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Solid-State ^{15}N NMR of Oriented Lipid Bilayer Bound Gramicidin A′ †

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ABSTRACT: Highly oriented samples of lipid and gramicidin A′ (8:1 molar ratio) have been prepared with the samples extensively hydrated (approximately 70% water v/w). These preparations have been shown to be completely in a bilayer phase with a transition temperature of 28 °C, and evidence is presented indicating that the gramicidin is in the channel conformation. An estimate of the disorder in the alignment of the bilayers parallel with the glass plates used to align the bilayers can be made from the asymmetry of the nuclear magnetic resonances (NMR). Such an analysis indicates a maximal range of disorder of ±3°. Uniformly ^{15}N-labeled gramicidin has been biosynthesized by *Bacillus brevis* grown in a media containing ^{15}N-labeled *Escherichia coli* cells as the only nitrogen source. When prepared with labeled gramicidin, the oriented samples result in high-resolution ^{15}N NMR spectra showing 12 resonances for the 20 nitrogen sites of the polypeptide. The frequency of the three major multiple resonance peaks has been interpreted to yield the approximate orientation of the N–H bonds in the peptide linkages with respect to the magnetic field. These bond orientations are only partially consistent with the extant structural models of gramicidin.

The spectroscopic study of lipid bilayer bound proteins and polypeptides has been severely hampered by the large size of the aggregated system. A variety of techniques have been employed to circumvent the resultant problems of long correlation times and light scattering. Chemical modifiers (e.g., lysolipids or detergents) or mechanical modifiers (e.g., sonication) of these samples alter the nature of the preparation. The consequences of such sample modifications for the conformational and dynamic properties of the protein or polypeptide are significant. Solid-state nuclear magnetic resonance (NMR)[1] spectroscopy is an approach that requires the very long correlation times associated with extensive lipid bilayers. Here, the pentadecapeptide gramicidin A′ has been isotopically labeled with ^{15}N, incorporated into bilayers of dimyristoylphosphatidylcholine (DMPC), hydrated, and oriented such that the lipid bilayers are uniformly parallel with each other, and then studied by ^{15}N and ^{31}P solid-state NMR.

Gramicidin A′ is a linear polypeptide produced by *Bacillus brevis* during the early stages of its sporulation cycle. In lipid bilayers, it forms a monovalent cation-selective channel that has been extensively studied. The channel is formed by a dimer of gramicidin, and in single-channel conductance studies, the lifetime of the dimer has been measured. The lifetime is highly dependent on the length of the fatty acyl chains of the lipids; in DMPC, the hydrophobic width of the bilayer coincides with the length of the gramicidin dimer, and long lifetimes are expected (Wallace et al., 1981). A model of the channel, which was developed by means of conformational analysis, has been extant since 1971 (Urry, 1971). However, confirmation by an atomic resolution structure determination has not been achieved for gramicidin in the presence of lipids, because of the difficulty of forming cocrystals of the polypeptide and lipid which diffract to high resolution (Wallace, 1986). Urry's model, which is generally accepted as an approximate description of how the polypeptide backbone is folded, predicts in the rigid limit a structurally uniform pore that may not completely account for cation binding sites (Urry et al., 1982) and potential energy barriers to cation passage (Eisenman & Sandblom, 1984).

Not only is a higher resolution structure determination of interest but also the dynamics of this molecule appear to be very interesting. It is thought that the peptide linkages that line the channel pore rotate so that the carbonyl oxygens can coordinate the cation during its passage across the membrane. While there have been several efforts to calculate the local motions of the peptide linkages (Fischer & Brickmann, 1983; Venkatachalam & Urry, 1984; MacKay et al., 1984), it has not been experimentally characterized. Consequently, gramicidin A still poses a number of interesting structural and dynamic questions.

Recently, the question of gramicidin dynamics has been approached with both ^{13}C (Smith & Cornell, 1986) and ^{2}H

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[1] Abbreviations: NMR, nuclear magnetic resonance; CD, circular dichroism; DSC, differential scanning calorimetry; DMPC, dimyristoylphosphatidylcholine; CSA, chemical shift anisotropy; ppm, parts per million; MSA, molecular symmetry axis.

