Biochimica et Biophysica Acta 1778 (2008) 2526–2534

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Lipid dynamics in fast-tumbling bicelles with varying bilayer thickness: Effect of model transmembrane peptides

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ARTICLE INFO

Article history:
Received 29 February 2008
Received in revised form 15 July 2008
Accepted 15 July 2008
Available online 22 July 2008

Keywords:
Bicelle
Peptide
Transmembrane
Dynamics
Relaxation
Diffusion

ABSTRACT

The morphology of $q=0.5$ fast-tumbling bicelles prepared with three different acyl chain lengths has been investigated by NMR. It is shown that bicelles prepared with DLPC (12 C) and DHPC are on average larger than those containing DMPC or DPPC (14 and 16 C) and DHPC, which may be due to a higher degree of mixing between DLPC and DHPC. The fast internal mobility of the lipids was determined from natural abundance carbon-13 relaxation. A similar dynamical behaviour of the phospholipids in the three different bicelles was observed, although the DPPC lipid acyl chain displayed a somewhat lower degree of mobility, as evidenced by higher generalized order parameters throughout the acyl chain. Carbon-13 relaxation was also used to determine the effect of different model transmembrane peptides, with flanking Lys residues, on the lipid dynamics in the three different bicelles. All peptides had the effect of increasing the order parameters for the DLPC lipid, while no effect was observed on the longer lipid chains. This effect may be explained by a mismatch between the hydrophobic length of the peptides and the DLPC lipid acyl chain.

1. Introduction

The action of membrane-associated peptides is often correlated with the membrane-induced structure of the peptide, but also on factors such as lateral diffusion and reorientational motion of both the peptide and lipids in the membrane. There are several NMR methods for elucidating the effect of peptides on membrane mobility and order. ^{2}H NMR of deuterated lipids and ^{31}P NMR have been used to determine the structure and phase properties of lipid mixtures [1–3], and the effect of bioactive peptides on lipid bilayers [4–8]. More recently ^{13}C–^{1}H dipolar couplings [9–11] of phospholipids in ordered bilayers or magnetically aligned bicelles have been reported. The effect of an antimicrobial model peptide on bicelle order has recently been determined [10] by the use of ^{13}C–^{1}H dipolar coupling in magnetically aligned bicelles.

It has been recognized since the 80s that mixtures of phospholipids and bile salts or detergents form soluble bilayers that at appropriate concentrations align in the magnetic field [12–16]. They have been used to characterize the interaction between peptides, proteins and the bilayer, including structural studies of integral membrane proteins [17]. Small ($q \leq 0.5$) isotropic bicelles have been used to investigate structure and membrane interaction of several peptides and as well as membrane proteins [18,19]. The bicelles have been shown to have several advantages as membrane mimetic media compared to e.g. micelles. For instance, the integral membrane protein diacylglycerol kinase retains its activity in bicelles, in contrast to in micelles [20]. Furthermore, the membrane protein bacteriorhodopsin refolds in both DMPC/CHAPS and DMPC/DHPC bicelles [21,22]. Bicelles have also been used successfully to crystallize membrane proteins, allowing X-ray crystallographic studies [23]. Hence, small isotropic bicelles (with low lipid to detergent ratios, q-values) are ideal for combining studies of structure and membrane interactions of peptides [24,25], since their tumbling is isotropic and the reorientational diffusion of the lipids is fast enough to give reasonable solution-state NMR spectra.

The morphology of small isotropic DMPC/DHPC bicelles has been studied in detail, including determination of geometry, dynamics as well as phase behaviour [26–29]. It has been shown that the small bicelles are disk-like objects, in contrast to the magnetically aligned nematic phases formed by lipid mixtures with higher q-values, for which more continuous phases are observed [30,31]. Several peptide structures in the presence of fast-tumbling bicelles have been reported [32–36], and it has been shown that peptides may adopt different conformations in the more disk-like bicelles as compared to in detergent micelles [33,34].

The effect of the peptides on the phospholipids in bicelles has, however, not been studied in that much detail. In most studies, bicelles with DMPC (14 C) as the phospholipid have been used. A few studies have been made on bicelles made with shorter or longer lipid chains, DLPC or DPPC, but in these cases it was of the magnetically aligned phase [37–40].

Abbreviations: NMR, nuclear magnetic resonance; CD, circular dichroism; NOE, nuclear Overhauser enhancement; DHPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphatidylcholine; DLPC, 1,2-dilauroyl-*sn*-glycero-3-phosphatidylcholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; TFE, trifuoroethanol

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doi:10.1016/j.bbamem.2008.07.010

The DMPC dynamics in bicelles [29] have previously been determined and it was shown that the lipid dynamics in the bicelle agree well with the dynamics in unilamellar vesicles obtained from EPR methods [41,42] as well as from NMR spin relaxation [43]. Recently we reported a carbon-13 relaxation study of the interaction between the lytic peptide melittin in small, fast-tumbling bicelles [44]. This study showed that natural abundance carbon-13 relaxation is a useful tool for investigating lipid reorientation. Spin relaxation rates carry information about dynamics on the ps–ns time-scale, which include overall lipid tumbling within the bicelle, as well as local dynamics for each measured site.

In this study we have investigated the lipid mobility in three different bicelles, containing phospholipids with varying chain length. In order to characterize the lipid chain dynamics we measured carbon-13 relaxation of the acyl chain carbons in bicelles containing DHPC as the detergent, and DLPC, DMPC or DPPC as the long-chained lipid. The three bicelle aggregates were further characterized with respect to their hydrodynamic sizes by NMR diffusion experiments.

