ORIENTATION OF GRAMICIDIN A TRANSMEMBRANE CHANNEL

Infrared Dichroism Study of Gramicidin in Vesicles

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ABSTRACT Polarized infrared spectroscopy has been used to investigate the orientation of gramicidin A incorporated in dimyristoylphosphatidylcholine liposomes. Dichroism measurements of the major lipid (C = O ester, PO_2^-, CH_2) and peptide (amide A, I, II) bands were performed on liposomes (with or without gramicidin) oriented by air-drying. The mean orientation of the lipid groups and of the π_{LD} helix chain in the gramicidin has been determined. It can be inferred from infrared frequencies of gramicidin that the dominant conformation of the peptide in liposomes cannot be identified to the antiparallel double-helical dimer found in organic solution. No shift in lipid frequencies was observed upon incorporation of gramicidin in the liposomes. However, a slight reorganization of the lipid hydrocarbon chains which become oriented more closely to the normal to the bilayer is evidenced by a change in the dichroism of the CH_2 vibrations. The infrared dichroism results of gramicidin imply a perpendicular orientation of the gramicidin transmembrane channel with the π_{LD} helix axis at <15° with respect to the normal to the bilayer.

INTRODUCTION

Gramicidin A is a linear polypeptide antibiotic consisting of 15 alternating L and D amino acids (1); in natural and artificial lipid bilayer membranes it forms ion conductive channels (2–4), each of which is composed of two molecules of gramicidin (3, 5–10). Several structural models have been proposed for the conformation of the dimer gramicidin transmembrane channel (7, 11, 12). Structure-function studies of gramicidin derivatives in lipid bilayers (7, 13–18) and more recently ^{13}C and ^{19}F NMR studies of gramicidin in phosphatidylcholine liposomes (19, 20) both argue that the major conformation of the gramicidin channel in lipid vesicles consists of two π_{LD} helices joined at their NH_2 terminals, as originally proposed by Urry et al. (7, 11).

Here, we report the first infrared (IR)[1] spectroscopic investigation of gramicidin A incorporated in dimyristoylphosphatidylcholine (DMPC) vesicles. Recently, polarized IR spectroscopy has been shown to be a useful technique for studying the orientation of intrinsic proteins in biological membranes (21–24). We have used it to obtain direct information on the molecular structure and on the orientation of gramicidin in DMPC vesicles. By measuring the IR dichroism of the amide and lipid absorption bands, we have estimated that the gramicidin transmembrane channel is tilted at <15° from the normal to the bilayer plane. Our data also indicate that the lipid hydrocarbon chains become oriented more closely to the normal to the bilayer in the presence of gramicidin.

[1] *Abbreviations used in this paper:* DMPC, dimyristoylphosphatidylcholine, DPPC, dipalmitoylphosphatidylcholine; IR, infrared.

MATERIALS AND METHODS

Preparation and Orientation of Liposomes

DMPC and gramicidin A were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification; they were dissolved in $CHCl_3$ at a molar ratio of 30 DMPC per gramicidin and evaporated to dryness by vacuum pumping at 30°C. The dry film was resuspended in 1.5 ml H_2O and incubated at 30°C for 30 min to hydrate the lipid. The sample was then sonicated for 10 min at 35°C. After sonication, the suspension was centrifuged at 2,000 *g* for 30 min. The lipid suspension (100 μl) was then applied to a CaF_2 disk. Stacking of the vesicles parallel to the disk plane was achieved by air-drying at room temperature (24).

Spectroscopic Measurements

IR spectra were recorded on a Perkin-Elmer 180 double beam spectrometer (Perkin-Elmer Corp., Norwalk, CT) equipped with a common beam Perkin-Elmer wire grid polarizer and linked to a Hewlett-Packard 9825 A computer (Hewlett-Packard Co., Palo Alto, CA). IR dichroism was measured with an angle of incidence of 60°. IR light was linearly polarized either perpendicular to the normal to the disk plane (to record $A_\perp$) or parallel to the plane of incidence (to record $A_{//}$), as described earlier (24). When recording $A_\perp$, the transitions parallel to the disk surface will absorb strongly, while the transitions oriented along the normal will not absorb. A blank CaF_2 disk was mounted with an identical geometry in the reference beam.

