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# Modulating Ion Channel Properties of Transmembrane Peptide Nanotubes through Heteromeric Supramolecular Assemblies

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Processes such as electrical signaling in nerve and muscle cells require appropriate transmembrane potentials established by different intra- and extracellular ion concentrations. Transmembrane channels involved in these processes display ion selectivity and rectification and are regulated (gated) by a variety of molecular, voltage, or mechanical signals.[1] Synthetic channels possessing similar properties have been described and are generally based either on fragments of naturally occurring ion channels[2] or on de novo designed amphiphilic α-helices.[2f] Here we describe a novel approach based on the self-assembling peptide nanotube architecture for the formation of heteromeric synthetic channels that display altered conductance and rectification properties.

The cyclic D,L-α-peptides employed in the present study were designed to modulate the inherent conductance properties of the transmembrane ion channels derived from the spontaneous self-assembly of peptide **1** in synthetic lipid bilayers.[3] The two subunits that reside at the ends of the channel assembly—termed "caps"—differ from other subunits by their mode of interaction and the microenvironment in which they reside (Figure 1). The cap subunits dwell at the lipid−water interface and participate in backbone−backbone hydrogen bonding from only one face of the peptide ring structure. We hypothesized that these characteristics can be exploited for selective formation of heteromeric nanotube assemblies by targeting appropriately designed cyclic peptides to the channel ends. Furthermore, modulation of ion selectivity and conductance was expected through local electrostatic perturbation brought about by the cap subunits bearing ionizable functionalities. It should be noted that, unlike α-helical peptides, self-assembling D,L-α-peptide nanotubes such as **1** owing to their pattern of *β*-sheetlike backbone hydrogen bonding do not possess a net dipole moment nor an inherent ion rectification capacity. However, it was thought that the channel asymmetry required for ion rectification can be attained when the cap subunit is present only on one side of the transmembrane channel assembly.

Two backbone N-alkylated cyclic peptides, c[-(L-Phe-*N*-(amino-eth-2-yl)-D-Ala-L-Phe-D-Ala)$_2$-] **2** and c[-(L-Phe-*N*-(hydroxycarbonylmethyl)-D-Ala-L-Phe-D-Ala)$_2$-] **3**, as well as two unmodified D,L-α-peptides, c[-(L-Arg-D-Leu)$_4$-] **4** and c[-(L-Glu-D-Leu)$_4$-] **5**, were designed to test the above hypothesis (Figure 1).[4] The amphiphilic character of peptides **2** and **3** was thought to steer them to the lipid−water interface where they could cap the transmembrane channel through backbone-directed hydrogen bonding interactions from only one face of the peptide ring structure, due to the directive effects of the two alkyl-substituted amide nitrogens.[5] Cyclic peptides **4** and **5**, each having four ionizable side chains, can neither dissolve in lipid bilayers nor self-assemble in neutral aqueous solutions due to side chain−side chain electrostatic repulsion. However, they were expected to assemble onto peptide channel **1** at its solvent exposed ends.

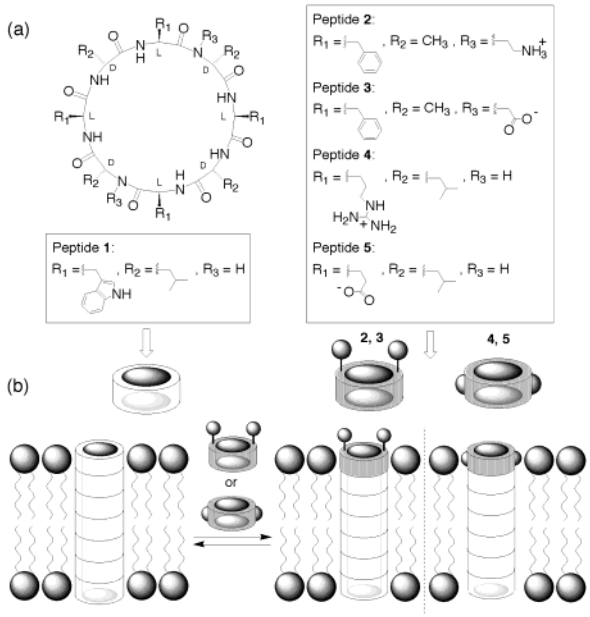


***Figure 1.*** (a) Chemical structures of the cyclic D,L-α-peptides employed in this study and (b) schematic representation of the mode of interaction of the tube caps in forming heteromeric transmembrane channels. Peptide **1** self-assembles in lipid membranes to form the core transmembrane channel structure, while the peptides **2**−**5** serve as the cap subunits creating the heteromeric channel assemblies with altered ion conductance properties.

As described previously, cyclopeptides with hydrophobic side chains form discrete and well-characterized transmembrane channels. Experiments performed with L-α-phosphatidyl-choline (L-α-lecithin) planar bilayers[4,6] showed the formation of discrete ion channels by **1** (open probability = 0.76, mean open time = 800 ms). Current dependence on voltage is ohmic in symmetric KCl solutions (Figure 2a), and conductance (Λ) values obtained at different KCl concentrations follow a Michaelis−Menten saturation model[1] ($\Lambda_{max}$= 21.5 ± 0.2 pS, $K_m$= 7.1 ± 0.8 mM). The same experiments with glyceryl-monooleate (GMO) planar bilayers gave larger values ($\Lambda_{max}$ = 44.2 ± 1.4 pS, $K_m$= 50.0 ± 7.1 mM), which can mostly be attributed to the dipole layers present at the interface in L-α-lecithin.[7]

