Insights on the Interactions of Synthetic Amphipathic Peptides with Model Membranes as Revealed by ^{31}P and ^{2}H Solid-State NMR and Infrared Spectroscopies

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ABSTRACT We studied the interaction between synthetic amphipathic peptides and model membranes by solid-state NMR and infrared spectroscopies. Peptides with 14 and 21 amino acids composed of leucines and phenylalanines modified by the addition of crown ethers were synthesized. The 14-mer and 21-mer peptides both possess a helical amphipathic structure. To shed light on their membrane interaction, ^{31}P and ^{2}H solid-state NMR experiments were performed on both peptides in interaction with dimyristoylphosphatidylcholine vesicles in the absence and presence of cholesterol, dimyristoylphosphatidylglycerol vesicles, and oriented bicelles. ^{31}P NMR experiments on multilamellar vesicles reveal that the dynamics and/or orientation of the polar headgroups are weakly yet markedly affected by the presence of the peptides, whereas ^{31}P NMR experiments on bicelles indicate no significant changes in the morphology and orientation of the bicelles. On the other hand, ^{2}H NMR experiments on vesicles reveal that the acyl chain order is affected differently depending on the membrane lipidic composition and on the peptide hydrophobic length. Finally, infrared spectroscopy was used to study the interfacial region of the bilayer. Based on these studies, mechanisms of membrane perturbation are proposed for the 14-mer and 21-mer peptides in interaction with model membranes depending on the bilayer composition and peptide length.

INTRODUCTION

The spreading of antibiotic resistance and the mechanisms related among bacteria are becoming an increasingly important global healthcare problem. Therefore, there is an urgent need for novel classes of antimicrobial compounds having new modes of action defying most of the known resistance mechanisms to antibiotics. Antimicrobial peptides have recently become the focus of much research (1,2). These peptides are an important component of the defense system of a variety of species such as mammals, plants, insects, viruses, and bacteria (1). Most of them are small (12–45 amino acids), cationic, amphipathic, and can adopt different secondary structures in interaction with membranes, such as α-helix and β-sheet conformations (3–5). Many natural antimicrobial peptides have been extensively studied (e.g., melittin, cecropin, magainin, gramicidin, etc.), and these studies have demonstrated that the lipidic membrane of prokaryotic and/or eukaryotic cells seems to be the primary or final target of such peptides. Their activities seem to be modulated both by the structural parameters of the peptides, such as helicity, charge, hydrophobicity, and the lipidic composition and physical state of the membranes (6,7).

Since the mechanisms of action by which antimicrobial peptides perturb membranes, as well as the structural features involved, are not well understood, many researches have relied on peptide synthesis of analogs and models of natural antimicrobial peptides to shed light on the mechanisms of membrane perturbation and on the structural features that drive these mechanisms. Several mechanisms of action have been proposed, such as the ''barrel-stave'', the ''carpet-like'', the ''toroidal'', the ''in-plane diffusion'', and the ''detergent-like'' models (8).

Solid-state nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectroscopies are techniques well suited to investigate the interactions between lipids and peptides since they provide information on the effects of the peptides on different regions of the lipid bilayer, such as the polar headgroup, the interfacial region, and the lipid acyl chains, and several antimicrobial peptides have been studied by these techniques. For example, many members of the magainin family, such as the antimicrobial peptide PGLa, have been shown to interact preferentially with bacterial and negatively charged model membranes via the ''carpet-like'' model, i.e., in a parallel orientation relative to the bilayer surface (9,10). Comparable studies have been done with melittin, alamethicin, gramicidin A and S, mastoparan, amphibian peptides (aurein, caerin, citropin, and maculatin), colicin Ia, equinatoxin II, human peptide LL-37, protegrin, and nisin, with a view to determining the effects of specific peptide structural parameters and the membrane lipidic composition in the mutual interactions between peptides and lipids (11–21).

Synthetic peptides have also been studied in detail to determine which parameters enhance the antimicrobial activity without increasing the hemolytic action, and the related

Submitted November 7, 2005, and accepted for publication February 24, 2006.

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0006-3495/06/06/4071/14 $2.00

doi: 10.1529/biophysj.105.077339

effects on the structure and conformation of the lipid bilayer (22–25). Although these studies provide a wealth of information about the factors that dominate peptide-membrane interactions, the specific role of structural parameters remains uncertain and imprecise. Therefore, the knowledge of the mutual influences of different structural parameters is essential for the design of novel classes of agents having effective antimicrobial and low hemolytic properties.

With the goal of better understanding the types of interactions involved in the membrane perturbation of amphipathic peptides, we have designed synthetic helical amphipathic peptides that are oligomers of a repeating unit of five leucine residues and two synthetic 21-crown-7-phenylalanine residues appropriately positioned so that the hydrophilic crown ethers align on one side of the hydrophobic helical axis, to form neutral amphipathic 14-mer and 21-mer peptides (Fig. 1). Crown ethers were chosen as hydrophilic side chains because once incorporated into membranes, the peptides can allow ion translocation from one crown ether to another and thus create a channel that could destabilize the electrochemical gradient on both sides of the bacterial membrane. It is interesting to note that many other ways have also been reported in the literature to confer amphipathic character to natural and synthetic antimicrobial peptides. For example, Thennarasu et al. reported the addition of octanoic acid to the N-terminal region of lipopeptide MSI-843 to enhance its hydrophobic character (26). Sitaram et al. also reported various examples of chemical modifications of natural antimicrobial peptides by addition, deletion, or substitution of amino acids (27).

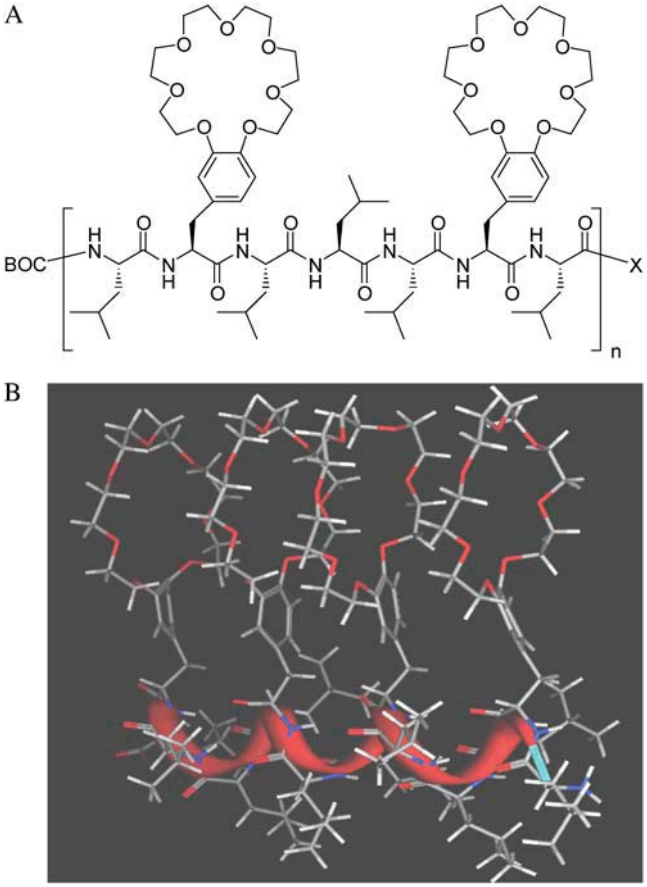

FIGURE 1 (*A*) Monoprotected 14-mer and diprotected 21-mer peptides used in this study. For the 14-mer peptide, $n = 2$ and $X = OH$. For the 21-mer peptide, $n = 3$ and $X = OCH_3$. (*B*) Three-dimensional view of the 14-mer peptide showing the aligned 21-crown-7-phenylalanines.

Previous studies have shown that both the 14-mer and 21-mer peptides adopt preferentially an amphipathic helicoidal conformation and act as membrane perturbers (28,29). The membrane activity of the 14-mer peptide has been determined by fluorescence and ^{23}Na NMR spectroscopies. These studies revealed that the 14-mer peptide promotes the rapid release of calcein and Na^+ from vesicles, and hemoglobin from erythrocytes (28). On the other hand, previous results with pH-stat and single-channel measurement techniques have shown that the 21-mer peptide can span a bilayer membrane and act as an artificial ion channel, allowing the flow of ions through the bilayer (29–32). These studies therefore indicate a high membrane activity for both peptides, making them very promising antimicrobial agents.

