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# Glycosphingolipid interdigitation in phospholipid bilayers examined by deuterium NMR and EPR

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**Glycosphingolipid fatty acids commonly have up to eight methylene carbons more than do their surrounding phospholipid-attached counterparts. The resultant 'extra' segment may very well modulate glycosphingolipid function as receptor and structural element. As part of an investigation of this phenomenon, galactosylceramide was prepared with a deuterated 18-carbon fatty acid chain. Deuterium-labelled galactosylceramide was assembled at 10 mol% into unsonicated phosphatidylcholine bilayers having all 14-carbon or all 18-carbon saturated fatty acid chains (DMPC and DSPC, respectively). The systems were studied by $^2$H-NMR spectroscopy above and below the phase transition temperatures, $T_m$, of the host matrices. At comparable reduced temperatures in fluid membranes the degree of motional order exhibited by the glycolipid fatty acid was significantly higher in the phospholipid host matrix that was four carbons shorter. The fatty acid chain segment least affected by the change from long to short chain host matrix was the terminal (deutero)methyl group (an increase of 8% in quadrupolar splitting for the terminal methyl vs. 16% for deuterons at $C_{17}$ and 23-28% for the remainder of the chain). Order parameter profiles for galactosylceramide were qualitatively very similar in the two host membranes, arguing against any major conformational difference between the arrangement of the 18-carbon glycolipid fatty acid in the 18-carbon vs. 14-carbon host matrices. Similarly a nitroxide spin probe covalently attached to carbon-12 of the galactosylceramide fatty acid gave clear indication of greater order in the fluid 14-carbon fatty acid phospholipid bilayer. These results are consistent with 'tethering' of the extra length of fatty acid via interdigitation into the opposing monolayer. There was no spectroscopic evidence of any intrinsic difference in glycolipid behaviour in the two fluid host matrices. $^2$H-NMR spectra of galactosylceramide at comparable reduced temperatures below $T_m$ of the phospholipid bilayer were very different for 14-carbon vs. 18-carbon host matrices. The glycolipid fatty acid showed evidence of relatively reduced mobility in the shorter chain matrix.**

## Introduction

The hydrophobic midplane of the cell (bilayer) membrane is frequently presented as a well-defined region at which two planar surfaces comprised of acyl chain methyl terminii contact one another. However, it is well known that there is considerable disparity in acyl chain length of membrane lipids: lengths as low as 14 carbons and as high as 24 carbons being widely observed. The concept of 'interdigitation' has been put forward to describe the phenomenon whereby a chain longer than half the membrane thickness might cross the midplane to protrude amongst acyl chains of the opposing monolayer [1–5]. This concept has been addressed most systematically for bilayer model membranes comprised of single pure phospholipids with one long and one short fatty acid chain (i.e., phosphatidylcholines exhibiting intramolecular fatty acid chain length disparity). Pure sphingomyelins with fatty acids of selected length [6,7] and pure galactosyl glycosphingolipids [8,9] have also been studied. In these situations the longer chains are seen as matching up stoichiometrically with the shorter chains of the opposing monolayer when chain length differences are not extreme.

Our interest has been to extend this concept to glycosphingolipids in cell membranes. The glycosphingolipid molecule possesses one fatty acid, typically

Abbreviations: GalCer, galactosylceramide; DMPC, L-α-dimyristoylphosphatidylcholine; DSPC, L-α-distearoylphosphatidylcholine; $T_m$, phase transition temperature.

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18–24 carbons in length. The sphingosine portion, which contributes the equivalent of a second acyl chain, extends to a membrane depth of only 13 to 15 carbons. Hence glycosphingolipids may be expected to behave as mixed-chain lipids. However, glycosphingolipids are a small fraction of the total lipids in most eucaryote membranes, and are restricted to the outer surface. Furthermore, since the single fatty acid is commonly up to 24 carbons long, acyl chain length mismatch within a given glycolipid is greater than that in surrounding phospholipids, most of which will have 16- or 18-carbon fatty acids. Thus the situation for glycolipids in cell membranes is different from that of the pure single-component systems so far studied [10]. We have proposed previously that glycosphingolipid long chain fatty acids exhibit a type of interdigitation in phospholipid membranes. The proposal was based on the results of our experiments with GalCer, LacCer, globoside and $GM_1$ bearing a long chain (24-carbon) spin-labelled fatty acid [11,12]. In these earlier experiments the nitroxide radical was at C-16 of the glycolipid fatty acid, and thus could be used to measure its motional anisotropy (order) in the region of the host phospholipid terminal methyl groups. The order at C-16 was much greater for a glycolipid 24-carbon fatty acid than for its 18-carbon analogue, suggesting that interdigitation of the longer chain results in 'tethering' of the upper portion. Alternative acyl chain arrangements were less consistent with spectral observations.

