Constant helical pitch of the gramicidin channel in phospholipid bilayers

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ABSTRACT X-ray diffraction has been applied in measuring the helical pitch of the gramicidin channel in oriented bilayers of dilauroylphosphatidylcholine (DLPC) and dimyristoylphosphatidylcholine (DMPC) at a polypeptide concentration of 9.1 mol %. The diffraction data show the helical pitch of gramicidin to be 4.7 ± 0.2 Å in both gel and liquid-crystalline phase bilayers, with and without monovalent cations. In addition, the width of the reflection due to the pitch of the helical gramicidin channel is consistent with a five turn helix.

INTRODUCTION

The bacterium *Bacillus brevis* produces a family of linear pentadecapeptide antibiotics known as gramicidins. In biological and model membranes, these gramicidins dimerize to form highly selective monovalent cation channels which seem to be impermeable to anions and divalent cations (1–3). In 1971, Urry proposed that the functional structure of gramicidin in synthetic and biological membranes is a transmembrane channel formed by the head-to-head dimerization of two left-handed gramicidin helices (4, 5). Since then, numerous experiments have been carried out to characterize the proposed channel's physical properties.

X-ray crystallographic studies have shown that a Cs^+-gramicidin complex crystallized from methanol forms a left-handed helical coil with a pitch of 10.4–11.2 Å and two cation binding sites separated by ≈5 Å (6–9). We now know that this helical coil is comprised of two left-handed polypeptide chains arranged as antiparallel β double helices and is commonly referred to as the "pore" conformer (6, 7). It has also been shown that binding of monovalent cations causes the length of the helix to decrease from ≈32 to 26 Å. However, the pore corresponds to the minor conducting form in lipid membrane preparations and can be distinguished quite readily from the channel conformer (predominant form in membranes) by circular dichroism (CD) (10).

In 1979, Weinstein et al. (11) proposed that gramicidin A in phospholipid vesicles forms an NH_2-terminal to NH_2-terminal helical dimer on the basis of ^{13}C and ^{19}F nuclear magnetic resonance (NMR) data. Recently, a two-dimensional ^1H NMR study of the Na^+ complex of gramicidin in detergent micelles has revealed gramicidin forming a head-to-head right-handed helix (12), and from whose data we have calculated an average helical pitch of 4.6 Å.

Here we report x-ray studies which show the helical pitch of gramicidin to be 4.7 ± 0.2 Å in both gel and liquid-crystalline bilayers of DLPC and DMPC, with and without monovalent cations.

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MATERIALS AND METHODS

Gramicidin D (a natural mixture of gramicidins A, B, and C which differ only in the eleventh amino acid of the pentadecapeptide) was purchased from Sigma Chemical Co. (St. Louis, MO). L-α-Dilauroyl phosphatidylcholine (DLPC) and L-α-Dimyristoyl phosphatidylcholine (DMPC) were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). The gramicidin and lipids were used as supplied. HEPES buffer and sodium chloride were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI), whereas thallium fluoride was purchased from K and K Laboratories (Cleveland, OH).

DLPC or DMPC, was codissolved with 9.1 mol % of gramicidin D in benzene:methanol, ≈95:5 (*v*/*v*) and lyophilized. The mixture was hydrated using 18 MΩ cm water to which an appropriate amount of HEPES buffer and salt (NaCl or TlF) were added. The concentration of buffer at 100% RH was ≈0.02 M and had a pH of 7.0. The sample was subsequently dried at 70°C (≈12 h) with the resultant powder mixture oriented as previously described (13). Using a similar methodology for the preparation of gramicidin and DLPC bilayers in a molar ratio of 1:10 (codissolved in benzene), Olah et al. using CD on oriented multilayers have shown that gramicidin is present in the channel conformation (14).

X-rays were produced using a Cu sealed x-ray tube in the line mode and a Philips generator (Model: PW1320/00) operating at approximately 1,000 W. Monochromation of the Cu K_α line was achieved by a combination nickel filter and nickel coated Franks mirror. Diffraction patterns were recorded using a B-OED-50S linear position sensitive detector (M. Braun, GmbH, Munich, Germany) and appropriate timing electronics (Tennelec, Oak Ridge, TN). The detector was pressurized (11.5 bar) using 95% Ar and 5% CH_4 and the cathode (beryllium window) held at a potential of 3.5 kV. The sample-to-detector distance was 83.8 mm and the detector calibration was checked by using potassium hydrogen pthalate as a reference.