We have thus investigated in this study, by solid-state NMR and FTIR spectroscopy, the interactions between the 14-mer and 21-mer peptides and zwitterionic dimyristoylphosphatidylcholine (DMPC), DMPC/cholesterol, and anionic dimyristoylphosphatidylglycerol (DMPG) model membranes that mimic eukaryote and bacterial cells. Experiments on magnetically oriented bicelles have also been performed to determine the effects of the 14-mer and 21-mer peptides on these oriented model membranes and to verify the utility of bicelles for the determination of peptide orientation by ^{15}N NMR. Since the lipids found in natural membranes are in the fluid phase, the NMR results presented here have been obtained at temperatures above the lipid phase transition. The 14-mer and 21-mer peptides constitute very good models to study the membrane interaction of membrane active peptides since they are relatively small, can be chemically modified, and adopt an amphipathic helical structure that seems to be essential for the interaction. In addition, their neutral nature allows study of the effect of hydrophobic forces in the interaction with lipids without the influence of strong electrostatic interactions.

MATERIALS AND METHODS

Materials

DMPC with protonated and deuterated (d_{54}) acyl chains, dihexanoylphosphatidylcholine (DHPC), DMPG, and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL) and used without purification. Oxime resin was prepared by a standard procedure using polystyrene beads (100–200 mesh 1% DVB, Advanced ChemTech, Louisville, KY) (33). Resins with substitution levels around 0.5 mmol per gram of oxime group were used. Boc-protected amino acids were purchased from Advanced ChemTech. All solvents were Reagent, Spectro, or high-performance liquid chromatography-grade quality purchased commercially and used without any further purification except for dimethyl formamide (degassed with N_2),

dichloromethane (distilled), and diethyl ether (distilled from sodium and benzophenone). Water used throughout the studies was distilled and deionized using a Barnstead NANOpurII system (Boston, MA) with four purification columns. All other reagents were purchased from Sigma-Aldrich (Milwaukee, WI).

Peptide synthesis

The monoprotected 14-mer and diprotected 21-mer peptides (Fig. 1) were synthesized and purified according to published procedures (32). For the 14-mer peptide, $n = 2$ and $X = OH$, and for the 21-mer peptide, $n = 3$ and $X = OCH_3$. Previous studies indicate that the monoprotected 14-mer peptide shows the greatest lytic activity, whereas the diprotected 21-mer peptide shows the greatest ion channel activity (E. Biron and N. Voyer, unpublished results).

Sample preparation: vesicles containing the 14-mer (21-mer) peptide

The DMPC, DMPG, and DMPC/cholesterol lipid vesicles were prepared by mixing 20 mg (15 mg) of phospholipids in 80 μL (60 μL) of water, giving a total proportion of 20% (w/w) lipids in water. For the DMPC/cholesterol system, the lipids and cholesterol in a molar ratio of 7:3 were codissolved in chloroform, the solvent was evaporated under nitrogen gas, and the samples were put into vacuum overnight to remove all traces of organic solvent. In the three lipidic systems, the peptide was added to the lipids before the addition of water to give lipid/peptide molar ratios of 60:1 and 20:1, and the pH was ~6.5 in all samples. In the bicelle preparation, DHPC and DMPC were mixed in a molar ratio of 1:3.55 before sample hydration. The peptide was added after the bicelle formation and hydration in a lipid/peptide molar ratio of 60:1. The vesicle and bicelle samples then underwent at least three freeze (liquid N_2)/thaw (lipid phase transition temperature of 23°C + 7°C)/vortex shaking cycles and were stored at −20°C until analysis. Three additional freeze/thaw/vortex cycles were performed on the samples before the acquisition of NMR and FTIR spectra. For ^{2}H NMR analyses on both multilamellar vesicles and bicelles, an equimolar portion of lipids with deuterated acyl chains was used to replace the undeuterated lipids. Deionized and deuterium-depleted water were used for the ^{31}P and ^{2}H NMR experiments, respectively. Deuterium oxide (D_2O) was used to eliminate the interference of water in the FTIR spectra. In all samples used in these NMR and FTIR studies, the use of buffer was avoided to limit the interference of salts in the interaction of the peptides with model membranes.

NMR experiments

The static ^{31}P and ^{2}H NMR spectra were acquired with a Bruker Avance 300 MHz spectrometer (Bruker Canada, Milton, Ontario, Canada), whereas the static ^{31}P spectra of bicelles were acquired with a Bruker Avance 400 MHz spectrometer (Bruker Canada). For the static ^{31}P and ^{2}H NMR experiments, the samples were placed into a 5-mm coil of a homebuilt probe. For the static ^{31}P experiments performed at 400 MHz, the samples were placed into a 4-mm NMR tube inserted into a magic-angle spinning (MAS) probe. The ^{31}P NMR spectra were obtained at 121.5 MHz (300 MHz) and 161.9 MHz (400 MHz) using a phase-cycled Hahn echo pulse sequence (34) with TPPM proton decoupling (35). Using 2048 data points, typically 1200 scans were acquired with a pulse length of 4 μs, an interpulse delay of 30 μs, and a recycle delay of 4 s. A line broadening of 50 Hz was applied to all static ^{31}P spectra. The chemical shifts were referenced relative to external H_3PO_4 85% (0 ppm). The ^{2}H NMR experiments were carried out at 46.1 MHz (300 MHz) using a quadrupolar echo sequence (36). Using 4096 data points, 3200 scans were acquired with a 90° pulse length of 5 μs, an interpulse delay of 60 μs, and a recycle time of 500 ms. A line broadening of 100 Hz was applied to all spectra and the quadrupolar splittings were measured on dePaked spectra (37). An 1800-s equilibration delay was allowed between each temperature in the ^{31}P and ^{2}H experiments.

FTIR experiments

The infrared spectra were recorded with a Nicolet Fourier transform spectrometer (Thermo-Nicolet, Madison, WI) equipped with a narrow-band mercury-cadmium-telluride detector and a germanium-coated KBr beam splitter. A 10-μL amount of the sample was placed between CaF_2 windows separated by a 6-μm Mylar spacer. A total of 100 interferograms was acquired with a resolution of 2 cm^{-1} in the spectral range of 4000–650 cm^{-1} at various temperatures and controlled by a home-made device. The spectra were corrected for the water vapor and CaF_2 contribution by subtraction of a reference spectrum. The data was processed with the Grams 386 software (Galactic Industries, Salem, MA). The spectral region corresponding to the ester carbonyl stretching vibrations was baseline-corrected using a cubic function. The ester carbonyl region of the spectra was deconvolved using the method proposed by Griffiths and Pariente (38) with a narrowing factor (γ) of ~1.93, 1.73, and 1.63, and an apodization filter of 48%, 50%, and 53%, for DMPC, DMPG, and DMPC/cholesterol vesicles, respectively, in the absence and presence of peptides.

RESULTS AND DISCUSSION

Solid-state NMR spectroscopy

Phosphorus-31 NMR

Since the phospholipid headgroup contains a phosphorus-31 atom with a 100% natural isotopic abundance, ^{31}P NMR is a powerful technique to monitor changes occurring in the polar region of the bilayer (39–41). We have used ^{31}P NMR spectroscopy to investigate changes occurring in the headgroup region of neutral and anionic multilamellar vesicles, and to bicelles upon addition of the 14-mer and 21-mer peptides. More specifically, static ^{31}P solid-state NMR spectra provide information on the dynamics and/or orientation of the polar headgroup and on the vesicle morphology by analyzing the changes in chemical shift anisotropy (CSA) and spectral lineshape. In addition, ^{31}P MAS experiments can be used to determine the effects of the peptides on the dynamics and/or orientation of the polar headgroup and on the shielding/deshielding of the phosphorus nucleus.

