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New Membrane Mimetics with Galactolipids: Lipid Properties in Fast-Tumbling Bicelles

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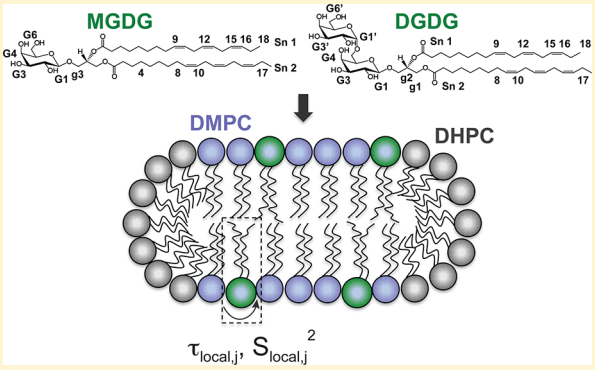
ABSTRACT: Galactolipids are the main structural component of plant chloroplastic (thylakoid) membranes and of blue-green algae cell membranes. The predominant lipids in this class are monogalactosyl-diacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG). We here present a method for the preparation of bicelles that contain these galactolipids together with a characterization of the bicelles, and the lipids within the bicelles. NMR diffusion data show that up to 30% of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) in a q = 0.5 DMPC/DHPC lipid matrix can be replaced with either monogalactosyl-diacylglycerol or digalactosyl-diacylglycerol and that these lipids incorporate into the bicelles. No evidence for phase separation is observed. Bicelles made with monogalactosyl-diacylglycerol are significantly larger than bicelles containing only DMPC, already with only 10% of the DMPC replaced with the galactolipid. The effect of digalactosyl-diacylglycerol on bicelle size is much smaller. These observations are likely to be correlated with the different bilayer-forming properties of the lipids. Monogalactosyl-diacylglycerol is a non-bilayer-forming lipid, while digalactosyl-diacylglycerol is a bilayer-forming lipid. Both galactolipids display extensive local motion within the bilayer, as evidenced by natural abundance carbon-13 relaxation of the lipid molecules. The sugar headgroup regions are motionally restricted and cannot be described by a model that does not take into account anisotropic reorientation of the sugar units. No significant effect of the galactolipids on DMPC dynamics was observed. Our results indicate that these bicelles may become useful as model membrane mimetic media for studies of galactolipid−protein interactions.



INTRODUCTION

Fast tumbling bicelles provide a versatile membrane mimetic model for structural and topological studies of membrane additives and membrane peptides.[1−4] Bicelles are mixtures of a variety of long-chain phospholipids and one type of short-chain lipids (or detergents). The ratio between the amounts of the two components in bicelles, i.e., long-chained lipid molecules and detergents, known as q-ratio, can be manipulated to give different bicellar size, shape and even morphology.[5,6] At high q-ratio (>2), a complicated phase diagram has been identified using a range of techniques, such as neutron and X-ray scattering,[7−11] cryotransmission electron microscopy,[12] and a variety of solution and solid-state NMR techniques.[13−16] At around or below room temperature, isotropic disklike objects persist, while a range of morphologies such as ribbon, lamellae, and perforated lamellae have been observed at higher temperatures as a function of charge density, concentration, and lipid properties.

Bicelles with a q-ratio below 1 resemble disklike aggregates, where the long-chained lipids mainly locate in the bilayered region and short-chained lipids, or detergents, in the rim. There are many reasons to choose bicelles as a membrane mimetic medium, in particular for high-resolution NMR.[1,3,4,17,18] Compared to large vesicles, small bicelles tumble fast in solution, so they fulfill the high-resolution requirement. Although micelles are also fast tumbling objects, there are problems associated with micelles and for example some peptide structures have been shown to be distorted in micelles as compared to bicelles.[17,19] More importantly, micelles do not allow for studies of membrane location of peptides and proteins in a bilayer, or for studies of the effects that they have on lipid properties. Bicelles can be made with a reasonable variety of lipids, and hence are attractive for investigations of the direct effects that lipids have on protein and peptide properties, and conversely the effects that proteins exert on lipid properties.[20,21]

Galactolipids are the main structural component of plant chloroplastic (thylakoid) membranes and of blue-green algae cell membranes. The predominant galactolipids are monogalactosyl-diacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG).[22] In particular MGDG is considered to be the most abundant polar lipid in nature.[23,24] MGDG and DGDG play important roles in many biological activities, e.g., cell growth and membrane lipid homeostasis under stress conditions.[24−26] Phospholipids are for instance substituted for DGDG under phosphate deprivation conditions, during which DGDG is also found in extraplastidial membranes.[24,27] Both lipids are found as cofactors in the crystal structure of the photosystem II core

