mental unit of association of the DCCD-protein in organic solutions is the dimer ($CHCl_3$:CH_3:H_2O=5:1:1), giving evidence that this hydrophobic protein can aggregate in low-polarity media. The maximum chord length of the dimer was found to be 61.5 ± 1.5 Å; however, at a concentration of 25% (vol/vol) of DMSO the maximum chord length was determined to be 48.5 Å. The weight-average mol wt was found to be 9,000, apparently the monomer of the DCCD-protein, suggesting that the conformation changes upon addition of DMSO. Small-angle x-ray scattering intensity curves obtained from the DCCD-protein in water show a discrete interference maximum indicative of a considerable degree of regularity in the two dimensional arrangement in the plane perpendicular to the DCCD-protein-axis. A well-orientated diffraction pattern of the DCCD-protein was obtained from which cylindrically average intensity distribution curves were calculated, revealing a π-left-handed helix rather than α-helix.

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THE EFFECTS OF LIPID ENVIRONMENT, ION-BINDING AND CHEMICAL MODIFICATIONS ON THE STRUCTURE OF THE GRAMICIDIN TRANSMEMBRANE CHANNEL

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Gramicidin A is a linear polypeptide antibiotic which forms ion channels in membranes. It is capable of adopting a number of different conformations, depending on its environment. Nuclear Magnetic Resonance (NMR) studies have indicated that its conformation in phospholipid vesicles is likely to be an N-terminal to N-terminal dimer of single helices (1). Circular dichroism (CD) and infrared spectroscopic studies of gramicidin in methanol and ethanol solutions have suggested that the molecule forms a family of intertwined double helical dimers in these hydrophilic organic solvents (2). The present CD study of gramicidin demonstrates that neither the structures which the molecule assumes in a variety of organic solvents, including such amphipathic solvents as decanol and decanoic acid, nor likely those formed in crystals prepared from hydrophilic solvents, are equivalent to its structure in phospholipid vesicles. Our data support the single helical channel as being the structure formed in membranes.

RESULTS AND DISCUSSION

Circular dichroism spectroscopy has also been used to ascertain those features necessary for maintenance of the active channel conformation of gramicidin A, and to examine this antibiotic's mechanism of action, alterations in its structure accompanying cation binding, chemical modification, and variations in lipid structure. X-ray diffraction of crystals formed from methanol and ethanol solutions have suggested that the cation binding mechanism might involve a widening of the channel and, consequently, foreshortening of the helix (3). Our CD study of gramicidin in membranes demonstrates that the helical

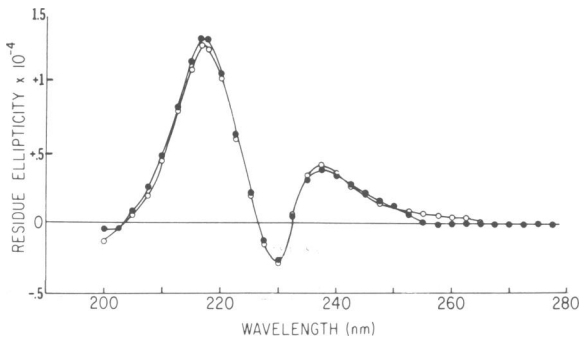

FIGURE 1 CD spectrum of gramicidin A in DMPC vesicles at 20°C, in presence (OOO) and absence (●●●) of 2M CsCl.

pitch (and, therefore, width and length) of the molecule remains unaltered upon binding of monovalent cations, and indicates that the ion channel binding mechanism must involve only small, local changes such as positions of side chains or hydrogen bonds near the cation binding site (Fig. 1). Studies of chemically-modified gramicidin molecules have been correlated with their conductance properties (Fig. 2). Modifications which do not alter the steady-state conductance properties of the molecule, i.e., *O*-acetylation, result in essentially no change in the CD spectrum, while removal and modification of the N-terminal formylvaline result in substantial reduction in