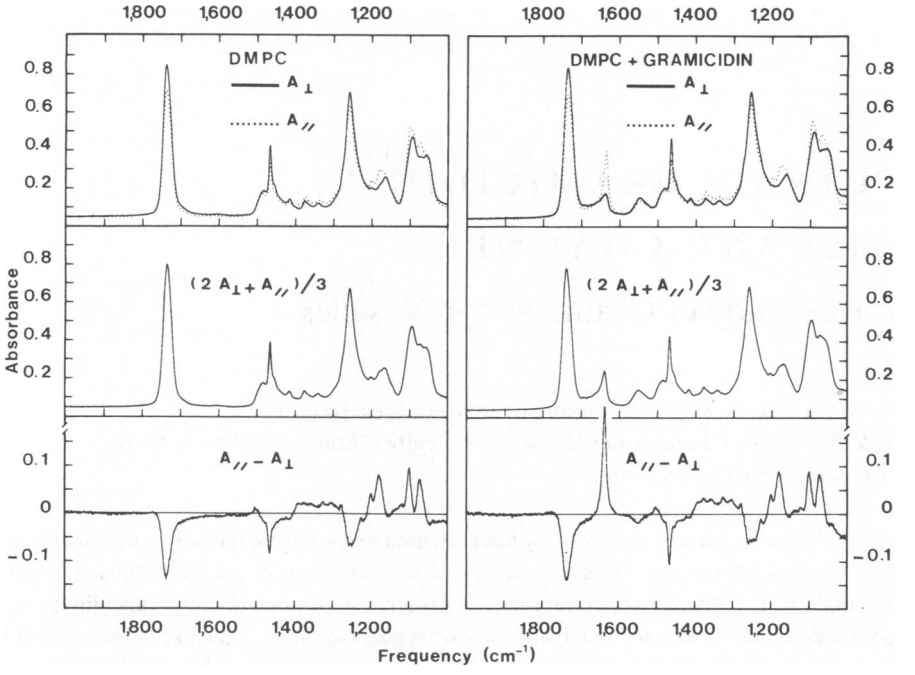

FIGURE 1 Polarized infrared spectra ($A_{//}$, $A_\perp$), absorbance spectrum [$A = (2A_\perp + A_{//})/3$] and difference spectrum ($A_{//} - A_\perp$) of air-dried dimyristoylphosphatidylcholine (DMPC) liposomes from 2,000 to 1,000 cm^{-1}. The angle of incidence was 60° and the polarization direction was perpendicular (—) to the normal to the disk ($A_\perp$) or parallel (· · ·) to the plane of incidence ($A_{//}$).

Data Analysis

The model used to estimate the orientation of the transition moments in our air-dried samples of liposomes (±gramicidin) has been described (24). Briefly, the distribution of a set of transition moments corresponding to a given absorption band can be characterized by an order parameter $S = (3\cos^2\phi - 1)/2$, where ϕ is the angle between the transition moment of the absorption peak and the normal to the disk. As described (24), S can be estimated from the band absorbance for parallel and perpendicular polarized light. The dichroic ratio $D = A_{//}/A_\perp$ measured at a tilt angle i is related to S by applying the relation (25, 26):

$$D = \frac{3S}{1 - S}\frac{\sin^2 i}{n^2} + 1 \qquad (1)$$

where n, the refractive index of the layer of air-dried lipids was taken as 1.55 (27). Then, from the measured dichroic ratio, we can calculate the mean orientation of the transition vector.

RESULTS AND DISCUSSION

Assignments of IR Absorption Bands

Fig. 1 displays typical IR spectra from 2,000 to 1,000 cm^{-1} for pure DMPC liposomes and DMPC liposomes containing gramicidin A in a 30/1 lipid-peptide mole ratio. Polarized IR spectra ($A_{//}$ and $A_\perp$), computed absorbance [$A = (2A_\perp + A_{//})/3$] and difference spectra ($A_{//} - A_\perp$) are shown in this figure. Many of the major peaks can be assigned to specific group vibrations, on the basis of previous IR studies of lipids and proteins (28–30). In particular, for pure DMPC, the ester carbonyl stretching vibration at 1,738 cm^{-1}, the CH_2 bending mode of hydrocarbon chains at 1,467 cm^{-1}, and the PO_2^- antisymmetric stretching transition at 1,258 cm^{-1} are easily identified (Fig. 1). Other assignments are listed in Table I. Note that the frequencies of the PO_2^- symmetric and antisymmetric stretching vibrations depend on hydration and intermolecular interaction: a shift to lower frequencies is observed after hydrogen bonding with water molecules (31, 32). In our experimental conditions, the absorption bands due to the PO_2^- group were observed at 1,258 and 1,095 cm^{-1}, which is comparable to the frequencies reported by Fookson and Wallach (32) for various phosphatidylcholines in a dry state.

For the gramicidin peptide, Figs. 1 and 2 show the amide I (C = O stretching), II (N—H bending) and A (N—H stretching) bands located at 1,638; 1,547; and 3,280 cm^{-1}; respectively.

IR Dichroism of DMPC Liposomes

Several lipid bands exhibit strong dichroism with their absorption more intense in the $A_\perp$ spectrum [for example, ν(C = O) ester, $\delta(CH_2)$, $\nu_{as}(PO_2^-)$, $\nu_s(CH_2)$ and $\nu_{as}(CH_2)$] when the light is polarized perpendicular to the normal to the disk plane than in the $A_{//}$ spectrum (Fig. 1). An opposite situation is observed for other vibrations, such as the CH_2 wagging mode of the hydrocarbon chains at 1,202 cm^{-1} and $\nu_s(PO_2^-)$ at 1,095 cm^{-1}. Therefore, the observed dichroism for the polar head groups of DMPC qualitatively indicates that the mean orientation of the PO_2^- and C = O stretching transition moments is rather parallel to the bilayer plane while the hydrocarbon chains are preferentially oriented along the normal to the bilayer plane.

