Biochimica et Biophysica Acta 1848 (2015) 760–766

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Membrane interactions in small fast-tumbling bicelles as studied by ^{31}P NMR

Andrea Bodor [a,*], Katalin E. Kövér [b], Lena Mäler [c,**]

[a] Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Loránd University, H-1117 Budapest, Hungary
[b] Department of Inorganic and Analytical Chemistry, University of Debrecen, H-4032 Debrecen, Hungary
[c] Department of Biochemistry and Biophysics, Stockholm University, 10691 Stockholm Sweden

ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form 11 November 2014
Accepted 1 December 2014
Available online 9 December 2014

Keywords:
Bicelle
Solution ^{31}P NMR spectroscopy
Relaxation study
Membrane protein
Dynamic light scattering

ABSTRACT

Small fast-tumbling bicelles are ideal for studies of membrane interactions at molecular level; they allow analysis of lipid properties using solution-state NMR. In the present study we used ^{31}P NMR relaxation to obtain detailed information on lipid head-group dynamics. We explored the effect of two topologically different membrane-interacting peptides on bicelles containing either dimyristoylphosphocholine (DMPC), or a mixture of DMPC and dimyristoylphosphoglycerol (DMPG), and dihexanoylphosphocholine (DHPC). KALP21 is a model transmembrane peptide, designed to span a DMPC bilayer and dynorphin B is a membrane surface active neuropeptide. KALP21 causes significant increase in bicelle size, as evidenced by both dynamic light scattering and ^{31}P T_2 relaxation measurements. The effect of dynorphin B on bicelle size is more modest, although significant effects on T_2 relaxation are observed at higher temperatures. A comparison of ^{31}P T_1 values for the lipids with and without the peptides showed that dynorphin B has a greater effect on lipid head-group dynamics than KALP21, especially at elevated temperatures. From the field-dependence of T_1 relaxation data, a correlation time describing the overall lipid motion was derived. Results indicate that the positively charged dynorphin B decreases the mobility of the lipid molecules – in particular for the negatively charged DMPG – while KALP21 has a more modest influence. Our results demonstrate that while a transmembrane peptide has severe effects on overall bilayer properties, the surface bound peptide has a more dramatic effect in reducing lipid head-group mobility. These observations may be of general importance for understanding peptide–membrane interactions.

1. Introduction

The membrane bilayer is a highly dynamic system with many motional modes. Lipid molecules undergo lateral diffusion within the bilayer [1–4], they rotate around their molecular axis, and possess several degrees of internal motion [5,6]. All these motions affect the interactions between a lipid molecule and a peptide or protein. Consequently it is of great interest to understand how proteins and peptides affect lipid properties including their dynamics. There are a variety of biophysical methods available that are suitable for studying these motions and the influence of proteins and peptides on lipid order and dynamics. NMR spin relaxation provides one way to examine the reorientational motion of individual lipid molecules, a parameter that may be critically altered by introducing components such as peptides to a bilayer.

Studies of dynamics, however, inevitably require suitable membrane mimicking models that are both realistic enough to properly reflect on true lipid bilayer motions, and at the same time provide simple enough systems to allow for detailed studies on a molecular level. Accordingly, small ($q \leq 0.5$) isotropic bicelles [7–9] have been developed for the purpose of investigating the behavior of peptides and membrane proteins in a membrane-like environment [10–15]. Bicelles are ideal for combining structural and membrane interaction studies, since their tumbling is isotropic and the reorientational diffusion of the lipids is fast enough to give reasonable solution-state NMR spectra for both the peptides and the lipids. In addition to the classical ^{1}H, ^{15}N and ^{13}C measurements used for structural studies of the membrane inserted (or bound) peptides, ^{31}P NMR [16] as well as ^{2}H NMR [17,18] have for a long time been used to determine the phase properties of lipid mixtures, including magnetically aligned bicelles [9,12,19–22], and to characterize the effect of bioactive peptides on lipid bilayers [11,23–25]. Several studies have been reported by Killian and coworkers [26,27] on the insertion of model transmembrane peptides into phospholipid bilayers in which peptide-induced membrane effects, such as hydrophobic mismatch and effect of flanking residues have been investigated [28–37]. The acyl chain dynamics in fast-tumbling bicelles and their dependence on interactions with peptides and proteins have earlier been studied by ^{13}C NMR relaxation methods [38,39] and the dynamics of phosphate head-groups in lipids have previously been studied by ^{31}P NMR relaxation [40–42] or a combination of NMR methods and molecular

* Corresponding author. Fax: +36 1 3722620.
** Corresponding author. Fax: +46 8 155597.
E-mail addresses: abodor@chem.elte.hu (A. Bodor), lena.maler@dbb.su.se (L. Mäler).

http://dx.doi.org/10.1016/j.bbamem.2014.12.001
0005-2736/

dynamics simulations [43,44]. To date, however, no attempts have been made to correlate the location of peptides with the effects that they have on lipid mobility or on assembly properties. To address this subject we have explored the approach of using a combination of ^{31}P relaxation and dynamic laser light scattering to characterize the effect of two topologically different peptides on the head-group dynamics and lipid assembly properties in zwitterionic DMPC/DHPC (hereafter denoted PC), and partly negatively charged (DMPC + DMPG)/DHPC (hereafter denoted PC/PG) bicelles. We examined the influence of two membrane-active peptides: the surface-bound neuropeptide dynorphin B (DynB) and the model transmembrane peptide KALP21.

Dynorphins, including DynB, derive from prodynorphin [45] and are primarily endogenous ligands to the κ-opioid receptor, but due to direct membrane interactions, they have non-opioid functions as well [46–50]. It has been demonstrated that the basic 13 residue long DynB, with the sequence YGGFLRRDFKVVT, interacts with different bilayers and resides on the bilayer surface, but remains relatively unstructured in the membrane-bound form [50–53]. The KALP peptide series were designed to contain a stretch of alternating leucine and alanine residues, providing a model hydrophobic transmembrane segment, flanked by lysine residues [26,32]. The 21 residue long KALP21, with the sequence Ac-GKKLALALALALALALALKKA-NH_2, has been shown to insert in a transmembrane fashion in DMPC bilayers, with no or little mismatch [27,37,54]. Both peptides contain a large number of positively charged residues, mostly arginines, and both have positive net charges (+2 for DynB and +4 for KALP21). Therefore, it is also of interest to examine the effects of these membrane-active protein fragments not only on zwitterionic (PC), but also on partly negatively charged bilayers (PC/PG).

