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Evidence that trans-bilayer interdigitation of glycosphingolipid long chain fatty acids may be a general phenomenon

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'Interdigitation' is a term coined to describe the phenomenon whereby pure phosphatidylcholines with intramolecular fatty acid chain length heterogeneity when hydrated to form bilayers may insert the methyl ends of long fatty acids from one side across more than half of the membrane thickness to protrude amongst the acyl chains of the opposite side of the bilayer (Keough, K.M.W. and Davis, P.J. (1979) Biochemistry 18, 1453–1459; Huang, C. and Mason, J.T. (1986) Biochim. Biophys. Acta 864, 423–470). In this article we address the fate of long fatty acid chains of glycosphingolipids present as minor components in membranes of non-interdigitating phosphatidylcholines. In this pursuit, derivatives of galactosyl ceramide, lactosyl ceramide, globoside and G_{M1} were synthesized having either 18-carbon or 24-carbon fatty acid with a spin label covalently attached at C-16. Labelled glycolipids were incorporated at 1–2 mol% into bilayers of synthetic phosphatidylcholines, their mixtures with cholesterol, or natural egg phosphatidylcholine. In each case the C-16 carbon of the glycolipid long chain fatty acid showed considerably greater 'order' and immobility than did C-16 of the fatty acid which was similar in length to the host matrix phospholipids. We interpret this as strong evidence that the long chain fatty acid interdigitates across the mid point of the bilayer in the systems studied. Clearly this phenomenon did not require that the phospholipid host matrix have mixed chain lengths. Furthermore it was totally independent of glycolipid family: for a given host matrix and (glycolipid) fatty acid chain length the order parameter values found were the same amongst all four glycolipid families tested.

Introduction

The concept of the phospholipid bilayer is of two monolayers whose hydrophobic surfaces are in apposition. Thus one tends to envisage a planar region of contact between two flat surfaces composed of fatty acid terminal methyl groups. It has been pointed out however that fatty acids within natural membranes vary widely in chain length so that some may protrude beyond the above-mentioned planar array of methyl termini, while others will fail to reach it. Hence methyl termini of long fatty acids may cross the mid plane of the membrane hydrophobic interior to interdigitate with fatty acids of the companion monolayer. This concept has been strengthened by studies of bilayers comprised of synthetic mixed-chain phos-

Abbreviations: G_{M1}, Galβ1 → 3GalNAcβ1 → 4Gal(3 ← 2αNeuAc)β1 → 4Glcβ1 → 1Cer; globoside, GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer; egg PC, egg phosphatidylcholine; DMPC, L-α-dimyristoylphosphatidylcholine; DPPC, L-α-dipalmitoylphosphatidylcholine; EPR, electron paramagnetic resonance.

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pholipids (phospholipids with one long and one shorter fatty acid) [1–4] and sphingomyelins with fatty acids of selected lengths [5,6].

The glycosphingolipid molecule possesses one fatty acid, typically 18–24 carbons in length. The sphingosine portion, which contributes the second acyl chain, extends to a membrane depth of only 14 or 15 carbons. Hence glycosphingolipids may be expected to manifest some characteristics of mixed-chain phospholipids and sphingolipids. However, they are a small fraction of the total lipids in most eucaryote membranes, and are restricted to the outer surface. Furthermore, since the sphingosine acyl chain penetrates the membrane to a depth of 14 or 15 carbons, while the fatty acid may penetrate to a depth of 16–24 carbons, acyl chain length discrepancy within a given glycolipid will be greater than that typically seen in surrounding phospholipids, most of which will have 16- or 18-carbon fatty acids. Thus the situation for glycolipids in cell membranes is not entirely analogous to that of pure mixed-chain phospholipids or sphingolipids in bilayer form. We recently proposed that glycosphingolipid long chain fatty acids exhibit a type of interdigitation in phospholipid membranes, based on the results of our experiments with a new long chain (24-carbon) spin labelled fatty acid attached to lactosyl ceramide [7]. The nitroxide radical was at C-16, and thus could be used to measure the degree of motional anisotropy (order) for the glycolipid fatty acid in the region of the host phospholipid terminal methyl groups. We found the order at C-16 to be much greater for a 24-carbon fatty acid than for an 18-carbon fatty acid attached to lactosyl ceramide, consistent with interdigitation of the longer chain. It is not possible, however, to simply assume the same result for other glycolipid families, since there is considerable debate in the literature as to the effect of headgroup sugars on glycolipid behaviour and arrangement [8]. We record here extension of the spin label approach to the simpler neutral glycolipid, galactosyl ceramide; the more complex neutral glycolipid, globoside; and the ganglioside, G_{M1}.

