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Comparison between the dynamics of lipid/gramicidin A systems in the lamellar and hexagonal phases: a solid-state ^{13}C NMR study

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Abstract

We have investigated the effect of gramicidin A on the dynamics of two model membranes: dimyristoylphosphatidylcholine (DMPC) in the lamellar phase at a lipid-to-peptide molar ratio of 10:1 and dioleoylphosphatidylcholine (DOPC) in the hexagonal H_{II} phase at a lipid-to-peptide molar ratio of 5:1. Natural abundance ^{13}C nuclear magnetic resonance (NMR) spectroscopy was used in combination with magic angle spinning to increase the spectral resolution, therefore allowing the different regions of the lipid bilayers to be investigated from the same spectra. ^{31}P NMR was also used to detect and confirm the formation of the DOPC H_{II} phase in the presence of gramicidin A. In order to examine the effect of gramicidin A on both the fast and slow motions of DMPC and DOPC, the ^{1}H spin-lattice relaxation times in the laboratory frame (HT_1) as well as the ^{1}H spin-lattice relaxation times in the rotating frame ($HT_{1\rho}$) were calculated for each resolved protonated lipid resonance in the ^{13}C spectra. For both DMPC and DOPC, we found that the presence of gramicidin A does not significantly affect the fast motions of the lipid acyl chains but increases slightly the fast motions of the polar head group. However, the $HT_{1\rho}$ are significantly decreased, this effect being more pronounced for DOPC most likely due to a decrease in the rate of the lipid lateral diffusion.

Keywords: ^{13}C nuclear magnetic resonance; ^{31}P nuclear magnetic resonance; Peptide; Relaxation; Lipid bilayer

1. Introduction

Several physical and biological functions of membranes are thought to depend upon the structure, organization and dynamics of the lipid molecules. In this respect, considerable attention has been given to the ability of lipids to adopt non-bilayer organizations in the presence of peptides or proteins [1–8]. In addition, this ability of lipids to adopt non-bilayer structures has been implied to be of importance for various functional membrane processes such as transbilayer movements of lipids and membrane fusion [1].

Gramicidin A, a 15 amino acid linear hydrophobic peptide which can form transmembrane channels that induce permeability to monovalent cations in biological membranes [9], is one of the obvious candidates to study the effect of a membrane peptide on the polymorphism and dynamics of lipids since it

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induces non-bilayer organizations in different phosphatidylcholine systems [1,10–14]. For example, gramicidin A at high concentration in unsaturated dioleoylphosphatidylcholine (DOPC) (lipid-to-peptide molar ratios of 25:1 and lower) induces H_{II} phase formation [11]. In addition, gramicidin is known to induce H_{II} phase formation in erythrocyte membranes [5]. On the other hand, Rajan and coworkers [15] have shown by ^{31}P nuclear magnetic resonance (NMR) that in saturated dimyristoylphosphatidylcholine (DMPC) systems, very high concentrations of gramicidin A (DMPC/gramicidin A molar ratio $<4:1$) can induce non-bilayer organization, namely isotropic structures. This observation was corroborated by small X-ray diffraction measurements [14]. At lower gramicidin A concentration, DMPC remains in the lamellar phase.

Static solid-state NMR studies of the interaction between gramicidin and DMPC bilayers in the lamellar phase have shown that the presence of the peptide significantly affects the slow motions of the lipid acyl chains [16]. On the other hand, the induction of a DOPC H_{II} phase by gramicidin A is accompanied with an aggregation of the peptide and this aggregation has been shown to be essential for the H_{II} phase formation. Moreover, the aggregation of gramicidin A may lead to a barrier for the lipid lateral diffusion in the induced H_{II} phase [1,4]. It is therefore of interest to investigate in detail the dynamics of lipid membranes in the presence of gramicidin A, both in the lamellar and hexagonal phases.

Several techniques have been used to study the dynamics of lipid membranes. Among these techniques, ^{13}C NMR spectroscopy [16–20] is well suited for dynamic studies since it allows the simultaneous investigation of different regions of the lipid molecules, i.e. the polar head group, the glycerol backbone and the acyl chains. Furthermore, a cross-polarization (CP) method has been developed in which the combined use of both magic angle spinning (MAS) and ramped-amplitude cross-polarization (RAMP-CP) suppresses Hartmann-Hahn mismatches and improves the signal-to-noise ratio of some of the carbon resonances [21,22], therefore facilitating the investigation of membrane systems by ^{13}C natural abundance NMR. Moreover, NMR spin-lattice relaxation measurements are valuable techniques to obtain information on both the rate and type of motions that occur in bilayers. The spin-lattice relaxation times in the laboratory frame (T_1) are sensitive to high frequency motions (10^8–10^{11} s^{-1}), such as the internal rotations in the acyl chains and the rapid reorientation of the lipid molecules about their long axis. In contrast, spin-lattice relaxation times in the rotating frame ($T_{1\rho}$) are sensitive to lower frequency motions (10^4–10^6 s^{-1}), such as the wobbling of the lipid molecules [23–25].

