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An Artificial Ion Channel Formed by a Macrocyclic Resorcin[4]arene with Amphiphilic Cholic Acid Ether Groups**

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Ion channels are naturally occurring molecular devices in the brains and nerve systems of all animals that generate large ionic fluxes with on – off gate control. An ion channel with a typical conductance of 10 pS allows the passage of 5×10^6 ions per second through a single molecular pore. Even at such high ionic fluxes, ion channels generally discriminate different ionic species, for example, cation/anion and Na^+/K^+.[1] These functions are of potential use as the basis for molecular ionic devices[2] that can transfer information across a thin membrane. If these functions are properly attributed to artificial ion channels, they should show great advantages with regard to handling, stability, and availability over large natural proteins, which are hardly expected to meet these demands. At the same time, construction of artificial ion channels by using simple molecular systems may provide important information with regard to understanding natural ion channels and to establishing structure – function relationships.

Several approaches to artificial ion channels have been reported by us[3] and others.[4] We have already obtained several nonpeptidic ion channels by two approaches: supramolecular and molecular half channels. Two important results were: 1) A macrocyclic resorcin[4]arene with four alkyl chains **1** (Figure 1) gave a single ion channel having only one conductance level.[3d] 2) A supramolecular ion channel obtained by using the rigid methyl cholate **2** with amphiphilic nature showed a long-lasting open state.[3e] We have now combined these two functions by synthesizing a macrocyclic resorcin[4]arene having four amphiphilic methyl cholate groups (**3**) in the hope of obtaining a fundamental molecular channel unit with a single conductance level and a stable open state. Then, refined design of the ion-transporting path by modifying the rigid molecular plane of the cholic acid residues is expected to modify the conductance and ion selectivities so that structure – function relationships can be established.

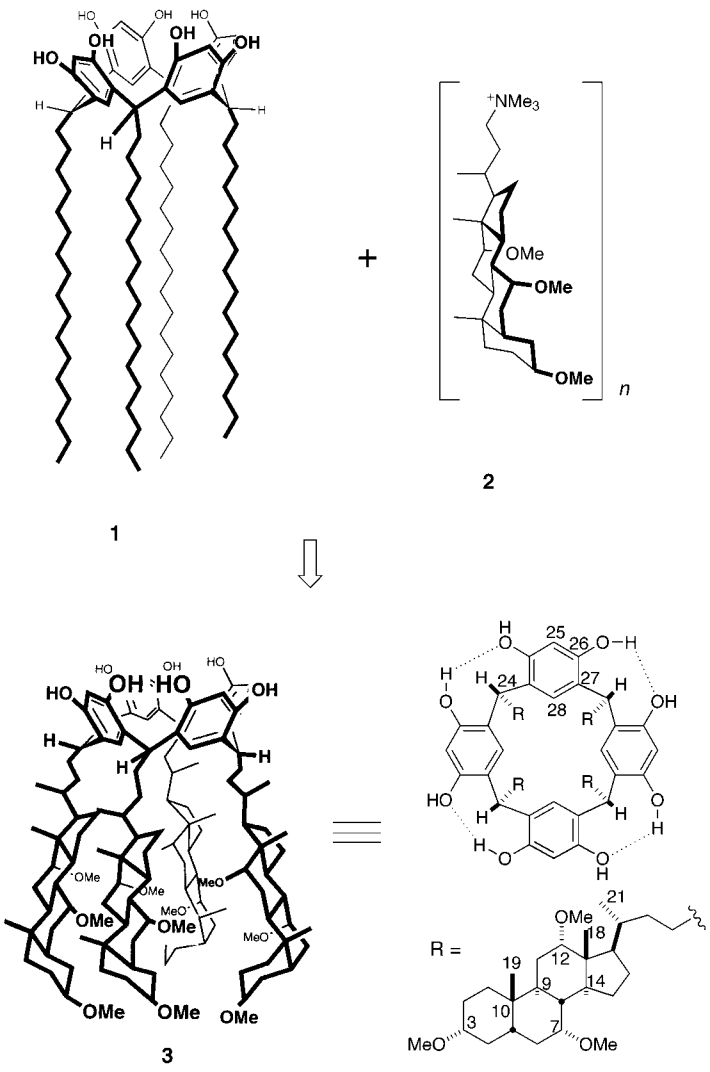

Figure 1. Structures of compounds **1** – **3**.