We first investigated the effects of the 14-mer and 21-mer peptides on zwitterionic DMPC membranes. The ^{31}P NMR static spectra and related spectral parameters are displayed in Fig. 2 *A* and Table 1. The spectra in the absence and presence of peptides are characteristic of a lamellar phase with axial symmetry (41). Upon peptide binding, a decrease in the DMPC CSA is observed for both peptides. More specifically, Table 1 shows that the CSA values (δ) of DMPC vesicles at a lipid/peptide molar ratio of 60:1 (20:1) are decreased by 3.1 (4.6) ppm for the 14-mer peptide and by 0.7 (2.0) ppm for the 21-mer peptide. This decrease is more pronounced for the vesicles containing the 14-mer peptide than for those containing the 21-mer peptide, and in both cases could be attributed to a change in the dynamics and/or orientation of the lipid polar headgroup (41). Picard et al.

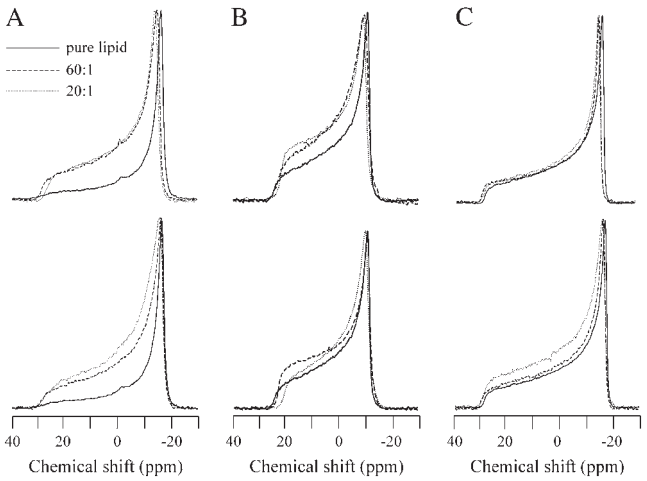

FIGURE 2 ^{31}P NMR spectra of (*A*) zwitterionic DMPC, (*B*) anionic DMPG, and (*C*) zwitterionic DMPC/cholesterol membranes at 37°C in the absence and presence of the 14-mer (*top row*) and 21-mer (*bottom row*) peptides at lipid/peptide molar ratios of 60:1 and 20:1.

proposed another way to quantify the effect of peptides on the CSA by evaluating a relative order parameter of the form

$$S_2 = \frac{\delta}{\delta_{\text{ref}}}, \tag{1}$$

in which δ_{ref} is the CSA of a reference system, such as the pure lipid system in the absence of peptide (42,43). The order parameter S_2 can vary from 1, for a system in which the dynamics is the same as that in the reference system, to 0 if the system becomes totally isotropic. As seen in Table 1, we obtained S_2 values of 0.91 (0.86) for the DMPC/14-mer peptide systems and 0.98 (0.94) for the DMPC/21-mer peptide system at a lipid/peptide molar ratio of 60:1 (20:1). These S_2 values again confirm the more perturbing effect of the 14-mer peptide compared to the 21-mer peptide on the DMPC headgroup dynamics and/or orientation.

The second effect observed upon peptide binding is a change in the downfield edge intensity of the spectra. The spectra displayed in Fig. 2 *A* clearly show that the intensity of the downfield edge increases upon peptide binding and they are indicative of a greater 0° orientation of the lipid membranes relative to the external magnetic field (44). An order parameter (S_1) has been calculated to assess the level of orientation of the phospholipid membranes, as described by Picard et al. (42,43). The first spectral moment (M_1) of ^{31}P NMR spectra is a good indicator of the orientation of a lipidic system with axial symmetry. This moment is related to S_1 as

$$S_1 = \frac{(M_1 - \delta_{\text{iso}})}{\delta}, \tag{2}$$

where δ_{iso} is the isotropic chemical shift, and δ is the CSA defined by

$$\delta = \delta_{//} - \delta_{\text{iso}} = -2\delta_{\perp} - \delta_{\text{iso}}. \tag{3}$$

The perpendicular chemical shift ($\delta_{\perp}$) can be obtained by measuring the chemical shift of maximum intensity at the 90° edge of the static ^{31}P NMR spectra. If $S_1 = 1$, the lipid axis is oriented parallel to the magnetic field, whereas a value of -0.5 is characteristic of perpendicular alignment. Table 1 reveals S_1 values -0.17 for the pure DMPC system, -0.02 (-0.01) for the DMPC/14-mer peptide system, and -0.06 (-0.05) for the DMPC/21-mer peptide at a lipid/peptide molar ratio of 60:1 (20:1). These results indicate that the peptide-containing systems tend to orient in the magnetic field in a different way compared to the pure lipid system, the vesicles being less 90° oriented in the presence of peptides.

The ^{31}P NMR spectra of the DMPG vesicles containing the 14-mer and 21-mer peptides are displayed in Fig. 2 *B*. As in the case of the DMPC vesicles, these spectra indicate that the 14-mer and 21-mer peptides both reduce the CSA at lipid/peptide molar ratios of 60:1 and 20:1. As shown in Table 1, the CSA values of the DMPG vesicles containing the 14-mer and 21-mer peptides are decreased by 2.6 (4.2) ppm and 0.0 (1.6) at a lipid/peptide molar ratio of 60:1 (20:1). This is also confirmed by the calculation of the S_2 order parameter as shown in Table 1. For the vesicles containing the 14-mer and the 21-mer peptides, the S_2 values obtained are 0.88 (0.81) and 0.99 (0.92) at a molar ratio of 60:1 (20:1). Even if the intensity of the component at 0° is greater upon peptide binding, the S_1 order parameters obtained for the DMPG vesicles containing the 14-mer and 21-mer peptides indicate that the orientation of the DMPG vesicles is not significantly affected by the presence of peptides. In fact, as shown in Table 1, vesicles containing the 14-mer peptide have an S_1 of 0.09 (0.03), whereas vesicles with the 21-mer peptide have an S_1 of 0.06 (-0.03) at a lipid/peptide molar ratio of 60:1 (20:1), compared to the S_1 value of 0.00 for the pure DMPG vesicles.

TABLE 1 ^{31}P NMR spectral parameters for membrane vesicles in the absence and presence of peptides at different molar ratios

	CSA (ppm)			S_1			S_2	
	Pure	(60:1) 14-mer (21-mer)	(20:1) 14-mer (21-mer)	Pure	(60:1) 14-mer (21-mer)	(20:1) 14-mer (21-mer)	(60:1) 14-mer (21-mer)	(20:1) 14-mer (21-mer)
DMPC	33.5	30.4 (32.8)	28.9 (31.5)	−0.17	−0.02 (−0.06)	−0.01 (−0.05)	0.91 (0.98)	0.86 (0.94)
DMPG	21.6	19.0 (21.6)	17.4 (20.0)	0.00	0.09 (0.06)	0.03 (−0.03)	0.88 (0.99)	0.81 (0.92)
DMPC/Chol.	34.9	32.1 (33.3)	31.4 (32.6)	−0.05	0.01 (−0.03)	−0.00 (−0.01)	0.92 (0.96)	0.90 (0.94)

Measurements were taken at 37°C for lipid/peptide molar ratios of 60:1 and 20:1.

As depicted in Fig. 2 *C* and listed in Table 1, the CSA of the DMPC/cholesterol vesicles containing the peptides at a lipid/peptide molar ratio of 60:1 (20:1) is reduced by 2.8 (3.5) ppm in the presence of the 14-mer peptide and by 1.6 (2.3) ppm in the presence of the 21-mer peptide. At a lipid/peptide molar ratio of 60:1 (20:1), the S_2 order parameter is 0.92 (0.90) in the presence of the 14-mer peptide and 0.96 (0.94) in the presence of the 21-mer peptide. The calculation of the S_1 order parameter reveals that, unlike with the DMPC vesicles, both peptides do not perturb significantly the orientation of the system in the magnetic field. This is in agreement with previous studies indicating that cholesterol molecules stabilize the bilayer in such a way that their hydrocarbon framework and the alkyl chain are inserted in the hydrophobic core, whereas their hydroxyl group is in the vicinity of the lipid ester carbonyl groups (45).