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complex, suggesting a role in photosynthesis.[28,29] MGDG is prone to form nonbilayer structures and is mainly located in thylakoid membranes while DGDG forms bilayers and can also be found in extraplastidial membranes. MGDG can form hexagonal liquid crystalline phases,[30] while, if incorporated into bilayers containing bilayer-prone lipids, these mixtures can form various types of structures. The presence of nonbilayer-prone lipids in biological membranes appears to be crucial for regulating, e.g., enzymes that are sensitive to bilayer properties such as curvature and stress.[31,32]

Although several structural studies of glycolipids in model bilayer systems have been performed,[33−37] properties including overall and local dynamics of galactolipids in bilayers are not well understood. It is becoming increasingly evident that, in order to understand how membrane proteins interact with bilayers, more detailed knowledge about the lipid molecules is needed. Moreover, to be able to study the specialized functions of MGDG and DGDG, interactions between the galactolipids and other essential components during the related biological processes are of great interest. Therefore, we have developed versatile membrane mimetic media by incorporating either MGDG or DGDG into small fast-tumbling bicelles composed of a 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) matrix. Earlier studies of MGDG or DGDG have focused on the lipid structure in a bilayer or on the morphology and properties of bilayers containing the galactolipids.[33,38−41] In this study, we have examined the physical properties of mixtures of MGDG or DGDG with DMPC in bicelles by NMR methods. The size distribution of the lipid assemblies was studied by pulsed field gradient NMR, and the fast dynamics of the lipids in these mixtures were determined by natural abundance carbon-13 spin relaxation. Our diffusion results indicate that both galactolipids integrate into the bicelle and that no significant amounts of other lipid assemblies are formed. The local dynamics of the lipids indicate that the sugar headgroups are relatively rigid and that the acyl chains display a significant amount of motion. The results are important in providing local motional parameters for further galactolipid-related interaction studies.

EXPERIMENTAL SECTION

Materials. Monogalactosyl-diacylglycerol (MGDG, 1,2-diacyl-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol) and digalactosyl-diacylglycerol (DGDG, 1,2-diacyl-3-*O*-[α-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl]-*sn*-glycerol) were purchased as a solution in chloroform−methanol from Larodan AB (Malmö, Sweden). Phospholipids, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), and chain-deuterated 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC-d_{22}) were purchased as powder from Avanti Polar Lipids (Alabaster, AL). All the lipids were used without further purification. The galactolipids were analyzed by electrospray ionization (ESI) mass spectrometry, using a LCQ Deca Ion Trap system equipped with an ESI ion source (Thermo Finnigan, San Jose, CA). Based on the analyses, it was concluded that the acyl chains in DGDG were all C18:3, while in MGDG, two different variants were observed, yielding a mixture of di-C18:3 and C18:3, C16:3, as found previously.[40] The results indicate a ratio of the amount of C18:3 to C16:3 of 3:1. The peaks in the carbon-13 NMR spectra for MGDG used for relaxation analyses were all assigned to the C18:3 chains, based on their relatively higher intensities and chemical shift similarities in MGDG compared to DGDG.

Preparation of Bicelles. The bicelle samples with q = ([DMPC] + [galactolipid])/[DHPC-d_{22}] were prepared with a 300 mM total concentration of lipids in 50 mM phosphate buffer (pD = 5.7) in D_2O. First, MGDG or DGDG in chloroform−methanol was dried under a stream of nitrogen gas. Then, DMPC and buffer were added. Subsequently, DHPC-d_{22} was added from a 1 M stock solution to the lipid mixture solution. The mixture was then lyophilized and dissolved in D_2O. Several cycles (>5) of vortexing and gentle centrifugation were performed until a clear nonviscous solution was obtained. The final concentration of DHPC-d_{22} was 200 mM while that of the long-chain lipids was 100 mM. The mole fractions of galactolipid (MGDG/DGDG) to DMPC were 0%, 10%, and 30%.

NMR Spectroscopy. NMR experiments were carried out using Bruker Avance spectrometers, operating at 9.4 and 14.1 T. ^{1}H translational diffusion experiments were recorded at 14.1 T using a modified Stejskal−Tanner spin−echo pulse sequence with a fixed diffusion time and a pulsed field gradient increasing linearly over 32 steps.[42−44] A standard sample of 0.01% H_2O in D_2O and 1 mg/mL $GdCl_3$ was used for gradient calibration at 25 °C. To avoid signal from DHPC, chain-deuterated DHPC-d_{22} was used in all bicelles. The ^{1}H signal of the methyl group in the acyl chains in DMPC (0.78 ppm) was used to monitor the diffusion of DMPC, while signals for five sites in the acyl chains of MGDG or DGDG (position 11/14, 8/17, and 18 at 2.68, 1.96, and 0.86 ppm, respectively) that did not overlap with DMPC and DHPC were used to monitor the diffusion of the galactolipids. The attenuation of the signal, as a result of the increasing gradient, was fitted using a modified version of the Stejskal−Tanner equation which takes into account possible field inhomogeneity.[45] Errors in the diffusion coefficients were estimated from several measurements using different samples with the same composition. Viscosities of the samples were estimated by measuring the H_2O diffusion coefficients (trace ^{1}HDO in D_2O) with the same pulse sequence but without water suppression and a much longer preacquisition delay time (6 s) and comparing these to the diffusion of H_2O. Normalized, i.e., viscosity-corrected, translational diffusion coefficients were then used to calculate hydrodynamic radii via the Stokes−Einstein equation.