In the present work we have investigated the effect of model transmembrane peptides on the lipid dynamics (on the ps–ns time-scale) in bicelles with varying bilayer thickness. The KALP series of peptides have been designed, originally by Killian and co-workers [45,46] to contain a stretch of alternating Leu and Ala residues, which provide model hydrophobic transmembrane segments of the peptide. The transmembrane segments are flanked by Lys residues. Detailed biophysical investigations of the effect of model transmembrane peptides on membrane properties have been performed in great detail. Several studies of the insertion of model transmembrane peptides into phospholipid bilayers have been reported, in which peptide-induced membrane effects, such as hydrophobic mismatch and effect of flanking residues have been investigated [47–55]. In particular ^{2}H and ^{31}P NMR methods in the ordered phase have been used to characterize effects of the model peptides on membrane properties.

In addition to having effect on acyl chain order and dynamics, peptides and transmembrane protein domains may have important effects on head-group orientation through interfacial interactions [54]. Typically aromatic (Trp) residues and positively charged amino acid residues (Lys) have been proposed to participate in head-group interactions [56]. The Trp residues have been shown to interact with the carbonyl groups in lipids, while Lys residues preferentially interact with the more polar region of the interface (e.g. phosphate group). In addition, it has been seen that Lys residues have less impact on lipid properties than Trp residues.

Here, we have studied the effect of two peptides with different hydrophobic length, KALP21 and KALP23 (Table 1), on the phospholipid dynamics in different bicelles. Natural abundance carbon-13 relaxation parameters were determined for DLPC, DMPC and DPPC in bicelles. Many peptides have severe effects on the integrity of membranes as well of vesicles, and may cause lipid phase transitions. Hence, we also investigated the effects of the peptides on bicelle size by pulsed field gradient diffusion experiments.

2. Materials and methods

2.1. Materials

The detergent, DHPC, and long chain lipids, DLPC, DMPC and DPPC were all purchased from Avanti Polar Lipids, Inc (Alabaster, AL, USA).

Table 1
Peptide sequences used

Peptide	Sequence
KALP21	Ac-GKK LALALALALALAL KKA-NH_2
KALP23	Ac-GKK LALALALALALALAL KKA-NH_2

Bicelles were prepared by mixing lyophilized phospholipids (either DLPC, DMPC or DPPC) with a stock solution of DHPC (1M) and phosphate buffer (final concentration 50 mM, pH 5). The samples were vortexed and heated to 50 °C, in several cycles, until all lipids were dissolved. All bicelle samples were prepared with a phospholipid/DHPC ratio of q=0.5 and the total lipid concentration (phospholipid+DHPC) was 300 mM.

The peptides used, KALP21 and KALP23, were made as custom solid phase syntheses. KALP21 was obtained from Neosystem, (Strasbourg, France), and KALP23 from EZBiolab Custom Peptide (Westfield, IN, USA). Lyophilized peptides were dissolved in TFE and added to the ready-made bicelle stock solutions. The samples were dried under a stream on nitrogen gas and were then freeze-dried and redissolved in H_2O in order to remove traces of TFE. This procedure was repeated twice. The TFE content in the samples was checked by carbon-13 NMR. After drying only, a significant amount of TFE was still present in the sample (comparable to the amount of peptide), but after two rounds of freeze-drying no traces of TFE were observed. The samples were prepared to give a peptide concentration of either 4 or 10 mM, which corresponds to a lipid/DMPC ratio of 1:25 or 1:10 respectively. For NMR samples, 10% D_2O was added for NMR field/frequency lock stabilization.

2.2. CD

CD spectra were recorded for samples containing peptide in 300 mM bicelle solution of either DLPC/DMPC, DMPC/DHPC or DPPC/DHPC (q=0.5). The CD spectra were obtained using a 0.2 mm quartz cuvette and measured over a temperature range of 13 to 51 °C in steps of 2 °C using a Jasco J-720 CD spectropolarimeter with a PTC-343 temperature controller. Wavelengths ranging from 200 to 250 nm were measured with a speed of 100 nm/min and a 0.2 nm step resolution. Background spectra of pure bicelle solution were also recorded and subtracted from the sample spectra.

2.3. NMR

Natural abundance carbon-13 relaxation measurements were performed on samples composed of pure bicelles (DLPC, DMPC or DPPC and DHPC) and on bicelle samples to which the KALP21 or KALP23 peptides had been added. For the DMPC/DHPC–peptide samples, the peptide concentration was 4 mM and 10 mM, while for the DLPC/DHPC and DPPC/DHPC–peptide samples the peptide concentration was 4 mM. To ensure that the lipids were in the liquid crystalline phase, the experiments for the DLPC and DMPC-containing samples were performed at 37 °C while the temperature was 45 °C for the DPPC-containing samples. The gel-to-liquid crystalline phase transition temperature, t_c, is −2 °C for DLPC, 24 °C for DMPC and 41 °C for DPPC [57].

The relaxation parameters T_1 and NOE were measured at two magnetic fields; 9.39 T (Bruker Avance spectrometer) and 14.09 T (Varian Inova spectrometer). T_1 was recorded using a standard one-dimensional direct carbon detection inversion-recovery experiment with a pre-acquisition delay of 5 s, and 10 relaxation delays ranging from 0.1 to 10 s. Steady-state NOE factors were measured from the intensity difference of spectra recorded with and without a 27 s pre-acquisition ^{1}H irradiation. The T_1 data were collected using 256–512 transients while typically 2000 transients were used for the NOE measurements. Relaxation data were evaluated for carbons 2, 3, 10 and 11 in DLPC, 2, 3, 12 and 13 in DMPC and for carbons 2, 3, 14 and 15 in DPPC. No attempt to separate the two acyl chains in the lipids was made, and average values for relaxation parameters for the two chains were used in the subsequent fit of the data.

