Comparison of Lipid/Gramicidin Dispersions and Cocrystals by Raman Scattering†

Kurt W. Short,‡,§ B. A. Wallace,‖,⊥ Richard A. Myers,‡,# Stephen P. A. Fodor,‡,○ and A. Keith Dunker*,‡

Biochemistry/Biophysics Program and Department of Chemistry, Washington State University, Pullman, Washington 99164-4630, and Department of Biochemistry and Molecular Biophysics, Columbia University, New York, New York 10032

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ABSTRACT: Gramicidin crystals, dimyristoylphosphatidylcholine (DMPC)/gramicidin dispersions, and DMPC/gramicidin cocrystals were examined by Raman scattering to determine lipid/gramicidin stoichiometries and lipid organization. Calibrations of the choline (716-cm^{-1}) and tryptophan (756-cm^{-1}) peaks indicate that the cocrystals contain two lipids for each gramicidin monomer, a result confirmed by chemical analyses of washed crystals. In dispersions with high lipid/gramicidin ratios (e.g., 25:1), the lipid is ordered but becomes increasingly disordered as the gramicidin content is increased. Paradoxically, the DMPC/gramicidin cocrystals have highly ordered lipids that possibly contain no gauche bonds at all, despite their low lipid/gramicidin ratio. In addition, the polypeptide amide I peak position near 1670 cm^{-1} is found to be independent of the lipid/gramicidin ratio in the complexes and may indicate a β-helix-type secondary structure at all ratios. However, the amide I peak broadens significantly at low lipid/gramicidin ratios and broadens still further in the cocrystals, suggesting that protein–protein interactions may induce band-broadening distortions of the polypeptide structure.

Raman scattering has been used to study lipids (Mendelsohn, 1972; Gaber & Peticolas, 1977), proteins (Yu, 1977; Williams & Dunker, 1981), and lipid/protein complexes (Dunker et al., 1979; Mendelsohn et al., 1981). A particularly useful feature of Raman scattering is its capacity to reveal protein and lipid conformations in solution, dispersions, and crystals (Yu, 1977).

Gramicidin,[1] an ion channel forming polypeptide that contains alternating L- and D-amino acids, has been crystallized from various alcoholic solutions (Koeppe et al., 1978; Wallace, 1983; Kimball & Wallace, 1984). More recently, this polypeptide has been cocrystallized with lipid molecules (Wallace, 1983, 1986). The availability of these crystals will make it possible to determine the detailed structure of the protein and perhaps the lipid as well, if the latter is well organized in the crystal lattice. The goal of this project was to use Raman scattering to compare gramicidin and associated lipids in various states, including DMPC/gramicidin dispersions, gramicidin crystals, and DMPC/gramicidin cocrystals. The feasibility of using Raman scattering to study gramicidin has been demonstrated from previous work (Rothschild & Stanley, 1974, 1975; Chapman et al., 1977; Weidekamm et al., 1977; Susi et al., 1979; Iqbal & Weidekamm, 1980; Naik & Krimm, 1984a,b). Here Raman scattering is used to quantitate the lipid/gramicidin molar ratios and to examine the influence of the lipid and protein components on each other. These studies demonstrate that Raman scattering is an especially useful tool for the study of membrane protein crystals.

In addition to gramicidin, crystals of several other membrane proteins and polypeptides have been prepared recently, including the following: photosynthetic reaction centers (Michel, 1982; Diesenhof et al., 1984); melittin, a detergentlike polypeptide from bee venom (Terwillinger et al., 1982); alamethicin, a voltage-dependent ion channel (Fox & Richards, 1982); bacteriorhdopsin, a light-driven proton pump (Michel & Osterhelt, 1980); porin, a pore-forming protein (Garavito & Rosenbusch, 1980). From our results on the lipid/gramicidin cocrystals, it is suggested that these other membrane protein crystals would be good candidates for Raman scattering experiments.

Materials and Methods

Sample Preparation. For the dispersions, DMPC from Sigma and gramicidin from Boehringer Mannheim were used without further purification. Gramicidin crystals were prepared from ethanolic solution in the presence of DMPC with initial lipid/protein mole ratios of 3:1 (Wallace, 1983, 1986). Formation of crystals took approximately 6 months. Crystals of gramicidin alone were prepared from ethanol by slow solvent evaporation.

For lipid/gramicidin dispersions, DMPC and gramicidin were dried under high vacuum and then cosolublized in 95:5 chloroform/methanol at lipid/gramicidin ratios ranging from 1:1 to 25:1. The solvent was evaporated with a nitrogen stream followed by high vacuum overnight. The samples were hydrated by the addition of glass-distilled water, incubated for

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* Address correspondence to this author.