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Macrocyclic resorcin[4]arene **3** was synthesized by one-pot condensation of 3,7,12-trimethylcholanal with resorcinol under acidic conditions. Crude **3** was obtained by simple filtration from the reaction mixture. Purification by column chromatography (SiO_2) afforded **3** in 32% yield. A FAB mass spectrum of **3** directly showed the existence of resorcin tetramer (*m*/*z* 2106.47 [*M*+H$^+$]). Proton and ^{13}C NMR spectra of **3** in [D$_6$]DMSO at 90°C gave only one set of signals for aromatic and steroidal components: sharp signals for one set each of methine protons α to methoxy groups (H^3, H^7, and H^{12}), methine protons α to two phenylene groups (H^{24}), and aromatic protons (H^{25} and H^{28}). Furthermore, all ^{13}C signals of the aromatic and steroidal components were observed separately. These results indicated that **3** has a C_{4v}-symmetrical structure. The four steroidal units are located at axial positions in a bowl-shaped conformation by intramolecular hydrogen bonding of adjacent resorcin units,[5] as shown in Figure 1. As resorcin[4]arene **3** has hydrophilic head groups and amphiphilic steroidal parts, a tail-to-tail coupled pair of molecules of **3** are expected to assemble in a bilayer membrane to afford a transmembrane channel (Figure 2). The hydrophilic molecular sides of the steroidal groups are expected to be aligned to form a hydrophilic pore near the central axis, while the hydrophobic sides are directed to the surrounding lipid alkyl groups.

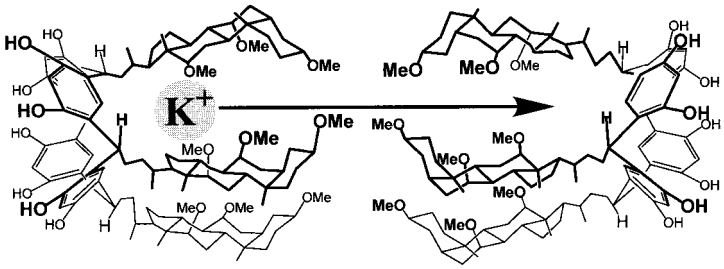

Figure 2. Tail-to-tail assembly of two molecules of **3** to give a bilayer membrane with an intramembrane channel.

The properties of single ion channels were examined according to established methods.[3b–g] A premixed solution of soybean lecithin and **3** in $CHCl_3$/*n*-decane (1/5) was applied to a hole in a partition separating two aqueous chambers to form a planar bilayer. Incorporation of 0.2 wt% of **3** in the lipid gave stable single-channel currents at various applied voltages. Figure 3a shows a typical record of single-channel currents at 70 mV with symmetrical 500 mM KCl at pH 7.2 (tris-hepes buffer). As shown in this example, ion channels formed from **3** usually showed long-lasting and stable open states. The ratio of open to closed states was about 95 to 5, and the average open times determined by histogram analysis of open durations were in the range of 2.5–4.5 s. In the case of the alkyl resorcin channel **1**,[3d] very rapid open–closed transitions were observed (Figure 3b) and their analysis gave an average open time of 35 ± 10 ms. Comparison of the two systems suggests that the rapid transitions in **1** are based on molecular motion of flexible alkyl side chains. In **3** with rigid steroidal anchors in the membrane, these rapid open–closed transitions are almost quenched. In the gramicidin A channel, intermolecular hydrogen bonding in a β-helical conformation results in stable pore formation across the bilayer membrane, and its open lifetime of 0.56 ± 0.06 s[6] has been ascribed to a head-to-head dimer. Interestingly, the average open time of **3** exceeds even this value. Therefore, fairly strong interactions are suggested to exist in the channel formed by two molecules of **3**. The higher stability of ion channels of **3** not only results from the rigid steroid framework, but also from the polar methoxy groups, which are absent from **1** and should hold the tails of **3** that face each other together by polar interactions and/or by cation complexation.

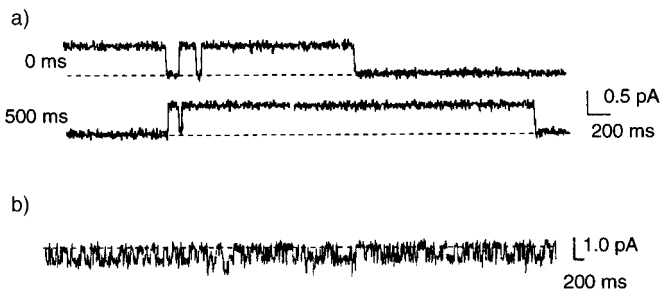

Figure 3. Time profiles for single-channel currents. a) **3**, 500 mM KCl, pH 7.2, 70 mV. b) **1**, 500 mM KCl, pH 7.2, −74.4 mV.

