 90° oriented sample ('depaked') spectra were calculated from the powder spectra as described previously [25].

^{31}P-NMR spectra were acquired at 121.5 MHz on a Bruker MSL-300 spectrometer. The spectra were recorded using a Hahn echo pulse sequence [25] with Waltz decoupling (gated on during acquisition). The π/2 pulse length was 4.0 μs (10 mm solenoid coil), the pulse spacing was 60 μs, and the recycle time was 7.5 ms.

Results and Discussion

Deuterium(^{2}H)-NMR is a powerful technique for probing the structure and dynamics of membrane systems [26–36]. Anisotropic molecular motion within the lipid bilayer results in incomplete averaging of the quadrupolar interaction, giving rise to a residual quadrupolar splitting ($\Delta\nu_Q$) in the spectrum. The quadrupolar splitting for 90° orientation of the motional director axis (the bilayer normal) with respect to the magnetic field, is directly related to the carbon-deuterium 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 [29]). The order parameter S_{CD} provides a measure of the time averaged angular fluctuations of the C-^{2}H bond with respect to the motional director axis. In the present study the acyl chains were perdeuterated so that the ^{2}H-NMR spectra are superpositions of spectra associated with each of the GSL fatty acid methylene groups.

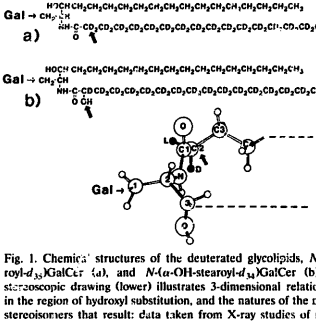

Fig. 1. Chemical structures of the deuterated glycolipids, *N*-(stearoyl-d_{35})GalCer (a), and *N*-(α-OH-stearoyl-d_{34})GalCer (b). The stereoscopic drawing (lower) illustrates 3-dimensional relationships in the region of hydroxyl substitution, and the natures of the D and L stereoisomers that result: data taken from X-ray studies of related crystal structures [31–33]. Arrows indicate the location of C-2 of the single GSL fatty acid. In the stereoscopic drawing carbons 1–3 of the sphingosine backbone are numbered.

Chemical structures for the deuterated forms of the glycosphingolipid studied in this work are indicated in Figs. 1a and b. In each case the single fatty acid was a saturated, 18-carbon chain: the basic structure being that of *N*-(stearoyl-d_{35})GalCer (1a). Modification of this molecule by replacement of a deuterium with a hydroxyl group at the α-carbon (C-2) of the fatty acyl chain leads to the two possible stereoisomers, D- and L-α-OH *N*-(stearoyl-d_{34})GalCer (1b). Suggested conformational stereochemistry of these structures is illustrated at the bottom of Fig. 1, based on X-ray crystallographic evidence for related compounds [31,32]). It should be emphasized though that the conformation displayed has not been proven to exist in bilayer membranes.

^{2}H-NMR spectra of the above three deuterated glycolipids at 10 mol% in bilayers of DMPC are shown in Fig. 2 for the temperatures, 30°C and 50°C. DMPC in pure bilayer form has a main transition of 23°C [34]. To the right of each spectrum is displayed the corresponding calculated 90° oriented sample (depaked)

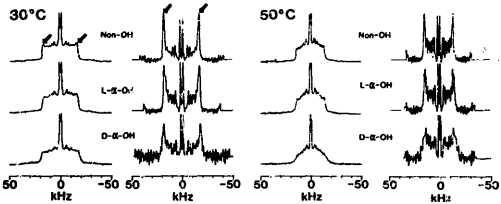

Fig. 2. ^{2}H-NMR spectra of galactosylceramide at 30°C and 50°C in unsonicated bilayers of DMPC. Spectra are shown from top to bottom for *N*-(stearoyl-d_{35})GalCer (the 'non-OH isomer') and the L and D isomers of *N*-(α-OH-stearoyl-d_{34})GalCer, respectively. Corresponding depaked spectra are displayed to the right of each powder spectrum. For the 30°C non-OH spectra arrows indicate spectral features associated with the plateau region referred to in the text. In each case the glycolipid comprised 10 mol% of total membrane lipid.

spectrum [30]. All spectra in Fig. 2 have overall appearances characteristic of deuterated lipids in fluid bilayers, and show no evidence of gel phase material. The observation of spectra attributable to deuterated lipid in a liquid crystalline phase, at temperatures well below the known phase transition temperatures of the glycolipid (70°C and 83°C for α-hydroxy and non-hydroxy GalCer, respectively [9,13]), argues that the GalCer molecules were uniformly dispersed throughout the host matrix. In agreement with this, ^{31}P-NMR spectra of such samples at the same temperature displayed the characteristic axially symmetric liquid crystal lineshape with a $\Delta\delta$ value of approx. 40 ppm [35], and spin-label derived melting curves [34] of the mixtures showed fluidization to be complete at 30°C (data not shown).