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(Datema et al., 1986) solid-state NMR. The results showed that the gramicidin channel rotates about an axis parallel with the normal to the bilayer when the sample temperature is above the gel to liquid-crystalline phase transition temperature. Details of either this global motion or possible local motions of the peptide linkages were not elucidated. As in the deuterium study above, the samples used here are multiply labeled in the polypeptide backbone and are therefore sensitive to the whole range of motions experienced along the length of the gramicidin channel. Our spectra have been recorded above the phase transition temperature, and our spectral interpretations assume the validity of channel rotation at a frequency greater than the ^{15}N chemical shift interaction in a 5.1-T field (approximately 3.5 kHz). Because the static ^{15}N chemical shift tensor for a peptide amide is nearly axially symmetric, it is difficult to prove that free rotation about the channel axis is taking place; however, much of the data presented is most easily explained by assuming such a motion. One of the approaches taken in this study has been to orient gramicidin-containing bilayers such that the channel axis, rotation axis, and bilayer normal are all parallel with the magnetic field. Oriented samples have been used with solid-state NMR for elucidating the orientation of protein side chain groups (Rothgeb & Oldfield, 1981a; Cross & Opella, 1983) and protein backbone torsion angles (Cross & Opella, 1985).

Materials and Methods

Uniformly ^{15}N-labeled gramicidin A′ was produced biosynthetically by *Bacillus brevis* (ATCC 10068). Because *B. brevis* does not grow well on a defined medium, where the only nitrogen source is either an ammonium or a nitrate salt (Stokes & Woodward, 1943), the following approach was used for the biosynthesis. *Escherichia coli* (K12 thi$^-$) was grown on a minimal mineral salts media where the only nitrogen source was $^{15}NH_4Cl$. These cells were harvested by centrifugation after reaching an OD_{600} = 5, resuspended in buffer, and autoclaved. The *B. brevis* cultures were grown on minimal media (Mach et al., 1963) lacking the normal nitrogen source, asparagine, but supplemented with 8 g of uniformly labeled *E. coli* cell paste. Tyrothricin, which is a mixture of the polypeptides gramicidin A′ and tyrocidine, was purified by a published procedure (Bartley et al., 1972). Treatment of this product with activated charcoal was an added step before it was layered onto a Sephadex LH-20 column for resolving the gramicidin from the tyrocidine (Bartley et al., 1972).

All samples containing gramicidin were prepared in a 1:8 molar ratio of gramicidin and DMPC (Sigma Chemical Co.; used without further purification) by dissolving both the gramicidin and lipid in spectroscopic-grade methanol, drying under vacuum, and hydrating with deionized water (70% water v/w). To avoid the formation of vesicles and liposomes with short correlation times (<0.1 s), the hydration step was conducted at 45 °C without agitation for 24–48 h (Seelig et al., 1985). The oriented samples were prepared by drying the methanol solution on glass microscope coverslips having dimensions of 5.8 × 12.0 mm. Fourteen microliters of methanol solution containing 3.8 mg of gramicidin and DMPC was spread on each coverslip; 28 of the coverslips were then stacked in a 12-mm-long section of square tubing with a 6-mm inner dimension, and enough deionized water was added to achieve 70% hydration. The ends of the tube were sealed with glass plates and a quick-setting epoxy. Again, hydration was allowed to proceed at 45 °C without agitation for 24–48 h before spectra were recorded. The total weight of gramicidin in such a sample is approximately 13.5 mg. This approach was developed in light of several related schemes (Rothgeb & Oldfield, 1981b; Seelig & Gally, 1976; Worcester & Franks, 1976) and discussions with B. A. Cornell (CSIRO, Australia).