The decrease of the CSA observed in DMPC, DMPG, and DMPC/cholesterol vesicles upon the addition of the 14-mer peptide could be explained by an interaction of the peptide at the polar headgroup. As illustrated in Fig. 1 *B*, the strategic positioning of the crown ethers at sites 2, 6, 9, and 13 for the 14-mer peptide, and sites 2, 6, 9, 13, 16, and 20 for the 21-mer peptide makes all the crown ethers well sequestered on one side of the α-helix. This confers an amphipathic nature to the peptides, and when inserted in a bilayer, the crown ethers are facing the aqueous phase, whereas the hydrophobic helix is facing the hydrophobic region of the bilayer. This interfacial interaction could result in a change in the dynamics and/or orientation of the polar headgroup. Bonev et al. have reported a decrease of the CSA of membranes composed of DMPC and sphingomyelin (10% mol) upon equinatoxin II addition, and they attributed this decrease to a perturbation of the lipid headgroup packing (13). Huster et al. studied the colicin Ia channel in interaction with palmitoyloleoylphosphatidylcholine/palmitoyloleoylphosphatidylglycerol vesicles and they concluded that colicin Ia interacts with the lipid bilayer since a smaller ^{31}P CSA was obtained (46). Several other groups have also performed ^{31}P NMR experiments on antimicrobial and human peptides (14,18,47).

The 21-mer peptide has a similar but less pronounced effect on the three lipidic systems. This could be explained by a different mode of interaction between the 14-mer and the 21-mer peptides since both peptides do not have the same length. Considering the peptides as ideal α-helices, the lengths of the 14-mer and the 21-mer peptides are 21.0 Å and 31.5 Å, respectively. Since the mean hydrophobic length of DMPC in the fluid phase is ~28.5 Å (48), the hydrophobic mismatch could play a role in the membrane topology and thus explain the differences observed between the 14-mer and the 21-mer peptides. In fact, considering the difference in length between the 14-mer peptide and the DMPC bilayers in the fluid phase, a hydrophobic mismatch of 7.5 Å could allow the 14-mer peptide to adopt preferentially a surface orientation, whereas the smaller mismatch of 3.0 Å between the 21-mer peptide and the DMPC bilayers could, but would not exclusively, favor a transmembrane orientation.

Another interesting phenomenon observed in the spectra presented in Fig. 2 *A* is the change in the downfield intensity of the spectra upon peptide binding. The ^{31}P NMR spectrum of the pure DMPC vesicles shows a partial alignment in the magnetic field. This behavior has already been reported and is related to the anisotropy in the diamagnetic susceptibility $\Delta\chi$ of the phospholipids (44). With a negative $\Delta\chi$, the lipid molecules tend to align their long axis perpendicular to the magnetic field. The facility by which vesicle deformation occurs in the magnetic field is a function of parameters such as the membrane shape, elasticity, curvature, fluidity, viscosity, and the strength of the magnetic field (49). By analyzing the spectral shape of DMPC, DMPG, and DMPC/cholesterol spectra, we note that DMPC vesicles are more susceptible to deformation in the magnetic field compared to DMPG and DMPC/cholesterol vesicles. The repulsive charges at the polar headgroup of DMPG molecules, as well as the stabilizing effect of the cholesterol molecules, could explain their relative spherical shape compared to the ellipsoidal shape of DMPC vesicles in the magnetic field (15,45,50).

An increase in the intensity of the downfield edge is observed upon addition of the two peptides. Two hypotheses could explain this change in the morphology of the vesicles, and they are well described by Pott et al. (21). First, the binding of the peptide to the bilayer could result in a change of the membrane elastic properties, resulting in less deformable vesicles. Second, this deformation could result from a change of the membrane magnetic susceptibility. In fact, most of the helical peptides and proteins possess a large and positive diamagnetic susceptibility due to the peptide bond, and the helix therefore tends to align its long axis parallel to the magnetic field (51). If a helix is bound to the bilayer with its axis parallel to the bilayer plane, a greater 90° orientation is expected. A similar case has been reported by Neugebauer et al. in the study of magnetic orientation of membranes by bacteriorhodopsin using optical measurements and neutron scattering (52). In our case, however, despite the presence of helical peptides, an opposite effect is observed on the magnetic orientation of the DMPC membranes. This observation could be explained by the presence of phenylalanine residues, which contain an aromatic ring whose plane is aligned perpendicular to the helix axis. According to Worcester (51), in such an arrangement, the diamagnetic susceptibility of the aromatic rings will tend to align their planes parallel to the magnetic field, which could partially overcome the perpendicular orientation of the phospholipids.

Finally, the magnetic orientation of bicelles in the presence of the 14-mer and 21-mer peptides was investigated. Such an experiment is of great interest since bicelles represent a good way to orient peptides in the magnetic field and to take advantage of the information contained in orientation-dependent anisotropic interactions. As previously

reported, bicelles are discoid bilayers or perforated lamellae that spontaneously orient with their normal perpendicular to the magnetic field (53–55). At temperatures in the range of bicelle existence, two resonances are seen. As described in previous reports, the most upfield and intense resonance is attributed to molecules (mostly DMPC) located in the planar section of the bicelles and aligned perpendicular to the direction of the magnetic field (42,53). The downfield resonance is attributed to molecules (mostly DHPC) on the highly curved region of the bicelle torus or pore (42,56). ^{31}P static NMR spectra and related spectral parameters of peptide-containing bicelles are shown in Fig. 3 and in Table 2. A lipid/peptide molar ratio of 60:1 was used and the spectra were recorded at 33°C. The integrity of the bicellar system appears to be preserved when the 14-mer and 21-mer peptides are added, since not only are the spectra typical of well-aligned bicelles, but the samples are optically clear. As mentioned previously, the sharp resonances at −4.5 ppm and −10.4 ppm are associated with the phosphorus atom present in the DHPC and DMPC headgroups and the linewidth is 1.1 ppm for both resonances. Upon the addition of the 14-mer peptide, the resonances of DHPC and DMPC are upfield shifted to −5.1 ppm and −10.6 ppm, respectively, and slightly broadened (1.5 ppm). In addition, the temperature range in which the bicelles align is not significantly changed in the presence of the 14-mer peptide (data not shown). Upon the addition of the 21-mer peptide, the DHPC and DMPC resonances are downfield shifted to −4.0 ppm and −9.5 ppm, respectively, and the linewidth is slightly changed, i.e., 0.7 ppm for DHPC and 1.3 ppm for DMPC. The temperature range in which the bicelles align is increased by 5° in the presence of the 21-mer peptide (data not shown).

The changes in chemical shifts observed for the DHPC and DMPC resonances in bicellar systems containing the peptides are more pronounced for the 21-mer peptide than for the 14-mer peptide. The upfield (downfield) shifts of the DHPC and DMPC resonances in the presence of the 14-mer (21-mer) peptide could both originate from a modification of the phosphorus atom environment upon interaction of the phospholipid headgroups with shielding (deshielding) moieties of the 14-mer (21-mer) peptides. In such cases, changes in the lipid isotropic chemical shift (δ_{iso}) are expected. The changes observed in the bicelle ^{31}P NMR spectra could also be due to changes in the mobility of the phospholipids, as well as the headgroup orientation. To verify the first hypothesis, we performed ^{31}P MAS NMR experiments on the bicellar system (data not shown). No change in the isotropic chemical shift (δ_{iso}) is observed when the peptides are added to the bicellar system. The lipid resonance linewidth is also unchanged when both peptides are added. Therefore, the chemical shift variation observed for both the DMPC and DHPC resonances induced by the interaction with both peptides cannot be attributed to a change in the phosphorus nucleus environment, but is most likely due to a small ordering (disordering) of the phosphate group by the 14-mer (21-mer) peptide or to a change in the headgroup orientation. As shown in Fig. 3 *A* (*top row*), the shape of the resonances is slightly distorted by the presence of the 14-mer peptide but