In our work with lactosyl ceramide, the natural fatty acid had been removed in a single step by base hydrolysis. An 18-carbon or 24-carbon fatty acid spin label could then be attached in a further one-step reaction. However in the case of globoside and G_{M1} a much more complex synthesis was necessary to arrive at the fatty acid-substituted species, since the hydrolysis step that removes the pre-existing fatty acid may also remove headgroup sugar *N*-acetyl functions. The latter difficulty in production of lyso derivatives from glycosphingolipids bearing *N*-acetyl groups was recently solved by Neuenhofer et al. who reported the synthesis of lyso G_{M1} and subsequent selective re-*N*-acetylation of amino sugars [9]. We have been able to use the same approach with globoside, and have reacylated the resultant lysoglycolipids with either 18-carbon or 24-carbon spin labelled fatty acids.

Materials and Methods

Egg phosphatidylcholine was from Avanti Polar Lipids, Birmingham, AL. L-α-Dimyristoyl- and L-α-dipalmitoylphosphatidylcholines were from Sigma, St. Louis, MO, as were galactosyl ceramide (type II) and *N*-lignoceroyldihydrolactocerebroside. Cholesterol was from Serdary Research, London, Canada. The above lipids were pure by the criterion of giving single spots on thin-layer chromatography plates (Merck silica gel 60) eluted with 65 : 25 : 4 (by vol.) $CHCl_3/CH_3OH/H_2O$, and developed with 1 : 2.75 sulfuric acid/ethanol spray. *N,N'*-dicyclohexylcarbodiimide was from Aldrich, Milwaukee, WI.

Globoside was isolated from porcine red blood cells according to the method of Hakomori and Siddiqui [10], except that the crude sphingolipid extract was run on a silicic acid column (Bio-Sil A, 3 × 70 cm eluted with 1 litre $CH_3OH/CHCl_3$ (1 : 9, v/v) and 1.5 litre $CH_3OH/CHCl_3$ (1 : 4, v/v), followed by a gradient of 1 : 4–2 : 3 (v/v)). The globoside fraction was further purified on a small silicic acid column eluting with $CHCl_3/CH_3OH/NH_4OH$ (60 : 40 : 4, by vol.). It ran as a single spot on TLC plates eluted with 55 : 25 : 4 (by vol.) $CHCl_3/CH_3OH/H_2O$. The ganglioside, G_{M1}, was prepared and purified following the procedure of Felgner et al. [11] as modified by Thompson et al. [12]. This involved isolation of a fraction consisting of G_{D1a}, G_{D1b} and G_{T1b} from total bovine brain gangliosides (prepared accord-

ing to Kanfer [13], and treatment with neuraminidase (*Clostridium perfringens* type VI from Sigma).