TABLE I
ASSIGNMENT OF MAIN PEAKS IN IR SPECTRA OF AIR-DRIED DMPC LIPOSOMES WITH OR WITHOUT INCORPORATED GRAMICIDIN A

Frequency (cm^{-1})		Assignment	Dichroism
3,280	Amide A	N—H stretching of peptide groups	$A_{//} > A_{\perp}$
2,955	ν_{as} (CH_3)	Antisymmetric stretching of hydrocarbon methyl groups	$A_{\perp} > A_{//}$
2,918	ν_{as} (CH_2)	Antisymmetric stretching of hydrocarbon methylene groups	$A_{\perp} > A_{//}$
2,849	ν_s (CH_2)	Symmetric stretching of hydrocarbon methylene groups	$A_{\perp} > A_{//}$
1,738	ν (C═O)	C═O stretching of fatty acid ester groups	$A_{\perp} > A_{//}$
1,638	Amide I	C═O double bond stretching of peptide groups	$A_{//} > A_{\perp}$
1,547	Amide II	N—H bending of peptide groups	$A_{\perp} > A_{//}$
1,467	δ (CH_2)	CH_2 bending of hydrocarbon chains	$A_{\perp} > A_{//}$
1,455	δ (CH_2) + δ (CH_3)	CH_2 bending + CH_3 asymmetric deformation of hydrocarbon chains methyl groups	$A_{\perp} > A_{//}$
1,415	δ (CH_2)	CH_2 deformation	
1,377	δ_s (CH_3)	CH_3 symmetric bending of hydrocarbon chain methyl groups	
1,258	ν_{as} (PO_2^-)	Antisymmetric stretching of PO_2^- group	$A_{\perp} > A_{//}$
1,202	γ_w (CH_2)	CH_2 wagging of hydrocarbon chains	$A_{//} > A_{\perp}$
1,165–1,180	ν (CO)	C—O stretching	$A_{//} > A_{\perp}$
1,095	ν_s (PO_2^-)	Symmetric stretching of PO_2^- group	$A_{//} > A_{\perp}$
1,075–1,060	ν (CO)	C—O stretching	$A_{//} > A_{\perp}$

Measurements of dichroism were performed individually for six lipid vibrational modes: ν(C = O) at 1,738 cm^{-1}; $\delta(CH_2)$ at 1,467 cm^{-1}; $\nu_{as}(PO_2^-)$ at 1,258 cm^{-1}; $\nu_w(CH_2)$ at 1,202 cm^{-1}; $\nu_{as}(CH_2)$ at 2,918 cm^{-1}; and $\nu_s(CH_2)$ at 2,849 cm^{-1}. Table II summarizes the IR dichroism of head groups and chain vibrations of DMPC in oriented vesicles.

First, it is shown that the transition moments of the C = O ester stretching and PO_2^- antisymmetric stretching bands are oriented nearly parallel to one another and deviate by 23° (C = O) and 27° (PO_2^-) with respect to the bilayer plane. These values are in good agreement with those measured by Akutsu et al. (31) by IR dichroism on dry oriented multilayers: these authors calculated that the ν(C = O) ester of dipalmitoylphosphatidylcholine (DPPC) deviates from the lipid film plane by 26° while the P—O bonds orient in the range 0–35° from the film plane.

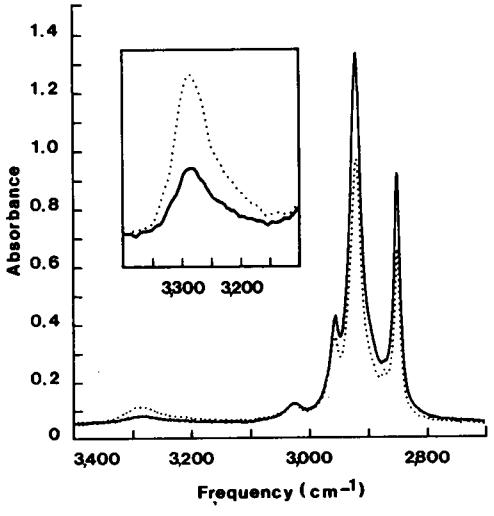

FIGURE 2 CH_2 and amide A region of air-dried DMPC liposomes with gramicidin. *Inset:* expansion 10 of amide A region. $A_{\perp}$ (—); $A_{//}$ (· · ·).

