Melittin-induced changes in lipid multilayers

A solid-state NMR study

Ross Smith,* Frances Separovic,‡ Fiona C. Bennett,* and Bruce A. Cornell‡

*Biochemistry Department, The University of Queensland, QLD 4072, Australia; and ‡Australian Membrane and Biotechnology Research Institute, Commonwealth Scientific and Industrial Research Organization, Division of Food Processing, North Ryde, NSW 2113, Australia

ABSTRACT Solid-state ^{1}H, ^{13}C, ^{14}N, and ^{31}P NMR spectroscopy was used to study the effects of the bee venom peptide, melittin, on aligned multilayers of dimyristoyl-, dilauryl- and ditetradecyl-phosphatidylcholines above the gel to liquid-crystalline transition temperature, T_c. Both ^{31}P spectra from the lipid headgroups and ^{1}H resonances from the lipid acyl chain methylene groups indicate that the peptide does not affect the mosaic spread of the lipid molecules at lipid:peptide molar ratios of 10:1, or higher. None of the samples prepared above T_c showed any evidence of the formation of hexagonal or isotropic phases. Melittin-induced changes in the chemical shift anisotropy of the headgroup phosphate and the lipid carbonyl groups, and in the choline ^{14}N quadrupole splittings, show that the peptide has effects on the headgroup order and on the molecular organization in the sections of the acyl chains nearest to the bilayer surface. The spin-lattice relaxation time for the lipid acyl chain methylene protons was found to increase and the rotating-frame longitudinal relaxation time to markedly decrease with the addition of melittin, suggesting that motions on the nanosecond time scale are restricted, whereas the slower, collective motions are enhanced in the presence of the peptide.

INTRODUCTION

Melittin is a 26-residue peptide from bee (*Apis mellifera*) venom with the sequence H_2N-G-I-G-A-V-L-K-V-L-T-T-G-L-P-A-L-I-S-W-I-K-R-K-R-Q-Q-$CONH_2$ (Habermann and Jensch, 1967). It is one of a family of water-soluble peptides that bind strongly to membranes, altering their permeability and, under some conditions, causing disruption that leads to cell lysis (DeGrado et al., 1982). It forms voltage-dependent, anion-selective pores in lipid membranes (Dempsey, 1990).

In the crystalline state (Terwilliger et al., 1982) and in methanol solutions (Bazzo et al., 1988; Pastore et al., 1989) melittin has been shown to be predominantly α-helical. Studies of the peptide bound to micelles of perdeuterated lysophosphatidylcholine (Inagaki et al., 1989) and to lipid vesicles (Vogel and Jahnig, 1986; Wakamatsu et al., 1986) indicate that the membrane-bound melittin is also largely helical. Major controversies remain, however, over the state of aggregation of the peptide in the membrane (monomeric [Schwarz and Beschiaschvili, 1989; Weaver et al., 1989] or tetrameric [Vogel and Jahnig, 1986]), the location with respect to the lipid membrane (on the surface [Terwilliger et al., 1982; Altenbach et al., 1988; Altenbach and Hubbell, 1988; Schulze et al., 1987; Dawson et al., 1978; Stankowski and Schwarz, 1990; Eibl and Woolley, 1986], partly looped into the lipid [Dawson et al., 1978; Brown et al., 1982; Coddington et al., 1983], or a transbilayer orientation [Vogel and Jahnig, 1986; Dawson et al., 1978; Vogel, 1987]), and the mechanisms by which it promotes both ion transport and cell lysis (Dempsey, 1990). Earlier experiments with gramicidin have shown that NMR studies of peptides in aligned lipid multilayers are useful in resolving such questions (Cornell et al., 1988*a*; Smith et al., 1989). Interpretation of these experiments, however, requires knowledge of the lipid organization in the presence of the peptide (Cornell et al., 1988*a*).

Melittin has been shown to affect the orientation and organization of lipid molecules. The effects are dependent on the lipid class: it causes negatively-charged lipids, but not zwitterionic lipids, to form inverted (H_{II}) phases at low lipid:peptide ratios. The phase properties of zwitterionic lipids are affected in a different way: at lipid:peptide ratios near 4, dipalmitoylphosphatidylcholine (DPPC) molecules in dispersions retain a bilayer arrangement but form small, rapidly-tumbling structures that manifest spectra characteristic of isotropic motion (Dufourc et al., 1986; Dempsey and Watts, 1987).