Spin labelled fatty acids employed in this work were prepared following the general method of Hubbell and McConnell [14]. The approach involves deriving long chain keto methyl esters of the general formula CH_3-$(CH_2)_m$-C(=O)-$(CH_2)_n$-C(=O)-OCH_3 via reaction of alkylcadmium compounds, $[CH_3$-$(CH_2)_m]_2Cd$, with ω-carbalkoxyacyl chlorides, ClC(=O)-$(CH_2)_n$-C(=O)-OCH_3. The spin label (*N*-oxyloxazolidine) ring is then produced at the location of the ketone by the method of Keana et al. [15]. The resultant spin labelled fatty acids may be named according to their (m,n) values as above, or according to the number of the fatty acid carbon carrying the spin label ring. Thus the long and short chain fatty acids synthesized for this work were the (7,14) and (1,14) spin labels respectively or the 16-nitroxylignoceric and 16-nitroxystearic acids respectively. The (1,14) species was first prepared by Philippe Devaux and Harden McConnell who communicated the synthetic and analytical procedures to one of us (CWMG). Briefly the 16-carbon monomethyl ester of hexadecanedioic acid was linked to the 2-carbon fragment of ethyl bromide to produce CH_3-$(CH_2)_1$-C(=O)-$(CH_2)_{14}$-C(=O)-OCH_3, which was converted to a spin label [15]. For the new ((7,14)) spin label, CH_3-$(CH_2)_7$-C(=O)-$(CH_2)_{14}$-C(=O)-$OCH_3$was produced by replacing ethyl bromide in the above procedure with octyl bromide (1-bromooctane). Details of the long chain synthesis have been reported elsewhere [7].

Incorporation of spin labelled fatty acids into lysoglycosphingolipids followed the method originally developed by our laboratory to synthesize spin labelled galactosyl ceramide [16]. Generation of the lysoglycosphingolipids for this purpose was performed using methanolic KOH according to the work of Neuenhofer et al. on G_{M1} [9]. For galactosyl ceramide and lactosyl ceramide this is a one-step procedure [7]. In our hands the method of Neuenhofer et al. for generating complex lysoglycosphingolipids worked precisely as described by the authors for G_{M1}, and was equally applicable to globoside (Mehlhorn et al., manuscript in preparation). It was readily demonstrated by ^{1}H-NMR that the initial base hydrolysis step removed the *N*-acetyl function from GalNAc of globoside. Subsequent selective replacement of the *N*-acetyl group was also demonstrated by NMR. Lysoglycolipids reacylated with spin labelled fatty acids ran with R_f values identical to those of the glycolipid starting materials.

Lipid bilayer membranes for these experiments were prepared by dissolving all components at the final desired ratio 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 prepared by hydration of such films with 10 mM phosphate-buffered normal saline (pH 7.4) containing Ca^{2+} and Mg^{2+}. Free Ca^{2+} and Mg^{2+} concentrations in the samples were 0.96 mM and 0.55 mM, respectively, as determined by chemical analysis. 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 the study.

EPR spectra of samples were run on a Varian E 12 spectrometer equipped with a TM_{110} cavity and variable temperature accessory (Varian Association, Palo Alto, CA). For this purpose vesicle suspensions were held in 50 μl Dade® disposable glass micropipettes sealed at one end and supported in a plastic sleeve that permitted gas flow.

Results and Discussion

Fig. 1 illustrates the concept of fatty acid interdigitation as applied to glycolipids that make up a small fraction of the total lipid in a membrane. It shows the basic options that exist with regard to fatty acid arrangement for both 18-carbon and 24-carbon chains. Note that there has been no attempt to arrange short chain gaps in one monolayer to accommodate long chain species of the opposing monolayer. If the glycolipid acyl chain length is roughly compatible with that of surrounding phospholipids, one might anticipate that it would simply behave much like another phospholipid with regard to the question of chain organization. Such a view is compatible with existing information on glycolipid physical behaviour [8]. However, a very long fatty acid attached to sphingosine would be faced with extending into