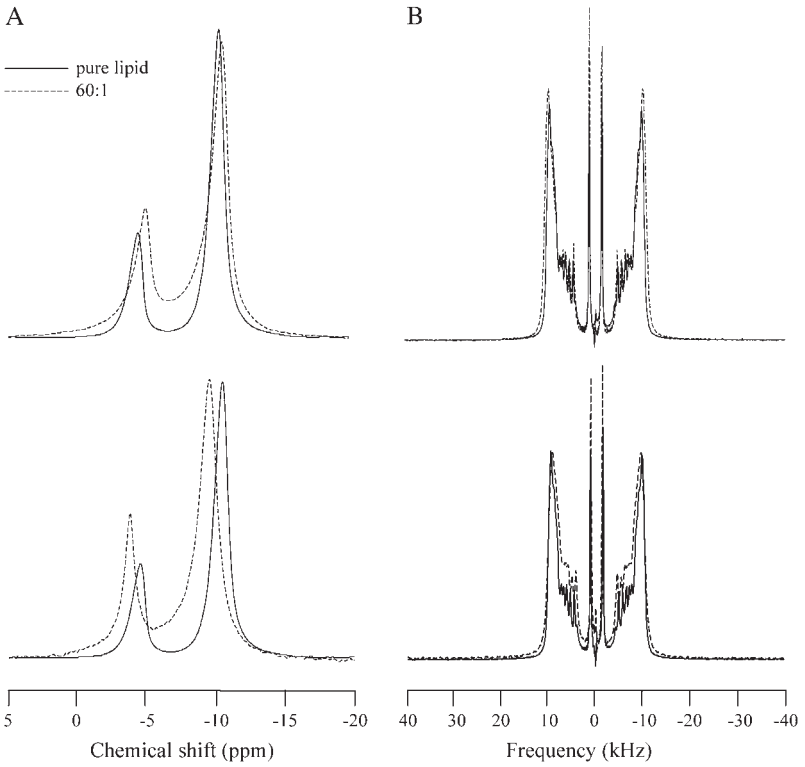

FIGURE 3 (*A*) ^{31}P static and (*B*) ^{2}H NMR spectra of bicelles at 33°C in the absence and presence of the 14-mer (*top row*) and 21-mer (*bottom row*) peptides at a lipid/peptide molar ratio of 60:1.

TABLE 2 ^{31}P NMR spectral parameters of bicelles in the absence and presence of peptides

System	DHPC (δ, ppm)	DHPC (FWHM, ppm)	DMPC (δ, ppm)	DMPC (FWHM, ppm)
Bicelles	−4.5	1.1	−10.4	1.1
+ 14-mer	−5.1	1.5	−10.6	1.5
+ 21-mer	−4.0	0.7	−9.5	1.3

Measurements were taken at 33°C for a lipid/peptide molar ratio of 60:1. FWHM, full width at half-maximum.

the sample is still clear. This could be explained by a small change in the magnetic orientation of the bicelles upon peptide binding. These results for the bicellar systems are in agreement with those obtained for the vesicular systems. Upon the 21-mer peptide addition, the spectra in Fig. 3 *A* (*bottom row*) show that the 21-mer peptide does not greatly affect the shape of the resonance, but it affects to a greater extent the orientation of the polar headgroup, since a large change in the CSA is observed. The effect of the 21-mer peptide on the bicelles is therefore most likely not attributed to a destabilizing effect of the polar headgroup but to a change in its orientation in the magnetic field. These results could explain the fact that the temperature range of bicelle alignment is unperturbed (increased) in the presence of the 14-mer (21-mer) peptides, since both peptides perturb the bicellar system to different extents.

In light of these results, it appears that both peptides interact in a different manner with bicelles, the 14-mer peptide slightly destabilizing the bicellar system, whereas the 21-mer peptide appears to have a pronounced effect on the polar headgroup orientation. Similar results were obtained by Marcotte et al., who studied the interaction of the neuropeptide methionine-enkephalin (Menk) with modified bicelles (Bic/PG and Bic/PS) (57) and attributed the differences observed in Bic/PG and Bic/PS to a different location of the peptide at the interfacial region of the bilayer. Our results therefore suggest that the 14-mer and 21-mer peptides have different membrane topologies in bicelles.

Deuterium NMR

We investigated the effect of the 14-mer and 21-mer peptides on the hydrophobic region of zwitterionic DMPC and DMPC/cholesterol systems, as well as bicelles, by ^{2}H NMR spectroscopy. This is achieved by using phospholipids with deuterated acyl chains. The deuteration of the lipid acyl chains does not significantly alter the properties of the membrane arrangement except for the main phase transition temperature, which is decreased by ~3–5°C (58). It is thus possible to determine variations in the lipid chain order by monitoring changes in the quadrupolar splitting ($\Delta\nu_Q$) values. The increase (decrease) in $\Delta\nu_Q$ for a C-D bond in a lipid bilayer system with axial symmetry is associated to order (disorder) in the deuterated chains and is related to an order parameter S_{CD}:

$$\Delta\nu_Q = \frac{3}{4}\frac{e^2qQ}{h}(3\cos^2\theta - 1)S_{CD}, \quad (4)$$

where (e^2qQ/h) is the quadrupole coupling constant for C-D bonds (~167 kHz) and θ is the angle between the bilayer normal and B_0 (39). We investigated both the plateau ($\Delta\nu_P$) and the terminal methyl ($\Delta\nu_M$) regions of the lipid acyl chains (59).

The ^{2}H NMR spectra obtained for the DMPC vesicles in the absence and presence of the 14-mer and 21-mer peptides are shown in Fig. 4 *A*. As also observed by static ^{31}P NMR, the spectral lineshapes are typical of lipids in the fluid phase. As illustrated in Fig. 4 *A* (*top row*) for the DMPC/14-mer vesicles, there is no significant change of $\Delta\nu_Q$ (kHz) at a lipid/peptide molar ratio of 60:1. However, at a molar ratio of 20:1, there is a slight increase in the quadrupolar splitting $\Delta\nu_P$ at the plateau region. As listed in Table 3, the $\Delta\nu_P$ is increased by 1.3 kHz at a lipid/peptide molar ratio of 20:1. This is reflected by an S_{CD} order parameter of 0.21 compared to the pure DMPC vesicles with an S_{CD} of 0.20. On the other hand, the spectra shown in Fig. 4 *A* (*bottom row*) indicate that the 21-mer peptide does not perturb the acyl chain ordering of DMPC at either of the lipid/peptide molar ratios used in this study since no significant changes in the quadrupolar splitting $\Delta\nu_Q$ and S_{CD} values are observed (Table 3).

The ^{2}H NMR spectra of the DMPC/cholesterol vesicles in the absence and presence of the 14-mer and 21-mer peptides at lipid/peptide molar ratios of 60:1 and 20:1 are presented in Fig. 4 *B*. As illustrated in Fig. 4 *B* (*top row*), there is an increase in the quadrupolar splitting at the plateau region in DMPC/cholesterol vesicles containing the 14-mer peptide. The $\Delta\nu_P$ of the DMPC/cholesterol/14-mer peptide system at lipid/peptide molar ratios of 60:1 and 20:1 are 43.2 kHz and 42.0 kHz, compared to 39.7 kHz for the pure DMPC/cholesterol system. This increased order is also reflected in the calculation of the S_{CD} parameter with values of 0.34 (0.33) for a molar ratio of 60:1 (20:1), compared to an S_{CD} of 0.31 for the pure system. The effect of the 21-mer peptide on the hydrophobic core of the DMPC/cholesterol vesicles is represented in the spectra of Fig. 4 *B* (*bottom row*). As observed on the spectra, there is an increase of the quadrupolar splitting at the plateau region, the $\Delta\nu_P$ being 41.1 (41.0) kHz at a lipid/peptide molar ratio of 60:1 (20:1). The S_{CD} values at the plateau region also reflect this tendency, varying from 0.31 for the pure system to 0.32 at lipid/peptide molar ratios of 60:1 and 20:1. On the other hand, the results shown in Fig. 4 indicate that the 14-mer and 21-mer peptides do not have any significant effect on the methyl region in either lipid system.