‡ Washington State University.

§ Present address: Howard Hughes Medical Institute, University of Washington, Seattle, WA.

‖ Columbia University.

⊥ Present address: Department of Chemistry, Rensselaer Polytechnic Institute, Troy, NY.

Present address: Department of Biochemistry, University of Utah, Salt Lake City, UT.

○ Present address: Department of Chemistry, University of California, Berkeley, CA.

[1] Abbreviations: DMPC, dimyristoylphosphatidylcholine; gramicidin, mixture of about 88% gramicidin A, 7% gramicidin B, and 5% gramicidin C; NMR, nuclear magnetic resonance; ESR, electron spin resonance; CD, circular dichroism.

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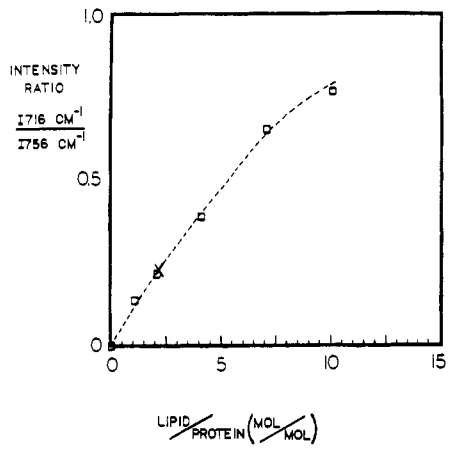

FIGURE 1: Lipid-to-gramicidin ratio by Raman scattering. The intensity ratio I_{715}/I_{758} is plotted vs. the gravimetrically determined lipid-to-protein ratio (□). The intersection of the standard curve by the I_{716}/I_{758} ratio of the cocrystals is indicated (×).

3 h at 68 °C, vortexed to disperse the contents, and incubated for an additional 9 h to ensure that the gramicidin was in the channel form (Urry et al., 1979; Masotti et al., 1980). These samples are homogeneous and are bilayers at high lipid-to-polypeptide ratios (Chapman et al., 1977).

Raman Scattering. Spectra of crystals and dispersions were obtained in sealed capillaries with and without mother liquor at 15 ± 0.5 °C by irradiating at 5145 Å with a Spectra-Physics Model 164 argon laser set at 100 mW. Data were collected on a Ramanor HG-2S double monochrometer with a Spex Digital Photometer at a scan rate of 1 cm^{-1}/s. Repetitive scans were averaged; typically 8–60 scans were used, except for the 25:1 lipid/gramicidin sample for which 180 scans were used to obtain a good signal/noise for the amide I peak. Base-line corrections, smoothing, and water subtractions were carried out as described previously (Williams & Dunker, 1981).

Digitally subtracted difference spectra were calculated from two criteria: (1) Environmentally induced shifts in some peaks lead to positive/negative doublets; subtractions were adjusted to give equal areas for such doublets. (2) Subtractions were adjusted to give an absence of excessive positive or negative peaks in any region of the difference spectrum.

Lipid/Gramicidin Mole Ratio Determined by Raman Scattering. A standard curve using the lipid choline head group stretch near 716 cm^{-1} and the polypeptide tryptophan peak near 758 cm^{-1} was constructed. Calibration of the ratio of the integrated peak intensities I_{716}/I_{758} against the gravimetrically determined molar lipid/polypeptide ratio yielded a determination of the lipid/polypeptide ratio in the cocrystals.

Chemical Estimate of Lipid/Gramicidin Mole Ratio. Individual crystals were washed in cold solvent and then dissolved in methanol. Gramicidin content was estimated from the molar extinction coefficient (ϵ = 22 000) at 286 nm. Lipid content was determined by a modified Fiske and Subbarow (1925) phosphate assay.

Results and Discussion

Lipid/Gramicidin Ratio. Determination of the structure in the cocrystals will be simplified by knowledge of the contents of the unit cell, which requires an accurate estimate of the lipid/polypeptide ratio. The choline head group stretch at 716 cm^{-1} and the tryptophan peak at 758 cm^{-1} provide a means to measure the lipid/polypeptide ratio.

The I_{716}/I_{758} ratio is plotted vs. the gravimetrically determined DMPC/gramicidin ratio (Figure 1). The cocrystal ratio intersects the standard curve at a lipid/gramicidin ratio of 2:1. Chemical determinations on washed cocrystals also give a value of 2:1.

