Biochimica et Biophysica Acta 854 (1986) 133–142
Elsevier

BBA 72880

Comparative ^{2}H- and ^{31}P-NMR study on the properties of palmitoyllysophosphatidylcholine in bilayers with gramicidin, cholesterol and dipalmitoylphosphatidylcholine

J. Antoinette Killian [a], François Borle [b], Ben de Kruijff [c] and Joachim Seelig [b]

[a] *Department of Biochemistry and* [c] *Institute of Molecular Biology, State University of Utrecht, Padualaan 8, Transitorium III, NL-3584 CH Utrecht (The Netherlands) and*
[b] *Department of Biophysical Chemistry, Biocentre of the University of Basel, Klingelbergstrasse 70, CH-4056 Basel (Switzerland)*

(Received August 12th, 1985)

Key words: Gramicidin; Cholesterol; Lysophosphatidylcholine; Bilayer structure; Order profile; NMR

The stoichiometric palmitoyllysophosphatidylcholine (lysoPC)/gramicidin (4:1, mol/mol) lamellar complex (Killian, J.A., De Kruijff, B., Van Echteld, C.J.A., Verkleij, A.J., Leunissen-Bijvelt, J. and De Gier, J. (1983) Biochim. Biophys. Acta 728, 141–144) is a useful model system to investigate the various aspects of lipid protein interactions. To study the effect of gramicidin on local order and motion of 1-palmitoyl-*sn*-glycero-3-phosphocholine (lysoPC) we employed ^{31}P and ^{2}H nuclear magnetic resonance (NMR) using selectively deuterated lysoPC's and we compared the results to those obtained for lysoPC in bilayers with cholesterol (1:1, mol/mol) and dipalmitoylphosphatidylcholine (DPPC) (1:4, mol/mol). ^{2}H-NMR experiments on acyl chain deuterated lysoPC showed similar quadrupole splittings in the liquid crystalline state for the lysoPC/DPPC and the lysoPC/gramicidin samples. In the lysoPC/cholesterol sample an increase of the quadrupole splitting was found. T_1 measurements showed that gramicidin decreases the lysoPC acyl chain motion, especially at the C12 position. In the lysoPC/cholesterol sample an increase of motion was observed as compared to lysoPC in fluid bilayers of DPPC. ^{31}P-NMR and ^{2}-H-NMR measurements of lysoPC, deuterated at the α- and β-position of the choline moiety, indicated an increase in headgroup flexibility in all samples as compared to the parent compound DPPC. In addition, a change in headgroup conformation was observed. The α- and β-segments in all samples exhibited concerted motion. It was found that also in the polar headgroup gramicidin induces a decrease of the rate of motion.

Introduction

Lipid-protein interactions play an intriguing role in membrane structure and functioning (for a recent review, see Devaux and Seigneuret [1]). In model membranes the effects of protein incorporation on local lipid structure and on macroscopic lipid organization have been subject to numerous investigations [1–8]. In many of these studies the hydrophobic pentadecapeptide gramicidin was used as a model for the membrane-spanning part of intrinsic membrane proteins [4–7]. Gramicidin is believed to form dimers which span the apolar part of the membrane [9]. Its known sequence and the ready commercial availability of the peptide furthermore contribute to its popularity.

Abbreviations: $\Delta\nu_q$, residual quadrupole splitting; $\Delta\nu_{1/2}$, linewidth at half height; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; EDTA, ethylenediaminetetraacetic acid; lysoPC, 1-palmitoyl-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance; T_1, spin-lattice relaxation time.

Upon incorporation of gramicidin in liquid-crystalline bilayers of dimyristoylphosphatidylcholine it was found by ^{2}H-NMR that, at low molar ratios of peptide to lipid (less than 1 : 15), the local lipid acyl chain order increases [4]. In accordance with this observation infra-red studies showed a decrease in the number of lipid chain gauche isomers upon incorporation of the peptide in a 1 : 50 molar ratio [7]. At high amounts of gramicidin (molar ratio greater than 1 : 15), both techniques consistently indicated a disordering of the lipids. In addition, and possibly related to these phenomena, the peptide has a dramatic effect on macroscopic lipid structure. When gramicidin is incorporated in bilayers of phosphatidylcholine, a hexagonal H_{II} phase is formed when the acyl chain length exceeds 16 carbon atoms [10]. For dioleoylphosphatidylcholine it has been shown that this H_{II} phase is very rich in gramicidin with a molar ratio of peptide to lipid of approx. 1 : 2.5 [11]. Also in phosphatidylethanolamine model membranes the peptide promotes H_{II} phase formation [12]. In contrast, lamellar structures are formed [13,14] with a fixed stoichiometry of 1 gramicidin per four lipid molecules [14] upon aqueous dispersion of gramicidin with lysophosphatidylcholine, which in the absence of the peptide organizes in micelles. As the lysoPC/gramicidin lamellar system contains only four acyl chains per gramicidin monomer it seems an ideal model to investigate the influence of a peptide on local lipid structure.