However, while nitroxide spin label ESR spectroscopy is ideally suited to monitoring membrane structures [13,14], a significant concern is that the spin label itself may present enough of a spatial perturbation to leave the final conclusion in doubt. In this article we describe an extension of our previous experiments to the non-perturbing deuterium probe. A limitation to this approach is the inherently low sensitivity of $^2$H-NMR, which made it necessary to use larger quantities of labelled glycolipid than in ESR studies.

## Materials and Methods

L-α-Distearoyl and L-α-dimyristoylphosphatidylcholines were from Avanti Polar Lipids, Birmingham AL. Octadecanoic-$d_{35}$ acid (98.8 atom% D) and deuterium-depleted water were from MSD Isotopes, Montreal Canada. Galactosylceramide was isolated from the non-polar residue after a Folch extraction [15] of lyophilized beef brain grey matter. The material was subjected to column chromatography on silicic acid (Bio-Sil A 200–400 mesh) using a gradient of $CH_3OH$ in $CHCl_3$. Stearic acid with nitroxide spin label at carbon 12 of the fatty acid chain was prepared following the general method of Hubbell and McConnell [16]. Stearic acid selectively dideuterated at carbon 17 was a generous gift from A.P. Tulloch. Lyso GalCer (GalCer with natural fatty acid removed) was produced from isolated GalCer by hydrolysis in stirred methanolic KOH at 97° C in a sealed glass culture tube [17] or by hydrolysis in refluxing butanolic KOH [18] and was ninhydrin positive. Probe-labelled GalCer was synthesized by coupling of spin-labelled or deuterated fatty acids to lyso GalCer as described previously [19]. Reactions were followed by thin-layer chromatography on Merck Silica gel 60 plates eluted with 65 : 25 : 4 $CHCl_3/CH_3OH/H_2O$ and developed with ninhydrin or sulfuric acid/ethanol spray. Probe-labelled galactosylceramides did not stain with ninhydrin and co-migrated with natural GalCer. Probe-labelled derivatives were stable to overnight treatment with aqueous KOH at 5 mg/ml, conditions which cleave ester bonds readily.

Lipid bilayer membranes for these experiments were prepared by dissolving all components at the final desired ratio (10 mol% glycolipid) in 1 : 1 $CHCl_3/CH_3OH$, and removing the solvent under a $N_2$ atmosphere. Resultant films were further dried by pumping in vacuum (rotary pump) for 2 h at 22° C. Liposomes were generated by hydration of such films with deuterium-depleted water. Samples were lyophilized three times from 100 μl of deuterium-depleted water, after which the hydrated samples were subjected to eight freeze-thaw cycles prior to the final hydration step. Each sample comprised 30–45 mg total lipid in a volume of 150–200 μl. All samples were incubated 10 C° above their transition temperatures for 15 min to assure diffusional equilibrium within the bilayer before being allowed to cool to the temperature of study.

ESR spectra were run on a Bruker ER 200D-SRC spectrometer equipped with a $TM_{110}$ cavity and variable temperature accessory. For this purpose vesicle suspensions were held in 50 μL Dade®disposable glass micropipettes sealed at one end.

$^2$H-NMR spectra were acquired at 30.7 MHz on a 'home-built' spectrometer operated by a Nicolet 1280 computer. Spectra were recorded using the quadrupolar echo pulse sequence [20] with full phase cycling [21] and quadrature detection. The $\pi/2$ pulse length was 2.2 μs (5 mm solenoid coil) the pulse spacing was 60 μs, and the recycle time was 800 ms. Spectra were not folded about the Larmor frequency.