The ^{2}H spectral features for the GSL, non-hydroxy GalCer, in DMPC (Fig. 2, uppermost spectra) are very similar to those well known for deuterated phospholipids in the liquid crystal phase [30]. This observation is in accord with the ^{2}H-NMR observations of Skarjune and Oldfield [36] who concluded that hydrocarbon

TABLE I

^{2}H-NMR spectral data corresponding to Fig. 2 for N-(stearoyl-d_{35})galactosylceramide, and D- *and* L-α-*hydroxy N-(stearoyl-d_{34})galactosylceramide isomers, respectively, in fluid bilayers of dimyristoylphosphatidylcholine*

Data are listed for 30°C and 50°C. Glycolipid comprised 10 mol% of membrane lipid. $\Delta\nu_Q$ refers to measured spectral quadrupolar splittings; S_{CD} is the calculated order parameter. 'Carbon number' refers to the location of the spectral probe on the glycolipid fatty acid chain (carboxyl carbon as C-1). Peak assignments are based on peak intensities relative to the integrated total, assuming a gradient of decreasing motional order to C-18. 'Plateau region' refers to unresolved resonances giving rise to the intense outermost spectral peaks. The plateau region covers C-3–C-10 at 30°C and C-3–C-9 at 50°C. The peak width at half-height of this spectral feature is listed as a measure of the spread in values of quadrupolar splitting for methylene groups in the plateau region.

Carbon number	Non-OH		D-α-OH		L-α-OH	
	$\Delta\nu_Q$ (kHz)*	S_{CD}	$\Delta\nu_Q$ (kHz)*	S_{CD}	$\Delta\nu_Q$ (kHz)*	S_{CD}
30°C C-18	2.9	0.022	2.4	0.019	2.6	0.020
C-17	12.5	0.098	11.8	0.093	12.7	0.100
C-16	17.0	0.133	15.8	0.124	17.2	0.135
C-15	21.6	0.171	20.5	0.161	21.8	0.171
Plateau region centre	35.2	0.276	35.6	0.279	34.2	0.268
Plateau region width	2.2		3.1		3.2	
50°C C-18	2.4	0.019	1.6	0.013	1.9	0.015
C-17	8.5	0.066	8.4	0.066	8.7	0.068
C-16	10.8	0.085	10.2	0.080	11.0	0.086
C-15	13.6	0.107	14.0	0.110	14.5	0.114
C-14	15.8	0.124	15.3	0.120	15.1	0.126
C-13	18.6	0.146	19.3	0.151	19.5	0.153
Plateau region centre	28.2	0.221	29.7	0.233	28.0	0.220
Plateau region width	2.0		4.1		2.7	

* Values obtained from depaked spectra. Estimated error ± 0.5 kHz.

chain organization in pure *N*-palmitoylGalCer at 90°C is similar to that in pure DMPC at the same reduced temperature. In particular, the plateau in methylene group orientational order as a function of acyl chain position, which is characteristic for phospholipids, is clearly observed in the powder spectrum of *N*-(stearoyl-d_{35})GalCer as a buildup of overlapping peak intensity at the outer edges. It is also apparent in the accompanying depaked spectra as intense outer peaks. This feature indicates that positions C-3 to approx. C-10 of the GSL non-hydroxy fatty acid chains exhibit very similar orientational order, and hence correspondingly similar quadrupolar splittings. Measured spectral splittings taken from the depaked spectra in Fig. 2 are listed in Table I along with the calculated order parameters. Tentative spectral assignments of clearly resolved spectral peaks were made based on literature information from pure phospholipids and GalCer deuterated in the fatty acid [27,29,30,36–38]: beginning with the sharp central doublet as C-18, and moving sequentially outward assuming a gradient of increasing order to C-17, C-16, C-15 and so on. More extensive assignments were possible at the higher temperature. Integrated intensities were consistent with the assignments shown in Table I. The outermost intense resonances resulting from peak overlap in the plateau region were treated as single broad resonances for the purpose of resonance position and peakwidth calculation. Integration showed the area involved to correspond to some 10 methylene groups at 30°C and 9 methylene groups at 50°C. Based on the quadrupolar splittings observed for the C^2H_2 units comprising the 'plateau region' (35.2 kHz at 30°C for the non-hydroxy GalCer), a value for S_{CD} of 0.28 is calculated, which is slightly higher than that obtained from DMPC [37] or DPPC [38] in the liquid crystalline phase (0.22–0.25). This finding is consistent with our previous observation that GSLs displayed higher order parameters than phospholipid fatty acids in a given phospholipid host matrix [4,29].