^{31}P NMR spectra were recorded on a home-built spectrometer operating at 62 MHz for phosphorus. Proton decoupling was accomplished with greater than 5 W of power at 150 MHz. A sweep width of ±25 kHz was used with 90° ^{31}P pulses and a 3-s recycle delay. ^{31}P chemical shifts are given relative to the isotropic average for hydrated DMPC. ^{15}N NMR spectra were recorded on a modified IBM/Bruker WP200 SY spectrometer with a solids package. A static (i.e., non-magic-angle spinning) probe with variable temperature control was constructed for observation of the powder patterns from unoriented samples and for spectra of oriented samples. Spectra of oriented samples were obtained with the bilayer normal aligned parallel with the magnetic field unless otherwise noted. Typical experimental conditions utilized a sweep width of 62.5 kHz, a preacquisition delay of 16 μs, and a recycle delay of 7 s. Spectra were obtained by cross-polarization with fields generated by 4.6-μs 90° pulse lengths, a mixing period of 1 ms, and an increased ^1H decoupling field of 2.0 mT. To generate these pulse conditions, kilowatt Henry Radio amplifiers have been installed, a Model 2002A instrument tuned for 200 MHz and a Model 2K2D instrument tuned for 20 MHz. ^{15}N chemical shifts are given relative to the resonance of a saturated solution of $^{15}NH_4NO_3$.

The gramicidin–DMPC samples were characterized in several ways. Differential scanning calorimetry (DSC) was performed on a Perkin-Elmer DSC to determine the primary phase transition temperature of the lipid bilayer samples (Janiak et al., 1976). Because the dynamics of the gramicidin channel are intimately dependent on this temperature, it was important to determine it for our own preparations. Some of the same samples used for NMR were used for the DSC experiments. Gramicidin concentrations were determined by using an extinction coefficient of 2.25×10^4 M^{-1} cm^{-1} at 280 nm (Spisni et al., 1983). The circular dichroism (CD) spectra were recorded with a Jasco J-500C spectropolarimeter and 1-mm path-length cells. The CD spectra provide evidence that the gramicidin molecules are in the channel conformation (Urry et al., 1979; Wallace et al., 1981). Characteristic of this channel conformation are maxima at 217 and 237 nm as well as a negative ellipticity below 205 nm and a minimum at 229 nm. Spectra of gramicidin in organic solvents where the gramicidin structure is altered yield very different CD spectra. A complication with using this approach for characterizing the channel conformation in the NMR samples is that the samples need to be modified before the CD spectra can be recorded. The samples for CD were diluted and sonicated for 30 min at 37 °C and at a high intensity using a Braun-Sonic 1510 sonicator equipped with a microtip probe. Prior to sonication, the sample for CD was incubated at 68 °C for 12 h to parallel the conditions used by Wallace (1984) and Shungu et al. (1986) in preparing gramicidin channels in DMPC vesicles. A final characterization of the lipid-solubilized gramicidin samples has been to record ^{31}P NMR spectra to ensure that the lipids are in a bilayer state and that there is no significant amount of either hexagonal phase or isotropic phase lipids (Cullis & de Kruijff, 1979).

Results

The DSC experiments provided a trace for the polypeptide-bound lipid bilayers that showed a very broad phase transition compared with the pure hydrated DMPC (see Figure 1). The presence of gramicidin at a molar ratio of 1:8 decreases the enthalpy of the transition and broadens the trace dramatically. The width of the main calorimetric endotherm

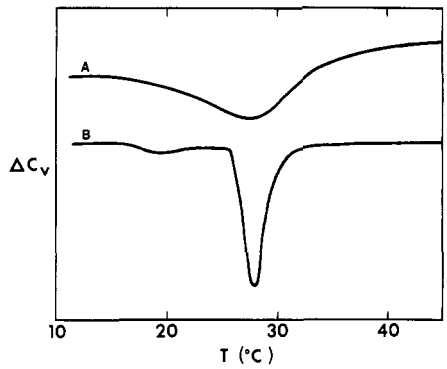

FIGURE 1: Differential scanning calorimetry thermograms of 70% (v/w) hydrated DMPC bilayer preparations. (A) Bilayers containing gramicidin; (B) DMPC without gramicidin. A heating rate of 5 °C/min was used for each trace. The sensitivity of the calorimeter was increased by a factor of 5 for the gramicidin-containing sample. Each sample contained approximately 5 mg of DMPC.

