Gramicidin Channel Function Does Not Depend on Phospholipid Chirality†

L. L. Providence,‡ O. S. Andersen,*,‡ D. V. Greathouse,§ R. E. Koeppe, II,*,§ and R. Bittman*,||

Department of Physiology and Biophysics, Cornell University Medical College, New York, New York 10021, Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, Arkansas 72701, and Department of Chemistry, Queens College of the City University of New York, Flushing, New York 11367

Received August 1, 1995⊗

ABSTRACT: Chiral interactions are often important determinants for molecular recognition in chemistry and biochemistry. In order to determine whether the phospholipid backbone could be important for the conformational preference of membrane-spanning channels, we made use of the linear pentadecapeptide antibiotic gramicidin A (gA^+) and a Trp → Phe-substituted gA^+ analogue, gramicidin M^+ (gM^+), as well as their enantiomers [gramicidin A^- (gA^-) and gramicidin M^- (gM^-), respectively]. All four analogues form conducting channels in planar bilayers formed from the dialkylphospholipids (*R*)- or (*S*)-dioleylphosphatidylcholine or from the diacylphospholipid (*R*)-dioleoylphosphatidylcholine. The characteristics of channels formed by the two gramicidin A enantiomers, or the two gramicidin M enantiomers, in membranes formed by either of the dioleylphosphatidylcholine enantiomers are indistinguishable. Similarly, channels formed by either pair of gramicidin enantiomers in dioleoylphosphatidylcholine bilayers are indistinguishable. We conclude that chiral interactions between gramicidin channels and the lipids in the host bilayer cannot be important determinants of gramicidin channel structure or function. The membrane/solution interface, therefore, seems to organize the channel structure because of the general characteristics of the nonpolar/polar transition at the interface rather than because of specific chemical interactions.

The three-dimensional structure and function of integral membrane proteins are determined by the primary amino acid sequences and by the organization of the lipid bilayer in which the proteins are imbedded. It is generally recognized that the membrane's hydrophobic core constitutes a critical organizing element in this respect. What is the role of the membrane/solution interfaces? We address this question through studies on channels formed by the linear gramicidins, a family of pentadecapeptide antibiotics.

Membrane-spanning gramicidin channels are dimers, formed by the transmembrane association (O'Connell et al., 1990) of two single-stranded right-handed $\beta^{6.3}$-helical (Arseniev et al., 1986; Ketchem et al., 1993) monomers, which are joined at their formyl NH-termini [see Andersen and Koeppe (1992), Killian (1992), and Busath (1993) for recent reviews]. Each monomer is stabilized by ten intramolecular (CO···HN) hydrogen bonds. The membrane-spanning dimer is stabilized by six intermolecular (CO···HN) hydrogen bonds at the formyl NH-terminal-to-formyl NH-terminal junction (Arseniev et al., 1986c; Ketchem et al., 1993). In addition, there are hydrogen bonds between peptide C=O and NH groups at the ethanolamide CO-termini and H_2O at the channel/solution interface and between the indole NH moieties and either H_2O or phospholipid polar groups at the membrane/solution interface (O'Connell et al., 1990; Becker et al., 1991; Takeuchi et al., 1990; Durkin et al., 1992; Hu et al., 1993; Koeppe et al., 1994).

The amino acid side chains project outward and interact with the lipid bilayer's hydrophobic core and membrane/solution interface, and gramicidin channel function (conductance, average duration, and channel-forming potency) varies with the chemical composition of the host bilayer (Neher & Eibl, 1977; Kolb & Bamberg, 1977; Froehlich, 1979a,b; Elliott et al., 1983; Sawyer et al., 1989; Fonseca et al., 1992; Lundbæk & Andersen, 1994). The importance of the thickness of the membrane's hydrophobic core is well-established (Kolb & Bamberg, 1977; Elliott et al., 1983). The role of the membrane/solution interface remains unknown, except that hydrogen bonds between the indole NH moieties and polar groups at the membrane/solution interface are important for channel structure and function (O'Connell et al., 1990; Becker et al., 1991; Durkin et al., 1992). It has been suggested that chiral interactions between the channel and the phospholipid backbone stabilize the channel structure (Wallace, 1986), and hydrogen-bonded interactions between the indole group of Trp^{15} and the ester C=O groups in membrane-forming lipids have been postulated to be important for channel function (Meulendijks et al., 1989; Hu et al., 1993; Seoh & Busath, 1995). The precise manner in which the gramicidin channel structure and function may be affected by such interactions remains unclear, however.

To assess the role of polar groups at the membrane/solution interface in gramicidin channel structure and function, we used four different gramicidin analogues: the standard gramicidin A, which will be denoted gA^+;[1] its enantiomer, gA^-; a Trp → Phe-substituted analogue [$Phe^{9,11,13,15}$]gA, also called gM (Tredgold et al., 1977) or gM^+ in the present study; and its enantiomer gM^- (Heitz et al., 1982). All four

† Supported by NIH Grants GM21342 (O.S.A.), GM34968 (R.E.K.), and HL16660 (R.B.). The peptide synthesizer was funded by NSF Grant RII-8922108.

* To whom correspondence should be sent.
‡ Cornell University Medical College.
§ University of Arkanses.
|| Queens College of the City University of New York.

⊗ Abstract published in *Advance ACS Abstracts,* December 1, 1995.

