Communication

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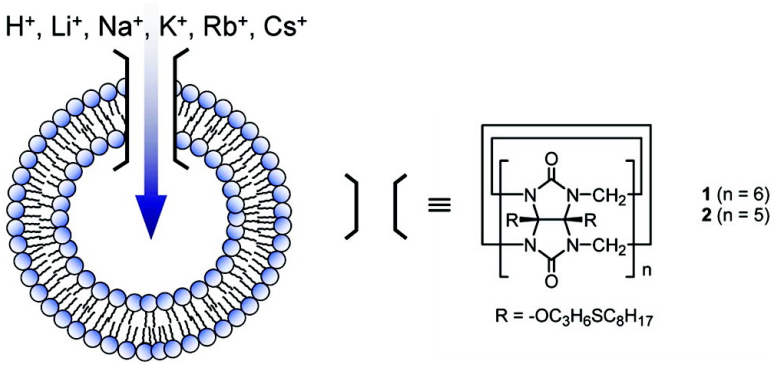

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Artificial Ion Channel Formed by Cucurbit[*n*]uril Derivatives with a Carbonyl Group Fringed Portal Reminiscent of the Selectivity Filter of K^+ Channels

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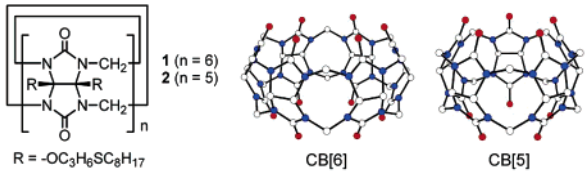
Studies of artificial ion channels[1] have received much attention not only because they contribute to the fundamental understanding of natural ion channels[2] but also because they may lead to applications such as in drug discovery[3] and sensors.[4] The X-ray crystal structure of a K^+ channel reported by MacKinnon and co-workers in 1998 revealed for the first time the structure of the selectivity filter that discriminates between K^+ and Na^+ ions.[5] The pore size of the selectivity filter, which is lined with the main chain carbonyl oxygen atoms, is just right for bare K^+ but too large for Na^+, which gives the K^+ ion selectivity. Although many synthetic ion channels[1] based on receptor molecules including cyclodextrins,[6a] crown ether,[6b] resorcin[4]arene,[6c] calix[4]arene,[6d] cyclic peptides,[6e] and others[1,6] have been reported, however, none of the nonpeptitic synthetic ion channels mimic the structural feature of the selectivity filter of K^+ channels.



Cucurbituril (CB[6]), a macrocyclic cavitand comprising six glycoluril units, has a hydrophobic cavity (diameter ≈ 5.5 Å) that is accessible through two identical carbonyl-fringed portals (diameter 3.9 Å).[7] The rigid structure and capability of forming complexes with molecules and ions make CB[6] attractive not only as a synthetic receptor but also as a building block for supramolecular assemblies.[8] Our recent synthesis of CB homologues, cucurbit[*n*]uril (CB[*n*], $n = 5$, 7, and 8) having five, seven, and eight glycoluril units has widened the scope of cucurbituril chemistry.[9] Among the CB homologues, CB[5] has the smallest cavity (diameter ≈ 4.4 Å) and portal (diameter ≈ 2.4 Å). Very recently, we also reported the direct functionalization of CB[*n*],[10] which now allows us to synthesize a wide variety of CB[*n*] derivatives and to study their applications. In particular, the structural resemblance of the carbonyl-fringed portals of CB[*n*] to the selectivity filter of K^+ channels prompted us to study artificial ion channels based on CB[*n*]. Herein we report novel artificial ion channels based on CB[*n*] ($n = 6$ and 5), which can transport proton and alkali metal ions across a membrane with ion selectivity.

To study the proton transport across membranes, large unilamellar vesicles were prepared from egg yolk L-α-phosphatidylcholine (EYPC), cholesterol, and dicetyl phosphate with and without **1** (1 mol %) as described in the literature.[12] A strong peak at 1765 cm^{-1} corresponding to the carbonyl stretching band of **1** in the FT-IR spectrum evidenced the incorporation of **1** into the vesicle membrane (Figure S1). The proton flux through the membranes was assessed by the change in fluorescence intensity of the pH-sensitive dye 8-hydroxypyrene-1,3,6-trisulfonate (HPTS) entrapped inside the vesicles. A sudden pH change of the extravesicular solution by adding HCl solution caused no change in fluorescence intensity of HPTS for the vesicles without **1**, but results in immediate quenching of the fluorescence for the vesicles with **1** (Figure 1). However, addition of 15 equiv of the neurotransmitter acetylcholine (Ach^+), which is known to form a stable host−guest complex ($K \approx 10^3$) with CB[6] derivatives in water,[11] to the vesicle solution completely inhibited the fluorescence quenching (Figure 1). These results support that **1** is involved in the proton transport across the membrane, which is blocked by Ach^+, reminiscent of the blocking of the K^+ channels by polyamines.[18]

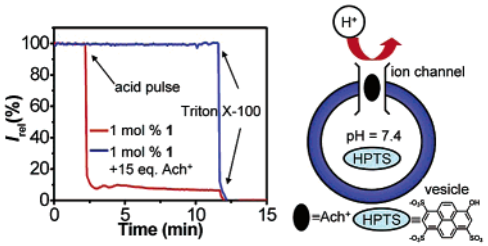


Figure 1. Changes in fluorescence intensity as a function of time for the vesicles with **1** (red line) and with **1** and 15 equiv of Ach^+ (blue line).