In the present study, we have investigated the dynamics of DMPC/gramicidin and DOPC/gramicidin systems in the lamellar and hexagonal phase, respectively, by ^1H relaxation time measurements from natural abundance ^{13}C NMR spectra. The spectra were acquired with the magic angle spinning technique in order to investigate the dynamics in the different regions of the lipid bilayers. Moreover, ^{31}P NMR has been used to confirm the phase behavior of DOPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 5:1. This molar ratio was chosen since it has been shown that at a DOPC-to-gramicidin molar ratio of 10:1, a lamellar component is also present in addition to the hexagonal phase [13,26]. Addition of gramicidin to DOPC at a lipid-to-peptide molar ratio of 5:1 allows the formation of a pure DOPC hexagonal phase and, therefore, the relaxation times measured solely reflect DOPC in this phase. The results obtained by ^{31}P NMR confirm the formation of a pure DOPC H_{II} phase in the presence of the peptide. In addition, the combination of HT_1 and $HT_{1\rho}$ measurements indicates that gramicidin A does not significantly affect the fast motions present in DMPC or DOPC acyl chains but reduces the rate and/or amplitude of slow motions present in both lipid systems, the effect being more pronounced for DOPC in the H_{II} phase, possibly due to peptide aggregation.

2. Material and methods

2.1. Materials

DMPC and DOPC were obtained from Sigma (St. Louis, MO) and used without any purification. Gramicidin A was purchased from Fluka Chemika-Biochemica (Ronkonkoma, NY) and trifluoroethanol (TFE) was purchased from Sigma.

2.2. Pure DMPC and DMPC/gramicidin A samples

The pure DMPC sample was prepared by adding H_2O (50 wt%) to the lipid. The DMPC/gramicidin A sample was prepared at a 10:1 molar ratio by codissolving the appropriate amount of peptide and lipid in TFE. To obtain a homogeneous peptide/lipid system, the sample was incubated a few minutes at 52°C and shaken on a vortex mixer at least a few times during the incubation. After the incubation, TFE was evaporated with a nitrogen stream followed by high vacuum pumping overnight to ensure the complete evaporation of the solvent. The sample was then hydrated (50 wt%) with pure H_2O. Both the pure DMPC and DMPC/gramicidin A samples were submitted to several cycles of heating (52°C), vortex shaking and cooling (0°C) after hydration.

2.3. Pure DOPC and DOPC/gramicidin A samples

The pure DOPC sample was prepared by adding H_2O (50 wt% of lipid) to the lipid and was submitted to several cycles of heating (52°C), vortex shaking, and cooling (0°C). The preparation of the DOPC/gramicidin A (5:1 molar ratio) sample was based on the method of Killian et al. [2]. More specifically, the appropriate amount of peptide in TFE was added to a lipid dispersion in H_2O and excess water was added to the sample. The sample was freeze-dried in liquid nitrogen and lyophilized for 2 days to ensure complete evaporation of TFE and water. The resulting powder was then hydrated (50 wt%) with H_2O and submitted to several cycles of heating (52°C), vortex shaking and cooling (0°C).

2.4. ^{13}C NMR measurements

The ^{13}C MAS NMR spectra were acquired on a Bruker ASX 300 solid-state NMR spectrometer (Bruker Canada, Milton, ON) operating at a frequency of 75.4 MHz for ^{13}C and a frequency of 300.0 MHz for ^{1}H. A broadband/^{1}H dual frequency 4 mm magic angle spinning probe head was used for all experiments (Bruker Canada). The spectra were acquired at a temperature of 30°C and with a spinning speed of 2.000 ± 0.002 kHz.

The ^{13}C spectra were acquired with the cross-polarization sequence [27] while the HT_1 measurements were carried out with the inversion recovery method [28] coupled to the cross-polarization sequence. The pulse sequence used for the $HT_{1\rho}$ experiments has been described elsewhere [22]. For all these experiments, the conventional cross-polarization was replaced by RAMP-CP on the proton channel [21]. More specifically, the matched spin-lock field period began with a 34 kHz proton spin-lock pulse and the amplitude was increased up to 69 kHz. The ^{13}C spin-lock field was set to 47 kHz. The CP contact time was 15 ms for the pure lipids and the lipid/GA samples, unless indicated otherwise [22]. The 90° proton pulse length was typically 5.5 μs and protons were decoupled during data acquisition. The spectra (4 K data points) were acquired with an acquisition time of 0.04 s and a spectral width of 50 kHz. The recycle delay was set to 8 s for the 1D spectra and the HT_1 experiments and to 10 s for the $HT_{1\rho}$ experiments. Between 400 and 720 scans were acquired for each 1D spectrum while 1200–1600 scans were recorded for each spectrum in the relaxation experiments. The spectra were zero-filled to 16 K points and a 10 Hz line broadening was applied to all spectra. The chemical shifts were referenced relative to external tetramethylsilane.