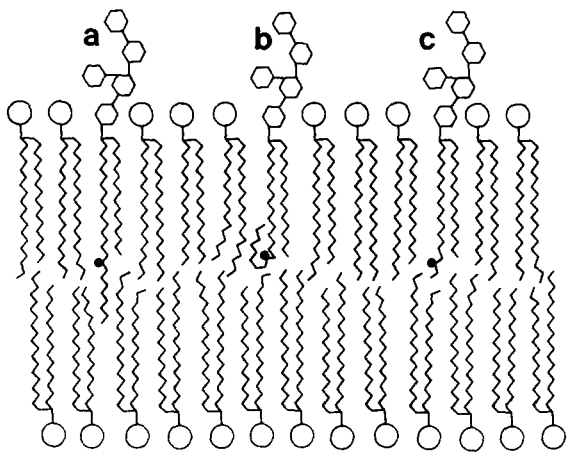

Fig. 1. Representation of the concept of glycosphingolipid interdigitation in a phospholipid bilayer membrane. Three G_{M1} molecules with 18-carbon or 24-carbon fatty acids are shown amongst host membrane phospholipids with 16-carbon and 18-carbon fatty acids. The G_{M1} fatty acid is shown as interdigitated (scenario *a*, 24-carbon chain), collapsed upon itself to form a focus of disruption (scenario *b*, 24-carbon chain), and packed similarly to the surrounding phospholipid fatty acids (scenario *c*, 18-carbon chain). G_{M1} headgroup sugars are drawn as hexagons. The nitroxide spin label attached to C-16 of the glycolipid fatty acid is indicated by a black dot: note that it is localized to a point roughly in the center of the hydrophobic interior.

and across the plane defined by the host matrix methyl terminii, or collapsing longitudinally via *trans* → *gauche* isomerization. The latter option would be expected to decrease the degree of alignment of the glycolipid acyl chain portion located in the region of the host matrix terminal methyl groups; which is the region probed by the spin labels described here (Fig. 2). Interdigitation on the other hand should reduce the tendency of this same portion of the glycolipid fatty acid to deviate from a position of alignment relative to surrounding acyl chains. These possibilities have been displayed for spin labelled G_{M1} in a host matrix composed of 16-carbon and 18-carbon fatty acid phospholipids (Fig. 1).

The concept of 'order parameter' was introduced to permit quantitation of alkyl chain anisotropic motion in bilayer membranes [14,17–20]. The order parameter, S, associated with a given fatty acid carbon in a membrane is a measure of the degree of motional alignment of that carbon relative to the plane of the membrane. It is not simply a function of membrane fluidity: a rigid rod that rotated rapidly about its long axis and

