DYNAMICS OF THE INTRINSIC MEMBRANE POLYPEPTIDE GRAMICIDIN A IN PHOSPHOLIPID BILAYERS

A Solid-state ^{13}C Nuclear Magnetic Resonance Study

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A series of polypeptides has been prepared that possesses the same primary sequence as gramicidin A yet includes ^{13}C labels at the C_o position of valine-7, or glycine-2, or alanine-3, or at the C_α and C_o position of glycine-2. These polypeptides have been codispersed with hydrated perdeuterated dimyristoylphosphatidylcholine (DMPC) and studied by solid-state, cross polarization ^{13}C nuclear magnetic resonance (NMR). The combination of NMR spectra derived from the ^{13}C nuclei in each of these molecules has been used to relate the proposed $\pi^{6.3}_{L\ D}$ structure of gramicidin A (1) with simple models for the motion undergone by this peptide in a phospholipid bilayer.

RESULTS

Fig. 1 shows the ^{13}C NMR spectrum derived from a 9 mg sample of the valine-labeled gramicidin A dispersed in perdeuterated DMPC. The labeled amino acids, enriched to 90%, were purchased from KOR Isotopes (Cambridge, MA) and Mercke Sharpe and Dohme (Montreal, Canada). The labeled polypeptides were dispersed in per-

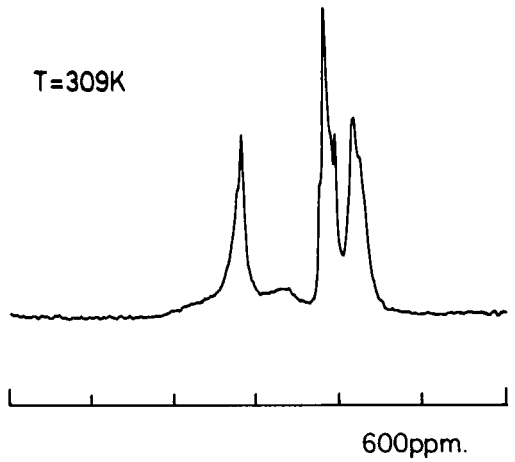

FIGURE 1 Carbon-13 cross-polarization NMR spectrum of valine-labeled gramicidin A in perdeuterated DMPC at a molar ratio of 1:15. The linebroading was 100 Hz, the sweep width 62 kHz, and the number of scans 20,000. The repetition delay was 2 s and the contact time under the Hartmann-Hahn condition 1 ms.