To model the lipid dynamics in a bicelle, we used an extension of the classical model-free approach, originally stated by Wennerström and co-workers [58,59] and by Lipari and Szabo [60]. A lipid molecule

Table 2
Diffusion coefficients and calculated hydrodynamic radii for the bicelles

Sample	Temp (°C)	D^a (10^{-11} m^2/s) bicelle	$r_H{}^b$ (nm) bicelle	D^a (10^{-11} m^2/s) bicelle+KALP21	$r_H{}^b$ (nm) bicelle+KALP21
DLPC	25	4.4±0.1	5.6±0.1	3.0±0.1	8.2±0.1
	37	5.2±0.1	6.3±0.1	3.7±0.1	9.0±0.1
	45	5.5±0.1	7.1±0.1	3.9±0.1	9.9±0.1
DMPC	25	5.2±0.1	4.7±0.1	4.1±0.1	6.0±0.1
	37	6.4±0.1	5.1±0.1	5.0±0.1	6.5±0.1
	45	6.8±0.1	5.8±0.1	4.9±0.1	8.0±0.1
DPPC	25	6.1±0.1	4.0±0.1	6.2±0.1	4.0±0.1
	37	5.5±0.1	6.0±0.1	4.8±0.1	6.8±0.1
	45	6.3±0.1	6.2±0.1	4.5±0.1	8.6±0.1

[a] Normalized according to the diffusion of HDO to account for viscosity differences.
[b] Hydrodynamic radius as calculated from the DLPC, DMPC or DPPC diffusion coefficients.

in a membrane is free to rotate around its principal axis but the rotation around the other two axes is strongly restricted by the membrane surface. In order to accurately describe its motion, an order parameter for the entire lipid molecule, S^2_{lipid}, is required. This concept is the same for the local motion. In principle, one needs also to consider the overall tumbling of the entire bicelle aggregate. The predicted correlation time for an aggregate of the size of these bicelles (hundreds of ns) is however far too large to affect the spectral density and can thus safely be neglected in the evaluation of T_1 and NOE data [61]. Accepting these assumptions, a minimal spectral density function, given by [61,62]:

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

in which $1/\tau' = 1/\tau_{\text{lipid}} + 1/\tau_{\text{local}}$ was thus used. τ_{lipid} is here the correlation time for the reorientation of the lipid molecule within the bicelle and S^2_{lipid} is the generalized order parameter squared for this motion. The dynamics of lipids in bilayers have been determined experimentally for different lipids by carbon-13 and deuterium relaxation [61,63,64] as well as by EPR [41,42,65], and at least a qualitative agreement between the findings has been seen. Hence, these two parameters were kept fixed in the calculation [29], with values of $S^2_{\text{lipid}} = 0.36$ [61] and $\tau_{\text{lipid}} = 1.9$ ns [66–68] as determined previously. τ_{local} is the correlation time for the local motion for each measured site and S^2_{local} is the corresponding generalized order parameter squared for the local motion of each site.

Thus, an order parameter squared for the internal motion of each measured site in the lipids, together with a local correlation time for the internal motion was fitted using the program Matlab (version 7.0) as described previously [29].

Translational diffusion experiments were recorded on a Varian Inova 600 MHz spectrometer equipped with a triple resonance probe head. Measurements were made at 25 °C, 37 °C and 45 °C. Diffusion constants were measured using a modified Stejskal–Tanner spin-echo experiment [69–71] with a fixed diffusion time and a pulsed field gradient increasing linearly over 32 steps. In order to correct for possible field gradient inhomogeneity the intensity decline for every experiment was fitted to a modified version of the Stejskal–Tanner equation [72]. Separate resonances for the methyl groups in the long-chained lipids and for DHPC (0.88 ppm and 0.93 ppm respectively) can be observed in the proton spectrum, and these resonances were used to measure diffusion for the lipids and for DHPC (data not shown). Since the solubility of the long-chained lipids in water is negligible (unlike DHPC), these measured diffusion constants can be assumed to represent the diffusion of the lipid aggregates. The rotation of a bicelle occurs on a much shorter time-scale than the diffusion delay during an experiment (typically 150 ms), hence all lipid diffusion within the bicelle will be averaged to zero and the only motion reflected in the diffusion constants is the translational diffusion of the lipid bicelle aggregates.

Viscosities of the samples were estimated by measuring the H_2O diffusion rates and comparing them to standard values in water [73]. Viscosity-corrected diffusion constants could then be used to calculate hydrodynamic radii via the Stoke–Einstein relation. By assuming oblate-shaped bicelles with a fixed bilayer thickness, the Perrin equations for a shape factor could be used iteratively to calculate the radii of the bicelle disks [74]. A hydration layer with an estimated depth of 2.8 Å was included in the calculations.

