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A Voltage-Responding Ion Channel Derived by C-Terminal Modification of Gramicidin A

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The proper function of biological ion channels requires defined modes of control (gating).[1] Voltage gating is the response of the ionic flux through the channel pore upon a change of the membrane potential. Neuronal signal propagation relies on voltage-gated ion channels.

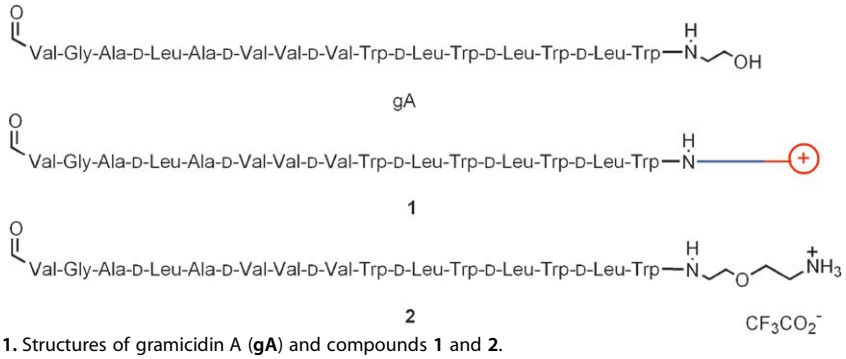

Figure 1. Structures of gramicidin A (**gA**) and compounds **1** and **2**.

Progress in the structural biology of voltage gated ion channels[2] and the design and functional studies[3] of voltage-modulated model channels helps in understanding voltage gating,[4] and is a first step towards the implantation of synthetic voltage-modulated channels into neurons. In a continuation of our studies on the implantation of synthetic gramicidin-hybrid channels into cells (trabecular meshwork cells,[5] CHO cells[6]), we turned to synthetic voltage-gated channels. Terminal-charged amphiphilic compounds,[7] can self-assemble into voltage-dependent pores and rigid push–pull rods[8] with permanent axial macrodipoles to give voltage-sensitive pores. Compared with these self-assembled pores, the channel-active $\beta^{6,3}$-helix of gramicidin A (**gA**) is structurally well defined.[9] (Figure 1)

The attachment of a positive charge at the C-terminal end of **gA** as a voltage-modulating element resulted in **gA** derivatives of type **1**; this work was pioneered by Läuger[10] and Woolley.[11] A schematic description for the voltage modulation is the ball-and-chain model that is shown in Figure 2.[1] A heterodimer channel of **1** and **gA** should lead to an open state if the positive end group of **1** is located at a negative-potential membrane site (Figure 2 A).

Upon switching to a positive membrane potential, electrostatic repelling forces should direct the positive charge into the channel entrance like a stopper in a bottle neck. The steric demand and the rigidity of the linker (in blue) between the C terminus and the positive charge is crucial for successful voltage gating. A sterically demanding linker allows the positive charge to come only near to the entrance, which results in a moderate decrease of the ion flux (Figure 2 B). A slender and flexible linker should allow a deeper diving of the positive charge into the channel entrance (Figure 2 C), which should ultimately lead to a complete blockage.

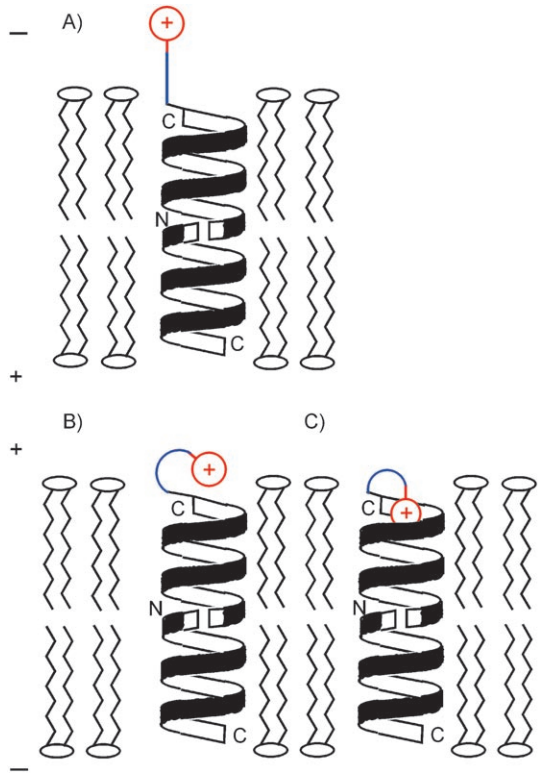

Figure 2. Ball-and-chain model for the voltage response of a **1**–**gA**-heterodimer channel. A) Negative potential at the positive end (in red) of the channel leads to an opened channel; B, C) Positive potential at the positive end of the channel leads to partly or fully blocked channel (linker in blue).

