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The membrane-induced structure of melittin is correlated with the fluidity of the lipids

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Abstract

The effect of the bee toxin melittin on DMPC dynamics in fast-tumbling bicelles has been investigated. The ^{13}C R_1 and ^{13}C–^{1}H NOE relaxation parameters for DMPC were used to monitor the effect of melittin and cholesterol on lipid dynamics. It was found that melittin has the largest effect on the DMPC mobility in DMPC/DHPC bicelles, while less effect was observed in cholesterol-doped bicelles, or in bicelles made with CHAPS, indicating that the rigidity of the membrane affects the melittin–membrane interaction. CD spectra were analysed in terms of cooperativity of the α-helix to random coil transition in melittin, and these results also indicated similar differences between the bicelles. The study shows that bicelles can be used to investigate lipid dynamics by spin relaxation, and in particular of peptide-induced changes in membrane fluidity.

Keywords: Bicelle; Melittin; Cholesterol; NMR; Relaxation; Helix transition

1. Introduction

Melittin is a 26 amino acid residue long peptide with the sequence GIGAVLKVLTTGLLPALISWIKRKRQQ, and is the major component of the European honey bee venom. The action of melittin on phospholipid membranes is well characterized [1] and the peptide is believed to induce a transient pore formation causing lysis of both natural and artificial membranes [2], and it can also cause phase transitions in bilayers to the inverse hexagonal phase. It has been shown that melittin disrupts the membrane by inducing micellization [3,4], which can lead to the formation of disk-shaped objects resembling bicelles.

The structure of melittin has been investigated under various conditions, and in aqueous solution the peptide adopts a random conformation. In the presence of phospholipids (e.g. vesicles or bilayers), the peptide adopts a helical conformation [1], and has the ability to form tetramers [5]. Although melittin is seen to adopt α-helical structure in almost all membranes and membrane models, the orientation of the peptide may differ under different conditions. Melittin adopts more or less a transmembrane helical structure in aligned fluid-phase bilayers [6], while, as shown by solid-state NMR, this orientation may differ in gel- or powder-like bilayers [7]. It has also been suggested that melittin does not fully penetrate the bilayer in a transmembrane structure, which may result in disruption of phospholipid vesicles [8].

The action of membrane-associated peptides may be correlated with the structure induction of the peptide, but also with factors such as lateral diffusion and reorientational motion of both the peptide and lipids in the membrane. Leakage experiments, both from vesicles and cells have been reported, which determine the action of lytic peptides on membranes, depending on many factors such as surface charge density and concentration [9]. There are several NMR methods for elucidating the effect of peptides on membrane mobility and order, including ^{2}H-NMR [10]. ^{31}P NMR has been used to determine the phase properties of lipid mixtures, including bicelles [11], and the effect of bioactive peptides [12] on lipid bilayers. More recently ^{13}C–^{1}H dipolar couplings [13,14] of phospholipids of ordered bilayers or bicelles have been reported. The effect of an antimicrobial model peptide on bicelle order has recently been determined [14] by the use of

Abbreviations: NMR, nuclear magnetic resonance; CD, circular dichroism; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine; DHPC, 1,2-dihexaoyl-*sn*-glycero-3-phosphatidylcholine; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate

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^{13}C–^{1}H dipolar coupling in magnetically aligned bicelles. These methods, however, need to be conducted on anisotropic samples, which make high-resolution NMR investigations of structure and dynamics in the same samples difficult.

Small isotropic bicelles appear ideal for combining studies of structure and membrane interactions of peptides [15,16], 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 bicelles has been studied in detail, including determination of geometry, dynamics as well as phase behaviour [11,17–19]. It has been established that the two-component mixtures of lipid and detergent form disk-like objects and that the lipids form a bilayer-like structure. It appears that these bicelles form a different phase altogether than the magnetically aligned bicelles, which explains why no ordering of either lipids or bound peptides is observed in the isotropic bicelles. Several peptide structures in the presence of fast-tumbling bicelles have been reported [20–24], and it has been shown that peptides may adopt different conformation in the more disk-like bicelles as compared to in detergent micelles [21 22]. The effect of the peptides on the phospholipids in bicelles has, however, not been studied in that much detail.