3. Results

3.1. Bicelle size

The hydrodynamic sizes of the three bicelles, with lipids of different acyl chain lengths, 12 C (DLPC), 14 C (DMPC and 16 C (DPPC), were estimated from NMR diffusion data, together with the Stokes–Einstein relationship (Table 2). The data indicate an increase in the size of all three bicelles with temperature. For DMPC and DPPC, measurements were conducted both below and above the melting temperature of the lipid. No dramatic effects on the apparent bicelle size due to acyl chain melting were, however, observed at the transition temperature for either lipid. The data further clearly show that the aggregates obtained

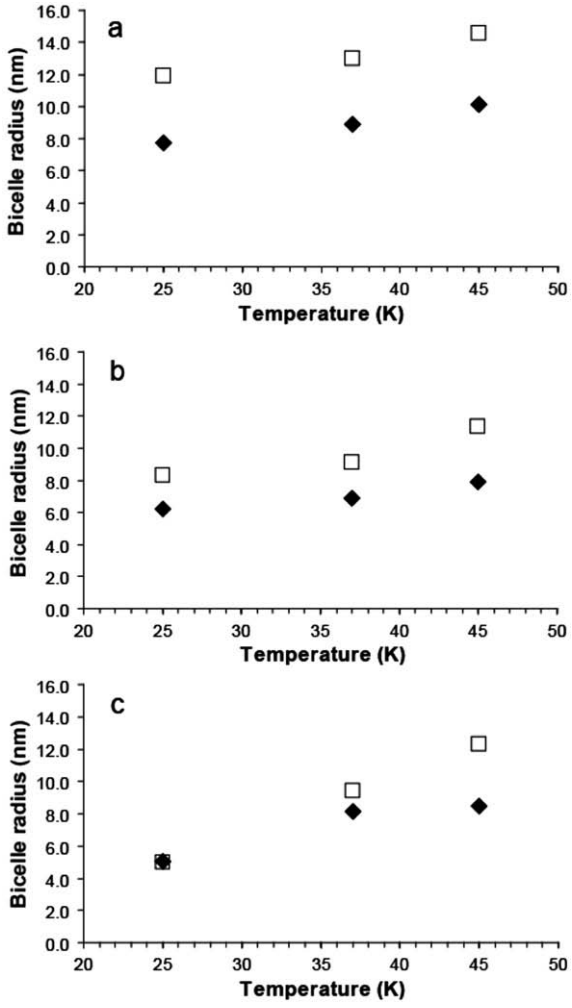

Fig. 1. Size of the bicelles as a function of temperature as determined by NMR diffusion with (open squares) and without (filled diamonds) the addition of 4 mM KALP21. (a) $q = 0.5$ DLPC/DHPC bicelles; (b) $q = 0.5$ DMPC/DHPC bicelles; (c) $q = 0.5$ DPPC/DHPC bicelles.

with the DLPC lipid are somewhat larger (slower diffusion, or larger hydrodynamic radius) than those obtained with DMPC and DPPC. This effect is likely to be related to the fact that the measurements for DLPC are made at a higher temperature relative to the gel-fluid phase transition temperature. Nevertheless, the measurements show that small well-defined bicelles are formed by all lipid mixtures, and that their size increases, as expected, with temperature.

The average values for the hydrodynamic radius over a temperature range of 25–45 °C for the bicelles are 6.3 nm (DLPC), 5.2 (DMPC) and 5.4 (DPPC). The values obtained at the temperatures used for the relaxation study, 37 °C for DLPC and DMPC and at 45 °C for DPPC bicelles, are 6.3 nm, 5.1 nm and 6.2 nm for the three bicelles respectively. Based on these values together with values for the bilayer thickness for the different lipids, we estimated the actual bicelle dimensions. The radii of the bicelles are presented in Fig. 1.

The effect on bicelle size of adding the KALP21 peptide, was also investigated by NMR diffusion measurements (Table 2, Fig. 1). The peptide makes all bicelles appear larger, especially the DLPC bicelles, but despite the increase in bicelle size, we still observe fairly small fast-tumbling bicelles after the addition of the peptide, which shows that the bicelles are stable after incorporation of the transmembrane peptide.

It should be mentioned that it is not straightforward to estimate true bicelle sizes, even though accurate measurements of diffusion coefficients can be made. Relating a measured diffusion rate to true bicelle size is complicated by the physical obstruction an aggregate experiences in its Brownian motion path due to the relatively high concentration of large neighbors. This obstruction effect can generally be dealt with by performing measurements at varying concentrations and extrapolating a diffusion constant at infinite dilution. This is however problematic for the complicated bicelle system where a change in concentration will affect the phase of the lipid mixture [26–28,30]. By normalizing measured values with respect to the diffusion of HDO in the sample, viscosity differences and obstruction are partly accounted for, and since the total lipid concentration is the same in all samples in this study the obtained diffusion constants can be used for a relative comparison between the samples.

Another property that affects the interpretation is that a non-spherical object will move slower than a perfect sphere of the same mass due to increased friction with the solvent, hence the symmetry of the aggregates should be accounted for. The coin-like shape of isotropic bicelles can be modeled by an oblate ellipse, and by applying the Perrin equations the deviation in motion from a sphere can be expressed as a shape factor [74]. In this model the bilayer thickness is held fixed with values taken from literature [75,76].

Despite these shortcomings, the diffusion coefficients here can be used to qualitatively define our bicelles as small, isotropic objects that agree with the previously defined disk-shape [26–28,30].

3.2. Bicelle–peptide interactions

To investigate the binding of the peptides to the different bicelles, CD spectra of the peptides in the three bicelle solutions were recorded (Fig. 2). Measurements were made at 37 and 45 °C, as these were the temperatures used for the relaxation studies. All peptides become highly helical, as expected, in all bicelle media, with some slight variations in signal intensity. The in general low CD signal for all samples is probably due to difficulties to incorporate the entire amount (4 mM) of peptide into the bicelles during the preparation step. The signal variations between the samples are neither dominant nor systematic and can be explained with concentration differences