0006-2960/95/0434-16404$09.00/0

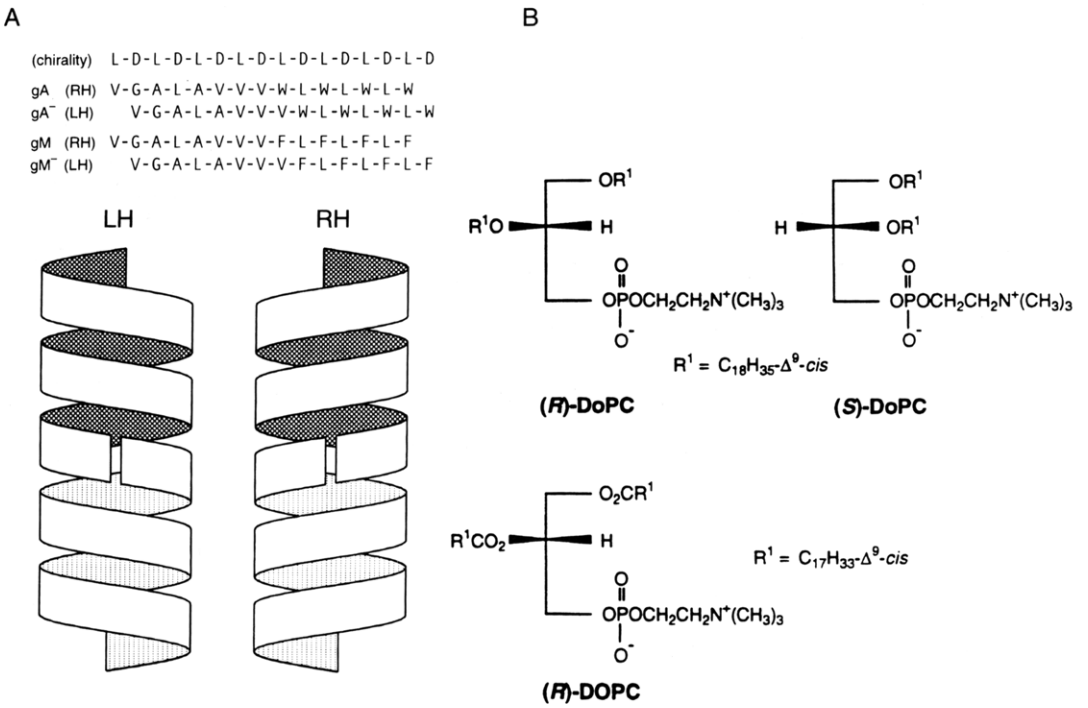

FIGURE 1: Structures of the phospholipids and gramicidin analogues used in this study. (A) Primary sequences (top) of the gramicidin analogues (one-letter code; the NH_2 terminal formyl group and the COOH terminal ethanol amide group are not shown) and schematic representation (bottom) of left-handed and right-handed single-stranded $\beta^{6.3}$-helical dimers. (B) Structures of (*R*)-DoPC (left), (*S*)-DoPC (right), and (*R*)-DOPC (bottom).

analogues form single-stranded $\beta^{6.3}$-helical channels. gA^- and gM^- form channels that have opposite handedness from the channels formed by gA^+ and gM^+ (Koeppe et al., 1992; M. D. Becker, O. S. Andersen, D. V. Greathouse, and R. E. Koeppe, II, unpublished observations). gA^+ and gM^+ form right-handed channels; gA^- and gM^- form left-handed channels. The gA (gA^+ and gA^-) indole NH moieties form hydrogen bonds with polar groups at the membrane/solution interface; a similar interaction is not possible for gM (gM^+ and gM^-), which has no Trp residues.

The membranes were formed from the enantiomeric ether phospholipids, (*R*)-dioleylphosphatidylcholine [(*R*)-DoPC] and (*S*)-dioleylphosphatidylcholine [(*S*)-DoPC], and from the ester lipid (*R*)-dioleoylphosphatidylcholine (DOPC). Figure 1 shows schematic representations of the gramicidin analogues, as well as of the phospholipids. This combination of gramicidin analogues and phospholipids allows for a determination of whether channel function is affected by the molecular characteristics of the membrane/solution interface. The results show no evidence for chiral interactions between either type of gramicidin analogue and the phospholipid backbone. Moreover, the average channel durations are longer in DoPC bilayers than in DOPC bilayers. We therefore conclude that hydrogen-bonded interactions between the Trp side chains and the ester C=O groups are not important for channel function.

A preliminary report of some of these results has appeared previously (Providence et al., 1991).

[1] Abbreviations: gA^+, gramicidin A; gA^-, gramicidin A enantiomer; gM^+, [Phe^{9,11,13,15}]gA^+; gM^-, gM^+ enantiomer; (*S*)-DoPC, (*S*)-dioleylphosphatidylcholine; (*R*)-DoPC, (*R*)-dioleylphosphatidylcholine; DOPC, (*R*)-dioleoylphosphatidylcholine; HPLC, high-performance liquid chromatography; tBOC, *tert*-butoxycarbonyl function; f_m, minichannel frequency; τ, average channel duration; DPPC, (*R*)-dipalmitoylphosphatidylcholine; DpPC, (*R*)-dipalmitylphosphatidylcholine.

MATERIALS AND METHODS

gA^+ was purified from the naturally occurring mixture of gramicidin A, B, and C by high-performance liquid chromatography (HPLC) as previously described (Koeppe & Weiss, 1981). gM^- (Heitz et al., 1982) was a generous gift from Dr. F. Heitz (CNRS, Montpellier, France) and Y. Trudelle (CNRS, Orleans, France). gA^- (Koeppe et al., 1992) and gM^+ were synthesized by solid-phase peptide synthesis using tBOC chemistry and purified by HPLC as previously described (Becker et al., 1991; Koeppe et al., 1992). (*R*)-DoPC and (*S*)-DoPC were synthesized in a synthetic scheme starting with (*R*)- and (*S*)-oxiranemethanol *tert*-butyldiphenylsilyl ethers, respectively (Guivisdalsky & Bittman, 1989). The epoxide ring was opened with oleyl alcohol in the presence of boron trifluoride etherate; the ring-opened product was alkylated with oleyl triflate using NaOH in tetrahydrofuran (at 0 °C to room temperature). Desilylation was carried out by using tetra-*n*-butylammonium fluoride in tetrahydrofuran at room temperature. The phosphocholine group was introduced by using 2-chloro-2-oxo-1,2,3-dioxaphosphalane at 0 °C, followed by the opening of the cyclic phosphate with trimethylamine in acetonitrile at 65−70 °C in a pressure tube. The enantiomeric excess was >94%. The purity was checked using thin-layer chromatography with $CHCl_3/CH_3OH/H_2O$ (65:25:4); R_f for either DoPC was 0.57. DOPC (>99% pure) was from Avanti Polar Lipids, Inc. (Alabaster, AL), and was used as supplied. *n*-Decane (>99.9% pure) was from Wiley Organics (Coshocton, OH) and was used without further purification. NaCl was analytical grade from EM Science (Cherry Hill, NJ) and was roasted at 500 °C for 24 h and stored over $CaSO_4$ in an evacuated desiccator. Water was deionized with Milli-Q water (Millipore Corp., Bedford, MA). Ethanol was from U.S. Industrial Chemicals (Tuscola, IL).