The effect of cholesterol on membrane order and dynamics, as well as on the membrane-action of melittin has been investigated by several groups. Cholesterol is known to reduce the lytic effect of melittin, by altering its membrane-interaction properties [25–27]. The majority of studies of cholesterol and melittin have been conducted in vesicles, but it has been shown that cholesterol has a stabilizing effect on magnetically aligned bicelles [28]. Furthermore, it has been observed that the melittin-induced disruption of magnetically aligned bicelles was suppressed by the addition of cholesterol. Similar observations have been made for the lytic peptide pardaxin, for which the solution structure and bilayer disruption properties have been studied [29,30].

In this study we have investigated the effect of melittin and cholesterol on the lipid mobility in fast-tumbling bicelles in the isotropic phase. NMR spin relaxation, in particular ^{13}C relaxation, offers the possibility to investigate local flexibility, and we have used natural abundance ^{13}C relaxation to characterize the effects of melittin as well as cholesterol on local dynamics of DMPC in different bicelles. Melittin was chosen since much effort has been put into understanding the action of this peptide on real as well as model membranes [31–33]. CD spectroscopy was used to investigate the secondary structural transition of melittin in different bicelles, and the interaction between the peptide and bicelles with and without cholesterol was further studied by fluorescence.

2. Materials and methods

2.1. Sample preparation

Phospholipids, dihexanoyl-*sn*-glycero-3-phosphatidylcholine (DHPC) and dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC), were obtained from Avanti Lipids Inc. (Alabaster, AL, USA). Melittin, 3-[3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) and cholesterol were purchased from Sigma Aldrich (http://www.sigmaaldrich.com) (St. Louis, USA).

DHPC/DMPC bicelle samples with $q=0.5$ were prepared using a stock solution of DHPC, which was added to a slurry of DMPC in water. The final concentration of the sample was 192 mM DHPC, 96 mM DMPC, 50 mM phosphate buffer (pH 5.5). Samples containing CHAPS ($q=0.5$) were produced analogously, from a 0.5 M stock solution of CHAPS. DMPC/DHPC bicelle samples containing [cholesterol]/[DMPC]=0.05 were prepared by first mixing DMPC and cholesterol, dissolving it in a volume of chloroform, followed by vortexing and drying under a stream of N_2. This mixture was subsequently dissolved in an appropriate amount of DHPC solution. The bicelle solution was lyophilized and then re-dissolved in H_2O to produce the desired concentration (300 mM total lipid). Samples containing melittin were produced by adding an amount of melittin corresponding to a concentration of 3 mM to the ready-made bicelle solution followed by vortexing. DHPC micelles (200 mM) in 50 mM phosphate buffer were prepared by dissolving DHPC in buffer solution. 10% D_2O was used in the NMR samples for field/frequency lock stabilization.

2.2. CD spectroscopy

CD spectra were recorded for melittin in aqueous solution, phosphate buffer solution, DHPC micelles, DMPC/DHPC and DMPC/CHAPS bicelles, and in cholesterol-doped DMPC/DHPC bicelle samples. The CD measurements were made on a Jasco J-720 CD spectropolarimeter with a 0.01 mm quartz cuvette. Wavelengths from 190 to 250 nm were measured, with a 0.5 nm step resolution and 100 nm/min speed. The response time was 4 s, with 50 mdeg sensitivity and a 5 nm band width. Measurements were conducted in the temperature range between 5 and 65 °C and the temperature was controlled by a PTC-343 controller. Spectra were collected and averaged over 5–20 scans. The CD spectra were interpreted using the JASCO program J-700 standard analysis for windows. The α-helical content was estimated from the amplitude at 222 nm [34], assuming that only α-helix and random coil conformations contribute to the CD-spectrum.

2.3. Fluorescence spectroscopy

All fluorescence spectroscopy measurements were carried out using a Jobin Yvon Horiba Fluorolog 3 spectrometer and analyzed with the Datamax software (version 2.20). All measurements were done at 25 °C using a 2×10 mm cuvette. The excitation wavelength was 280 nm and the emission was scanned from 300 to 500 nm. Scans were taken with 1 nm excitation and emission bandwidths, at a scan speed of 600 nm/min.

