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Effect of acyl chain length on the structure and motion of gramicidin A in lipid bilayers

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The transmembrane ion transport properties of gramicidin A have previously been shown to be dependent on the nature of its lipid environment. Solid-state NMR spectroscopic studies of ^{13}C-labelled analogues of gramicidin in oriented multilayers of phosphatidylcholine have shown that variation of the lipid hydrocarbon chain length has no effect on the structure or orientation of the peptide backbone.

The conductivity of membrane-incorporated ion channels formed by gramicidin A is influenced by the nature of the lipid forming the bilayers (reviewed in Refs. 1 and 2). Changes in the lipid headgroup structure [3–6] and membrane thickness [7,8] alter the channel characteristics. Increasing the extent of headgroup methylation from phosphatidylethanolamine to phosphatidylcholine results in a decrease in channel lifetime and an increase in the single-channel conductance [3]. Compared with zwitterionic lipids, gramicidin in negatively charged lipids manifests a higher single-channel conductance [6]. Variation of the membrane thickness, either by use of lipids with different acyl chain lengths, or incorporation of hydrocarbon solvent into black lipid membranes, results in complex effects on the single-channel conductance and channel lifetime. Both of these parameters fall as the chain length of the lipid in the membrane increases [1,8]; however, the single-channel conductance does not vary when the membrane thickness is adjusted by addition of solvent to membranes formed from a single lipid [8]. Variation of the relative dimensions of the lipid bilayer and the channel by addition or deletion of amino acids to the gramicidin sequence also affects the conductance in a fashion which suggests that shortening of the channel reduces the probability of both ion-binding sites within the channel being simultaneously occupied [9].

The gramicidin channel is not only affected by chemical changes in the molecules forming the lipid matrix, but also by the physical state of the membrane. The conductance falls a few degrees below the temperature at which the lipid undergoes the transition from the liquid-crystalline to the gel state [10].

The molecular mechanisms by which the lipid matrix modulates the channel characteristics are unknown. To address this aspect of the properties of the peptide we have examined its structure and dynamics in several lipid matrices using a ^{13}C, solid-state NMR approach described earlier [11–13].

Analogues of gramicidin A, labelled with ^{13}C in selected peptide carbonyl groups, were synthesized by the solid-phase method [12]. The syntheses were initiated with PAM-trp(CHO) resin (Applied Biosystems, Foster City, U.S.A.), and used standard *t*-BOC protection for the amino groups and formyl protection on the imidazole group of the tryptophan residues. The assembled peptide was cleaved from the resin using ethanolamine [14], which removes the side-chain protecting group of the tryptophans and introduces the required carboxy-terminal ethanolamide group. The amino-terminal amino acid was formylated and then the peptide was purified by chromatography on Sephadex LH-20 (Pharmacia, Uppsala, Sweden) and isocratic, reverse-phase HPLC. Each peptide gave a single peak accounting for over 95% of the integrated intensity on HPLC:

Abbreviations: CSA, chemical shift anisotropy; DCPC, dicaprylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DTPC, ditetradecylphosphatidylcholine (ether-linked chains); DHPC, dihexadecylphosphatidylcholine (ether-linked chains); DOPC, dioleoylphosphatidylcholine.

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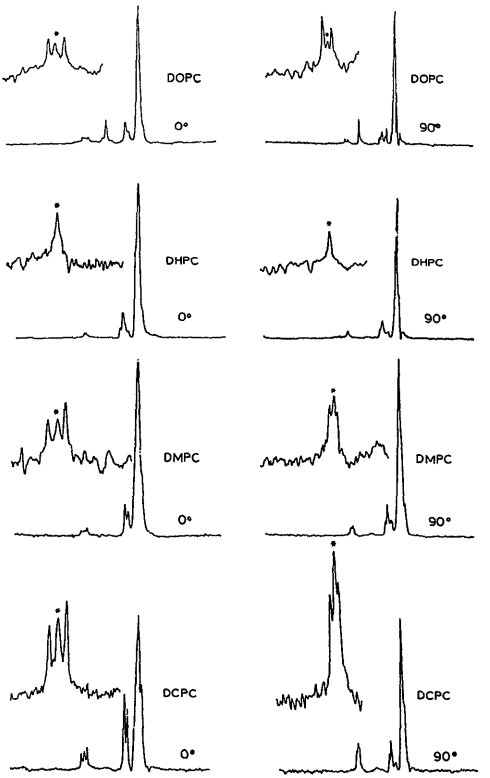

