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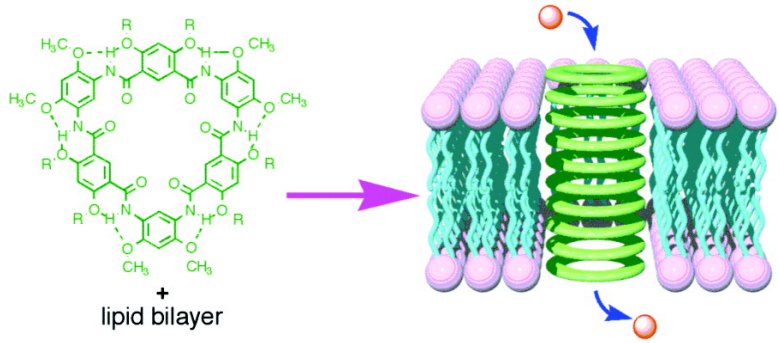
J. Am. Chem. Soc., **2008**, 130 (47), 15784-15785 • DOI: 10.1021/ja807078y • Publication Date (Web): 31 October 2008



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Published on Web 10/31/2008

Highly Conducting Transmembrane Pores Formed by Aromatic Oligoamide Macrocycles

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Naturally occurring membrane channels and pores are formed from different proteins, peptides, and organic secondary metabolites with vital biological functions.[1] Channel- and pore-forming peptides and proteins,[2] which function by creating pores within the plasma membrane of a target cell, have attracted intense interest in the development of transmembrane molecular gatekeepers with potential applications. Inspired by natural systems, progress has been made in the designs of various artificial channels.[3,4] Few examples[5] of artificial transmembrane pores with large lumens and conductance are known. We report here transmembrane pores formed by shape-persistent oligoamide macrocycles with large transmembrane conductances rivaling those of protein toxins[2f] that form large transmembrane pores.

We recently reported macrocycles **1** that formed in high yields from a one-step condensation of the corresponding diamines and diacid chlorides (Figure 1a).[6] Macrocycles **1** contain a large (~8 Å across), noncollapsible hydrophilic cavity defined by six introverted amide oxygen atoms. These macrocycles were found to aggregate strongly, forming fibrous assemblies.[7] Given their relatively flat oligoamide backbone with a large aromatic surface area, the aggregation of **1** is most likely mediated by face-to-face stacking, which presumably aligns the macrocycles into nanotubes containing a large channel (or nanopore) (Figure 1b).[8] Such a nanotube, when having a membrane-compatible exterior, may act as a transmembrane channel by partitioning into a lipid bilayer.

The possibility for macrocycles **1** to form transmembrane channels was first assessed using ^{23}Na NMR. While **1a**−**1c** were not manageable due to solubilities that are either too low or too high in aqueous media, macrocycle **1d** was found to be membrane-active. The ^{23}Na NMR method[9] relies on a shift reagent[10] composed of sodium tris(polyphosphate) and dysprosium chloride hexahydrate, which leads to two ^{23}Na NMR peaks when added to a lipsomal solution: an intravesicular one that is not affected, and an extravesicular one that shifts upfield. These two signals were observed in the presence of **1d** in a wide range of concentrations,[11] indicating that **1d** did not lyse the POPC/PS (80/20) LUVs. Upon increasing the concentration of **1d** or gamicidin, the intravesicular ^{23}Na peaks broadened due to transmembrane exchange of Na^+ ions. The rate of transport was quantified by measuring concentration-dependent peak broadening.[11] Plotting the rate values (in Hz) against the concentration of test compound leads to the determination of the relative rate constant of sodium transport, which is equal to the slope of the line (Figure 2). Macrocycle **1d** had an exchange rate constant of 5.65 ± 1.62 s^{-1}, about half of that of gramicidin (12.2 ± 3.02 s^{-1}). In contrast, the exchange rate constant of ion carrier valinomycin was smaller (0.42 ± 0.28 s^{-1}) than that of **1d** for over an order of magnitude. Thus, similar to gramicidin, it is likely that **1d** also acted by forming transmembrane channels.

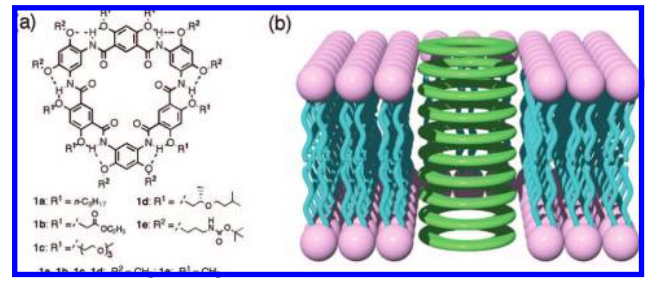

Figure 1. (a) Shape persistent macrocyles **1**, with their large aromatic surfaces, (b) could assemble anisotropically into a tubular structure that acts as a transmembrane channel or pore in the hydrophobic environment of a lipid bilayer.

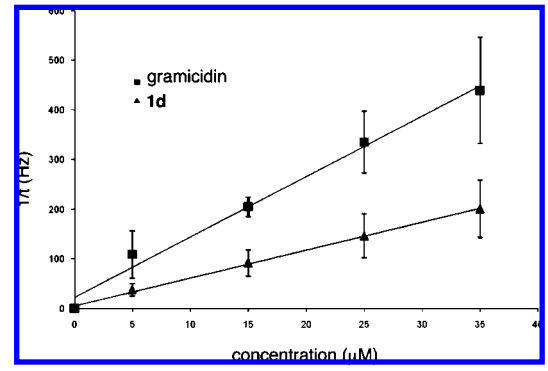

Figure 2. Rate [$k = 1/\tau = \pi(\Delta\nu - \Delta\nu_o)$] of sodium efflux from the liposomes vs the concentration of gramicidin and macrocycle **1d**. The sodium exchange rate constant is equal to the slope of each line.