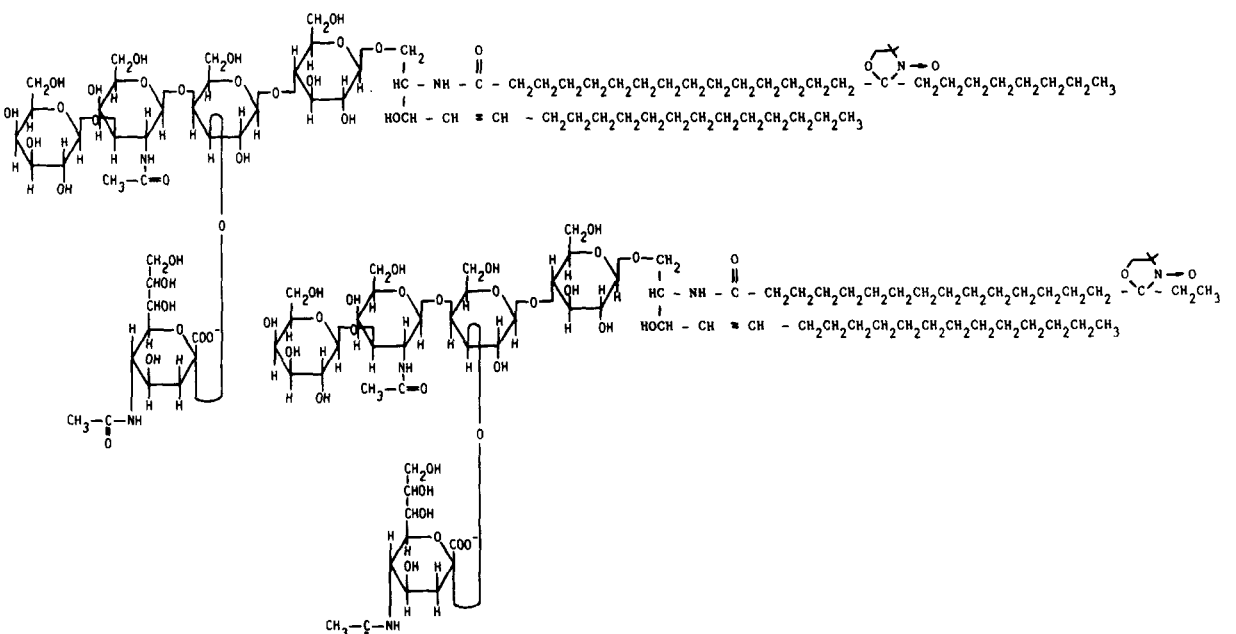

Fig. 2. Chemical structures of the spin-labelled G_{M1} used in work described here: lignoceroyl-16-nitroxy-G_{M1} and stearoyl-16-nitroxy G_{M1}, respectively. The galactosyl ceramide, lactosyl ceramide, and globoside spin labelled derivatives differed in oligosaccharide component, and lactosyl ceramide also lacked the double bond in the ceramide portion – being a dihydro derivative. Note that in each case the nitroxide free radical is covalently attached at C-16 of the fatty acid chain.

diffused rapidly in the plane of the bilayer would exhibit perfect order in a fluid membrane, while a more slowly moving molecule whose motion was random (isotropic) would exhibit disorder. The structures of the spin labelled glycolipids employed in this work are illustrated in Fig. 2. Note that the spin label ring is attached rigidly to the C-16 carbon in the glycolipid fatty acids so that it monitors the motional alignment ('order') of this region of the molecule. Information concerning this alignment is available in the EPR spectrum in that, with high order, a maximum outer peak separation and a minimum inner peak separation are evident (illustrated for example in Fig. 3) while low order makes the outer and inner peak separations approach one another in magnitude. Calculation of S then reduces to measuring the experimental difference in outer and inner spectral splitting. The ratio of the observed number to that obtained with a sample of known perfect alignment is the order parameter, S. S varies between 0 and 1. Small corrections have been devised for the measured minimum splitting, and to account for polarity effects, however, an approximate order parameter, S^{app}, is readily arrived at and is included in our data along with the corrected value. A complete description of order parameter calculations may be found in the original works of Hubbell and McConnell [14] and Seelig [16]. The concept has also been dealt with in more recent reviews [18–20].

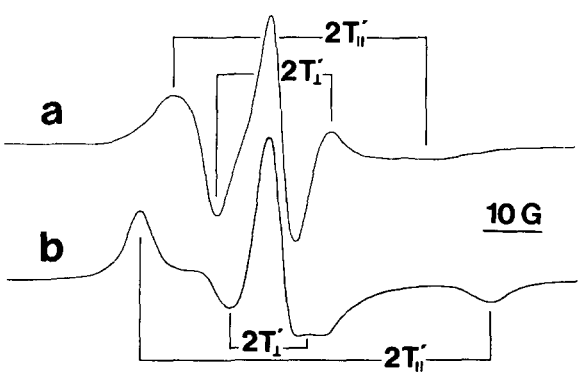

Fig. 3. Typical comparative features of the short vs. long chain spin labelled glycolipids: *N*-stearoyl G_{M1} (a); and *N*-lignoceroyl G_{M1} (b) at 2 mol% in bilayers of dipalmitoylphosphatidylcholine. Spectral features used to calculate order parameters are illustrated. Samples were in phosphate buffered isotonic saline containing Ca^{2+} and Mg^{2+}, and were run at 22° C.

It is clear from Fig. 3 that the spectra obtained are sensitive to factors related to dynamic and organizational differences between short and long chain glycolipids. Our hypothesis was that, by placing a spin probe at the point where interdigitation is determined, we could test the effect of the 'extra' length of hydrocarbon chain on local order. We examined the phenomenon in membranes with different properties: the highly fluid matrix, egg phosphatidylcholine (egg PC); the rigid matrix, dipalmitoylphosphatidylcholine (DPPC); the system of intermediate fluidity, 2 : 1 DPPC/cholesterol; and the short chain matrix, dimyristoylphosphatidylcholine (DMPC). The ratio of glycolipid to host matrix lipid has been kept in the range 1 : 50 to 1 : 100 for all our experiments.