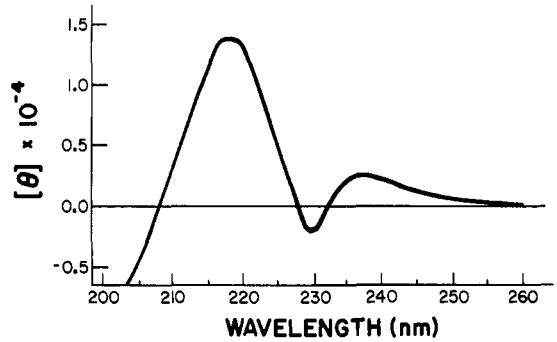

FIGURE 2: Circular dichroism spectrum of a gramicidin–DMPC preparation heated to 68 °C for 12 h and vesicularized by sonication. The molar ellipticity is on a per residue basis. The concentration of gramicidin in the sample was 0.05 mM.

of 9 °C is in close agreement with Chapman et al. (1977). The CD spectrum (Figure 2) shows a spectrum very similar to that reported by Wallace (1984) and universally referred to as the spectrum characteristic of the channel conformation. Because of the long lifetimes expected for gramicidin in DMPC, it is not likely that another conformation is in fast exchange with the channel. For the concentration of gramicidin used in the NMR samples, it is not expected that the interval between breaking and forming a dimer will be significant. The ^{31}P NMR spectra of Figure 3 show that both pure hydrated DMPC and the hydrated 1:8 molar ratio of gramicidin to DMPC were prepared in the bilayer phase with no detectable amounts of either hexagonal or isotropic phases (i.e., <1%). The width of the DMPC powder pattern is in agreement with that found in the literature as is the reduced width of the spectrum obtained from the gramicidin–lipid sample (Rajan et al., 1981).

The ^{15}N powder pattern spectra in Figure 4 represent the overlapping powder patterns of 20 atomic sites in the gramicidin monomer. Sixteen of these sites are in the polypeptide backbone and should have similar static chemical shift tensors with approximate σ_{ii} values of 212, 58, and 40 ppm as observed in Figure 4A,B. Four of the nitrogen sites in gramicidin are in the indole rings of the tryptophan residues. The static chemical shift powder pattern for these will be substantially different from the backbone sites since the polycrystalline amino acid has σ_{ii} values of 151, 105, and 38 ppm (Gall et al., 1982; Cross & Opella, 1983).

While the powder pattern spectra of Figure 4A,B appear almost axially symmetric (η = 0.21), the spectrum in Figure 4C appears at first glance to be an axially asymmetric powder

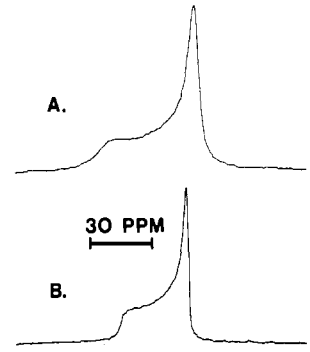

FIGURE 3: ^{31}P NMR spectra of hydrated DMPC bilayers recorded with ^{1}H decoupling. (A) Hydrated DMPC alone, 1000 acquisitions. (B) Bilayers containing gramicidin, 1000 acquisitions. Both samples contained approximately 100 mg of DMPC.

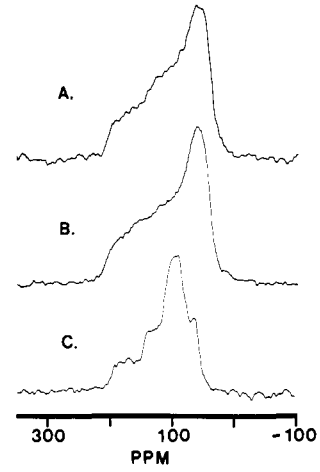

FIGURE 4: ^{15}N NMR powder patterns of uniformly ^{15}N-labeled gramicidin at 27 °C. (A) 8 mg of gramicidin alone, 21 000 acquisitions. (B) Lipidated gramicidin, 30 mg of gramicidin, 2100 acquisitions. (C) Gramicidin lipidated and hydrated with 70% (v/w) distilled water, 22 mg of gramicidin, 8200 acquisitions.