0006-3495/92/03/827/04 $2.00

RESULTS AND DISCUSSION

Fig. 1 shows x-ray diffraction patterns of oriented bilayers of DMPC + 9.1 mol % Na^+-gramicidin (gramicidin: Na^+, 1:1) at 0% and 100% relative humidity (RH) and a temperature of 35°C. At 0% RH (Fig. 1 *b*) the bilayers are present in the lamellar gel phase having a repeat- or *d*-spacing of 50.8 Å. The broad reflection centered at about channel number 660 is consistent with a five-turn helix of pitch 4.7 ± 0.2 Å. The uncertainty in the pitch of the helix is due to (*a*) the difficulty in finding the absolute center of the reflection due to the pitch of the helix and (*b*) the nonlinearity of the detector. Although at 100% RH (Fig. 1 *a*) the bilayers undergo a gel to liquid crystalline phase transition with a corresponding 2.8-Å decrease in *d*-spacing, the helical pitch remains unaltered. This is consistent with Krasne et al., who in 1971 observed no change in the gramicidin channel conductance for K^+ ions across bilayers of glyceryl dipalmitate and glyceryl distearate experiencing a liquid crystalline to gel phase transition (15).

Crystallographic studies of native gramicidin crystals found that the binding of Cs^+ or K^+ decreases the coil length from 32 to 26 Å (8, 9). In Fig. 2 we present data for gel phase DMPC bilayers containing 9.1 mol % of either gramicidin (Fig. 2 *a*) or Na^+-gramicidin (Fig. 2 *b*), at 35°C and 20% RH. The *d*-spacing for the two systems is approximately 53 Å. From the figure, it is evident that the pitch of the helix and thus the length of the channel remains unchanged in the presence or absence of the monovalent cation. Similar results were obtained using the Tl^+-gramicidin complex (data not shown). Experiments using CD have found changes in the conformation of gramicidin A in methanol upon binding with Cs^+ which suggest changes in folding motif and handedness of the helix (10, 16). In contrast, the binding of Cs^+ to gramicidin incorporated in DMPC vesicles produced no overall changes in the polypeptide's backbone structure (10). The x-ray diffraction pattern in Fig. 2 *a* also contains a peak centered at channel number 740 corresponding to a *d*-spacing of 4.2 Å which is indicative of gel phase bilayers. The weak equatorial wide-angle reflection in the meridional plane demonstrates the presence of some disorder in the sample (13, 17).

Ion-conductance studies of the gramicidin A channel in lipid bilayers of varying hydrocarbon thickness (26–64 Å) have not produced any detectable change in the single-channel conductance, implying that the backbone structure of the channel is independent of the membrane in which it is incorporated (1, 18). In Fig. 3 we present data for gramicidin doped DMPC (Fig. 3 *a*) and DLPC (Fig. 3 *b*) bilayers having *d*-spacings of 52.8 and 47.2 Å, respectively. Although the DLPC + 9.1 mol % gramicidin bilayers were hydrated to a greater extent than the DMPC bilayers containing gramicidin (40%

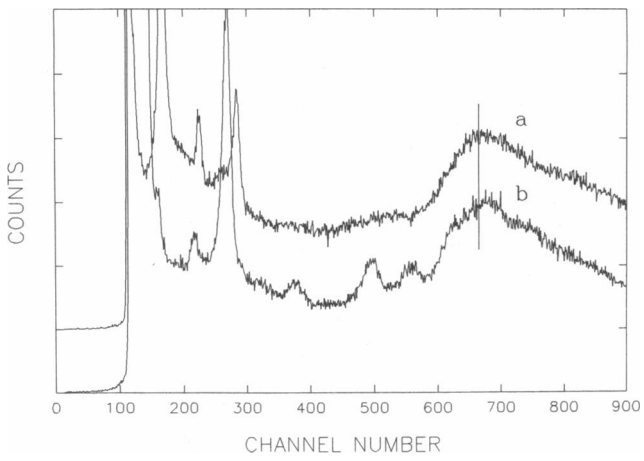

FIGURE 1 X-ray diffraction patterns showing the constancy of the helical pitch of the Na^+-gramicidin complex in DMPC bilayers at (*a*), 100% RH and (*b*), 0% RH and temperature of 35°C. In addition to the diffraction peak due to the pitch of the helix, there exist other peaks which define the lamellar stacking of bilayers, of which some are superimposed on the helical pitch reflection. From the position of these peaks one can obtain the d-spacing of the sample which is inversely related to channel number. For example, the 100% RH sample (*a*), has a fourth order peak centered at channel number 283 corresponding to a *d*-space of 48.0 Å. The 0% RH sample (*b*), has a fourth-order peak centered at channel number 269, equivalent to a 50.8 Å *d*-spacing. Even though the 100% RH sample contains more water, it has a smaller *d*-space as a result of the gel to liquid crystalline phase transition. These diffraction patterns were obtained only after the samples were annealed from 1–2 wk in a 100% RH environment at 20°C. Patterns were collected in 1,250 s.