2.4. NMR spectroscopy

NMR experiments were carried out on a Bruker Avance spectrometer operating at 9.39 T, equipped with a probe-head for direct ^{13}C detection. The ^{13}C 90° pulse-length was typically 7.5 μs. Natural abundance ^{13}C R_1 relaxation measurements were performed with the standard inversion recovery pulse sequence (direct ^{13}C detection), using 10–18 relaxation delays ranging from 0.01 s to 10 s. A pre-acquisition delay of a minimum of 4 s was used. Errors were evaluated by recording two or more time-points twice. Steady-state NOE factors were measured by taking the ratio of intensities in a spectrum acquired with and without employing a long (>10 s) ^{1}H irradiation prior to detecting ^{13}C. A minimum pre-acquisition delay of 40 s was used in the NOE experiment. Errors in the NOE factors were evaluated from duplicate experiments. The number of scans ranged from 500 to 700 in the R_1 measurements, and was 2048 in the NOE measurements. All experiments were recorded at 37 °C and the temperature was calibrated using an external thermocouple, inserted in an NMR tube containing water.

2.5. α-helix to random coil transition theory

The Zimm–Bragg theory [35] for the α-helix to random coil transition was used to describe the temperature dependence of the amount of α-helix structure of melittin in different samples as a function of temperature. The theory was originally developed for homopolymers in solution. Lately, however, it has been applied to model peptides that interact with biological membranes [36].

In this model the cooperativity of the helix-formation ($0 \le \sigma \le 1$), the transition temperature (T_{trans}) and the growth parameter (s) are fitted to the data. The cooperativity is zero when $\sigma = 1$ and if $\sigma < 1$ the cooperativity is high. The relative amounts of random coil and α-helix are equal at the transition temperature. The growth parameter may be used to calculate the enthalpy of the helix-to-coil transition according to $s = \exp[-\Delta H_{helix}/(R(T - T_{trans}))]$, where R is the gas constant and T is the absolute temperature. The α-helix formation is assumed to be nucleated by three sequential helix-forming residues. The fitting was done by analogous procedures as published elsewhere [37].

3. Results

3.1. Melittin-bicelle binding

In order to estimate the membrane binding of melittin, Trp emission spectra were recorded in different media (Fig. 1). A distinct blue-shift of the emission maximum was observed for Trp20 in melittin in the presence of DMPC/DHPC bicelles as compared to in aqueous solution (from 356 nm in water to 336 nm in bicelles). The intensity was seen to decrease, consistent with internal quenching due to helix–helix interactions. Both these observations are typical for a melittin–membrane interaction [38].

A similar shift in emission maximum (from 356 nm to 337 nm) was observed for Trp20 in melittin in cholesterol-doped bicelles, but in this case an increase in intensity was observed, indicating a difference in the membrane interaction. The increase in emission intensity could be due to several factors, such as difference in solvent polarity around the Trp residue. The difference in Trp fluorescence intensity in the two bicelles could also be due to differences in membrane location. These results are qualitatively in agreement with previous findings [39].

3.2. Structure of melittin

The secondary structure of melittin in different model membranes was investigated by CD spectroscopy. In order to characterize the structural conversion, spectra were recorded at several different temperatures (ranging from 5 to 65 °C). Typical α-helix to random coil transitions were observed for melittin in all membrane models (DHPC micelles, DMPC/DHPC bicelles, cholesterol-doped DMPC/DHPC bicelles and DMPC/CHAPS bicelles). The temperature-dependent CD spectra for melittin in DMPC/DHPC bicelles are shown in Fig. 2a, and very similar trends were observed for melittin also in the other membrane mimetic solvents. The presence of other structural elements is expected to be low, since clear isodichroic points at 203 nm are observed. For melittin in water the temperature dependent structure changed only little with temperature, and indicated a small amount of stable α-helical structure at all temperatures.

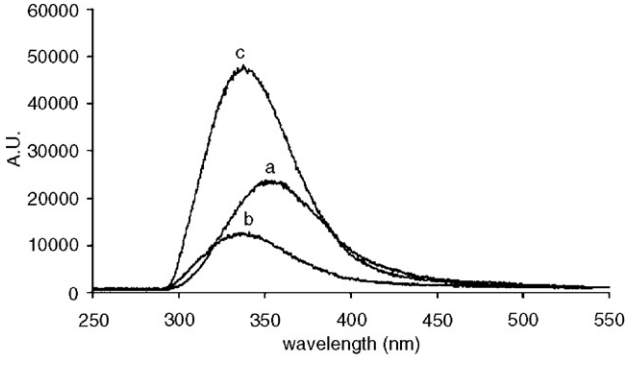

Fig. 1. Intrinsic Trp fluorescence spectra for melittin at 25 °C in water (a), DMPC/DHPC bicelles (b) and DMPC/DHPC bicelles with 5% of the DMPC replaced by cholesterol (c). The total lipid concentration was 300 mM and the peptide concentration was in all cases 3 mM, (peptide:lipid = 1:100).