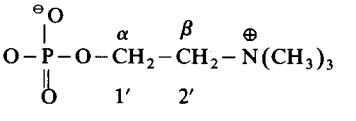
An additional, interesting feature of the gramicidin/lysoPC lamellar system is the highly irregular contour of the peptide, which is the result of the presence of four bulky tryptophan residues, all located at the carboxy-terminal part of the molecule [15]. This could lead to a relatively large effect on the acyl chain segments adjacent to the tryptophan-containing part of the gramicidin molecule and thus might permit an elucidation of the orientation of the gramicidin molecule in these gramicidin/lysoPC (4 : 1, mol/mol) bilayers, over which still different ideas exist [2].

In the present study we used ^{2}H-NMR to investigate the effect of gramicidin on local order and motion of lysoPC which was selectively deuterated at different positions in the acyl chain and in the polar headgroup. Since comparison with peptide-free lysoPC in a liquid-crystalline lamellar phase is not possible we used as reference systems lysoPC incorporated in DPPC bilayers in a molar ratio of 1 : 4 (at this ratio the bilayer configuration is still fully retained [16]) and lysoPC/cholesterol mixtures, which form bilayers in a 1 : 1 molar ratio [16–18]. The results obtained were related to literature data on DPPC bilayers [19–22]. In addition, we investigated the thermodynamic and structural parameters of the various lysoPC-containing bilayers using differential scanning calorimetry and ^{31}P-NMR.

Materials and Methods

Chemicals

1-[2-$^2H_2$]Palmitoyl-*sn*-glycero-3-phosphocholine (1-[2-$^2H_2$]lysoPC), 1-[4-$^2H_2$]palmitoyl-*sn*-glycero-3-phosphocholine (1-[4-$^2H_2$]lysoPC), 1-[7-$^2H_2$]palmitoyl-*sn*-glycero-3-phosphocholine (1-[7-$^2H_2$]lysoPC), 1-[12-$^2H_2$]palmitoyl-*sn*-glycero-3-phosphocholine (1-[12-$^2H_1$lysoPC) and the headgroup labeled 1-palmitoyl-*sn*-glycero-3-phospho[1′-$^2H_2$]choline (α-lysoPC) and 1-palmitoyl-*sn*-glycero-3-phospho[2′-$^2H_2$]choline (β-lysoPC), for which the following nomenclature was used:



were obtained by hydrolysis of the corresponding deuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholines (DPPC's) via the action of phospholipase A_2 [23] and purified as described elsewhere [24]. Acyl chain and headgroup deuterated DPPC's were synthesized according to Seelig and Seelig [19] and Gally et al. [21], respectively. For all lyso compounds HPTLC with chloroform/methanol/water (65 : 25 : 4, by vol.) as the eluent, showed one single spot, indicating the purity of the lipids and the lack of any significant chain migration.

Unlabeled DPPC for the control experiments was purchased from Fluka (Buchs, Switzerland) and used without further purification. The highest available grade of cholesterol was obtained from Merck (Darmstadt, F.R.G.). Gramicidin was purchased from Sigma (St. Louis, MO, U.S.A.) and used as such. All chemicals were of analytical grade.

Sample preparation

In all measurements the following molar ratios were used: lysoPC/ DPPC (1 : 4), lysoPC/ gramicidin (4 : 1) and lysoPC/cholesterol (1 : 1). NMR samples were prepared as follows. 50–100 μmol of lysoPC and the appropriate amount of gramicidin, cholesterol or DPPC were dissolved in dichloromethane/methanol (2 : 1, v/v) and the samples were evaporated to dryness under N_2. After further drying under high vacuum a defined amount of buffer (100 mM NaCl, 25 mM Tris/ acetic acid, 0.2 mM EDTA (pH 7.0) made up with deuterium-depleted water) was added, corresponding to twice the weight of the dry sample. The samples were dispersed by vigorously vortexing at room temperature or at 45–50°C in case of DPPC-containing samples. Subsequently the samples were centrifuged (10 min, 20000 × *g*, 4°C) and the pelleted samples were used for NMR measurements.

Nuclear magnetic resonance

^{2}H-NMR measurements were performed on a Bruker CXP-300 spectrometer, operating at 46.1 MHz. The quadrupole echo technique was used [25] employing a 90° pulse (3.6 μs), an echo pulse separation of 30 μs, an interpulse time of 250 ms, and a 2 K memory. Acyl-chain-labeled lipids were measured with a spectral width of 125 kHz. 20 K–200 K transients were accumulated and prior to Fourier transformation exponential multiplication was applied, resulting in a 200 Hz linebroadening. For headgroup deuterated lipids a spectral width of 62.5 kHz was used. 10 K free induction decays were accumulated and a 100 Hz linebroadening was applied.

T_1 measurements were performed by the conventional 190°-τ-90° pulse sequence modified to include the quadrupole echo. The experimental error in the T_1 values was estimated to be 2–3 ms.

^{31}P-NMR measurements were performed at 121.4 MHz. A gated decoupling technique was used, with an interpulse time of 7 s, a 2.3 μs 90° pulse, a 31.25 kHz sweep width and a 2 K memory. 500 free induction decays were accumulated. Prior to Fourier transformation exponential multiplication was applied, resulting in a 100 Hz linebroadening. The chemical shift anisotropy was measured as the distance between the low-field shoulder and the high-field peak. Since the low-field shoulder of lysoPC in a fluid DPPC bilayer was not visible, we measured the residual chemical shift anisotropy of lysoPC in this sample by subtracting 3-times the distance between the high-field peaks of both lipid components from the residual chemical shift anisotropy of the DPPC component. This procedure is based on the fact that the residual chemical shift anisotropy is equal to 3-times the chemical shift difference between the high-field peak and the position of isotropically moving lipid molecules. The maximum error in the chemical shift anisotropy measurements was estimated to be 1 ppm.

