Ion Transport

DOI: 10.1002/anie.200804099

A Synthetic Ion Channel Derived from a Metallogallarene Capsule That Functions in Phospholipid Bilayers**

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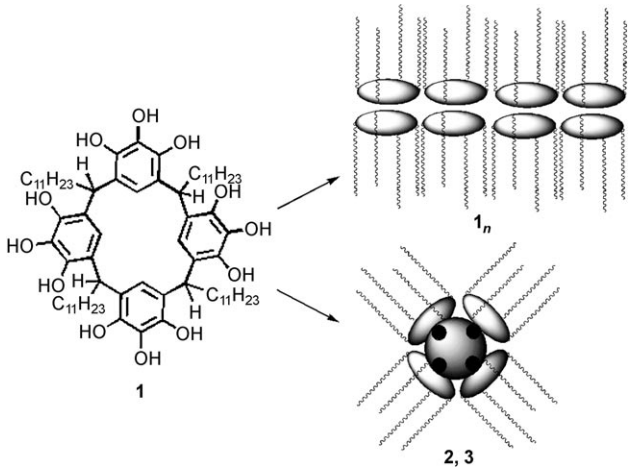
The transport of ions into and out of cells is essential to vitality. This flow of ions within cellular systems is exquisitely regulated by a variety of transporters including channels, carriers, and pumps.[1] Synthetic ion channels are now known that permit the selective transport of cations[2] or anions[3] across the phospholipid bilayer. These abiotic systems are models of protein carriers and channels but they are also functional channels. They are certainly simpler and less selective than protein channels but they transport ions and often show considerable selectivity. Many different structures have shown channel activity and the variations continue to expand.[4] In addition to a recently reported example,[5] a unique metal–organic framework that functions as a channel reported by Yaghi and co-workers,[6] and our own expectation that pyrogallolarene capsules could also exhibit channel activity prompts us to report the efficacy of pyrogallarene[7] capsules as cation channels.



The reaction of dodecanal with pyrogallol produced the known[8] tetramer, **1**, in 48% yield. This tetramer was then recrystallized from ethanol to afford the known self-assembled bilayer.[9] The structure of the bilayer, $\mathbf{1}_n$, was confirmed by ^{1}H NMR spectroscopy and FAB mass spectrometry. Crystallization of **1** from EtOAc, as previously reported by Atwood and co-workers gave the hexameric, hydrogen-bonded capsule **2** ($\mathbf{1}_6$).[10] Crystallization from a mixture of CH_3CN and EtOAc also afforded **2**. Atwood and co-workers have recently revealed the remarkable property of C-alkylpyrogallol[4]arenes to undergo addition of metal ions and form metal–organic nanocapsules.[11] We followed the procedure reported for this conversion (that is, **1**→**3**) and obtained the copper-network capsule **3** in 91% yield. The structure of **3** appears to be identical to that reported and both FTIR spectroscopy and MALDI mass spectrometry data are in accord with the assigned structure. The structure of **1** is shown along with schematic representations of the bilayer ($\mathbf{1}_n$) and the hydrogen-bonded (**2**) and copper-network capsules (**3**).

The solid-state structure of **2** has been reported previously and was also independently obtained by us.[10] The minor differences between our structure and that published arise from slight variations in the undecyl chain positions; our result is therefore not shown here. The crystal structure did reveal that the capsule has six openings of approximately 3.8 Å diameter on opposite faces. The capsule is approximately 17 Å in diameter, exclusive of side chain extensions. The fact that the capsule is rather like a barrel that has a hydrophobic interior with circular orifices and numerous hydrocarbon chains extending from it suggested that it might insert into a phospholipid bilayer and that the hydrocarbon chains of the capsule could interact with, and be stabilized by, the fatty acid chains of the phospholipid. We anticipated that this compound would insert in a bilayer membrane and function as a channel.