So far, only sterically demanding linkers have been used by Läuger[10] (aromatic) and Woolley[11] (carbamate). Both groups were restricted in their linker choice because they used the

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wild-type **gA** as their starting material. The recently reported segment-coupling synthesis of **gA** derivatives makes novel linkers accessible.[12] Here, we introduce compound **2**, which has a small and flexible ether linker, and report on its voltage-gating/rectifying properties. The **gA** channel is permeant for methylammonium ions,[13] so the primary ammonium group in **2** could be suitable stopper.

Compound **2** was synthesized via solution segment coupling.[12] The C-terminal ammonium group was generated by *t*-butyloxycarbonyl (Boc)-deprotection in the final step. Details for the synthesis and for the analytical data of **2** and all synthetic intermediates are given in the Supporting Information.

Single-channel currents of compound **2** were measured by the planar lipid bilayer method (Supporting Information). Heterodimer channels of **gA** and **2** were formed by the addition of each channel from opposite sites of the membrane. Diphytanoyl phosphatidyl choline (DPhPC) was used as a relatively rigid lipid to assure heterodimer stability and to avoid flip-flop. All channel measurements were done at pH 7 to assure the predominant formation of the ammonium group, and to match the physiological conditions.

Representative single-channel traces for the conductance of Cs^+ ions are summarized in Figure 3. The **2**–**gA** heterodimer exhibited a voltage-dependent effect that increased with increasing voltage. The difference in single-channel currents for different membrane potentials can be quantified by a voltage-asymmetry factor. At 50 mV this factor is 38%; it increases to 51% at 200 mV. A potential that is higher than 200 mV results in membrane disturbances and damage. Although a 51% asymmetry is a considerable voltage response, no complete voltage gating (asymmetry > 90%) was observed.

The **2**–**gA**-heterodimer was studied in comparison with the **gA**-homodimer and the **2**-homodimer (Figure 4).

Homodimer channels of **gA** and **2** were formed by the addition of each channel from both sites of the membrane. The absence of a positive charge in **gA** led to the linear current voltage relationship for the **gA**-homodimer (Figure 4A) with no rectified current behaviour.[9]

In contrast, the **2**-homodimer exhibited a pronounced nonlinearity in the current–voltage relationship (Figure 4B). The **2**–**gA**-heterodimer showed a current–voltage behaviour that corresponds to the **gA**-homodimer in the positive voltage region, and to the **2**-homodimer in the negative voltage region. The heterodimer channel exhibits an additive behaviour compared to the behaviour of its components. These results indicate that the positive charge at the channel entrance leads to the voltage-responding effect. The positive charge at the channel exit has no effect on the ionic flux through the pore. Both observations are consistent with the ball-and-chain model, as discussed above. However, even with the slender ether linkage in **2**, a maximum voltage response of 51% only was obtained and no complete blockage of the ionic flux as in Figure 2C was possible.

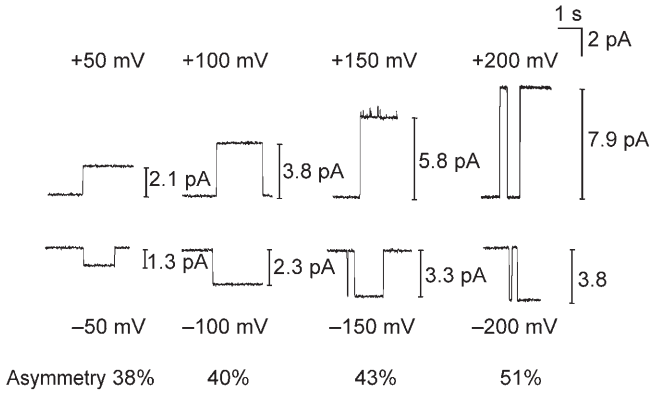

Figure 3. Single-channel currents of **2**–**gA**-heterodimer for different membrane potentials show an increasing voltage asymmetry with increasing potential (DPhPC, 1 M CsCl).

