Artificial Channels

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Selective Artificial Transmembrane Channels for Protons by Formation of Water Wires**

Wen Si, Lei Chen, Xiao-Bo Hu, Gangfeng Tang, Zhenxia Chen, Jun-Li Hou, and Zhan-Ting Li*

Natural proton channels are passages formed by proteins that allow selective transport of protons across membranes.[1,2] One of the most important functions of the channels is that they can prevent cells from microbial invasion, which is realized with the cooperation of NADPH oxidase.[3] The channels are activated by the low pH environment of the endosome and exhibit voltage-dependent gating that is modulated by the extra- and intracellular pH values.[4] The first mechanism proposed for the transport concerns a process mediated through the hydrogen-bonded networks formed by amino acid residues of the channel proteins,[5,6] which is supported by both natural[7] and synthetic models.[8] Further investigations on structurally simple membrane proton channels, including gramicidin A and influenza A M2, have revealed that they can induce the formation of a water wire associated through hydrogen bonds, which can also conduct protons in a selective way.[9–12] In the past decades, a number of artificial systems have been developed which are capable of delivering metal cations and anions across membranes.[13–20] However, to the best of our knowledge, there is only one report[8] of synthetic structures that can realize the selective transport of protons.[21] In view of the straightforward water wire transport mechanism revealed in natural proton channels, we have been inspired to develop simple artificial channels for efficient transmembrane proton transport. In addition, the new systems can also be used as simple models to investigate the factors that affect the transport process.[22] We herein report the first class of water-wire-based artificial transmembrane proton channels, which are constructed from pillar[5]arene monomeric and dimeric derivatives. Remarkably, the new channels display a channel activity which is as high as 68%.

We recently reported that pillar[5]arene[23,24] **1** (Figure 1a) can stack in a face-to-face manner to form infinite organic nanotubes (Figure 1b) in the crystal structure, which induce water molecules to form ordered water wires (Figure 1b).[25] Hydrogen-deuterium exchange experiments revealed that the encapsulated water wires in the tubular structures can serve as a pathway for proton transport in the crystals. We conjectured that **1** and its analogues might form transmembrane proton channels by portioning into lipid bilayers. We thus prepared two series of new dimeric compounds **2a–e** and **3a–e** to investigate their potentials in transmembrane proton conductance (Figure 1c).

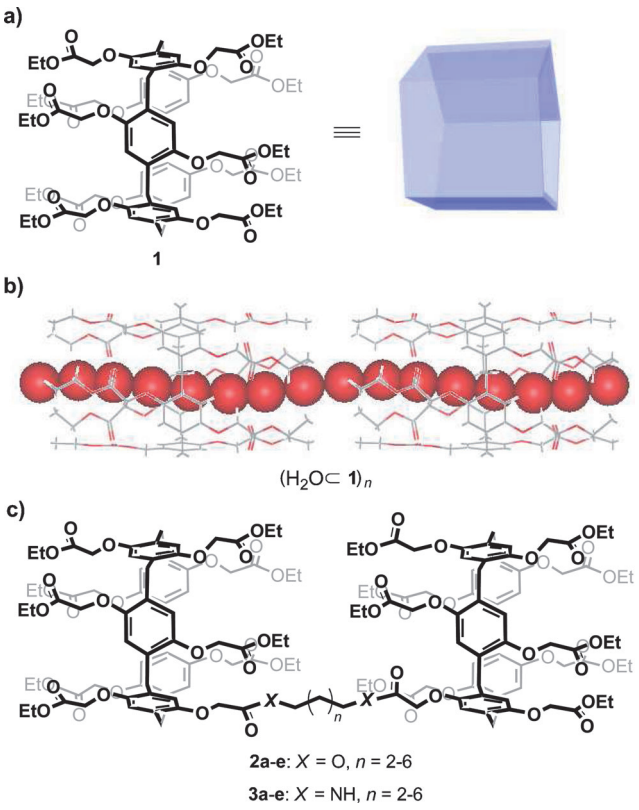

Figure 1. a) Structure of **1**. b) Partial X-ray crystal structure of **1**, indicating that water molecules (CPK model) were induced to form linear water wires in organic nanotubes (stick model). c) Structures of **2a–e** and **3a–e**.