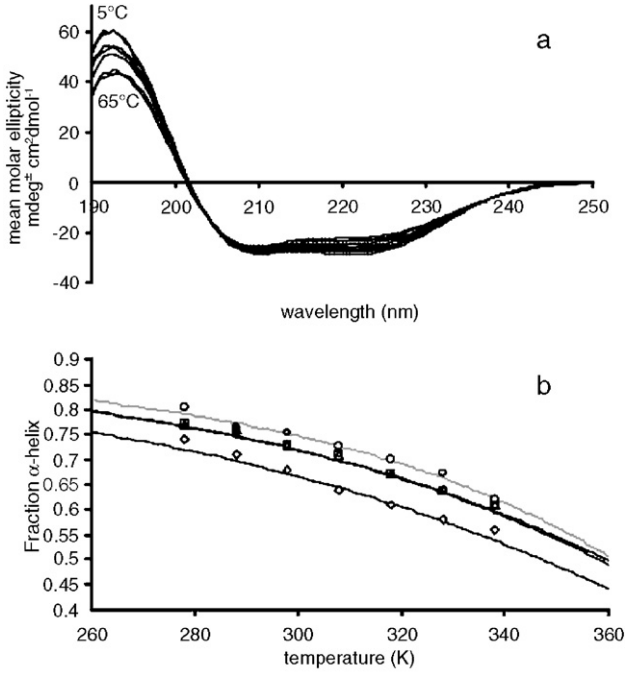

Fig. 2. (a) CD spectra for melittin in DMPC/DHPC bicelles at 5, 15, 25, 35, 45, 55 and 65 °C. (b) Fraction α-helix plotted against temperature for melittin in DMPC/DHPC bicelles (circles), DMPC/DHPC bicelles with 5% cholesterol, DHPC micelles (diamonds) and DMPC/CHAPS bicelles (triangles). The concentrations were for all bicelles the same as in Fig. 1. For the sample containing DHPC micelles, the DHPC concentration was 200 mM and the peptide concentration was 3 mM (peptide:lipid = 1.5:100). Solid curves are the fits from the α-helix to random coil transition theory.

The cooperativity of the α-helix to random coil transition was modeled by the Zimm–Bragg theory (Fig. 2b). The fitted parameters are collected in Table 1. It should, be noted that the observed transition temperatures in this study are higher than the temperature interval for which measurements were

Table 1
Fitted parameters from the temperature-induced unfolding of melittin in different membrane mimetics

	σ [a]	T_{trans} (K)	ΔH_{helix} (kJ mol^{-1} per residue)
DMPC/DHPC bicelles	0.206	365	−13.8
DMPC/DHPC bicelles with 5% cholesterol	0.047	373	−6.6
DMPC/CHAPS bicelles	0.049	374	−6.5
DHPC micelles	0.046	350	−5.3

[a] Cooperativity factor for the helix-coil transition.

conducted. Nevertheless, the results can be interpreted qualitatively, and are certainly useful for comparing the different membrane media. It is evident that the results for melittin in DMPC/DHPC bicelles differ significantly from the results in bicelles containing either CHAPS or cholesterol. In pure DMPC/DHPC bicelles, a much lower degree of cooperativity in the helix-to-coil transition for melittin was seen, as compared to the other systems. Furthermore, a higher transition temperature was observed for melittin in the cholesterol-doped bicelles and the DMPC/CHAPS bicelles. This is not unexpected since both cholesterol [10] and CHAPS [19] are known to make membranes stiffer. In DHPC micelles, melittin has a lower transition temperature as in the DMPC/DHPC bicelles, but a much higher degree of cooperativity is observed.