The aims of the experiments reported here were first to determine whether at low lipid:peptide ratios the peptide retained a well oriented bilayer structure, and secondly to determine the influence of the peptide on the structure and dynamics of the lipid molecules. Experiments have been performed with ester- and ether-linked lipids; although the former are more biologically abundant, the latter have significant advantages in ^{13}C solid-state NMR studies of peptide-lipid complexes, as they do not have carbonyl resonances that overlap with those of the peptide (Cornell et al., 1988a).

MATERIALS AND METHODS

Melittin synthesis and purification

Natural melittin (Sigma Chemical Co., St. Louis, MO) was purified by reverse-phase high performance liquid chromatography to remove traces of phospholipase A_2, which is commonly found associated with

Address correspondence to Dr. R. Smith, Biochemistry Department, The University of Queensland, QLD 4072, Australia.

Abbreviations used in this paper: PC, *sn*-glycero-3-phosphocholine; DLPC, dilaurylPC; DMPC, dimyristoylPC; DTPC, ditetradecylPC; CSA, chemical shift anisotropy.

0006-3495/92/08/469/06 $2.00

the commercial peptide. The purified sample was assayed for residual lipase activity (Batenburg et al., 1987*a*) and revealed no lipid breakdown. Many of the experiments were performed with ^{13}C-labeled melittin synthesized by standard solid-phase methods using either t-butyloxycarbonyl (t-Boc) or fluorenylmethoxycarbonyl (Fmoc) amino protecting groups; details will be published elsewhere.

Dilauryl-*sn*-glycerophosphorylcholine (DLPC) and dimyristoyl-*sn*-glycerophosphorylcholine (DMPC) were purchased from Calbiochem-Behring Corp. (La Jolla, CA) and Sigma Chemical Co., respectively. DitetradecylPC (DTPC) was a gift from Dr. Ruthven Lewis (University of Alberta).

Preparation of aligned multilayers

For solid-state NMR spectroscopy aligned multilayers of lipid containing melittin (~50 mg total weight) were deposited on glass slides. The lipids and melittin were dissolved in methanol, and aliquots applied to each of a series of glass plates. The solvent was removed by heating to 323 K under vacuum in a dessicator before the plates were placed under vacuum overnight. The lipid:peptide mixtures were hydrated by addition of 50 μl of distilled water.

The stack of glass plates was sealed in a 10-mm glass tube containing an additional 10 μl of water, which was then mounted in a probe that allowed measured rotation of the sample about an axis perpendicular to the magnetic field without removal of the probe from the spectrometer (Cornell et al., 1988*a*).

NMR spectroscopy

^1H NMR spectra were recorded at 300.066 MHz on a Bruker CXP-300 spectrometer (Bruker Instruments Inc., Karlsruhe, Germany). Typical operating conditions were: 90° pulse, 7 μs; repetition delay, 10 s; acquisition time, 8.5 ms; sweep width, 62.5 kHz; number of scans, 10. Spectra were acquired using a simple 90° pulse.

^{31}P spectra were recorded at 121.46 MHz using the Ganapathy pulse sequence (Ganapathy et al., 1985). Typical operating conditions were: 90° pulse duration, 7 μs; repetition delay, 2 s; sweep width, 62.5 kHz; spin-locking time, 10 μs; number of scans 100.

^{14}N quadrupole spectra were recorded at 21.67 MHz using the Ganapathy pulse sequence with a 90° pulse time of 8 μs, a spin-lock period of 100 μs, and recycle time of 0.3 s. Typically, 40,000 scans were recorded for each spectrum.

Proton-enhanced ^{13}C spectra (Pines et al., 1973) were recorded at 75.46 MHz. Typical operating conditions were: Hartman-Hahn 90° pulse, 8 μs; contact time, 1.5 ms; acquisition time, 8.5 ms; repetition delay, 2 s; sweep width, 62.5 kHz.

Spin-lattice relaxation

Laboratory frame spin-lattice relaxation times, T_1, were measured by the inversion-recovery method. The rotating frame longitudinal relaxation times, $T_{1\rho}$, were measured using the cross-polarization method of Pines et al. (1973).

