A Conformational Rearrangement in Gramicidin A: From a Double-Stranded Left-Handed to a Single-Stranded Right-Handed Helix†

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ABSTRACT: A conformational transition is described for the polypeptide, gramicidin A, in which a dimer that forms a left-handed intertwined antiparallel helix is converted to a single-stranded amino terminus to amino terminus right-handed helix. The starting structure is determined here by solution NMR methods while reference is made to the well-established folding motif of gramicidin in a lipid bilayer for the ultimate conformation of this transition. Furthermore, an organic solvent system of benzene and ethanol in which gramicidin has a unique conformation is identified. This conformation is shown to be very similar to that derived from X-ray diffraction of crystals prepared from a similar solvent system.

Yet another approach can be taken for attacking the protein folding question. Here a study of the solvent dependent structure of gramicidin A is presented. It is well-known that this molecule forms a variety of monomeric and dimeric structures depending on solvent conditions. In fact, it is thought that gramicidin has two native conformations; one of these is the extensively studied ion channel conformation (Wallace, 1990; Cornell, 1987), and the other is a protein-bound conformation in gramicidin's regulatory role of interacting with the σ-subunit of RNA[1] polymerase (Fisher & Blumenthal, 1982). This paper focuses on the formation of the ion channel conformation from an intertwined double-helical structure observed in a mixed solvent system of benzene/ethanol that has been used to prepare the channel conformation in lipid bilayers.

Gramicidin A is a polypeptide of 15 amino acid residues with an alternating sequence of L and D stereochemistry: HCO-LVal$_1$-Gal$_2$-LAla$_3$-DLeu$_4$-LAla$_5$-DVal$_6$-LVal$_7$-DVal$_8$-LTrp$_9$-DLeu$_{10}$-LTrp$_{11}$-DLeu$_{12}$-LTrp$_{13}$-DLeu$_{14}$-LTrp$_{15}$-NHCH$_2$CH$_2$-OH. An N-terminal to N-terminal dimer of this polypeptide forms a monovalent cation selective channel in lipid bilayers. The folding motif, originally proposed by Urry in 1971, is a strand of β-sheet that has been wrapped into a helix with all C_α–C_β bonds directed radially away from the channel axis. Recently, this β-helix was determined by solid-state NMR methods in lipid bilayers to be right-handed (Nicholson & Cross, 1989). Although there is no crystal structure for this conformation of the molecule, there have been recent X-ray studies of oriented bilayer preparations of the channel form that document the helical pitch as that of a single-stranded helix (Katsaras et al., 1992) and another study that determined the separation for monovalent and divalent cation binding sites in the gramicidin channel (Olah et al., 1991). Furthermore, numerous computational approaches have been taken to achieve reasonable molecular models of the channel conformation. One of these models has been substantially verified by comparison of model-based and observed ^{15}N chemical shifts from oriented preparations of gramicidin in dimyristoylphosphatidylcholine (DMPC) bilayers (Chiu et al., 1991). Such models have 6 intermolecular hydrogen bonds and 10 intramolecular hydrogen bonds in each monomer and approximately 6.3 residues per turn of the helix. A detailed structure determination is underway via solid-state NMR by means of a process of analyzing orientational constraints (Brenneman & Cross, 1990). Recently, the first experimentally determined backbone torsion angles have been published (Teng et al., 1991).

In DMPC bilayers, the monomer/dimer equilibrium for the channel conformation very strongly favors the dimer, presumably because there is a good match between the hydrophobic width of the bilayer and the hydrophobic length of the channel. When lipids with longer fatty acyl chains are used, the dimerization equilibrium shifts toward the monomeric state; i.e., the lifetime of the conducting dimers becomes much shorter (Kolb & Bamberg, 1977). Furthermore, in these longer chain fatty acyl lipids the phase behavior of the lipids is modulated by gramicidin, and possible mechanisms for this effect have recently been published (Killian & deKruijff, 1988; Cornell & Separovic, 1988; Watnick et al., 1990). For the purposes of this study, it is important to recognize that the hydrophobic dimension of the bilayer affects the stability of the channel state.