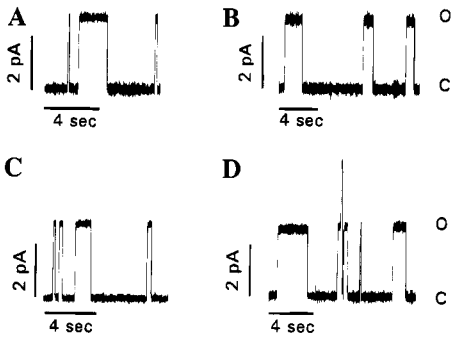

FIGURE 2: gA channels in DoPC membranes. Single-channel current traces obtained with gA^+ (top row) and gA^- (bottom row) in (*S*)-DoPC/*n*-decane membranes (left column) and (*R*)-DoPC/*n*-decane membranes (right column). The different appearance of the current traces is due to uncontrollable mechanical vibrations: 200 mV, 100 Hz, 1.0 M NaCl.

Planar bilayers were formed from *n*-decane solutions (2.5% w/v) of the different phospholipids across a hole (~1.6 mm diameter) in a Teflon partition that separates two aqueous solutions of unbuffered 1.0 M NaCl (for gA^+ and gA^-) or CsCl (for gM^+ and gM^-). The salt solutions were prepared fresh each day. Single-channel experiments were done at 25 ± 1 °C using the bilayer punch technique with pipet tip diameters ~30 μm (Andersen, 1983) and a Dagan 3900 patch clamp amplifier (Dagan Instruments, Minneapolis, MN). The gramicidins were added to the electrolyte solution on both sides of the membrane from ethanolic stock solutions. The amount of gramicidin added was adjusted to give a channel appearance rate of about one event per second. Single-channel current transitions were detected using a PC/AT-compatible computer using AxoBasic (Axon Instruments, Foster City, CA) and employing the algorithm described by Andersen (1983). The applied potential was 200 mV. Single-channel current transition amplitude histograms and duration histograms were constructed as previously described (Andersen, 1983; Sawyer et al., 1989; Durkin et al., 1990).

The distribution of single-channel current transitions can be characterized by the minichannel frequency (f_m), the percentage of transitions that fall outside the major peak in the histogram [cf. Busath et al. (1987)]:

$$f_m = \frac{100 t_m}{t_m + t_s} \quad (1)$$

where t_m and t_s denote the number of transitions that fall outside and within the major peak in the histogram, respectively. The duration distributions were determined only for the channel events in the major peak of the current transition amplitude histograms. The duration histograms were transformed into survivor distributions, and the average durations (τ) were determined by fitting a single exponential distribution, $N(t) = N(0) \exp(-t/\tau)$, where $N(t)$ denotes the number of channels with a duration longer than time t, to each histogram (Sawyer et al., 1989; Durkin et al., 1990).

RESULTS

Experiments with Dioleylphosphatidylcholines. For either pair of gramicidin analogues, gA^+ and gA^- or gM^+ and gM^-, the single-channel characteristics are very similar in bilayers formed from either (*S*)-DoPC or (*R*)-DoPC (Figures 2–6).

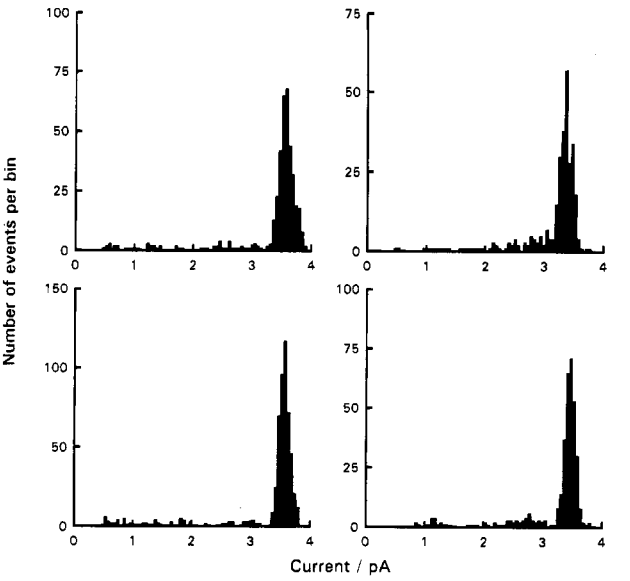

FIGURE 3: gA channels in DoPC membranes. Current transition amplitude histograms for gA^+ channels (top row) and gA^- channels (bottom row) in (*S*)-DoPC/*n*-decane membranes (left column) and (*R*)-DoPC/*n*-decane membranes (right column). The average currents and standard deviations for the channels in the main peak and minichannel frequencies are summarized in Table 1A: 200 mV, 100 Hz, 1.0 M NaCl.

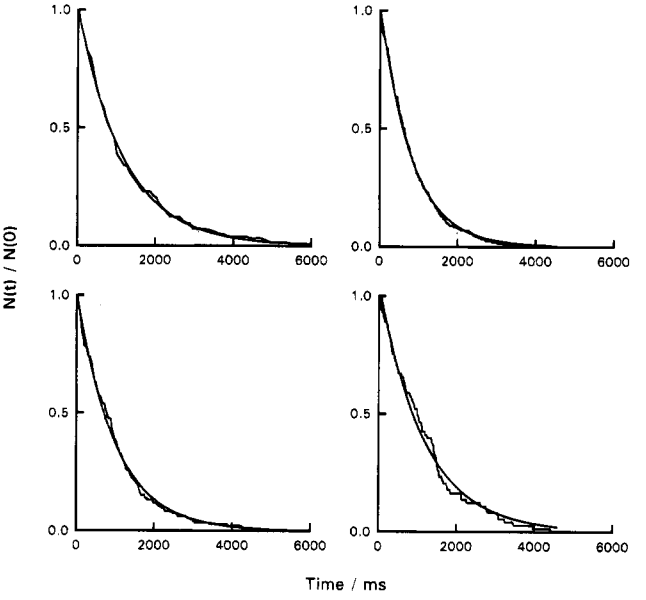

FIGURE 4: gA channels in DoPC membranes. Duration distributions, represented as normalized duration histograms for gA^+ channels (top row) and gA^- channels (bottom row) in (*S*)-DoPC/*n*-decane membranes (left column) and (*R*)-DoPC/*n*-decane membranes (right column). The jagged curves denote the experimental results, and the smooth curves denote the fit of a single exponential distribution to the results. The average durations and standard deviations are summarized in Table 1B: 200 mV, 100 Hz, 1.0 M NaCl.