RESULTS

^1H NMR

Fig. 1 shows the ^1H resonances arising from water, the lipid headgroup methyls, and the lipid acyl chain methylene groups for an aligned multilayer sample containing a 1:15 molar ratio of melittin:DMPC. Parallel results were obtained with the other two lipids. The samples were aligned with the lipid bilayer normal oriented at the "magic angle" to the spectrometer magnetic field, resulting in the collapse of the dipolar interactions that would

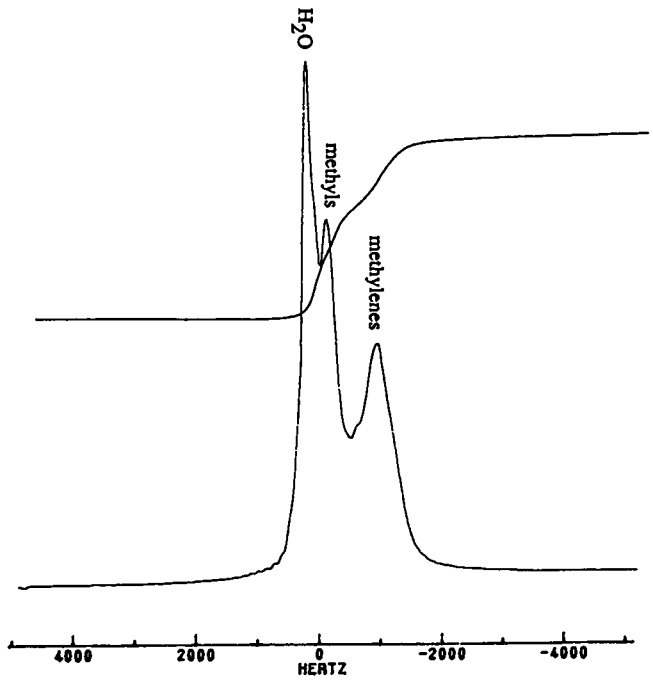

FIGURE 1 300.066 MHz ^1H spectrum obtained with chemically synthesized (^{13}C-Leu-6) melittin in aligned multilayers of dimyristoylPC at a 15:1 lipid:peptide mole ratio. The experimental parameters were: temperature, 303 K; linebroadening, 1 Hz; number of scans, 10. The multilayers were aligned with the bilayer normal at the "magic angle" (54°47′) to the spectrometer magnetic field.

otherwise cause these resonances to overlap. Integration of the water resonance yields the extent of hydration of the samples, which was always ≥20 molecules/lipid molecule. About 10 water molecules are needed to fully hydrate the headgroup of each lipid molecule. The sample tubes also contained excess bulk water that was not taken up by the lipid-peptide complexes over several days at room temperature.

The width of the lipid resonances reveals that these molecules are well aligned. Had a significant proportion of the molecules varied from the magic angle orientation by more than 2° the resultant chemical shift anisotropy and dipolar interactions would have substantially broadened the lipid peaks. Although previous experiments had always shown a mosaic spread of less than 1° for diacylphosphatidylcholine molecules in the presence of the uncharged peptide gramicidin A, it was not known whether this alignment would be maintained in the presence of melittin. The latter peptide has a carboxy-terminal segment that includes the tetrapeptide Lys-Arg-Lys-Arg, resulting in the introduction of positive charge at the bilayer surface. Whereas zwitterionic lipid bilayers maintain a fixed separation in the presence of excess water, bilayers of charged lipids tend to increase their separation continuously because of the electrostatic repulsion between bilayers (Hauser, 1984), with a resultant deterioration in the degree of molecular alignment within the bilayers. It appears, however, that introduc-