A comparison of the spectra in Fig. 2 reveals that the most striking difference associated with spectra of the α-OH derivatives is in the appearance of the 'plateau region'. Whereas the methylene groups that give rise to this spectral region in the case of non-hydroxy GalCer have very similar ordering leading to sharp shoulders in the powder spectra, the corresponding powder spectra of the hydroxylated GalCer derivatives show varying degrees of rounding to their shoulders at both 30°C and 50°C. This is more evident in the depaked spectra: inspection of the depaked spectra in Fig. 2 and the derived parameters in Table I reveals, for the hydroxy lipids, a decrease in intensity in the 'plateau region' peak and increase in its width relative to that obtained for the non-hydroxy analogue. There is little change in the resolved inner splittings. These results indicate that peak overlap for deuterons associated with C-3–C-10 (30°C) and C-3–C-9 (50°C) is less complete – i.e. that these methylene groups are no longer displaying such uniform orientational order as was the case for the non-hydroxy fatty acid. The observation is consistent with a locally disruptive influence of the hydroxyl group on acyl chain packing near the membrane surface.

In spite of the potential for impact on lipid dynamics that one might anticipate from introduction of a hydroxyl group in place of a hydrogen atom on the fatty acid chain, the data in Table I provide little

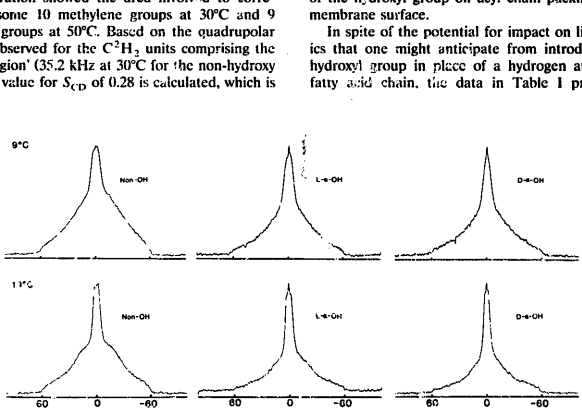

Fig. 3. Gel phase ^{2}H-NMR spectra of galactosyl ceramide at 9°C and 19°C in unsonicated bilayers of DMPC. From left to right: *N*-stearoyl-d_{35}GalCer, *N*-(L-α-OH-stearoyl-d_{34})GalCer, and *N*-(D-α-OH-stearoyl-d_{34})Galcer. In each case the glycolipid comprised 10 mol% of the membrane lipid.

quantitative evidence of major effects. To a first approximation the tabulated values of $\Delta\nu_Q$ and S_{CD} are notably similar for assignable spectral features of α-OH and non-hydroxy GalCer at a given temperature. There is some evidence of decreased $\Delta\nu_Q$ (and thus S_{CD}) values for methylene groups toward the methyl terminus of the naturally occurring D-α-OH isomer at the lower (more physiological) temperature, but the differences found in the table are close to experimental error. For the L-α-OH derivative average values of quadrupolar splittings attributable to the 'plateau region' (i.e. the centre of the plateau region peak) show no obvious systematic change relative to the non-hydroxy form. However, the naturally-occurring D-α-OH stereoisomer of GalCer shows a slightly larger plateau region splitting than does the non-hydroxy or L-α derivative at both 30°C and 50°C (Table I). This feature is visually apparent in both the powder and depaked spectra (Fig. 2).