The results obtained in ^{2}H NMR experiments for the 14-mer peptide in DMPC and DMPC/cholesterol vesicles suggest that the peptide is located at the surface of the bilayer in both systems, but appears to be closer to the interface in the DMPC/cholesterol system than in DMPC, since a greater

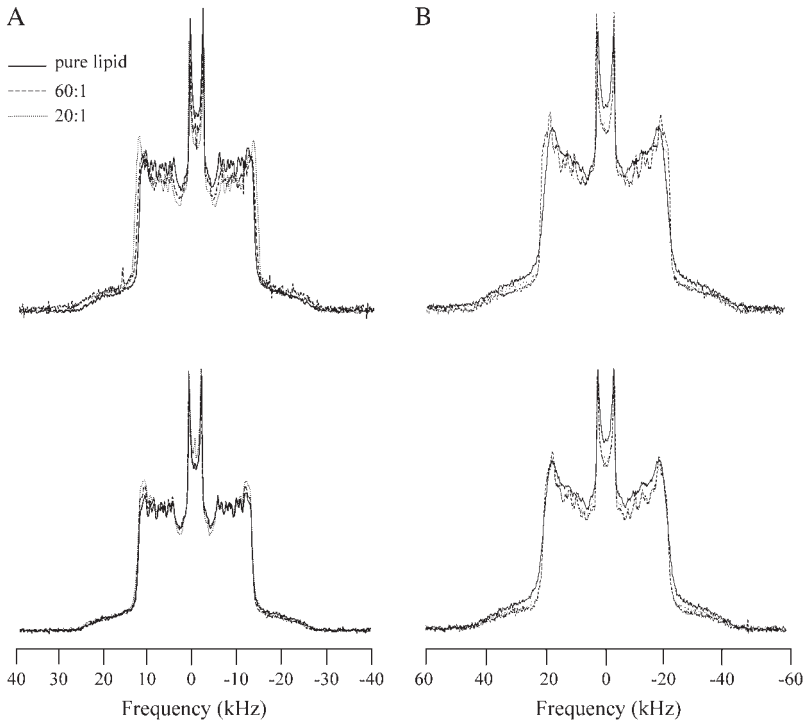

FIGURE 4 ^{2}H NMR spectra of the zwitterionic (*A*) DMPC and (*B*) DMPC/cholesterol membranes at 37°C in the absence and presence of the 14-mer (*top*) and 21-mer (*bottom*) peptides at lipid/peptide molar ratios of 60:1 and 20:1.

increase in the $\Delta\nu_Q$ of the plateau region is observed for the DMPC/cholesterol system. Similar observations have been made by Dufourc et al., who investigated the interaction of melittin with phospholipid membranes (20). They observed that the melittin location in the bilayer depends on the temperature and thus the fluidity of the bilayer. More specifically, they attributed an ordering of the acyl chains to a location of melittin at the surface of the bilayer, capping the lipid headgroups and leading to a greater chain packing. A deeper insertion of melittin in the bilayer results in a disordering effect of the hydrophobic core. Henzler-Wildman et al. observed the same temperature dependence of the degree of insertion with the human antimicrobial peptide LL-37 (12). Since the cholesterol molecule is located in the hydrophobic core of the bilayer, it could prevent the insertion of the 14-mer peptide in the bilayer, sequestering the peptide at the surface and then enhancing its capping effect at the polar headgroup and its ordering effect on the lipid acyl chains.

Interesting points of view are reported in the literature concerning the formation of rich- and poor-cholesterol domains and the influence of such domains in lipid-peptide interactions. In particular, it has been suggested that amidated pardaxin induces the formation of rich- and poor-cholesterol domains and that it interacts preferentially with poor-cholesterol regions (60). Pokorny et al. observed a similar behavior with the δ-lysin peptide that preferentially binds to eukaryotic cells and strongly interacts with liquid-disordered domains poor in cholesterol (61). These studies indicate that cholesterol reduces the ability of the peptides to disrupt rich-cholesterol domains, whereas peptides accumulate in poor-cholesterol domains, where they disrupt the lipid bilayer.

As seen in the spectra presented in Fig. 4 *A* (*bottom row*), no change is observed on the packing of the DMPC acyl chains in the presence of the 21-mer peptide. This observation could be explained either by a transmembrane orientation of the peptide, since the hydrophobic lengths of the lipid

TABLE 3 Quadrupolar splittings and S_{CD} order parameters of DMPC-d_{54} for the plateau and methyl regions in DMPC and DMPC/cholesterol systems

	$\Delta\nu_P$ (kHz)			$\Delta\nu_M$ (kHz)			$S_{CD\ (P)}$			$S_{CD\ (M)}$		
System	Pure	(60:1) 14-mer (21-mer)	(20:1) 14-mer (21-mer)	Pure	(60:1) 14-mer (21-mer)	(20:1) 14-mer (21-mer)	Pure	(60:1) 14-mer (21-mer)	(20:1) 14-mer (21-mer)	Pure	(60:1) 14-mer (21-mer)	(20:1) 14-mer (2-1mer)
DMPC	25.6	25.9 (25.6)	26.9 (25.5)	3.3	3.4 (3.2)	3.7 (3.2)	0.20	0.20 (0.20)	0.21 (0.20)	0.03	0.03 (0.03)	0.03 (0.03)
DMPC/Chol.	39.7	43.2 (41.1)	42.0 (41.0)	5.8	6.6 (5.9)	6.3 (5.9)	0.31	0.34 (0.32)	0.33 (0.32)	0.05	0.05 (0.05)	0.05 (0.05)

Measurements were taken at 37°C in the absence and presence of 14-mer and 21-mer peptides at lipid/peptide molar ratios of 60:1 and 20:1. The quadrupolar splittings have been measured on dePaked spectra (37).

bilayer and the peptide are approximately identical, or by a location of the peptide at the bilayer surface, with no direct effect on the acyl chain ordering. De Planque et al. studied a series of synthetic peptides of different lengths with model membranes of varying acyl-chain lengths and concluded that a hydrophobic match between the bilayer and the peptide results in a nonperturbed bilayer, since no adjustment has to be made to compensate for hydrophobic mismatch (16,23). In addition, spectral simulations performed by Belohorcova et al. indicate that a membrane-spanning peptide has little effect on lipid chain order if its hydrophobic length closely matches the lipid hydrophobic thickness (22). On the other hand, the results obtained by Banerjee et al. on the antimicrobial peptide alamethicin are very similar to those obtained with the 21-mer peptide (18). In fact, they also observed a change in the dynamics and/or orientation at the polar headgroup of the lipid molecules without any change in the quadrupolar splitting. They associated these observations with an interaction of the peptide near the interface of the bilayer.

In the presence of cholesterol, the hydrophobic thickness of the bilayer increases by ~5.0 Å (50). If we suppose a transmembrane orientation for the 21-mer peptide in DMPC bilayers, this membrane topology is susceptible to change by the presence of cholesterol, which rigidifies the hydrophobic core of the bilayer and makes the bilayers less flexible and elastic. A surface orientation of the 21-mer peptide is possible since cholesterol makes the bilayer more rigid and the 21-mer peptide less susceptible to penetrate the DMPC/cholesterol vesicles. If the 21-mer peptide is oriented at the bilayer surface like the 14-mer peptide, it could cap the lipid headgroups and increase the acyl chain packing. A change in membrane orientation could be observed for peptides that do not have the length required to closely span a lipid bilayer without hydrophobic mismatch, as reported by Bechinger and Nezil (17,62).

We also performed ^{2}H experiments on perdeuterated bicelles containing the 14-mer and 21-mer peptides to support the previous ^{31}P NMR results. The spectra shown in Fig. 3 *B* are typical of aligned bilayers with the normal perpendicular to the magnetic field, with well-resolved resonances for most of the deuterium positions. The addition of the 14-mer peptide (Fig. 3 *B* (*top row*)) results in a small increase of the quadrupolar splitting (0.3 kHz) but the deuterium resonances are still well resolved. This is consistent with the increase of the quadrupolar splitting observed in DMPC vesicles upon peptide binding. A surface location of the 14-mer peptide in bicelles could explain both the ^{31}P and ^{2}H NMR results. In fact, a surface location could cap the polar headgroup and stabilize the hydrophobic region of the bicelles while perturbing the choline headgroup dynamics and/or orientation. The spectra of the bicelles in the absence and presence of the 21-mer peptide are shown in Fig. 3 *B* (*bottom row*). As with the 14-mer peptide, the addition of the 21-mer peptide does not perturb the bicelle orientation. In addition, there is no significant change in the quadrupolar splitting. However, unlike the 14-mer peptide-containing bicelles, the deuterium resonances are broadened upon 21-mer peptide binding. This could originate from a decrease of the T_2 relaxation time that could be due to a transmembrane orientation of the peptide. This is consistent with the stabilizing effect of the 21-mer peptide on bicelles as revealed by the ^{31}P static NMR spectra.