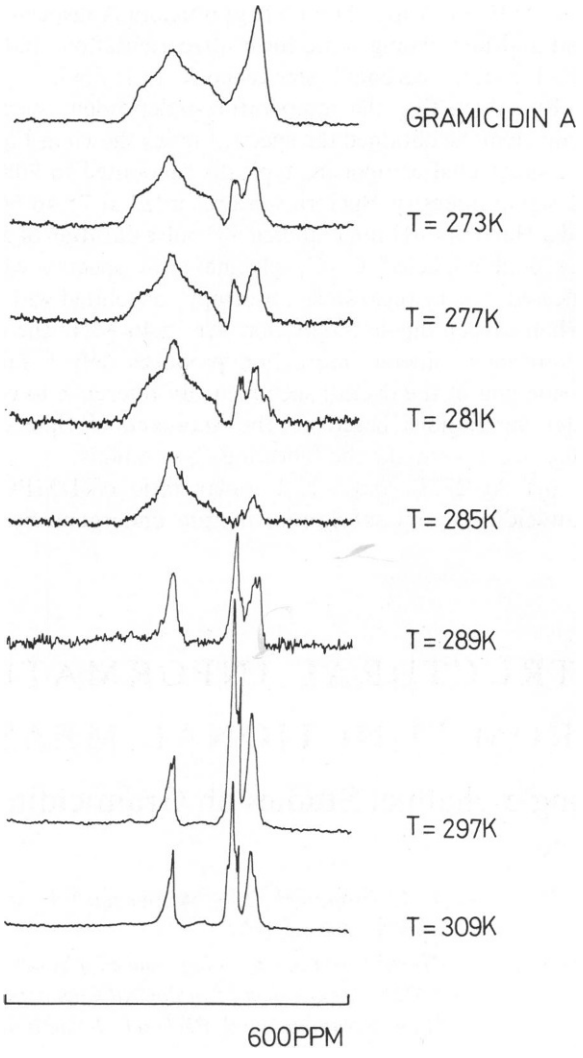

FIGURE 2 Difference spectra derived by subtracting the temperature-independent component from spectra obtained at the temperatures shown. The linebroadening was 50 Hz, the sweep width, on acquisition, 62 kHz, and the number of scans typically 30,000. The repetition delay was 25 and the contact time under the Hartmann-Hahn condition 1 ms at low temperatures to 2 ms for the higher-temperature studies.

deuterated phospholipid by hydrating lyophilized powders formed from benzene-methanol solutions of lipid and gramicidin A in the molar ratio 15:1. The carbonyl region of the resonance was studied over a range of temperatures spanning the phase transition temperature of a hydrated lipid dispersion. The use of perdeuterated DMPC in combination with cross polarization minimizes the contribution of the natural-abundance ^{13}C signal to the resultant spectrum. Parallel studies were performed using a Hahn echo sequence.

There were three distinct spectral components. They were interpreted as arising from: (*a*) aggregated or undispersed gramicidin A, which results in an essentially rigid lattice powder pattern and is independent of temperature from 273K to 310K; (*b*) mobile gramicidin A dispersed in lipid and undergoing some form of reorientation; and (*c*) signal from the carbonyl ester carbons of the lipid.

By subtracting the temperature-independent spectral component we obtained the spectral series shown in Fig. 2. The subtracted component typically amounted to 20% of the signal intensity. Spectra were recorded at 75.46 MHz and a Hartmann-Hahn matched 90° pulse duration of 5 μs. The double-labeled C_o–C_α glycine gave spectra which reflected the chemical shift anisotropy convoluted with the carbon-carbon dipole interaction. At 75.46 MHz the carbon-nitrogen dipolar interaction produces only a minor broadening of the overall spectrum. By reference to computer simulations based on the approach of Spiess, in reference 2, we make the following observations:

(*a*) At 273K and ~15:1 molar ratio of DMPC to gramicidin A any substantial motion undergone by the backbone of the polypeptide occurs on a timescale which is very much longer than 100 μs.

(*b*) At temperatures between 280K and the phase transition temperature of 289K the gramicidin A undergoes random reorientations about an axis close to the long axis of the helix. The rate of this reorientation progressively increases over this temperaure interval and is fast on the 100 μs timescale before reaching 289K.

(*c*) At temperatures well above the phase transition (310K) both the lipid and the gramicidin A undergo rapid reorientation about the bilayer normal.

(*d*) Using the orientation of the shielding tensor obtained from glycine-glycine (3), in which σ_{22} is approximately parallel to the carbonyl bond, the experimental spectra are consistent with a random fluctuation of the azimuthal angle about an axis parallel to both the bilayer normal and the long axis of the peptide helix. These observations are consistent with any conformation in which the carbonyl C = 0 bond is close to the direction of the reorientation axis. This geometry is found in the $\pi_{LD}^{6.3}$ helix.

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STRUCTURAL INFORMATION FROM FUNCTIONAL MEASUREMENTS

Single-channel Studies on Gramicidin Analogues

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The linear gramicidins form dimeric transmembrane channels permeable to monovalent cations and H_2O (for a recent review see 1). The three-dimensional structure of the membrane channel is known. One can therefore reasonably hope to understand the molecular determinants of channel conductance, lifetime, and ion selectivity by making defined changes in the sequence and measuring the resultant changes in channel function. A fundamental

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