Second, analysis of the polarization of the CH_2 vibrations at 2,918; 2,849; 1,467; and 1,202 cm^{-1} allows calculation of the mean orientation direction of the hydrocarbon chains (perpendicular to the CH_2 plane). As shown in Table II, the hydrocarbon chains of DMPC are tilted from the bilayer normal by ~20°, which is comparable to previous results on DPPC multilayers (19°). It must be

TABLE II
INFLUENCE OF GRAMICIDIN A ON IR PARAMETERS OF AIR-DRIED DMPC LIPOSOMES

Assignment	DMPC		DMPC + Gramicidin A	
	D^*	ϕ	D^*	ϕ‡
ν (C═O)	0.80 ± 0.02	67°	0.83 ± 0.02	64°
ν_{as} (PO_2^-)	0.85 ± 0.02	63°	0.85 ± 0.02	63°
δ (CH_2)	0.78 ± 0.02	69°	0.74 ± 0.02	74°
γ_w (CH_2)	7 ± 1	17°	>15	<12°
ν_{as} (CH_2)	0.77 ± 0.02	70°	0.70 ± 0.02	82°
ν_s (CH_2)	0.75 ± 0.02	72°	0.70 ± 0.02	82°

*D, dichroic ratio (average obtained from four different air-dried samples).
‡ϕ, angle between the transition moment of a given vibration and the normal to the disk plane.

noticed that a small (or even negligible) chain tilt was also detected in oriented multilayers of other lipids by using different techniques such as IR (33), attenuated total reflection IR (27), spin labeling (34), neutron diffraction (35), and x-ray diffraction (36–38) analysis; in particular, it was reported that in the DMPC crystal, the hydrocarbon chains tilt from the bilayer normal by 12° (36).

Incorporation of gramicidin A into DMPC liposomes does not influence significantly the IR frequencies of the lipids (Fig. 1) nor the dichroism of the polar groups (Tables I and II). However, Table II shows that the dichroic ratios of $\delta(CH_2)$, $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ vibrations are all slightly decreased while that of the $\gamma_w(CH_2)$ is greatly increased, as is also seen in Fig. 3. For this last vibration, the absorption nearly vanishes in the $A_\perp$ spectrum of DMPC with incorporated gramicidin (Fig. 3). It therefore seems that the tilt angle between the hydrocarbon chains of DMPC and the normal to the bilayer is decreased (from 20° to ~10°) in the presence of gramicidin. A similar effect was observed by Fringeli et al. (39) after incorporation of the antibiotic alamethicin in dry DPPC bilayers.

Conformation of Gramicidin A in DMPC Liposomes

Different models for the structure of the dimeric gramicidin channel have already been proposed:

(*a*) A head-to-head (NH_2 terminal to NH_2 terminal) association of two π_{LD} helical monomers proposed by Urry (7, 11). Dimerization occurs via six intermolecular hydrogen bonds with antiparallel hydrogen bonding.

(*b*) An antiparallel double helix in which all the hydrogen bonds are intermolecular. This structure isolated from nonpolar solvents, has been discussed by Veatch et al. (12).

In proteins, correlations between amide band frequencies and molecular conformation exist and have been well resolved for α-helix, β-sheets, and unordered structures (30, 40–43). However, only limited IR data are available for π_{LD} helix (44, 45). The amide I frequency of gramicidin in liposomes at 1,638 cm^{-1} (Fig. 1) could indicate some β-sheet structure (the π_{LD} helix is essentially a rolled parallel β-structure in its intramolecular hydrogen bonding) but the position of the amide II band at 1,547 cm^{-1} is out of the range characteristic for β or antiparallel pleated sheets (~1,530 cm^{-1}) (40); furthermore, the absence of any shoulder at 1,685 cm^{-1} usually associated with antiparallel hydrogen bonding (40, 41, 43) seems to exclude antiparallelism of the polypeptide chain, and therefore is not consistent with the double helix model of Veatch et al. (12). In contrast, the IR spectrum of gramicidin dried from a $CHCl_3$ solution shows the amide I and amide II peaks at 1,632 and 1,535 cm^{-1}, respectively and a shoulder at 1,685 cm^{-1} (spectrum not shown) which indicates an antiparallel structure and is in agreement with previous IR and Raman studies in nonpolar solvents (12, 46, 47). This demonstrates that the dominant conformation of the antibiotic is different in the liposomes and in the solid state or in organic solution.

Furthermore, recent NMR studies (19, 20) of gramicidin in DMPC vesicles (same experimental conditions as ours) argue strongly for a NH_2 terminal to NH_2 terminal arrangement of the gramicidin dimer in vesicles. We therefore suggest that the observed amide I and amide II frequencies of gramicidin in liposomes at 1,638 and 1,547 cm^{-1}, respectively, may be characteristic of the NH_2 terminal to NH_2 terminal helical dimer, i.e., the model of Urry (7). It should be, however, noted that the calculated amide I frequency (at 1,655 cm^{-1}) for a gramicidin dimer with a head-to-head π_{LD} helix (48) appears quite far from that observed for gramicidin in liposomes (at 1,638 cm^{-1}). This discrepancy suggests that refinements in the calculations of IR frequencies for π_{LD} helix dimers are probably required. It is also possible that the contribution of other conformations and/or interactions between the antibiotic and the lipids have to be taken into account.