3.3. Effect of melittin and cholesterol on lipid dynamics

The effect of melittin and cholesterol on DMPC dynamics was investigated by carbon-13 relaxation measurements (R_1 and $^{13}C–^{1}H$ NOE factors) in DMPC/DHPC bicelles. NOE is a sensitive measure of local dynamics, and can be used to observe changes in mobility throughout the lipid molecule. R_1, on the other hand, reflects changes in both local dynamics as well as parameters influencing the motion of the entire lipid molecule [40]. Due to complete overlap between the head-group resonances for DMPC and DHPC, we only evaluated the dynamics for the acyl chains in DMPC. No attempts were made to distinguish between the *sn*-1 and *sn*-2 chains in DMPC. NOE factors and R_1 values for non-overlapping carbons in the acyl chains in DMPC are shown in Fig. 3.

Cholesterol is seen to influence the NOE factors closest to the head-group region (carbons 2 and 3), indicating that the low amount of cholesterol is sufficient to affect the local dynamics (Fig. 3a). No significant effect on the R_1 values can, however, be observed for any of the carbons (Fig. 3b). This indicates that cholesterol affects the local dynamics but does not influence the overall mobility of the lipids. This is in agreement with ^{2}H-NMR studies on magnetically aligned bicelles, which showed an increased order in the lipids [41].

Melittin, on the other hand, is seen to affect both the NOE values and the R_1 values of pure bicelles, indicating that the peptide alters the local dynamics of the acyl chains of the lipids, as well as the overall tumbling of the DMPC molecule. It can also be noted that while cholesterol has only little effect on the NOE factors for carbons in the end of the acyl chains (carbons 12, 13 and 14), melittin has an effect on NOE factors throughout the acyl chain (Fig. 3a).

To investigate the effect of melittin on bicelles containing cholesterol, we added the peptide to the ready-made bicelles containing cholesterol. In this case, melittin did not have the same effect as it had on DMPC relaxation parameters in bicelles without cholesterol. The NOE factors for DMPC in the cholesterol-doped bicelles were not changed significantly upon addition of peptide, and the effect on R_1 values was smaller than in pure DMPC/DHPC bicelles.

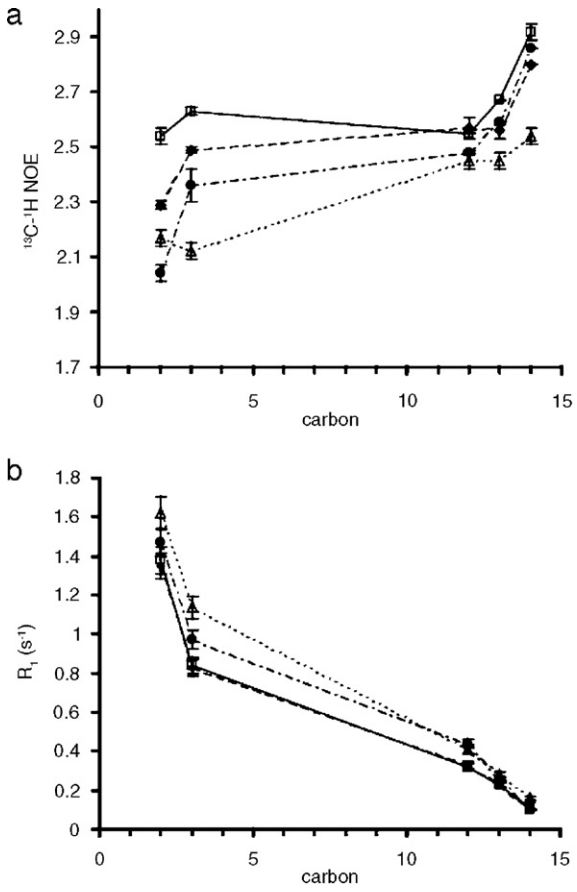

Fig. 3. The effect of cholesterol and melittin on $^{13}C–^{1}H$ NOE factors (a) and R_1 values (b) for the acyl chains in DMPC in DMPC/DHPC bicelles. The data were recorded for $q=0.5$ DMPC/DHPC bicelles at 9.4 T and at 37 °C. Open squares show relaxation data for DMPC in DMPC/DHPC bicelles, filled diamonds for DMPC in DMPC/DHPC bicelles with 5% of the DMPC replaced by cholesterol, open triangles for DMPC in DMPC/DHPC bicelles with 3 mM melittin, and filled circles show data for DMPC in DMPC/DHPC bicelles with 5% of the DMPC replaced by cholesterol and with 3 mM melittin. The error bars are obtained from at least two measurements (NOE), or duplicate data points (R_1). The concentrations were the same as in Fig. 1.