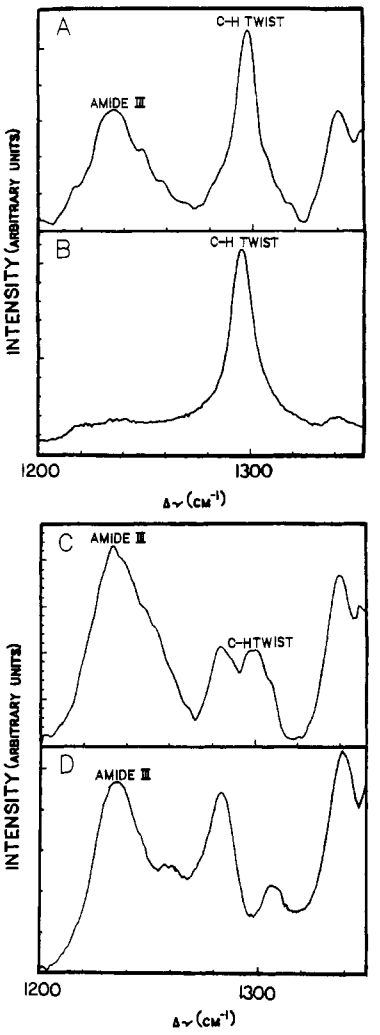

FIGURE 2: Raman spectra of the 1300-cm^{-1} region. Four spectra are compared: (A) DMPC/gramicidin cocrystals; (B) DMPC/gramicidin dispersion, 25:1 lipid-to-polypeptide; (C) DMPC/gramicidin dispersion, 2:1 lipid-to-polypeptide; (D) gramicidin crystals from ethanol.

The chemical approach assumes that washing the cocrystals does not alter the lipid/gramicidin ratio and that the lipid-dependent and polypeptide extinction coefficients are accurate and not affected by the presence of the other component. The chemical method requires distruction of large amounts of valuable, difficult to obtain crystalline material. The Raman method depends on the assumptions that the scattering intensities of tryptophan and choline have the same relative values in the cocrystals and dispersions and that contributions from the noncrystalline portions of the sample (e.g., the mother liquor) are negligible. The principle advantage of the Raman method is that it provides a nondestructive means for estimating the lipid/polypeptide ratio. The consistency of the Raman and chemical determinations increases confidence in the 2:1 lipid/polypeptide ratio obtained.

Lipid Structure. The conformationally sensitive vibration at 1300 cm^{-1} has been attributed to CH_2 twisting vibrations (Mendelsohn, 1972). An increase in molecular motion and/or gauche isomers broadens the 1300-cm^{-1} band and decreases its intensity (Mendelsohn et al., 1975). The cocrystals (Figure 2A) and the 25:1 lipid/gramicidin dispersion (Figure 2B) both have narrow intense bands at 1300 cm^{-1}, indicating a high degree of lipid order. However, the 2:1 dispersion (Figure 2C) shows a broad, low-intensity band in this region, indicative of

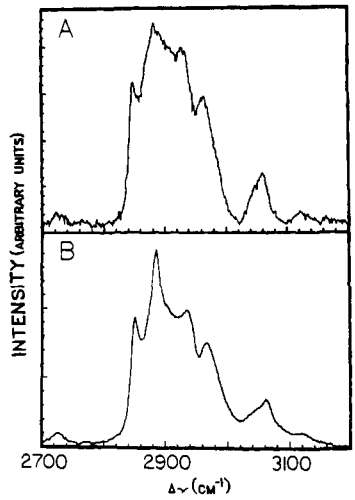

FIGURE 3: Raman spectra of the 2900-cm^{-1} region. Two spectra are compared: (A) DMPC/gramicidin cocrystals; (B) DMPC/gramicidin dispersion, 2:1 lipid-to-polypeptide.

highly disordered hydrocarbon chains.

The methylene symmetric C–H stretch at about 2850 cm^{-1} and the asymmetric stretch at about 2890 cm^{-1} are also sensitive to lipid structure (Gaber & Peticolas, 1977). Near the pretransition temperature of the lipid, the intensity ratio I_{2890}/I_{2850} decreases, representing a loss of lateral order in the lipid. At the main phase transition temperature, the intensity ratio I_{2890}/I_{2850} decreases further, and the 2890-cm^{-1} peak shifts to a value 8–10 cm^{-1} higher, evidently due to "rotamer broadening".

The intensity ratio I_{2890}/I_{2850} is 1.37 for the 2:1 dispersion (Figure 3A), compared to 1.51 for the cocrystals (Figure 3B), indicating that the lipids in the cocrystals are more ordered than those in the dispersions at the same lipid/gramicidin ratio. Contrary to expectations, the 2890-cm^{-1} band of the dispersions does not shift to a higher frequency. It is possible that differences in underlying C–H stretching bands of gramicidin may be responsible for differences between the dispersion and cocrystals. However, we believe that this is unlikely since the dispersion and cocrystals are at the same 2:1 lipid/protein ratio. Thus in dispersions at low lipid/polypeptide ratios, gramicidin seems to cause loss of lateral order of the lipid without causing rotamer broadening.