Recording currents across bilayers at the single-channel level is the best way to test whether a molecule or an assembled system forms ionic channels.[26] However, the determination of the single-channel conductance of proton channels has been a challenge because single-channel currents can only be observed at low pH values and the used lipid bilayers must therefore withstand the low pH conditions. Lipids composed of phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and cholesterol (molar ratio, 4:1:1:2) has been proved to fulfill these requirements,[27,28] and was thus employed in this research. Compound **1** was first investigated as a model. The single-channel conductance,

[*] W. Si, L. Chen, X.-B. Hu, G. Tang, Z. Chen, Prof. J.-L. Hou, Prof. Z.-T. Li
Department of Chemistry, Fudan University
220 Handan Road, Shanghai 200433 (China)
E-mail: houjl@fudan.edu.cn

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using the planar lipid bilayers with 0.50 μM of **1** in the subphases, was first measured with two symmetrical HCl solutions of pH 4.4. The currents recording at various voltages showed square ion channel currents (Figure 2a,b) and fell

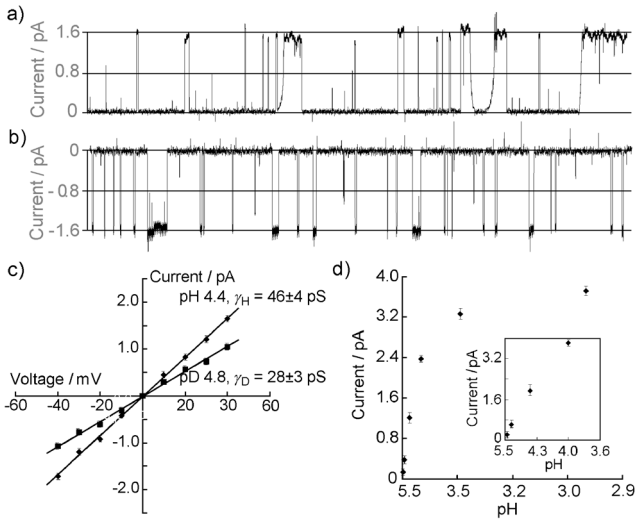

Figure 2. Current traces (50 seconds) of **1** in the planar lipid bilayer at a) 40 and b) −40 mV in a symmetrical HCl solution (pH 4.4), indicating the formation of transmembrane channels. c) Plots of single-channel currents versus voltages at pH 4.4 and pD 4.8, from which an isotope effect of 1.6 was deduced. d) Plot of the current versus the pH value, suggesting that protons were the main current carriers and that the channel was stable in the pH range 4.0–5.5. Error bars in (c) and (d) are standard deviations of the three experiments.

into a linear current–voltage relationship in the measured range (Figure 2c), indicating the formation of the channel in the planar lipid bilayer. The conductance (γ_H) calculated from the linear current–voltage relationship was 44 pS. The current traces at 40 mV at various pH values showed that the channel at pH > 5.5 did not display any activity because of the lowered proton concentration. Within the range of pH 4.0–5.5, the current magnitude increased linearly with decreasing pH (Figure 2d), suggesting that the proton was the principal current carrier.[28] Further decreasing the pH caused only a slight increase of the current, probably because of the hydrolysis of **1** under more acidic conditions. The length of **1** was 1.6 nm as calculated from the X-ray structure.[25] In view of the thickness (≈3.7 nm) of the hydrophobic part of the lipid bilayer, we proposed that two molecules of **1** randomly distributed in the lipid bilayer would stack to form a channel matching the thickness of the bilayer, as shown in Figure 3.