## Results and Discussion

$^2$H-NMR has proven to be one of the most effective spectroscopic techniques available for probing the highly anisotropic membrane structure [22–26]. Constraints imposed on molecular fluctuations by the liquid crystal membrane matrix lead to anisotropic motion of the lipid molecules, which is reflected in incomplete averaging of deuterium quadrupolar interactions [25]. For glycolipids in membranes (as for phospholipids) motion

occurs about the bilayer normal, which projects the average quadrupolar interaction along this axis. For membranes in which the bilayer normal is 90° with respect to the magnetic field direction the residual quadrupolar splitting is given by

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

where $e^2qQ/h$ is the quadrupolar coupling constant (170 kHz [25]) and $S_{CD}$ is the C-$^2$H bond order parameter [25]. For acyl chains, $S_{CD}$ measures the time average of the angular fluctuations of the C-$^2$H bond with respect to the director axis resulting from molecular motion and acyl chain conformational isomerization. In the case of paramagnetic spin labels anisotropic motion is reflected in a corresponding order parameter for the nitroxide radical [16,27–30].

The experimental system utilized in the present study is represented in Fig. 1. GalCer, containing either spin-labelled or $^2$H-labelled stearic acid, was examined at a concentration of 10 mol% in bilayers of DSPC (Fig. 1A) or DMPC (Fig. 1B). In the case of DSPC the glycolipid had the same fatty acid chain length as its surrounding host matrix, while in DMPC the glycolipid carried an 'extra' four carbons relative to its host matrix. In principle GalCer with perdeuterated fatty acid has the potential to probe its membrane arrangement at all depths. In practice most of the spectral features cannot be assigned unambiguously to individual methylene residues due to peak overlap. Nevertheless, it is well known that in fluid membranes $^2$H peaks can typically be resolved for the methyl terminal carbon and for several of the methylene groups immediately proximal to it. In addition, methylene units located in the 'plateau' region (C-2 to C-8) may be assigned as a unit. In the case of the spin labelled glycolipid derivative the spectral probe

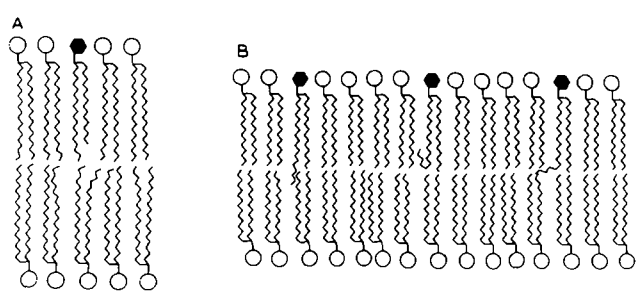


Fig. 1. Possible arrangements of galactosylceramide in fluid phosphatidylcholine bilayer membranes having the same chain length (DSPC) (A) or four carbons shorter chain length (DMPC) (B). GalCer is distinguished from phospholipids by a shaded hexagonal headgroup: in each case the single glycolipid fatty acid is stearic acid (18 carbons). The three major possibilities shown for GalCer fatty acid organization in the 14-carbon fatty acid DMPC host matrix are (1B from left to right): extended so as to interdigitate with phospholipid fatty acids of the opposing monolayer, collapsed via *trans* → *gauche* isomerization into a focus of disorder, and bent at 90° into the plane defined by host matrix methyl terminii.

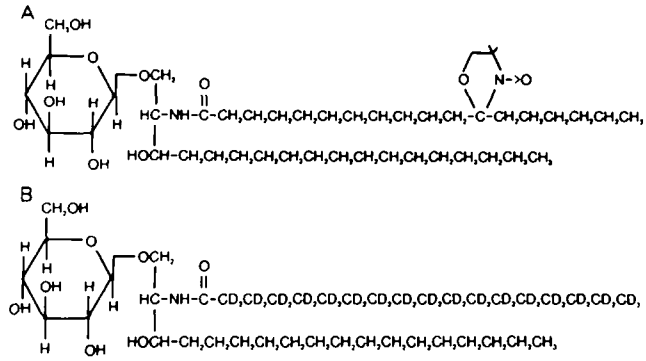


Fig. 2. Chemical structures of the major probe-labelled glycolipids used in this work: (12-nitroxystearoyl)GalCer (A) and (stearoyl-$d_{35}$)GalCer (B).

was attached to C-12 of the glycolipid fatty acid so that it monitored the motional alignment ('order') of a region six carbons from the methyl terminus of the fatty acid (Fig. 2). It was hoped that this choice would minimize steric interference of the nitroxide ring system with the putative interdigitating fatty acid segment. In the ESR experiments, probe-labelled GalCer comprised only one-fifth of the total glycolipid in the sample to avoid excessive spin exchange broadening of spectral features [31].