Repurification of deuterated lysoPC

For some of the control experiments lysoPC was repurified from NMR samples as follows. The sample was sonicated in chloroform/methanol (1 : 1, v/v) until clear). LysoPC was separated by partition chromatography using a column (150 × 15 mm) filled with Polygosil 60, particle size 63–100 μm (Macherey-Nagel, Düren, F.R.G.) with chloroform/methanol/ammonia/water (68 : 28 : 2 : 2, by vol.) as the eluent. Fractions with pure lysoPC, as judged from HPTLC using the same eluent, were combined and dried.

Differential scanning calorimetry

Differential scanning calorimetry measurements were performed on a Perkin-Elmer DSC 4, using the procedure described by Van Echteld et al. [26].

Results

The lysoPC/gramicidin (4 : 1, mol/mol) complex can be visualized by freeze-fracture electron microscopy as large multilamellar structures with rather closely stacked bilayers [14]. Small-angle X-ray diffraction experiments, carried out as described elsewhere [27], did not show any sharp diffraction lines (data not shown), indicating that in these systems no long range order occurs.

In order to obtain insight into the effect of gramicidin on the chain order of lysoPC as compared to cholesterol and the control situation of lysoPC incorporated into DPPC bilayers, it is necessary to know whether the acyl chains of the lysoPC are in the gel or in the liquid-crystalline

state. The thermodynamic properties of aqueous dispersion of lysoPC/DPPC mixtures have been investigated previously [26]. While pure lysoPC exhibits a gel (lamellar) to liquid-crystalline phase (micellar) transition at about 3°C, it was shown that upon incorporation of lysoPC in DPPC in a 1 : 4 molar ratio the acyl chains of both lipids melt at 41°C, which corresponds to the gel to liquid-crystalline phase transition temperature of pure DPPC. We reproduced these results and in addition we studied lysoPC/gramicidin and lysoPC/cholesterol mixtures (data not shown). In both systems we were unable to detect any phase transition in the temperature range of 0–24°C.

Acyl-chain ordering and motion

^{2}H-NMR measurements of quadrupole splittings were performed over a temperature range of 15–55°C on the various lysoPC-containing system, with ^{2}H labels at various positions along the acyl chain.

Fig. 1 shows the spectra obtained for the C4-labeled lipid at 45°C. At this temperature the lysoPC/DPPC mixture is in the liquid-crystalline state and shows a $\Delta\nu_q$ of 24.0 kHz. In the lysoPC/gramicidin sample a remarkably similar $\Delta\nu_q$ of 25.4 kHz is observed. In contrast, a much larger quadrupole splitting (45.4 kHz) is found in the lysoPC/cholesterol system.

A similar behavior is observed for the other acyl chain positions measured. Fig. 2 shows a comparison of the corresponding order profiles. LysoPC/DPPC and lysoPC/gramicidin mixtures at 45°C give rise to similar quadrupole splittings, which are close to the values obtained for pure DPPC (data from Ref. 19), for which an approximately constant value of $\Delta\nu_q$ was obtained up till the C9 position, followed by a progressive decrease towards the terminal part of the acyl chain [19]. The increased value of $\Delta\nu_q$ in the lysoPC/cholesterol samples shows that the ordering effect of the sterol, as found before upon incorporation into fluid bilayers [28–30] also holds for bilayers with lysoPC.

In all samples $\Delta\nu_q$ appeared to be slightly temperature-dependent. With increasing temperature a decrease of $\Delta\nu_q$ was observed, mostly in the order of 1 kHz/10 Cdeg. In the lysoPC/DPPC system such a dependency was found only at temperatures above the gel to liquid-crystalline phase transition. Below the phase transition ^{2}H-NMR spectra of acyl-chain deuterated lysoPC in DPPC bilayers showed only a broad signal, typical

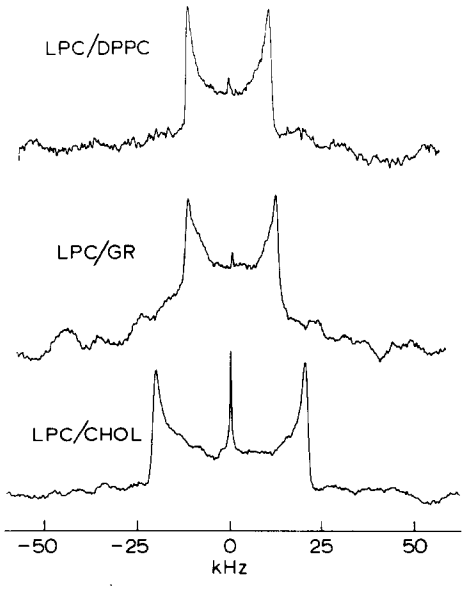


Fig. 1. 46.1 MHz ^{2}H-NMR spectra at 45°C of aqueous dispersions of 1-[4-$^2H_2$]lysoPC/DPPC (1 : 4, mol/mol) (A), 1-[4-$^2H_2$]lysoPC/gramicidin (4 : 1, mol/mol) (B) and of 1-[4-$^2H_2$]lysoPC/cholesterol (1 : 1, mol/mol) (C).