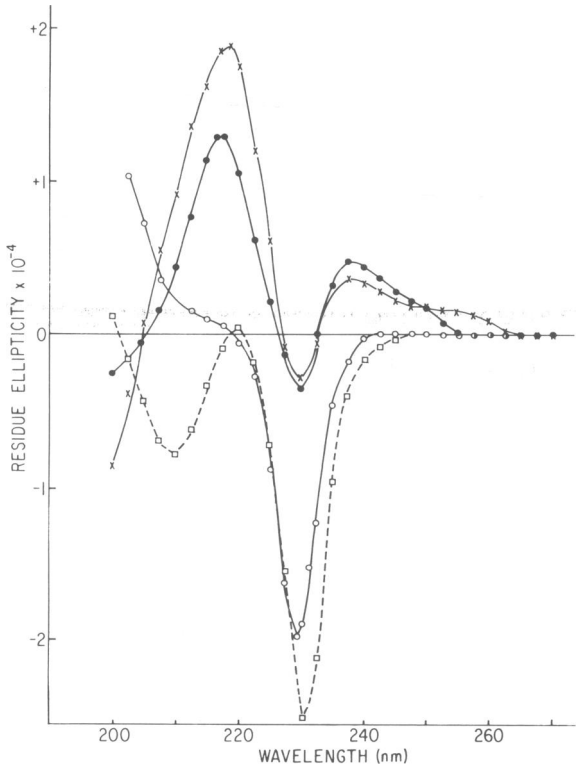

FIGURE 2 CD spectrum of *O*-Acetyl-(●●●), N-Acetyl-(XXX), desformyl-(OOO) and desformylvaline-(□□□) gramicidin in DMPC vesicles at 20°C.

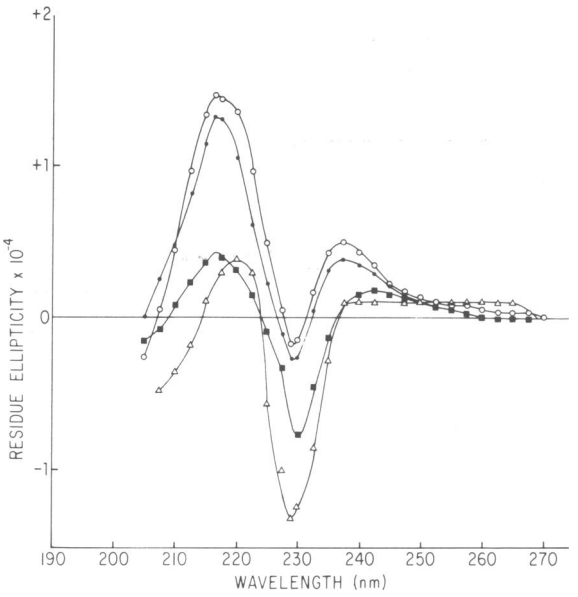

FIGURE 3 CD spectrum of gramicidin A in DLPC (OOO), DMPC (●●●), DPPC (■■■), and DSPC (ΔΔΔ) at 20°C (lipid:peptide ratio is 30:1).

conductance and spectra consistent with decreased capacity of the molecule to form conducting dimers.

The effects of lipid organization and bilayer thickness on the transmembrane channel conformation have been determined by altering the lipid phase states and the fatty acid chain lengths. The structure is independent of the lipid phase transition in dimyristoyl phosphatidylcholine (DMPC) vesicles, and does not require bulk fluid lipids to maintain its integrity. A family of curves (Fig. 3) is obtained for gramicidin in bilayers of different thicknesses. The spectra in relatively thin membranes are very similar, suggesting that the molecule does not change pitch and thus does not elongate to accommodate the thicker bilayers. The spectral changes which occur with very thick bilayers may reflect a decreased dimerization constant for the molecule in these membranes. Studies using various peptide:lipid ratios and phospholipid molecules also indicate that specific peptide-lipid interactions are important in maintaining the channel conformation.

Further studies are now underway to determine more details of the channel conformation by x-ray diffraction of three-dimensional co-crystals of gramicidin and lipid, formed under conditions which may mimic the bilayer environment.[1]

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[1]B. A. Wallace and M. R. Kimball, unpublished results.