Since long relaxation experiments are particularly sensitive to changes in experimental conditions, short and long τ values were alternated in the relaxation experiments. Furthermore, a minimum of 12 different delay values were used in each experiment. The uncertainty in the T_1 values arising from this procedure is approx. 5%, estimated from replica experiments.

2.5. ^{31}P NMR measurements

The ^{31}P spectra were acquired at 121.5 MHz on a Bruker ASX-300 (Bruker Canada) operating at a ^{1}H frequency of 300.0 MHz. Experiments were carried out with a broadband/^{1}H dual frequency 4 mm probe head (Bruker Canada) under conditions of proton decoupling. The free induction decays (1 K data points) were recorded with a spin echo sequence with a 90° pulse length of 5.0 μs, a delay between pulses of 30 μs and a 5 s recycle delay. The temperature was controlled to within ± 0.5°C and the chemical shifts expressed in parts per millions (ppm) were

referenced relative to the signal of phosphoric acid at 0 ppm.

3. Results and discussion

3.1. Dynamics of the DMPC/gramicidin A system in the lamellar phase

We have first investigated the effect of gramicidin A at a lipid-to-peptide molar ratio of 10:1 on the dynamics of DMPC in the lamellar phase. Fig. 1 shows the ^{13}C CPMAS NMR spectra of the pure DMPC multilamellar dispersion (A) and the DMPC/gramicidin A mixture (10:1 molar ratio) (B) at 30°C in the liquid-crystalline lamellar phase. For each spectrum, well resolved ^{13}C peaks can be observed and assigned to the three regions of the lipid molecules, i.e. the polar head group, the glycerol backbone and the acyl chains. The complete chemical shift assignments for pure DMPC and DMPC in the presence of gramicidin A are given in Table 1 and are in agreement with the assignments of Forbes et al. [29] and Le Guernevé and Auger [22]. In the spectrum of DMPC in the presence of gramicidin A,

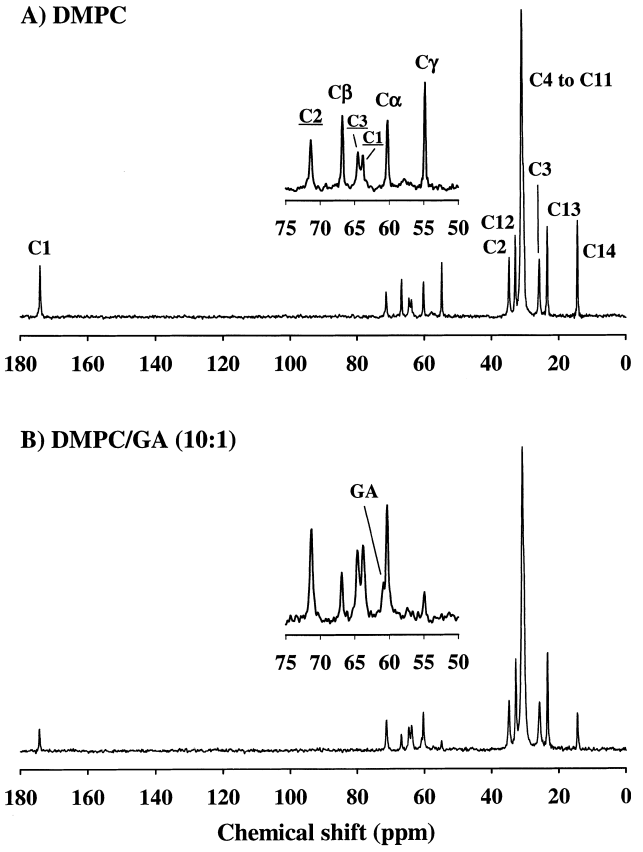

Fig. 1. ^{13}C NMR spectra at 30°C of (A) pure DMPC and (B) DMPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 10:1. The inset represents the expansion of the spectral region between 50 and 75 ppm. The choline head group carbons are indicated by Greek letters, the glycerol backbone carbons are underlined and the remaining carbons belong to the acyl chains.

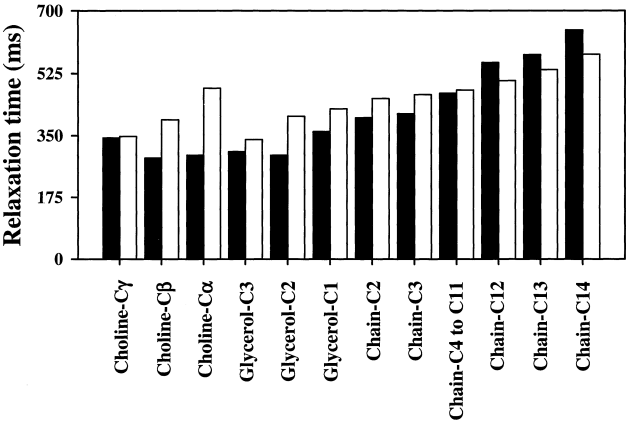

Fig. 2. HT_1 at 30°C for the DMPC resonances in pure DMPC (black bars) and DMPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 10:1 (open bars). The abscissa represents the different protonated carbons as defined in Fig. 1.