pattern. However, there is not conceivable way to average the static backbone tensor elements to yield σ_{ii} values of 202, 95, and 58 ppm, which furthermore yields an inconsistent σ_{iso} value of 118 ppm. An axially symmetric powder pattern is expected, because this sample has been hydrated and free rotation of the gramicidin channel is anticipated about the the bilayer normal. The rotation about a molecular axis will average the powder pattern depending on the orientation of the chemical shift tensor with respect to the axis of rotation. Consequently, the tensors for the 20 different sites will be averaged differently, resulting in a spectrum with many overlapping axially symmetric powder patterns. Despite this anticipated complexity, the observed spectrum appears to be dominated by two powder patterns having axially symmetric $\sigma_{\parallel}$ and $\sigma_{\perp}$ values of 202 and 58 ppm, respectively, for one powder pattern and 146 and 86 ppm, respectively, for the other. To identify just two powder patterns is an oversimplification, but it will be useful to consider two classes of backbone sites. The intensity of the highly averaged powder pattern is more than could be accounted for by the indole nitrogens.

The temperature dependence of the powder pattern spectra (Figure 5) of the lipidated and hydrated gramicidin sample further supports the contention of channel rotation above and during the lipid phase transition temperature range. At 7 °C, the powder pattern is virtually superimposable with the lipidated but unhydrated powder pattern (Figure 4B). Spectra obtained at 27 and 37 °C are very similar in that they clearly show the two different classes of backbone sites. The low-

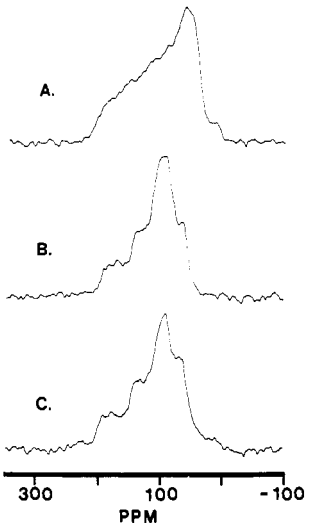

FIGURE 5: Temperature dependence of the ^{15}N powder pattern spectra of uniformly ^{15}N-labeled gramicidin in hydrated DMPC bilayers. (A) 6 °C, 20 mg of gramicidin, 2300 acquisitions. (B) 27 °C, as in Figure 4C. (C) 37 °C, 20 mg of gramicidin, 6400 acquisitions.

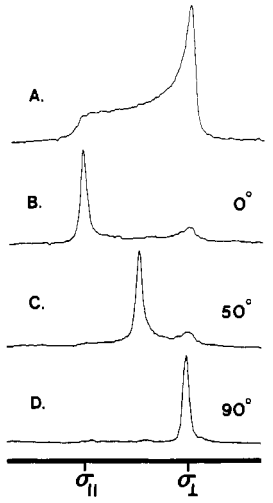

FIGURE 6: ^{31}P NMR spectra of hydrated DMPC bilayers containing gramicidin. (A) As in Figure 3B. (B–D) Spectra of samples oriented as indicated by the approximate angle of the bilayer normal with respect to the magnetic field. The oriented samples represent 300 acquisitions obtained on a sample containing 40 mg of DMPC.

temperature spectrum strongly indicates that the motional averaging of the powder patterns observed at higher temperatures is a result entirely of the global rotational motion of gramicidin in the lipid bilayer. It is reasonable to expect that the local motions of the peptide backbone would not be sensitive to the lipid phase transition temperature, while the overall rotational motion is highly sensitive to this transition.

Figure 6 shows the ^{31}P NMR spectra of both unoriented and oriented samples of gramicidin A in a hydrated lipid bilayer. The uniformity of the sample orientation is described by the mosaic spread, θ, a measure of the variation in the orientation of the bilayer normal with respect to the mean orientation of the bilayer normal. Here, the asymmetry in the width at half-height for the 0° orientation spectrum can be used to estimate a maximum value for θ of ±3°. This estimate is based on the assumption that the downfield half-width of the resonance results from processes other than mosaic spread such as T_2 relaxation, while the upfield half-width will also have contributions from a range of frequencies caused by the mosaic spread. Utilizing the angular dependence of the frequencies for an axially symmetric powder pattern, ($3\cos^2\theta - 1)/2$, a maximum range of θ can be determined.