The relaxation parameters T_1 and NOE were measured at two fields, 9.4 and 14.1 T, using natural abundance carbon-13 samples. T_1 measurements were performed with a standard direct carbon-13 detection inversion recovery pulse sequence, using 10−14 relaxation delays ranging from 0.01 to 10 s. A preacquisition delay of a minimum of 4 s was used. Steady-state NOE factors were obtained by taking the ratio of intensities in a spectrum acquired with and without a long (25 s) preacquisition ^{1}H irradiation. The T_1 and NOE data were collected using typically about 2000 transients. All NMR experiments were carried out at 25 °C.

NMR Relaxation Data Evaluation. R_1 and heteronuclear NOE data were evaluated by using an extension of the classical model-free approach.[46−49] In this model, three types of motions contribute to spin relaxation in bicelles: overall bicelle reorientation, overall lipid reorientation, and local motion of individual ^{13}C−^{1}H bond vectors.[6,50,51] The overall motion of the entire bicelle was considered to be too slow to affect R_1 and heteronuclear NOE, as described previously.[6] Therefore, the contribution of overall tumbling of the entire bicelle to the spectral density function can be neglected for R_1 and heteronuclear NOE evaluation. The overall motion of a lipid

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can be described by a squared generalized order parameter and a correlation time for the entire lipid, S^2_{lipid} and τ_{lipid}. For the local motion of each $^{13}C-^{1}H$ vector, $S^2_{local,j}$ and $\tau_{local,j}$ are the corresponding squared generalized order parameter and correlation time. Hence, relaxation data were fitted to the spectral density function given by

$$J_j(\omega) = \frac{2}{5}\left[\frac{(S^2_{local,j} - S^2_{local,j}S^2_{lipid})\tau_{lipid}}{1 + \omega^2\tau^2_{lipid}} + \frac{(1 - S^2_{local,j})\tau'}{1 + \omega^2\tau'^2}\right] \quad (1)$$

in which j indicates each measured site, $1/\tau' = 1/\tau_{lipid} + 1/\tau_{local,j}$ and $\tau_{lipid} \gg \tau_{local,j}$. Moreover, the relaxation is dominated by dipole−dipole interactions for carbons with one or more directly attached protons, so the contribution of other relaxation mechanisms can safely be neglected.[52] In the fitting procedure, S^2_{lipid} and τ_{lipid} were kept fixed at 0.36 and 1.9 ns, in agreement with previous estimates for lipid molecules.[6,51,53−56] It can be noted that, although these estimates may not be valid for galactolipids, small variations in, e.g., S^2_{lipid} and τ_{lipid} have been demonstrated previously to only have the effect of shifting the local motional parameters in a minor and consistent way that does not have any effect on the overall trend in the parameters.[21] Although there may exist different or more complex motions in the molecules, a comparison between the different samples is still possible. In this way, R_1 and NOE were fitted for each measured site to give the corresponding local motional parameters, $S^2_{local,j}$ and $\tau_{local,j}$. The fitting routine was performed with in-house Matlab (version 7.13) routines as described previously.[6]

RESULTS AND DISCUSSION

Galactolipids Are Incorporated into Small Fast-Tumbling Bicelles. To examine whether the galactolipids, MGDG and DGDG, integrate into bicelles, translational diffusion coefficients were measured for bicelle samples with different amounts of galactolipids. The diffusion data for bicelles with different mole ratio (0%, 10%, 30%) of galactolipids are collected in Table 1. Several expressions relating the thickness of the bicelles to the radii of the long axes have been developed.[6,16,57,58] Here, the hydrodynamic radii were calculated based on the Stokes−Einstein equation and a Perrin shape factor of 1.22 for bicelles with q = 0.5.[59] One should note, however, that the present lipid mixtures contain lipids with acyl chains of different length, which may affect the bilayer thickness as well as the shape of the bicelles. Hence, bilayer thickness and bicelle shape have an impact on the estimated hydrodynamic radius. It has furthermore been demonstrated that mixtures with higher q-values display a great variety in morphology as a function of temperature, a finding that was in part argued to be due to a variation in DHPC−lipid miscibility.[16]