We now turn our attention to the gel phase spectra of GalCer in DMPC (Fig. 3). These spectra were acquired at 9°C (below the 15°C pretransition of pure DMPC) and 19°C (between the pretransition and the 23°C gel-to-liquid crystalline transition of pure DMPC). The spectra of non-hydroxy GalCer at 9°C are broad, with considerable intensity in the region of ±63 kHz. The latter results are reminiscent of the spectral features observed for dipalmitoylphosphatidylcholine with perdeuterated acyl chains in the gel phase [38]; and indicate that the lipid molecules are still rotating about their long axes, and that there is some chain disorder present. The gel phase spectra of L- and D-α-hydroxy GalCer are identical to one another within experimental error, but they differ from that of the non-hydroxy compound: the shoulders reach baseline at ±60 kHz, suggesting that the glycolipid rotational rate is slower for the non-hydroxy analogue and/or that there is a slight increase in *gauche* population for the α-OH species. As well, there is a sizeable increase of spectral intensity in the center of the spectrum, which may indicate increased chain motion or disordering. Warming the samples to 19°C led to narrowing of the spectra and to increase in the central intensity consistent with increased molecular freedom without transition to a fully fluid phase. There remained no apparent difference between the α-OH stereoisomers in this matrix since the spectra were indistinguishable from one another; and once again the linewidth was substantially narrower than that of *N*-(stearoyl-d_{35})GalCer.

Conclusions

One might anticipate that in phospholipid membranes the presence of a fatty acid OH group could lead to interlipid attraction and hence to increased order within the bilayer. Indeed the hydroxyl function at C-2 has been considered a contributor to increased order seen for membranes containing GSLs (reviewed in Refs. 1 and 2), and as a possible source of GSL-GSL attraction for GalCer in fluid DMPC [12]. Indeed a network of inter- and intramolecular H-bonds involving the carbohydrate portion, ceramide functional groups, and bound water, has been demonstrated to play an important role in determining the physical properties of pure glycolipid bilayers [1,2]. X-ray studies of D-α-OH sphingolipid crystals noted intramolecular H-bonding to the fatty acid hydroxyl function, which has been suggested to have the potential for controlling GSL conformation [31–33,44]. Another consideration is the possible steric effect of the hydroxyl group.

At the 10 mol% GSL concentrations utilized in our experiments, it was expected that GalCer would be dispersed in a liquid crystalline (DMPC) matrix at 30°C and above, although perhaps somewhat phase separated in the gel phase [40–42]. Our observation of only liquid crystal spectra for the deuterated forms of GalCer at 30°C and 50°C is consistent with such an expectation, and echoes the earlier similar finding of Skarjune and Oldfield for deuterated glucosylceramide at 17% in DMPC [40]. Thus our spectral findings at 30°C and above relate to the behaviour of GSLs at low concentration in fluid phospholipid host matrices. The situation with regard to lipid lateral arrangement below 23°C is less clear, although these temperatures provided systems with gel phase character. In the fluid membranes it would appear that insertion of a hydroxyl group at the α-position of the glycolipid fatty acid led to some dispersion of the normally highly uniform methylene group order near the GSL headgroup (i.e. breakdown of the 'plateau' of uniform order). The plateau region of the natural (D) isomer displayed a generally slightly higher degree of order than did either the L isomer or the non-hydroxy form. Apart from this, the dynamic and orientational behaviour of the hydroxy and non-hydroxy GalCer species, examined in a fluid phase phospholipid host matrix, were very similar. In the gel phase the result of hydroxylation was an increase in motion and/or decrease in order.

A reasonable partial explanation of the above observations would seem to be that GSL fatty acyl chains in which a hydrogen has been replaced by the bulkier and more polar OH group have greater local area requirements in the membrane. The acyl group plateau region of the α-hydroxy GalCer appears not to pack as uniformly into the lipid matrix as does its non-hydroxy analogue. This is consistent with previous studies of pure GalCer which found that the presence of an α-OH group in the fatty acid lowered the temperature and enthalpy of the phase transition, and reduced the order in the gel phase [8–10, 43]. We cannot exclude the possibility that the narrower gel phase spectra, and the wider plateau regions in the liquid crystal spectra,

seen for the α-OH derivatives in this work, were mediated by conformational differences amongst the three GalCer species, arising from intramolecular H-bonding suggested in x-ray studies [31–33,44] rather than from a direct steric effect of the OH group. Interlipid attractive forces involving the α-OH group appeared not to play a very large role in the systems studied.

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