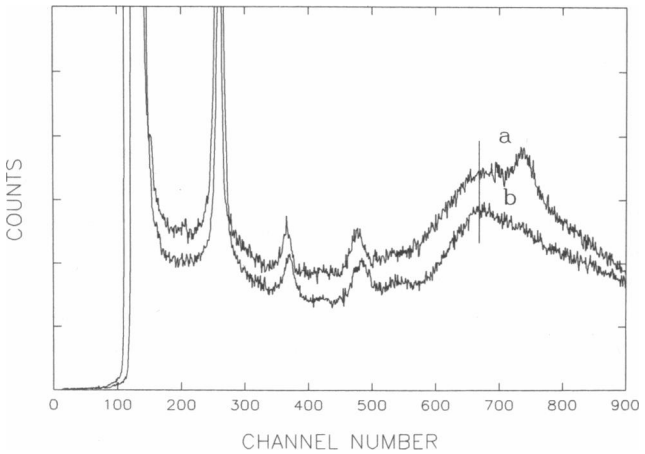

FIGURE 2 X-ray diffraction patterns of (*a*), DMPC + 9.1 mol % gramicidin bilayers and (*b*), DMPC + 9.1 mol % Na^+-gramicidin bilayers at 35°C and ≈20% RH.

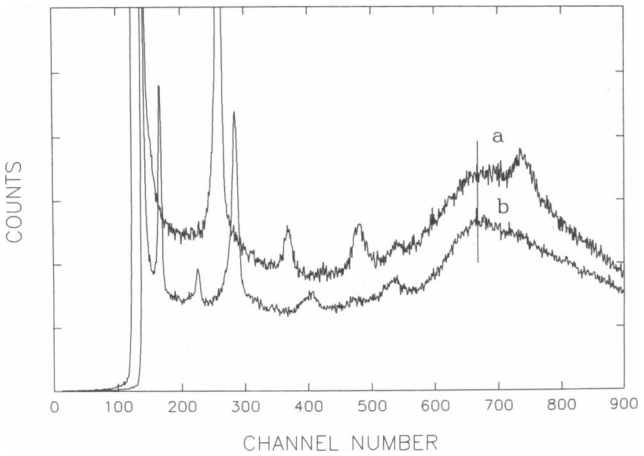

FIGURE 3 X-ray diffraction patterns of (*a*), DMPC + 9.1 mol % gramicidin at 35°C and (*b*), DLPC + 9.1 mol % gramicidin at 20°C.

RH vs 10% RH), they (DLPC) possessed a *d*-spacing which was smaller by 5.6 Å, attributable for the most part to their shorter hydrocarbon chains. Despite the difference in bilayer thickness between the two lipid bilayers, the pitch of the gramicidin helix remained constant with the diffraction peak due to the helix centered at about channel 660 for the two different membranes.

Using two-dimensional proton NMR, Arseniev et al. have proposed a right-handed helical dimer of gramicidin in sodium dodecyl-d_{25} sulfate micelles (12). From their data we calculated a pitch of 4.6 ± 0.3 Å for the gramicidin channel. We have also calculated the pitch of the gramicidin channel for the right-handed Urry model (19) to be 4.9 ± 0.3 Å and the relaxed right-handed Urry model (20) to be 4.9 ± 0.1 Å. The relaxed Urry model was obtained by an 80-ps molecular dynamics simulation in which water molecules were incorporated inside the channel, whereas lipid molecules were accounted for by constraints imposed on the motions of the polypeptide (20). For each of the models, the pitch was determined by unwrapping the helix and calculating the slope of the backbone atoms that lay on the resulting straight line; the uncertainty in the pitch is therefore a measure of the uncertainty in the slope of the line.

CONCLUSIONS

Here we have presented direct evidence of the magnitude (4.7 ± 0.2 Å) and constancy of the helical pitch of gramicidin in gel and liquid crystalline bilayers of varying hydrophobic thickness using x-ray diffraction. From this we may conclude that the average backbone structure of the channel is independent of the membrane type in which it is situated. The peak width predicted for a finite five-turn helix is consistent with the experimental observation. Further verification for some of the present results may be forthcoming now that highly ordered cocrystals of gramicidin A and phospholipid have been produced (21).

The authors would like to thank Professors Barry M. Millman and Martha G. Shepherd for useful discussions.

This research was supported by a grant from the National Sciences and Engineering Research Council of Canada.

Received for publication 14 June 1991 and in final 25 October 1991.

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