The very different peptides chosen in this study are representatives for two ways in which peptides can interact with membranes, either located at the surface or inserted into the bilayer in a transmembrane configuration. Results based on ^{31}P T_1 and T_2 relaxation time data and dynamic light scattering demonstrate that there are large differences in how they affect lipid properties, such as overall reorientational dynamics of the lipid molecules and bicelle size. The data clearly suggest a correlation between the location of a peptide with respect to the bilayer and the effect that it has on lipid properties. Moreover, this correlation demonstrates that ^{31}P relaxation of phospholipids can be used to discriminate between a transmembrane and a peripheral configuration of a membrane-interacting peptide.

2. Materials and methods

2.1. Materials

Synthetic peptides corresponding to either the KALP21 or DynB amino acid sequence were obtained from PolyPeptide Group (Strasbourg, France) and used without further purification. Phospholipids, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (DMPG) and 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) were purchased as powder from Avanti Polar Lipids (Alabaster, AL, USA). All the lipids were used without further purification.

2.2. Preparation of bicelles

Solutions containing fast-tumbling bicelles were produced by mixing the appropriate amount of DMPC, or DMPC/DMPG (8:2) carefully with H_2O by vortexing and centrifuging the sample repeatedly until a homogeneous slurry was formed, and then adding an aliquot of a 1 M DHPC stock solution to obtain the molar ratio [DMPC] / [DHPC] = 0.5 (for PC bicelles) or [DMPC(0.2) + DMPG(0.8)] / [DHPC] = 0.5 (for PG bicelles) as described earlier [15,55,56]. This mixture was subjected to several cycles of heating (up to 45 °C) and cooling (to room temperature) and to gentle vortexing, until a clear non-viscous solution was obtained. The total concentration of DMPC and DHPC, or DMPC + DMPG and DHPC, was 150 mM (c_L (w/v) ≈ 8%). The pH was in all cases adjusted to 6.5–6.7 with 50 mM HEPES buffer.

For the samples containing the peptide, the necessary amount of lyophilized peptide (powder) was measured by weight and mixed with the bicelle solution. This mixture was subjected to freezing and lyophilization, after which it was dissolved in distilled H_2O. DynB samples resulted in clear, transparent solutions under all experimental conditions, while KALP21 samples of higher than 1 mM concentration could not be prepared with the charged bicelles, due to a permanent formation of precipitate. According to the GRAVY (Grand average of hydropathicity) solubility index of proteins, DynB has a value of −0.108, while KALP21 has 1.371. The positive number indicates a requirement of a hydrophobic environment, and the negative value a hydrophilic environment. Therefore the highly hydrophobic KALP21 was difficult to solubilize in negatively charged (PC/PG) bicelles above a concentration of 1 mM. NMR samples had the typical volume of 550 μl containing 10% D_2O.

2.3. Dynamic laser light scattering measurements

The size of the bicelles was determined with dynamic light scattering over a temperature interval of 25–45 °C. Measurements were performed on an ALV/CGS-3 instrument equipped with a Light Scattering Electronics and Multiple Tau Digital correlator ALV/LSE-5004, using a 5 mm glass cuvette. Light scattering data were collected for 10–30 s and repeated 10–30 times and were exported as autocorrelation functions. The results are presented as hydrodynamic radii, R_h, by relating derived decay constants via the Stokes–Einstein relationship:

$$R_h = \frac{k_B T}{6\pi\eta D} \quad (1)$$

where k_B is the Boltzmann's constant, T is the absolute temperature, η is the viscosity of the sample and D is the diffusion coefficient of the particles. For monodisperse particles undergoing Brownian motion D is obtained from the decay rate, Γ, of the autocorrelation function from DLS, given by $\Gamma = Dq^2$, where q (not to be confused with q = [DMPC] / [DHPC]) is the magnitude of the scattering wave vector. $q = (4\pi n/\lambda)\sin(\theta/2)$ is determined by the wavelength of the light source, λ, the refractive index of the medium, n, and the scattering angle θ. Data were processed with the software provided by the manufacturer.

2.4. ^{31}P NMR measurements

Measurements were performed on a Bruker Avance spectrometer (9.4 T) operating at 162.0 MHz for ^{31}P, equipped with a 5-mm BBO probe-head and a 500 DRX spectrometer (11.7 T) operating at 202.5 MHz frequency for ^{31}P using a 5-mm BBI probe-head. ^{31}P chemical shifts were referenced to an external 85% H_3PO_4 standard and temperatures were checked with the ethylene glycol standard. Chemical shift values and integrated intensities were determined from one-dimensional inverse gated $^{31}P\{^1H\}$ spectra.

T_1 measurements were recorded using the standard inversion recovery pulse-sequence with power gated decoupling in pseudo-2D mode. A typical set of 14 spectra with delays varying between 1 ms–6 s was acquired, and at least 3 sets of experiments were performed. T_2 measurements were performed using the Carr–Purcell–Meiboom–Gill pulse-sequence with power gated decoupling in pseudo-2D mode. Typically 12 spectra were collected with delays between 0.03 s and 0.58 s, and at least 3 sets of experiments were acquired. The delay between scans was 8 s and 32 scans were recorded using a 20 ppm spectral window for both T_1 and T_2 measurements. All relaxation measurements were performed at three temperatures: 25 °C, 42 °C and 59 °C.

Data evaluation was done using the relaxation module of the TopSpin software. Intensity values were fitted to the $I(t) = I(0)[1-2A\exp(-t/T_1)]$ equation for T_1 evaluation, and integrated intensities were fitted to

the $I(t) = I(0)\exp(-t/T_2)$ equation for T_2 evaluation. For all relaxation times the error is given by the standard deviation of the values obtained in the different experiments.

3. Results

3.1. Effect of KALP21 and DynB on bicelle size

Dynamic laser light scattering was used to measure the relative sizes of the two different bicelles, PC and PC/PG (Fig. 1). Both of the bicelle types were observed to have a hydrodynamic radius between 3.5 and 4.5 nm in the 25–45 °C temperature region. Above the melting temperature (around 24 °C) of the acyl chains in both PC and PG bicelles, a minor increase in bicelle size was detected with elevating temperature. This phenomenon can be attributed to the increased motion of the acyl chains leading to the formation of larger bicelles, and it is in accordance with previous observations [57]. Upon addition of 2 mM DynB to the zwitterionic/neutral PC bicelles (Fig. 1A), a modest increase of the apparent hydrodynamic radius (from 3.4 to 3.8 nm) was observed at 25 °C, but at higher temperatures the difference in bicelle size with and without peptide became negligible. This is an indication that only minor rearrangements of the lipids occur in this temperature range, and therefore that DynB has only small effect on the bicelle size. Contrary to these results, the addition of KALP21 had a more dramatic effect on bicelle size, as an increase of around 70% in size was detected (at 25 °C from 3.3 to 5.6 nm, and at 45 °C from 4.4 to 7.3 nm, respectively). This finding is in agreement with previous studies conducted on the influence of KALP peptides on bicelle size [54].