In examining Fig. 1A (glycolipid acyl chain length identical to that of the phospholipid host matrix, DSPC), one might anticipate that GalCer would behave much like surrounding phospholipids with regard to chain organization. However, as illustrated diagramatically in Fig. 1B, conceptually at least, the terminal carbons of GalCer in the short chain host matrix, DMPC, have various options. Important conformational possibilities include interdigitation, collapse via *trans* → *gauche* isomerization into a focus of disorder, and bending into the plane of the bilayer at 90° to the rest of the chains. Another option is that the disproportionately long glycolipid fatty acid might result in a tendency for the entire molecule to sit slightly higher in the membrane, leading to greater headgroup protrusion at the surface. Of course this could also occur in association with any of the other major options illustrated. While we have in past considered protrusion from the bilayer surface to be a less likely explanation for certain aspects of glycosphingolipid fluidity gradient profiles [32], it has been proposed by Alving [33,34] and more recently by Esmann et al. [35] and by Crook et al. [36] as a modifier of glycolipid receptor function.

A separate consideration in experimental design was the difference in phase transition temperatures between short and long chain host matrices: 23°C for DMPC and 54–55°C for DSPC [37–39]. To allow for this and to assure similar host matrix fluidities, spectra were compared for samples at comparable reduced temperatures [40]. We have considered our experimental results

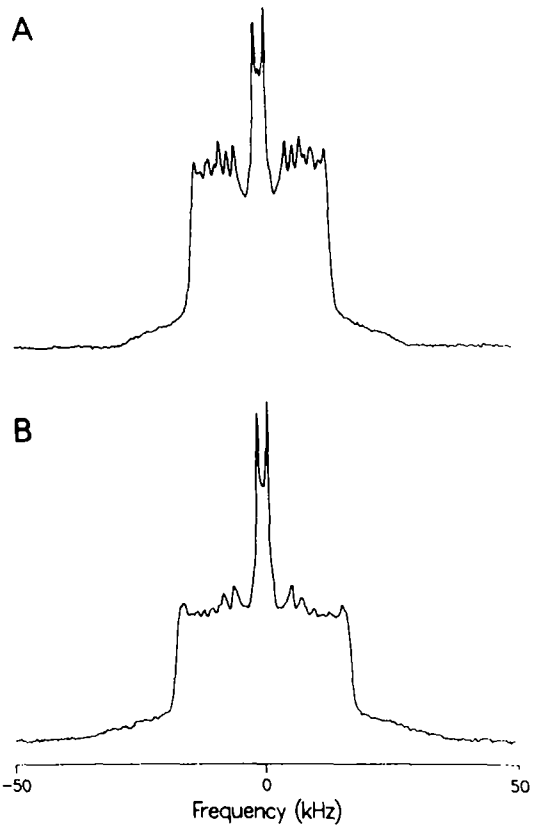


Fig. 3. $^2$H-NMR spectra at comparable reduced temperatures for (stearoyl-$d_{35}$)GalCer in fluid phospholipid bilayers of the same acyl chain length (A) and four carbons shorter acyl chain length (B). Glycolipid comprised 10 mol% of total lipid in fully hydrated multilamellar vesicles of distearoylphosphatidylcholine at 65°C (A) and dimyristoylphosphatidylcholine at 30° C (B).

in light of the various molecular possibilities described above. Spectra are illustrated in Fig. 3 for the 18-carbon perdeuterated GalCer derivative at comparable reduced temperatures in the host matrix, DSPC (same chain length), and DMPC (shorter chain length). Fig. 3A shows the spectrum in DSPC at 65° C. It demonstrates the well known increase in methylene group motional disorder with increasing membrane depth. Comparison with known systems permits assignment of the resolved innermost intense spectral doublet as arising from the methyl terminus C-18 [23,25,26]. Moving outward from this point, its neighbouring spectral features are associated with (methylene) deuterium nuclei at C-17, C-16 and C-15, respectively. Measured splittings and calculated order parameters are listed in Table I. An estimate of $\Delta\nu_Q$ for methylene groups in the plateau region was obtained from the outermost spectral peaks, and these values are also listed. Spectra for deuterated GalCer with 18-carbon fatty acid in the 14-carbon fatty acid DMPC host matrix at 30° C are shown in Fig. 2B for comparison. Assignment of the C-17 to C-15 peaks was less trivial in this case since the expectation that $\Delta\nu_Q$ should increase monotonically from methyl terminus to