Table 1
Chemical shift assignments (in ppm) for pure DMPC and DMPC in the presence of gramicidin A at 30°C at a lipid-to-peptide molar ratio of 10:1

	DMPC	DMPC/gramicidin A
Polar head group		
Choline-Cγ	54.8	54.9
Choline-Cβ	66.8	66.9
Choline-Cα	60.3	60.3
Interfacial region		
Glycerol-C3	64.5	64.6
Glycerol-C2	71.4	71.9
Glycerol-C1	63.8	63.8
Hydrocarbon chains		
Chain-C1	174.2	174.3
Chain-C2	34.8	34.9
Chain-C3	25.9	25.8
Chain-C4 to C11	31.2	30.8
Chain-C12	33.0	32.8
Chain-C13	23.5	23.4
Chain-C14	14.5	14.6
Gramicidin A		
Cα-ethanolamine		60.9

a new small resonance at 61 ppm can be observed (Fig. 1) and has been assigned to the Cα of the peptide ethanolamine group [26].

The proton spin-lattice relaxation times in the laboratory frame (HT_1) at 30°C of the DMPC resonances for pure DMPC and DMPC in the presence of gramicidin A are given in Table 2 and illustrated in Fig. 2. For pure DMPC, the HT_1 for the head group and the glycerol backbone are approx. 300 ms with slightly higher values (350 ms) for the Cγ carbon of the head group and the C1 carbon of the glycerol backbone. Higher values are also observed for the acyl chain carbons. In addition, the relaxation time values increase along the chain from the interfacial region to the terminal methyl group. These results are in agreement with results of previous groups and suggest that the fast motions responsible for spin-lattice relaxation are more restricted for the polar head group and glycerol backbone nuclei than for the carbons in the acyl chains [22,23,30,31]. Furthermore, the results obtained confirm the idea of a motional gradient extending from the glycerol backbone region in both directions. Moreover, previous ^1H relaxation measurements carried out on DMPC at higher temperature (50°C) showed that longer HT_1 values are obtained with increasing temperature, indicating that the frequencies of the motions contributing to the HT_1 relaxation are higher than the ^1H Larmor frequency (300 MHz) [22].

When gramicidin A is added to the lipid dispersion, no significant change in the HT_1 is observed for the majority of the protonated carbons of DMPC, with the exception of the choline Cα and Cβ carbons, as well as the glycerol C2 carbon. This indicates that gramicidin A has no significant effect on the fast motions of the DMPC acyl chains but that the fast motions of the polar head group are slightly enhanced, most likely due to the increased head group spacing caused by the incorporation of the peptide between the lipid acyl chains. These results are in agreement with previous studies that showed that proteins and peptides have little or no effect on high frequency motions of lipid acyl chains in membranes [16,32–35].

The proton spin-lattice relaxation times in the rotating frame ($HT_{1\rho}$) of the DMPC resonances in the absence and presence of gramicidin A at 30°C are given in Table 2 and illustrated in Fig. 3A. Fig. 3B also gives the relative variation of the $HT_{1\rho}$ observed for the DMPC atom sites in the presence of gramicidin A $(((HT_{1\rho}$ DMPC/gramicidin A$-HT_{1\rho}$ DMPC)/$HT_{1\rho}$ DMPC)$\times 100$). It can be observed that the incorporation of gramicidin A into the DMPC bilayer causes a decrease of the $HT_{1\rho}$ for all the carbon nuclei with the exception of the Cγ carbon of the polar head group. Moreover, the decrease in the $HT_{1\rho}$ values is more pronounced for the carbons of the acyl chains and the glycerol backbone

Table 2
HT_1 and $HT_{1\rho}$ for pure DMPC and DMPC in the presence of gramicidin A at 30°C at a lipid-to-peptide molar ratio of 10:1

	HT_1 (ms)		$HT_{1\rho}$ (ms)	
	DMPC	DMPC/GA	DMPC	DMPC/GA
Polar head group				
Choline-Cγ	344	348	141	157
Choline-Cβ	287	395	89	62
Choline-Cα	295	484	86	65
Interfacial region				
Glycerol-C3	305	339	39	16
Glycerol-C2	295	405	41	21
Glycerol-C1	362	426	33	17
Hydrocarbon chains				
Chain-C2	401	455	54	27
Chain-C3	412	466	58	28
Chain-C4 to C11	470	479	64	34
Chain-C12	556	505	78	47
Chain-C13	578	536	95	59
Chain-C14	647	579	109	62

(about 40–50%), compared to the effect observed in the polar head group (about 20–30%).