We also measured $^{13}C–^{1}H$ NOE factors for DMPC in DMPC/CHAPS bicelles with and without the peptide. In this case it is also possible to study effects on the head-group dynamics. Fig. 4 shows the $^{13}C–^{1}H$ steady-state NOE factors for DMPC with and without melittin. We first note that the lowest NOE factors are observed for carbons in the glycerol part of DMPC (1.2−1.8), indicating that this part of the molecule is the least flexible. As expected, the methyl groups at the end of the acyl chain, together with the choline head-group, have the largest NOE, indicating a high degree of flexibility. These results are in agreement with previous findings [19]. The restriction of local dynamics correlates well with the known lateral pressure profile for phospholipids, where a large interfacial tension is observed for the glycerol positions. We may also note that the NOE factors for the DMPC acyl chain in pure DMPC/DHPC bicelles are substantially higher than the corresponding values for DMPC in DMPC/CHAPS bicelles. This is in agreement with previous results showing that DMPC

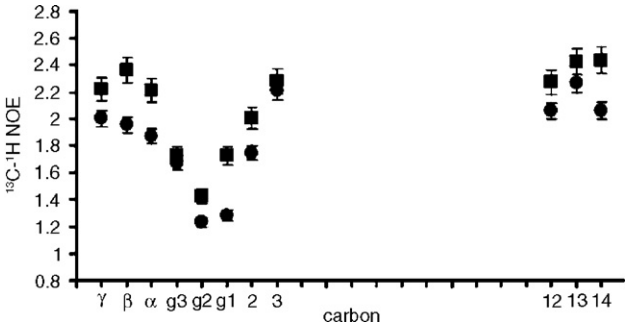

Fig. 4. The effect of melittin on ^{13}C–^{1}H NOE factors for DMPC in DMPC/CHAPS bicelles. The data were recorded for $q=0.5$ DMPC/CHAPS bicelles at 9.4 T and at 37 °C. Squares show NOE factors for DMPC without melittin, and circles show NOE factors for DMPC in bicelles after the addition of 6 mM melittin. The error bars are obtained from at least two measurements. The concentrations were the same as in Fig. 1.

in DMPC/CHAPS bicelles is less flexible than in DMPC/DHPC bicelles [19].

A drop in NOE factors throughout the DMPC molecule in the presence of melittin is observed, indicating that the local dynamics are affected by the peptide. This effect is, however, not as pronounced as seen in DMPC/DHPC bicelles, indicating that the peptide has a larger effect on the local dynamics in DMPC/DHPC bicelles than in bicelles made with CHAPS.

4. Discussion

Melittin is a well-studied membrane-interacting peptide, known to induce hemolysis, possibly by several mechanisms, including bilayer micellization [1,2]. One of the possible lytic effects of melittin on model membranes is to induce formation of disk-shaped, bicelle-like particles in vesicles, and in this way disrupt the membrane [25]. The peptide is known to adopt a helical structure in a variety of membranes, and aqueous melittin has been shown to crystallize in tetramers [5]. This property may be correlated with the pore-forming abilities of the peptide [42].

We find that melittin interacts with bicelles using either DHPC or CHAPS as detergents, and adopts an α-helical structure in all membrane mimetics. There are, however, significant differences in the interaction between melittin and the different bicelles. A much lower degree of cooperativity in the structural conversion is observed in pure DMPC/DHPC bicelles as compared to the DMPC/CHAPS or cholesterol-doped DMPC/DHPC bicelles. The enthalpy changes for melittin undergoing a helix-to-coil transition in bicelles with CHAPS or cholesterol are in the range of values previously observed for peptides undergoing helix formation [36,43,44], while it differs for melittin in pure DMPC/DHPC bicelles. The transition temperature for melittin in DMPC/DHPC bicelles differs significantly from in the CHAPS- and cholesterol-containing bicelles. Even though no general conclusion may be drawn from the analysis, it is evident that melittin interacts with the more dynamic DMPC/DHPC bicelles differently than with the more rigid CHAPS or cholesterol-containing bicelles.

It is important to emphasize that several parameters may influence the transition dynamics. One may be the occurrence of coiled-coils. These structure are known to affect CD spectra and it is not known how the super-coiling influences the cooperativity. In this paper no special attempts have been made to account for these effects.