The conductance values in KCl and NaCl under symmetrical conditions were obtained from the linear relationship between voltages and current for several experimental runs (Figure 4). In each case, only one conductance value, 9.9 ± 0.1 and 5.3 ± 0.1 pS, was obtained in 500 mM solutions of KCl and

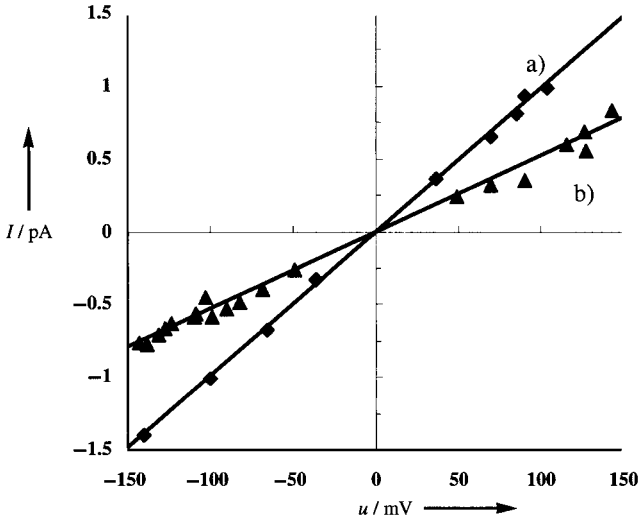

Figure 4. Current–voltage plots of single ion channel **3**. a) Symmetrical 500 mM KCl, pH 7.2; b) symmetrical 500 mM NaCl, pH 7.2.

NaCl, respectively. The value in KCl solution (9.9 pS) was higher than that of the alkyl resorcin channel **1** (6.1 pS in 500 mM KCl). Cation/anion selectivity was examined with asymmetrical salt concentrations: *cis*, 100 mM KCl; *trans*, 500 mM KCl. Reversal potential values E_{rev} from several experimental runs converged at 27.9 ± 1.8 mV (Figure 5). The permeability ratio $P_K/P_{Cl} = 8.2 \pm 1.6$ was then determined from the Goldman–Hodgkin–Katz equation.[1] The value was significantly lower than that of **1** ($P_K/P_{Cl} = 20$). Compar-

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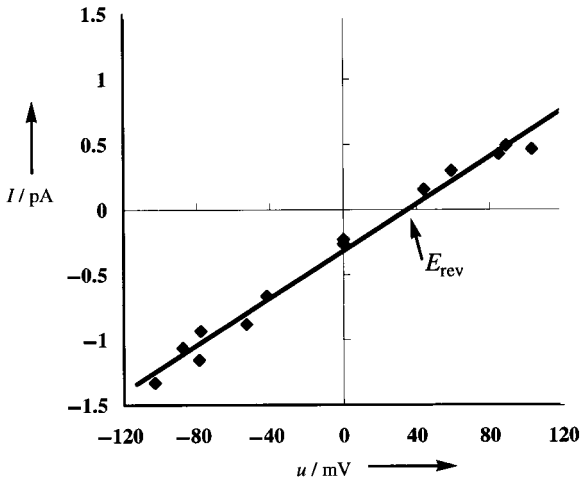

Figure 5. Current–voltage plot of single ion channel **3** under asymmetric salt concentrations: *cis* 100 mM KCl and *trans* 500 mM KCl, pH 7.2.

ison of conductance values and cation/anion selectivities between resorcin channels **1** and **3** showed clearly the effect of the steroidal units, which resulted in the formation of a more hydrophilic environment at the central pore. The conductance value increased by 60% but was accompanied by a 60% loss of cation/anion selectivity.