Results from the 1300-cm^{-1} region and also from the 1100-cm^{-1} region (see below) suggest that the lipid dispersions with low lipid/polypeptide ratios are in a disordered state similar to that found in pure lipid above the phase transition temperature. The lack of a shift of the 2890-cm^{-1} band to a higher frequency would seem to contradict this. One likely explanation is that the lipid is highly disordered but immobilized within the polypeptide matrix. A similar explanation has been evoked previously to explain electron spin resonance (ESR) results (Chapman et al., 1977).

In pure DMPC below its phase-transition temperature, when the lipid hydrocarbon tail groups are in a highly ordered state with a large proportion of trans isomers, the C–C stretches at 1065 and 1130 cm^{-1} are strong and the C–C stretch near 1090 cm^{-1} is weak. Disordering of the lipid tail groups above the phase transition temperature is accompanied by a decrease in the 1065- and 1130-cm^{-1} bands and an increase in a broad peak near 1090 cm^{-1} (Lippert & Peticolas, 1971, 1972; Gaber & Peticolas, 1977).

For the dispersions, decreasing the lipid/gramicidin ratio from 25:1 to 1:1 causes a substantial decrease in the 1065- and 1130-cm^{-1} peaks and a corresponding increase in the peak

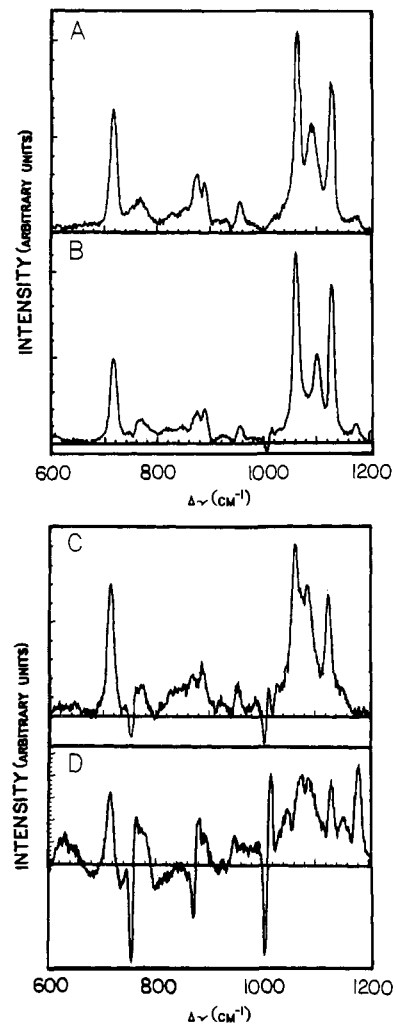

FIGURE 4: Skeletal optical bands near 1100 cm^{-1} for DMPC/gramicidin dispersions. Spectra of the 25:1 (B), 7:1 (C), and 1:1 (D) DMPC/gramicidin dispersions with the gramicidin contributions subtracted out are shown. A spectrum of pure DMPC (A) at 15 °C is also shown for comparison.

near 1090 cm^{-1}. For purposes of comparison, a spectrum of DMPC below its phase-transition temperature is shown (Figure 4A). The gramicidin-induced changes are more clearly evident if the gramicidin contributions are removed by subtraction (Figure 4B–D).

The finding that incorporation of proteins leads to loss of lipid order as measured by Raman scattering has been reported previously for other small, hydrophobic membrane proteins (Dunker et al., 1979; Tarascki & Mendelsohn, 1980; Mendelsohn et al., 1981). Thus, the reduction of lipid order by proteins similar to gramicidin may be a relatively general phenomenon.

In contrast to this trend, the lipids in the cocrystals are highly ordered despite their low 2:1 lipid/gramicidin ratio. Raman spectra of the 2:1 dispersion in the 1050–1150-cm^{-1} region with protein subtraction (Figure 5A) shows a very low degree of lipid order, whereas a comparable spectrum of the cocrystals (Figure 5B) indicates highly ordered lipid. This is in agreement with our interpretation of the 1300-cm^{-1} (Figure 2) and the 2850/2890-cm^{-1} bands (Figure 3).