While there are numerous approaches for generating the channel state, a typical method involves the cosolubilization of gramicidin and lipid in an organic solvent followed by drying the sample and then hydration. The ease with which the channel state is achieved is dependent on the organic solvent used and presumably on the conformation of the gramicidin within the organic solvent. This channel conformation is difficult to characterize functionally in the samples that are useful for structural studies. Circular dichroism (CD) methods require either sonication or the addition of detergent to vesicularize lipid bilayers. Sonication has been shown by CD (LoGrasso et al., 1988) and HPLC (Bano et al., 1989) to induce the formation of the channel state. Furthermore, detergents such as SDS are known to favor a channel-like

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[1] Abbreviations: NMR, nuclear magnetic resonance; RNA, ribonucleic acid; DMPC, dimyristoylphosphatidylcholine; CD, circular dichroism; HPLC, high-performance liquid chromatography; SDS, sodium dodecyl sulfate; NOE, nuclear Overhauser enhancement; RH-NN, single-stranded right-handed N-terminus to N-terminus helix; LH-aDS; left-handed antiparallel double-stranded helix; THF, tetrahydrofuran.

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conformation (Arseniev et al., 1986). However, resonance line widths and chemical shifts from ^{23}Na NMR have been used in both vesicle preparations (Urry et al., 1979) and extensive bilayers (LoGrasso et al., 1988) to demonstrate the presence of gramicidin that either binds or does not bind Na^+. Using these approaches with due caution, it has been shown that a solvent history dependence exists upon forming the channel conformation out of an organic solution (LoGrasso et al., 1988; Killian et al., 1988). Fluorescence studies have recently confirmed this solvent history dependence (Cox et al., 1992), and novel HPLC studies by Bano and co-workers (1988, 1989) have confirmed the presence of intertwined dimers and monomers of gramicidin in vesicles shortly after incorporation of gramicidin into the lipid structures. They have also shown that the ratio of monomer (or single-stranded helix) to intertwined dimer varies depending on the solvent history of the vesicle preparation.

To understand the conformational rearrangement that occurs in such a procedure, both the initial and final conformations need to be well characterized. Veatch et al. (1974) documented the presence of four gramicidin conformers in ethanolic solution. These conformers are in rapid equilibrium in this solvent and other hydrogen bond donating solvents. They are discrete dimeric structures representing left- and right-handed species that are parallel or antiparallel intertwined helices and that may or may not be staggered. The folding pattern for these various structures has been modeled by high-resolution NMR (Bystrov & Arseniev, 1988; Arseniev et al., 1986). Recently, a more detailed analysis of one of these conformers has been achieved (Pascal & Cross, 1992). However, recent studies have sometimes ignored the diversity of these structures in discussions of channel formation (Bano et al., 1989, 1991).

The mixed organic solvent system chosen for this study is benzene/ethanol. This solvent system has been used to generate the channel conformation (Moll & Cross, 1990) and used to prepare crystals for an X-ray structure determination (Langs, 1988). Other crystal structures (Wallace & Ravikumar, 1988; Langs et al., 1991) have been obtained from methanol where a mixture of conformations is known to exist and less than 50% of the gramicidin in solution may have been in the form that crystallized. Consequently, an effort was made to characterize the conformation or conformations present in benzene/ethanol solution. Furthermore, for relatively small polypeptides the reliability of a crystal structure as an accurate representation of the structure in solution is less than for larger proteins where there is a smaller surface to volume ratio.

This paper describes the solution structure determination of gramicidin in benzene/ethanol by high-resolution NMR methods as well as speculation on the pathway for the solvent-dependent conformational rearrangement from an intertwined helix in organic solvent to the channel conformation in a lipid bilayer.

METHODS AND MATERIALS

(*a*) *Sample Preparation: Choice of Solvent.* Gramicidin D was obtained from Sigma Chemical Co. (St. Louis, MO) and used without isolation of gramicidin A for one-dimensional spectra only. Two-dimensional spectra were obtained with gramicidin A prepared by solid-phase peptide synthesis as previously described (Fields et al., 1989). Synthetic gramicidin when cleaved from the solid-phase support was greater than 98% pure as judged by HPLC and was used without further purification. Solutions were prepared as a function of gramicidin concentration and solvent ratio of benzene-d_6/ethanol-d_6 (Cambridge Isotope Lab, Woburn, MA) in order to identify conditions creating a single conformational species. Conformational purity was assayed by the observation of the formyl ^1H resonance. In pure solvents such as ethanol or methanol, four or more resonances can be identified with the formyl proton representing the known variety of conformations in these solvents. In benzene/ethanol, solvent ratios (by volume) from 96:4 to less than 50:50 yield a single dominant conformation as indicated by a single formyl ^1H resonance. This, however, does not preclude rapid local conformational averaging to yield a single chemical shift, but the dimeric equilibria observed in ethanol and methanol are known to be slow on the nanosecond time scale. At a concentration of 80 mg/mL in an 80:20 mixture, only a single resonance is observed for the formyl proton, strongly suggesting the presence of a single species. Two-dimensional ^1H NMR of this sample confirms this conclusion as described in the Results section.

