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Nanoscale Ionic Diodes with Tunable and Switchable Rectifying Behavior

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This paper reports a modular strategy for constructing self-assembled ionic diodes in membranes using semisynthetic ion channel-forming peptides with internal diameters of less than 1 nm. Methods for constructing circuit elements for incorporation into nanofluidic devices are attracting interest for applications in biosensing,[1] constructing artificial cells,[2] and engineering biological batteries.[3] The majority of approaches to engineering ionic diodes employ top-down etching and lithographic techniques[1,4] to produce rectifying nanopores with aperture diameters greater than 2.5 nm.[1] Recently, ion channel proteins have been engineered to exhibit permanent rectifying behavior in a membrane by exploiting environmental factors such as pH[5] or incorporating genetically introduced modifications on the protein.[6] Here we demonstrate that synthetic modification of the ion channel-forming peptide gramicidin A (gA) allows the generation of self-assembled pores with subnanometer aperture diameters and controlled, rectified conductance behavior across a planar lipid bilayer. In contrast to previously reported rectifying gA channels,[10,12a−c,13a] we show that this ionic diode-like behavior can be tuned by simple replacement of one gA derivative with another in the bilayer and that rectification can also be "turned on" in response to an external stimulus (here, an enzyme in solution). Since the internal pore dimensions of the gramicidin channels (0.4 nm)[7] are approximately equal to the diameter of the monovalent cations transported across the membrane (e.g., Cs^+ has a diameter of 0.34 nm),[7] these semisynthetic channels represent the lower limit in terms of pore size for generating ionic diodes.

Gramicidin A is a fifteen amino acid peptide (produced by the bacterium *Bacillus brevis*)[7] that spontaneously incorporates into a lipid bilayer and reversibly dimerizes (Figure 1C) via its N-termini to generate ion channels that facilitate a flux of monovalent cations across the membrane.[8] We[9−11] and others[12a−c,13] have previously shown that charged groups attached to the C-terminus of gA can markedly affect its single ion channel conductance properties across bilayers. Here, we hypothesized that we could exploit the dependence of conductance on the charge of functional groups presented on derivatives of gA in order to engineer asymmetric channels[10,12] with diode-like conductance properties in planar lipid bilayers. To achieve this goal, we synthesized and examined the single ion channel conductance properties of the following three gA derivatives carrying either negatively or positively charged groups on the C-terminal entrance of the gA pore (Figure 1D): taurinyl gramicidin (gA-T, charge = −1), gramicidin phosphate (gA-P, charge = −2),[14] and trimethylgramicidamine (gA-NMe_3, charge = +1).

Figure 2A and Figure S1 in the Supporting Information show that under conditions of relatively low ionic strength (here, 100 mM CsCl), the negatively charged derivatives of gA (gA-T and gA-P) exhibit increased single channel conductance relative to native gA, while the positively charged derivative of gA (gA-NMe_3) exhibits decreased

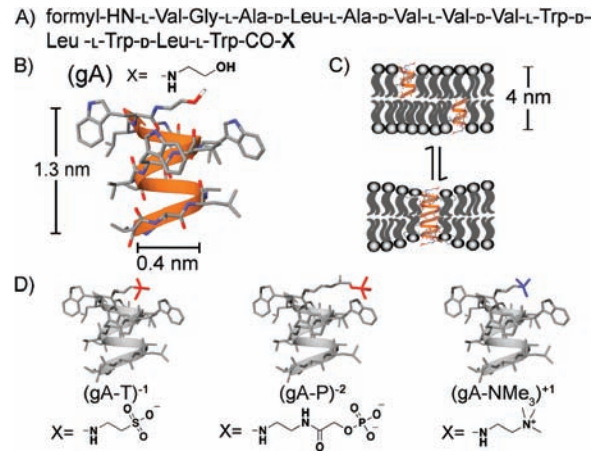

Figure 1. Sequence and structure of native and derivatized gramicidin A. (A) Sequence of gA. (B) Dimensions of a helical gA monomer (PDB entry 1GRM). (C) Reversible dimerization of gA in a bilayer; dimerization of gA induces compression of the bilayer near the pore.[8] (D) Computed structures of charged derivatives of gA: taurinyl gramicidin (gA-T), gramicidin phosphate (gA-P), and trimethylgramicidamine (gA-NMe_3).