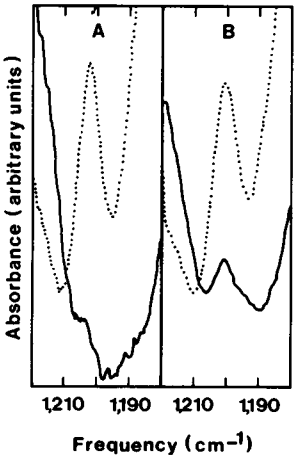

FIGURE 3 Comparison of CH_2 wagging region (expansion 20) of dried DMPC liposomes with (*A*) or without gramicidin (*B*); $A_\perp$ (—); $A_{//}$ (· · ·).

Orientation of Gramicidin Peptide Chain in DMPC Liposomes

Figs. 1 and 2 show that gramicidin in DMPC liposomes gives a high dichroism in the amide I and amide A bands with their absorption more intense in the $A_{//}$ spectrum than in the $A_\perp$ spectrum; the opposite situation is found for the amide II band. Qualitatively, this argues for an alignment of the C = O and N—H bonds approximately parallel to the normal to the disk plane, suggesting a preferential orientation of the peptide groups with respect to the bilayer.

In a π_{LD} helix, the backbone C=O groups are positioned rather parallel or antiparallel to the helix axis (7) in contrast to the usual α-helix in which the C=O groups

form a parallel array. Note that parallel and antiparallel bonds are not distinguable by IR dichroism. For the C=O stretching amide I and the N—H stretching amide A vibrations, the transition moment lies approximately parallel to the C=O or the N—H bond respectively; in contrast, for the N—H bending amide II mode, it lies rather perpendicular to the N—H bond (49). Therefore, it appears that the π_{LD} helices in the gramicidin dimer are preferentially oriented along the normal to the bilayer with the amide I transition dipole moment oriented predominantly parallel to the hydrocarbon chains of DMPC and the transition moment of amide II directed perpendicular to the lipid chains.

In order to calculate the tilt angle ϕ_π of the helix relative to the bilayer normal, we must take into account the angle ϕ_M between the transition moments of the amide vibrations and the helix axis. In a π_{LD} helix, the two peptide units of the LD repeat do not have the same orientation with respect to the helical axis. Investigations of poly-γ-benzyl-D-L-glutamate (which is regarded as a stereochemical model of gramicidin A) show that this polypeptide can adopt a great variety of conformations (44, 45) such as single-stranded $\pi_{LD}^{4.4}$ helix and double-stranded π_{LD} π_{LD} helices (with 5.6, 7.2, 9.0, and 10.8 amino acid residues per helical turn of the helix). For each of these structures, angles between amide transition moments and the helical axis were calculated (44, 45). However, no data are available for the $\pi_{LD}^{6.3}$ helix of gramicidin proposed by Urry (7, 11). In these conditions, we use the transition moments data available for the $\pi_{LD}^{4.4}$ helix to calculate ϕ_π. Heitz et al. (44, 45) reported that within each LD repeat of a $\pi_{LD}^{4.4}$ helix, the individual amide I vibration vectors make an angle ϕ_{MI} of about 21°8 and 23°4 with respect to the helical axis. In the following analysis, we calculated the average order parameter $\overline{S_{MI}} = \frac{1}{2}[S(21°8) + S(23°4)] = 0.778$ leading to the average angle $\overline{\phi_{MI}} = 22°6$; for amide II, $\phi_{MII} = 81°0$ and 46°0 (44, 45) and $\overline{\phi_{MII}} = 59°8$; for amide A, $\phi_{MA} = 13°3$ and 30°3 (44, 45) and $\overline{\phi_{MA}} = 23°1$. Finally, the distribution S_π of the gramicidin π_{LD} helix axis with respect to the bilayer normal can be calculated from the following equation (24):

$$S = S_\pi \times S_M, \qquad (2)$$

where S is estimated from the amide band dichroic ratio (Eq. 1) and S_M is the average order parameter for the amide transition moment.

The IR dichroism results of gramicidin are summarized in Table III; it is shown that the dichroic ratio of the amide I band leads to $\phi_\pi = 15°$, whereas from the dichroic ratio of the amide A band, a value $\phi_\pi = 24°$ is obtained. It therefore appears that gramicidin polypeptide chain is approximately perpendicular to the bilayer plane and nearly parallel to the DMPC hydrocarbon chains. Notice that a ϕ_π value cannot be calculated from the amide II data ($S_\pi > 1$). Table III shows that the accuracy on the dichroism measurement is worse for the amide II band than for the other bands. We calculate that the amide II dichroic ratio would have to be increased to 0.91 to give a ϕ_π value of 15°. Nevertheless, the measured dichroic ratio values for the amide II band are always <0.88. Accordingly, the possibility of some inconsistancy in the choice of ϕ_M angles ought to be taken into account. For example, an average ϕ_{MII} value of 71° (instead of 59°8) does lead to $\phi_\pi = 15°$. It must be also emphasized that no ϕ_π value can be obtained from our IR data (for any amide band) if we use the transition moments data given for an antiparallel double $\pi_{LD}^{5.6}$ helix model (44, 45). This again confirms that the conformation of gramicidin in liposomes cannot be identified to the model of Veatch et al. (12). Further calculations for a $\pi_{LD}^{6.3}$ helix dimer may be required in order to improve our results on the orientation of gramicidin in liposomes.