Order parameters were determined by the method summarized in the caption to Table I. They have been arranged in groups corresponding to the different glycolipid families. Within each family of glycolipids, for example galactosyl ceramides, the order parameters reflect the anticipated relative fluidities of the various host lipids. Thus for both 18-carbon fatty acid galactosyl ceramide and 24-carbon fatty acid galactosyl ceramide the order at C-16 is highest in the crystalline DPPC host matrix ($S = 0.42$ and 0.76, respectively). These values drop in going to the DPPC/cholesterol matrix which has intermediate fluidity, and are lowest in the natural, highly fluid egg phosphatidylcholines ($S = 0.08$ and 0.22, respectively). However, the striking aspect is that each value of S for the long chain species is some 2 or more times that for its shorter analogue. This is certainly inconsistent with any model that would view the long chains of glycolipids as collapsed at the plane of the host methyl termini, and argues in favour of interdigitation as follows. Situation b, of Fig. 1 (long chain fatty acid forming a focus of local disorder) should lead to an S value similar to or less than that of the short chain analogue. Instead, as mentioned, we observed that S values for the long chain spin labelled glycolipid are much greater than those of the short chain analogue in fluid, rigid, and cholesterol-containing matrices. Such a result would be expected if interdigitation were occurring (situation a of Fig. 1). A third possibility, which is not shown, might also

TABLE I

ORDER PARAMETER DATA FOR GALACTOSYL CERAMIDE, LACTOSYL CERAMIDE, GLOBOSIDE, AND G_{M1} WITH SPIN LABEL AT C-16 OF THE FATTY ACID CHAIN

Data are given for the long-chain (24 carbons) and short-chain (18 carbons) derivatives for comparison. Glycolipid was incorporated at 2 mol% in liposomes of host matrix phospholipid, either egg phosphatidylcholine (egg PC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), or DPPC/cholesterol (2:1, mol ratio). Sample preparation as in Materials and Methods. Sample buffer was phosphate buffered normal saline pH 7.4 containing Ca^{2+} and Mg^{2+}. $T'_{\parallel}$ is half the measured outer spectral splitting (as shown for DPPC bilayers in Fig. 3), and is an accurate reflection of the desired matrix element, $T_{\parallel}$ [14,17,19]. $T'_{\perp}$ is half the measured inner spectral splitting (also shown as an example for DPPC in Fig. 3). A better estimate of the true value of the desired matrix element is $T_{\perp} = T'_{\perp} + 1.4(1 - S^{app})$ where

$$S^{app} = (T'_{\parallel} - T'_{\perp})/\left[T^{c}_{zz} - (T^{c}_{xx} + T^{c}_{yy})/2\right]$$

is a first approximation to the order parameter and $T^{c}_{zz} = 32.9$ gauss, $T^{c}_{xx} = 5.9$ gauss, $T^{c}_{yy} = 5.4$ gauss are parameters from a crystal in which $S = 1$. A better value for the order parameter is then obtained as

$$S = (T_{\parallel} - T_{\perp})/\left[T^{c}_{zz} - (T^{c}_{xx} + T^{c}_{yy})/2\right] \cdot \left(a_0^{crystal}/a_0^{bilayer}\right)$$

where $a_0^{bilayer} = (T_{\parallel} + 2T_{\perp})/3$ and $a_0^{crystal} = (T^{c}_{xx} + T^{c}_{yy} + T^{c}_{zz})/3$ correct for polarity differences between crystal and bilayer.