Table 1. Normalized[a] Translational Diffusion Coefficients and Hydrodynamic Radii for Lipids in q = 0.5 Bicelles

samples	D (10^{-11} m^2 s^{-1})		R_H (nm)[b]
	DMPC	galactolipid	
DMPC/DHPC	5.2	–	3.9
(10% MGDG + 90% DMPC)/ DHPC	4.4 ± 0.3	4.1 ± 0.2	4.6 ± 0.3
(10% DGDG + 90% DMPC)/ DHPC	4.9 ± 0.2	4.7 ± 0.4	4.1 ± 0.2
(30% MGDG + 70% DMPC)/ DHPC	3.7 ± 0.2	3.6 ± 0.2	5.4 ± 0.3
(30% DGDG + 70% DMPC)/ DHPC	4.3 ± 0.3	4.2 ± 0.3	4.7 ± 0.3

[a]Normalized according to the diffusion of HDO in D_2O and comparing to H_2O diffusion. [b]Based on the diffusion coefficients of DMPC.

For all bicelles containing MGDG and DGDG, the diffusion coefficients of the galactolipids are within the error margin the same as those of DMPC, indicating that the galactolipids and DMPC experience the same overall translational motion. The diffusion data for q = 0.5 DMPC/DHPC bicelles agree well with previously published results,[60] and compared to these bicelles, incorporation of 10% MGDG results in significantly larger bicelles (an increase in hydrodynamic radius from 3.9 to 4.6 nm). Contrary to this, incorporation of 10% DGDG does not result in an evident change in bicelle size (3.9 nm compared to 4.1 nm). However, when more galactolipid is added, the increase of bicelle sizes is apparent for both 30% MGDG and 30% DGDG containing bicelles, although the increase is significantly larger for MGDG. This indicates that the bicelle size depends on the galactolipid content, and especially so for the nonbilayer prone MGDG.

The fact that DMPC and the galactolipids diffuse with the same diffusion coefficients indicates that they do not phase separate under our conditions. Previous solid-state NMR studies have shown that high amounts of MGDG in bilayers induced a hexagonal liquid crystalline phase and also an isotropic phase, which was attributed to a cubic phase or the formation of micelles.[40,61] However, a fraction as high as 60% (of the total lipids) was required for this to happen, which further supports that at our concentrations (10−30% MGDG), all of the MGDG molecules are incorporated into bicelles. Bicelle size increases with increasing MGDG concentration, an effect that is not as pronounced for DGDG, indicating that the increase in size is most likely related to the shape of MGDG. A large amount of MGDG may distort the shape of the bicelle, which in turn leads to differences in hydrodynamic dimensions. At high galactolipid concentrations (30%), a modest increase in dimension is, however, also observed for DGDG. Both lipids have unsaturations in the acyl chains, and it is expected that introducing polyunsaturated chains increases the chain flexibility, and hence also the chain mean area of the lipids.[62,63] Hence, the increase in bicelle size for DGDG is most likely related to the fact that longer polyunsaturated chains are introduced, while the additional increase observed for MGDG is due to the shape and non-bilayer-forming properties of this lipid.

One may also note that, while both systems contain the same amount of DHPC and show no phase separation, bicelles that contain MGDG have a larger hydrodynamic radius. This may have two reasons: a different degree of mixing between DHPC and long-chained lipids,[16] and/or a larger amount of free DHPC in solution. A higher degree of mixing results in fewer DHPC molecules available for covering the rim, leading to an effectively larger q-value. This has previously been argued to be the case in bicelles with DLPC, with 12 carbons in the acyl chains,[60] as well as for bicelles with CHAPS as the detergent,[6] for which a higher degree of mixing is expected. A larger amount of free DHPC would also increase the effective q-value leading to larger bicelles. Further studies using, e.g., ^{31}P NMR in either aligned or isotropic lipid bicelle mixtures could shed

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further light on the size variations observed with the two galactolipids.