TABLE III
ESTIMATION OF D AND ϕ_π FROM GRAMICIDIN IR DICHROISM DATA

IR Parameters	Amide I	Amide II	Amide A
D*	3.10 ± 0.20	0.78 ± 0.10	2.25 ± 0.20
ϕ_π‡	$15°\ ^{+3°}_{-1°}$	§	$24°\ ^{+3°}_{-2°}$

*Average obtained from four different air-dried samples.
‡ϕ_π calculated assuming perfect orientation of the liposomes parallel to the disk surface.
§See Results and Discussion for explanation. Otherwise as in Table II.

The tilt angles for the lipid and peptide chains were calculated, assuming that the liposome membranes are oriented perfectly parallel to the disk surface. If the mosaic spread of our samples is >0°, i.e., if the extent of orientation of the vesicles is not perfect, then the hydrocarbon chains and the helix axis would even be oriented more closely parallel to the normal to the bilayer plane. Stamatoff et al. (37) have measured by x-ray diffraction the tilt of DPPC chains in phospholipid bilayers and observed different mosaic spreads for various samples but each was <15°. If we assume this 15° mosaic spread value, the DMPC lipid chains and the π_{LD} helix axis are calculated to be essentially perpendicular to the membrane plane.

The IR dichroism study presented here shows that addition of gramicidin to DMPC vesicles does not influence the polar regions of the lipid but seems to induce a slight reorganization of the acyl chains: the tilt angle between the hydrocarbon chains of the lipids and the normal to the bilayer (about 20° for DMPC alone) is decreased by almost 10° upon interaction with gramicidin (Table II). This result appears consistent with the hydrophobic character of gramicidin. Different types of lipid-peptide interactions were recently suggested by IR and Raman spectroscopic investigations, by monitoring the lipid thermal phase transition (50, 51) or the IR dichroism of lipid bands (39, 52). In particular, it has been shown

that melittin can influence both the polar and apolar regions of phosphatides arranged in multilayers (52), while alamethicin incorporated in dry DPPC bilayers only changes the hydrocarbon chain ordering (39).

The transmembrane orientation of the gramicidin channel has previously been inferred from functional and structural studies (7, 13–20). However, a tilt value for the axis of the channel had never been estimated. Our data quantify the transmembrane orientation of the gramicidin channel at $<15°$ with respect to the normal to the bilayer.