To further investigate the proton conductance mechanism, the isotope effect was explored by substituting deuterium oxide (D_2O) and deuterium chloride (DCl) for water and hydrogen chloride,[29] respectively, for the single-channel conductance experiment at a pD of 4.8.[30] Under this condition, the measured conductance was $\gamma_D = 28$ pS (Figure 2c), which corresponded to an isotope effect (γ_H/γ_D)[29] of 1.6. The isotope effect value was comparable to that of natural proton channels,[29] supporting that the proton migration occurred along the water wire in the Grotthus mechanism.[31]

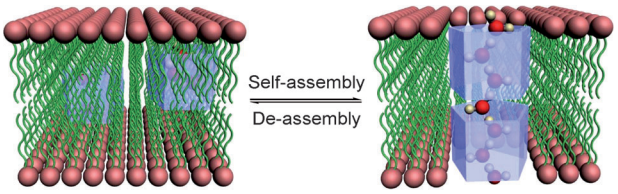

Figure 3. Channel formed from **1**. Two molecules of **1** randomly distributed in lipid bilayer (left) undergo self-assembly to form a water-wire-based proton channel (right).

The formed water wire might also account for the stabilization of the channel in the planar lipid bilayer. The channel switching from the open to closed states was probably due to the disassembly of the water wire or the channel.

It was envisioned that connecting two pillar[5]arene units with a linker of suitable length would generate a channel of increased stability. We thus further prepared **2a–e** and **3a–e**. The channel activities of compounds **2a–e** were first investigated using the same method. The results are provided in Figure 4a–e, and the related open probability and conductance data are listed in Table 1. The single-channel conduc-

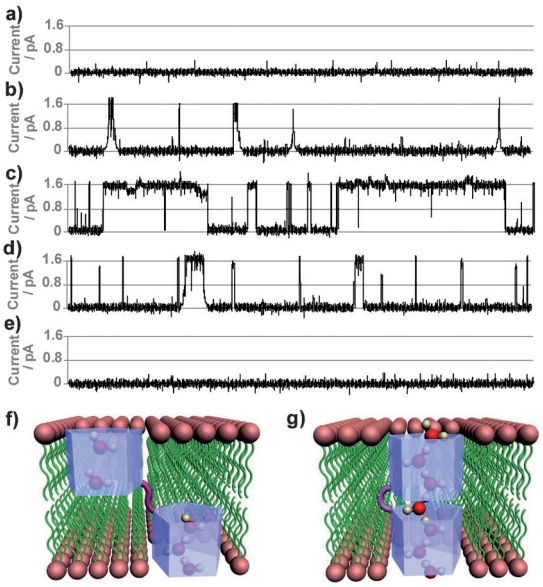

Figure 4. a–e) Current traces (50 s) of **2a–e** (in the planar lipid bilayer at 40 mV in a symmetrical HCl solution at pH 4.4), showing that the compounds of series **2** display different channel activities. f) The linkers of **2a** and **2e** minimized the intramolecular stacking interactions of the two pillar[5]arene units. g) However, the linker of **2c** maximized the stacking interactions.

tance measurement for **2c** was also performed in the solution of DCl in D_2O, and the related isotope effect data are also included in Table 1. It can be seen that the shortest **2a** displayed no channel activity. The longer **2b–d** all displayed the channel activities, with the channel open probability being 7, 64, and 12%, respectively, while the longest **2e** again did not exhibit any activity. The value of **2c** is more than three times higher than that of **1**, indicating that the channel formed by **2c** was more stable than the assembled dimeric channel of

Table 1: Measured channel properties of **1**–**3**.[a]

Compound	Open Probability [%]	Conductance[b] (γ_H [pS])	Isotope Effect (γ_H/γ_D)
1	18	44 ± 2	1.6 ± 0.1
2b	7	42 ± 3	–
2c	64	42 ± 2	1.6 ± 0.1
2d	12	41 ± 2	–
3b	11	42 ± 2	–
	5	18 ± 2	
3c	45	40 ± 3	1.6 ± 0.1
	15	17 ± 2	2.0 ± 0.1
3d	13	42 ± 3	–
	6	17 ± 2	

[a] Based on 100 observed opening events. [b] The uncertainties derived from the uncertainties of the slop of the current–voltage plots.