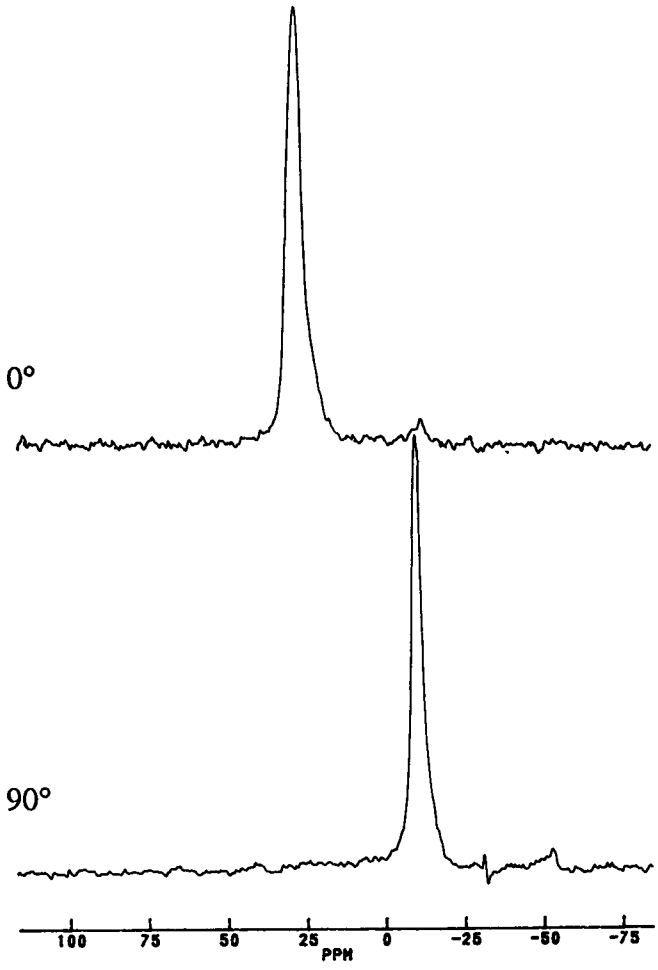

FIGURE 2 121.46 MHz ^{31}P NMR spectrum with ^{13}C-Leu-16-labeled melittin at a 19:1 lipid:peptide molar ratio in aligned multilayers of dilaurylphosphatidylcholine. The multilayers were oriented with the bilayer normal at 0° (*top*) and 90° (*bottom*) to the spectrometer magnetic field. The experimental parameters were: temperature, 300 K; sweep width, 62.5 kHz; 90 pulse, 6.5 μs; repetition delay, 2 s; linebroadening, 50 Hz; number of scans, 100.

tion of the charged peptide has not resulted in greater lipid disorder within the bilayers.

^{31}P NMR

As in the ^{1}H NMR spectra, the ^{31}P NMR peaks obtained with aligned samples are narrow and there is only a small powder resonance under the aligned signal; a typical result is shown in Fig. 2. The peak widths in the spectra obtained at angles of 0° and 90° are, within the experimental errors, equal to those of aligned bilayers of pure PC. These results reinforce the conclusion, drawn from the proton data, that melittin, even at a lipid:peptide molar ratio of 10:1, does not significantly alter the mosaic spread of the lipid molecules, which is ~1° for pure PC. Although melittin promotes formation of the hexagonal II phase by negatively charged lipids (Batenburg et al., 1987*b*) it is apparent that PC remains in a lamellar

TABLE 1 **Comparison of ^{31}P and ^{13}C chemical shift anisotropy of melittin-phosphatidylcholine aligned samples at 303 K**

	^{31}P	^{13}C (*sn*-1 carbonyl)
DMPC	−45 ppm	−27 ppm
DMPC-Melittin (15:1)	−38 ppm	−27 ppm
DMPPC-Melittin (10:1)	−26 ppm	−22 ppm
DTPC	−46 ppm	
DTPC-Melittin (15:1)	−40 ppm	

All chemical shifts have an estimated error of ±1 ppm.

phase as hexagonal, cubic, and isotropic phases yield markedly different spectra from those observed. From the angular dependence of the chemical shift of the ^{31}P resonances reduced chemical shift anisotropies of −42 to −46 ± 1 ppm were determined for the PCs in the absence of the peptide. These values were slightly reduced at a lipid:peptide ratio of 15:1 and more markedly reduced at a ratio of 10:1 (Table 1). Variations in the order of the lipid molecules, the amplitude of the headgroup motions, the headgroup orientation, and the electronic environment of the phosphorus nucleus can all lead to alterations in the CSA. The absolute magnitude of the changes observed with melittin are similar to those resulting from the addition of basic proteins to acidic lipids (Smith et al., 1983) or the addition of gramicidin A to PC (Cornell et al., 1988*b*), molecules that are believed to induce only minor changes in the amplitude of motion of the headgroup (Smith et al., 1983).

^{14}N NMR

The magnitude of the splitting of the ^{14}N quadrupolar signal is determined by the amplitude of the motions experienced by the choline segment of the headgroup. The observed reduction in the ^{14}N splitting upon the addition of melittin is ~25% (Table 2). From Rothgeb and Oldfield (1981) the S_z of the ^{14}N group is 0.08, which is principally assigned to the overall disorder (S_{mol}) of the choline group (Petersen and Chan, 1977). The 25% reduction in S_{mol}, thus, suggests an increase in the angular range of tumbling of this group by ~5°.