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REFERENCES

1. Sarges, R., and B. Witkop. 1965. Gramicidin A. V. The structure of valine- and isoleucine-gramicidin A. *J. Am. Chem. Soc.* 87:2011–2020.
2. Chappell, J. B., and A. R. Crofts. 1965. Gramicidin and ion transport in isolated liver mitochondria. *Biochem. J.* 95:393–402.
3. Hladky, S. B., and D. A. Haydon. 1970. Discreteness of conductance change in bimolecular lipid membranes in the presence of certain antibiotics. *Nature (Lond.).* 225:451–453.
4. Krasne, S., G. Eisenman, and G. Szabo. 1971. Freezing and melting of lipid bilayers and the mode of action of nonactin, valinomycin and gramicidin. *Science (Wash., D.C.).* 174:412–415.
5. Tosteson, D. C., T. E. Andreoli, M. Tieffenberg, and P. Cook. 1968. The effect of macrocyclic compounds on cation transport in sheep red cells and thin and thick lipid membranes. *J. Gen. Physiol.* 51:373–384.
6. Goodhall, M. C. 1970. Structural effects in the action of antibiotics on the ion permeability of lipid bilayers. III. Gramicidins "A" and "S" and lipid specificity. *Biochim. Biophys. Acta.* 219:471–478.
7. Urry, D. W., M. C. Goodhall, J. D. Glickson, and D. F. Mayers. 1971. The gramicidin A transmembrane channel: characteristics of head-to-head dimerized $\pi_{(LD)}$ helices. *Proc. Natl. Acad. Sci. U.S.A.* 68:1907–1911.
8. Bamberg, E., and P. Laüger. 1973. Channel formation kinetics of gramicidin A in lipid bilayer membranes. *J. Membr. Biol.* 11:177–194.
9. Veatch, W. R., R. Mathies, M. Eisenberg, and L. Stryer. 1975. Simultaneous fluorescence and conductance studies of planar bilayer membranes containing a highly active and fluorescent analogue of gramicidin A. *J. Mol. Biol.* 99:75–92.
10. Veatch, W. R., and L. Stryer. 1977. The dimeric nature of the gramicidin A transmembrane channel: conductance and fluorescence energy transfer studies of hybrid channels. *J. Mol. Biol.* 113:89–102.
11. Urry, D. W. 1971. The gramicidin A transmembrane channel: a proposed $\pi_{(L,D)}$ helix. *Proc. Natl. Acad. Sci. U.S.A.* 68:672–676.
12. Veatch, W. R., E. T. Fossel, and E. R. Blout. 1974. The conformation of gramicidin A. *Biochemistry.* 13:5249–5256.
13. Bamberg, E., and K. Janko. 1977. The action of a carbon-suboxide dimerized gramicidin A on lipid bilayer membranes. *Biochim. Biophys. Acta.* 465:486–499.
14. Apell, H. J., H. Bamberg, H. Alpes, and P. Laüger. 1977. Formation of ion channels by a negatively charged analog of gramicidin A. *J. Membr. Biol.* 31:171–188.
15. Bamberg, E., H. J. Apell, and H. Alpes. 1977. Structure of the gramicidin A channel: discrimination between the $\pi_{L,D}$ and the β helix by electrical measurements with lipid bilayer membranes. *Proc. Natl. Acad. Sci. U.S.A.* 74:2402–2406.
16. Bradley, R. J., D. W. Urry, K. Okamoto, and R. Rapaka. 1978. Channel structures of gramicidin: characterization of succinyl derivatives. *Science (Wash., D.C.).* 200:435–437.
17. Szabo, G., and D. W. Urry. 1979. *N*-acetyl gramicidin: single-channel properties and implications for channel structure. *Science (Wash., D.C.).* 203:55–57.
18. Morrow, J. S., W. R. Veatch, and L. Stryer. 1979. Transmembrane channel activity of gramicidin A analogs: effects of modification and deletion of the amino-terminal residue. *J. Mol. Biol.* 132:733–738.
19. Weinstein, S., B. A. Wallace, E. R. Blout, J. S. Morrow, and W. Veatch. Conformation of gramicidin A channel in phospholipid vesicles: a ^{13}C and ^{19}F nuclear magnetic resonance study. *Proc. Natl. Acad. Sci. U.S.A.* 76:4230–4234.
20. Weinstein, S., B. A. Wallace, J. S. Morrow, and W. R. Veatch. 1980. Conformation of the gramicidin A transmembrane channel: a ^{13}C nuclear magnetic resonance study of ^{13}C-enriched gramicidin in phosphatidylcholine vesicles. *J. Mol. Biol.* 143:1–19.
21. Rothschild, K. J., and N. A. Clark. 1979. Polarized infrared spectroscopy of oriented purple membrane. *Biophys. J.* 25:473–488.
22. Michel-Villaz, M., H. R. Saibil, and M. Chabre. 1979. Orientation of rhodopsin α-helices in retinal rod outer segment membranes studied by infrared linear dichroism. *Proc. Natl. Acad. Sci. U.S.A.* 76:4405–4408.
23. Rothschild, K. J., R. Sanches, T. L. Hsiao, and N. A. Clark. 1980. A spectroscopic study of rhodopsin alpha-helix orientation. *Biophys. J.* 31:53–64.
24. Nabedryk, E., and J. Breton. 1981. Orientation of intrinsic proteins in photosynthetic membranes. Polarized infrared spectroscopy of chloroplasts and chromatophores. *Biochim. Biophys. Acta.* 635:515–524.
25. Morita, S., and T. Miyasaki. 1971. Dichroism of bacteriochlorophyll in cells of the photosynthetic bacterium *Rhodopseudomonas palustris*. *Biochim. Biophys. Acta.* 245:151–159.
26. Breton, J., M. Michel-Villaz, and G. Paillotin. 1973. Orientation of pigments and structural proteins in the photosynthetic membrane of spinach chloroplasts: a linear dichroism study. *Biochim. Biophys. Acta.* 314:42–56.
27. Fringeli, U.P. 1977. The structure of lipids and proteins studied by attenuated total reflection (ATR) infrared spectroscopy. II. Oriented layers of a homologous series: phosphatidylethanolamine to phosphatidylcholine. *Z. Naturforsch.* 32*c*: 20.45.
28. Chapman, D., R.M. Williams, and B.D. Ladbrooke. 1967. Physical studies of phospholipids. VI. Thermotropic and lyotropic mesomorphism of some 1, 2-diacyl-phosphatidylcholines (lecithins). *Chem. Phys. Lipids.* 1:445–475.
29. Wallach, D.F.H., S.P. Verma, and J. Fookson. 1979. Application of laser raman and infrared spectroscopy to the analysis of membrane structure. *Biochim. Biophys. Acta.* 559:153–208.
30. Susi, H. 1969. Infrared spectra of biological macromolecules and related systems. *In* Structure and Stability of Biological Macromolecules. S.N. Timasheff and G.P. Fasman, editors. Marcel Dekker, Inc., New York. 575–663.
31. Akutsu, H., M. Ikematsu, and Y. Kyogoku. 1981. Molecular structure and interaction of dipalmitoyl phosphatidylcholine in multilayers. Comparative study with phosphatidylethanolamine. *Chem. Phys. Lipids.* 28:149–158.
32. Fookson, J.E., and D.F.H. Wallach. 1978. Structural differences among phosphatidylcholine, phosphatidylethanolamine, and mixed phosphatidylcholine/phosphatidylethanolamine multilayers: an infrared absorption study. *Arch. Biochem. Biophys.* 189:195–204.
33. Akutsu, H., Y. Kyogoku, H. Nakahara, and K. Fukuda. 1975.