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ONE KIND OF INTRAMEMBRANE PARTICLE IS WATER SOLUBLE

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Mammalian urothelial cell membranes contain paracrystalline plaques 0.3–0.5 μm Diam consisting of particles 120 Å Diam in a hexagonal lattice with a constant of 160 Å. Each particle in negative stain consists of a ring of twelve subunits as determined by image analysis (1, 2). These surround a central depression or pore. The membrane between the plaques is smooth and referred to as the "hinge" membrane. In thin transverse sections the hinge membrane appears as a unit membrane ~ 75 Å thick, whereas the plaque membrane is ~ 130 Å thick. In freeze-fracture-etch (FFE) preparations, the external fracture (EF) faces display particles about the same size as the external ones, also in a hexagonal array with a lattice constant of 160 Å. The PF faces have corresponding plaque regions with the same lattice constant, but there are no pits to correspond to the particles in the EF face. Instead, the PF pattern consists of repeating domains, each made of a ring of metal surrounding a slight depression with a spot of metal in the center. The PF pattern is easily destroyed by etching but the EF pattern is very resistant. We have recently published (3) a study focused mainly on the EF faces in which we presented evidence that the EF particles are artifacts. We believe our findings indicate that the particles are plastic deformation and decoration artifacts.

The isolated membranes were deposited on a glass coverslip, covered with a copper sheet and fractured under liquid nitrogen (LN_2). We replicated fractured membranes on the glass surface and measured the height of the EF particles above the glass surface. It was greater than the total thickness of the membrane and thus the particles

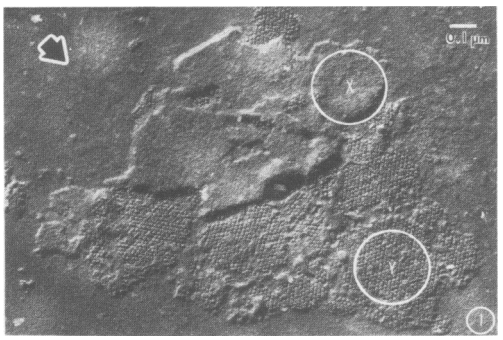
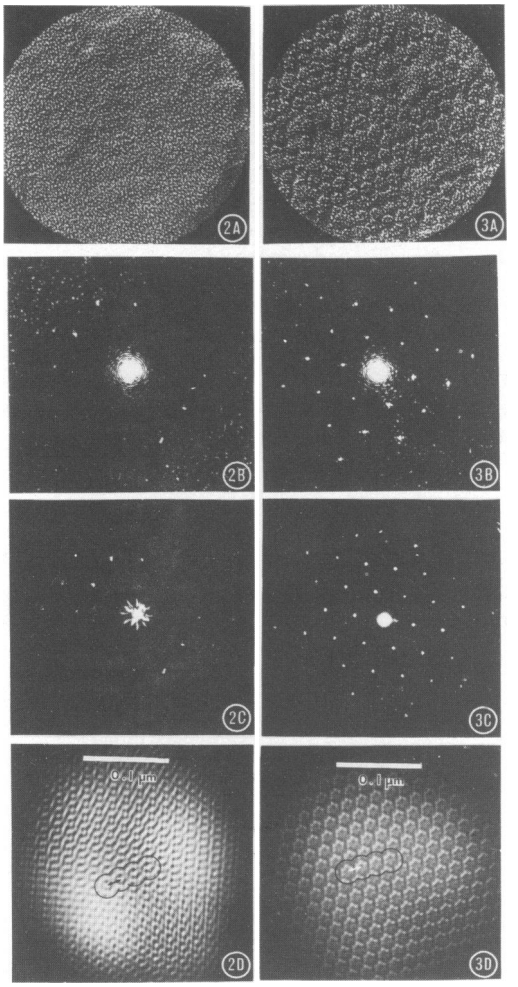


FIGURE 1 FFE preparation of mammalian urothelial membranes deposited on glass. Two superimposed membranes are shown, both of which are fractured to reveal parts of their EF faces. Magnification as indicated.



FIGURES 2 AND 3 FFE preparation of mammaliam urothelial membranes deposited on glass. 2 *A* and 3 *A* are enlargements of areas *X* and *Y*, respectively, from Fig. 1. 2 *B–D* and 3 *B–D* are areas *X* and *Y* from Fig. 1 analyzed by image filtration. 2 *A* and 3 *A* are at the same magnification as 2 *D* and 3 *D*.