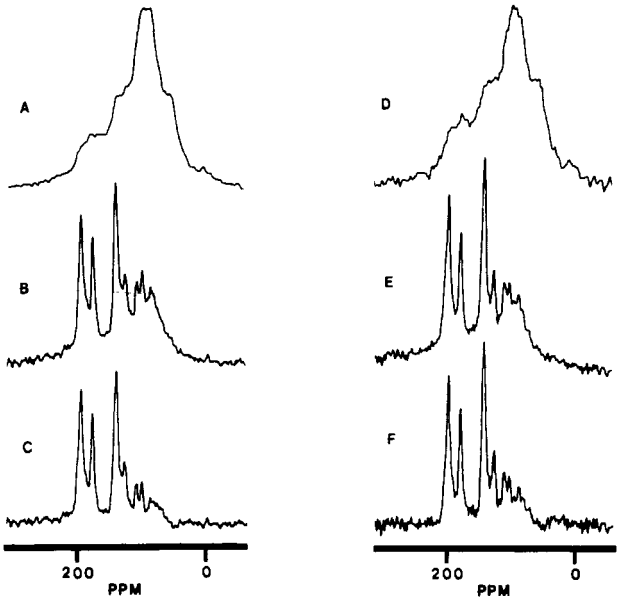

FIGURE 7: ^{15}N NMR spectra of hydrated DMPC bilayers containing gramicidin. (A–C) represent spectra of samples that have not been incubated at high temperature, while (D–F) are spectra of samples incubated at high temperature. (A) and (D) are powder pattern spectra of unoriented samples. (B) and (E) are spectra of oriented samples, and (C) and (F) are the "oriented spectra" [(B) and (E), respectively] minus the powder pattern intensity [(A) and (D), respectively]. (A) 50 mg of gramicidin, 4900 acquisitions; (B) 14 mg of gramicidin, 12 200 acquisitions; (D) sample heated to 68 °C for 7 h, 25 mg of gramicidin, 4600 acquisitions; (E) sample heated to 60 °C for 3 h, 14 mg of gramicidin, 4400 acquisitions.

While the mosaic spread indicates a very high degree of order in these samples, there is an underlying powder pattern intensity in all of the spectra of the oriented samples, most clearly evident in the ubiquitous $\sigma_{\perp}$ resonance. This presumably arises from sample that has seeped beyond the bounds of the glass plates which fit with less than 0.2-mm tolerance within the sample tube. However, this seepage is not a continuing process, because the intensity ratio seen in Figure 6B is stable for months whether the sample is maintained at 45, 30, or 4 °C. Also, the line width of the oriented resonance is stable, indicating that there is no deterioration of the mosaic spread over extensive periods of time.

The narrow resonances observed in Figure 6C,D demonstrate very clearly the axial rotation of the lipid molecules about an axis parallel with the bilayer normal. Furthermore, there is not significant time dependence for these spectra. The sample orientation used for obtaining the spectrum of Figure 6C was maintained for several hours in the magnetic field without seeing any increase in intensity at $\sigma_{\parallel}$. This means that the interaction between the magnetic field and the diamagntic anisotropy of the bilayer system is insignificant, resulting in no magnetic orientation (Seelig et al., 1985; van Echteld et al., 1982). Under a variety of different sample conditions, magnetic orientation takes place, and the spectrum in Figure 6A would be replaced by a distorted powder pattern in which the intensity at $\sigma_{\parallel}$ is substantially increased.