Conformational analysis of phosphatidylethanolamine in multilayers by infrared dichroism. *Chem. Phys. Lipids.* 15:222–242.

34. Birrell, G.B., and O.H. Griffith. 1976. Angle of tilt and domain structure in dipalmitoyl phosphatidylcholine multilayers. *Arch. Biochem. Biophys.* 172:455–462.
35. Büldt, G., H. U. Gally, A. Seelig, J. Seelig, and G. Zaccai. 1978. Nutron diffraction studies on selectively deuterated phospholipid bilayers. *Nature Lond.* 271:182–184.
36. Pearson, R.H., and I. Pascher. 1979. The molecular structure of lecithin dihydrate. *Nature Lond.* 281:499–501.
37. Stamatoff, J.B., W.F. Graddick, L. Powers, and D.E. Moncton. 1979. Direct observation of the hydrocarbon chain tilt angle in phospholipid bilayers. *Biophys. J.* 25:253–262.
38. McIntosh, T.J. 1980. Differences in hydrocarbon chain tilt between hydrated phosphatidylethanolamine and phosphatidylcholine bilayers. A molecular packing model. *Biophys. J.* 29:237–246.
39. Fringeli, U.P., and M. Fringeli. 1979. Pore formation in lipid membranes by alamethicin. *Proc. Natl. Acad. Sci. U.S.A.* 76:3852–3856.
40. Miyazawa, T., and E.R. Blout. 1961. The infrared spectra of polypeptides in various conformations: amide I and II bands. *J. Am. Chem. Soc.* 83:712–719.
41. Chirgadze, YU.N., and N.A. Neskaya. 1976. Infrared spectra and resonance interaction of amide I vibration of the antiparallel-chain pleated sheet. *Biopolymers.* 15:607–625.
42. Nevskaya, N.A., and YU.N. Chirgadze. 1976. Infrared spectra and resonance interactions of amide I and II vibrations of α-helix. *Biopolymers.* 15:637–648.
43. Eckert, K., R. Grosse, J. Malur, and K.R.H. Repke. 1977. Calculation and use of protein-derived conformation-related spectra for the estimate of the secondary structure of proteins from their infrared spectra. *Biopolymers.* 16:2549–2563.
44. Heitz, F., B. Lotz, and G. Spach. 1975. α_{DL} and π_{DL} helices of alternating poly-δ-benzyl-D-L-glutamate. *J. Mol. Biol.* 92:1–13.
45. Lotz, B., F. Colonna-Cesari, F. Heitz, and G. Spach. 1976. A family of double helices of alternating poly (γ-benzyl-D-L-glutamate), a stereochemical model for gramicidin A. *J. Mol. Biol.* 106:915–942.
46. Rothschild, K.J., and H.E. Stanley. 1974. Raman spectroscopic investigation of gramicidin A′ conformations. *Science (Wash., D.C.).* 185:616–618.
47. Iqbal, Z., and E. Weidekamm. 1980. Raman and infrared study of gramicidin A conformations. *Arch. Biochem. Biophys.* 202:639–649.
48. Sychev, S.V., N.A. Nevskaya, S.T. Jordanov, E.N. Shephel, A.I. Miroshnikov, and V.T. Ivanov. 1980. The solution conformations of gramicidin A and its analogs. *Bioorg. Chem.* 9:121–151.
49. Miyazawa, T. 1960. Perturbation treatment of the characteristic vibrations of polypeptide chains in various configurations. *J. Am. Chem. Soc.* 32:1647–1652.
50. Verma, S.P., and D.F.H. Wallach. 1976. Effect of melittin on thermotropic lipid state transitions in phosphatidylcholine liposomes. *Biochim. Biophys. Acta.* 426:616–623.
51. Lavialle, F., I.W. Lewin, and C. Mollay. 1980. Interaction of melittin with dimyristoyl phosphatidylcholine liposomes. Evidence for boundary lipid by Raman spectroscopy. *Biochim. Biophys. Acta.* 600:62–71.
52. Verma. S.P., D.F.H. Wallach, and I.C.P. Smith. 1974. The action of melittin on phosphatide multibilayers as studied by infrared dichroism and spin labeling. A model approach to lipid-protein interactions. *Biochim. Biophys. Acta.* 345:129–140.