Glycolipid fatty acid chain length and host matrix phospholipid	Temperature (°C)	$T'_{\parallel}$ ($\simeq T_{\parallel}$) (gauss)	$T'_{\perp}$ (gauss)	S^{app}	$T_{\perp}$ (gauss)	S
Galactosyl ceramide						
Long in egg PC (fluid)	10	20.7	9.8	0.40	10.7	0.39
Short in egg PC (fluid)	10	16.4	12.0	0.16	13.1	0.12
Long in egg PC (very fluid)	22	17.8	11.0	0.25	12.0	0.22
Short in egg PC (very fluid)	22	15.9	12.6	0.12	13.8	0.08
Long in DMPC (rigid)	22	28.1	8.4	0.72	8.8	0.69
Short in DMPC (rigid)	22	20.0	10.3	0.36	11.2	0.33
Long in DPPC (rigid)	22	28.7	7.6	0.78	7.9	0.76
Short in DPPC (rigid)	22	21.1	9.5	0.42	10.3	0.42
Long in DPPC/CHOL (intermediate fluidity)	22	26.0	8.4	0.65	8.9	0.64
Short in DPPC/CHOL (intermediate fluidity)	22	19.9	10.3	0.35	11.2	0.34
Lactosyl ceramide						
Long in egg PC (fluid)	10	21.0	9.7	0.41	10.5	0.40
Short in egg PC (fluid)	10	16.5	11.9	0.17	13.1	0.13
Long in egg PC (very fluid)	22	18.0	10.9	0.26	11.9	0.24
Short in egg PC (very fluid)	22	15.9	12.5	0.12	13.8	0.08
Long in DMPC (rigid)	22	27.7	8.3	0.71	8.7	0.68
Short in DMPC (rigid)	22	19.8	10.4	0.34	11.3	0.32
Long in DPPC (rigid)	22	28.6	6.8	0.80	7.1	0.81
Short in DPPC (rigid)	22	20.8	9.4	0.42	10.2	0.42
Long in DPPC/CHOL (intermediate fluidity)	22	26.2	8.3	0.66	8.8	0.64
Short in DPPC/CHOL (intermediate fluidity)	22	19.7	10.2	0.35	11.1	0.33
Globoside						
Long in egg PC (fluid)	10	21.3	9.7	0.43	10.5	0.42
Short in egg PC (fluid)	10	16.5	12.0	0.17	13.1	0.13
Long in egg PC (very fluid)	22	18.2	10.9	0.27	11.9	0.24
Short in egg PC (very fluid)	22	16.0	12.5	0.13	13.7	0.09

TABLE I (Continued)

Glycolipid fatty acid chain length and host matrix phospholipid	Temperature (°C)	$T_{\parallel}'$ ($\simeq T_{\parallel}$) (gauss)	$T_{\perp}'$ (gauss)	S^{app}	$T_{\perp}$ (gauss)	S
Globoside (continued)						
Long in DMPC (rigid)	22	27.3	8.8	0.68	9.2	0.64
Short in DMPC (rigid)	22	19.5	10.5	0.33	11.4	0.31
Long in DPPC (rigid)	22	29.1	6.1	0.84	6.3	0.89
Short in DPPC (rigid)	22	21.1	9.5	0.43	10.3	0.42
Long in DPPC/CHOL (intermediate fluidity)	22	26.4	8.3	0.66	8.8	0.65
Short in DPPC/CHOL (intermediate fluidity)	22	19.7	10.2	0.35	11.1	0.33
G_{M1}						
Long in egg PC (fluid)	10	21.4	9.8	0.43	10.6	0.41
Short in egg PC (fluid)	10	16.6	12.0	0.17	13.1	0.13
Long in egg PC (very fluid)	22	18.3	10.9	0.27	11.9	0.25
Short in egg PC (very fluid)	22	16.0	12.6	0.13	13.8	0.08
Long in DMPC (rigid)	22	28.0	8.3	0.72	8.7	0.69
Short in DMPC (rigid)	22	19.9	10.3	0.35	11.2	0.33
Long in DPPC (rigid)	22	28.9	6.4	0.83	6.6	0.86
Short in DPPC (rigid)	22	20.9	9.4	0.42	10.3	0.42
Long in DPPC/CHOL (intermediate fluidity)	22	26.3	8.2	0.66	8.7	0.65
Short in DPPC/CHOL (intermediate fluidity)	22	19.8	10.3	0.35	11.2	0.33

