Synthesis of Minigramicidin Ion Channels and Test of Their Hydrophobic Match with the Membrane

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KEYWORDS:

hydrophobic mismatch · ion channels · membranes · peptides · synthesis design

HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu- L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHCH$_2$CH$_2$OH

gramicidin A

HCO-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHCH$_2$CH$_2$OR

1 R = SiPh$_2$*t*Bu
2 R = H

L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHCH$_2$CH$_2$OR
L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHCH$_2$CH$_2$OR

3 R = SiPh$_2$*t*Bu
4 R = H

Scheme 1. *Gramicidin A and the minigramicidins **1** – **4**.*

The question of how the function of an integral membrane protein depends on its phospholipid-bilayer environment is important in biological chemistry.[1] In particular, the interplay of the lipid-bilayer thickness and a membrane protein in terms of their hydrophobic match or mismatch is of current interest.[2] Ion-channel-active proteins are especially suited to correlate biological function (for example, single-channel characteristics) with a change in membrane properties.[3] Here we present the synthesis of novel ion channels derived from the gramicidin A motif[4] and report on a remarkable change in channel behavior upon variation of the lipid-bilayer thickness.

Gramicidin A (gA) is a peptide formed from 15 amino acid residues with formyl and aminoethanol as the N and C termini, respectively (Scheme 1).[5] The alternation of L- and D-amino acids favors the formation of β-helical secondary structures.[6] The accepted ion-channel-active conformation in the membrane is a hydrogen-bridged, head-to-head dimer of two gA molecules, each having a right-handed, single-stranded $\beta^{6.3}$-helical conformation.[7] The gA channel has a size of 26 Å with a hydrophobic length of 22 Å.[7] With the goal of investigating smaller L-/D-peptides as potential ion channels, we chose the minigramicidins **1** – **4** as synthetic targets. In these compounds the functionally important D-Leu-L-Trp domain of gA is conserved, while four amino acids from the N terminus have been omitted. We report on the first synthesis and functional analysis of these novel minigramicidins.[8]

Compounds **1** and **2** consist of 11 amino acids which lead to a β-helical channel of 20 Å total length and a hydrophobic part of 17 Å based on the gA channel model. The *tert*-butyldiphenylsilyl (TBDPS) group in **1** should increase the lipophilic length and could contribute to the hydrophobic stabilization of the channel within the membrane. In compounds **3** and **4** the two channel halves are covalently linked by succinic acid.[9] This link should stabilize the active channel form relative to the monomers **1** and **2** under conditions of severe hydrophobic mismatch.

The synthesis of the minigramicidin channels used the undecamer **5**[4c] as a common building block (Scheme 2). Hydrogenolytic deprotection of **5** to **6** was best accomplished in the solvent system MeOH/DMF (10/1) at 40 °C. *N*-Formylation of **6** with HOBT/EDC afforded the monomer minigramicidin **1**. The fluoride-mediated deprotection of **1** to **2** was accomplished in the biphasic solvent system $H_2O/CH_3CN/CHCl_3$. Treatment of **6** with an excess of succinic anhydride gave the carboxylic acid **7** in 92% yield, which was coupled with the undecamer **6** to afford the covalently linked minigramicidin **3**. The deprotection of **3** to **4** was first complicated by low reactivity which may arise from aggregation phenomena.[6] But with MeOH as the solvent, removal of TBDPS occurred smoothly to give **4** in 92% yield. Compounds **1** – **4** were stringently purified by semipreparative RP8-HPLC (CH_3CN/*i*PrOH/H_2O) before their ion-channel activity was quantified.[10]

R-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHCH$_2$CH$_2$OSiPh$_2$*t*Bu

a: **5** R = Cbz → **6** R = H; b: → **1**; c: → **2**

d:

L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHCH$_2$CH$_2$OSiPh$_2$*t*Bu
OH

7

e:

3

f: → **4**

Scheme 2. *a) H_2, Pd/C (5%), MeOH/DMF (10/1), 40 °C, 4 h, 96%; b) HCOOH (6 equiv), cat. HOBT, EDC (6 equiv), EtNiPr$_2$ (6 equiv), $CHCl_3$, 0 → 20 °C, 3 h, 87%; c) 48% HF/CH_3CN/$CHCl_3$ (1/25/75), 0 → 20 °C, 4 h, 59%; d) succinic anhydride (10 equiv), cat. pyridine, CH_2Cl_2/DMF (10/1), 0 °C, 1 h, 92%; e) **6**, HATU (2 equiv), HOAT (5 equiv), EtNiPr$_2$ (4 equiv), CH_2Cl_2/DMF (3/1), 0 → 20 °C, 4 h, 89%; f) 48% HF/CH_3CN/MeOH (1/20/80), 20 °C, 16 h, 92% after silica gel filtration to remove TBDPSOH; 35% after preparative RP(8)-HPLC. Cbz = benzyloxycarbonyl, HOBT = 1-hydroxy-1H-benzotriazole, EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride, HATU = O-(7-azabenzotriazol-1-yl)-N,N-N′,N′-tetramethyluronium hexafluorophosphate, HOAT = 7-aza-1-hydroxy-1H-benzotriazole.*

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The ion-channel activity of compounds **1** – **4** in membranes of different thickness was investigated with the planar lipid-bilayer technique.[11] Three lipids, *Z*-unsaturated at C9, were used: 1,2-dioleoylphosphatidylcholine or DOPC(18:1), 1,2-dipalmitoleoylphosphatidylcholine or DPPC(16:1), and 1,2-dimyristoleoylphosphatidylcholine or DMPC(14:1). Compound **1** showed no typical single-channel events in DOPC(18:1) (Figure 1 a). With

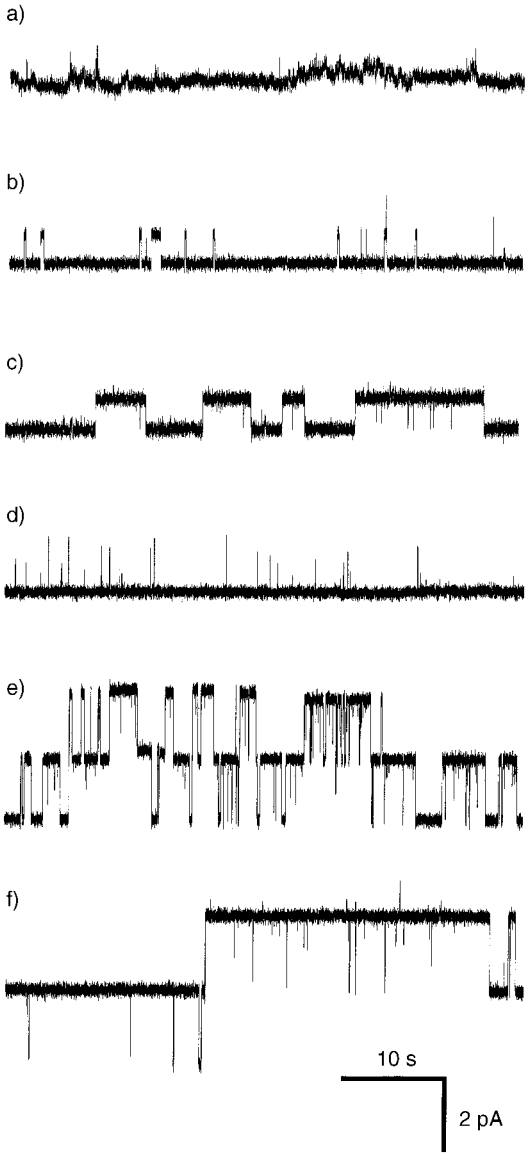

Figure 1. *Representative current traces of compounds **1** and **3** in planar lipid bilayers. a) **1** in DOPC(18:1); b) **1** in DPPC(16:1); c) **1** in DMPC(14:1); d) **3** in DOPC(18:1); e) **3** in DPPC(16:1); f) **3** in DMPC(14:1). For further details, see the Experimental Section.*

DPPC(16:1) single-channel events were observed for **1** with a mean dwell time of 211 ms and a conductance of 16 pS (Figure 1 b). The dwell time of **1** increased to 1020 ms when the lipid was changed to DMPC(14:1) but the conductance was left nearly unaffected at 17 pS (Figure 1 c). In the case of the covalently-linked compound **3** short-lived channels (15 ms, 21 pS) were detected in DOPC(18:1) (Figure 1 d). The mean channel life-times and conductances increased to 630 ms and 33 pS in DPPC(16:1), and to 3670 ms and 41 pS in DMPC(14:1) (Figures 1 e and f, respectively).