The addition of 2 mM DynB to the negatively charged PG bicelles had a different effect than on PC bicelles (Fig. 1B). The size of the PG bicelles increased at all temperatures with 12–13%. This shows that although it has previously been established that DynB interacts with both types of lipids [51], the interaction hugely differs, and is more accentuated with bicelles containing anionic lipids. It was not possible to incorporate KALP21 into PC/PG bicelles at higher concentrations than around 1 mM, but also at this concentration the PC/PG bicelles increased in size, although not as much as when adding 2 mM to the PC bicelles (data not shown).

In conclusion, the DLS measurements revealed that there is a difference in the way that the two peptides influence PC and PC/PG bicelles. The surface-bound DynB has a larger effect on the size of PC/PG bicelles than on PC, but overall the size increase caused by DynB is modest. In contrast, the transmembrane KALP21 induces a much larger increase in bicelle size.

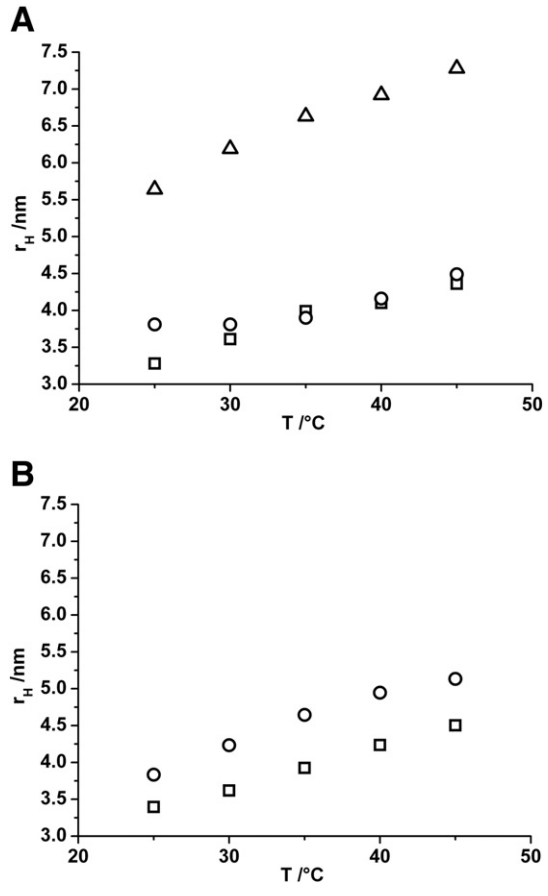

Fig. 1. Hydrodynamic radii measured from dynamic laser light scattering for PC (A) and PC/PG (B) bicelles, where squares indicate bicelles without peptides, spheres bicelles with 2 mM DynB and triangles bicelles with 2 mM KALP. The total lipid concentration (DMPC/DMPG and DHPC) was 150 mM and q = 0.5. Errors were estimated from at least 20 individual measurements for each temperature, and in most cases the errors are smaller or comparable to the symbol sizes.

3.2. Effect of KALP21 and DynB on lipid motion–^{31}P longitudinal relaxation

To investigate the motion of the phosphate head-group in the lipids, and the effect of different membrane interacting peptides on this motion, ^{31}P longitudinal relaxation measurements were performed. These parameters carry information about the effective overall motion of the phosphate moiety in individual lipid molecules, and in order to characterize it in a more complex way both the temperature and the magnetic field dependence of relaxation properties were investigated.

Under the given experimental conditions all systems show distinct and well defined resonances in the one-dimensional $^{31}P\{^1H\}$ spectrum (Fig. 2). Typical values of the phosphate chemical shifts at room temperature for a system containing all three phospholipids, DHPC, DMPC and DMPG, were: −0.83, −0.89 and 0.22 ppm, respectively. ^{31}P-chemical shift values are very sensitive to the environment, and even though both molecules have the same polar head group, the other parts differ: 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC). Moreover, previous studies have shown that the chemical shift difference is also evidence that the two molecules are separated in the bicelles, i.e. DHPC on the rim and DMPC mainly in the bilayer part of the bicelle [9]. Therefore, a small, but clearly detectable 0.06 ppm difference is observed between the two head groups. At higher temperatures bicelles containing KALP21 show an accentuated line broadening, and as a consequence the DMPC and DHPC resonances partly overlap. For this reason, integrated intensity values are used for data evaluation. The results of the

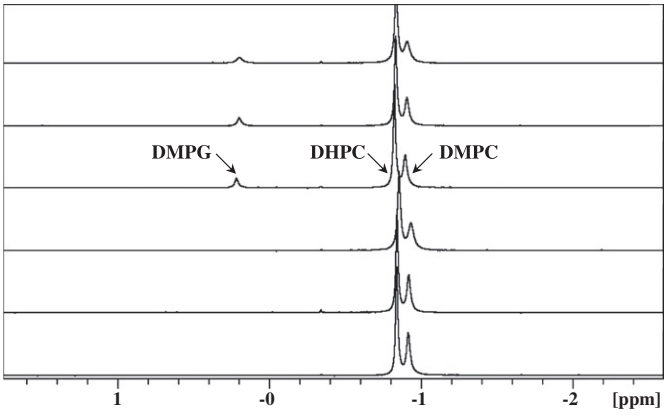

Fig. 2. $^{31}P\{^1H\}$ NMR spectra of (from bottom): PC bicelles, 2 mM Dyn in PC bicelles, 2 mM KALP in PC bicelles, PC/PG bicelles, 2 mM Dyn in PC/PG bicelles, and 1 mM KALP in PC/PG bicelles.

T_1 measurements are shown in Figs. 3 (PC bicelles) and 4 (PC/PG bicelles), and in Table S1 (Supplementary data). As expected, the T_1 values were for all samples longer at lower fields and at higher temperatures. The DHPC head-group showed the slowest relaxation in all cases, i.e. longest T_1 values, while DMPG and DMPC had very similar relaxation properties. These characteristics indicate that the presence of either anionic or zwitterionic head-groups does not influence motions that affect T_1.

The addition of DynB or KALP21 to zwitterionic PC bicelles caused only minor changes in the T_1 relaxation parameters (Fig. 3). A small decrease in T_1 values was induced by both peptides at the highest temperature, 59 °C (from 1.43 s to 1.32 s for DMPC, and from 1.82 to 1.70s for DHPC) indicating only a modest change in the overall dynamics of the lipid head-groups. For anionic PC/PG bicelles, however, there was a larger effect on the T_1 values for all lipids at the highest temperature for both peptides, but mostly so from DynB added (Fig. 4). The shorter T_1 values upon the addition of the peptides indicate a restriction of the phosphate head-group dynamics, which is not surprising since both peptides contain positively charged residues that interact favorably with anionic lipids [53,58–60]. Moreover, the ^{31}P longitudinal relaxation is somewhat faster for bicelles containing DynB than those containing KALP21, especially so for the DMPG ^{31}P moiety. This result indicates that despite the fact that DynB contains only three positively charged residues, with an overall net charge of +2, compared to the four positively charged residues of KALP21, the surface-associated DynB has a greater effect on lipid mobility than the transmembrane KALP21.