Three sets of experiments were undertaken to determine if capsules **2** and/or **3** exhibited channel function. The assessment was made by planar bilayer conductance measurements. In this technique, two salt solutions are separated by a barrier that has an opening of approximately 200 μm. A phospholipid membrane is formed in the opening, which prevents contact of the two solutions until or unless a

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[**] We gratefully acknowledge support of this work by the National Science Foundation (NSF, CHE-9805840) and the National Institutes of Health (NIH, GM-36262).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200804099.

functional channel is present in the membrane. A potential is applied and channel activity is observed as classic open–close behavior. The bilayer ($\mathbf{1}_n$), hydrogen-bonded capsule (**2**), and copper-seamed capsule (**3**) were all assessed as possible channel-forming structures. It seemed unlikely that the extended bilayer structure of $\mathbf{1}_n$ would form a conductance pathway orthogonal to the phospholipid bilayer. Compound $\mathbf{1}_n$ is indeed inactive as a channel and serves effectively as a control.

Compound **3** was added to DMSO so that the resulting turbid solution had a concentration of 1 mM. The cuvette (*trans*) and chamber (*cis*) were charged with KCl solutions (3.0 M (*cis*), 0.45 M (*trans*), pH 7). The membrane was formed from asolectin (from soybean) dissolved in *n*-decane (25 mg mL^{-1}). The solution of the potential channel-forming structure (9 μL) was added to the cuvette (*cis*) and the mixture was allowed to equilibrate. The membrane conductance was then assessed at the applied voltages specified. Both $\mathbf{1}_n$ and **2** were inactive when assayed by this technique. Figure 1 shows the results of planar bilayer conductance experiments for **3**.

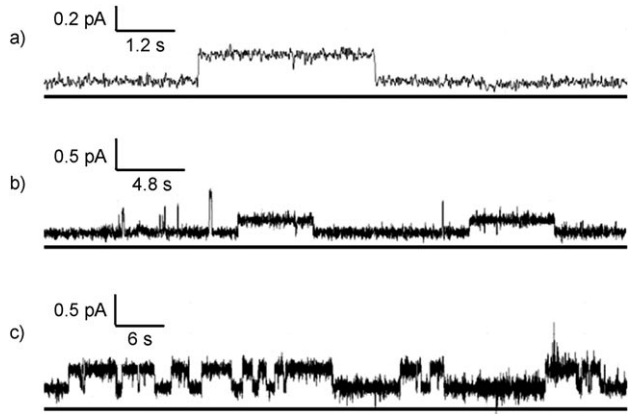

Figure 1. Planar bilayer voltage clamp conductance data (KCl) for **3**, determined at a) 30 mV, b) 50 mV, and c) 60 mV. The concentrations of KCl solutions were 3.0 M and 0.45 M for *cis* and *trans*, respectively, at pH 7.

The traces in Figure 1 show that **3** conducts ions at 30, 50, and 60 mV and that the open states are increasingly more stable. Longer opening at increasing voltage is referred to as voltage-dependent gating. As noted above, no characteristic open–close behavior was apparent when either the bilayer structure $\mathbf{1}_n$ or the hydrogen-bonded capsule **2** was added to the planar bilayer (data not shown), although minor membrane disruption was apparent in both cases.

The graph shown in Figure 2 is the current–voltage (*I–V*) plot that was obtained for KCl. By using the Goldman–Hodgkin–Katz equation, we calculated a K^+/Cl^- permeability ratio of 2.4:1. This is a modest selectivity but the fact that these channels function is remarkable, and the fact that they exhibit even this selectivity is significant. When symmetric KCl buffer solutions (0.45 M) were examined, the average conductance was approximately 12 pS (data not shown). The ionic diameter of K^+ is reported to be approximately 2.7 Å.[12]

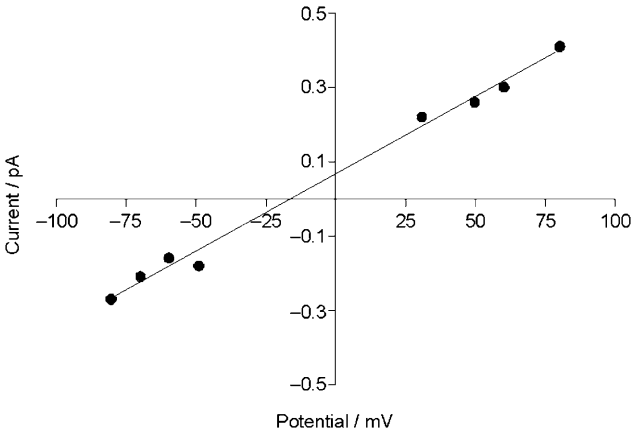

Figure 2. *I–V* plot for **3**.