Fourier transform infrared spectroscopy

Since the different molecular groups composing the phospholipids vibrate at different frequencies, FTIR spectroscopy is a useful tool for probing several regions of the bilayer simultaneously. In this study, we investigated the interfacial region of the lipid bilayers by monitoring the ester carbonyl stretching mode in the spectral region 1700–1750 cm^{-1}. The carbonyl stretching mode has been extensively described by Mendelsohn and Mantsch (63), Blume et al. (64), and Mantsch and McElhaney (65), and was reviewed more recently by Tamm and Tatulian (66) and Lewis and McElhaney (67). Deconvolution of the broad C=O stretching band centered at ~1735 cm^{-1} reveals the superposition of two narrower bands at 1742 and 1728 cm^{-1}. The high-frequency band is associated with the nonhydrogen-bonded carbonyl groups, whereas the band at 1728 cm^{-1} corresponds to the hydrogen-bonded carbonyl groups (64,65,67).

FTIR was used in this study to investigate the effect of the peptides occurring at the polar/apolar interface of the bilayer by monitoring changes in the ester carbonyl stretching intensities of the deconvolved band at 1742 and 1728 cm^{-1} in the infrared spectra as a function of the concentration of the 14-mer and 21-mer peptides at temperatures below (15°C) and above (50°C) the main phase transition. For DMPC and DMPG phospholipids, the main phase transition occurs at 23°C, and cholesterol is known to attenuate this phase transition (68,69).

The deconvolved FTIR spectra of the phospholipid carbonyl stretching mode of DMPC, DMPG, and DMPC/cholesterol vesicles are displayed in Fig. 5. At temperatures above the gel-to-fluid phase transition temperature, the DMPC and DMPG bilayers are more hydrated compared to the DMPC/cholesterol system. For DMPC and DMPG, this is reflected by a more intense band at 1728 cm^{-1} characteristic of hydrogen-bonded ester carbonyls, and this is consistent with the looser packing of the lipids in the fluid phase and a greater access of water molecules to the ester carbonyls (67,70). However, the band at 1728 cm^{-1} in DMPC/cholesterol vesicles is still less intense in comparison to the one at 1742 cm^{-1} even if the sample is heated to 50°C. This behavior indicates that the location of the cholesterol molecules in the lipid bilayer decreases the hydration of the carbonyl groups. The spectral lineshapes also indicate that the DMPG vesicles are less hydrated than the DMPC and DMPC/cholesterol systems at 15°C. Above the phase transition temperature however, the DMPC and DMPG

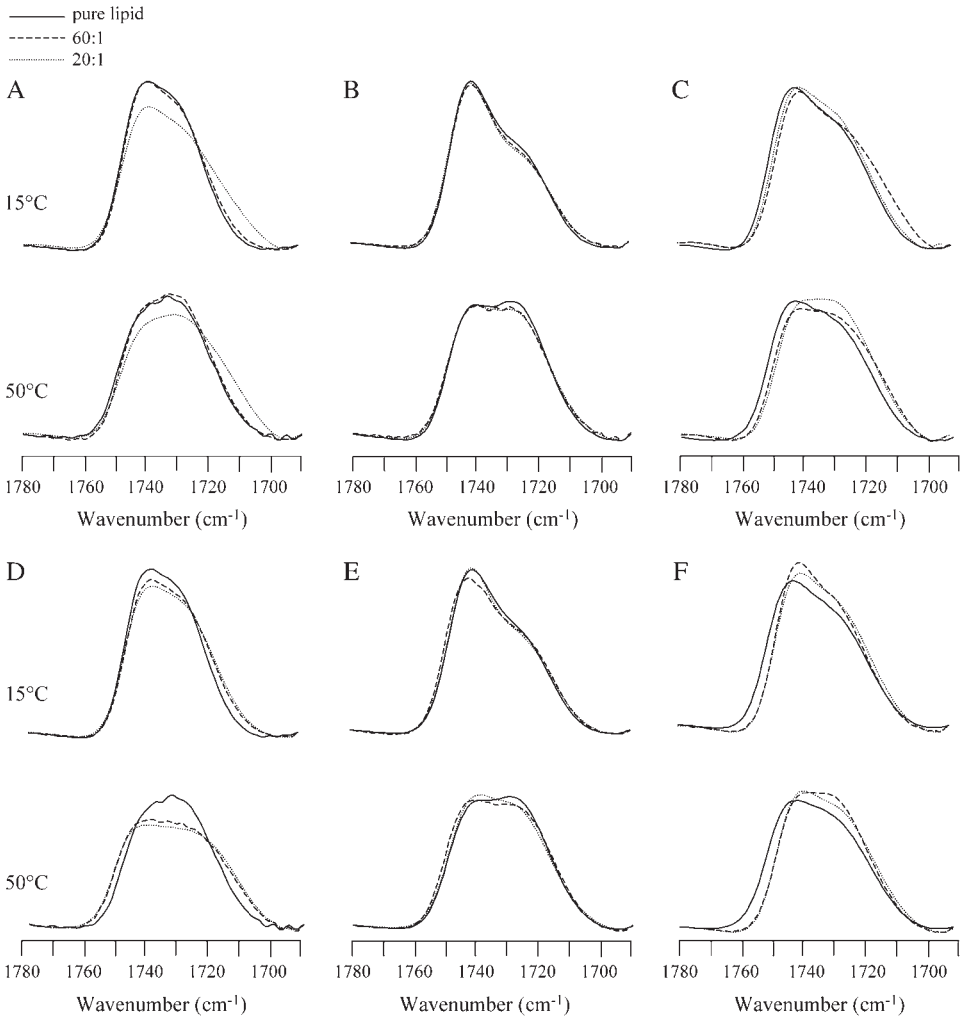

FIGURE 5 FTIR spectra of the C=O stretching bands of zwitterionic and anionic membranes at 15°C and 50°C: (*A* and *D*) DMPC, (*B* and *E*) DMPG, and (*C* and *F*) DMPC/cholesterol vesicles in the absence and presence of the 14-mer (*top*) and 21-mer (*bottom*) peptides at lipid/peptide molar ratios of 60:1 and 20:1.

vesicles become well hydrated since the intensity of the 1728-cm^{-1} band is greater than that of the 1742-cm^{-1} band.

The effects of the 14-mer and 21-mer peptides on the hydration profile of the DMPC, DMPG, and DMPC/cholesterol systems below and above the gel-to-fluid phase transition temperature are shown in Fig. 5. For the DMPC/14-mer vesicles at a lipid/peptide molar ratio of 60:1, there are no changes in the spectral lineshape or in the intensity of the components at 1742 cm^{-1} and 1728 cm^{-1} at either temperature. However, there is a broadening of the band at 1728 cm^{-1} at a lipid/peptide molar ratio of 20:1. This suggests that the lipid molecules in interaction with the 14-mer peptide undergo greater motion than the pure lipid molecules (71) but that the hydrogen bonding of the carbonyl groups does not seem to be perturbed. Arrondo et al. have reported a similar effect of glycophorin on DPPC membranes, which they attributed to an increase of lipid motion in the presence of the protein (72). A similar effect is observed for the DMPC/21-mer vesicles, as shown in Fig. 5 *D*. At 15°C, there is a broadening of the band at 1728 cm^{-1}, whereas both bands are broadened at 50°C, suggesting that the lipid molecules undergo rapid molecular motion in the bilayer with no change in the hydrogen-bonding pattern. These results suggest that the 14-mer and 21-mer peptides make the polar region of the DMPC molecules more mobile upon peptide binding. This is consistent with the decrease of the CSA observed for both peptides in ^{31}P NMR spectra of DMPC vesicles.