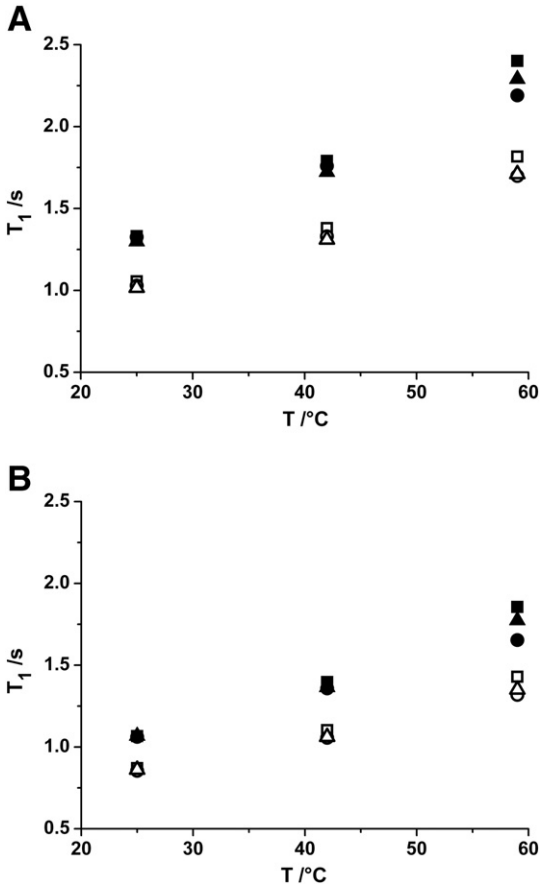

Fig. 3. T_1 relaxation time constants for ^{31}P in 150 mM, q = 0.5 PC bicelles at three temperatures and two magnetic fields. Filled symbols represent values at 9.4 T, open symbols 11.7 T; squares indicate bicelles without peptide, circles bicelles with 2 mM DynB and triangles bicelles with 2 mM KALP21; A) shows relaxation times for the DHPC head-group and B) for the DMPC head-group.

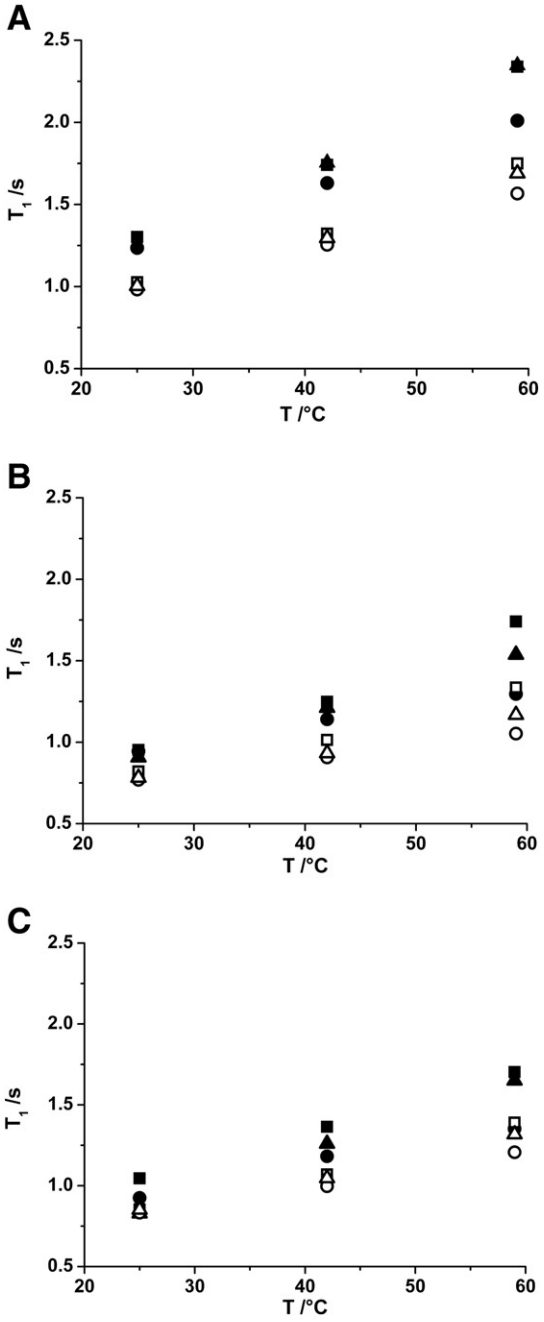

Fig. 4. T_1 relaxation time constants for ^{31}P in 150 mM, q = 0.5 PC/PG bicelles at three temperatures and two magnetic fields. Filled symbols represent values at 9.4 T, open symbols 11.7 T; squares indicate bicelles without peptide, circles bicelles with 2 mM DynB and triangles bicelles with 1 mM KALP21; A) shows relaxation times for the DHPC head-group, B) for the DMPC head-group and C) for the DMPG head-group.

3.3. Effect of KALP21 and DynB on lipid motion–^{31}P transverse relaxation

In addition to being sensitive to the overall motion of the lipid molecules, transverse relaxation also reports on the size of bicelle assemblies. ^{31}P transverse relaxation time values were determined for the two bicelle systems with and without the model peptides. The data are collected in Table S2 and in Figs. 5 (PC bicelles) and 6 (PC/PG bicelles). The transverse relaxation rates measured for bicelles alone were almost tenfold faster than the longitudinal relaxation rates, indicating a significant effect of bicelle size on T_2. When adding either of the peptides to zwitterionic bicelles, we noted significant line-broadening in the spectra indicating a change in T_2, and mostly so