Several different Raman order parameters have been used to quantitate structural changes of lipids in bilayer environments (Marsh, 1974; Gaber & Peticolas, 1977; Pink et al., 1980, 1981). The 10-state model of Caille et al. (1980) seems to correlate with the experimental results, which are expressed as the order parameter I_{1130}/I_{716} (Pink et al., 1980, 1981). The

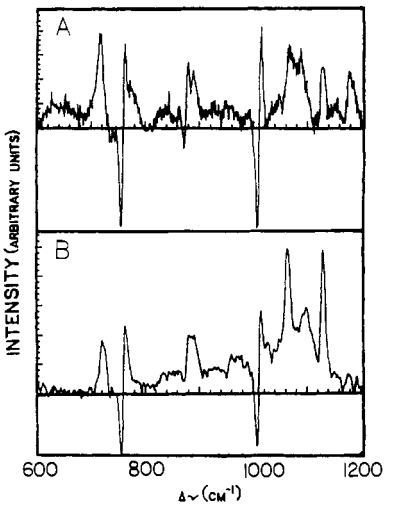

FIGURE 5: Comparison of dispersions and cocrystals. Spectra with the gramicidin component subtracted out are compared for the dispersion at 2:1 lipid-to-polypeptide (A) and the cocrystals (B).

experimental order parameter and the number of gauche bonds from the model are graphed vs. the temperature (Pink et al., 1980). Thus, temperature provides the means to connect the order parameter to the 10-state model. To make this connection for our data, the order parameters were estimated and then adjusted for chain length as described in Pink et al. (1981). Next, the adjusted order parameters were used to estimate the apparent temperature from the curves in Pink et al. (1980). Finally, the apparent temperature was used to estimate the number of gauche bonds from the appropriate curves in Pink et al. (1980).

For our DMPC spectrum collected at 15 °C (Figure 4A), an order parameter of 1.16 was estimated; the chain length correction transforms this value to 0.81 ± 0.04, corresponding to an apparent temperature of 15 to −8 °C, or to 1.4–0.9 gauche bonds/lipid. Note that the temperature we actually used (15 °C) is within the error range of the extrapolated apparent temperature (15 to −8 °C), which provides a control indicating that our data and the results of Pink et al. (1980) are fairly consistent with each other.

From the spectrum of the cocrystals (Figure 5B), an order parameter of 1.49 was estimated, which becomes chain length corrected to 1.04 ± 0.05. This corrected order parameter is indistinguishable from the limiting value of 1.0, which, for pure lipid, is reached at a temperature between −150 and −200 °C, corresponding to lipids with no gauche bonds at all.

Although our analysis suggests that the cocrystals contain lipids with no gauche bonds, two caveats should be noted. First, the order parameter estimation requires subtraction of the gramicidin contribution, but the reference gramicidin spectrum might not match perfectly that in the cocrystals. Second, environmental effects on the Raman scattering intensities could alter the relationship between lipid structure and order parameter in pure lipids as compared to the dispersions and cocrystals. This is an especially significant uncertainty since the 10-state model was developed in a pure lipid system but is being applied here to systems containing high polypeptide concentrations. Yet the similarity of the gramicidin-subtracted lipid spectrum of the cocrystals to that of pure lipid indicates that these two caveats may not seriously alter our conclusions.

There is one other interesting observation here involving the 1100-cm^{-1} region. When the spectra in panels A and B of Figure 4 are compared, it is noted that the band around 1100 cm^{-1} shifts from 1090 cm^{-1} in the pure lipid dispersion (Figure 4A) to 1101 cm^{-1} in the 25:1 dispersion. This band has been assigned to the PO_2^- symmetric stretch and is believed to be sensitive to the solvent environment around the lipid head group (Brown et al., 1973). At the lipid phase trasition this band shifts to a lower frequency, which implies that there is a greater exposure of the head group to the solvent. For the 25:1 dispersion, the 1100-cm^{-1} band is shifted upward in frequency from that of the pure lipid. This implies that the head group is less exposed to the aqueous environment at this DMPC-to-gramicidin ratio.

Lipid Participation in Cocrystals. The formation of gramicidin crystals in the presence of lipid molecules does not ensure that the lipids will be incorporated into the crystal lattice nor that the lipids, if incorporated, will be ordered within the crystal. Lipids, like ordinary solvent, could simply fill spaces in a disorganized way or, even if at discrete positions, could be rotationally or otherwise disordered. However, these studies show the lipid acyl chains to be highly ordered and in a stoichiometric ratio different from the input crystallization mixture. These two observations suggest that the lipids may occupy specific sites in the crystal lattice in an ordered fashion. If so, solving the cocrystal structure will provide a detailed picture of interactions between lipid and an intrinsic membrane polypeptide.