TABLE 2 **Nitrogen-14 quadrupolar splittings for melittin-phosphatidylcholine aligned samples, at 303 K, with the bilayer normal at 0° to the magnetic field**

DMPC	19.6 kHz	
DTPC	24.4 kHz	
		$\Delta\nu/\Delta\nu_{lipid}$
Melittin + DMPC (1:15)	16.0 kHz	0.82
Melittin + DTPC (1:15)	16.8 kHz	0.69
Melittin + DMPC (1:10)	14.2 kHz	0.72

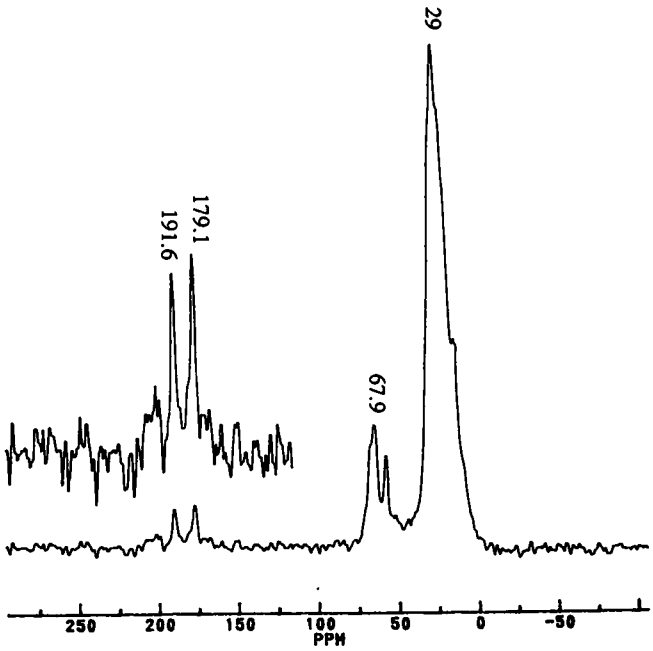

FIGURE 3 75.46 MHz ^{13}C NMR spectrum recorded with ^{13}C-Leu-16-labeled melittin at a 10:1 dimyristoylPC:peptide molar ratio. The multilayers were aligned with the bilayer normal at 0° to the spectrometer magnetic field. The resonances are: 191.6 ppm, lipid *sn*-1 chain carbonyl; 179.1 ppm, lipid *sn*-2 chain carbonyl; 67.9 ppm glycerol carbons; 29 ppm lipid chain methylenes. The spectral parameters were: temperature, 303 K; sweep width, 62.5 kHz; 90° pulse, 8 μs; repetition delay, 2 s; linebroadening 50 Hz; number of scans, 38,000.

^{13}C NMR

At the highest concentrations employed, melittin caused a reduction in the CSA of the lipid *sn*-1 carbonyl resonance (Fig. 3 and Table 1), showing that it not only affects the headgroup, but also induces minor changes in the molecular organization at the hydrocarbon/water interface. No change was observed in the CSA of the resonance of the *sn*-2 carbonyl. As this carbonyl normally has a small CSA, which will be very sensitive to the orientation of this group, it appears that the peptide has not altered the organization of this segment of the lipid molecule.

Relaxation rates

The effects of added molecules on the motion of lipid bilayers may be monitored by measurement of the relaxation rates for the protons in the acyl chain methylene groups.

Both the laboratory- and rotating-frame spin-lattice relaxation rates were measured for the methylene protons of aligned samples of DMPC and DLPC in the presence and absence of the peptide, with the bilayer normal at 0° to the spectrometer field (Table 3). The relaxation rates were identical for pure DMPC and DLPC multilayers. With increasing concentrations of melittin, T_1 increased whereas $T_{1\rho}$ was reduced up to eightfold. $T_{1\rho}$ also lost its orientation dependence (checked at 90°). Both relaxation times are dependent on the modulation of dipole–dipole interactions which results from molecular motion. T_1 is dominated by nuclear (and hence atomic) reorientations that occur on the timescale of ω_0, i.e., ~300 MHz, motions that primarily involve rotation about bonds within the acyl chain. Rotating frame relaxation is dominated by motions on the 30–40 kHz timescale (2 ω_1); reorientations in lipids in this time frame involve movement of substantial molecular segments (i.e., collective motions) (Peng et al., 1988). Thus, the changes recorded in Table 3 are indicative of an increase in the spectral density of collective, segmental motions and a decline in the spectral density of the faster motions attributable to intramolecular rearrangements.

DISCUSSION

The longer term objective of our research is the direct delineation of the location and structure of melittin in lipid membranes. Application of solid-state NMR methods to such a problem requires the use of high peptide:lipid ratios. It is necessary to establish that under these conditions the lipid remains in the bilayer phase and that there is no resultant phase change or significant mosaic spread introduced into its molecular alignment.