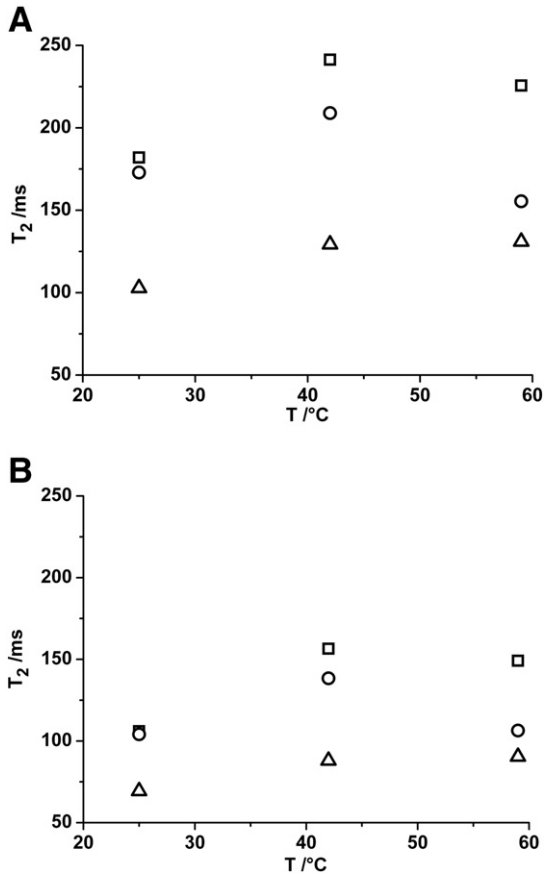

Fig. 5. T_2 relaxation time constants for ^{31}P in 150 mM, q = 0.5 PC bicelles. Squares indicate bicelles without peptide, circles bicelles with 2 mM DynB and triangles bicelles with 2 mM KALP21; A) shows relaxation times for the DHPC head-group and B) for the DMPC head-group.

when adding KALP21 (Figs. 2, 5 and 6). A dramatic decrease in T_2 values was induced by KALP21 in both zwitterionic as well as in anionic bicelles, a finding that clearly supports the DLS results, which showed that the model transmembrane helix had a large effect on bicelle size (Fig. 1). As for T_1, DynB was observed to affect T_2 of the lipids in PC/PG bicelles more than in PC bicelles (Fig. 6), again in agreement with the DLS results that indicated an increase in PC/PG bicelle size, but not in PC bicelle size. Moreover, the relaxation of the phosphorus moiety in DMPG in anionic bicelles is greatly affected by both peptides.

4. Discussion

By applying the two methods, dynamic light scattering and ^{31}P NMR spectroscopy we characterized the behavior of membrane bound model peptides and their effect on lipid motion. ^{31}P NMR longitudinal relaxation can be used as a method to characterize the local environment of the phosphorus moieties, i.e. the lipid head-groups, and the dynamics of the bicelle system in which the phosphorus nucleus is embedded. It has previously been demonstrated that dynorphins interact with lipid bilayers in a charge-dependent way, and that they reside on the surface of the bilayer. Qualitatively our present results indicate that this surface-interaction of DynB is coupled to a decrease in the lipid head-group motion, while it has negligible effect on the overall morphology of the bicelles. Contrary to the surface-active peptide DynB, KALP21 induces a significant increase in bicelle size and at the same time has a much smaller effect on lipid mobility in the bilayer, demonstrating that a transmembrane peptide that does not induce a mismatch, does not affect lipid head-group motion to the same extent as a surface-bound peptide.

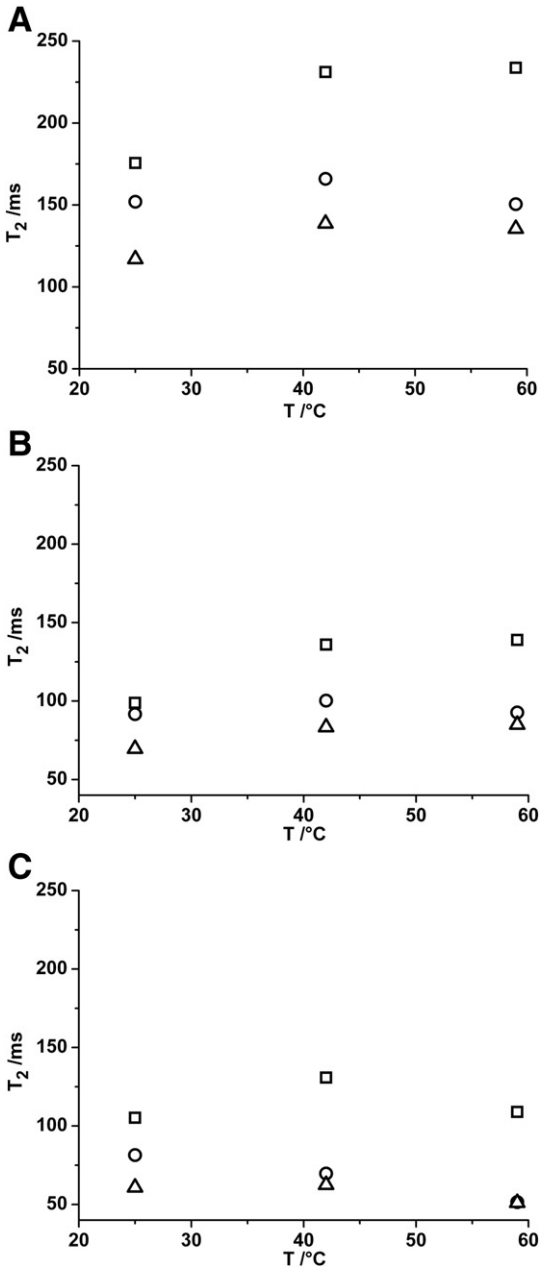

Fig. 6. T_2 relaxation time constants for ^{31}P in 150 mM, q = 0.5 PC/PG bicelles. Squares indicate bicelles without peptide, circles bicelles with 2 mM DynB and triangles bicelles with 1 mM KALP21; A) shows relaxation times for the DHPC head-group, B) for the DMPC head-group and C) for the DMPG head-group.

To obtain further insights into the dynamics and thermodynamics of motions at molecular level relaxation data were analyzed in more details. Under the applied experimental conditions different relaxation mechanisms contribute to the relaxation process. For the spin half ^{31}P nucleus the following relaxation mechanisms need to be considered: (a) the nuclear dipole–dipole (DD) interaction, (b) the anisotropic chemical shielding (CSA), (c) spin rotation (SR) and (d) scalar coupling (SC) between the ^{31}P nucleus and the magnetic nuclei in the neighborhood/solvation shell. If several of these routes are operative, they assumed to be additive:

$$\frac{1}{T_1} = \frac{1}{T_1^{DD}} + \frac{1}{T_1^{CSA}} + \frac{1}{T_1^{SR}} + \frac{1}{T_1^{SC}}. \quad (2)$$

The contribution by spin-rotation and scalar coupling mechanisms can safely be neglected. Milburn and coworkers proved that at higher frequencies i.e. 145.7 MHz and above, thus under our experimental conditions (being 162 and 202 MHz, respectively), ^{31}P relaxation is dominated by the chemical shielding anisotropy mechanism [61]. This assumption is also supported by the fact that with increasing field strength at constant temperature a decrease in relaxation time is observed (see Figs. 3 and 4).