single channel conductance. We attribute the observed conductance behavior to the electrostatic effect of the charged groups on the local concentration of ions near the entrance of the pore;[9−12c] these electrostatic effects are especially pronounced at total CsCl concentrations of ≤100 mM in the bulk electrolyte buffer.[10] As conductance is diffusion-limited at these relatively low salt concentrations, the local concentration of permeable cations near the entrance of the pore significantly affects the conductance through these charged derivatives of gA relative to native gA (Figure 2A). Additionally, potential binding of the trimethylammonium cation in gA-NMe_3 to the cation binding site at the entrance of the gA channel may significantly contribute to the reduced conductance of gA-NMe_3 relative to native gA.[13] Increasing the salt concentration in the electrolyte buffer is expected to reduce the Coulombic effects on conductance that are introduced by the

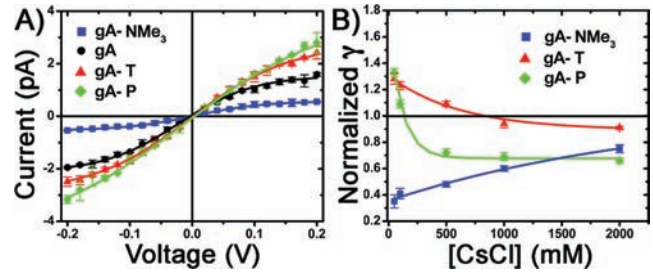

Figure 2. (A) Current−voltage ($I-V$) curves for positively charged, negatively charged, and native gA pores in recording electrolyte (100 mM CsCl, 1 mM HEPES, pH 7). (B) Single channel conductance (γ) of charged derivatives of gA (normalized to the conductance of native gA) as a function of the Cs^+ concentration (i.e., the ionic strength).

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charged groups on the gA derivatives.[12] Thus, under conditions of high ionic strength, the local ion concentration near the opening of the gA pore should be similar to the bulk ion concentration of the recording electrolyte, regardless of the charge on the group presented on the gA derivative. The results shown in Figure 2B support this hypothesis since they demonstrate that the conductance values of the charged derivatives of gA approach the conductance value of native (uncharged) gA with increasing ionic strength of the recording electrolyte. The results in Figure 2B also suggest that as the ionic strength increases, the Coulombic effects on conductance relative to gA are outweighed by other factors introduced by the functional groups and linkers attached to gA. These non-Coulombic effects on the conductance appear to correlate most strongly with the steric bulk introduced by the functional groups attached to the C-terminus of gA (Figure 1D). Similar conclusions were drawn for another gA derivative described by Apell et al.[12a]

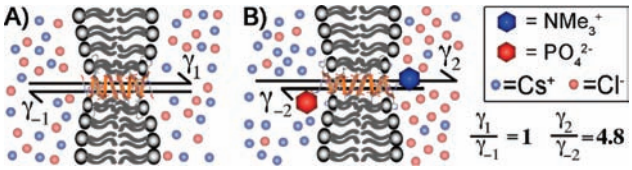

Figure 3. Schematic illustration of homodimeric and heterodimeric gramicidin channels in a membrane. (A) Nonrectified conductance of monovalent cations through a symmetric gA channel. (B) The charged groups on an asymmetric channel comprising gA-P and gA-NMe_3 presumably have different effects on the local concentration of cations near the pore,[10] resulting in diode-like rectifying behavior.