Fig. 1. Cross polarization spectra of ^{13}C-labelled D-Leu^{14} analogues of gramicidin in aligned multilayers of various lipids. The spectra were recorded with the bilayer normal aligned along (0°) or perpendicular to (90°) the direction of the spectrometer magnetic field at temperatures above the phase transition. The asterisks mark the gramicidin carbonyl resonances. The spectra, which were plotted with a 50 or 100 Hz line broadening, were obtained with 20000–40000 scans. Typical spectrometer parameters are given in the text. The plot widths are 600 ppm, with a 3-fold expansion for the carbonyl resonances.

the purity was confirmed by ^{1}H- and ^{13}C-NMR in solutions of perdeuterated methanol or dimethylsulphoxide, and by circular dichroism spectroscopy.

Samples of gramicidin in aligned phosphatidylcholine bilayers, at a 1:15 peptide/lipid molar ratio, were prepared as previously described [12].

TABLE I

Reduced chemical shift anisotropies from the labelled carbonyl sites of gramicidin analogues

Measurements were performed on samples containing a 1:15 peptide/lipid molar ratio at 307° K in all lipids except DHPC, for which the temperature was 323° K. The asterisked analogue is the gramicidin labelled in both the alpha and carbonyl carbons. Ditetradecyl- and dihexadecyl-PC contain ether-linked hydrocarbon chains rather than the ester-linkage extant in the other lipids.

	Gly^2	Gly^2 (^{13}C–^{13}C) *	Ala^3	D-Leu^4	Val^7	D-Leu^{10}	D-Leu^{12}	D-Leu^{14}
DCPC (C_{10})			16±1					14±1
DMPC (C_{14})	11±2	12±2	14±1	12±2	16±1	9±1	11±1	13±1
DOPC ($C_{18:1}$)			16±1					13±1
DTPC (C_{14})	11±1	10±1	15±1					
DHPC (C_{16})	12±2		16±1	12±1		9±1		12±1

Proton-enhanced, ^{13}C spectra were recorded at 75.46 MHz using a Bruker CXP-300 spectrometer. Typical operating conditions were: 90° pulse, 8 μs; contact time, 2 ms; acquisition time, 8.5 ms; repetition time, 2 s; sweep width, 62.5 kHz. The tube was mounted in a probe which allowed the sample to be rotated about an axis perpendicular to the magnetic field, without removal of the probe from the spectrometer.

^{13}C spectra were recorded for aligned lipid multilayers of the ester-linked lipids dicaoryl-, dimyristoyl- and dioleoylphosphatidylcholine, and the ether-linked lipids ditetradecyl- and dihexadecylphosphatidylcholine (DCPC, DMPC, DOPC, DTPC, and DHPC, respectively) at temperatures above their gel-to-liquid-crystalline transition temperatures, T_c, i.e. 307 K except for DHPC, for which the temperature was 323 K. Spectra were also obtained for DMPC and DTPC at 273 K, and for DHPC at 285 K, temperatures which are below T_c. Measurements of the resonance position as a function of membrane orientation were used to derive the reduced chemical shift anisotropies (CSA) for each of the gramicidin analogues.

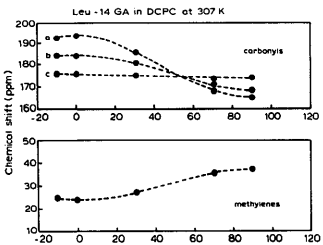

Fig. 2. Representative data from measurements of the chemical shift as a function of the direction of orientation of aligned samples. Results are presented for lipid (a, c) and peptide (b) carbonyls, and for the lipid methylene resonance.

Spectra recorded at 0° angle show no dependence of the peak position or line width on the chemical composition of the lipid matrix. Results obtained for the D-Leu^{14} analogue are shown in Fig. 1. Nor do the CSAs show any dependence on the lipid: within the experimental errors the CSAs are identical for the Ala^3 and D-Leu^{14} analogues in bilayers formed from C_{10}, C_{14}, C_{16} and $C_{18:1}$ lipids (Table I and Fig. 2). Similarly, the D-Leu^{10} analogue has identical CSAs in C_{14} and C_{16} lipid.