Planar bilayer (POPC/PS = 80/20) conductance measurements provided diagnostic evidence for a channel mechanism. At 50 mV across the membrane separating two KCl (0.5 M) solutions in the presence of **1d** (0.32 μM), the recorded current profile is shown in Figure 2a. A linear current−voltage relationship was found (Figure 2b), corresponding to a specific conductance of 770 ± 30 picosiemens (pS). The switching from open to closed states was probably due to the assembly and disassembly of the macrocycles. Consistent with such a model, a single channel was detected at 0.32 μM; at higher concentrations, multiple channels of a similar conductance could be detected.

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10.1021/ja807078y CCC: $40.75

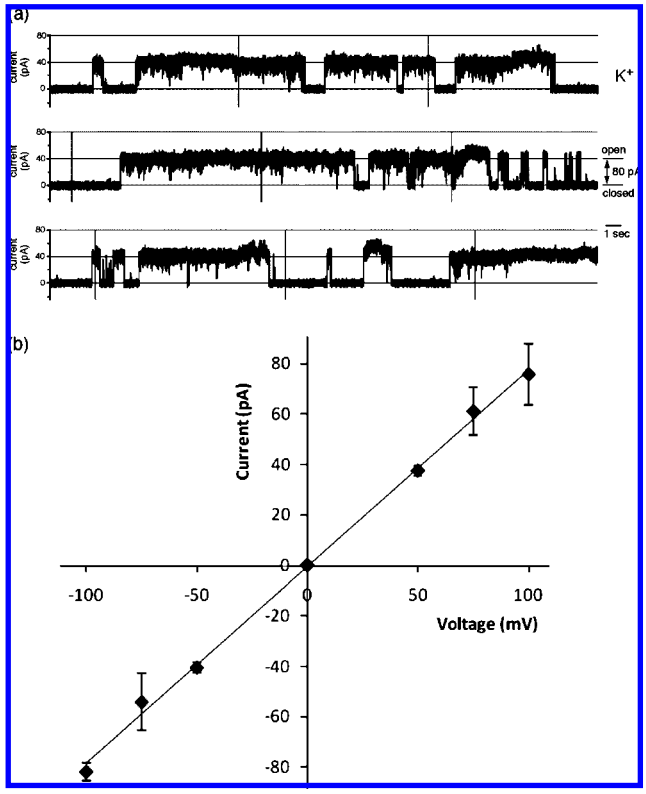

Figure 3. (a) A 108-s continuous K^+ single channel conductance recording at 50 mV with macrocycle **1d** (0.32 μM). (b) The current−voltage diagram of the channel formed by macrocycle **1d** (0.32 μM).

The transmembrane conductances of macrocycle **1e** also showed a linear current−voltage relationship,[11] based on which a specific conductance of 890 ± 52 pS (0.5 M KCl) was obtained. Thus, in spite of their different side chains, macrocycles **1d** and **1e** formed pores with very similar, if not identical, conductance, which implies that the observed ion-conducting activities are due to the cyclic backbone shared by both **1d** and **1e**, which defines a pore of the same diameter.

The conductances of **1d** and **1e** are much larger than those of natural and synthetic ion channels (a few to tens of pS), and are comparable to those of pore-forming protein toxins such as α-hemolysin (800 to 1000 pS) under similar conditions. The observed high conductances may result from self-assembling pores consisting of macrocyclic molecules aligned via intermolecular stacking interaction (Figure 1b). The shape persistency of the constituent macrocycles lead to robust pores, allowing efficient passage of ions.

To examine such a self-assembling model, the pore size formed by **1d** was estimated[11] based on the measured single channel conductance by using the Hille's equation as described before.[12] To account for the observed conductance, a diameter of 8.5 Å was found when the transmembrane pore was assumed to have the length (40 Å) to span the entire lipid bilayer. If the transmembrane pore could only reach across the hydrophobic core of the lipid bilayer, its length would be 27 Å, which necessitates a diameter of 7.2 Å. Given that the exact pore length could not be determined at present, the estimated diameters are well within that (∼8.3 Å) revealed by the structure of macrocycle **1** optimized by molecular modeling.

In summary, we have demonstrated that macrocycles **1d** and **1e** form transmembrane channels with very large conductances. Given the aggregation tendency of macrocycles **1** and the initial evidence presented herein, the transmembrane pores as shown in Figure 1b is a very likely model. Efforts are being made to elucidate and establish the detailed mechanism behind the formation and ion-transport activities of the transmembrane pores. The ready availability and easy tunability of these shape-persistent macrocycles have presented a new system for developing well-defined, stable nanopores by incorporating additional noncovalent and covalent interactions, which should lead to a new series of highly conducting nanopores with many applications.

Acknowledgment. This work is supported by the National Science Foundation (CHE-0701540 to B.G.), National Institutes of Health (GM68729 to Z.F.S.), and National 973 Project of China (2007CB936003 to Z.F.S.).

Supporting Information Available: Procedures and results for ^{23}Na NMR, single channel recordings, and Hille analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA807078Y