TABLE I

*$^2$H-NMR order parameter data corresponding to Fig. 3 for (stearoyl-$d_{35}$)GalCer in fluid phospholipid bilayers of the same fatty acid chain length (DSPC) and shorter fatty acid chain length (DMPC)*

Glycolipid concentration was 10 mol% of membrane lipid. $\Delta\nu_Q$ refers to the measured spectral quadrupolar splitting. $S_{CD}$ is the calculated order parameter. 'Carbon number' refers to location of spectral probe on the glycolipid fatty acid chain (carboxyl carbon as 1); the plateau region covers C-2 to C-10. Samples were run at comparable reduced temperatures: 65° C for the DSPC host matrix vs. 30° C for DMPC. A sample specifically dideuterated at C-17 was used to verify peak assignment. Errors in measurement of $\Delta\nu_Q$ were on the order of ±0.5 kHz, giving rise to errors in the calculated $S_{CD}$ for methylene positions ranging from 1.4 to 4%.

| Carbon number | GalCer/DMPC (30° C) | | GalCer/DSPC (65° C) | |
|---|---|---|---|---|
| | $\Delta\nu_Q$ (kHz) | $S_{CD}$ | $\Delta\nu_Q$ (kHz) | $S_{CD}$ |
| C-18 | 2.8 | 0.022 | 2.6 | 0.020 |
| C-17 | 12.3 (11.7 [a]) | 0.096 (0.092 [a]) | 10.7 | 0.08 |
| C-16 | 16.7 | 0.13 | 13.6 | 0.11 |
| C-15 | 21.3 | 0.17 | 16.6 | 0.13 |
| Plateau region | 34.8 | 0.27 | 27.3 | 0.21 |

[a] Values found for GalCer specifically dideuterated at C-17.

polar headgroup is based upon comparison with systems of homogeneous fatty acid chain length that would not permit interdigitation or its alternative possibilities. The assumption that peak assignment (i.e., relative peak position) remains the same in the shorter chain matrix seems reasonable based on the spectral similarities apparent for GalCer in the DSPC (Fig. 2A) and DMPC (Fig. 2B). However, it was verified by running the $^2$H-NMR spectrum of GalCer with 18-carbon saturated fatty acid chain specifically dideuterated at C-17 (see Table I). Measured values of $\Delta\nu_Q$ for GalCer in the DMPC matrix and calculated order parameters are also recorded in Table I. At comparable reduced temperatures each assignable carbon of GalCer in the short chain host matrix showed a significantly larger quadrupole splitting (i.e., a higher order parameter) than did its corresponding carbon on the same glycolipid in DSPC. This effect was most marked at methylene groups not in close proximity to the terminal methyl: thus $\Delta\nu_Q$ for the terminal (deutero)methyl group was 8% higher in the short vs. long chain matrix, 16% higher for the C-17 deutrons, 23% for the C-16 deutrons, and 28% for C-15 and the plateau region. The basic result is reminiscent of previously published results obtained by ESR spectroscopy using glycolipids with spin label at C-16 of the fatty acid chain [11,12]. In earlier spin-label experiments glycolipid fatty acids that were longer than those of their host phospholipid bilayer matrix were found to exhibit higher order at C-16 than did glycolipid fatty acids having similar length to those of their host membrane. This was felt to be consistent with interdigitation of the extra long fatty acid [11,12]. Simi-