Cooling the samples below their lipid phase transition temperatures caused no change in the position of the 0° angle peaks and, except for the D-Leu^{10}, D-Leu^{12} and D-Leu^{14} labelled analogues, little change in peak width [12]. Spectra obtained for the D-Leu^{14} analogue

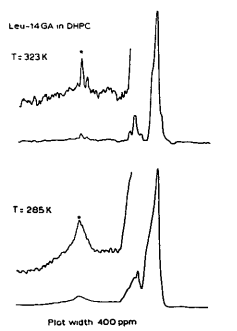

Fig. 3. Comparison of spectra recorded for a sample of D-Leu^{14} gramicidin in DHPC aligned with the bilayer normal along the direction of the spectrometer magnetic field, above and below the lipid phase transition temperature. The asterisks mark the peptide carbonyl resonances. The spectral acquisition parameters were as for Fig. 1. The top spectrum was obtained with 24000 scans and the bottom spectrum with 32000 scans.

are shown in Fig. 3, in which the presence of a small amount of unaligned peptide is evident [15].

Several conclusions may be drawn from these observations. First, in all lipid liquid-crystalline phases the peptide rotates about the bilayer normal: this is consistent with the observed invariance of the CSA for the 0° orientation on passage from the liquid crystalline to the gel state, where the peptide molecules become immobile on the milli- to microsecond timescale of the NMR experiment. Secondly, the orientations of the labelled peptide bonds are insensitive to lipid acyl chain length, and therefore to membrane thickness. As argued earlier [12], the values of the reduced CSAs are dependent on the carbonyl bond orientation; the observed values indicate alignment close to the bilayer normal, consistent with a $\beta^{6,3}$ single-helix structure for the gramicidin. We can therefore conclude that the peptide retains this helical structure, with no detectable distortion, in all of the lipid phases we have examined.

The D-Leu[10], D-Leu[12] and D-Leu[14] residues are positioned close to the membrane surface and they appear to possess greater motional freedom than the groups closer to the bilayer centre [13]: this motion is eliminated in the gel phase but it appears to be unaffected by alteration of membrane thickness from 2.53 nm (C_{10}) to 3.06 nm (C_{18}) [16], figures which may be compared with the 2.6 nm end-to-end length for the $\beta^{6,3}$ dimeric helix of gramicidin [9].

The weak interdependence of lipid and peptide motion in the liquid-crystalline state was indicated by the observation [12] that the lipid methylene order in the presence of a 1:15 peptide/lipid ratio is barely distinguishable from that in pure lipid bilayers. This view is reinforced by comparison of the results from aligned samples of the Ala[3] and D-Leu[14] analogues in multilayers of the unsaturated C_{18} DOPC and the saturated lipids, which suggests that changes in the order of the bilayer methylene chains do not influence the gramicidin. By contrast, the changes in lipid order which occur below T_c do affect the peptide. In the gel phase, the lipid chain C–C bonds are in the all-*trans* configuration with maximum extension of the chains, closer chain packing and markedly reduced lateral molecular motion. There are corresponding marked changes in the motion and function of the gramicidin channels: the peptide becomes immobile a few degrees below T_c [11] and the conductivity falls [10]. The observed dependence of the channel lifetime on lipid chain length [1] may be reconciled with the present results by viewing the formation of a dimerized channel as dependent on a distortion of the membrane lipid. Changing the thickness of the lipid bilayer thus changes the frequency of the channel formation without changing the conformation of the gramicidin A or the ionic conductance in the dimerized state.

As noted earlier [12,13], the replacement of the usual ester-linked lipids with ether-linked acyl chains does not affect gramicidin, although these lipids differ in their conformation near the headgroup region.

We have not explored the effects of introduction of charged lipids, as it is difficult to prepare bilayers with an acceptably small mosaic spread from such lipids. Electrostatic repulsion between headgroups in adjacent bilayers results in a greater separation of bilayers than for zwitterionic lipids, with a consequent reduction in the long-range order of the bilayers.

In conclusion, the structural characteristics of the gramicidin ion channel appear to be relatively insensitive to the state of their lipid environment. Changes in membrane thickness, the introduction of unsaturated bonds, and the substitution of ester for ether linkages produce little observable change in the ^{13}C-NMR spectra of the gramicidin analogues. Only when the lipid acyl chain motion is frozen, several degrees below the T_c for the pure lipid, are changes detected in the motion of the molecule.

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