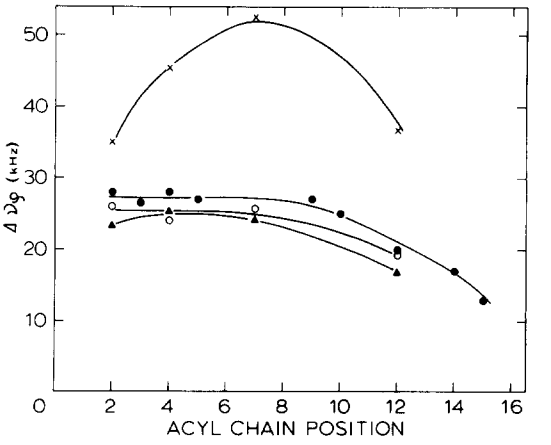


Fig. 2. Quadrupole splitting at 45°C as a function of the labeled carbon atom in the acyl chain, in aqueous dispersions of DPPC* (●———●), lysoPC/DPPC (1 : 4, mol/mol) (○———○), lysoPC/gramicidin (4 : 1, mol/mol) (▲———▲) and lysoPC/cholesterol (1 : 1, mol/mol) (×———×). * means data are interpolated from Ref. 19.

TABLE I

SPIN-LATTICE RELAXATION TIMES OF ACYL-CHAIN DEUTERATED lysoPC IN BILAYERS WITH DPPC, GRAMICIDIN AND CHOLESTEROL

Relaxation times T_1 are in ms. GR, gramicidin.

Acyl chain position	lysoPC/DPPC (1:4)	lysoPC/GR (4:1)		lysoPC/Chol (1:1)	
	45°C	25°C	45°C	25°C	45°C
C2	21.9	12.6	16.9	12.9	24.5
C4	23.2	15.2	22.8	19.4	31.4
C12	38.0	22.0	25.6	40.0	59.6

for gel state lipids. In the gramicidin/lysoPC sample most of the signal intensity was lost in the temperature range of 15°C to −5°C.

We next performed ^{2}H spin-lattice relaxation time measurements to investigate the motional properties of the lysoPC acyl chain segments in the various samples. In the lysoPC/DPPC sample at 45°C the T_1 relaxation times were of the order of 13–30 ms and an increase of T_1 towards the terminal part of the acyl chain was observed (Table I). A similar behavior was found in pure DPPC samples in which the decrease of the relaxation rate R $(= 1/T_1)$ paralleled the decrease in lipid chain order [20]. Also in the lysoPC/gramicidin and in the lysoPC/cholesterol sample such a decrease of the T_1 relaxation rate was found, both at 25°C and at 45°C. In all samples T_1 values increased with temperature, as was shown previously for pure DPPC [20], indicating that motions occur in the fast correlation time region. The lysoPC/gramicidin sample showed smaller T_1 values compared to the lysoPC/DPPC mixture, particularly at the C12 position, indicating that gramicidin decreases the rate of fast motions of the methylene segments, while in the lysoPC/cholesterol sample an increased T_1 value was observed. This latter effect, a simultaneous increase of $\Delta\nu_q$ and T_1 appears to be paradoxal at first sight, but has been observed before in other cholesterol-containing systems [31].

Polar-headgroup ordering and motion

The various lysoPC-containing bilayers were further characterized by ^{31}P-NMR and ^{2}H-NMR using lysoPC with selectively deuterated methylene segments in the choline moiety of the polar headgroup. Fig. 3 shows ^{31}P-NMR spectra of the three systems studied. All spectra exhibit similar lineshapes with a low-field shoulder and a high-field peak, characteristic for lipids in a bilayer organization [32,33]. In the lysoPC/DPPC sample two spectral components are visible with residual chemical shift anisotropies of 46 and 34 ppm (Table II) which are assigned to DPPC and lysoPC, respectively. These assignments are based on the argument, that for DPPC a residual chemical shift anisotropy of about 47 ppm has been observed before [18,22], while in lysoPC a decreased residual chemical shift anisotropy has been reported [16,18,34], suggesting an increased motional freedom of the phosphate moiety due to the larger flexibility of the glycerol backbone in the absence of the acyl chain at the glycerol C2 position. At temperatures below 40°C a lineshape typical for lipids in a gel state was observed, with broad edges

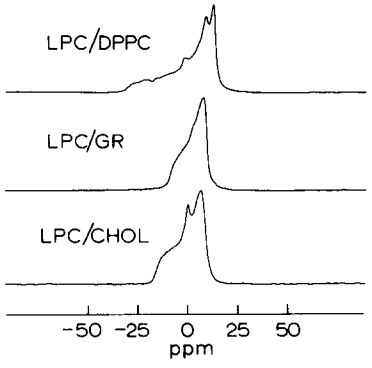


Fig. 3. 121.4 MHz ^{31}P-NMR spectra at 45°C of aqueous dispersions of lysoPC/DPPC (1:4, mol/mol) (A), lysoPC/gramicidin (4:1, mol/mol) (B) and lysoPC/cholesterol (1:1, mol/mol) (C). The samples are the same as used in Fig. 1.