The spectra of the DMPG/14-mer vesicles shown in Fig. 5 *B* indicate that there are no significant changes in the relative intensities of the 1742-cm^{-1} and 1728-cm^{-1} bands either below or above the phase-transition temperatures. Very similar results are also obtained for the DMPG/21-mer vesicles, as shown in Fig. 5 *E*. These results indicate that there is no significant change in the hydrogen-bonding profile of the DMPG vesicles upon peptide binding at molar ratios of 60:1 and 20:1 and at temperatures below and above the lipid phase transition. Marcotte et al. obtained very similar results with the neuropeptide Menk in interaction with DMPG vesicles (73). More specifically, they observed no change in the hydrogen bonding profile, whereas a reduction in the CSA was seen on the DMPG ^{31}P NMR spectra. These results were attributed to a surface location of Menk in DMPG vesicles.

The FTIR spectra of the DMPC/cholesterol/14-mer system are shown in Fig. 5 *C*. At 15°C, there are no significant changes in the 1728-cm^{-1} and 1742-cm^{-1} bands upon the 14-mer peptide binding at molar ratios of 60:1 and 20:1. Upon sample heating, there is a shift of the band to lower frequencies and the component at 1728 cm^{-1} becomes more intense. According to Lewis et al., this shift to lower frequencies could be attributed to a decrease in the polarity of the environment around the ester carbonyl groups (74). Fig. 5 *F* illustrates the case of the DMPC/cholesterol/21-mer system. At 15°C and at lipid/peptide molar ratios of 60:1 and 20:1, there is a slight narrowing of the component at 1742 cm^{-1} and no significant change for the component at 1728 cm^{-1}. The narrowing could be explained by a decrease in the motion of lipid molecules upon peptide binding. As the temperature is raised until it reaches 50°C, the intensity of the component at 1728 cm^{-1} increases and becomes identical to that at 1742 cm^{-1}. In summary, the 14-mer peptide reduces the polarity of the environment and favors the formation of hydrogen bonds at the ester carbonyl groups. The 21-mer peptide reduces the mobility of the lipid molecules and also favors the formation of hydrogen bonds to the ester carbonyl groups. Since the component at 1728 cm^{-1} is more broadened in the presence of the 14-mer peptide compared to that in the presence of the 21-mer peptide, the 14-mer peptide seems to increase to a greater extent the mobility of the polar region of the lipid molecules, again supporting the data obtained by ^{31}P NMR spectra, which showed a more pronounced effect of the 14-mer peptide on the dynamics and/or orientation of the DMPC headgroups compared to the effect of the 21-mer peptide.

Proposed mechanisms of membrane perturbation

The aim of this study was to shed light on the interactions between synthetic amphipathic peptides and model membranes. A better understanding of such interactions is very important for the design of active antimicrobial peptides with the desired specificity against bacteria. We have studied the interactions of synthetic 14-mer and 21-mer peptides with DMPC, DMPC/cholesterol, and DMPG vesicles, and with bicelles, by varying the lipid/peptide molar ratio. Solid-state NMR and Fourier transform infrared spectroscopies have been shown to be very useful to probe the effects of the 14-mer and 21-mer peptides at three different regions of the bilayer, namely the polar headgroup, the interfacial region, and the hydrophobic core. Based on these results, mechanisms of interaction between the 14-mer and 21-mer peptides and model membranes are proposed below. More specifically, since the perturbing effect induced by the peptide binding to the bilayer is more pronounced for the 14-mer peptide than for the 21-mer peptide, we suggest different modes of interaction between these peptides and lipid bilayers.

The effect of the 14-mer peptide on the dynamics and/or orientation of the polar headgroup has been shown to be slightly more pronounced on DMPC than on DMPG. The morphology of the DMPC vesicles is also affected to a greater extent than that of the DMPG and DMPC/cholesterol vesicles. The polar headgroups of bicelles are slightly destabilized by the 14-mer peptide, since the shape of the DHPC and DMPC resonances are slightly modified. As shown by ^{2}H NMR experiments on DMPC vesicles, the 14-mer peptide induces order in the hydrophobic core of the bilayer, and the presence of cholesterol increases this ordering effect. At the interfacial region of DMPC vesicles, the lipid molecules in interaction with the 14-mer peptide undergo rapid motions, but the hydrogen bonding pattern at the ester carbonyl is not modified. In the DMPC/cholesterol vesicles, the interfacial region is less exposed to a polar environment and more intermolecular hydrogen bonds are created with the surrounding water molecules at the ester carbonyl groups.

Combined with previous calcein and ^{23}Na leakage studies done by fluorescence and NMR (28), our results strongly suggest that the 14-mer peptide interacts at the bilayer surface with an in-plane orientation. The inverse-cone shape of the 14-mer peptide could explain the perturbation induced in the bilayer upon peptide binding. According to Epand et al., this peptide belongs to structural class A, in which the region occupied by the hydrophilic part on the amphipathic helix is larger than the hydrophobic region, and this shape is recognized as inducing positive curvature in the bilayer (6). Such an effect has been reported by Ramamoorthy et al., who studied the disruption of the lipid bilayer induced by an analog of magainin, MSI-78 (75). The induction of a positive curvature by the 14-mer peptide is also supported by a net decrease of calcein leakage of vesicles composed of phosphatidylcholine and phosphatidylethanolamine lipids (28). More specifically, the cone shape of the phosphatidylethanolamine lipids counterbalances the inverse-cone shape of the 14-mer peptide, therefore inhibiting the positive curvature imposed on the lipid bilayer. The presence of cholesterol only affects the surface interaction of the peptide with the polar headgroups.

The results obtained by ^{31}P NMR experiments indicate that the 21-mer peptide has an effect on the dynamics and/or orientation of the DMPC headgroups, and the presence of cholesterol does not perturb this pattern. The morphology of the DMPC vesicles is also affected by peptide binding. At a lipid/peptide molar ratio of 60:1, the dynamics and/or orientation of the DMPG polar headgroup are not affected. The 21-mer peptide has a pronounced stabilizing effect on bicelles, since the temperature range of orientation is increased. The order of the DMPC acyl chains in the DMPC system is not significantly affected upon peptide binding, whereas it is slightly increased in the DMPC/cholesterol system. The hydrogen-bonding patterns of the DMPC and DMPG systems are very similar to those obtained with the 14-mer peptide, whereas in DMPC/cholesterol vesicles, the presence of the 21-mer peptide increases the number of

hydrogen bonds at the ester carbonyl groups. Even though previous single-channel measurements and attenuated total reflectance experiments suggest a partial transmembrane alignment for the 21-mer peptide in DMPC bilayers (29–31), we still cannot draw conclusions about the exact membrane topology of the 21-mer peptide in lipid bilayers.

CONCLUSIONS

In this study, we investigated the interactions between synthetic amphipathic peptides and model membranes by solid-state NMR and FTIR spectroscopy. The model membranes investigated include zwitterionic vesicles composed of DMPC and DMPC/cholesterol, anionic DMPG vesicles, and DMPC/DHPC bicelles. The results obtained by ^{31}P and ^{2}H NMR, and by FTIR spectroscopy, suggest that the 14-mer peptide is located at the bilayer surface of DMPC, DMPG, and DMPC/cholesterol vesicles. Our results also indicate that the presence of cholesterol confines the peptide closer to the surface of the bilayer and makes the vesicles less deformable in the magnetic field. The 21-mer peptide seems to interact differently with the different lipid systems. As revealed by ^{2}H NMR spectroscopy, the presence of cholesterol affects the interaction between the 21-mer peptide and DMPC bilayers, which could be due to a change in membrane topology. Our results also indicate that neither peptide significantly alters the orientation and morphology of bicelles in the magnetic field.

Experiments are in progress to further confirm the membrane orientation and topology of the 14-mer and 21-mer peptides. These studies should provide useful information on the mechanisms of action of the 14-mer and 21-mer peptides. In addition, we are synthesizing a series of novel synthetic peptides that possess positive charges to probe the influence of these charges on the membrane interactions of the peptides. This will be of primary importance for the design of antimicrobial agents targeted toward negatively charged bacterial membranes.

The authors thank Pierre Audet for his technical assistance and helpful discussions.

This work was supported by the Natural Science and Engineering Research Council of Canada, the Fonds Québécois de la Recherche sur la Nature et les Technologies, the Centre de Recherche sur la Structure, la Fonction et l'Ingénierie des Protéines, and the Centre de Recherche en Sciences et Ingénierie des Macromolécules. M.O. also thanks the Natural Science and Engineering Research Council for the award of a postgraduate scholarship.

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