(*b*) *Two-Dimensional Spectra.* Spectra were recorded at a sample temperature of 30 °C on a 500-MHz Varian VXR500 spectrometer. The data were collected in a phase-sensitive mode using the hyper-complex method (States et al., 1982). A total of 4096 $t2$ points (2048 real) and 512 $t1$ increments (1024 spectra) were collected. Recycle times were set to approximately 1.4 s. The HOHAHA (Braunschweiler & Ernst, 1983) spectrum used a 50-ms MLEV-17 mixing scheme (Bax & Davis, 1985) with a transmitter power set to give a 90° proton pulse of 20.2 μs. The NOESY spectrum (Jeener et al., 1979; Kumar et al., 1980) was obtained with a 300-ms mixing time. The residual benzene peak was referenced to 7.17 ppm.

RESULTS

(*a*) *Peak Assignments.* The conformational purity of gramicidin A in an 80:20 mixture of benzene/ethanol is shown by the absence of an additional network of peaks in Figure 1. Individual spin systems were assigned via the HOHAHA (Figure 1A) and DQCOSY (not shown) spectra. Sequential NHαH NOEs from the NOESY spectrum (Figure 1B) were used to make sequence-specific assignments (Table I). Only the ethanolamine OH and tryptophan ring protons were not assigned. A total of 52 backbone NOEs, corresponding to 104 distance constraints for the dimer, were unambiguously identified.

Intensities of NOE cross-peaks were determined by volume integration using Varian VNMR sys4 software version 2.2. Linear buildup rates clearly demonstrated in studies of related conformations [e.g., Pascal and Cross (1992)] were assumed here. The self-consistent results reported in this paper verify this assumption. The NOEs were sorted into three categories: strong (2.0–2.5 Å), medium (2.5–4.0 Å), weak (4.0–5.0 Å). A backbone NOE pattern very closely resembling that found for species 3 in dioxane (Arseniev et al., 1984) emerged (Table II). NOEs on the diagonals of Table II represent intrachain NOEs, and those on the antidiagonals represent interchain NOEs. It therefore appeared that the conformation was an antiparallel intertwined double-helix as in Arseniev's model. It is antiparallel because the interchain NOEs are between protons at opposing ends of the polypeptide, and it is intertwined because there is contact between the two chains over their entire length. In order to confirm this conclusion and to obtain the helix sense, as well as more detailed information about the backbone fold, the following analysis was undertaken.