[For technical reasons, the experiments with gM^+ and gM^- were done in CsCl, as the single-channel conductance in CsCl is much higher than it is in NaCl (M. D. Becker and O. S. Andersen, unpublished observations).]

Figure 2 shows current traces for gA^+ channels (Figure 2A,C) and gA^- channels (Figure 2B,D) in (*S*)-DoPC/*n*-decane (Figure 2A,B) or (*R*)-DoPC/*n*-decane (Figure 2C,D) membranes (similar results were obtained with gM^+ and gM^- but are not shown). The general similarity among the four different experimental situations was further substantiated

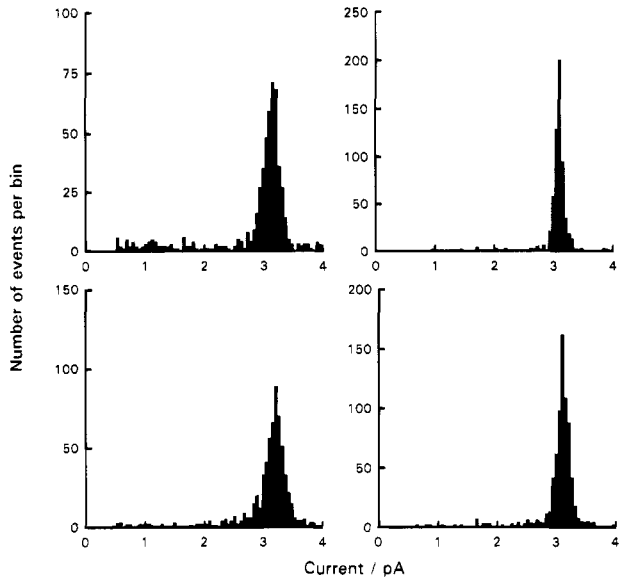

FIGURE 5: gM channels in DoPC membranes. Current transition amplitude histograms for gM^+ channels (top row) and gM^- channels (bottom row) in (*S*)-DoPC/*n*-decane membranes (left column) and (*R*)-DoPC/*n*-decane membranes (right column). The average currents and standard deviations for the channels in the main peak and minichannel frequencies are summarized in Table 2A: 200 mV, 100 Hz, 1.0 M CsCl.

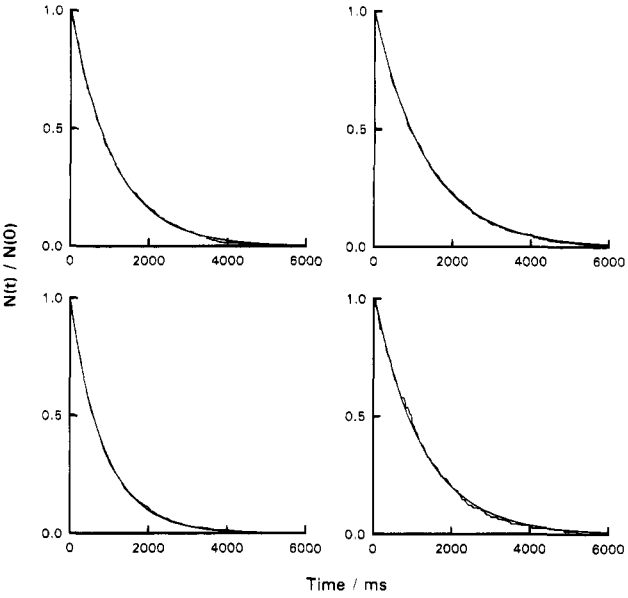

FIGURE 6: gM channels in DoPC membranes. Duration distributions, represented as normalized duration histograms for gM^+ channels (top row) and gM^- channels (bottom row) in (*S*)-DoPC/*n*-decane membranes (left column) and (*R*)-DoPC/*n*-decane membranes (right column). The jagged curves denote the experimental results, and the smooth curves denote the fit of a single exponential distribution to the results. The average durations and standard deviations are summarized in Table 2B: 200 mV, 100 Hz, 1.0 M CsCl.

in current transition amplitude histograms (Figures 3 and 5) and duration distributions (Figures 4 and 6).

For either pair of analogues, the channels are indistinguishable (as is also evident from Tables 1 and 2 that summarize the results). In addition, the channel-forming potency does not vary among the different gramicidin/lipid combinations that we tested because, for a given gramicidin, the concentration needed to achieve a channel appearance rate of $\sim 1\ s^{-1}$ varied by less than a factor of 2 between

Table 1: gA Channels in DoPC Membranes

gramicidin analogue	phospholipid	
	(*S*)-DoPC	(*R*)-DoPC
	A. Single-channel currents[a]	
gA^+	3.58 ± 0.12 pA (86%)	3.36 ± 0.12 pA (83%)
gA^-	3.55 ± 0.09 pA (84%)	3.44 ± 0.09 pA (80%)
	B. Average durations[b]	
gA^+	1200 ± 60 ms	1070 ± 60 ms
gA^-	1000 ± 120 ms	1190 ± 180 ms

[a] Mean ± standard deviation for the channels in the main peak (minichannel frequency). [b] Mean ± standard deviation, 1.0 M NaCl, 200 mV.

Table 2: gM Channels in DoPC Membranes

gramicidin analogue	phospholipid	
	(*S*)-DoPC	(*R*)-DoPC
	A. Single-channel currents[a]	
gM^+	3.18 ± 0.14 pA (76%)	3.15 ± 0.08 pA (90%)
gM^-	3.23 ± 0.17 pA (84%)	3.16 ± 0.12 pA (86%)
	B. Average durations[b]	
gM^+	1240 ± 180 ms	1370 ± 260 ms
gM^-	920 ± 150 ms	1190 ± 210 ms

[a] Mean ± standard deviation for the channels in the main peak (minichannel frequency). [b] Mean ± standard deviation, 1.0 M CsCl, 200 mV.

Table 3: Gramicidin Channels in DOPC Membranes

enantiomer	DOPC	
	gA	gM
	A. Single-channel currents[a]	
g^+	2.70 ± 0.06 pA (87%)	1.55 ± 0.06 pA (97%)
g^-	2.73 ± 0.06 pA (83%)	1.46 ± 0.10 pA (96%)
	B. Average durations[b]	
g^+	480 ± 90 ms	560 ± 70 ms
g^-	450 ± 120 ms	430 ± 60 ms

[a] Mean ± standard deviation for the channels in the main peak (minichannel frequency). [b] Mean ± standard deviation, 1.0 M NaCl (gA) or 1.0 M CsCl (gM), 200 mV.

membranes formed from either (*S*)-DoPC or (*R*)-DoPC (not shown). Taken together, these results therefore show no evidence for specific interactions between gramicidin channels and the phospholipids in the bilayer.