Cap peptide **2** and **4** were found to interact with transmembrane channel assembly of peptide **1** with similar binding constants regardless of the identity of lipid bilayers employed as determined in symmetric 100 mM KCl by measuring the decrease of conductance at different cap peptide concentrations (peptide **2**, $K_a$ = 22200 ± 2600 $M^{-1}$ in L-α-lecithin and 27300 ± 2100 $M^{-1}$ in GMO; peptide **4**, $K_a$ = 29300 ± 1100 $M^{-1}$ in L-α-lecithin and 26600 ±

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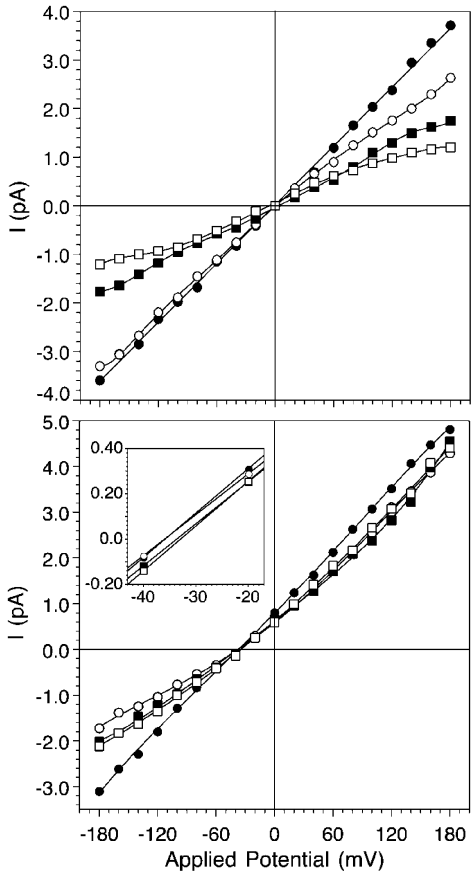


***Figure 2.*** Current−voltage relationships: (a) ion channel **1** alone (●) and with 36 $\mu$M **2** in both chambers (■), 18 $\mu$M **4** in *trans* side (○) and 2.4 mM $CaCl_2$ in both sides (□) in L-α-lecithin bilayers, 100 mM KCl, 5 mM MOPS, pH 7.5. (b) Ion channel **1** (●) and with 12 $\mu$M **2** (□), 12 $\mu$M **4** (■), and 1.2 mM $CaCl_2$ in both sides (○) in L-α-lecithin bilayers, 100 mM and 500 mM KCl in *cis* and *trans* chambers, respectively. Inset shows a less negative $E_r$ for capped channels.

600 $M^{-1}$ in GMO, all at +100 mV). Non-ohmic current−voltage relationships (rectification) were also observed when either peptide **2** or **4** was added to only one side of the bilayer (Figure 2a). The electrical distance ($\delta$), defined as the ratio of potential drop at the position where binding or inhibition occurs, was estimated to be 0.04 ± 0.01 for **4** and 0.10 ± 0.02 for **2**.[8] These values are consistent with the binding of the cap peptides at the channel mouth. Interestingly, while transmembrane channel **1** is completely cation selective—as determined by the measured reverse potential ($E_r$) and the Goldman−Hodgkin−Katz equation[1]—reverse potentials were found to be less negative in the presence of peptide caps **2** or **4**, accounting for the loss of the complete charge selectivity of channel **1** (permeability ratio of $K^+$ vs $Cl^-$ of 20 and 30 for **2** and **4**, respectively, Figure 2b). The observations that these two structurally distinct cyclic peptide cap subunits, one backbone substituted and the other not, display similar association constants with the transmembrane channel assembly **1**, insensitivity to lipid composition, estimated short electrical distances, and diminution of cation selectivity support their backbone−backbone hydrogen bond-directed assembly at the channel termini.

The measured similar affinity of peptides **2** and **4** with the channel assembly **1** either in zwitterionic L-α-lecithin or the uncharged GMO bilayers strongly rules out nonspecific interactions with the lipid headgroups as the source of the observed changes in conductance. However, it is known that divalent cations such as calcium ions interact nonspecifically with the lipid headgroups, albeit with drastically different affinity depending on the lipid composition employed, to produce an electrical barrier resulting in changes in conductance.[9] Nevertheless, such nonspecific interaction while also placing the dication at the level of the channel mouth (calculated $\delta$ = 0.08 ± 0.01 in L-α-lecithin) does not alter the charge selectivity of the channel assembly **1** (Figure 2b).

Negatively charged tube caps also modify the properties of transmembrane ion channel **1**. An increase in conductance (10−15% in 20 mM KCl) was observed due to the inherent cation selectivity of **1** and the negative charges of the tube cap. Binding constants again proved to be independent of the lipid employed ($K_a$ = 3500 ± 100 $M^{-1}$ in L-α-lecithin and 4900 ± 200 $M^{-1}$ in GMO, for **3** and $K_a$ = 4500 ± 200 $M^{-1}$ in L-α-lecithin and 9000 ± 500 $M^{-1}$ in GMO for **5**, at 20 mM KCl and −100 mV).

In summary, we have shown the validity of the supramolecular approach in the design of transmembrane channels with altered transport characteristics. Formation of specific and directed heteromeric noncovalent assemblies at transmembrane channel openings using structurally distinct cyclic D,L-α-peptide cap subunits are supported by the confluence of various experimental observations derived from single channel conductance measurements. We suggest that the approach described above may find utlity in the design of ligand-gated channels and stochastic sensing.

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**Supporting Information Available:** Saturation curves, current/voltage relationships, and electrical distance calculation plots (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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