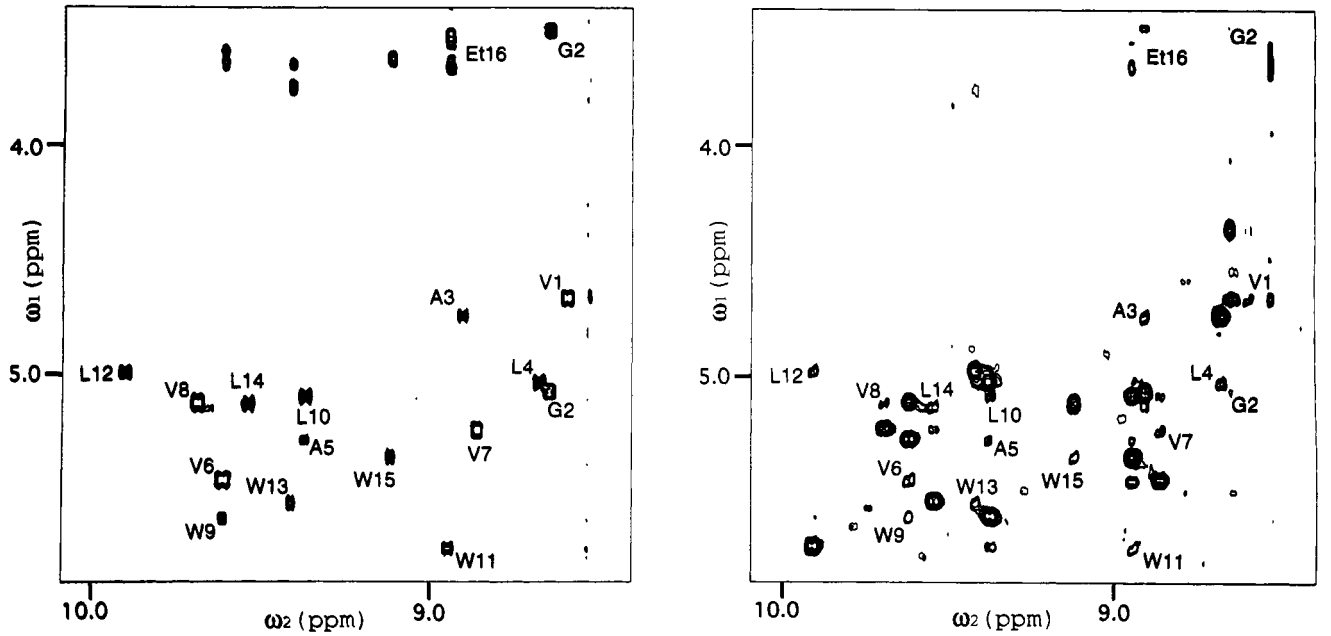

FIGURE 1: NHαH region of (A, left) 50-ms mixing time HOHAHA spectrum and (B, right) 300-ms mixing time NOESY spectrum of gramicidin A in the species 3 conformation in benzene/ethanol. Both spectra were recorded at 30 °C and 500 MHz. Intraresidue cross-peaks are identified. Backbone NOEs are summarized in Table II.

Table I: ^{1}H NMR Chemical Shift Assignments[a] for Gramicidin Species 3 in Benzene/Ethanol

residue	HN	Hα	Hβ	Hγ	Hδ
Val_1	8.57	4.69	2.26	1.49, 1.12	
Gly_2	8.63	5.08, 3.56			
Ala_3	8.91	4.77	1.41		
Leu_4	8.67	5.04	1.70	1.54	0.84, 0.70
Ala_5	9.38	5.28	1.50		
Val_6	9.60	5.47	2.55	1.28, 0.80	
Val_7	8.87	5.25	2.48	1.23, 1.17	
Val_8	9.69	5.13	2.43	1.35, 1.35	
Trp_9	9.61	5.62	3.64, 3.60		
Leu_{10}	9.37	5.09	1.24	1.12	0.74, 0.67
Trp_{11}	8.94	5.76	3.52, 3.39		
Leu_{12}	9.89	5.00	1.87	1.81	1.02, 0.86
Trp_{13}	9.40	5.57	3.74, 3.65		
Leu_{14}	9.53	5.12	1.67	1.44	1.19, 0.77
Trp_{15}	9.09	5.36	3.62, 3.37		
Et_{16}	8.93	3.70	3.60, 3.05		
formyl	8.53				

[a] Chemical shifts expressed at 30 °C relative to benzene at 7.17 ppm.

(b) Hydrogen Bond Pattern. The hydrogen bonds present in the helix can be determined from the backbone NOE pattern. An analysis of observed NOEs using a stack of four "β-circles" (Pascal & Cross, 1992) is pictured in Figure 2. Each circle is composed of alternating L and D amino acids with torsion angles of $\phi_L = -120°$, $\psi_L = +120°$ and $\phi_D = 120°$, $\psi_D = -120°$. Circles 1 and 3 have their "amino termini" on the left and represent an approximation to the separate turns of one molecule. Circles 2 and 4 have their "amino termini" on the right and represent helical turns of the second molecule. The left half of the stereo pairs shows intermolecular NHαH distances which closely match the observed NOEs. The right half of the stereo pair shows the hydrogen bonding pattern that these distances imply: an antiparallel β-sheet pattern of hydrogen bonding between the circles, consisting of alternating 14- and 10-membered rings. A listing of these hydrogen bonds for this conformational species is presented in Table III. All hydrogen bonds are intermolecular and symmetric between monomers. Each hydrogen bond was converted into two distance constraints: $d_{HO} = 1.9$ Å, $d_{NO} = 2.9$ Å (Baker & Hubbard, 1984; Mitchell & Price, 1990).

Table II: NHαH NOEs for Species 3 in Benzene/Ethanol[a]

αH \ NH	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	w	s														
2		m	s*												m*	
3			m	s												
4				m	s*								m*			
5					w	s										m
6						m	s				m					
7							m	s						m*		
8								m*	s†							
9									m	s		m				
10							m			m*	s*					
11										m	m	s				
12					m*							m	s*			
13								m					m	s		
14		m*												m*	s	
15						m									m	s
16	w															m

[a] Abbreviations and symbols: s, strong (2.0–2.5 Å); m, medium (2.5–4.0 Å); w, weak (4.0–5.0 Å); *, intensity estimated due to spectral overlap; †, combined contributions from intra- and intermolecular NOE.