Experiments with Dioleoylphosphatidylcholine. In order to determine whether the lack of specific interactions could be due to the use of DoPC (and the absence of the ester C=O group), we also examined the behavior of channels formed by the two pairs of gramicidin analogues, gA^+ and gA^- and gM^+ and gM^-, in bilayers formed by the ester phospholipid (*R*)-DOPC (Figures 7 and 8).

Again, the current traces (Figures 7A and 8A), current transition amplitude histograms (Figures 7B and 8B), and duration distributions (Figures 7C and 8C) are indistinguishable for channels formed by either set of enantiomeric analogues (see also Table 3). Moreover, the channel-forming potencies for either set of enantiomers in DoPC and DOPC bilayers are similar (results not shown).

We have results only for (*R*)-DOPC. Nevertheless, the indistinguishable behavior of gA^+ and gA^- channels, and of gM^+ and gM^- channels, provides no support for specific interactions between gramicidin channels and the phospholipid backbone.

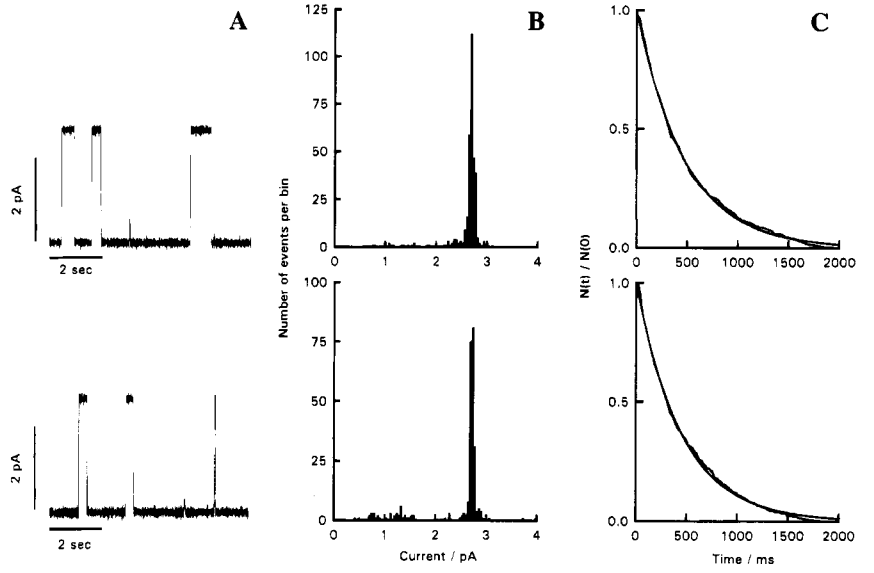

FIGURE 7: gA channels in DOPC membranes. Top row, gA^+; bottom row, gA^-. (A) Single-channel current traces. The different appearance of the current traces is due to uncontrollable mechanical vibrations. (B) Current amplitude transition histograms. The average currents, standard deviations, and minichannel frequencies are summarized in Table 3A. (C) Duration distributions. The jagged curves denote the experimental results, and the smooth curves denote the fit of a single exponential distribution to the results. The average durations and standard deviations are summarized in Table 2B: 200 mV, 100 Hz, 1.0 M NaCl.

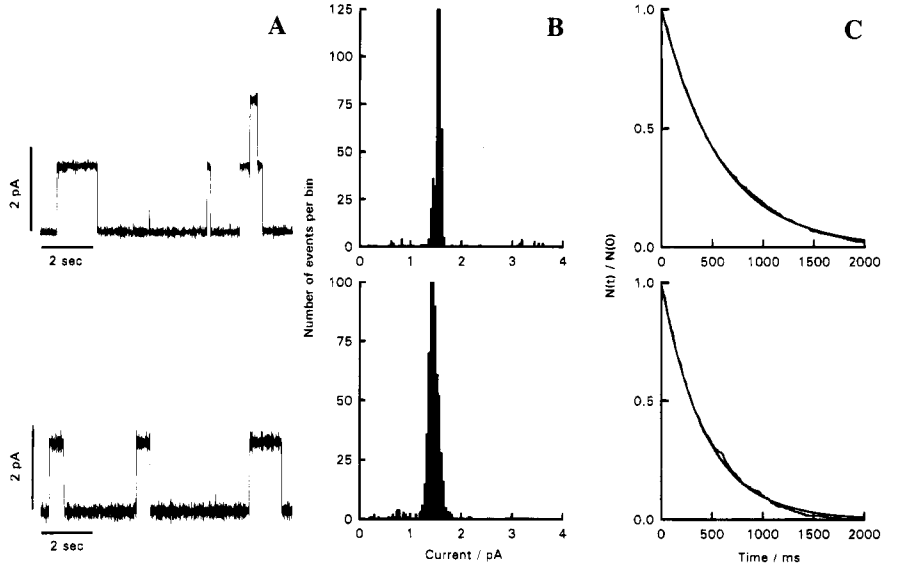

FIGURE 8: gM channels in DOPC membranes. Top row, gM^+; bottom row, gM^-. (A) Single-channel current traces. The different appearance of the current traces is due to uncontrollable mechanical vibrations. (B) Current amplitude transition histograms. The average currents and standard deviations for the channels in the main peak and minichannel frequencies are summarized in Table 3A. (C) Duration distributions. The jagged curves denote the experimental results, and the smooth curves denote the fit of a single exponential distribution to the results. The average durations and standard deviations are summarized in Table 2B: 200 mV, 100 Hz, 1.0 M CsCl.

DISCUSSION

We have compared the behavior of channels formed by enantiomeric gramicidin analogues in lipid bilayers formed by enantiomeric dialkylphosphatidylcholines. We tested all combinations of peptide and lipid chirality, and there were no systematic changes in channel function that could be ascribed to chiral interactions between gramicidin channels and the phospholipid backbone. This conclusion is in general agreement with that reached by Wade et al. (1990), who compared the antibiotic and channel-forming potencies of the natural L enantiomers with those of the synthetic D enantiomers of cecropin A, magainin 2 amide, and mellitin in bilayers formed from (*R*)-phospholipids and concluded that there was no stereoselective interaction between the antibacterial peptides and the membrane lipids (or some other receptor). Moreover, by comparing the behavior of gA and gM channels in membranes formed by DoPC and DOPC, we conclude that the fatty acid/glycerol ester C=O groups cannot be important for channel stability.