TABLE II

STRUCTURAL AND MOTIONAL CHARACTERISTICS OF THE VARIOUS HEADGROUP SEGMENTS OF lysoPC AT 45°C IN BILAYERS WITH DPPC, GRAMICIDIN AND CHOLESTEROL

Sample	$^{31}P/\Delta\sigma$ (ppm)	α-$^2H_2$			β-$^2H_2$		
		$\Delta\nu_{1/2}$ (kHz)	T_1 (ms)	E_{akt} (kJ/mol)	$\Delta\nu_q$ (kHz)	T_1 (ms)	E_{akt} (kJ/mol)
lysoPC/DPPC	34/36	1.5	34.5	18.1	4.5	37.6	20.1
lysoPC/GR	20	1.3	23.8	17.3	2.4	26.2	19.3
lysoPC/Chol	26	≅ 3.3	28.2	21.3	1.9	33.8	21.7

due to residual dipolar coupling. In these type of spectra the two lipid components did not give rise to separate ^{31}P NMR signals.

In the lysoPC/gramicidin as well as in the lysoPC/cholesterol sample the lineshape appeared to be independent of temperature in the range of 15–55°C. Residual chemical shift anisotropies were measured of 21 and 26 ppm, respectively, which values are slightly smaller than the residual chemical shift anisotropy observed for lysoPC in the lysoPC/DPPC mixture at 45°C (Table II).

Figs. 4A and 4B show the ^2H-NMR spectra at 45°C of the various systems in which lysoPC was deuterated at the α- and β-position of the polar headgroup. In pure DPPC dispersions well-defined quadrupole splittings of 5.9 kHz and 5.0 kHz at 50°C have been observed earlier for the α- and β-segments of the choline moiety, respectively [21,22]. In contrast, the α-segment of lysoPC in

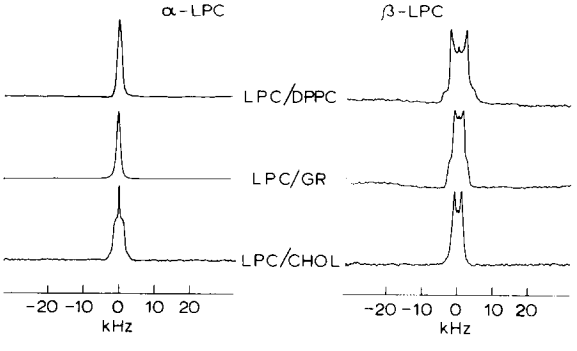


Fig. 4. 46.1 MHz ^2H-NMR spectra at 45°C of α-lysoPC (A) and β-lysoPC (B) in aqueous dispersions of lysoPC/DPPC (1:4, mol/mol), lysoPC/gramicidin (4:1, mol/mol) and lysoPC/cholesterol (1:1, mol/mol).

both the lysoPC/DPPC fluid bilayer and the lysoPC/gramicidin system shows an almost isotropic signal, without any resolved quadrupole splitting. Since the β-segments of the same systems give rise to a distinct quadrupole splitting (see Table II) the possibility of an isotropic motion of the lipids is ruled out.

In the lysoPC/cholesterol sample the α-segment gives rise to a broad ^2H-NMR spectrum with a small isotropic component superimposed. At 25°C a quadrupolar splitting of approx. 2.1 kHz could be estimated. For the β-segment at this temperature a quadrupole splitting of 1.9 kHz was observed.

To gain information concerning the rate of segmental motions of the headgroup regions we determined the deuterium spin-lattice relaxation times of the α- and β-segments. In Table II the values of T_1 at 45°C are given. In all samples T_1 of the β-segment is slightly increased as compared to the α-segment. This was also reported for pure DPPC, where T_1 values of 30 and 38 ms were measured for the α- and β-segment, respectively, at 51°C [22].

For all samples the activation energies are approximately similar and their signs shows that motions determining T_1 occur in the fast correlation time region. Thus, the decreased value of T_1 in the lysoPC-gramicidin sample in both segments as compared to lysoPC/DPPC mixtures indicates that the peptide induces a small decrease in the rate of motion of the lysoPC headgroup moiety. This also holds true for the lysoPC/cholesterol system. The similarity of T_1 in the α- and β-segment of the various samples indicates a concerted motion of both segments.

Discussion

Thermodynamic behavior

Differential scanning calorimetry measurements have shown that pure lysoPC exhibits a gel (bilayer) to fluid (micellar) transition at about 3°C (Ref. 16) and this study). If lysoPC is incorporated into DPPC bilayers the thermodynamic behavior is now dominated by DPPC, since all palmitic acid chains melt at 41°C. On the other hand, lysoPC in contact with gramicidin or cholesterol shows no phase transition by differential scanning calorimetry. Therefore, these measurements suggest that in all samples the lysoPC acyl chain interacts with the other membrane constituent.

In the lysoPC/DPPC sample the cooperative melting of both lipid components was also reflected in the ^{2}H-NMR measurements, which showed a gel type of spectrum for both the acyl chain and headgroup deuterated lysoPC at temperatures below the gel to liquid crystalline phase transition of DPPC.