(c) Structure Calculation. The hydrogen bond information and backbone NOEs were entered as constraints into a distance geometry/simulated annealing routine using DSPACE version 4.0. The routine was identical to a previously published procedure (Pascal & Cross, 1992) with the exception that only backbone NOEs were used here. This procedure involves conjugate gradient minimization applied first to local structure and then to increasingly larger portions of the dimer. This is followed by simulated annealing with shake enabled, in order to correct bond lengths and bond angles. One hundred runs were performed. Nine structures, each with a penalty function less than 0.25, are shown in Figure 3. All are left-handed antiparallel double helices with an average of 5.6

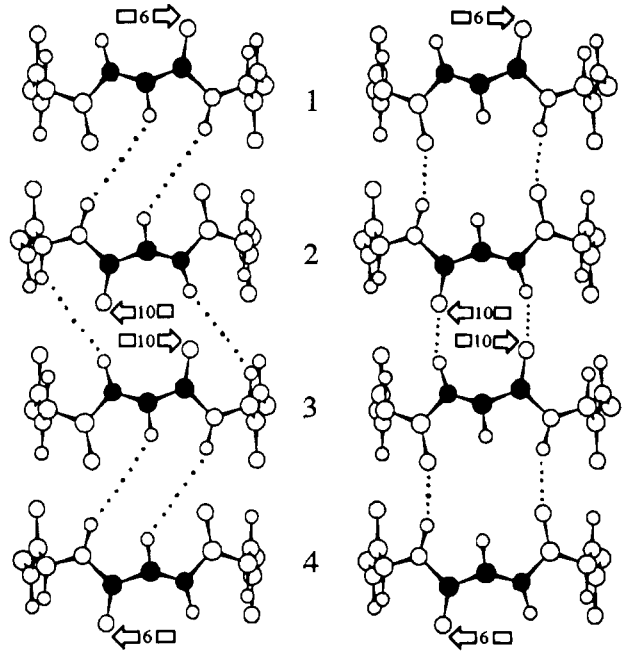

FIGURE 2: Stereo view of a portion of four "β-circles" centered on Val_6 or Leu_{10}. Interchain NHαH approaches close enough to cause NOEs are shown on the left stereo projection. These distances, all of approximately 3.7 Å, match the observed NOE pattern (Table II). The antiparallel β-sheet hydrogen bonding pattern these NOEs imply is shown in the projection on the right (Table III).

Table III: Residue Numbers of the Intermolecular Hydrogen Bonds for Species 3 in Benzene/Ethanol

even		odd	
NH	CO	NH	CO
6	14	1	15
8	12	3	13
10	10	5	11
12	8	7	9
14	6	9	7
16	4	11	5
		13	3
		15	1

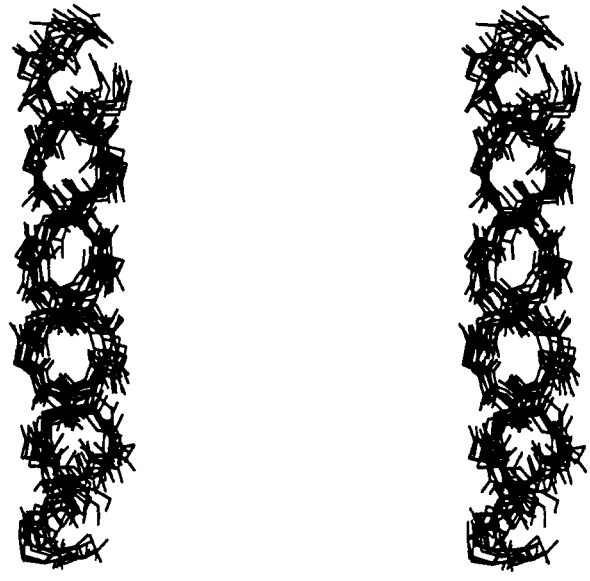

FIGURE 3: Backbone superposition (stereo view) of nine distance geometry/simulated annealing structures calculated by DSPACE. Note the lack of fraying at the helical ends.

residues per turn. Average helix length (Val_1N–Val_1N) is 33.2 Å, and the average width (backbone van der Waals to van der Waals distance) is near 2.5 Å. The rms violation of distance constraints, including converted hydrogen bond

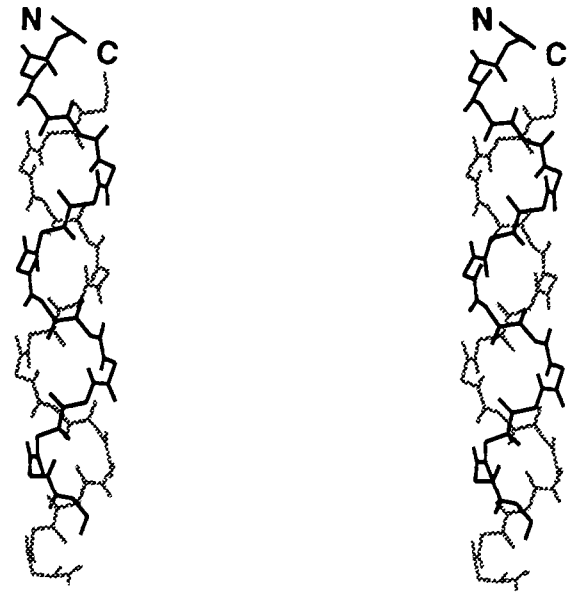

FIGURE 4: The rms-averaged conformation (stereo view) of the nine structures from Figure 3. For clarity, one monomer is shown as a solid line while the other is shown as a broken line.

information, is 0.013 Å. The rms difference between an rms average structure (shown in Figure 4) and the structures of Figure 3 is 0.96 Å for the backbone atoms.