Lipid Dynamics in Bicelles from Carbon-13 Relaxation. To investigate the local dynamics of the lipids in detail, we measured natural abundance carbon-13 relaxation for MGDG, DGDG, and DMPC lipids in the 30% MGDG or DGDG bicelle samples. The assignment of the MGDG and DGDG carbon-13 spectra was made by a combination of prediction values and previous assignments for these lipids in bilayers.[40,64−66] Nonoverlapping resonances that were assigned for MGDG and DGDG are indicated in Figure 1. For MGDG,

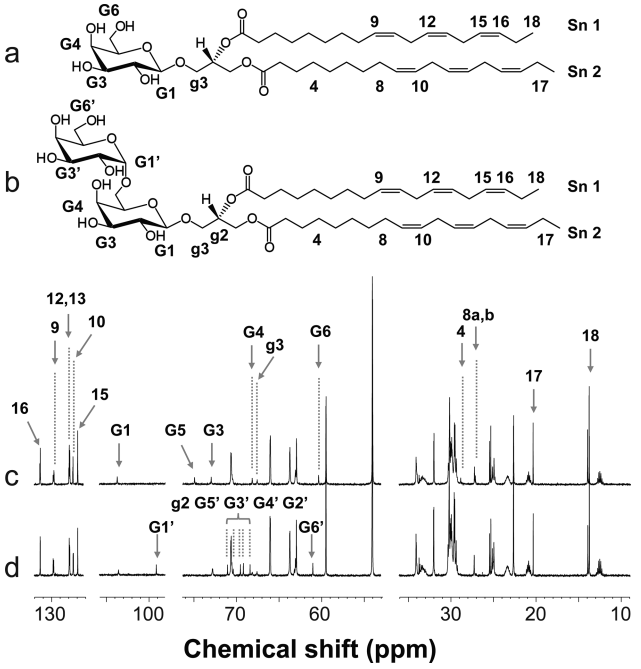

Figure 1. Carbon-13 spectra for bicelles containing the galactolipids MGDG or DGDG. The chemical structures of MGDG and DGDG with the atom nomenclature are shown in (a) and (b), respectively. A carbon-13 spectrum for [MGDG + DMPC]/DHPC bicelles is shown in (c) and a carbon-13 spectrum for [DGDG + DMPC]/DHPC bicelles in (d). The assignments are indicated by arrows. Assignments that are the same for both lipids are only indicated in (c). Both spectra were acquired at 25°C and at a magnetic field strength of 14.1 T.

G1, G3, G4, G5, and G6 of the galactosyl headgroup, g3 of the glycerol and positions 4, 8, 9, 10, 12, 13, 15, 16, 17, and 18 in the C18:3 acyl chains had nonoverlapping resonances and were used in the relaxation analyses. For DGDG, G2 and G6 in the second galactose moiety were not assigned and G3 and G5 overlapped. For the rest of the molecule, the same resonances as in MGDG, with the addition of g2 were used for relaxation analyses. In some instances the two C18:3 chains, sn-1 and sn-2, were seen to have separate resonances (e.g., C8) but most others overlapped in the spectra obtained under our experimental conditions.

The R_1 relaxation rates and NOE values were collected for MGDG and DGDG as well as DMPC at two fields (9.4 and 14.1 T). The data for the two galactolipids are shown in Figures 2 and 3. There are two distinct dynamic regions in both molecules, the first one being the sugar headgroup together with the glycerol part, and the second comprising the acyl chains. R_1 relaxation rates for the first region (headgroup carbons) in both lipids are much higher than for the acyl chains,

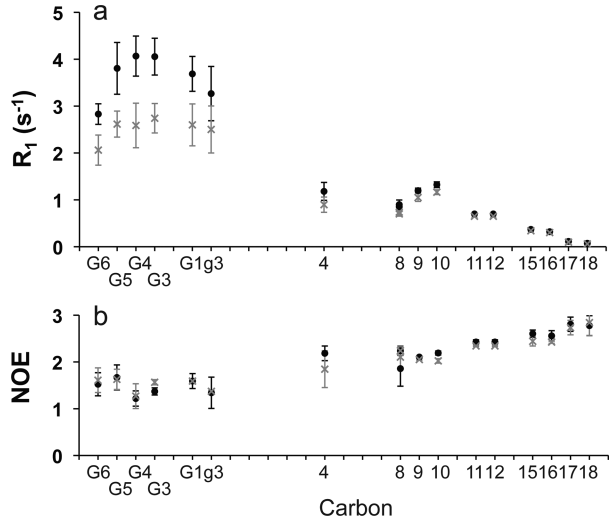

Figure 2. Relaxation data for MGDG. R_1 (a) and NOE (b) parameters measured at 25 °C at two different magnetic field strengths. Circles indicate 9.4 T and crosses 14.1 T. The labeling of the carbon atoms is done according to Figure 1.