In order to engineer rectifying behavior in gA channels, we generated heterodimeric channels by adding gA-NMe_3 to one side (the cis side) of the membrane and either gA-T or gA-P to the other side (the trans side) of the membrane (Figure 3B). For these asymmetric channels, we expected the positive and negative charges on the gA derivatives to affect (depending on the sign and magnitude of the charge) the local concentration of cations near the pore on either side of the membrane. This effect would result in an overall decrease in single channel conductance of ions from the cis side of the membrane relative to the conductance of ions originating from the trans side of the membrane. Figure 4A shows that asymmetric channels comprising gA-NMe_3 and gA-T exhibited at pH 7 a (3.4 ± 0.1)-fold difference in single channel conductance of monovalent cations across the membrane in one direction relative to the conductance in the other direction (i.e., with higher conductance observed when gA-T was at the entrance of the pore). To demonstrate the modular nature of these gA-based diodes, we found that replacing gA-T with gA-P (to generate asymmetric channels comprising gA-NMe_3 and gA-P) resulted in a (4.8 ± 0.1)-fold difference in single-channel conductance across the membrane

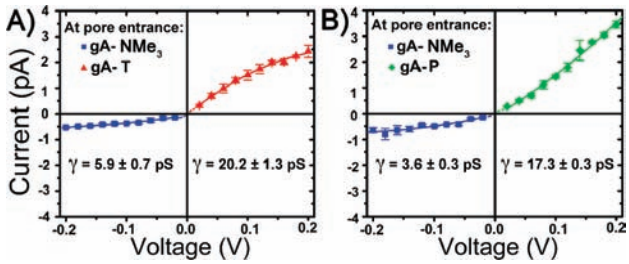

Figure 4. Single channel conductance (γ) profiles of asymmetric channels. (A) $I-V$ curves for an asymmetric channel consisting of gA-NMe_3 and gA-T in recording electrolyte (100 mM CsCl, 1 mM HEPES, pH 7). (B) $I-V$ curves for an asymmetric channel consisting of gA-NMe_3 and gA-P in recording electrolyte (50 mM CsCl, 0.5 mM Tris, pH 9). The bilayers were composed of DiPhyPC lipids. pS = picosiemens.

at pH 9, with high conductance observed when gA-P was at the entrance of the pore (Figure 4B). Since these charged derivatives of gA do not cross the membrane by themselves (see Figure S2), the rectifying behavior of these gA derivatives was stable over several hours in a membrane. Additionally, we demonstrated the capability to controllably "turn on" the rectifying behavior of a channel in situ by adding the enzyme alkaline phosphatase (which cleaves the phosphate group off gA-P to produce an uncharged gA derivative)[11c] to only one side of the membrane in the bilayer setup. Before addition of this enzyme, this setup contained only nonrectifying homodimeric channels comprised of gA-P (see Figure S3). The possibility of changing the conductance of gA derivatives in real time in response to a variety of external stimuli[9,11] may present opportunities for sensing and for controlling the "turn on" or "turn off" rectifying properties of these channels.

These results demonstrate the modular and tunable nature of a gA platform for generating ionic diodes in a membrane. The dimeric characteristic of gramicidin-based channels makes it possible to access each half of the channel independently in order to incorporate the channel asymmetry required for the desired rectification. Thus, the modular nature of gramicidin channels makes it possible to tune the diode-like behavior of these pores. Additionally, the possibility of changing the conductance properties of gA derivatives in the presence of external stimuli[9−11] may be advantageous for manipulating the rectifying behavior of gA-based ionic diodes after incorporation of these pores into a nanofluidic device. Finally, since these gA-based diodes are the smallest fluidic circuit elements reported to date, they may represent an important step toward the realization of miniaturized devices that rely on control of ionic flow within fluidic networks.

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Supporting Information Available: Synthesis, experimental methods, and $I-V$ curves and traces of single channel recordings. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) The charge on phosphate is expected to be roughly −1.5 at pH 7 and −2 at pH 9.

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