We first discuss the single-channel conductances and then the changes in average duration. Finally, we discuss previous proposals for specific interactions between gramicidin channels and the host bilayer that invoke indole NH to ester C=O hydrogen bonding.

Conductance Changes. The side chains of gramicidin channels extend 5−10 Å from the ion permeation path (Arseniev et al., 1986; Ketchem et al., 1993; Koeppe et al., 1994). Polar amino acid substitutions can induce large changes in the channels' permeability characteristics (Heitz et al., 1982; Mazet et al., 1984; Russell et al., 1986; Koeppe

et al., 1990; Becker et al., 1991; Fonseca et al., 1992) and average durations (Mazet et al., 1984; Russell et al., 1986; Becker et al., 1991; Fonseca et al., 1992), which result primarily from electrostatic interactions between the side chain dipoles and the permeant ions (Koeppe et al., 1990). The magnitude of these electrostatic interactions depends on the average orientation of the side chains relative to the pore (Etchebest & Pullman, 1985; Sancho & Martinez, 1991), and presumably also on the side chain dynamics [the ability of the side chains to readjust in response to ion movement through the pore (Mazet et al., 1984)]. Changes in the average orientation will be reflected as changes in the single-channel conductance.

The Trp residues at positions 9, 11, 13, and 15 are particularly important, as the single-channel conductance may vary (by up to 20-fold) when one or more of the Trp residues are replaced by Phe (Heitz et al., 1982; Becker et al., 1991; Fonseca et al., 1992). The Trp indole NH moieties form hydrogen bonds with groups at the membrane/solution interface (O'Connell et al., 1990; Takeuchi et al., 1990). This hydrogen-bonded link to the interface is important for the channels' conformational preference (O'Connell et al., 1990; Becker et al., 1991; Durkin et al., 1992). The single-channel conductances do not vary among the four combinations of gA and ether phospholipids (Table 1) or between gA^+ and gA^- channels in DOPC membranes (Table 3). The average orientations of the Trp side chains with respect to the pore axis are therefore similar among the four gA/ether phospholipid combinations and the two gA/ester phospholipid combinations. Hydrogen bond interactions between the indole NH's and the phospholipid C=O groups would depend on the channels' helix sense, because the stereochemical relation of the gramicidin helix with the phospholipid would be different for left-handed and right-handed channels. The invariant single-channel conductance within each of the respective experimental sets shows that such interactions cannot be important for channel structure and function.

Even though the indole NH moieties do not appear to form hydrogen bonds with the ester C=O groups, the C=O groups are important for the permeability characteristics of gA (and gM) channels, as shown by the higher conductances of channels in DoPC bilayers as compared to those of channels in DOPC bilayers (cf. Tables 1−3). These conductance changes arise from an interfacial dipole potential that is ~100 mV more positive for monolayers and bilayers of diacylphospholipids as compared to dialkylphospholipids (Paltauf et al., 1971; Gawrisch et al., 1992). The difference in interfacial dipole potential arises because the dipole moment associated with the C=O group of the *sn*-2 chain in diacylphosphatidylcholines is directed away from the hydrocarbon chains (Pearson & Pascher, 1979) toward the aqueous phase, where the oxygen can form hydrogen bonds with water (Schmidt et al., 1977). [The dipole moment of the C=O group of the *sn*-1 chain is in the plane of the membrane (Pearson & Pascher, 1979), and the C=O group is less accessible to water (Schmidt et al., 1977).] The more positive dipole potential in bilayers formed from diacylphosphatidylcholines will increase the energy barrier for cation movement through the channel interior (Jordan, 1983), which will reduce the single-channel conductance. In addition, the relative conductance decrease (associated with a DoPC → DOPC exchange) is determined by the relative importance of the energy barrier for ion movement through the channel interior, as compared to the barrier for ion dissociation from the channel. This accounts for the larger conductance changes seen with gM channels, which are due to a higher energy barrier for ion movement through the interior of gM channels as compared to ion movement throught the interior of gA channels [cf. Fonseca et al. (1992)].

Duration Changes. For both gA and gM channels, the average durations are less in DOPC bilayers than in DoPC bilayers (Tables 1−3). Remarkably, the relative decreases are similar for gM and gA channels, which shows that the alterations in average duration cannot result from different interactions between the indole NH moieties and the backbone of DOPC or DoPC. Instead, these results suggest instead that the change in channel duration is a general effect, which could arise from altered lipid packing in the membrane. But we cannot exclude that the decreased average channel duration in DOPC bilayers may be due to unfavorable interactions between the π-electrons of the aromatic residues in the gramicidin channels and the C=O groups (in particular the *sn*-1 C=O groups, which are localized deeper in the membrane).

In any case, there is little evidence for altered packing of diacylphosphatidylcholine and dialkylphosphatidylcholine bilayers in the liquid−crystalline phase. The gel → liquid crystalline transition temperature is ~4 °C higher for diacylphosphatidylcholine bilayers than for dialkylphosphatidylcholine bilayers having the same hydrocarbon chain length (Caffrey, 1993). In the liquid−crystalline phase, however, DoPC and DOPC bilayers should be very similar. The limiting area/molecule in monolayers at the air/electrolyte interface is indistinguishable (Paltauf et al., 1971); the general organization of the polar head groups in dialkyl- and diacylphosphatidylcholines is very similar (Hauser et al., 1981), and the permeability properties of phospholipid vesicles made from dialkylphospholipids or diacylphospholipids are comparable (Clejan et al., 1979; Bittman et al., 1981).[2]

The question of gramicidin channel function in membranes formed from ester or ether lipids has been examined by Seoh and Busath (1995) using glyceromonooleate and glyceromonooleyl bilayers. In that system, the average duration of gA^+ channels was higher in glyceromonooleate bilayers than in glyceromonooleyl bilayers, a result that is in contrast with the present findings. We do not understand why, but note that gA is likely to interact differently with phospholipids than with monoglycerides [as may be inferred from the results of Fonseca et al. (1992)] and that an ester → ether transformation may affect the physical properties of bilayers formed from monoglycerides and phospholipids differently.