be considered for arrangement of the extra length of the 24-carbon fatty acid: the long chain might bend sharply by 90° at the depth of the surrounding phospholipid terminal methyl groups. The upper portion of the fatty acid could then remain hexagonally packed with surrounding orderly acyl chains, while the extra length might lie in the plane of the membrane, extended amongst the phospholipid methyl termini. This is a significant possibility because, conceptually at least, if the 90° bend were below the spin label (i.e. at carbon 17 or greater) a higher S value might be predicted for the longer chain glycolipid fatty acid, as observed. However, the result with the dimyristoylphosphatidylcholine host matrix seems to rule out such a possibility since the 14-carbon fatty acids in the latter case would dictate that the 90° bend be above the spin label, forcing the nitroxide ring to take on a relatively random arrangement with a low S value, whereas in fact the values of S observed for short and long chains of all four glycolipids in dimyristoylphosphatidylcholine fit into the same pattern as the other host matrices.

It should be noted that the systems studied here are very different from the pure mixed chain bilayers studied by other workers in that only a small percentage of the lipids are capable of interdigitation (i.e. the few % glycolipid in a host matrix of phospholipid or phospholipid/cholesterol). However, the results are consistent with what has been observed by others. For instance Shipley and co-workers have remarked on the striking degree of order conferred upon the region of a sphingomyelin fatty acid acyl chain that interdigitates [5]. They suggest that it arises from favourable van der Waals interactions gained by such interdigitation. Boggs and Rangaraj [21] and Boggs and Mason [22] have utilized 18-carbon free fatty acids with spin label at the C-16 or C-5 position as probes to make the interesting observation that spectra of the species with spin

label at C-16 became similar to those of the C-5 spin label (i.e. highly immobile and ordered) when interdigitation of the surrounding homogeneous mixed chain phospholipids occurred. Note, however, that in their systems this is thought to be caused by the spin label itself moving physically across the bilayer mid point due to interdigitation of the surrounding phospholipids (decreased bilayer thickness) to assume a new position near the bilayer surface on the opposite side of the membrane. In our systems the host phospholipid membrane presumably does not change thickness by having 2% 18-carbon vs. 24-carbon fatty acid glycolipid so that the spin label remains at the same position in the membrane. With regard to glycolipid interdigitation, Bunow and Levin [23] recorded Raman spectroscopic evidence that in lamellar structures of pure galactosyl ceramide the long chain fatty acids were extended, consistent with their interdigitating across the membrane.

A feature of Table I that should be noted in passing is that the order parameters for a given host matrix type (e.g. DPPC) and a given spin labelled fatty acid (e.g. 18-carbon) are essentially identical amongst all four glycolipid families. This may be somewhat surprising, given current views that the nature of the headgroup may exert a strong influence upon glycolipid physical behaviour (e.g. reviewed in Refs. 8, 24, 25). However, order parameter, especially at the center of the bilayer, is likely not the most sensitive parameter to differences in the glycolipid headgroup. The important question of possible headgroup sugar effects upon glycolipid physical behaviour will require more systematic investigation, however, to date we have seen no difference amongst the spin labelled families studied in this manuscript in terms of spectral features and variation with temperature or concentration (unpublished observation).

Conclusions

Examples of interdigitation in the literature have mainly involved bilayers comprised of pure synthetic phospholipids or sphingomyelins, with mismatched fatty acid chains (Refs. 1–6, 22 and references therein). In such cases there is conceptually the possibility that long chains of one monolayer interdigitate by matching with short chains of the opposing monolayer, or that short chains on opposing sides pair up allowing long chains to cross the bilayer mid point. In our experiments, chain length discrepancy involves only 2% of the total lipid (i.e. only the glycolipid), and interdigitation by 'matching up' of shorter and longer chains is not a likely possibility. We used the order parameter function, S, to sample motional alignment at C-16 of glycolipid fatty acids which were the same length as, or significantly longer than, those of the phospholipid bilayer matrix. Our results argue strongly that the 'extra' length of a 24-carbon fatty acid attached to a sphingosine backbone, extends across the midplane of the membrane to interdigitate with acyl chains of the opposing monolayer. Apparently this can occur in membranes of natural phospholipids, as well as in rigid, synthetic phospholipids.

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