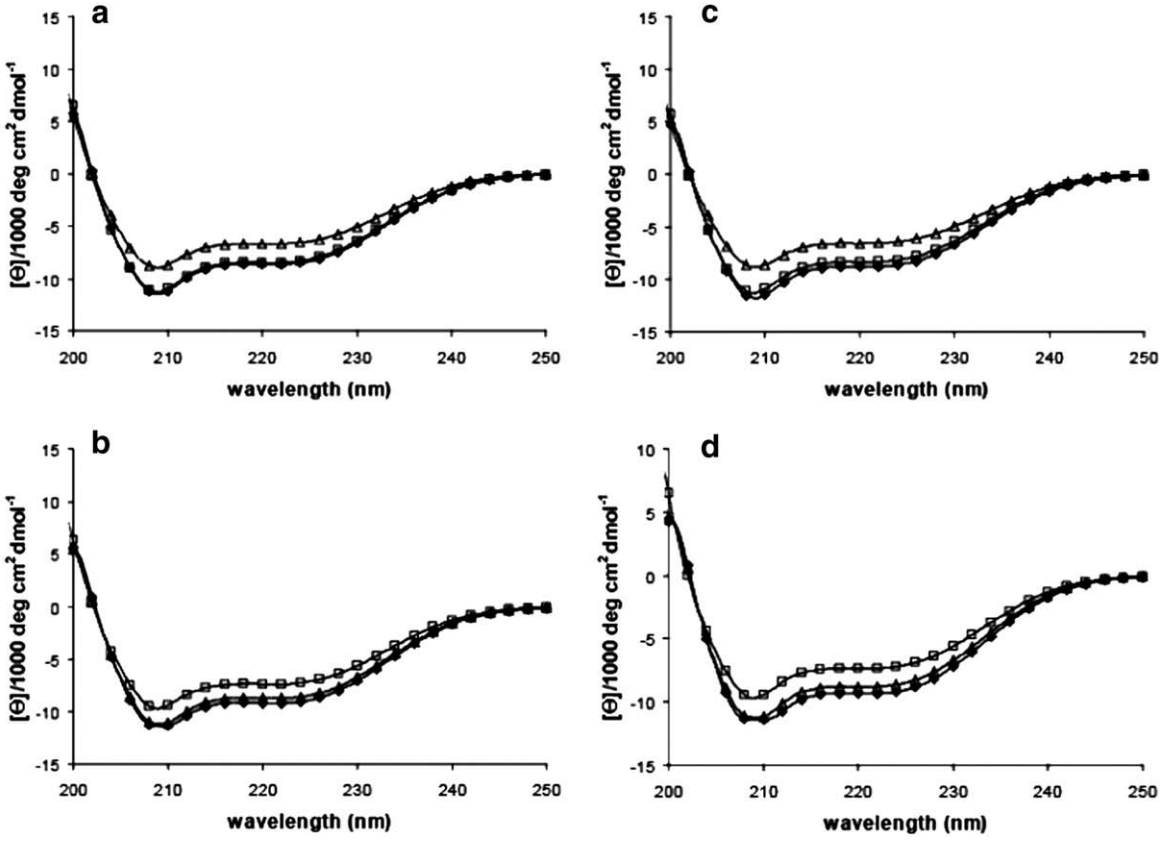

Fig. 2. CD spectra of the peptides (4 mM) in three $q=0.5$ phospholipid bicelle solvents (300 mM total lipid concentration) at 37 °C (a and b) and 45 °C (c and d). Panels a and c show the spectra for KALP21 in DLPC/DHPC (open squares), DMPC/DHPC (open triangles) and in DPPC/DHPC $q=0.5$ (open diamonds); panels b and d show spectra for KALP23 in DLPC/DHPC (open squares), DMPC/DHPC (open triangles) and in DPPC/DHPC $q=0.5$ (open diamonds).

Table 3
Model-free parameters[a] for the long-chained lipids in the different bicelles

Carbon[b]	DLPC/DHPC		DMPC/DHPC		DPPC/DHPC	
	S^2_{local}	τ_{local} (ps)	S^2_{local}	τ_{local} (ps)	S^2_{local}	τ_{local} (ps)
2	0.15±0.02	43±10	0.14±0.03	36±5	0.20±0.04	29±5
3	0.08±0.02	36±10	0.09±0.03	38±10	0.11±0.03	31±10
10/12/14[c]	0.03±0.02	11±5	0.03±0.03	11±5	0.03±0.02	10±2
11/13/15[c]	0.01±0.03	9±3	0.02±0.02	9±5	0.01±0.02	8±5

[a] $S^2_{lipid}=0.34$ and $\tau_{lipid}=1.9$ ns were kept constant during the fit.
[b] Since the chemical shifts of the *sn1* and *sn2* chains have not been assigned individually, average values are reported.
[c] Positions 10 and 11 refer to DLPC, 12 and 13 to DMPC, and 14 and 15 to DPPC.

rather than variations in secondary structure. All CD spectra indicate that the peptides contain only α-helical and random coil structures, as evidenced by a clear isodichroic point at 203 nm, in all bicelle media. Further, they show that the structure is stable over the temperature range 13 °C–51 °C (data not shown).

3.3. Lipid dynamics

To investigate the effect of the peptides on lipid dynamics we measured natural abundance carbon-13 relaxation of selected carbons in the lipids within the bicelle. The hydrodynamic radius of a $q=0.5$ bicelle, as determined from PFG-NMR diffusion, varies depending on the composition, between 5.1 and 6.3 nm at the temperatures used in the NMR relaxation study. Together with an estimate of the lipid size we can conclude that there are around 100–200 lipid molecules per bicelle. This gives us a bicelle concentration somewhere in the range of 0.2–0.5 mM, and thus, in the case of a peptide concentration of 4 mM, the bicelle to peptide ratio is around 1 to 10. As we could not in the PFG diffusion measurements detect any tendencies for the peptides to aggregate, or cause severe bicelle aggregation, we conclude that each bicelle contains a few peptide molecules that have the possibility to influence the lipid mobility.