The time constant of longitudinal relaxation by chemical shielding anisotropy is given by:

$$\frac{1}{T_1^{CSA}} = \frac{2}{15}\gamma^2(\Delta\sigma)^2 B_0^2 \tau_c \quad (3)$$

where $\Delta\sigma$ is the chemical shielding anisotropy, γ is the ^{31}P magnetogyric ratio, B_0 is the magnetic field and τ_c is the effective local correlation time for the reorientation of the phosphate group. On the basis of Eq. (3) and using $\Delta\sigma = 50$ ppm [61], the τ_c rotational correlation time of the phosphate head-group can be estimated (Table S3). Assuming that the molecular motion responsible for phosphorus relaxation is isotropic and so described by a single τ_c, this should present an Arrhenius type temperature dependence, allowing the assessment of the activation energy for the reorientation of the phosphate moiety with respect to the glycerol backbone (Table S4). Due to the relatively high errors in the estimates of the correlation times for PG because to the higher errors in the relaxation data (since PG is only present in relative amounts of 0.2:0.8) we limited this analysis to PC bicelles.

Our results for PC bicelles indicate that the rotational correlation time values decrease with temperature increase, as is also expected. At 298 K there is no significant difference among τc values obtained for pure bicelles, or after adding either of the two peptides, all being around 1 ns, meaning that the head-group behavior is very similar for all systems. Calculating the activation energy values for the reorientation of the head-groups we observe that the reorientation of the DHPC head-group necessitates a higher energy in all cases. The difference may be related to a different positioning of the two molecules within the bicelle (DHPC on the rim and DMPC in the bilayer region), although this is not evident from an analysis of relaxation data alone. No big changes in the activation energies for the lipids in PC bicelles are observed upon adding KALP21 (from 9.2 to 8.7 kJ/mol for DHPC and 6.4 to 5.7 kJ/mol for DMPC respectively). By adding DynB, on the other hand, the corresponding values become higher (11.8 and 9.4 kJ/mol). This is in accordance with the earlier observation that the surface-bound DynB peptide alters the dynamics. Previous studies performed at lower fields and broader temperature ranges indicate an activation energy of 16.0 kJ/mol for larger lipid molecules with different types of acyl chains than used here [61]. However, it has to be noted that this approach is an oversimplification, as it does not include other than isotropic reorientational motion of the head-group of the lipid molecule. It also assumes that the extreme narrowing condition prevails, which is reasonable at low fields.

Regarding the other limit of the motional time-scale for bicelles, DLS measurements shed light on the motion of the entire bicelle through the estimated hydrodynamic radii. In addition, using the Stokes–Einstein formula, the effective global correlation time of the bicelle can be calculated. This value falls in the 35–182 ns range depending on the investigated peptide–bicelle system. The lowest values are obtained for neat bicelles, and the highest for the KALP21–bicelle system. Note that in all cases the spherical assumption was used. The ^{31}P T_2 relaxation data also support the observations made from the DLS data. The large decrease in T_2 observed when adding e.g. the transmembrane KALP21 indicates a much larger change in dynamics than that observed in the T_1 data, supporting an increase in bicelle size. However, it is not trivial to draw quantitative conclusions about the size increase, but both methods provide direct evidence of this phenomenon, suggesting that the main effect of the transmembrane peptide is to alter the morphology of the bicelle.

Two important conclusions can be made from this study. The first is that the peptides may alter either the lipid dynamics (as does DynB) or the morphology of the bicelle (as evidenced by the increase in size caused by KALP21). This has previously been observed, although not directly, for a surface-associated cell penetrating peptide, transportan [14].

The second conclusion is of a more general nature and concerns the effect of surface-bound peptides versus transmembrane peptides. DynB has previously been demonstrated by a variety of methods to be located at, or on, the surface of model bilayers [51]. KALP21, on the other hand, has been designed to span a DMPC bilayer without introducing any transmembrane mismatch [26,27]. While a surface-associated peptide, like DynB, has the effect of limiting the overall motion of individual lipid molecules, a transmembrane peptide does not have this effect to the same degree. Intuitively, a surface-associated peptide will on average interact with several lipid head-groups, inducing a change in the overall dynamics of the lipid molecules, while this is not the case for transmembrane segments, like KALP21. This second conclusion is important for understanding membrane interactions, but further studies on similar peptides and proteins should be performed to verify this. The present study does, however, clearly indicate a correlation between the location of a surface-active peptide and its effects on lipid dynamics, and at the same time demonstrates that a transmembrane configuration does not influence these dynamics. This observation was also made previously for the cell penetrating peptide transportan, although based on ^{13}C relaxation, indicating that transportan exerts its influence on lipid bilayers by locating to the surface, and restricting the overall motion of lipid molecules [14]. On the other hand, a transmembrane configuration may induce morphological changes in the bicelle structure, observed as a size increase here. Results obtained from a study of the glycosyltransferase MGS from *Acholeplasma laidlawii* showed that the entire protein had the effect of altering bicelle morphology (as evidenced by large influences on ^{31}P T_2 values) while only limited effects on lipid mobility were observed, in agreement with the present results for KALP21 [62]. Hence we suggest that the relatively simple measurements presented here may be useful to elucidate the overall mode for bilayer interaction.

Acknowledgements

This work was funded by the Swedish Research Council (contract # 621-2011-5964) and the Wenner-Gren Foundation. Financial support by the Access to Research Infrastructures activity in the 7th Framework Programme of the EC (Contract 228461, EAST-NMR) for conducting the research is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2014.12.001.

References

[1] G. Lindblom, G. Orädd, NMR studies of translational diffusion in lyotropic liquid crystals and lipid membranes, Prog. Nucl. Magn. Reson. Spectrosc. 26 (1994) 483–516.
[2] G. Orädd, G. Lindblom, Lateral diffusion studied by pulsed field gradient NMR on oriented lipid membranes, Magn. Reson. Chem. 42 (2004) 123–131.
[3] A. Filippov, G. Orädd, G. Lindblom, Lipid lateral diffusion in ordered and disordered phases in raft mixtures, Biophys. J. 86 (2004) 891–896.
[4] H.C. Gaede, K. Gawrisch, Lateral diffusion rates of lipid, water, and a hydrophobic drug in a multilamellar liposome, Biophys. J. 85 (2003) 1734–1740.
[5] J.F. Ellena, L.S. Lepore, D.S. Cafiso, Estimating lipid lateral diffusion in phospholipid vesicles from carbon-13 spin–spin relaxation, J. Phys. Chem. 97 (1993) 2952–2957.
[6] C. Mayer, G. Grobner, K. Muller, K. Weisz, G. Kothe, Orientation-dependent deuteron spin-lattice relaxation times in bilayer membranes: characterization of the overall lipid motion, Chem. Phys. Lett. 165 (1990) 155–161.