Gramicidin Structure. Two major peptide bands, the amide I (1630–1680 cm^{-1}) and the amide III (1230–1300 cm^{-1}), exhibit strong Raman scattering intensities and have been used extensively for the investigation of protein structure [reviewed in Yu (1977) and Tu (1982)]. Due to the presence of alternating L- and D-amino acids, previous investigations correlating amide I and III band structure with protein conformation are of uncertain value. However, a recent vibrational analysis of the various structural forms of gramcidin has been carried out suggesting that Raman scattering can be used to distinguish the various structural forms of this molecule (Naik & Krimm, 1986).

The amide III bands of the cocrystals (Figure 2A), the 2:1 lipid/gramicidin dispersion (Figure 2C), and the crystals grown from ethanol (Figure 2D) are all near 1235 cm^{-1} with a rather similar overall sideband structure on the high-frequency side. In the dispersions, a second amide III peak is clearly evident near 1280 cm^{-1}. This sideband might also be present in the cocrystals, but the much higher intensity of the lipid peak at about 1300 cm^{-1} overlaps this amide III sideband thus making its presence uncertain. In the ethanolic crystals this band is obscured by an ethanol peak at 1276 cm^{-1}. Both the main band and the sideband are obscured in the 25:1 dispersions because of the high lipid concentration (Figure 2B). The position of the amide III bands is indicative of a β-type-structure (Naik & Krimm, 1986).

Spectra of the amide I region for the dispersions, cocrystals, crystals grown from ethanol, and CsCl crystals grown from ethanol are shown in Figure 6. All of these different samples exhibit amide I peaks near 1670 cm^{-1}. There are, however, distinct differences in the peak shapes. For samples containing lipid, the width of the amide I band decreases as the DMPC/gramicidin ratio increases. Since it would take about eight lipid molecules to surround a gramicidin single-stranded β-helix (Chapman et al., 1977), extensive gramidicin–gramicidin interactions probably occur at the lower lipid/gramicidin ratios (Chapman & Cornell, 1976). These interpolypeptide interactions evidently cause band-broadening disortions of the amide I peak.

For samples in the absence of lipid (Figure 6D,E), the differences in amide I band shape may once again be due to

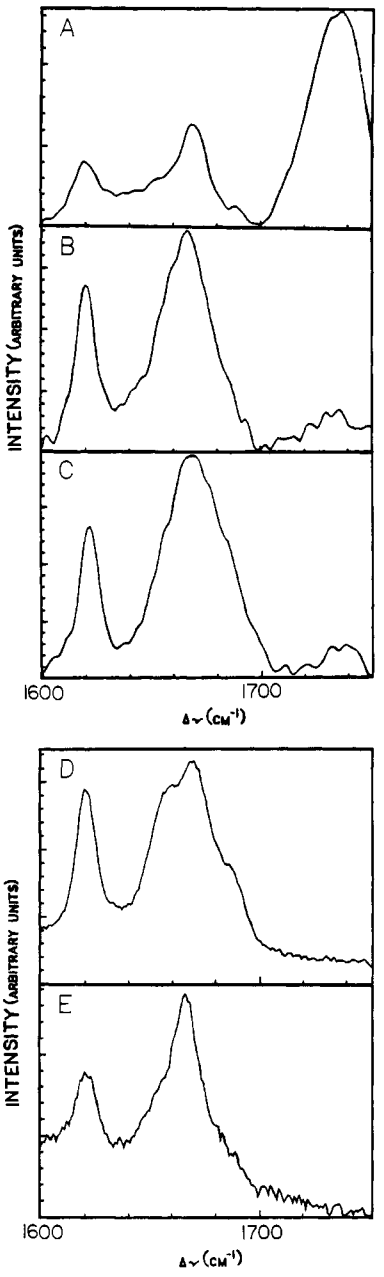

FIGURE 6: Raman spectra of amide I region. Five spectra are compared: (A) DMPC/gramicidin dispersion, 25:1 lipid-to-polypeptide; (B) DMPC/gramicidin dispersion, 2:1 lipid-to-polypeptide; (C) DMPC/gramicidin cocrystals; (D) gramicidin crystals formed from ethanol; (E) gramicidin crystals formed from ethanol in the presence of CsCl.

changes in the local environment of the gramicidin channel. When crystallized in the presence of CsCl, gramicidin has recently been shown to form a series of tubes of intertwined double helices that are joined end-to-end by hydrogen bonds (Wallace, 1986). Gramicidin crystals formed in the absence of ions also appear to form tubelike structures, whose helical type has not yet been determined (Koeppe et al., 1978); they may not be hydrogen bonded end-to-end. It is thus possible that hydrogen bonds formed to the solvent at the ends of these helices could give rise to the band splitting (Van Wart & Scheraga, 1978) seen for the gramicidin crystals grown from ethanol in the absence of ions (Figure 6).