The observed decrease of the $HT_{1\rho}$ values in the presence of gramicidin A could either reflect a decrease of the frequency of a motion near or above 10^8 Hz or an enhancement and/or the presence of a new low frequency motion. However, previous studies have shown that a reduction in the $HT_{1\rho}$, not matched by a similar reduction in the HT_1, is indicative of a change in the amplitude of a motion within the membrane at low rate which was present but not to the same extent in the pure lipid membrane as opposed to a decrease in the rate of a high frequency motion [16]. The results obtained in the present study are in agreement with previous static ^{13}C NMR studies on the effect of gramicidin on DMPC [16,36,37]. In particular, Cornell and coworkers [16] have shown that the incorporation of the peptide in DMPC bilayers decreases significantly the $HT_{1\rho}$ of the broad CH_2 resonance, indicating an increase of the lipid slow motions.

One of the considerable advantages of the magic angle spinning technique used in the present study is the increased spectral resolution, therefore allowing the distinction of the dynamics in different regions of the lipid bilayer. Hence, our results indicate that the slow motions in the polar head group region of DMPC bilayers are less affected by the interaction with gramicidin A while the fast motions are the most affected in this region. On the other hand, the most important decrease in the $HT_{1\rho}$ values in the presence of gramicidin A is observed for the carbons of the glycerol backbone and the first two protonated carbons of the acyl chains. This is most likely due to the formation of hydrogen bonds between the lipid carbonyl groups and the indole NH of the tryptophan residues in the peptide [38,39], which would restrict motions such as the wobble of the lipid molecules in the interfacial region of the bilayer.

3.2. Dynamics of the DOPC/gramicidin A system in the hexagonal phase

We have also investigated the dynamics of unsaturated phosphatidylcholine bilayers in the inverse hexagonal phase, induced by the presence of gramicidin A at a lipid-to-peptide molar ratio of 5:1. Solid-state ^{31}P NMR spectroscopy, a technique well suited to study the phase behavior of lipid membranes [1,40–43], was first used to detect and confirm the formation of a hexagonal H_{II} phase in this system. Fig. 4 presents the ^{31}P NMR spectra at 30°C of pure DOPC (A) and DOPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 5:1 (B). The spectrum of pure DOPC, with a chemical shift anisotropy (CSA) of 49 ppm, is typical of lipids in the lamellar phase [40,43]. Addition of gramicidin A at a lipid-to-peptide molar ratio of 5:1 gives rise to a spectrum with a lineshape characteristic of a pure hexagonal lipid phase, with a low-field peak and a high-field shoulder as well as a CSA of 25 ppm. This reduction of the chemical shift anisotropy by a factor of two compared to the lamellar phase is due to the additional motional averaging experienced by the lipids in the hexagonal phase, namely the lateral diffusion around the axes of the tubes [1,40,43]. The ^{31}P NMR spectra obtained in the present study are in full agreement with previous results obtained by Killian and coworkers on the study of hexagonal phase formation induced by gramicidin A [1,4].

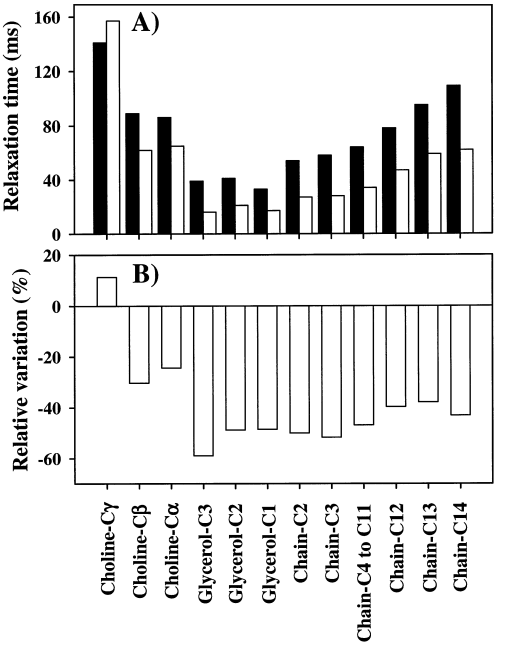

Fig. 3. (A) $HT_{1\rho}$ at 30°C for the DMPC resonances in pure DMPC (black bars) and DMPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 10:1 (open bars). (B) Relative variation $(((HT_{1\rho}$ DMPC/gramicidin A$-HT_{1\rho}$ DMPC)/$HT_{1\rho}$ DMPC)$\times 100)$ of the $HT_{1\rho}$ for the DMPC resonances. The abscissa represents the different carbons as defined in Fig. 1.