DISCUSSION

In contrast to the dogma that the amino acid sequence dictates protein conformation, gramicidin A has a conformation which is dependent upon its solvent environment (Milton et al., 1992). Here, it is shown that a mixed solvent system can be identified that yields a single conformation for this peptide. Other solvents, such as dioxane, have been used to trap specific conformations of gramicidin in a nonequilibrium situation [e.g., Veatch et al. (1974) and Pascal and Cross (1992)]. However, the mixed solvent system used here is unique in that the very complicated equilibrium of

conformations present in some solvents, such as methanol or ethanol, is shifted so that a single conformation predominates at equilibrium in this solvent.

The hydrogen bonding scheme that yields a backbone folding motif determined here in benzene/ethanol is the same as species 3 previously described by Arseniev (1984) in dioxane and by the crystal structure of Langs (1988). As in the recently determined species 4 structure (Pascal & Cross, 1992), this conformation is remarkably well-defined in solution. Most small polypeptide structures that have been determined depend upon disulfide linkages or prolines to provide stability for a well-defined three-dimensional conformation. Here, the chain termini are not significantly frayed as shown both by the NOE intensity that is uniform along the helix and by the structural models derived from the distance geometry/simulated annealing routines. The stability of this polypeptide is even more remarkable when the absence of long-range structural constraints is recognized. There is only a single helical domain so that the polypeptide does not fold back on itself. Consequently, there is no tertiary structure, but only a mixture of primary, secondary, and quaternary structure. The quaternary structural aspect of this conformation may add considerably to its stability, since a stable monomeric gramicidin conformation has not been observed (Roux et al., 1990).

The formation of the single-stranded channel conformation from an intertwined helical state has been considered in several studies. Urry et al. (1975) described a possible unwinding mechanism for the generation of an intertwined helix of the same helical sense as the channel conformation. Urry referred to this mechanism as a "double-helical zipper mechanism". In its description, they recognized that many steps involving the breaking and formation of numerous hydrogen bonds would have to occur. This process is referred to here as a multistep unidirectional reaction path. Others, such as Wallace (1990) and Salemma (1988), have noted that while many reaction steps would be needed, each step should involve only a small energy barrier. In unwinding an antiparallel double helix, two possible structures are possible: a C-terminus to C-terminus structure or an N-terminus to N-terminus structure. Killian and deKruijff (1988) suggested that C-terminal to C-terminal single-stranded helices do not form because of steric hindrance due to the tryptophan and leucine side chains that would be at the dimer junction. However, it was not recognized until the present study that the helix sense also had to change, that the difference in the length of the two structures might be significant, and that both of these factors may be important for the insertion mechanism of gramicidin into a lipid bilayer.

Recently, a clever study by O'Connell et al. (1990) using single channel conductance observations has provided evidence for the formation of the gramicidin A channel from an intertwined helix. Their studies utilized the difference (a factor of three) in conductance of [Val^1]gramicidin A and [4,4,4-F_3-Val^1]gramicidin A homodimers. When gramicidin A and its analog are added to opposite sides of a membrane, heterodimers are formed almost exclusively. These heterodimers have a conductance which is distinct from either homodimer. Because gramicidin is added to the lipid environment at very low concentrations (nanomolar in ethanol), the polypeptide is almost completely monomeric. In fact, according to the equilibrium constant measured by Veatch and Blout (1974), approximately 0.1% of the gramicidin should be in a dimeric form when ethanol is used as the solvent. When gramicidin is added to these membranes in this asymmetric way, a small concentration of homodimers is