Additional studies showed that the selectivity order among cations was $Na^+ > K^+ >> Cs^+$ (data not shown). The experiments involving Cs^+ were conducted with CsCl, it is presumed that the anion was transported. Moreover, shortly after the experiment was begun, transport shut down. We speculate that this is due to pore blockage by the large Cs^+ ion.

In order for any compound to function as a channel, it must create a conductance pathway through the bilayer and there must be a mechanism that permits gating. There are two obvious possibilities to explain the function of **3**. A single capsule may insert into the bilayer and its undecyl chains could extend into the hydrocarbon motif of the bilayer. A single capsule can easily fit within the hydrocarbon slab of the membrane, even including its alkyl chains. The hydrophobic contacts between the hydrocarbon chains and the bilayer could stabilize the position of the capsule. It is presumed that the phospholipid headgroups would reorganize to provide an entry portal of the channel. Even with hydrocarbon–hydrocarbon interactions that stabilize the capsule in the bilayer, a rocking motion of the capsule could serve as a gating mechanism that would change accessibility to either the entry or exit portal, or both.

Alternatively, two molecules of **3** could stack, one in each bilayer leaflet, to form a conductance pathway. The insulator layer of a membrane is thought to be 30–35 Å in thickness and a stack of two capsules would protrude from the insulator layer but be fully embedded in the bilayer itself. A movement of one molecule with respect to the other could either offset or align the orifices leading to closed or open gating behavior, respectively. Furthermore, at increased voltages, the enhanced flow of ions through the two capsules would make it more difficult for the pair to misalign. This could account for the voltage-dependent gating that is apparent in Figure 1 and favors the dimer mechanism. A schematic representation of the possible single-capsule and double-capsule mechanisms is shown in Figure 3 a and b, respectively.

It is also possible that ions flow over or around the capsule. This seems unlikely because the capsule exterior is oxygen-rich. Oxygen atoms would interact with transient K^+ ions and bind them, at least for a finite time. It may seem counterintuitive that transport is best through a hydrophobic channel such as that presented by the capsule interior. We

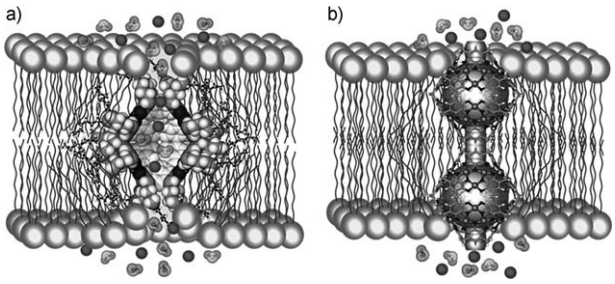

Figure 3. Postulated a) single-channel and b) double-channel organization of copper-seamed capsule **3** within an asolectin bilayer.

found that when the dodecyl chains of hydraphiles were replaced by ethyleneoxy units, the transport efficacy was diminished.[13] When Mackinnon and co-workers reported the first structure of a protein channel, they found that most of the channel interior was hydrophobic and described it as a "tunnel 18 Å in length (the internal pore)".[14] The interior of the pyrogallarene capsule is similar in dimension and in hydrophobicity.

The ability of these capsules to organize within bilayers and to mediate the transport of cations is a remarkable property. Other metallocapsules may also mediate ion transport and differences in the metals that are used to seam the capsules may alter association and transport properties. These possibilities present a vast landscape for future study.

Received: August 20, 2008
Revised: October 26, 2008
Published online: December 3, 2008

Keywords: capsules · hydrogen bonding · ion channels · membranes · phospholipids

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