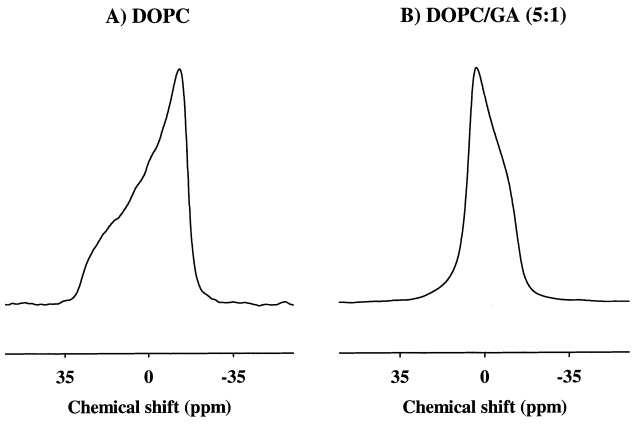

Fig. 4. ^{31}P NMR spectra at 30°C of (A) pure DOPC and (B) DOPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 5:1.

Fig. 5 presents the ^{13}C NMR spectra of pure DOPC (A) and DOPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 5:1 (B) at 30°C. Those spectra were acquired with contact times of 15 ms and 5 ms for pure DOPC and DOPC:GA, respectively. As for pure DMPC, carbon resonances from the different regions of the phospholipid molecule can be observed and assigned to specific protonated carbons of the lipid. The spectral attribution has been determined according to the assignments of Batchelor et al. [44] for oleic acid and those of Murari et al. [45] for the DOPC head group and glycerol backbone. Table 3 presents our complete ^{13}C chemical shift assignments for DOPC.

The peak associated to the Cα carbon of the ethanolamine group of gramicidin A can also be observed in the DOPC/gramicidin spectrum (Fig. 5B). Moreover, we notice in this spectrum a large decrease of the intensity of the three lipid resonances associated to the glycerol carbons (C1, C2 and C3) and the resonances of the C2 and C3 carbons of the

Table 3
Chemical shift assignments (in ppm) for pure DOPC and DOPC in the presence of gramicidin A at 30°C at a lipid-to-peptide molar ratio of 5:1

	DOPC	DOPC/gramicidin A
Polar head group		
Choline-Cγ	54.8	54.9
Choline-Cβ	66.8	66.8
Choline-Cα	60.2	60.3
Interfacial region		
Glycerol-C3	64.5	
Glycerol-C2	71.4	71.1
Glycerol-C1	63.8	
Hydrocarbon chains		
Chain-C1	174.2	174.2
Chain-C2	34.8	34.7
Chain-C3	25.8	25.4
Chain-C4 to C7	30.3	30.1
Chain-C8 and C11	28.0	27.9
Chain-C9 and C10	130.3	130.2
Chain-C12 to C15	30.3	30.1
Chain-C16	32.8	32.7
Chain-C17	23.4	23.4
Chain-C18	14.7	14.7
Gramicidin A		
Cα-ethanolamine		60.8

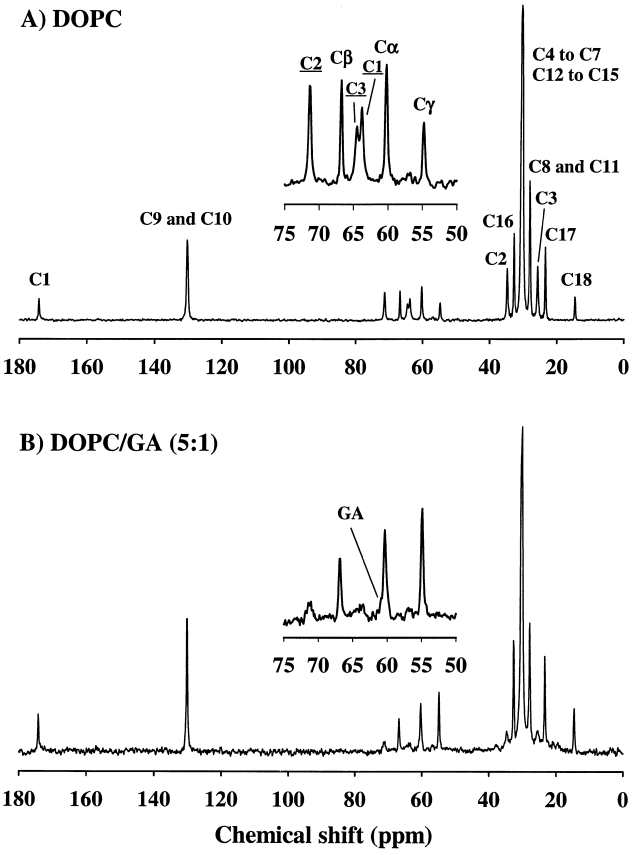

Fig. 5. ^{13}C NMR spectra at 30°C of (A) pure DOPC and (B) DOPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 5:1. The inset represents the expansion of the spectral region between 50 and 75 ppm. The choline head group carbons are indicated by Greek letters, the glycerol backbone carbons are underlined and the remaining carbons belong to the acyl chains.