The Zimm–Bragg analysis has to our knowledge only been applied once to membrane interacting peptides [36]. In that case different analogues of magainin were investigated in charged small unilamellar vesicles. Much higher degrees of cooperativity ($\sigma \approx 0.0003$) were observed as compared with the present results. It is not clear if this reflects differences in surface charge, differences between bicelles and vesicles, or if the peptides are different.

Carbon-13 relaxation has previously been used successfully to investigate phospholipid dynamics in both vesicles [45–47] and bicelles [19,40]. From these data, one can in principle calculate generalized order parameters using extended "model-free" approaches [48,49]. These order parameters can be compared with those obtained from analysis of ^{2}H quadrupolar splittings in deuterated phospholipids by assuming a value for the order parameter for the entire lipid molecule. This was recently done for DMPC dynamics in different bicelles, and the generalized order parameters obtained from carbon-13 relaxation data for DMPC were seen to be in good agreement with those obtained from ^{2}H quadrupolar splittings [19]. In the present study we limit ourselves to comparing R_1 and NOE relaxation data for the lipids. It is well known that the NOE relaxation parameter is clearly influenced by changes in local mobility for each ^{13}C–^{1}H vector. For melittin we see that the apparent order, as judged from NOE factors, in the membrane increases, which is in agreement with what has been observed with the antimicrobial peptide LL-37 in DMPC bilayers at approximately the same temperature [50]. These results are more in agreement with those presented for transmembrane helical peptides with a slight hydrophobic mismatch [24,51], indicating a thickening of the bilayer.

Cholesterol is known to broaden the sharp transition between the gel phase and the fluid, liquid crystalline phase of phospholipids. Here it is seen that it has the effect of decreasing the local mobility of the carbons in the acyl chains in DMPC, which is in agreement with early results using ^{2}H NMR [52,53]. Adding melittin to the cholesterol-containing bicelles, on the other hand, does not dramatically influence the local motion of the acyl chains in DMPC. However, in contrast it appears that melittin does interact with the cholesterol-containing bicelles, as seen from the structure-induction and the blue-shift in the fluorescence emission wave-length. The reason for these observations may in part be explained by melittin inducing cholesterol-rich "domains" within a few bicelles, and that melittin preferentially interacts with bicelles containing no, or little cholesterol. Hence melittin would only partially insert in the cholesterol-containing bicelles. Similar arguments have been presented for the interaction of the antimicrobial peptide pardaxin with cholesterol-containing POPC bilayers [29].

The addition of melittin to bicelles clearly influences carbon-13 relaxation data and hence local dynamics of the acyl

chains in DMPC. In agreement with the CD analysis, we see that melittin has the greatest effect on dynamics in bicelles made with DHPC. Lesser effects are seen on the more rigid cholesterol-doped DMPC/DHPC bicelles, as well as on bicelles made with CHAPS. There are several possible reasons for the differences in relaxation effects in the different bicelles. Melittin may adopt a different location in the DMPC/DHPC bicelles as compared to in the other bicelles. The largest effect of adding melittin to DMPC/DHPC bicelles are observed close to the head-group region, indicating that this region is mostly affected by the peptide. As it is well known that CHAPS and cholesterol induces rigidity in the bilayer, these differences are likely to be correlated with the more rigid bilayer. This supports the conclusion that the melittin–membrane interaction is influenced by the rigidity of the membrane, in agreement with previous results [26].

The finding that melittin has similar effects on bicelles made with CHAPS as on cholesterol-doped DMPC/DHPC bicelles, indicates that DMPC/detergent mixing occurs to a higher degree in CHAPS containing bicelles than in DHPC containing bicelles, resulting in a more rigid bilayer. A larger degree of rigidity in CHAPS-containing bicelles has previously been suggested based on carbon-13 relaxation of DMPC in different bicelles [19]. It should be noted that the bicelles may have different shape and curvature, depending on the composition, which could also influence the peptide–bicelle interaction. It has been reported that bicelles made with CHAPS are smaller than bicelles made with DHPC, using the same molar amount of detergent [19].

In summary we conclude that we have successfully used small isotropic bicelles to investigate the action of melittin on membranes, and that cholesterol can be incorporated into the bilayer. The incorporation of cholesterol in turn results in a change in the membrane-interaction of melittin. This report also shows that ^{13}C relaxation of phospholipids in bicelles is an excellent method to probe local dynamics as a function of membrane constitution, and in particular of the influence of different membrane-bound peptides.

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