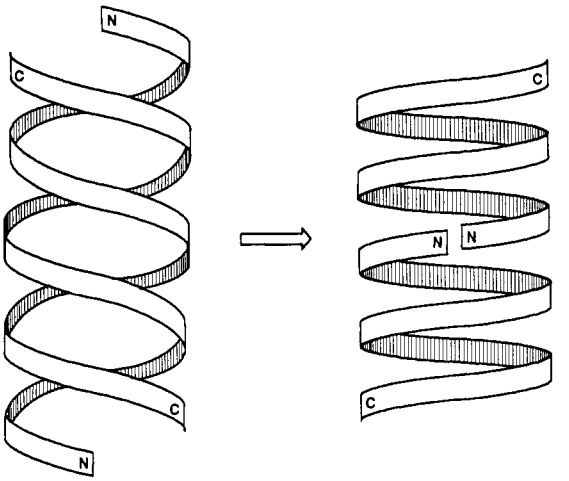

FIGURE 5: A schematic drawing of the gramicidin backbone illustrating some of the salient features for the conversion of a left-handed intertwined antiparallel helix to a right-handed N-terminus to N-terminus single-stranded dimer.

observed. The homodimers formed were observed very early in the conductance measurements, and in light of the relative abundance of dimer versus monomer, channels appear to form much more quickly from dimers than from monomers. Over the 20 min of observation, the number of heterodimer channel events per minute increases dramatically while the number of homodimers remains essentially constant. Consequently, the gramicidin in the two bilayer leaflets mixes at a very slow rate. Therefore, it is not reasonable to expect that the homodimers are formed by a monomeric migration across the bilayer. The authors (O'Connell et al., 1990) suggest that intertwined helices are inserted into the membrane and that this structure unwinds to form the channel conformation. Furthermore, it is likely that the rearrangement pathway for the formation of homodimers has maintained the integrity of the dimer; in other words, the specific monomers of a given intertwined helix are the monomers that formed the initial conducting homodimers. This is because the rate of formation of homodimers far exceeds the formation of heterodimers from monomeric gramicidin.

As mentioned before, the channel conformation is an N-terminus to N-terminus single-stranded helical dimer with a right-handed helical sense (RH-NN). This conformation is readily formed when gramicidin and DMPC are cosolubilized in benzene/ethanol followed by solvent evaporation and hydration with approximately 50% by weight water. In this organic solvent system at millimolar concentrations, the dominant conformation (>90%) as described in this study is an antiparallel intertwined helix with a left-handed helical sense (LH-aDS). In ethanol at millimolar concentrations, gramicidin is almost entirely dimeric and the most abundant conformation (41%) is species 3 (Veatch & Blout, 1974). Therefore, because of the relative abundance of the species, it is likely that the homodimers in O'Connell et al (1990) result from the conformational rearrangement of species 3 to form the channel conformation (Figure 5). Furthermore, the conclusion in Moll and Cross (1990) that the benzene/ethanol conformation, now recognized as species 3, can be readily converted to the channel conformation is supporting evidence that the homodimers observed by O'Connell et al. (1990) were formed from species 3. Therefore, the rearrangement pathway must convert a left-handed species to a right-handed one; it must convert an intertwined helix to a single-stranded one; and it appears to do so without going through a monomeric state.

A discussion of this pathway that leads from a LH-aDS structure to a RH-NN helix can be dissected into an analysis of various structural features. First, consider the antiparallel versus parallel nature of the intertwined helices. It is very difficult to envision how a parallel intertwined helix can dissociate under the above restrictions to form an NN helix. However, in an antiparallel structure if the monomers were unwound from one another they could end up as an NN dimer. Of course, they could also end up as the CC dimer. Secondly, consider the helix sense. If the pathway includes an unwinding of the intertwined monomers then it would appear that the starting and ending structures should have the same helical sense, as suggested by the previous efforts (O'Connell et al., 1990; Urry et al., 1975; Killian & de Kruijff, 1988). This is not the case, but the difference between left- and right-handed helices involves an interesting structural property: an approximate 180° rotation of each peptide plane (Nicholson & Cross, 1989). This is a property of polypeptides with β-helical torsion angles; like β-sheets, adjacent peptide planes alternate in orientation and the helical sense can be characterized by whether the LD planes or the DL planes have their carbonyl groups aligned parallel or antiparallel with respect to the helical axis. As mentioned before, this process would require a considerable unidirection reaction path. In other words, many separate reaction steps involving the breaking and forming of hydrogen bonds are needed to proceed from the starting structure to the final channel structure. However, if the terminal peptide planes that become liberated during each step of this process were to flip by 180°, then reversal of the process would first require the reorientation of these peptide planes. This reorientation may be hindered by intramolecular hydrogen bond formation. Flipping the peptide plane could, in effect, quench the reverse reaction, thereby enhancing the probability for the continuation of the forward reaction. Consequently, it is possible not only to envision a pathway for the conversion of a LH-aDS structure to a RH-NN helix but also to rationalize why such a pathway that involves a change in helical sense might be favored.