[7] R.R. Vold, R.S. Prosser, A.J. Deese, Isotropic solutions of phospholipid bicelles: a new membrane mimetic for high-resolution NMR studies of polypeptides, J. Biomol. NMR 9 (1997) 329–335.
[8] P.A. Luchette, T.N. Vetman, R.S. Prosser, R.E.W. Hancock, M.P. Nieh, C.J. Glinka, S. Krueger, J. Katsaras, Morphology of fast-tumbling bicelles: a small angle neutron scattering and NMR study, Biochim. Biophys. Acta 1513 (2001) 83–94.
[9] K.J. Glover, J.A. Whiles, G. Wu, N. Yu, R. Deems, J.O. Struppe, R.E. Stark, E.A. Komives, R.R. Vold, Structural evaluation of phospholipid bicelles for solution-state studies of membrane-associated biomolecules, Biophys. J. 81 (2001) 2163–2171.
[10] C.R. Sanders, G.C. Landis, Reconstitution of membrane proteins into lipid-rich bilayered mixed micelles for NMR studies, Biochemistry 34 (1995) 4030–4040.
[11] J.A. Whiles, R. Brasseur, K.J. Glover, G. Melacini, E.A. Komives, R.R. Vold, Orientation and effects of mastoparan X on phospholipid bicelles, Biophys. J. 80 (2001) 280–293.
[12] J.A. Whiles, K.J. Glover, R.R. Vold, E.A. Komives, Methods for studying transmembrane peptides in bicelles: consequences of hydrophobic mismatch and peptide sequence, J. Magn. Reson. 158 (2002) 149–156.
[13] E. Bárány-Wallje, A. Andersson, A. Gräslund, L. Mäler, NMR solution structure and position of transportan in neutral phospholipid bicelles, FEBS Lett. 567 (2004) 265–269.
[14] E. Bárány-Wallje, A. Andersson, A. Gräslund, L. Mäler, Dynamics of transportan in bicelles is surface charge dependent, J. Biomol. NMR 35 (2006) 137–147.
[15] L. Mäler, Solution NMR studies of peptide-lipid interactions in model membranes, Mol. Membr. Biol. (2012) 1–22.
[16] J. Seelig, 31P nuclear magnetic resonance and the head group structure of phospholipids in membranes, Biochim. Biophys. Acta 515 (1978) 105–140.
[17] J. Seelig, Deuterium magnetic resonance: theory and application to lipid membranes, Q. Rev. Biophys. 10 (1977) 353–418.
[18] J. Seelig, A. Seelig, Lipid conformation in model membranes and biological membranes, Q. Rev. Biophys. 13 (1980) 19–61.
[19] M.N. Triba, D.E. Warschawski, P.F. Devaux, Reinvestigation by phosphorus NMR of lipid distribution in bicelles, Biophys. J. 88 (2005) 1887–1901.
[20] M.N. Triba, P.F. Devaux, D.E. Warschawski, Effects of lipid chain length and unsaturation on bicelles stability. A phosphorus NMR study, Biophys. J. 91 (2006) 1357–1367.
[21] K.P. Howard, J.H. Prestegard, Conformation and dynamics of membrane-bound digalactosyldiacylglycerol, J. Am. Chem. Soc. 118 (1996) 3345–3353.
[22] M. Ottiger, A. Bax, Characterization of magnetically oriented phospholipid micelles for measurement of dipolar couplings in macromolecules, J. Biomol. NMR 12 (1998) 361–372.
[23] H. Biverståhl, A. Andersson, A. Gräslund, L. Mäler, NMR solution structure and membrane interaction studies of the N-terminal sequence (1–30) of the bovine prion protein, Biochemistry 43 (2004) 14940–14947.
[24] H. Biverståhl, J. Lind, A. Bodor, L. Mäler, Biophysical studies of the membrane location of the voltage-gated sensors in the HsapBK and KvAP K channels, Biochim. Biophys. Acta 1788 (2009) 1976–1986.
[25] S. Unnerståle, F. Madani, A. Gräslund, L. Mäler, Membrane-perturbing properties of two Arg-rich paddle domains from voltage-gated sensors in the KvAP and HsapBK K channels, Biochemistry 51 (2012) 3982–3992.
[26] J.A. Killian, Synthetic peptides as models for intrinsic membrane proteins, FEBS Lett. 555 (2003) 134–138.
[27] M.R.R. de Planque, J.A. Killian, Protein-lipid interactions studied with designed transmembrane peptides: role of hydrophobic matching and interfacial anchoring, Mol. Membr. Biol. 20 (2003) 271–284.
[28] J.A. Killian, I. Salemink, M.R.R. de Planque, G. Lindblom, R.E. Koeppe II, D.V. Greathouse, Induction of nonbilayer structures in diacylphosphatidylcholine model membranes by transmembrane α-helical peptides: importance of hydrophobic mismatch and proposed role of tryptophans, Biochemistry 35 (1996) 1037–1045.
[29] S. Morein, E. Strandberg, J. Killian, S. Persson, G. Arvidson, R. Koeppe, G. Lindblom, Influence of membrane-spanning alpha-helical peptides on the phase behavior of the dioleoylphosphatidylcholine/water system, Biophys. J. 73 (1997) 3078–3088.
[30] J.A. Killian, Hydrophobic mismatch between proteins and lipids in membranes, Biochim. Biophys. Acta 1376 (1998) 401–415.
[31] M.R.R. de Planque, D.V. Greathouse, R.E. Koeppe II, H. Schäfer, D. Marsh, J.A. Killian, Influence of lipid/peptide hydrophobic mismatch on the thickness of diacylphosphatidylcholine bilayers. A 2H NMR and ESR study using designed transmembrane α-helical peptides and gramicidin A, Biochemistry 37 (1998) 9333–9345.
[32] M.R.R. de Planque, J.A.W. Kruijtzer, R.M.J. Liskamp, D. Marsh, D.V. Greathouse, R.E. Koeppe, B. de Kruijff, J.A. Killian, Different membrane anchoring positions of tryptophan and lysine in synthetic transmembrane α-helical peptides, J. Biol. Chem. 274 (1999) 20839–20846.
[33] J.A. Killian, G. von Heijne, How proteins adapt to a membrane–water interface, Trends Biochem. Sci. 25 (2000) 429–434.
[34] M.R.R. de Planque, E. Goormaghtigh, D.V. Greathouse, R.E. Koeppe II, J.A.W. Kruijtzer, R.M.J. Liskamp, B. de Kruijff, J.A. Killian, Sensitivity of single membrane-spanning α-helical peptides to hydrophobic mismatch with a lipid bilayer: effects on backbone structure, orientation, and extent of membrane incorporation, Biochemistry 40 (2001) 5000–5010.