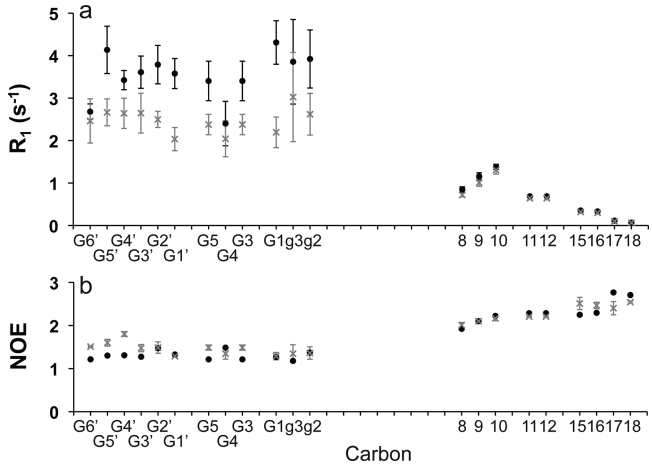

Figure 3. Relaxation data for DGDG. R_1 (a) and NOE (b) parameters measured at 25 °C at two different magnetic field strengths. Circles indicate 9.4 T and crosses 14.1 T. The labeling of the carbon atoms is done according to Figure 1.

indicating less extensive local motion in the first region. Moreover, a clear field dependence in the R_1 data can be observed for the first region, indicating limited local motion, while this is not evident for the acyl chain carbons. The acyl chain R_1 relaxation data are very similar for both lipids, indicating that the chains display dynamics that are uncoupled from the headgroup. The more rigid nature of the headgroup and glycerol region is also evident from low $^{13}C-^{1}H$ NOE factors for both lipid molecules. For the galactose moieties, the NOEs take on values between 1.2 and 1.7 for MGDG, and 1.3 and 1.5 for DGDG at 9.4 T, while the NOEs for $^{13}C-^{1}H$ bond vectors toward the ends of the acyl chains take on values above 2.5.

With the exception of the exocyclic carbon G6, the R_1 relaxation data for the galactose carbons in MGDG are rather uniform. The NOE factor, on the other hand, does not differ between G6 and the ring carbons, and instead a somewhat lower value is observed for carbon G4, indicating different dynamics (Figure 2). As for MGDG, the exocyclic carbon G6′

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in DGDG appears to have somewhat different dynamics, giving rise to slower R_1 relaxation rates than the ring carbons (Figure 3a). The NOE values for the G6′ carbon, on the other hand, are as low as for the ring carbons. The only other main difference in galactose relaxation data in DGDG is observed for G4, which has slower R_1 relaxation rates compared to the other carbons.

To investigate if the galactolipids had any effect on DMPC and DHPC dynamics, we also evaluated relaxation data for DMPC/DHPC in the two bicelles in the presence of either MGDG or DGDG (Figure 4). The resonances originating from

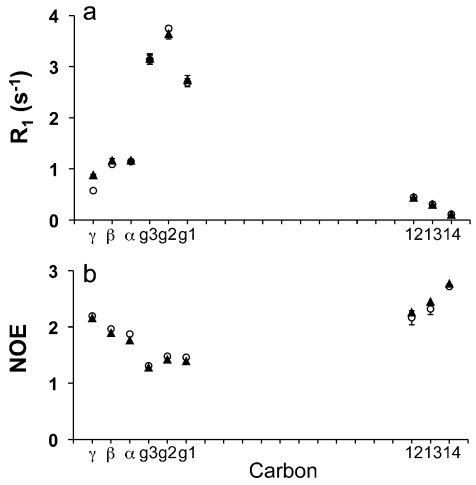

Figure 4. R_1 (a) and NOE (b) parameters measured at 9.4 T for DMPC in bicelles containing 30% MGDG (circles) and 30% DGDG (triangles).

the headgroup and glycerol part of DMPC and DHPC overlap completely, and therefore the data stem from a mixture of both molecules. This overlap precludes an analysis of the relaxation behavior of the $^{13}C-^{1}H$ vectors in DMPC alone, since an average of DMPC and the more abundant DHPC is observed. The overall appearance of the relaxation data as a function of carbon position for this region is similar to what has previously been found for DHPC,[21] and for DMPC in DMPC/CHAPS bicelles,[6] but the values are somewhat different. In accordance with DMPC dynamics in DMPC/CHAPS bicelles, the glycerol $^{13}C-^{1}H$ bond vectors are the most rigid, the choline carbons are relatively flexible, and the end of the tail is the most flexible region in the lipid. Overall, the R_1 and NOE values for DMPC/DHPC in the presence of MGDG or DGDG are very similar. This implies that the presence of either galactolipid affects the relaxation-related dynamics of DMPC and DHPC in the same manner. Resonances in the acyl chains stem exclusively from DMPC, as chain-deuterated DHPC was used in the samples. In this case there is a slight difference between the NOE values measured for DMPC in the presence of MGDG as compared to DGDG, but this difference is within the error limits of the measurements (Figure 4b). Also, compared to previous results for DMPC in DMPC/DHPC bicelles, there is very little influence of adding either of the two galactolipids on the dynamics of the DMPC acyl chains.[6] Taken together, we observe only minor differences on DMPC relaxation when adding MGDG or DGDG. This is in agreement with the modest effect of MGDG on segmental order parameters observed in simulations of MGDG in DMPC bilayers.[40]