Gramicidin Phospholipid Interactions. Wallace (1986) proposed that the conformational stability of gramicidin channels varied between diacyl- and dialkylphospholipids. It was proposed that channel stability was determined, in part, from specific interactions between the ethanolamide CO-termini (at the membrane/solution interface) and the membrane lipids. The present results, however, suggest that

[2] Clejan et al. (1979) and Bittman et al. (1981) also found that cholesterol had very similar effects on the permeability properties of dialkylphospholipid and diacylphospholipid vesicles, which indicates that the cholesterol OH group does not form a hydrogen bond with the C=O group in diacylphospholipids.

specific interactions between gramicidin channels and their lipid environment cannot be of major importance (with the possible exception of an electrostatic repulsion between the aromatic π-electrons and the C=O groups in diacylphosphatidylcholines). The near identity of the gA structure in sodium dodecyl sulfate micelles (Arseniev et al., 1986) and phospholipid bilayers (Ketchem et al., 1993) similarly argues against specific channel/lipid interactions.

Meulendijks et al. (1989) proposed, on the basis of a molecular-modeling argument, that the rotameric state of Trp^{15} could be determined by the formation of hydrogen bonds between the indole NH moiety and the C=O group of the *sn*-2 acyl chain. It was further proposed that this interaction is important for channel function. To test this proposal, they measured the rate of Na^+ loss (Na^+/Li^+ exchange) through gA^+ channels in large unilamellar vesicles (diameter, ~100 nm) formed from either dipalmitoylphosphatidylcholine (DPPC) or dipalmitylphosphatidylcholine (DpPC). The apparent Na^+ permeability coefficient was 30% less in vesicles formed from the DpPC than in vesicles formed from the DPPC (Meulendijks et al., 1989). This result was interpreted to mean that hydrogen bonding between the indole NH moiety (of Trp^{15}) and the *sn*-2 C=O is important for channel stability.

This interpretation is problematic. As shown in the Appendix, the rate constant for Na^+ loss from the population of lipid vesicles is only $^1/_{10^5}$ of that for Na^+ movement through gramicidin channels. The ensemble-averaged time course of Na^+ efflux from phospholipid vesicles therefore is determined by the slow formation of gA channels [due to a slow transfer of gA across phospholipid bilayers (O'Connell et al., 1990)], as well as from a slow exchange of gA between the vesicles (Loew et al., 1983). The slower efflux rate constant in DpPC vesicles, therefore, is most likely due to a slower exchange of gA between DpPC vesicles than between DPPC vesicles, presumably because of more favorable interactions between gA and DpPC than between gA and DPPC. This conclusion is in qualitative agreement with our finding that the average channel duration is longer in DoPC/*n*-decane than in DOPC/*n*-decane bilayers.

Recently, Chen and Gross (1995) have suggested that gA^+ may interact differently with plasmenylcholine, as compared to phosphatidylcholine bilayers, on the basis of measurements of the rate of K^+/Li^+ exchange through lipid vesicle-incorporated gA^+ channels. Using stopped flow methods, the rate constant for K^+/Li^+ exchange was found to be ~50% higher for channels incorporated into vesicles composed of the plasmenylcholine 1-hexadec-1′-enyl-2-oleoylphosphatidylcholine (~18 s^{-1}) as compared to 1-palmitoyl-2-oleoylphosphatidylcholine (~12 s^{-1}). This difference in exchange rate could result from a difference in single-channel conductance or in ion selectivity (which would alter the membrane potential and thus the exchange rate). In order to resolve this question further, one would need to examine the properties of gA channels in planar bilayers formed by these different lipids.

Conclusions and Implications for Membrane Protein Function. The major conclusion of this study is that phospholipid chirality has no effect on gramicidin channel function. Moreover, the ester C=O groups in diacylphospholipids do not interact with the indole NH moieties in gramicidin channels. The membrane/solution interface therefore seems to organize the channel structure because of general characteristics of the nonpolar/polar transition at the interface rather than because of specific channel/lipid backbone interactions. The interfacial localization of Trp residues in integral membrane proteins [cf. Andersen & Koeppe (1992)] is likely to result from a similar organizing principle.

ACKNOWLEDGMENT

We thank J. Girshman for a critical reading of the manuscript and D. D. Busath for helpful discussions. We also wish to acknowledge helpful comments and feedback on the gramicidin listserver (c/o D. D. Busath, Brown University).

APPENDIX

Analysis of Meulendijks et al. (1989). The radius of large unilamellar phospholipid vesicles is ~50 nm, and the internal volume is $\sim 5 \times 10^{-16}$ cm^3. The Na^+ concentration was 0.1 M, such that each vesicle contained $\sim 3 \times 10^4$ Na^+ ions. The single-channel conductance (g) is ~5 pS in 0.1 M NaCl and ~1 pS in 0.1 M LiCl. The unidirectional flux (j) of Na^+ or Li^+ through the channel can be approximated by (Hodgkin, 1951)

$$j = \frac{kT}{e^2} \times g \qquad (2)$$

where k is Boltzmann's constant, T the temperature in Kelvin, and e the elementary charge. (We neglect the diffusion potential that is established by the different ion compositions of the extracellular and intravesicular spaces). $kT/e^2 \approx 1.6 \times 10^{17}$ s^{-1}, and j will be $\sim 8 \times 10^5$ ions/s (for a channel with $g = 5$ pS). The formation of one gramicidin channel in a phospholipid vesicle therefore will equilibrate the intravesicular solution with the extracellular solution in less than 0.1 s, which should be compared to the average duration of gA channels in hydrocarbon-depleted phosphatidylcholine membranes, ~300 s (Sawyer et al., 1990a).

Meulendijks et al. (1989) estimated that there were about four gA channels per vesicle; but the Na^+ efflux observed by Meulendijks et al. (1989) occurred over many minutes (the rate constants were $\sim 4 \times 10^{-4}$ s^{-1} in DPPC and $\sim 3 \times 10^{-4}$ s^{-1} in DpPC vesicles), after a poorly resolved early phase. The numerical discrepancy between the observed and the predicted Na^+ efflux rates is $\sim 10^5$, which shows that the time course of Na^+ efflux cannot be determined by the rate constant for Na^+ movement through gA channels.