Previous differential scanning calorimetry studies have shown that, as a result of the interaction of gramicidin and cholesterol with diacylphosphatidylcholines, the gel to liquid-crystalline phase transition of the lipids was broadened and their transition enthalpy decreased [6,35–37]. It is likely that a similar interaction takes place in the lysoPC bilayer, when relatively high amounts of either gramicidin or cholesterol are present. In the lysoPC/gramicidin as well as in the lysoPC/cholesterol system both ^{31}P-NMR and ^{2}H-NMR measurements indicate that the chains are in the liquid-crystalline state in the temperature range of 15–55°C. The loss of intensity in the ^{2}H-NMR spectra in the temperature range of 15°C to −5°C in the lysoPC/gramicidin sample corresponds very well with the presence of a broad phase transition.

Order profile of the hydrocarbon chains

The order parameter S_{CD} is a measure of the angular fluctuations of the methylene segments. To a first approximation S_{CD} increases with increasing ordering of the hydrocarbon region. The order profile then describes the variation of the segmental order parameters with the position of the segment in the hydrocarbon chain.

Detailed order profiles are now available for a variety of different systems (for a review, see Ref. 38), including intact biological membranes derived from *Acholeplasma laidlawii* [39] and *Escherichia coli* [40] as well as a large number of model membrane systems. Of the pure lipid model membrane systems studied, the order profiles of DPPC [19] and lysoPC/palmitic acid (1 : 1, mol/mol) mixtures [34] are of particular interest in the present context. In the liquid-crystalline state for both lipid systems a similar order profile was found showing almost identical and approximately constant values of the quadrupole splitting up till the C9 position and a progressive decrease of $\Delta\nu_q$ towards the methyl terminal. The order profile of lysoPC in liquid-crystalline bilayers of DPPC reported here is similar to that of both other pure lipid systems. However, if cholesterol is incorporated in lysoPC the lipid chain order increases. Such an effect of cholesterol was observed before in model membranes of diacylphospholipids [28,29] and is in accordance with the condensing effect of the sterol in general [37]. Thus, the observed increase in $\Delta\nu_q$ in bilayers of lysoPC with cholesterol indicates that the system is sensitive to changes in the molecular order of the C^{2}H bonds.

Detailed order profiles also have been obtained for a number of lipid-protein systems [4,28,45] and it has been found that in general proteins do not significantly affect the acyl chain order. The unique features of the lipid-protein system studied in the present investigation are: (1) the well-defined high stoichiometry of one gramicidin molecule in contact with four lysophospholipids forming a stable bilayer, and (2) the highly irregular contour of the peptide. These properties might be expected to result in unusual features of the order profile. However, in the lysoPC/gramicidin bilayer a lipid chain order profile, similar to that of DPPC or of lysoPC in bilayers with DPPC was obtained. In particular the order profile showed the typical increase in chain flexibility towards the methyl terminal and did not provide any indication of the location of the tryptophan residues. It is known that gramicidin can adopt various conformations, including the N-N terminal dimer, proposed by Urry et al. [9,41] to be the channel structure. If such dimers are formed with the dimensions given by Urry et al. [9], it can be calculated that, of the acyl chain positions measured, the four tryp-

tophans would be located very close to the C2 and C4 position of the lipid. On the other hand, if a C-C terminal dimer is formed, as was suggested before [14], based on the assumption that both gramicidin and lysoPC molecules are cone shaped and that, in order to form stable bilayers, gramicidin and lysoPC should be oriented in such a way that their shapes are complementary, or if gramicidin is otherwise oriented with its bulky tryptophan containing moiety located at the center of the membrane, the peptide might be expected to affect the local order of the C^2H bond at the C12 position. The observed decrease of T_1 at this position might possibly suggest such an orientation. Still another possibility is that gramicidin molecules are present as parallel or antiparallel double helices [43,44], which would complicate an estimation of the location of the tryptophan residues with respect to the deuterated segments of the acyl chain.