Model-Free Analysis. In order to further elucidate the local motion of the galactolipids in bicelles, the R_1 and NOE relaxation data from two fields were fitted using a model-free analysis that yielded two local motional parameters: a generalized local order parameter squared $S^2_{\mathrm{local},j}$ and a local correlation time $\tau_{\mathrm{local},j}$ for the selected carbons of the two galactolipid molecules (Tables 2 and 3, Figure 5). The

Table 2. MGDG Local Motional Parameters Obtained from Model-Free Analysis

carbon	$S^2_{\mathrm{local},j}$	$\tau_{\mathrm{local},j}$ (ps)	carbon	$S^2_{\mathrm{local},j}$	$\tau_{\mathrm{local},j}$ (ps)
G6	0.68	82	9	0.19	34
G5	0.62	270	10	0.21	40
G4	0.76	730	12	0.07	24
G3	0.68	420	13	0.07	24
G1	0.68	260	15	0.03	13
g3	0.88	180	16	0.03	12
4	0.21	27	17	0.01	4
8a	0.15	20	18	0.003	3
8b	0.12	26			

Table 3. DGDG Local Motional Parameters Obtained from Model-Free Analysis

carbon	$S^2_{\mathrm{local},j}$	$\tau_{\mathrm{local},j}$ (ps)	carbon	$S^2_{\mathrm{local},j}$	$\tau_{\mathrm{local},j}$ (ps)
G6′	0.77	57	8	0.15	20
G5′	0.71	460	9	0.18	33
G3′	0.81	410	10	0.19	48
G1′	0.94	120	12	0.09	22
G5	0.90	140	13	0.09	22
G4	0.68	38	15	0.04	11
G3	0.90	140	16	0.04	11
g3	0.72	660	17	0.01	4
g2	0.75	460	18	0.004	3

correlation time for the overall tumbling of the bicelle can safely be assumed to be too large to influence R_1 and NOE relaxation.[1,51] Therefore, the spectral density function given in eq 1 was used. During the fitting procedure, the two reorientational parameters for the entire lipid were kept

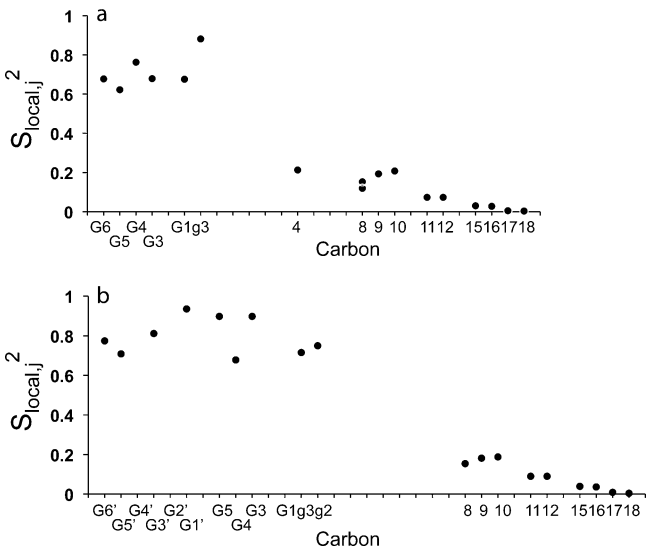

Figure 5. $S^2_{\mathrm{local},j}$ for MGDG (a) and DGDG (b). The labeling of the carbon atoms is done according to Figure 1.

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constant (the order parameter S_{lipid} and the correlation time τ_{lipid}). Although no estimates have been made specifically for galactolipids, reorientational parameters for other lipids, such as DMPC, in bilayers have been determined by deuterium and carbon-13 NMR[50,51,67] and EPR[55,68,69] and the value for S_{lipid} for DMPC has been found to be around 0.6, while τ_{lipid} has been estimated to 1.9−2.0 ns.[6,51] Varying the lipid reorientational parameters has earlier been shown to shift the local motional parameters in a minor and consistent way that does not have any effect on the overall trend in the parameters, as previously discussed.[21] Here, we therefore used the same lipid reorientational parameters for MGDG and DGDG in the model-free fitting assuming that the reorientational dynamics of the entire galactosyl lipid are the same as of DMPC in the bicelle. The local motional parameters of the selected $^{13}C-^{1}H$ vectors for MGDG and DGDG from the model-free fitting are provided in Tables 2 and 3.