REFERENCES

Andersen, O. S. (1983) *Biophys. J. 41*, 119−133.
Andersen, O. S., & Koeppe, R. E., II. (1992) *Physiol. Rev. 72*, S89−S158.
Arseniev, A. S., Lomize, A. L., Barsukov, I. L., & Bystrov, V. F. (1986) *Biol. Membr. 3*, 1077−1104.
Becker, M. D., Greathouse, D. V., Koeppe, R. E., II, & Andersen, O. S. (1991) *Biochemistry 30*, 8830−8839.
Bittman, R., Clejan, S., Jain, M. K., Deroo, P. W., & Rosenthal, A. F. (1981) *Biochemistry 20*, 2790−2795.
Busath, D. D. (1993) *Annu. Rev. Physiol. 55*, 473−501.
Busath, D. D., Andersen, O. S., & Koeppe, R. E., II. (1987) *Biophys. J. 51*, 79−88.
Caffrey, M. (1993) *Lipidat. A database of thermodynamic data and associated information on lipid mesomorphic and polymorphic transitions*, CRC Press, Boca Raton, FL.
Chen, X., & Gross, R. W. (1995) *Biochemistry 34*, 7356−7364.

Clejan, S., Bittman, R., Deroo, P. W., Isaacson, Y. A., & Rosenthal, A. F. (1979) *Biochemistry 18*, 2118−2125.
Durkin, J. T., Koeppe, R. E., II, & Andersen, O. S. (1990) *J. Mol. Biol. 211*, 221−234.
Durkin, J. T., Providence, L. L., Koeppe, R. E., II, & Andersen, O. S. (1992) *Biophys. J. 62*, 145−159.
Elliott, J. R., Needham, D., Dilger, J. P., & Haydon, D. A. (1983) *Biochim. Biophys. Acta 735*, 95−103.
Etchebest, C., & Pullman, A. (1985) *J. Biomol. Struct. Dyn. 2*, 859−870.
Fonseca, V., Daumas, P., Ranjalahy-Rasoloarijao, L., Heitz, F., Lazaro, R., Trudelle, Y., & Andersen, O. S. (1992) *Biochemistry 31*, 5340−5350.
Froehlich, O. (1979a) *J. Membr. Biol. 48*, 365−383.
Froehlich, O. (1979b) *J. Membr. Biol. 48*, 385−401.
Gawrisch, K., Ruston, D., Zimmerberg, J., Parsegian, V. A., Rand, R. P., & Fuller, N. (1992) *Biophys. J. 61*, 1213−1223.
Guivisdalsky, P. N., & Bittman, R. (1989) *J. Org. Chem. 54*, 4637−4642.
Hauser, H., Guyer, W., & Paltauf, F. (1981) *Chem. Phys. Lipids 29*, 103−120.
Heitz, F., Spach, G., & Trudelle, Y. (1982) *Biophys. J. 40*, 87−89.
Hodgkin, A. L. (1951) *Biol. Rev. 26*, 339−409.
Hu, W., Lee, K.-C., & Cross, T. A. (1993) *Biochemistry 32*, 7035−7047.
Jordan, P. C. (1983) *Biophys. J. 41*, 189−195.
Ketchem, R. R., Hu, W., & Cross, T. A. (1993) *Science 261*, 1457−1460.
Killian, J. A. (1992) *Biochim. Biophys. Acta 1113*, 391−425.
Koeppe, R. E., II, & Weiss, L. B. (1981) *J. Chromatogr. 208*, 414−418.
Koeppe, R. E., II, Mazet, J.-L., & Andersen, O. S. (1990) *Biochemistry 29*, 512−520.
Koeppe, R. E., II, Providence, L. L., Greathouse, D. V., Heitz, F., Trudelle, Y., Purdie, N., & Andersen, O. S. (1992) *Proteins 12*, 49−62.
Koeppe, R. E., II, Killian, J. A., & Greathouse, D. V. (1994) *Biophys. J. 66*, 14−24.
Kolb, H. A., & Bamberg, E. (1977) *Biochim. Biophys. Acta 464*, 127−141.
Loew, L. M., Rosenberg, I., Bridge, M., & Gitler, C. (1983) *Biochemistry 22*, 837−844.
Lundbæk, J. A., & Andersen, O. S. (1994) *J. Gen. Physiol. 104*, 645−673.
Mazet, J. L., Andersen, O. S., & Koeppe, R. E., II. (1984) *Biophys. J. 45*, 263−276.
Meulendijks, G. H. W. M., Sonderkamp, T., Dubois, J. E., Nielen, R. J., Kremers, J. A., & Buck, H. M. (1989) *Biochim. Biophys. Acta 979*, 321−330.
Neher, E., & Eibl, H. (1977) *Biochim. Biophys. Acta 464*, 37−44.
O'Connell, A. M., Koeppe, R. E., II, & Andersen, O. S. (1990) *Science 250*, 1256−1259.
Paltauf, F., Hauser, H., & Phillips, M. C. (1971) *Biochim. Biophys. Acta 249*, 539−547.
Pearson, R. H., & Pascher, I. (1979) *Nature 281*, 499−501.
Providence, L. L., Andersen, O. S., Bittman, R., & Koeppe, R. E., II. (1991) *Biophys. J. 59*, 321a.
Russell, E. W. B., Weiss, L. B., Navetta, F. I., Koeppe, R. E., II, & Andersen, O. S. (1986) *Biophys. J. 49*, 673−686.
Sancho, M., & Martinez, G. (1991) *Biophys. J. 60*, 81−88.
Sawyer, D. B., Koeppe, R. E., II, & Andersen, O. S. (1989) *Biochemistry 28*, 6571−6583.
Sawyer, D. B., Oiki, S., & Andersen, O. S. (1990) *Biophys. J. 57*, 100a.
Schmidt, C. F., Barenholz, Y., Huang, C., & Thompson, T. E. (1977) *Biochemistry 16*, 3948−3954.
Seoh, S.-A., & Busath, D. (1995) *Biophys. J. 68*, 2271−2279.
Takeuchi, H., Nemoto, Y., & Harada, I. (1990) *Biochemistry 29*, 1572−1579.
Tredgold, R. H., Hole, P. N., Sproule, R. C., & Elgamal, M. (1977) *Biochim. Biophys. Acta 471*, 189−194.
Wade, D., Boman, A., Wahlin, B., Drain, C. M., Andreu, D., Boman, H. G., & Merrifield, R. B. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 4761−4765.
Wallace, B. A. (1986) *Biophys. J. 49*, 295−306.

BI9517897