The observation that gramicidin, like many proteins [8,28,45], does not significantly affect the acyl chain order can have several possible explanations. Firstly, it has been suggested that proteins have a fluid-like surface [46], which does not interfere with the motions of the acyl chain. Secondly, it has been suggested that the proteins are rigid but have an irregular contour which allows motions of the lipids similar to those in the protein-free bilayer [47,48]. Thirdly, in a recent model the ^{2}H-NMR data were interpreted in terms of hydrophobic matching of the membrane-spanning part of the protein with the bilayer thickness [49]. This last model could well be applied to our systems, since in phosphatidylcholine systems gramicidin does not affect the bilayer thickness [27,35], while cholesterol incorporation is known to increase the bilayer thickness [36], which, according to this model, would lead to an increase of the quadrupole splitting as indeed we have observed. A fourth possibility, of particular interest in the case of the lysoPC/gramicidin bilayer is that self-assembly of the peptide occurs, thus reducing the amount of peptide interacting with the lipids. Such clustering has been previously suggested to occur in lysoPC/gramicidin bilayers [50]. Based on fluorescence quenching experiments it was proposed that in these aggregates tryptophan-tryptophan interactions occur. Moreover, it has been shown that, also in other lipid systems gramicidin easily forms aggregates [6,11,35]. To get insight into this possibility a more detailed study is required. In a separate series of experiments we have synthesized gramicidin selectively deuterated in the four tryptophan rings. The incorporation of the deuterium label was verified with ^{1}H- and ^{2}H-NMR. When membranes were formed with lysoPC and the deuterated gramicidin analog practically no ^{2}H-NMR signal could be obtained with the quadrupole echo technique in spite of a sufficient deuterium content (work in preparation). Such a behavior would be typical for slow motions with motional correlation times of the order of the reciprocal static quadrupole splitting ($\tau_c = 10^{-5}$ s). Since the membrane lipids are highly fluid ($\tau_c = 10^{-10}$ s for the segmental motions as judged from T_1) under the same experimental conditions, we conclude that the gramicidin molecules must be aggregated to larger structures and that this aggregation eliminates tryptophan side chain motions as well as the rotation of the individual molecules. A possible picture which would be consistent with the experimental data would be a partially aggregated network of gramicidin molecules, as was proposed by Spisni et al. [50], in which open regions between the peptide moieties are filled with lysoPC molecules. In agreement with this model, freeze-fracture electron microscopy experiments of gramicidin/lysoPC mixtures revealed the presence of highly ordered structures (data not shown) similar to those observed by Spisni et al. [50].

The lipid polar headgroup

The parent compound DPPC forms liquid-crystalline bilayers at temperatures above T_c = 41°C. The chemical shielding anisotropy in the fluid phase is about 46 ppm. In contrast, the residual chemical shift anisotropy is much smaller for the lysoPC systems studied in this work, ranging between 21 ppm for the lysoPC/gramicidin system to 34 ppm for lysoPC/DPPC. This finding is in agreement with previous results [14,16,18,34] and can most probably be attributed to the additional motional averaging resulting from rapid rotation of the LPC polar head group around the C(1) – C(2) glycerol bond [16,18].

It may be noted that cholesterol has no large

influence on the polar group. This is in contrast to its effects on the fatty-acyl chain region where the addition of cholesterol almost doubled the residual quadrupole splitting, indicating a considerable stiffening of the hydrocarbon chains. The absence of any significant effect of cholesterol on the polar groups has been observed previously [22,31] and has been explained by a cholesterol positioning such that the OH-group of cholesterol is located below the level of the phospholipid polar groups.

Next, we proceed to the α- and β-segment of the phosphocholine headgroup. DPPC membranes exhibit quadrupole splittings of $\Delta\nu_q = 5.9$ kHz and $\Delta\nu_q = 5.0$ kHz (at 50°C). In all lysoPC-containing systems, including the lysoPC/palmitic acid mixture studied previously [34], both quadrupole splittings are distinctly reduced, generally by more than a factor of two. On the other hand, the differences between the various lysoPC-containing membranes are not too large, suggesting the following molecular picture. The lysoPC headgroup conformation is approximately similar in all lysoPC-containing membranes. No distinct differences between the effect of cholesterol and gramicidin can be detected. Compared to the parent compound DPPC the headgroup is more flexible, since all anisotropies are reduced. Furthermore, it follows from the observation that the extent of anisotropy reduction varies from segment to segment that the increase in headgroup flexibility is accompanied by a change in headgroup conformation.

Acknowledgements

J.A.K. gratefully acknowledges the support of a short-term fellowship (ASTF 4202) awarded by the European Molecular Biology Association (EMBO). The authors would like to thank P. Ganz and R. Jenni for the skillful synthesis of selectively deuterated lysophospholipid.