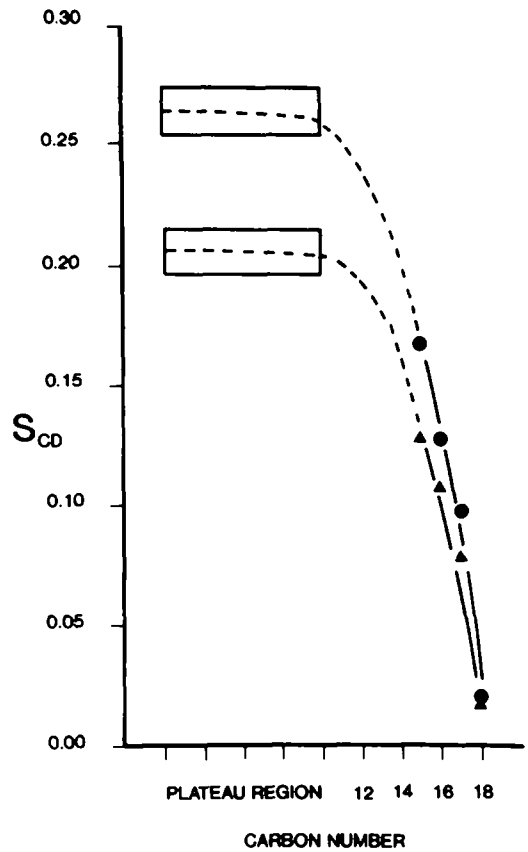


Fig. 4. Order parameter profiles derived from the $^2$H-NMR spectra shown in Fig. 3 for (stearoyl-$d_{35}$)GalCer in a fluid phospholipid matrix of the same chain length (18-carbon DSPC) (▲); and shorter chain length (14-carbon DMPC) (●). Samples were compared at similar reduced temperatures (65°C for DSPC and 30°C for DMPC). Error bars, where absent, are smaller than the symbols. Error boxes indicated for the plateau region approximate the actual spread of $\Delta\nu_Q$ values of C-2 to C-10.

larly the present results could be explained readily by assuming that the 18-carbon GalCer fatty acid remains aligned with the 14-carbon fatty acids of fluid phase DMPC and is thus 'tethered' by interdigitation of its methyl terminus.

Order parameter profiles estimated from the $^2$H-NMR data (Fig. 4) are qualitatively very similar for the two lipid systems: there is no break or deviation such as might be expected if the 18-carbon glycolipid acyl chain assumed a very different conformation in the region of its contact with the midpoint of the 14-carbon phospholipid acyl chain host matrix. Interestingly, order parameters for methylene groups near the polar headgroup (ones represented in the spectral plateau region and plotted on the upper portions of the curves in Fig. 4) are particularly affected in the short chain matrix. Even when warmed 42 C° above host matrix $T_m$ (i.e., at 65°C) $S_{CD}$ for the plateau region of GalCer in DMPC was 0.20 (spectrum not shown) which is similar to the value of 0.21 seen for the same glycolipid only 10 C° above host matrix $T_m$ in DSPC at 65°C. A possible effect of interdigitation would be to reduce fluctuations of the lipid molecule as a whole, leading to a uniform increase in the entire $S_{CD}$ profile. Inspection of Fig. 4 and Table I, however, reveals that, as already noted, the effect was least dramatic at the (deutero)methyl terminus. This might be rationalized as follows. Although interdigitation 'tethers' the whole chain, thus reducing molecular fluctuation, it also leads to some degree of local disruption (disordering of chain packing at the point of interdigitation).

Although cell membranes are generally considered to be of intermediate fluidity, we have examined the same system in the gel state. The gel phase spectra of [$^2H_{35}$]GalCer in DMPC and DSPC proved to be significantly different from one another at comparable reduced temperatures (Fig. 5). Unfortunately, interpretation of these spectra is complicated because line shape is particularly sensitive to both ordering and dynamic effects in the gel phase; and dynamics may differ at similar reduced temperatures. Nevertheless several observations may be made. The spectra differ in two regards: (i) in the intensity at ±63 kHz, and (ii) in the central region where methyl group contributions dominate. The $^2$H-NMR spectrum of GalCer in DSPC at 45°C (Fig. 5C) bears a striking resemblance to that of [$^2H_{62}$]DPPC at 20°C [41]. It is very broad, indicating a distribution of quadrupolar splittings, and suggesting