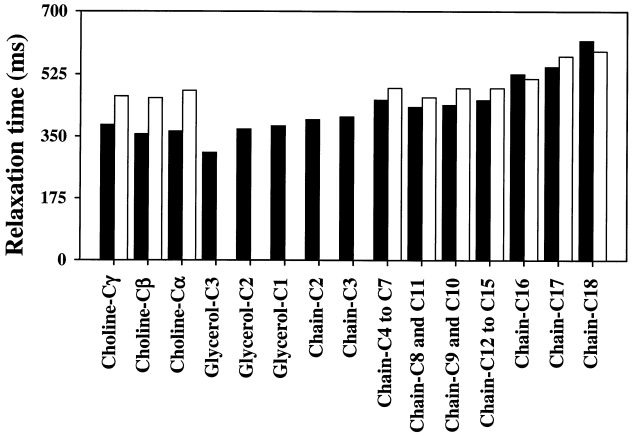

Fig. 6. HT_1 at 30°C for the DOPC resonances in pure DOPC (black bars) and DOPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 5:1 (open bars). The abscissa represents the different protonated carbons as defined in Fig. 5.

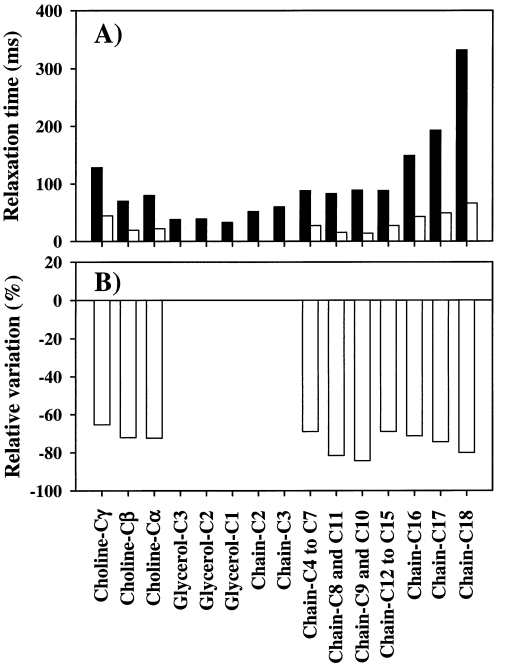

Fig. 7. (A) $HT_{1\rho}$ at 30°C for the DOPC resonances in pure DOPC (black bars) and DOPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 5:1 (open bars). (B) Relative variation $(((HT_{1\rho}$ DOPC/gramicidin A$-HT_{1\rho}$ DOPC)/$HT_{1\rho}$ DOPC)$\times 100)$ of the $HT_{1\rho}$ for the DOPC resonances. The abscissa represents the different carbons as defined in Fig. 5.

acyl chains compared to the pure DOPC spectrum. This decrease in intensity is most likely associated to a large decrease of the spin-lattice relaxation times in the rotating frame ($HT_{1\rho}$) for the lipids in the presence of gramicidin A, as discussed below. In fact, the spectrum obtained with a longer contact time of 15 ms did not show any signal for these carbon resonances, indicating the complete decay of the signal during the contact time.

Table 4
HT_1 and $HT_{1\rho}$ for pure DOPC and DOPC in the presence of gramicidin A at 30°C at a lipid-to-peptide molar ratio of 5:1

	HT_1 (ms)		$HT_{1\rho}$ (ms)	
	DOPC	DOPC/GA	DOPC	DOPC/GA
Polar head group				
Choline-Cγ	383	464	128	44
Choline-Cβ	357	459	70	20
Choline-Cα	365	479	80	22
Interfacial region				
Glycerol-C3	305	–	38	–
Glycerol-C2	371	–	39	–
Glycerol-C1	380	–	33	–
Hydrocarbon chains				
Chain-C2	398	–	52	–
Chain-C3	406	–	60	–
Chain-C4 to C7	453	487	88	27
Chain-C8 and C11	433	461	83	15
Chain-C9 and C10	439	487	89	14
Chain-C12 to C15	453	487	88	27
Chain-C16	526	513	149	43
Chain-C17	546	576	193	49
Chain-C18	619	590	332	66

Table 4 presents the ^1H spin-lattice relaxation times in the laboratory frame for pure DOPC and DOPC in the presence of gramicidin A at a lipid-to-peptide molar ratio of 5:1 at 30°C. The results are also illustrated in Fig. 6. The small intensities of the resonances attributed to the glycerol carbons (C1, C2 and C3) and to the C2 and C3 carbons of the acyl chains in the DOPC/GA system, even at small contact times, did not permit the measurements of the relaxation times for these atom sites. The HT_1 values of the pure DOPC atom sites show a similar behavior as the HT_1 of pure DMPC and the gradient extending from the glycerol backbone to both directions (polar head group and acyl chains) can also be observed in the unsaturated system, indicating that the fast motions are more restricted in the interfacial region of the bilayer. However, the HT_1 values obtained for the resonances of the polar head group are slightly higher in the unsaturated system.