Having observed the transmembrane proton transport mediated by **1**, we turned our attention to alkali metal ion transport. The alkali metal ion transport activities of **1** and **2** were evaluated by fluorometry using a well-established protocol.[6d] A THF solution of **1** or **2** was added to a suspension of EYPC vesicles containing entrapped HPTS. Addition of NaOH solution to the extravesicular solution to create a pH gradient (approximately one unit) caused proton efflux from the liposomes, which is compensated by influx of alkali metal ions into the vesicles as mediated by the exogenous ligand. The increase in the intravesicular pH, which reflects the alkali metal ion transport across the membrane, was monitored by increase in relative fluorescence (I_{460}/I_{403}). Figure 2a shows that the transport activity of **1** follows the order of $Li^+ > Cs^+ \approx Rb^+ > K^+ > Na^+$,[16,17] which is opposite to the binding affinity of CB[6] toward alkali metal ions.[13] A similar opposite relationship between transport activity and binding affinity has been observed in ion channel forming synthetic receptors.[1b] On the other hand, the transport

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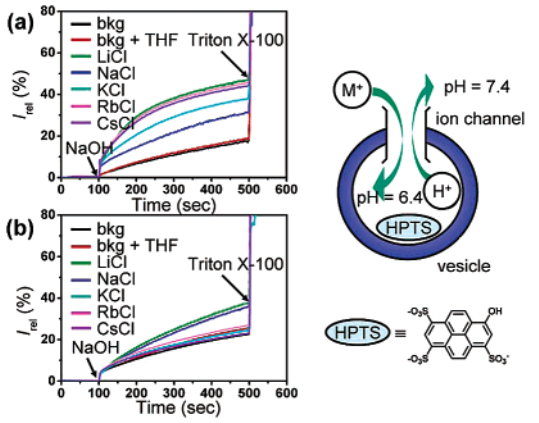

Figure 2. Changes in the fluorescence intensity ratio (I_{460}/I_{403}) as a function of time for EYPC vesicles. Alkali metal ion transport in the presence of (a) **1** and (b) **2** (1 mol % each case).

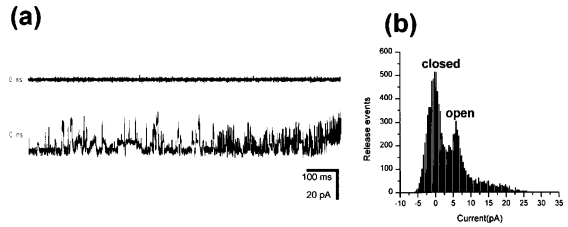

Figure 3. (a) Planar bilayer conductances measured with and without **1** and (b) a histogram of the currents at a transmembrane potential of +80 mV. (Symmetric 300 mM cesium methanesulfonate, 10 mM Tris-HEPES, 500 μM $CaCl_2$, and 490 μM EGTA.)

activity of **2** follows the order of $Li^+ > Na^+$ (Figure 2b), which is also opposite to the binding affinity of **2** toward these metal ions, but virtually no transport above the background was observed for K^+, Rb^+, and Cs^+. It is presumably because the carbonyl-fringed portal size of **2** (diameter 2.4 Å) is smaller than the diameters of these alkali metal ions. These results also suggest that **1** and **2** mediate the alkali metal ion transport across the membrane by a channel mechanism.

To determine whether the transport occurs via a channel or carrier mechanism, planar bilayer conductance measurements were made.[14,15] Figure 3a shows the current profiles with and without **1** at an applied voltage of +80 mV across the membrane separating two 0.3 M Cs^+ solutions. A histogram of the currents at open and closed states (Figure 3b) shows a single-channel current of ~5 pA, which corresponds to an ion flux of $\sim 3 \times 10^7$ ions/s. It is comparable to that of gramicidin.[19] These results are consistent with an ion channel mechanism.[1,14] On the other hand, **2** does not show any current flows under the same conditions, which coincides with the above results of the fluorometric experiments showing virtually no transport of Cs^+ across the membrane.

In summary, we have shown that the novel artificial ion channels based on CB[*n*] ($n = 6$ and 5) can transport proton and alkali metal ions across a lipid membrane with ion selectivity. Not only the structural resemblance to the selectivity filter of K^+ channels but also the remarkable ion selectivity makes this model system unique. At the moment, however, it is not clear how **1** and **2** form an ion channel in membranes, which is currently under investigation (see Supporting Information). Nevertheless, the novel ion channel system may find useful applications such as in drug discovery, detection of small molecules, ion separation, and construction of molecular devices.

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Supporting Information Available: Experimental details for synthesis of **1** and **2**, and for FT-IR, fluorescence, and planar bilayer lipid membrane voltage-clamp experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Alkylated cucurbit[*n*]uril ($n = 6$, 5), **1** and **2**, are synthesized from allyloxyCB[*n*].[9b] See Supporting Information.
(16) Pseudo-first-order rate constant (k in s^{-1}) for **1**: 10×10^{-3} for LiCl, 2.5×10^{-3} for NaCl, 5.9×10^{-3} for KCl, 8.1×10^{-3} for RbCl and CsCl; and for **2**: 2.1×10^{-3} for LiCl, 1.3×10^{-3} for NaCl. Rate constants for the KCl, RbCl, CsCl are no more than the background.
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