With the exception of one previous study (Naik & Krimm, 1984b), our data showing the coincidence of the amide I peak in the presence and absence of lipid agree with all previous Raman studies. In the 1984 study the amide I peak in the presence of lipid was reported to occur at about 1656 cm^{-1} but shifted to a value near 1670 cm^{-1} when the sample was dehydrated. The reasons for this disagreement with our results are unclear. Possibilities include differences in the method of sample preparation. An alternative explanation is that the 1984 study did not account for the 1635-cm^{-1} water peak and so the shift of the amide I peak in that study might have resulted simply from the underlying water peak. For our samples, we find no shift in the amide I peak in hydrated as compared to dehydrated dispersions at a 25:1 lipid/gramicidin ratio, but we cannot rule out such a shift for samples prepared differently.

Coincidence of the amide I peaks reported here could indicate that the structure of the gramicidin molecule is similar in all of the samples. This would suggest that all samples contain gramicidin in the double-helical form found in the crystals grown in the presence of CsCl. However, such an interpretation conflicts with two pieces of data. Previous NMR experiments provide strong evidence supporting end-to-end single-chain β-helices as the active form of the gramicidin channels in bilayers (Weinstein et al., 1979), and CD spectra indicate that the structures of the polypeptide in organic solvents (presumably in double-helical forms) are distinctly different from that of gramicidin in bilayers (end-to-end single-helical form) (Wallace, 1983).

The most likely explanation of our results is that the various double- and single-stranded forms of gramicidin give unexpectedly similar Raman spectra despite the large difference in the CD spectra. This apparently contradicts the recent vibrational analysis of Naik and Krimm (1986). However, as the authors themselves point out, "The intensities of Raman bands are more difficult to predict at this point than those of infrared bands" (Naik and Krimm, 1986). Furthermore, the calculations depend on assumed coordinates for the single-stranded β-helical forms, and it has already been shown that predicted Raman frequencies of helices are very sensitive to very slight alterations in the assumed coordinates; for example, a very slight shift in the parameters of the α-helix leads to a greater than 10-cm^{-1} shift in its predicted position (Krimm & Dwivedi, 1982). Our data suggest that Raman scattering is probably not the method of choice for distinguishing between the various β-helical forms of gramicidin.

Possible Use of Raman Scattering for Study of Other Membrane Protein Crystals. The ability of small, hydrophobic polypeptides to form multiple structures, depending on the environment, is not limited to gramicidin. The fd coat protein, the preproparathyroid hormone, glucagon, and several other hydrophobic peptides exhibit either α-helix or β-sheet, depending on the environment (Nozaki et al., 1976; Rosenblatt et al., 1980; Wu et al., 1981; Fodor et al., 1981; Fodor, 1982; Dunker et al., 1982). In crystals of alamethecin, there are three forms of the molecule with distinctive hydrogen bonding in the vicinity of the proline moiety (Fox & Richards, 1982), and the conformation of this molecule has been shown to be very sensitive to solvent environment (Cascio & Wallace, 1984). Given this ability of small, hydrophobic polypeptides to form different types of secondary structure and given the recent success in crystallizing a number of these, it would be very useful to develop a test that permits comparison of the structures of these molecules in their crystal forms with their structures in active, membrane-bound forms. Previous studies suggest that Raman scattering could be very useful for such comparisons, especially if the different environments lead to shifts between α- and β-structure (Dunker et al., 1982).

Summary. Raman scattering has shown that the cocrystals of gramicidin and lipid contain a 2:1 lipid/gramicidin mole ratio. The lipid components in the cocrystals are much more ordered than those found in dispersions at the same 2:1 molar ratio.

Registry No. DMPC, 18194-24-6; gramicidin, 1405-97-6.

References

Brown, K. G., Peticolas, W. L., & Brown, E. (1973) *Biochem. Biophys. Res. Commun. 54*, 358–364.

Caille, A., Pink, D., de Verteuil, F., & Zuckermann, M. S. (1980) *Can. J. Phys. 58*, 581–611.

Cascio, M., & Wallace, B. A. (1984) *Ann. N.Y. Acad. Sci. 435*, 527–529.

Chapman, D., & Cornell, B. A. (1977) *Nobel Symp. No. 34*, 85–94.

Chapman, D., Cornell, B. A., Eliasz, A. W., & Perry, A. (1977) *J. Mol. Biol. 113*, 517–538.

Dunker, A. K., Williams, R. W., Gaber, B. P., & Peticolas, W. L. (1979) *Biochim. Biophys. Acta 553*, 351–357.