Figure 7 shows a comparison of the ^{15}N spectra of gramicidin in a lipid bilayer for which the samples used for spectra D–F have been heat treated and those for spectra A–C have not. Neither the unoriented powder patterns nor the spectra of oriented samples show any reproducible changes upon heat treatment. The same underlying powder pattern that was noted with the ^{31}P spectra is present in the ^{15}N spectra of B and E. Furthermore, at higher temperature (>60 °C) and

longer periods of time (>3 h), the amount of powder pattern does increase when the sample viscosity lowers, and it might be anticipated that the gramicidin and lipid could drain from between the glass plates. The spectra in C and F result from subtracting this powder pattern intensity, A and D, from the spectra of the oriented samples, B and E, respectively. The sharp resonances in the oriented spectra are an indication of a very small mosaic spread. The resonance at 180 ppm which arises from at least two labeled sites is just 4 ppm wide while the powder pattern for these sites is approximately 114 ppm wide. An analysis of the intensities is made difficult by a number of experimental considerations. As an example, these are cross-polarization spectra, and consequently, it can be anticipated that a variation in the dipolar interaction for the different ^{15}N sites will result in a variation in the cross-polarization efficiency. Despite these concerns, the resonances at 115 and 104 ppm probably result from single sites while most of the intensity in spectra C and F is in the resonances at 198, 180, and 146 ppm. Integration and knowing that there are 20 nitrogen sites in gramicidin provide that these multiresonance peaks are derived from four, two, and six nitrogens, respectively.

Discussion

The characterization experiments have shown that the gramicidin–lipid samples are indeed lipid bilayer preparations with the gramicidin having a conformation consistent with that expected for the channel conformation. The ^{31}P NMR powder pattern spectra of Figure 3 unambiguously document the bilayer phase for the lipid preparations with and without gramicidin. The reduction in the ^{31}P CSA upon incorporation of gramicidin into the lipid bilayers indicates an intimate and uniform association between the lipid and gramicidin. The DSC traces show that the gramicidin is incorporated into the bilayer by the dramatic decrease in the enthalpy of the phase transition. The ^{15}N NMR spectra of the oriented samples show that the gramicidin is oriented by the lipid bilayer. When the bilayer is not oriented (^{31}P powder pattern), neither is the gramicidin (^{15}N powder pattern), and when the bilayer is oriented (^{31}P "oriented resonance"), so is the gramicidin (^{15}N "oriented resonances"). Spectra of a single site labeled gramicidin, [Ala$_3$-^{15}N]gramicidin, show just a single resonance (Fields et al., unpublished results), indicating that our sample preparations result in a uniform conformation of the gramicidin. Upon hydration of the gramicidin–lipid samples, substantial motional averaging takes place, resulting in ^{15}N powder patterns which are consistent with axial symmetry. Moreover, all of the chemical shifts of the "oriented resonances" appear to be associated with the $\sigma_\parallel$ components in the powder pattern spectra, providing evidence that free rotation of the gramicidin molecule takes place about a unique axis parallel with the bilayer normal. This result is consistent with the gramicidin channel conformation.

The ^{15}N NMR spectra of oriented samples remain unaffected by storage for months at temperatures of 45, 30, or 4 °C. The chemical shifts and relative intensities of the peaks do not change as a function of time. This time independence of our spectra is in contrast to the time-dependent ^{205}Tl chemical shift for samples of gramicidin and DMPC prepared as vesicles for solution NMR studies by Shungu et al. (1986). By heating their samples at 60 °C for 12 h, they ensured complete conversion to the channel state. The ^{15}N spectra of Figure 7 show no time dependence on incubating the samples at high temperature. Within the limitations of the spectral resolution and sensitivity, increasing the temperature to 68 °C and increasing the incubation time to 12 h also do not have any effect on the frequency or the relative intensity of the "oriented resonances". The CD spectrum under such circumstances is that which is routinely considered indicative of the channel conformation. Furthermore, separate samples have been prepared and studied (results not shown here) using a different molar ratio (1:15 gramicidin:DMPC ratio) and a different organic solvent (chloroform instead of methanol) for the cosolubilization of the gramicidin and DMPC. Within the spectral resolution and sensitivity limitations, the spectra of these samples all show the same frequencies and relative intensities for the "oriented resonances". From all of the spectroscopic data described here, the gramicidin in the samples used for this study has a uniform conformation in the lipid bilayer with a unique motional axis consistent with the channel conformation.