Finally, there is a question of the driving force. Why is the intertwined helix not stable in the bilayer while the channel state is very stable in DMPC bilayers? There may be two reasons; first, there is a very considerable length difference between these two structures (33 Å versus 25 Å for the channel). And it is known that the match of the hydrophobic dimension of the channel state with the DMPC bilayer is close to optimal (Kolb & Bamberg, 1977). Secondly, in the intertwined antiparallel helix the tryptophan side chains are dispersed uniformly along the helical axis. In the channel structure, the indole rings are clustered near the bilayer surface. Cox et al. (1992) has now demonstrated that it is possible to detect differences in the fluorescence spectra of the tryptophans in the nonchannel and channel conformations. The difference in overall shape that the tryptophan distribution imparts to the molecular conformation has been viewed as one of the driving forces for gramicidin induction of the H_{II} phase in dioleoylphosphatidylcholine preparations (Killian & deKruijff, 1988). However, it has recently been suggested, and evidence is mounting, that the indole NH groups are hydrogen bonded to the bilayer surface (Meulendijks et al., 1989; O'Connell et al., 1990; Scarlatta, 1991). If so, it would be energetically expensive to bury an indole group in the center of a lipid bilayer. This may very well explain why CC helices are not observed. Furthermore, the tethering of the C-terminus to the bilayer surface explains the lack of migration of the gramicidin between the bilayer leaflets (O'Connell et al., 1990). These two factors, conformer length and indole hydrogen bonding, may form the basis for a driving force to form the channel state from species 3.

Not only is the conformational distribution of gramicidin dependent upon the solvent environment, but the stability or rate of interconversion of the conformers is also dependent upon the solvent environment. In methanol or ethanol, the various intertwined helices interconvert rapidly enough so that the various conformers cannot be separated by thin-layer chromatography (Veatch et al., 1974). Furthermore, the monomer/dimer equilibrium shifts more toward the monomer state in polar solvents such as trifluoroethanol or dimethyl sulfoxide than it does in nonpolar solvents (Veatch & Blout, 1974). In dioxane or tetrahydrofuran, the interconversion rate is much slower and, in fact, species 4 is stable for 2–3 weeks in dioxane (Pascal & Cross, 1992). The addition of a small proportion of water to a gramicidin solution in THF induces a time-dependent monomerization (Bano et al., 1988). In other words, a solvent capable of donating hydrogen bonds can act as a catalyst for the interconversion of these species, presumably through an ability to promote hydrogen bond exchange. In DMPC bilayers, the presence of water at the ends of the gramicidin helices and in the channel or pore of the intertwined helix is likely to facilitate the interconversion of the LH-aDS conformation to the RH-NN structure.

It has been documented that there is a solvent history dependence in the formation of the channel conformation (LoGrasso et al., 1988; Killian et al., 1988; Bano et al., 1988, 1989; Cox et al., 1992): that the ease with which the channel conformation can be generated is dependent on the gramicidin conformation in the organic solvent. This solvent history dependence has been contested (Sawyer et al., 1990) through conductance studies of gramicidin. An explanation for the observed conductance from ostensibly "nonchannel" preparations has been put forward by Cox et al. (1992) in suggesting that the conductance measurements observed only a small fraction of the gramicidin present. An alternative explanation may be more likely. The conductance studies, as mentioned above, are performed under extremely dilute solution conditions where the gramicidin monomer/dimer equilibrium heavily favors the monomer, while the studies where the solvent history dependence has been observed have been conducted at a high concentration that heavily favors the dimer state. Consequently, the solvent history dependence is associated with the dimeric state of gramicidin (LoGrasso et al., 1988; Wallace, 1990). Moreover, the variability in the ease with which various conformations are converted to the channel state reflects the variability in the structure in organic solvents. Apparently other conformations, such as species 1, 2, or 4, may be much more difficult to convert to the channel state. In fact, these species are all parallel intertwined dimers, conformations that would require conversion to a monomeric state prior to channel formation. Furthermore, species 4 is a right-handed helix, and this feature may also be an impediment to channel formation because of the lengthy unidirectional reaction path.

This paper has defined the starting structure for the known conversion of gramicidin in benzene/ethanol into the channel state in a lipid bilayer. In this organic solvent, the predominant structure is species 3, a left-handed antiparallel double helix. The backbone structural dimensions are very similar to that of the X-ray diffraction structure of gramicidin A crystallized from benzene/ethanol. While it is surprising that both the nature of the dimeric form and the handedness of the helix should change, a plausible conformational rearrangement

pathway has been described for the formation of the channel state. Furthermore, this pathway is consistent with the conductance observations of O'Connell et al. (1990) as well as with the solvent history dependence for the formation of the channel state of gramicidin.

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