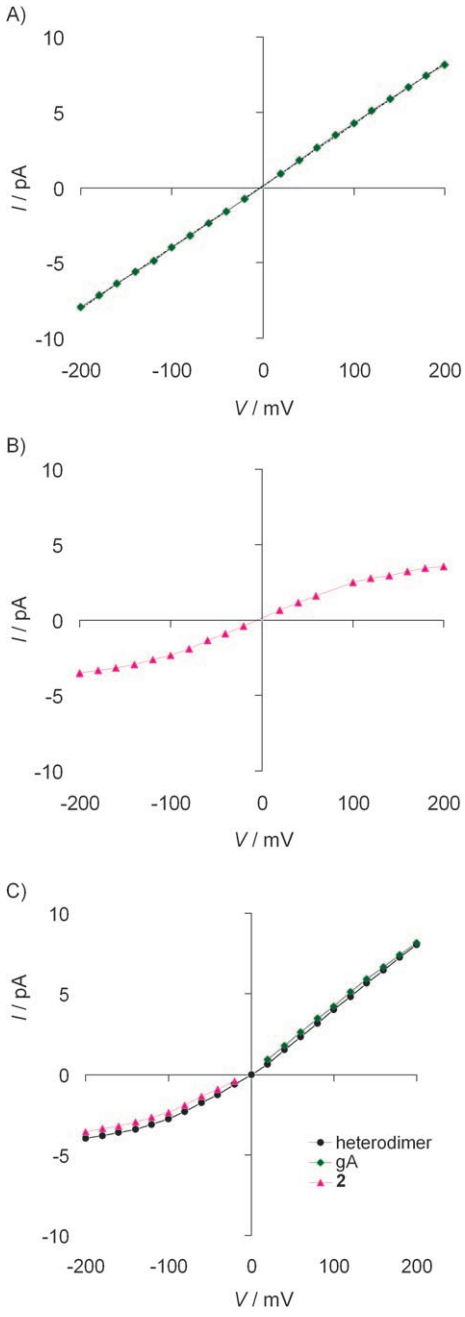

Figure 4. Current–voltage plots for A) **gA**-homodimer, B) **2**-homodimer, and C) **2**–**gA**-heterodimer in comparison with the homodimer plots.

The voltage-dependence channel activity of the **2**–**gA**-heterodimer for various alkaline cations was investigated next (Figure 5). A **gA**-typical Eisenman I selectivity[14] ($Cs^+ > K^+ > Na^+$) was found for the **2**–**gA**-heterodimer too. The ionic flux through the channel decreases with increasing dehydration energy because the passage of the cation through the channel needs a partial removal of the hydration shell. The rectified current behavior was strongest for Cs^+ and decreased over K^+ to Na^+.

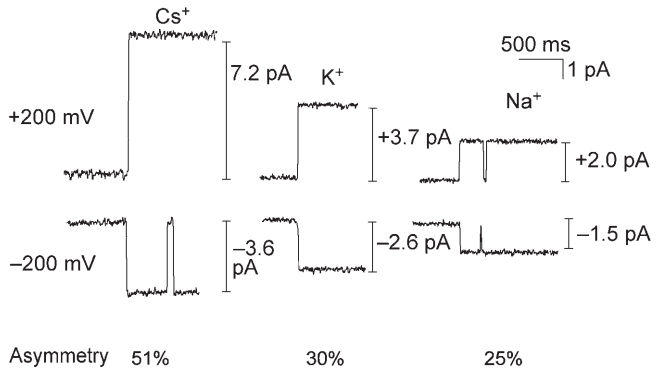

Figure 5. Single-channel currents of **2**–**gA**-heterodimer for different alkaline cations show an increasing voltage asymmetry from Na^+ to K^+ to Cs^+.

In a DPhPC membrane, the ammonium group of **2** is surrounded by the zwitterionic choline end-groups of the lipid. By gradually adding diphytanoylphosphatic acid (DPhPA), the environment of the ammonium group should be populated with anionic phosphate end-groups, which could interact with the ammonium group of **2**. This interaction might suppress the voltage-modulating action of the ammonium group.

Current–voltage relationships for the **2**-homodimer in membranes with different lipid composition (DPhPC + DPhPA) were recorded (Figure 6). By going from pure DPhPC (red curve) to 30% DPhPA (blue curve) the rectified current behaviour strongly decreases. Thus, the presence of anionic phosphate end-groups compensates the voltage-modulating action of the ammonium group.

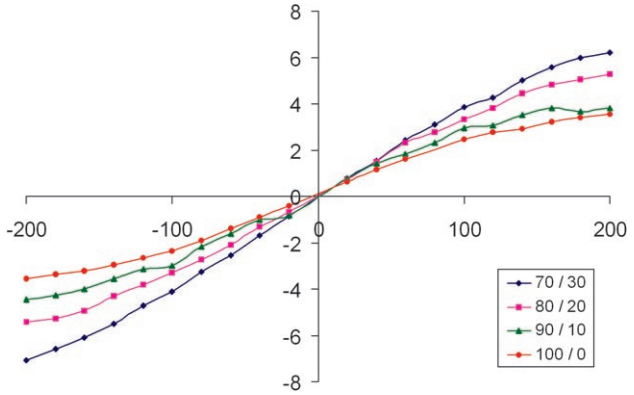

Figure 6. Current–voltage plots for **2**-homodimer in membranes with different lipid composition (DPhPC/DPhPA).

In conclusion a novel gramicidin A derivative **2** with an ammonium group that is linked to the C terminus via an ether linkage has been synthesized. Compound **2** showed a response of the ionic flux to the sign of the membrane potential (rectified current behaviour). The resulting voltage asymmetry increases with membrane potential and was strongest for Cs^+. Although the ether linkage should be small enough for a complete channel blockage (Figure 2C) only a 51% of blockage was obtained. An increasing amount of DPhPA in the lipid bilayer diminished the effect of the ammonium group. Compound **2** exhibits its voltage response under physiological pH and is therefore a suitable candidate for implantation into cells (e.g., neurons). Because heterodimer formation in vivo is unlikely, a covalent linkage of **2** and **gA**, for example by a succinic acid group, has to be accomplished.[15]

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Keywords: gramicidin · membranes · peptides · voltage-gated channels

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