Carbon-13 relaxation parameters depend on the molecular motion [77]. For a lipid this motion becomes quite complex, with the reorientation of the entire lipid, lateral motion within the bicelle, and tumbling of the entire bicelle [61]. Furthermore, the motion is clearly not isotropic. Nevertheless, attempts have been made to characterize lipid tumbling within bilayers, both from NMR relaxation data [29,43,61,63,64] as well as from EPR [41,42,65]. The R_1 and NOE relaxation data at two fields (9.4 and 14.1 T) are collected in Table 1, Supplementary material. The errors in the measured relaxation rates are judged to be less than 5% for both the NOE values and T_1 (as determined from at least two measurements of signal intensities).

As expected, the lowest NOE factors are observed for the carbons closest to the head-group region in all bicelles, indicating more restricted local motion at these sites [29]. Already from the relaxation data, we see a clear similarity in the dynamical behaviour of the three different lipids in the bicelles (Table 1, Supplementary material), with the notable exception of carbon two in DPPC, for which lower NOE values were seen than in the other lipids.

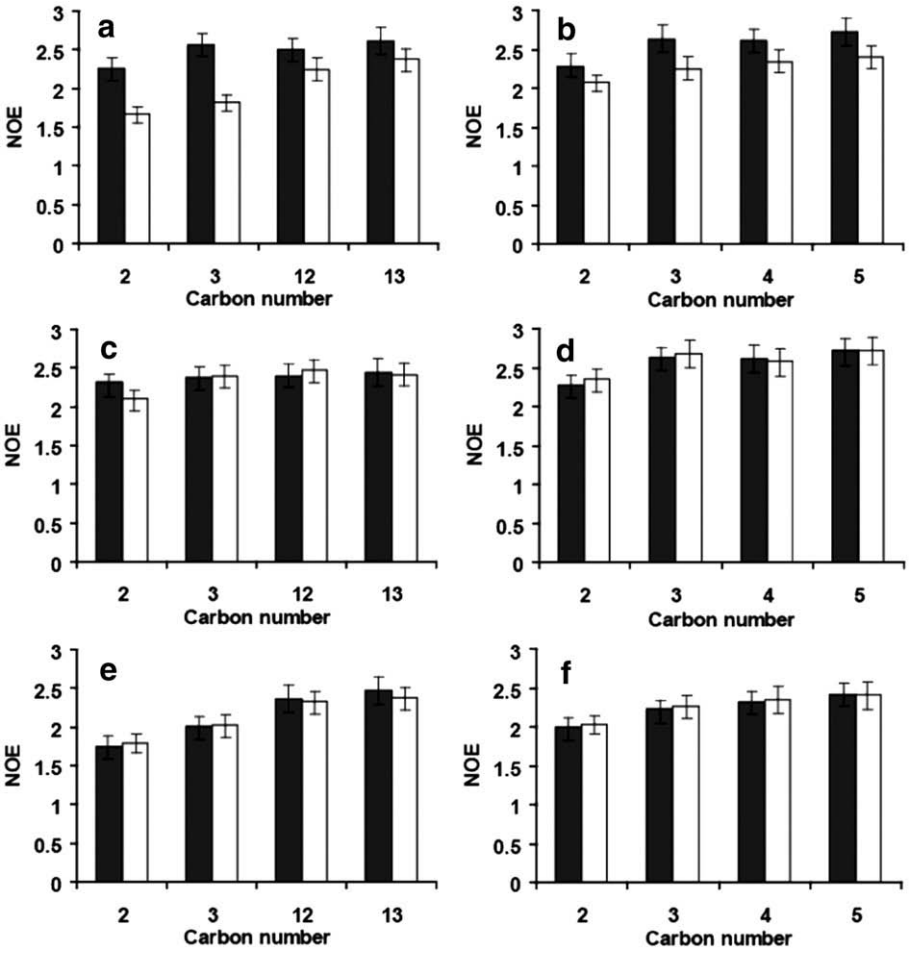

Fig. 3. $^{13}C-^{1}H$ NOE parameters recorded at 9.4 T for DLPC, DMPC, DPPC and for DHPC in $q=0.5$ bicelles with (filled bars) and without (open bars) the addition of 4 mM KALP21. Data for DLPC and DMPC were obtained at 37 °C and data for DPPC at 45 °C. Panels a and b show data for DLPC (a) and DHPC (b) in bicelles; panels c and d show data for DMPC (c) and DHPC (d) in bicelles; panels e and f show data for DPPC (e) and DHPC (f) with and without the peptide. Average values for the two acyl chains are reported.

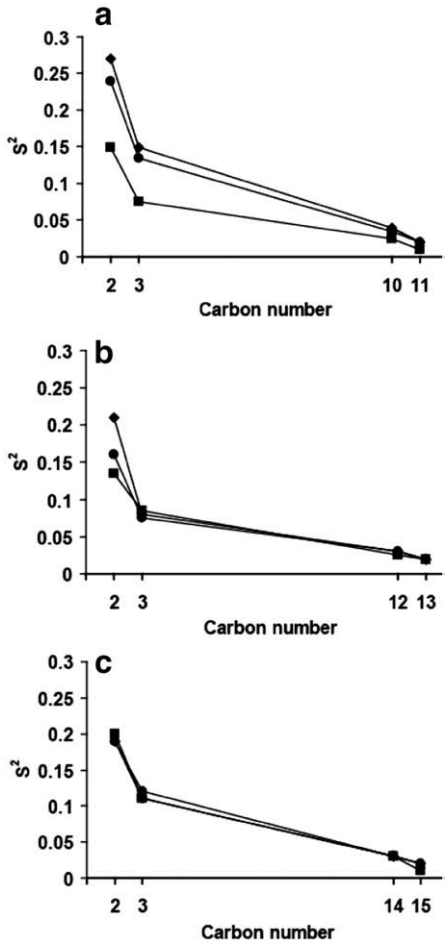

Fig. 4. Generalized order parameter squared, S^2_{local}, for selected positions in the acyl chain for DLPC (a), DMPC (b) and DPPC (b). Order parameters for pure bicelles are shown as squares, bicelles with the addition of 4 mM KALP21 as diamonds and 4 mM KALP23 as circles. Measurements for DLPC and DMPC bicelles were performed at 37 °C and at 45 °C for DPPC-containing bicelles.