Melittin at high concentrations has previously been shown to induce phase changes that depend on the lipid species. Whereas melittin causes negatively charged lipids to prefer the hexagonal II phase, it induces the formation of an isotropic phase in egg phosphatidylethanolamine at temperatures well above its gel-to-liquid crystalline phase transition (Dufourc et al., 1989). It has been reported that at extreme lipid:peptide ratios (near 4) melittin disrupts fluid phase (L_α) zwitterionic (PC) lipid multilayers, causing them to form small, rapidly-tumbling structures (Dufourc et al., 1986; Dempsey and Watts, 1987; Dufourc et al., 1989). At lower ratios it also causes negatively charged lipids to form an isotropic phase (Batenburg et al., 1987*b*). Under the conditions employed in these experiments (L_α zwitterionic lipid with a lipid:peptide molar ratio ≥ 10) the ^1H, ^{14}N, ^{31}P, and ^{13}C spectra were all consistent with the retention of a

TABLE 3 **Comparison of proton $T_{1\rho}$ and T_1 for melittin-phosphatidylcholine aligned samples with the bilayer normal at 0° to the magnetic field**

	$T_{1\rho}$	T_1	$T_1/T_{1\rho}$
	ms	*ms*	
DMPC	40.0	210	5.2
DMPC-Melittin (15:1)	8.3	365	44.0
DMPC-Melittin (10:1)	5.3	455	86.0
DTPC	30.0	285	9.5
DTPC-Mellitin (15:1)	7.0	385	55.0

multilamellar lipid phase by both the ester- and the ether-linked lipids.

Dempsey and Watts (1987) observed a decrease in the ^{31}P CSA of DMPC from -54 ± 2 ppm for the pure lipid to -46 ± 2 ppm at a 25:1 lipid:peptide molar ratio at 300 K. Although the CSAs in Table 1 are slightly smaller (possibly because of the different methods used to measure the CSA),[1] we observed a comparable decrease in the anisotropy at a 19:1 DLPC:melittin ratio. With POPC, the CSA has been observed to be approximately constant at -48.5 ppm down to a 50:1 lipid:peptide molar ratio (Kuchinka and Seelig, 1989), a result that is in accord with our observations. These changes in the ^{31}P CSA, which are only apparent at high melittin concentrations, are compatible with the conclusion, drawn from the experiments with metal ions (Akutsu and Seelig, 1981) and selectively headgroup-deuterated lipids (Dempsey and Watts, 1987; Kuchinka and Seelig, 1989), that minor conformational changes in the headgroup result from the increased charge density at the bilayer surface (Kuchinka and Seelig, 1989).

Changes in the motion and organization of the lipid acyl chains are also apparent. The marked reduction in $T_{1\rho}$ and increase in T_1 (Table 3) indicates that the intramolecular motion of the methylene groups is restricted in the presence of the peptide with an accompanying increase in the collective motions of the lipids. These observations parallel those in earlier studies of the effects of membrane proteins, gramicidin A, and cholesterol (Cornell et al., 1983; Cornell and Keniry, 1983). Others have also noted melittin-induced changes in the lipid hydrocarbon chain dynamics and order. The average orientation for the *cis* double bond of 1-palmitoyl-2 (9′, 10′, $^2H_2$) oleoylPC is altered by melittin (Dufourc et al., 1989). Coddington et al. (1983) noted a decrease in the acyl chain methylene ^{13}C T_1s of chloroplast lipids. Small reductions have also been noticed above T_c in the ^2H T_1s of DPPC labeled at the 3 position and with DMPC containing perdeuterated chains (Dufourc et al., 1989). By contrast, in our experiments there was a very clear increase in T_1 for the acyl chain methylene protons. The variable effects of the peptide on the T_1s for these different nuclei may arise from differences in their relaxation mechanisms.

In conclusion, down to 10:1 lipid:peptide ratios, multilamellar ester- and ether-linked PCs retain their bilayer structure. The peptide does however induce changes in the conformation of the lipid headgroups and modifies the spectral density of the lipid acyl chain motions, favoring low frequency motions.

This work was partly supported by an Australian Research Council grant to Ross Smith and a grant for Industrial Research and Development to Bruce A. Cornell.

[1] The CSAs reported here were derived from observations of the difference in the ^{31}P peak position in samples oriented at 0° and 90° to the spectrometer magnetic field, a method that is inherently more reliable than measurement of the shoulder positions in powder spectra.