1, and, as a result, the water wire formed in the channel of **2c** was also more stable, leading to the highest proton conductance activity. The fact that the other four dimers showed no activity or were less efficient than **1** implied that their linkers could not enable good stacking matching for the connected pillar[5]arene units (Figure 4 f). Both the conductance and isotope effect of **2c** are also similar to that of **1**, further suggesting that proton transport in the channel of **2c** also operated through the water wire formed in the well-stacked pillar[5]arene units (Figure 4 g).

The channel activities of compounds **3a**–**e** were also investigated. The related data are listed in Table 1 and the current traces of **3c** are provided in Figure 5 a as an example.

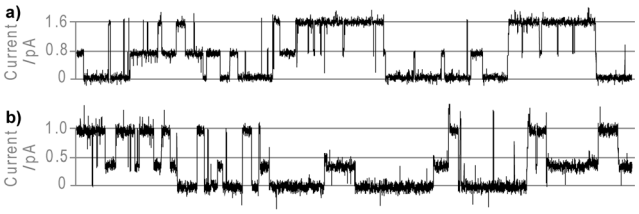

Figure 5. Current traces (50 seconds) of **3c** in the planar lipid bilayer at 40 mV in symmetrical a) HCl/H_2O (pH 4.4) and b) DCl/D_2O (pD 4.8) solutions.

Similar to **2a** and **2e**, compounds **3a** and **3e** did not display the activity either, while the activity of **3b**–**d** was comparable to that of the corresponding **2b**–**d**. Notably, different from **1** and **2b**–**d** which exhibited one uniform conductance state around 40 pS, **3b**–**d** all displayed two conductance states[32] around 40 and 17 pS (Figure 5 a for **3c** for example). The isotope effect experiment of **3c**, which showed the highest activity, was further carried out to explore the mechanism of the two conductance states, and current traces in DCl/D_2O are dispalyed in Figure 5 b. The isotope effect values of the two states were calculated by comparing the corresponding conductance states under HCl/H_2O and DCl/D_2O conditions. Isotope effects of 1.6 and 2.0 were obtained for the states at 40 and 17 pS, respectively. The facts that both the conductance and the isotope effect of the first state of **3c** were close to that of **1** and **2c** suggest that the mechanism of this conductance state was similar to that of the latter two. The smaller conductance at 17 pS might be attributed to the existence of the two amide units in **3c**, which might form hydrogen bonding with and also undergo hydrogen exchange with the neighboring hydrogen atoms in the water wires. These additional interactions should also lead to a larger isotope effect, as revealed in natural proton channels, like rat alveolar epithelium.[29] The fact that the first conductance state had a higher open probability than the second indicates that the first process contributed to a larger extent. Compounds **3b** and **3d** showed a similar tendency, even though their activity was relatively low.

The competitive effect[33] of Li^+, Na^+ and K^+ over H^+ ions was investigated for **1**, **2c,** and **3c** by measuring the conductance in symmetrical HCl baths (pH 4.4) in the presence of related alkali chloride salt. For the case of **1**, adding LiCl (5.0 mM) or KCl (5.0 mM) to the solution did not cause a discernible change of the current magnitude, while increasing NaCl to the concentration of 3.0 mM could lead to complete inhibition of the channel activity (Figure S12a–d, Supporting Information). ^{1}H NMR experiments in [D_8]toluene revealed that **1** was able to complex Na^+ but not Li^+ and K^+ ions (see Figure S13 in the Supporting Information). Thus, this inhibition might be ascribed to the complexation of the ether and ester units of **1** toward Na^+ [34a] or to the cation-π interaction between Na^+ and phenyl groups.[34b] Similar complexation might not exist for Li^+ and K^+ ions probably because of the size mismatching of the cations with the cavity surrounded by the ether and ester units. The investigation of **2c** gave rise to similar results, while, for **3c**, all the three cations did not impose important influence. Adding neutral diols (0.2 mM), including 1,5-pentanediol, 1,6-hexanediol, and 1,7-heptanediol, could also lead to partial blocking (40–65 %) of the currents (see Figure S12e in the Supporting Information), which should be attributed to the encapsulation of the diols by **1**, **2c**, and **3c**, as evidenced by the ^{1}H NMR experiments.[35,36] Further addition of the diols destroyed the lipid bilayer. Clearly, the complexation of Na^+ or diols to **1**, **2c**, or **3c** broke the water wire, leading to the loss or weakening of the channel activity.