Finally, K^+/Na^+ selectivity was examined under asymmetric salt concentration conditions: *trans* 450 mM NaCl+50 mM KCl; *cis* 450 mM KCl+50 mM NaCl. The permeability ratio P_K/P_{Na} was determined as 2.8 ± 0.6, which was similar to that of **1** ($P_K/P_{Na} = 3$). Therefore, steroidal units have negligible influence on the K^+/Na^+ selectivity. The result confirms the previous conclusion that the resorcin[4]arene macroring may provide the selectivity filter of the channel. The aromatic moiety provides only a weak electric field, which can desolvate the K^+ ion ($r = 1.33$ Å) with its lower dehydration energy (355 kJ mol^{-1}) but only partly desolvate the Na^+ ion ($r = 0.95$ Å; 438 kJ mol^{-1}), which therefore finds it hard to pass through the bottleneck of the resorcin[4]arene. On the other hand, the Rb^+ ion ($r = 1.48$ Å), with its smaller dehydration energy of 330 kJ mol^{-1}, but with a slightly larger ionic radius stops K^+ currents by atomic blockage.

Resorcin[4]arene channels have potential utilities for establishing the structure–function relationship in ion channels because of their single conductance level and facile variation of the tail unit. The steroidal tails introduced here have the advantages of a long-lasting open state and modification of the hydrophilic pore without changing the overall structural features, as evidenced here by conductance and selectivity values of cation/anion and metal-ion species. We are now investigating modification of the cholic acid tails of resorcin[4]arene channels.

Experimental Section

3: Concentrated HCl (0.185 mL) was added to a mixture of resorcinol (127 mg, 1.15 mmol) and 3,7,12-trimethylcholanal (500 mg, 1.15 mmol) in ethanol (1.15 mL) at 0 °C. The mixture was heated at 70 °C for 10 h. The resulting mixture was cooled to room temperature, and precipitates were filtered off. Water was added to the filtrate and the resulting precipitate was collected by filtration. The combined solids were washed with hot water (80 °C, 3 × 10 mL), and dried under reduced pressure. The crude solid was subjected to column chromatography (SiO_2, EtOAc/MeOH = 19/1) to give **3** (196 mg, 32%). ^{1}H NMR (600 MHz, [D$_6$]DMSO, 90 °C, TMS): $\delta = 8.69$ (br, 8H; OH), 7.18 (s, 4H; H^{28}), 6.18 (s, 4H; H^{25}), 4.21 (t, $J = 7.3$ Hz, 4H; H^{24}), 3.35–3.33 (m, 4H; HC(OMe)), 3.22 (s, 24H; 2MeO), 3.17 (s, 12H; MeO), 3.13–3.11 (m, 4H; HC(OMe)), 2.96–2.93 (m, 4H; HC(OMe)), 2.03–1.90 (m, 16H), 1.85–1.55 (m, 36H), 1.48–1.23 (m, 20H), 1.21–0.85 (m, 24H), 0.96 (d, $J = 6.6$ Hz, 12H; H^{21}), 0.86 (s, 12H; Me), 0.63 (s, 12H; Me); ^{13}C NMR (150 MHz, [D$_6$]DMSO, 90 °C, TMS): $\delta = 152.25$ (C(Ar), 2C), 124.90 (CH(Ar)), 124.22 (C(Ar)), 124.05 (C(Ar), 103.36 (CH(Ar)), 82.26 (CH, CH(OMe)), 80.42 (CH, CH(OMe)), 77.49 (CH, CH(OMe)), 55.95 (CH_3, MeO), 55.68 (CH_3, MeO), 55.18 (CH_3, MeO), 47.51 (CH), 46.52 (C), 42.84 (CH), 41.97 (CH), 39.85 (CH), 35.58 (CH), 35.41 (CH_2), 35.02 (C), 34.93 (CH_2), 34.72 (CH_2), 33.80 (CH, CH(Ar)$_2$), 32.09 (CH_2), 28.29 (CH_2), 28.19 (CH), 27.41 (CH_2), 27.19 (CH_2), 23.27 (CH_2), 23.04 (CH_3), 22.43 (CH_2), 18.66 (CH_3, C^{21}), 12.82 (CH_3). FAB-MS (NBA matrix) calcd for $C_{132}H_{200}O_{20}$: 2105.46; found (intensity): 2104.43 (23), 2105.44 (72, $[M]^+$), 2106.47 (100, $[M+H]^+$), 2107.54 (80), 2108.55 (46), 2109.56 (23), 2128.44 (17, $[M+Na]^+$); analysis calcd for $C_{132}H_{200}O_{20} \cdot 4H_2O$: C 72.76, H 9.62; found: C 72.45, H 9.61.

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