Received for publication 17 September 1991 and in final form 6 April 1992.

REFERENCES

Akutsu, H., and J. Seelig. 1981. Interaction of metal ions with phosphatidylcholine bilayer membranes. *Biochemistry.* 20:7366–7373.

Altenbach, C., W. Froncisz, J. S. Hyde, and W. L. Hubbell. 1989. Conformation of spin-labeled melittin at membrane surfaces investigated by pulse saturation recovery and continuous wave power saturation electron paramagnetic resonance. *Biophys. J.* 56:1183–1191.

Altenbach, C., and W. L. Hubbell. 1988. The aggregation state of spin-labeled melittin in solution and bound to phospholipid membranes: evidence that membrane-bound melittin is monomeric. *Proteins Struct. Funct. Genetics.* 3:230–242.

Batenburg, A. M., J. C. L. Hibbeln, and B. de Kruijff. 1987*a*. The specific penetration of melittin into phospholipid model membranes. *Biochim. Biophys. Acta.* 903:155–165.

Batenburg, A. M., J. H. van Esch, J. Leunissen-Bijvelt, A. J. Verkleij, and B. Kruijff. 1987*b*. Interaction of melittin with negatively charged phospholipids: consequences for lipid organization. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 223:148–154.

Bazzo, R., M. J. Tappin, A. Pastore, T. S. Harvey, J. A. Carver, and I. D. Campbell. 1988. The structure of melittin. A ^1H-NMR study in methanol. *Eur. J. Biochem.* 173:139–146.

Brown, L. R., W. Braun, A. Kumar, and K. Wüthrich. 1982. High resolution nuclear magnetic resonance studies of the conformation and orientation of melittin bound to a lipid-water interface. *Biophys. J.* 37:310–328.

Coddington, J. M., S. R. Johns, R. I. Willing, J. R. Kenrick, and D. G. Bishop. 1983. Monolayer and carbon-13 NMR studies on the interaction between melittin and chloroplast lipids. *Biochim. Biophys. Acta.* 727:1–6.

Cornell, B. A., R. G. Hiller, J. Raison, F. Separovic, R. Smith, J. C. Vary, and C. Morris. 1983. Biological membranes are rich in low-frequency motions. *Biochim. Biophys. Acta.* 732:473–478.

Cornell, B. A., and M. Keniry. 1983. The effect of cholesterol and gramicidin A on the carbonyl groups of dimyristoylphosphatidylcholine dispersions. *Biochim. Biophys. Acta.* 732:705–710.

Cornell, B. A., F. Separovic, A. T. Baldassi, and R. Smith. 1988*a*. Conformation and orientation of gramicidin A in oriented phospholipid bilayers measured by solid-state carbon-13 NMR. *Biophys. J.* 53:67–76.

Cornell, B. A., L. E. Weir, and F. Separovic. 1988*b*. The effect of gramicidin A in lipid bilayers. *Eur. Biophys. J.* 16:113–119.

Dawson, C. R., A. F. Drake, J. Helliwell, and R. C. Hider. 1978. The interaction of bee melittin with lipid bilayer membrane. *Biochim. Biophys. Acta.* 510:75–86.

De Grado, W. F., G. F. Musso, M. Lieber, E. T. Kaiser, and F. J. Kedzy. 1982. Kinetics and mechanism of hemolysis induced by melittin and by a synthetic melittin analogue. *Biophys. J.* 37:329–338.

Dempsey, C. E. 1990. The action of melittin on membranes. *Biochim. Biophys. Acta.* 1031:143–161.

Dempsey, C. E., and A. Watts. 1987. A deuterium and phosphorus-31 nuclear magnetic resonance study of the interaction of melittin with dimyristoylphosphatidylcholine bilayers and the effects of contaminating phospholipase A_2. *Biochemistry.* 26:5803–5811.

Dufourc, E. J., J.-M. Bonmatin, and J. Dufourcq. 1989. Membrane structure and dynamics by ^2H- and ^{31}P-NMR. Effects of amphipathic peptidic toxins on phospholipid and biological membranes. *Biochimie.* 71:117–123.

Dufourc, E. J., I. C. P. Smith, and J. Dufourcq. 1986. Molecular details

of melittin induced lysis of phospholipid membranes as revealed by deuterium and phosphorus NMR. *Biochemistry.* 25:6448–6455.