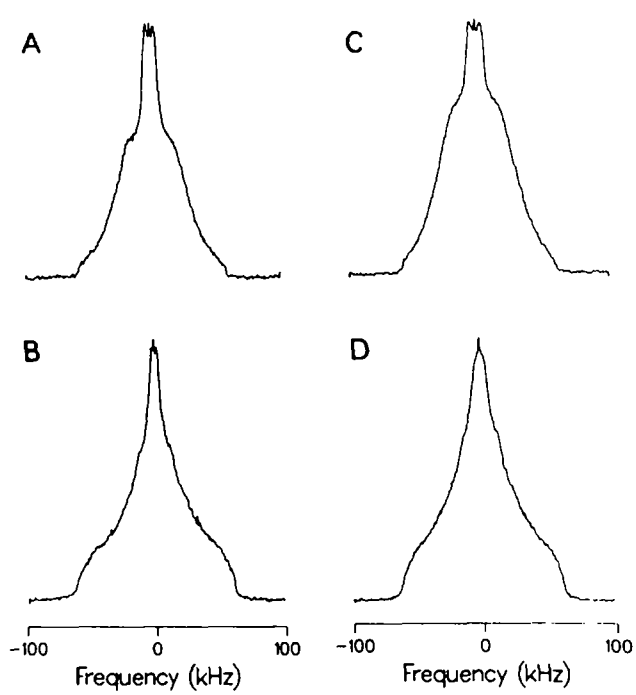


Fig. 5. $^2$H-NMR spectra at comparable reduced temperatures for (stearoyl-$d_{35}$)GalCer in gel phase phospholipid bilayers of the same acyl chain length (A,C) and four carbons shorter acyl chain length (B,D). Glycolipid comprised 10 mol% of total lipid in fully hydrated multilamellar vesicles of distearoylphosphatidylcholine at 52°C and 45°C (A and C, respectively), and dimyristoylphosphatidylcholine at 19° and 8.5°C (B and D, respectively).

that most of the glycolipid molecules are still rotating rapidly about their long axes on the NMR timescale [41]. For [$^2H_{35}$]GalCer in the shorter chain host matrix, DMPC, there is an increase in intensity at $\pm 63$ kHz (Fig. 5B,D). Since 126 kHz is the maximum possible splitting for a deuterated methylene group, an increase in intensity at $\pm 63$ kHz suggests a decrease in rotation rate about the GalCer long axis. Thus for the DMPC matrix in the gel phase, glycolipid molecular motion was reduced relative to that in DSPC, perhaps reflecting a consequence of interdigitation. Note, however, that the slight reduction in spectral splitting in the central region in the rigid DMPC matrix suggests that the terminal methyl group of GalCer is more disordered than it is in the 18-carbon fatty acid DSPC host matrix. Thus the gel phase results, although considerably more difficult to interpret, are also consistent with a model in which interdigitation may occur, leading to disruption of chain packing near the acyl chain terminus.

In previous spin label studies [11,12] the host phospholipid chain length was kept constant while the acyl chain length of the glycolipids was varied (either C-18 or C-24). In the present study the opposite approach was taken: ESR spectra of GalCer having 18-carbon spin-labelled fatty acid were compared in DMPC vs. DSPC (Fig. 6). The order parameter, $S$, for the carbon to which the spin-label ring is attached (C-12 in this case) was calculated from the inner and outer peak separations as described previously [27–30]. Table II lists measured spectral values and derived spin label order parameters at various temperatures. It should be borne in mind however that at temperatures below $T_m$ of the host matrix it is not clear that the order parameter is meaningful since motional rates are severely restricted.

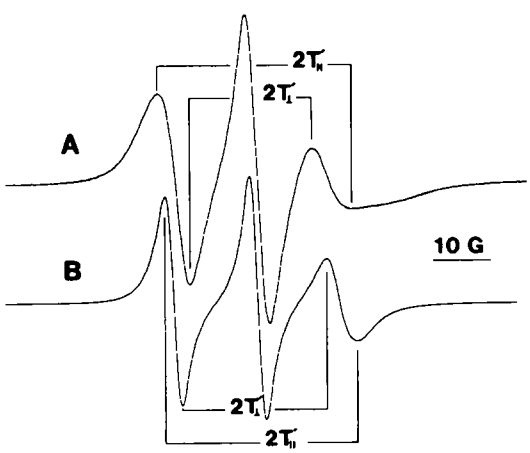


Fig. 6. EPR spectra of spin-labelled GalCer in fluid phospholipid membranes similar to those in Fig. 3. (12-Nitroxystearoyl)GalCer comprised 2 mol% of the membrane lipid while unlabelled (stearoyl)GalCer comprised 8 mol%, with phospholipid making up the remainder. (A) Distearoylphosphatidylcholine host matrix (same chain length) at 65°C, (B) dimyristoylphosphatidylcholine (four carbon shorter chain length) at 30°C. Spectral features used to calculate order parameters are illustrated.