The interpretation of the chemical shift for the "oriented resonances" is complicated. In the rigid limit, the precise orientation of the chemical shift (CSA) tensor with respect to the molecular symmetry axis (MSA) frame is not completely defined. With limited experience (Harbison et al., 1984; Cross & Opella, 1985) for determining the orientation of the ^{15}N CSA frame in the peptide linkage, σ_{11} is well-defined as the normal to the peptide plane that is parallel with the x axis of the MSA frame. However, σ_{22} and σ_{33} orientations appear to be more susceptible to environmental factors. In the single-crystal study of a dipeptide (Harbison et al., 1984), these latter CSA tensor elements are rotated 20° with respect to the y and z axes of the MSA frame. In the oriented protein study (Cross & Opella, 1985), the rotation of the axes was only 3°. Whether crystal packing interactions or "protein packing" interactions are more significant in distorting the CSA orientation from an in vacuo orientation state is unknown. The result of such difficulties is that it is still reasonable as a first approximation to assume that the MSA frame and the CSA axis frame are collinear (Nall et al., 1981; Cross et al., 1983).

Given the collinearity of the MSA and CSA axis frames and the rigid powder pattern tensor elements (Figure 4), the tensor elements for a site in gramicidin undergoing rotational motion can be calculated. A maximum width for the motionally averaged powder pattern occurs when the σ_{33} or z axis (also the axis of the N–H bond) is parallel with the motional axis. For such a situation, the σ_{33} axis becomes $\sigma_\parallel$, and σ_{11} and σ_{22} become averaged to $\sigma_\perp$. Intensity at the maximal $\sigma_\parallel$ frequency is not observed when spectra of gramicidin are recorded under conditions when rotation about the channel axis is expected (lipidated, hydrated, and at or above the phase transition temperature). The observed powder patterns directly reflect the orientation of the ^{15}N site with respect to the motional axis, provided that local motions of the peptide backbone are insignificant as suggested by the temperature dependence of the ^{15}N powder pattern spectra. The motionally averaged powder patterns for deuteriated sites in the backbone of gramicidin were recently interpreted by Bloom and colleagues as showing that the N–D primarily made an angle of 16° with respect to the bilayer normal, while two other groups of resonances were indicative of average orientations of 25° and 35° (Datema et al., 1986).

Here, instead of interpreting powder pattern discontinuities, oriented samples have been prepared resulting in improved sensitivity and resolution. The three most prominent ^{15}N resonance envelopes have chemical shifts of 198, 180, and 146 ppm. Despite having to assume collinearity of the axis frames and free rotation about the bilayer normal, the calculated orientations of the N–H bonds with respect to the field for

these resonances are 15°, 25°, and 39°, in very close agreement with the deuterium study noted above. These results also seem to be in agreement with the preliminary results of Davis (1986), who studied the deuteriated sites in a magnetically oriented lyotropic liquid-crystalline preparation of gramicidin. However, Davis did not attempt to interpret the spectra quantitatively.

In the rigid limit, the Urry model (1971) for the channel conformation predicts two different orientations for the backbone ^{15}N sites. The N–H bonds make an angle of either 15° or 21° with respect to the channel axis. The 39° orientation which accounts for a high percentage of the "oriented intensity" is not predicted by the model. Datema et al. (1986) proposed that the intensity from terminal, non-hydrogen-bonded peptide linkages in gramicidin monomers accounted for the intensity at 35 °C. For this publication, the samples are characterized by a wide range of spectroscopic measurements all of which indicate the presence of the dimerized channel conformation and still the intensity at an orientation greater than 30° persists. While, in part, the Urry model for the gramicidin channel conformation has been supported by the findings reported here, the results indicate an inconsistency with the structurally uniform pore of this model taken in its rigid limit. Experiments to further document the presence of the channel state in our samples are under way as are studies with specific site ^{15}N-labeled gramicidins which will soon identify all of the resonances in the spectra of uniformly labeled gramicidin. Furthermore, the possibility of variations in local mobility throughout the channel pore is being investigated with these specific site labeled samples.

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