References

1 Devaux, P.F. and Seigneuret, M. (1985) Biochim. Biophys. Acta 822, 63–125
2 De Kruijff, B., Cullis, P.R., Verkleij, A.J., Hope, M.J., Van Echteld, C.J.A., Taraschi, T.F., Van Hoogevest, P., Killian, J.A., Rietveld, A. and Van der Steen, A.T.M. (1985) in Progress in Protein-Lipid Interactions (Watts, A. and De Pont, J.J.H.H.M., eds.), pp. 89–142, Elsevier Science Publishers
3 Marsh, D. (1983) Trends Biochem. Sci. 8, 330–333
4 Rice, D. and Oldfield, E. (1979) Biochemistry 18, 3272–3279
5 Rajan, S., Kang, S.-Y., Gutowski, H.S. and Oldfield, E. (1981) J. Biol. Chem. 256, 1160–1166
6 Chapman, D., Cornell, B.A., Eliasz, A.W. and Perry, A. (1977) J. Mol. Biol. 113, 517–538
7 Lee, D.C., Durrani, A.A. and Chapman, D. (1984) Biochim. Biophys. Acta 769, 49–56
8 Tamm, L.K. and Seelig, J. (1983) Biochemistry 22, 1474–1483
9 Urry, D.W., Goodall, M.C., Glickson, J.D. and Mayers, D.F. (1971) Proc. Natl. Acad. Sci. USA 68, 1907–1911
10 Van Echteld, C.J.A., De Kruijff, B., Verkleij, A.J., Leunissen-Bijvelt, J. and De Gier, J. (1982) Biochim. Biophys. Acta 692, 126–138
11 Killian, J.A. and De Kruijff, B. (1985) Biochemistry, in the press
12 Van Echteld, C.J.A., Van Stigt, R., De Kruijff, B., Leunissen-Bijvelt, J., Verkleij, A.J. and De Gier, J. (1981) Biochim. Biophys. Acta 648, 287–291
13 Pasquali-Ronchetti, I., Spisni, A., Casali, E., Masotti, L. and Urry, D.W. (1983) Biosci. Rep. 3, 127–133
14 Killian, J.A., De Kruijff, B., Van Echteld, C.J.A., Verkleij, A.J., Leunissen-Bijvelt, J. and De Gier, J. (1983) Biochim. Biophys. Acta 782, 141–144
15 Sarges, R. and Witkop, B. (1965) J. Am. Chem. Soc. 87, 2011–2020
16 Van Echteld, C.J.A., De Kruijff, B., Mandersloot, J.G. and De Gier, J. (1981) Biochim. Biophys. Acta 649, 211–220
17 Rand, R.P., Pangborn, W.A., Purdon, A.D. and Tinker, D.O. (1975) Can. J. Biochem. 53, 189–195
18 Wu, W., Stephenson, F.A., Mason, J.T. and Huang, C. (1984) Lipids 19, 68–71
19 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839–4845
20 Brown, M.F. and Seelig, J. (1979) J. Chem. Phys. 70, 5045–5053
21 Gally, H.U., Niederberger, W. and Seelig, J. (1975) Biochemistry 14, 3647–3652
22 Brown, M.F. and Seelig, J. (1978) Biochemistry 17, 381–384
23 Cubero Robles, E. and Van den Berg, D. (1969) Biochim. Biophys. Acta 187, 520–526
24 Chakrabarti, P. and Khorana, H.G. (1975) Biochemistry 14, 5021–5033
25 Davis, J.H., Jeffrey, K.R., Bloom, M., Valic, M.I. and Higgs, T.P. (1976) Chem. Phys. Lett. 42, 390–394
26 Van Echteld, C.J.A., De Kruijff, B. and De Gier, J. (1980) Biochim. Biophys. Acta 595, 71–81
27 Killian, J.A., Leunissen-Bijvelt, J., Verkleij, A.J. and De Kruijff, B. (1985) Biochim. Biophys. Acta 812, 21–26
28 Oldfield, E. (1982) in Membranes and Transport, Vol. 1 (Martonosi, E.N., ed.), pp. 115–123, Plenum Press, New York
29 Davis, J.H., Bloom, M., Butler, K.W. and Smith, I.C.P. (1980) Biochim. Biophys. Acta 597, 477–491
30 Gally, H.U., Seelig, A. and Seelig, J. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 1447–1450

31 Gosh, R. and Seelig, J. (1982) Biochim. Biophys. Acta 691, 151–160
32 Seelig, J. (1978) Biochim. Biophys. Acta 515, 105–140
33 Cullis, P.R. and De Kruijff, B. (1979) Biochim. Biophys. Acta 559, 399–420
34 Allegrini, P.R., Van Scharrenburg, G., De Haas, G.H. and Seelig, J. (1983) Biochim. Biophys. Acta 731, 448–455
35 Killian, J.A. and De Kruijff, B. (1985) Biochemistry, in the press
36 Ladbrooke, B.D., Williams, R.M. and Chapman, D. (1968) Biochim. Biophys. Acta 50, 333–340
37 Demel, R.A. and De Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109–132
38 Seelig, J. and Seelig, A. (1980) Q. Rev. Biophys. 13, 19–61
39 Stockton, G.W., Johnson, K.G., Butler, K., Tulloch, A.P. Boulanger, Y., Smith, I.C.P., Davis, J.H. and Bloom, M. (1977) Nature 269, 267–268
40 Gally, H.U., Pluschke, G., Overath, P. and Seelig, J. (1979) Biochemistry 18, 5605–5610
41 Urry, D.W., Trapane, T.L. and Prasad, K.U. (1983) Science 211, 1064–1067
42 Bradley, R.J., Urry, D.W., Okamoto, K. and Rapaka, R. (1978) Science 200, 435–436
43 Veatch, W.R., Fossel, E.T. and Blout, E.R. (1974) Biochemistry 13, 5249–5256
44 Arseniev, A.S., Baroukov, I.L. and Bystrov, V.F. (1985) FEBS Lett. 180, 33–39
45 Bloom, M. and Smith, I.C.P. (1985) in Progress in Protein-Lipid Interactions (Watts, A. and De Pont, J.J.H.H.M., eds.), pp. 61–88, Elsevier Science Publishers BV, Amsterdam
46 Bloom, M. (1979) Can. J. Phys. 57, 2227–2230
47 Seelig, J. and Seelig, A. (1980) Quant. Rev. Biophys. 13, 19–61
48 Seelig, A., and Seelig, J. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 1747–1756
49 Mourtisen, O.G. and Bloom, M. (1984) Biophys. J. 46, 141–153
50 Spisni, A., Pasquali-Ronchetti, I., Casali, E., Lindner, L., Cavatorta, P., Masotti, L. and Urry, D.W. (1983) Biochim. Biophys. Acta 732, 58–68