From R_1 and NOE relaxation data at two fields (9.4 and 14.1 T), we fitted the model-free parameters S^2_{local} and τ_{local} according to Eq. (1). As noted in the Material and methods section, the fitting procedure may not be valid as relevant order parameters and correlation times for the reorientation of the entire lipid molecule in the bilayer may differ depending on the acyl chain length. A qualitative comparison between the different lipids can however be made. These parameters are collected in Table 3 for the bicelles containing the three different lipids, DLPC, DMPC and DPPC. The results show that the local dynamics are very similar for the DLPC and DMPC lipids, indicating that the acyl carbon chain length does not influence the extent of local motion displayed by the different lipids. Slightly higher order parameters are observed for DPPC, as seen already in the relaxation data (Table 1, Supplementary material). It should, however, be noted that the measurements for DPPC were conducted at a temperature closer to the gel-fluid phase transition temperature (45 °C compared to 41 °C) than what was the case for DMPC (37 °C compared to 24 °C) and DLPC (37 °C compared to −2 °C). The order parameters are low and decrease along the acyl chain, indicating extensive motion along the lipid chain in all lipids. This is in agreement with earlier results for DMPC-containing bicelles [29].

The effect on lipid dynamics of adding two KALP peptides with different length of the hydrophobic stretch (KALP21 and KALP23, Table 1) was also determined from relaxation data. The heteronuclear NOE parameter carries information about relative amplitudes of the internal mobility. In Fig. 3, the ^1H–^{13}C NOE parameters, measured at 9.4 T are shown for the lipids together with DHPC in the three different bicelles with and without the addition of KALP21. Starting with the DPPC/DHPC bicelles, we see no or little effect on the NOE parameter for any of the carbons in DPPC or for any of the carbons in DHPC when adding the peptide, indicating that the peptide does not have any significant effect on the internal motion of the lipid acyl chain.

For the DMPC-containing bicelle, we observe a decrease in the NOE for carbon 2, indicating a small perturbation of the DMPC dynamics. No significant effect is observed for any of the carbons in DHPC. In contrast, the NOE factors for carbons in DLPC as well as for carbons in DHPC in the DLPC/DHPC bicelles decrease significantly upon addition of KALP21. The effects are largest for carbons 2 and 3. These results clearly show that the peptide affects the dynamics of DLPC and to a certain extent also of the DHPC acyl chains.

Taking the analysis one step further, we fitted relaxation data from two magnetic field strengths in the same way as described for the pure bicelles above. As the NOE parameters indicated, the generalized order parameters for the carbons in DLPC are significantly increased by both the KALP21 and KALP23 peptides (Fig. 4a). For DMPC- and DPPC-containing bicelles, the order parameters are much less affected (Fig. 4b,c). An increase in S^2 for carbon 2 can however be observed in DMPC, most significantly by the addition of KALP21.

As the relaxation data indicated that the peptides only had a limited effect on the local dynamics in DMPC and DPPC, we increased the peptide concentration to 10 mM in DMPC-containing bicelles (Fig. 5). This resulted in a more pronounced increase in order parameters in the acyl chain. Both peptides were seen to affect the dynamics in a similar way, and significant increases in order parameters were found throughout the lipid chain. Hence, we conclude that the peptides indeed do affect also lipid dynamics in these bicelles, but to a much lower degree, since a higher peptide concentration is required.

We also evaluated relaxation data for DHPC in the three bicelles (Table 2, Supplementary material). The data indicate that the dynamics of DHPC are similar in all bicelles, and furthermore that the two KALP peptides have very little effect on the order parameters,

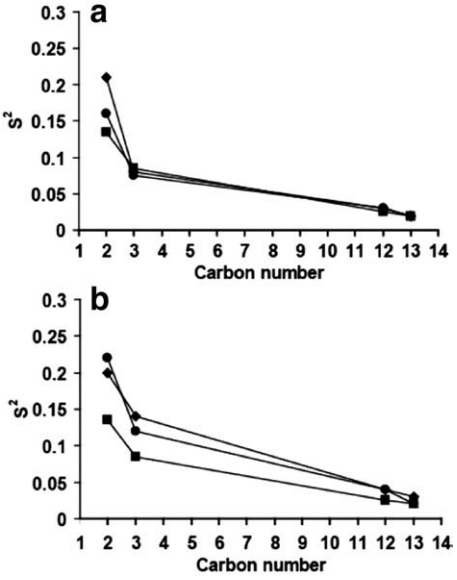

Fig. 5. Generalized order parameter squared, S^2_{local}, for selected positions in the acyl chain for DMPC in $q=0.5$ bicelles with and without the addition of 4 mM (a) and 10 mM (b) KALP21 and KALP23. The order parameters for pure bicelles are shown as squares and order parameters for bicelles with KALP21 as diamonds and with KALP23 as circles. The order parameters were calculated from relaxation data obtained at 9.4 and 14.05 T at 37 °C.

even if decreases in the NOE parameters were observed for DHPC acyl chain carbons in DLPC/DHPC bicelles (Fig. 3).