Dunker, A. K., Fodor, S. P. A., & Williams, R. W. (1982) *Biophys. J. 37*, 201–203.

Fiske, C. H., & Subbarow, Y. (1925) *J. Biol. Chem. 66*, 375–400.

Fodor, S. P. A. (1982) Masters Thesis, Washington State University.

Fodor, S. P. A., Dunker, A. K., Ng. Y, C., Carsten, D., & Williams, R. W. (1981) In *Bacteriophage Assembly*, pp 441–455, Liss, New York.

Fox, R. O., & Richards, F. M. (1982) *Nature (London) 300*, 325–330.

Gaber, B. P., & Peticolas, W. L. (1977) *Biochim. Biophys. Acta 465*, 260–274.

Garavito, R. M., & Rosenbusch, J. P. (1980) *J. Cell Biol. 86*, 327–329.

Kimball, M. R., & Wallace, B. A. (1984) *Ann. N.Y. Acad. Sci. 435*, 551–554.

Koeppe, R. E., II, Hodgson, K. O., & Stryer, L. (1978) *J. Mol. Biol. 121*, 41–54.

Krimm, S., & Dwivedi, A. M. (1982) *Science (Washington, D.C.) 216*, 407–408.

Lippert, J. L., & Peticolas, W. L. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 1572–1576.

Lippert, J. L., & Peticolas, W. L. (1972) *Biochim. Biophys. Acta 282*, 8–17.

Marsh, D. (1974) *J. Membr. Biol. 18*, 145–162.

Masotti, L., Spisni, A., & Urry, D. W. (1980) *Cell Biophys. 2*, 241–251.

Mendelsohn, R. (1972) *Biochim. Biophys. Acta 290*, 15–21.

Mendelsohn, R., Sunder, S., & Bernstein, H. J. (1975) *Biochim. Biophys. Acta 413*, 329–340.

Mendelsohn, R., Dluhy, R., Taraschi, T., Cameron, D. G., & Mantsch, H. H. (1981) *Biochemistry 20*, 6699–6706.

Michel, H. (1982) *J. Mol. Biol. 158*, 567–572.

Michel, H., & Oesterhelt, D. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1283–1285.

Naik, V. M., & Krimm, S. (1984a) *Biophys. J. 45*, 109–112.

Naik, V. M., & Krimm, S. (1984b) *Biochem. Biophys. Res. Commun. 125*, 919–925.

Naik, V. M., & Krimm, S. (1986) *Biophys. J. 49*, 1131–1154.

Nozaki, Y., Chamberlain, B. K., Webster, R. E., & Tanford, C. (1976) *Nature (London) 259*, 335–337.

Pink, D. A., Green, T. J., & Chapman, D. (1980) *Biochemistry 19*, 349–356.

Pink, D. A., Green, T. J., & Chapman, D. (1981) *Biochemistry 20*, 6692–6698.

Rosenblatt, M., Beaudetti, N. V., & Fasman, G. P. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 3983–3987.

Rothschild, K. J., & Stanley, H. E. (1974) *Science (Washington, D.C.) 185*, 616–618.

Rothschild, K. J., & Stanley, H. E. (1975) *Am. J. Clin. Pathol. 63*, 695–713.

Susi, H., Sampugna, J., Hampson, J. W., & Ard, J. S. (1979) *Biochemistry 18*, 297–301.

Taraschi, T., & Mendelsohn, R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2362–2366.

Terwillinger, T. C., Weissman, L., & Eisenberg, D. (1982) *Biophys. J. 37*, 353–361.

Tu, A. T. (1982) *Raman Spectroscopy in Biology*, Wiley, New York.

Urry, D. W., Spisni, A., & Khaled, A. (1979) *Biochem. Biophys. Res. Commun. 88*, 940–949.

Van Wart, H. E., & Scheraga, H. A. (1978) *Methods Enzymol. 49*, 67–149.

Wallace, B. A. (1983) *Biopolymers 22*, 397–402.

Wallace, B. A. (1986) *Biophys. J. 49*, 295–306.

Weidekamm, E., Bamberg, E., Brdiczka, D., Wildermuth, G., Macco, F. Lehmann, W., & Weber, R. (1977) *Biochim. Biophys. Acta 464*, 442–447.

Weinstein, S., Wallace, B. A., Blout, E. R., Morrow, J. S., & Veatch, W. R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4230–4234.

Williams, R. W., & Dunker, A. K. (1981) *J. Mol. Biol.* 152, 783–813.

Wu, C.-S., Ikeda, K., & Yang, J. T. (1981) *Biochemistry 20*, 566–570.

Yu, N.-T. (1977) *CRC Crit. Rev. Biochem. 4*(3), 229–280.