Eibl, H., and P. Woolley. 1986. Synthesis of enantiomerically pure glyceryl esters and ethers. I. Methods employing the precursor 1,2-isopropylidene-sn-glycerol. *Chem. Phys. Lipids.* 41:53–63.

Ganapathy, S., T. M. Eads, V. P. Chacko, and R. G. Bryant. 1985. Elimination of baseline artifacts in solid-state NMR spectra by spin locking. *J. Magn. Reson.* 62:314–315.

Habermann, E., and J. Jensch. 1967. Sequenzanalyse des melittins aus den tryptischen und peptischen spaltstücken. *Hoppe-Seyler's Z. Physiol. Chem.* 348:37–50.

Hauser, H. 1984. Some aspects of the phase behaviour of charged lipids. *Biochim. Biophys. Acta.* 772:37–50.

Inagaki, F., I. Shimada, K. Kawaguchi, M. Hirano, I. Teresawa, T. Ikura, and N. Go. 1989. Structure of melittin bound to micelles as studied by two-dimensional NMR and distance geometry calculations. *Biochemistry.* 28:5985–5991.

Kuchinka, E., and J. Seelig. 1989. Interaction of melittin with phosphatidylcholine membranes. Binding isotherm and lipid headgroup conformations. *Biochemistry.* 28:4216–4221.

Pastore, A., T. S. Harvey, C. E. Dempsey, and I. D. Campbell. 1989. The dynamic properties of melittin in solution: investigations by NMR and molecular dynamics. *Eur. Biophys. J.* 16:363–367.

Peng, Z.-Y., V. Simplaceanu, I. J. Lowe, and C. Ho. 1988. Rotating frame relaxation studies of slow motions in fluorinated phospholipid model membranes. *Biophys. J.* 54:81–95.

Petersen, N. O., and S. I. Chan. 1977. More on the motional state of lipid bilayer membranes: interpretation of order parameters obtained from nuclear magnetic resonance experiments. *Biochemistry.* 16:2657–2667.

Pines, A., M. C. Gibby, and J. S. Waugh. 1973. Proton enhanced nuclear magnetic resonance of dilute spins in solids. *J. Chem. Phys.* 59:569–590.

Rothgeb, T. M., and E. Oldfield. 1981. N-14 nuclear magnetic resonance spectroscopy as a probe of lipid bilayer headgroup structure. *J. Biol. Chem.* 256:6004–6009.

Schulze, J., U. Mischeck, S. Wigand, and H.-J. Galla. 1987. Incorporation of highly purified melittin into phosphatidylcholine bilayer vesicles. *Biochim. Biophys. Acta.* 901:101–111.

Schwarz, G., and G. Beschiaschvili. 1989. Thermodynamic and kinetic studies on the association of melittin with a phospholipid bilayer. *Biochim. Biophys. Acta.* 979:82–90.

Smith, R., B. A. Cornell, M. A. Keniry, and F. Separovic. 1983. ^{31}P nuclear magnetic resonance studies of the association of basic proteins with multilayers of diacyl phosphatidylserine. *Biochim. Biophys. Acta.* 732:492–498.

Smith, R., D. E. Thomas, F. Separovic, A. R. Atkins, and B. A. Cornell. 1989. Determination of the structure of a membrane-incorporated ion channel. Solid-state nuclear magnetic resonance studies of gramicidin A. *Biophys. J.* 56:307–314.

Stankowski, S., and G. Schwarz. 1990. Electrostatics of a peptide at a membrane/water interface. The pH dependence of melittin association with lipid vesicles. *Biochim. Biophys. Acta.* 1025:164–172.

Terwilliger, T. C., L. Weissman, and D. Eisenberg. 1982. The structure of melittin form I crystals and its implication for melittin's lytic and surface activities. *Biophys. J.* 37:353–361.

Vogel, H. 1987. Comparison of the conformation and orientation of alamethicin and melittin in lipid membranes. *Biochemistry.* 26:4562–4572.

Vogel, H., and F. Jähnig. 1986. The structure of melittin in membranes. *Biophys. J.* 50:573–582.

Wakamatsu, K., A. Okada, T. Higashijima, and T. Miyazawa. 1986. NMR analysis of the conformations of membrane-bound peptides. *Biopolymers.* 25:S193–S200.

Weaver, A. J., M. D. Kemple, and F. G. Prendergast. 1989. Characterization of selectively ^{13}C-labelled synthetic melittin and melittin analogues in isotropic solvents by circular dichroism, fluorescence, and NMR spectroscopy. *Biochemistry.* 28:8614–8623.