4. Discussion

In this study we have used bicelles composed of three different lipids with varying acyl chain lengths together with DHPC. All three mixtures form relatively small fast-tumbling aggregates, as shown by NMR diffusion experiments. The DLPC/DHPC aggregates are larger than the bicelles containing phospholipids with longer acyl chains. This observation may in part be due to the fact that the measurements for DLPC were carried out at higher temperatures relative to the gel-to-fluid phase transition temperature. However, the DLPC-containing bicelles are larger than what is observed for the DMPC/DHPC bicelles at any temperature, indicating that this cannot be the only explanation. It is also reasonable to assume that the size difference is related to a higher degree of mixing between the DLPC and DHPC than between DMPC or DPPC and DHPC. Several authors have indicated that there might be considerable mixing of DMPC and DHPC in small isotropic bicelles [28,78] and it is likely that an even higher degree of mixing would be observed for a lipid with shorter acyl chain length. Differences in mixing, and consequently in phase morphology, due to varying chain length has been implied in a study of DLPC, DMPC and DPPC bicelles, although this study was of magnetically aligned mixtures [40]. A higher degree of mixing would lead to a larger amount of molecules potentially participating in the bilayered part of the aggregate, which can make the aggregates larger.

The three lipids display somewhat different internal dynamics, as evidenced by differences in relaxation parameters (and in the generalized order parameters). There seems to be less flexibility in the DPPC acyl chain than what is observed for the other two lipids. It should however be noted that the measurements in this case were performed closer to the gel–liquid crystalline phase transition temperature.

It has previously been shown that the effect of the model transmembrane peptides, KALP, and WALP, on lipid order is highly dependent on the flanking residues [52]. For instance, the induced hydrophobic mismatch is much larger for the peptides, with flanking Trp residues (WALP) than what is seen for the Lys-containing peptides (KALP). Here we see that the KALP peptides only have a limited effect on the ps–ns dynamics of the acyl chains in DMPC and DPPC. A small increase in generalized order parameters for the DMPC molecules can be observed, most clearly for carbons close to the head-group region of the lipid (carbons 2 and 3, Fig. 4). This effect becomes more pronounced at elevated peptide concentrations. Similar effects are observed for all three peptides. The internal dynamics for the carbons in DPPC seem more or less unaffected by the addition of any of the peptides, which may indicate a different mode of association.

The bilayer thickness for the three lipids has been reported to be 19.5, 23 and 26 Å for DLPC, DMPC and DPPC [75,76]. The lengths of the central hydrophobic stretch in the two KALP peptides are 22.5 and 25.5 Å [50]. The shorter one, KALP21, matches almost perfectly with the bilayer thickness of a DMPC bilayer, while it is too short for the DPPC bilayer. The longer peptide, KALP23, matches well with the thickness of a DPPC bilayer, while it is somewhat too long for the DMPC bilayer.

Both peptides have too long hydrophobic stretches to match the thickness of the DLPC bilayer. The hydrophobic mismatch between a bilayer and a peptide with a longer hydrophobic stretch has previously been shown to induce an ordering in the lipids, as evidenced by ^{2}H NMR [48,51,79]. Killian and co-workers have shown that WALP peptides (with flanking Trp residues) induce a large degree of ordering in DLPC bilayers, while smaller effects were observed in thicker bilayers [49]. Here, we see an increase in the generalized order parameter for the acyl chain carbons in DLPC as a response to the incorporation of the KALP peptides, which is indicative of a reduction of the lipid internal dynamics. A recent study of two transmembrane cell-signaling peptides revealed that the tilt of the helices increased, rather than having an effect on lipid order and bilayer structure [80].

It is interesting to compare the order parameters derived from the present relaxation data and those obtained from analyzing ^{2}H quadrupolar splittings. This order parameter corresponds to $S_{CD} = S_{local} \cdot S_{lipid}$, and it has previously been observed that the generalized order parameters determined from relaxation (S_{local}) together with $S_{lipid} = 0.6$ agree well with what has been determined from ^{2}H quadrupolar splittings [1,29,36]. The increase in apparent order is in agreement with results for the antimicrobial peptide LL-37 in DMPC bilayers at approximately the same temperature, indicating a hydrophobic mismatch [81].

Virtually no effects were observed on the DPPC bilayer, which shows that the fast internal dynamics in the lipid is not affected by introducing the KALP peptides. Small increases in order parameters were seen for DMPC, which clearly shows that the peptides affect the bilayer less with increasing bilayer thickness. This implies that increasing the positive mismatch between the hydrophobic length of the peptide and the bilayer thickness has the effect of reducing the internal dynamics in the acyl chains.

In this study we have used natural abundance carbon-13 relaxation to investigate lipid acyl chain dynamics in three different bicelles with varying bilayer thickness. All lipid mixtures form stable fast-tumbling aggregates, which remain isotropic after the incorporation of model transmembrane peptides. This shows the potential for using bicelles with varying bilayer thickness as models for investigating peptide- and protein-membrane interactions. The dynamics of the lipids are clearly affected by the transmembrane peptides in the DLPC bilayer, in which the peptides have a longer hydrophobic stretch. The effect is smaller in DMPC bilayers while no effect is observed in DPPC. Carbon-13 relaxation provides an excellent tool for determining the extent of local dynamics, and in this study we have demonstrated the potential of using relaxation for investigating the internal dynamics of lipids, and the effect of membrane-associated peptides on the fast internal dynamics.

Acknowledgements

We wish to thank August Andersson for his help with the fitting routines. This work was supported by grants from the Swedish Research Council, The Carl Trygger Foundation and the Magnus Bergvall Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbamem.2008.07.010.

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