These results clearly show that **1** and **3** are potent channel formers in thin membranes, whilst they remain virtually "silent" in thick DOPC(18:1) bilayers. The covalent linker in **3** leads to a twofold increase in the channel conductivities. The deprotected compounds **2** and **4** showed a comparable activity pattern in the three phospholipids studied (**2**: no channels in DOPC(18:1), 82 ms/21 pS in DPPC(16:1), 346 ms/26 pS in DMPC(14:1); **4**: 9 ms/33 pS in DOPC(18:1), 214 ms/40 pS in DPPC(16:1), 7160 ms/47 pS in DMPC(14:1); see the Supporting Information). Again the longest dwell times for **2** and **4** were observed in DMPC(14:1). Gramicidin A (gA) formed channels in all three membranes with increased dwell times and conductivities in the shorter lipids (245 ms/39 pS in DOPC(18:1), 2445 ms/43 pS in DPPC(16:1), 20 800 ms/50 pS in DMPC(14:1); see the Supporting Information).[3a, 12]

Figure 2 gives a schematic rationalization for the results obtained. DOPC(18:1) has a hydrophobic thickness of 27 Å.[13] In this case, the hydrophobic mismatch with **1** is so strong, that no stable channel-active dimer $(\mathbf{1})_2$ is formed (Figure 2 a). Fitting the hydrophobic surface of $(\mathbf{1})_2$ in its $\beta^{6.3}$-helical conformation into the hydrophobic interior of the lipid bilayer would induce enormous strain in the membrane and is therefore energetically disfavored. By contrast, the longer gA is able to dimerize to $(gA)_2$, however with some hydrophobic mismatch leading to short dwell times (Figure 2 b).[14] The hydrophobic thickness of DMPC(14:1) can be estimated as 22 Å. In DMPC(14:1) **1** forms the channel-active dimer $(\mathbf{1})_2$ but still with some hydrophobic mismatch (Figure 2 c). For $(gA)_2$ a good hydrophobic match is now found between the channel and the lipid-bilayer environment (Figure 2 d).

The covalent linker in **3** and **4** avoids the monomer/dimer equilibrium and stabilizes the channel structure against hydrophobic mismatch. This leads to longer dwell times and a remarkable increase in the channel conductance.

The strong improvement in the performance of the minigramicidins **1** – **4** in thinner membranes shows the significance of hydrophobic matching between the peptide and its phospholipid environment. Compounds of this type could be valuable models for the biologically important process of protein sorting, for example, by selectively targeting thin-walled cell organelles.[2b] Investigations into the effect of the hydrophobic mismatch on the ion selectivity as well as structural studies to clarify the membrane-bound structure of the minigramicidins are important future topics.

Experimental Section

Planar lipid bilayers were prepared by painting a solution of 1,2-diacyl-*sn*-3-glycerophosphocholine (Avanti Polar Lipids, Alabaster, AL) and cholesterol (4.5/1 w/w) in *n*-decane over the aperture of a polystyrene cuvette with a diameter of 0.15 mm.[10] Lipid concentration in the painting solution: 25 mg of lipid per ml of *n*-decane;