Addition of gramicidin A to pure DOPC does not

affect significantly the HT_1 values of the lipid acyl chains but the values obtained for the polar head group are slightly increased, as observed in DMPC bilayers. This suggests that even though the incorporation of gramicidin A into DOPC bilayers affects the phase behavior of the lipid membrane (vide supra), it has little effect on the lipid acyl chain high frequency motions, as was also observed for the DMPC/gramicidin A system. This is in agreement with several studies which have suggested that the fast motional order parameter is independent of the lipid organization [46–49].

Fig. 7A shows the $HT_{1\rho}$ for the DOPC resonances for pure DOPC and for DOPC in the presence of gramicidin A while Fig. 7B gives the relative variation $(((HT_{1\rho}$ DOPC/gramicidin A$-HT_{1\rho}$ DOPC)/$HT_{1\rho}$ DOPC$)\times 100)$ of the $HT_{1\rho}$ for the DOPC carbon sites in the presence of gramicidin A. The results show the behavior of the $HT_{1\rho}$ for the two different DOPC lipid organizations studied: the lamellar phase and the hexagonal H_{II} phase. Changing the phase of DOPC by adding gramicidin A greatly reduces the $HT_{1\rho}$ values by about 60–70% for both the head group and acyl chain resonances. This suggests that the motion of the whole lipid molecule is affected by the presence of gramicidin A, as opposed to the more pronounced effect observed on the acyl chains in the DMPC/gramicidin A system. As discussed before, a reduction in the $HT_{1\rho}$, not matched by a decrease in the HT_1 values, indicates the introduction and/or enhancement of a low frequency motion. However, since the addition of gramicidin A at a lipid-to-peptide molar ratio of 5:1 induces a change in the phospholipid organization, it cannot be concluded that the observed reduction in the $HT_{1\rho}$ is solely due to the interaction between the lipids and gramicidin A.

It is believed and well accepted that the induction of a DOPC H_{II} phase by gramicidin A is accompanied by an aggregation of the peptide and that this aggregation is essential for the H_{II} phase formation. A model was proposed in which gramicidin in its channel conformation spans the distance between two adjacent tubes in the H_{II} phase so that the tryptophan residues are located at the lipid-water interface and the peptide molecules can form linear aggregates in a direction parallel to the tubes [1]. A similar model has also been proposed for a membrane-spanning α-helical peptide (WALP16) [2,3] and this has been shown to be a consequence of a hydrophobic mismatch between the peptide and the phosphatidylcholine molecules. The tryptophan residues, because of their preference for the lipid-water interface, were shown to be crucial for these effects of hydrophobic mismatch on lipid organization [1–3].

In this model, the aggregation of gramicidin A has been suggested to lead to a barrier for the lipid lateral diffusion in the induced H_{II} phase [1,2,4]. In addition, it has been shown that the proportion of ^{31}P NMR lineshape characteristic of the H_{II} phase in the DOPC/gramicidin A system gradually decreases upon decreasing temperature due to the rate of lateral diffusion of the lipids about the axes of the tubes becoming too slow on the NMR time scale to result in motional averaging [1]. This effect is, however, not observed for pure lipid systems in the hexagonal phase, indicating that the loss of the hexagonal phase lineshape observed in the DOPC/gramicidin spectrum at low temperature is solely due to the presence of the peptide.

Even though the lineshape of the DOPC/gramicidin ^{31}P NMR spectrum at 30°C is still characteristic of a H_{II} phase, a decrease in the rate of lipid lateral diffusion down to about 10^4–10^5 s^{-1} could increase significantly the contribution of this motion to the spin-lattice relaxation in the rotating frame and therefore explain the large decrease in the $HT_{1\rho}$ values observed in the present study, compared to those obtained for the pure DOPC multilamellar dispersions. In addition, the presence of the tryptophan residues at the lipid-water interface could account for the very significant decrease in the $HT_{1\rho}$ values observed in this region of the bilayer.

4. Conclusions

We have shown in the present study that ^{13}C natural abundance NMR spectroscopy can give detailed information about the dynamics of lipid-peptide systems in both the lamellar and hexagonal phases. Both the fast and slow motions were investigated through the measurements of proton spin-lattice relaxation times in the laboratory and rotating frames, respectively. In addition, the use of the magic angle spinning technique increases the spectral resolution

and allows the different regions of the lipid bilayers to be investigated from the same spectrum. The results indicate that the presence of gramicidin A does not significantly affect the fast motions of the acyl chains in lipid systems, both in the lamellar and hexagonal phases, but increases slightly the fast motions of the polar head group. On the other hand, the rate and/or amplitudes of slow time scale motions are significantly decreased, the effect being more important for the lipid-peptide system in the hexagonal H_{II} phase in which the aggregation of the peptide has been shown to act as a barrier for the lipid lateral diffusion. Since the presence of membrane proteins has been shown to affect predominantly the lipid slow motions [24,25,33,35–37], it would be interesting to apply the approach presented in this paper to the study of lipid dynamics in the presence of other intrinsic membrane proteins.

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