[35] M.R.R. de Planque, J.W.P. Boots, D.T.S. Rijkers, R.M.J. Liskamp, D.V. Greathouse, J.A. Killian, The effects of hydrophobic mismatch between phosphatidylcholine bilayers and transmembrane α-helical peptides depend on the nature of interfacially exposed aromatic and charged residues, Biochemistry 41 (2002) 8396–8404.
[36] T.M. Weiss, P.C.A. van der Wel, J.A. Killian, R.E. Koeppe II, H.W. Huang, Hydrophobic mismatch between helices and lipid bilayers, Biophys. J. 84 (2003) 379–385.
[37] M.R.R. de Planque, B.B. Bonev, J.A.A. Demmers, D.V. Greathouse, R.E. Koeppe II, F. Separovic, A. Watts, J.A. Killian, Interfacial anchor properties of tryptophan residues in transmembrane peptides can dominate over hydrophobic matching effects in peptide–lipid interactions, Biochemistry 42 (2003) 5341–5348.
[38] A. Andersson, L. Mäler, Magnetic resonance investigations of lipid motion in isotropic bicelles, Langmuir 21 (2005) 7702–7709.
[39] A. Andersson, H. Biverståhl, J. Nordin, J. Danielsson, E. Lindahl, L. Mäler, The membrane-induced structure of melittin is correlated with the fluidity of the lipids, Biochim. Biophys. Acta 1768 (2007) 115–121.
[40] E.J. Dufourc, C. Mayer, J. Stohrer, G. Althoff, G. Kothe, Dynamics of phosphate head groups in biomembranes. Comprehensive analysis using phosphorus-31 nuclear magnetic resonance lineshape and relaxation time measurements, Biophys. J. 61 (1992) 42–57.
[41] M.F. Roberts, A.G. Redfield, High-resolution ^{31}P field cycling NMR as a probe of phospholipid dynamics, J. Am. Chem. Soc. 126 (2004) 13765–13777.
[42] M.F. Roberts, A.G. Redfield, Phospholipid bilayer surface configuration probed quantitatively by ^{31}P field-cycling NMR, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17066–17071.
[43] J.B. Klauda, M.F. Roberts, A.G. Redfield, B.R. Brooks, R.W. Pastor, Rotation of lipids in membranes: molecular dynamics simulation, ^{31}P spin-lattice relaxation, and rigid-body dynamics, Biophys. J. 94 (2008) 3074–3083.
[44] M.A. Swairjo, M.F. Roberts, M. Campos, J.R. Dedman, B.A. Seaton, Annexin V binding to the outer leaflet of small unilamellar vesicles leads to altered inner leaflet properties: ^{31}P- and ^{1}H-NMR studies, Biochemistry 33 (1994) 10944–10950.
[45] K.F. Hauser, J.V. Aldrich, K.J. Anderson, G. Bakalkin, M. Christie, E.D. Hall, P.E. Knapp, S.W. Scheff, I.N. Singh, B. Vissel, Pathobiology of dynorphins in trauma and disease, Front. Biosci. 10 (2005) 216–235.
[46] V. Höllt, Opioid peptide processing and receptor selectivity, Annu. Rev. Pharmacol. Toxicol. 26 (1986) 59–77.
[47] R. Caudle, A. Mannes, Dynorphin: friend of foe? Pain 87 (2000) 235–239.
[48] K. Tan-No, G. Cebers, T. Yakovleva, B. Hoon Goh, I. Gileva, K. Reznikov, M. Aguilar-Santelises, K.F. Hauser, L. Terenius, G. Bakalkin, Cytotoxic effects of dynorphins through nonopioid intracellular mechanisms, Exp. Cell Res. 269 (2001) 54–63.
[49] M. Wollemann, S. Benyhe, Non-opioid actions of opioid peptides, Life Sci. 75 (2004) 257–270.
[50] Z. Marinova, V. Vukojevic, S. Surcheva, T. Yakovleva, G. Cebers, N. Pasikova, I. Usynin, L. Hugonin, W. Fang, H.D. Hallberg, T. Bergman, Ü. Langel, K.F. Hauser, A. Pramanik, J.V. Aldrich, A. Gräslund, L. Terenius, G. Bakalkin, Translocation of dynorphin neuropeptides across the plasma membrane: a putative mechanism of signal transmission, J. Biol. Chem. 280 (2005) 26360–26370.
[51] J. Lind, A. Gräslund, L. Mäler, Membrane interactions of dynorphins, Biochemistry 45 (2006) 15931–15940.
[52] L. Hugonin, A. Barth, A. Gräslund, A. Perálvarez-Marín, Secondary structure transitions and aggregation induced in dynorphin neuropeptides by the detergent sodium dodecyl sulfate, Biochim. Biophys. Acta 1778 (2008) 2580–2587.
[53] J. Björnerås, A. Gräslund, L. Mäler, Membrane interaction of disease-related Dynorphin A variants, Biochemistry 52 (2013) 4157–4167.
[54] J. Lind, J. Nordin, L. Mäler, Lipid dynamics in fast-tumbling bicelles with varying bilayer thickness: effect of model transmembrane peptides, Biochim. Biophys. Acta 1778 (2008) 2526–2534.
[55] L. Mäler, A. Gräslund, Artificial membrane models for the study of macromolecular delivery, Methods Mol. Biol. 480 (2009) 129–139.
[56] L. Mäler, A. Gräslund, NMR studies of three-dimensional structure and positioning of CPPs in membrane model systems, Methods Mol. Biol. 683 (2011) 57–67.
[57] W. Ye, J. Lind, J. Eriksson, L. Mäler, Characterization of the morphology of fast-tumbling bicelles with varying composition, Langmuir 30 (2014) 5488–5496.
[58] B. de Kruijff, Anionic phospholipids and protein translocation, FEBS Lett. 346 (1994) 78–82.
[59] W. van Klompenburg, I. Nilsson, G. von Heijne, B. de Kruijff, Anionic phospholipids are determinants of membrane protein topology, EMBO J. 16 (1997) 4261–4266.
[60] K. Hristova, W.C. Wimley, A look at arginine in membranes, J. Membr. Biol. 239 (2011) 49–56.
[61] M.P. Milburn, K.R. Jeffrey, Dynamics of the phosphate group in phospholipid bilayers. A ^{31}P nuclear relaxation time study, Biophys. J. 52 (1987) 791–799.
[62] C. Ariöz, W. Ye, C. Ge, J. Liebau, H. Götzke, A. Barth, Å. Wieslander, L. Mäler, Anionic lipid binding to the foreign protein MGS provides a tight coupling between phospholipid synthesis and protein overexpression in *Escherichia coli*, Biochemistry 52 (2013) 5533–5544.