Starting with MGDG (Figure 5 and Table 2), we see that the overall appearance of the order parameters as a function of carbon position agrees very well with the trends in the relaxation parameters (Figure 2). The order parameters for the galactose unit and the glycerol are relatively high, with $S^2_{local,j}$ ranging between 0.6 and 0.9, while the acyl chains have very low order parameters, with $S^2_{local,j}$ in the range of 0.003−0.2. It is worth noting that the $^{13}C-^{1}H$ bond vector at position G4 in the galactosyl moiety is equatorial, while all other vectors are axial, which could potentially reveal information about the orientation of the galactose unit. Notably, the carbon at G4 has the highest order parameter, indicating that this carbon experiences less fluctuations coupled to relaxation. For this bond vector the fitting was in fact problematic, leading to either a very high order parameter, close to unity, or a very long local correlation time, close to the nanosecond time scale. This indicates the need to describe the motion for the equatorial bond vector in a different way, with a model that takes into account the anisotropy of the overall motion of the molecule, and/or the incorporation of additional, slower local motion. The higher order parameter of the G4 bond vector is due to lack of motional modulation of the dipole−dipole interaction, indicating that it is oriented along the main axis of the molecule. This orientation implies a small tilt of the sugar headgroup, in agreement with experimental and modeling data.[39,41] The differences in relaxation parameters or order parameters within the ring are, however, not large, which indicates a substantial variation in the position of the galactose ring.

For DGDG, the fitting procedure was unsuccessful (unstable) for several of the galactose ring carbons (G1, G2′, and G4′) leading to varying and unphysical results, i.e., high order parameters (close to or 1) and very long local correlation times. This indicates a more complex dynamic behavior, which most likely involves anisotropic motion of the sugar unit. Nevertheless, comparing $S^2_{local,j}$ for DGDG (Figure 5, Table 3) with the relaxation data (Figure 3), one can clearly see also for this molecule that the overall trends closely follow the trends in relaxation parameters, indicating that the fitting procedure is a reasonable approach. The order parameters of the $^{13}C-^{1}H$ bond vectors vary significantly and together with the fact that data for three of the vectors could not be fitted in a stable way, this indicates that the motion of the digalactose unit is much more complex, which is most likely related to anisotropic headgroup motion, as also seen in MGDG. As for MGDG, $S^2_{local,j}$ for the acyl chains are very low, indicating substantial flexibility.

The order parameters for the galactose units and the glycerol are in both MGDG and DGDG relatively high, with $S^2_{local,j}$ ranging between 0.6 and 0.9. The different relaxation behavior observed for the exocyclic carbons in both MGDG and DGDG translates into relatively high order parameters (due to the small NOE) but very short correlation times for the local motion (Tables 2 and 3), indicating fast but restricted local motion. It is also worth noting that no significant differences in relaxation behavior or order parameters are observed for the exocyclic carbon G6 in MGDG compared to G6′ in DGDG. The acyl chains have very low order parameters, with $S^2_{local,j}$ in the range of 0.003−0.2 in both lipids. On the basis of our results, we cannot see substantial differences in the relative rigidity of the headgroup sugars or the acyl chains in the two lipids. Hence, differences in dynamics are not crucial for their membrane-forming properties.

CONCLUSIONS

We have carried out solution-state NMR analyses of novel small fast-tumbling bicelles with two types of galactolipids incorporated into the bilayer. Both the bilayer-forming DGDG and the nonbilayer prone MGDG are incorporated into DMPC/DHPC mixtures and they form relatively small (R_H < 5.4 nm) bicelles. Both the galactolipids and DMPC diffuse with the same rate in all bicelles, demonstrating that there is no phase separation under the conditions used here (0, 10, and 30% galactolipid at 25 °C).

Both galactolipids display varied local dynamics in bicelles, with two distinct dynamic regions: the headgroup and glycerol moieties on the one hand, and the acyl chains on the other. The galactose headgroups undergo limited local motion with order parameters ranging between 0.6 < $S^2_{local,j}$ < 0.9. Also the glycerol moiety is rigid on the nanosecond−picosecond time scale. Contrary to this, the acyl chains display extensive local motions (0.003 < $S^2_{local,j}$ < 0.2), as expected. For MGDG, it is possible to distinguish motional properties of the equatorial $^{13}C-^{1}H$ bond vector from the axial ones, while such a difference is not easily identified for DGDG. The exocyclic carbons in the galactose units of MGDG and DGDG have surprisingly high order parameters. Finally, the dynamics of the DMPC chains do not appear to be greatly affected by the incorporation of the galactolipids, and there is no difference between bicelles containing MGDG or DGDG. The bicelles that have been studied here, therefore, appear to be very well suited for use as model membrane mimetics for studies of, e.g., interactions between galactolipids and proteins.

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Notes

The authors declare no competing financial interest.

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