TABLE II

*Spin label ESR order parameter data for (12-nitroxystearoyl)GalCer in phospholipid bilayers of the same fatty acid chain length (DSPC) and shorter chain length (DMPC)*

Glycolipid concentration was 10 mol% (2 mol% spin labelled) of the membrane lipid. 'Host matrix' refers to the phosphatidylcholine component. $T'_{\parallel}$ is half the measured outer spectral splitting (as illustrated in Fig. 6) and accurately reflects the matrix element, $T_{\parallel}$. $T'_{\perp}$ is half the measured inner spectral splitting, and $T_{\perp}$ is a corrected value that more accurately approximates the true matrix element [27–29]. The error involved in estimating the centres of outer and inner spectral peaks ranged from 0.1 gauss to 0.3 gauss, giving rise to a standard error of some $\pm 0.02$–$\pm 0.04$ in calculated order parameter.

| Host matrix | Temp. (°C) | $T'_{\parallel}$ ($\cong T_{\parallel}$) (gauss) | $T'_{\perp}$ (gauss) | $T_{\perp}$ (gauss) | $S$ ($\pm 0.02$–$0.04$) |
|---|---|---|---|---|---|
| DMPC | 9 | 29.1 | 8.9 | 9.2 | 0.68 |
| DMPC | 19 | 27.4 | 8.8 | 9.3 | 0.64 |
| DMPC | 30 | 16.5 | 10.5 | 11.6 | 0.20 |
| DMPC | 65 | 16.2 | 12.5 | 13.7 | 0.10 |
| DSPC | 45 | 18.0 | 9.7 | 10.7 | 0.30 |
| DSPC | 52 | 17.3 | 11.0 | 12.0 | 0.21 |
| DSPC | 65 | 16.4 | 12.2 | 13.4 | 0.11 |

ESR- and NMR-derived order parameters cannot be quantitatively compared since the techniques are sensitive to very different motional timescales [14]. The overall observation via ESR is, however, the same as that via NMR: at equivalent reduced temperatures order at a given glycolipid fatty acid carbon is markedly higher in a situation that would permit interdigitation. Thus the value of $S$ for C-12 in DMPC at 30°C was 0.20 while that for C-12 in DSPC at a comparable reduced temperature (65°C) was 0.11. Note that even at 65°C the value of $S$ in DMPC was 0.10 which is very close to the DSPC value at the same temperature, and that in the gel phase the value calculated for $S$ (although not a true motional reflection in these cases) was consistent with much greater immobility in the short chain matrix. We did not investigate the possibility that the gel phase systems may well be expected to exhibit metastability with regard to interdigitation [4,7,9].

## Conclusions

The present study demonstrates that it is possible to follow deuterated glycosphingolipids by $^2$H-NMR at concentrations in the membrane that mimic those found naturally. Of the various possible models for organization of the neutral glycosphingolipid, GalCer, within a fluid membrane, the $^2$H-NMR results are clearly consistent with partial acyl chain interdigitation when the glycolipid fatty acid chain is longer than those of its surrounding phospholipids. In such systems acyl chain motions are more restricted than when phospholipid and glycolipid chain lengths are the same. This effect appeared to be transmitted all the way up to the carbo-

xyl portion of the chain, suggesting that interdigitation 'tethers' the chain. The $^2$H-NMR results are consistent with spin label results on the same system, and with previous conclusions drawn from studies of four glycolipids having 18-carbon vs. 24-carbon spin-labelled fatty acids [11,12]. The present results suggest that interdigitation of an 'extra' segment of glycolipid fatty acid may lead to some disruption of local acyl chain packing in the opposing monolayer. Although our data have been interpreted to favour interdigitation, they do not rule out the possibility of coexistent protrusion of a long-chain glycolipid from the membrane surface. On the other hand, if the glycolipid is indeed tethered by interdigitation, an alternative explanation of the improved receptor function sometimes seen for long chain simple glycosphingolipids [33,34,36,42] may be advanced: that the effect may result from altered headgroup dynamics and orientation.

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