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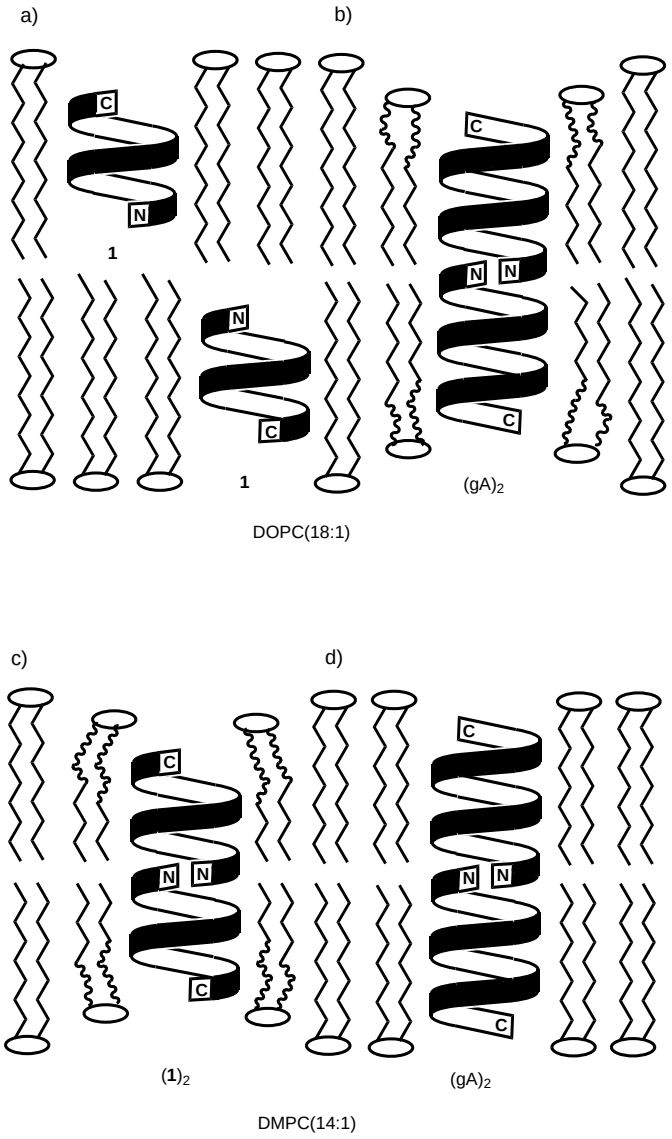

Figure 2. *Schematic representation of the channel formation of* ***1*** *and gA in membranes of different thickness. a)* ***1*** *is too short to dimerize and form* $(\mathbf{1})_2$ *in DOPC(18:1), while in (b) the longer gA forms the channel active dimer* $(gA)_2$ *with some hydrophobic mismatch. c) In DMPC(14:1)* ***1*** *forms the channel active dimer* $(\mathbf{1})_2$ *with some hydrophobic mismatch, while in (d) a good match is realized for* $(gA)_2$.

22 °C; electrolyte solution: 1 M CsCl; membrane potential: 50 mV. The samples, dissolved in methanol, were added to the trans side of the recording chamber. Single-channel recording was started after the system had reached equilibrium (15 min). Generally the linked compounds **3** and **4** required concentrations which were 2 – 3 orders of magnitude lower than their monomeric counterparts **1** and **2**. The end concentration in the chamber was adjusted to the appearance of a few single-channel events and covered a range from 10^{-8} – 10^{-14} M. Current detection and recording was performed with a patch-clamp Axopatch 200 amplifier, a DigiData A/D converter, and pClamp6 software (Axon Instruments, Foster City, MA). The acquisition frequency was 5 kHz. The data were filtered with an analogue filter at 100 or 500 Hz for further analysis. Mean dwell times were determined by fitting the dwell-time histograms of single-channel events with a simplex least-squares algorithm.

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[10] Compounds **1** – **4** have been characterized by ^{1}H NMR spectroscopy and MALDI-TOF or ESI mass spectrometry (**1**: $[M+Na]^+$ calcd 1802.0, found 1801.8; **2**: $[M+Na]^+$ calcd 1563.9, found 1563.9; **3**: $[M+Na]^+$ calcd 3607.0, found 3607.0; **4**: $[M+Na]^+$ calcd 3130.7, found 3130.9). The ^{1}H NMR spectra in organic solvents indicate a strong preference for a secondary structure, which is subject of current investigations (for selected spectra, see the Supporting Information).

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