The proton selectivity of the channels over Cl^- was also investigated by employing compound **1** as model.[28,37] To do this, asymmetric baths (*trans* chamber: [HCl] = 0.02 mM, *cis* chamber: [HCl] = 0.1 mM) were used to measure the currents at different voltages. The uncorrected reversal potential (E_{rev}) was determined to be −73 mV (see Figure S14 in the Supporting Information), which corresponded to a channel reversal potential of −38 mV after correction.[28] From the Goldman–Hodgkin–Katz equation,[38] the permeability ratio between H^+ and Cl^- ions (P_{H^+}/P_{Cl^-}) was calculated to be around 32, indicating a very substantial preference for protons. Under the identical conditions, the permeability ratios of **2c** and **3c** were measured to be 24 and 18, respectively, showing that they are also selective in conducting protons.

In summary, we have developed a new class of artificial transmembrane proton channels from mono- and dimeric

pillar[5]arene derivatives. The new channels conduct protons through water wires formed in the pillar[5]arene backbones, which are the first examples of water-wire-based proton channels built from synthetic systems. The new channels can work in less acidic environments. Moreover, the two dimeric channels with the hexamethylene linker show channel activities that are even higher than those of reported natural proton channels.[28] The two states of conductance displayed by the amide-bearing channels indicate that the proton conduction process of the new systems may be tuned by additional noncovalent interactions around the water wire. In principle, the new synthetic systems may also be incorporated into the membranes of vesicles and cells, and these systems might find a variety of chemical[39] and biological applications.[40]

Experimental Section

Method: The pH values were measured using a glass pH electrode. For pD measurement, the pH value was corrected by adding 0.04 M HCl solution (1.0 mL) to H_2O (0.1 L) and D_2O, respectively.[30] The pH meter reading in D_2O solution is 0.41 unit higher than in H_2O solution, which ascribe for a change of electrode potential in D_2O compared to H_2O.[30] Therefore, the pD values in D_2O solutions were corrected by adding 0.41 to the measured pH value.

Description for the conductance measurements of the planar lipid bilayers: The chloroform solution of L-α-phosphatidylethanolamine (Sigma, lyophilized powder from egg yolk), L-α-phosphatidylcholine (Sigma, from egg yolk), L-α-phosphatidyl-L-serine (Sigma, from Glycine max), and cholesterol (Aldrich) were mixed in a molar ratio of 4:1:1:2, and then the mixture was evaporated with nitrogen gas to form a thin film. The resulted lipid was suspended in *n*-decane by sonicating for five seconds. Planar lipid bilayers were formed by painting the lipids dispersed in *n*-decane around a 200 μm diameter aperture of the Delrin cup (Warner Instruments, Hamden, CT). The chambers were filled with either symmetrical or asymmetrical HCl solution, 1.0 mL for each side. Formation of membrane was monitored by measuring the membrane capacitance. In a typical experiment, a 5 μL solution of the test compound in DMSO (1 mM) was added to the *cis* chamber and the solution was stirred for 1 min. Ag-AgCl electrodes were applied directly to the solutions instead of connecting to salt bridges to protect the solution from contamination of metal ions.[28] Membrane currents were measured using a Warner BC-535D bilayer clamp amplifier and then, filtered with a 8-pole Bessel filter at 1 kHz, and digitized (Digitizer Digidata 1322A from Axon Instruments, Foster City, CA). The data were collected and analyzed by using the pClamp suit software (version 9.2; Axon Instruments, Foster City, CA). All experiments were performed at 20 °C. Parallel experiments were carried out to accumulate enough opening events. The open probabilities and conductances were derived from 100 openings. For compounds with two conductance states, the openings of the two states were separated by hand from all openings (100 events) and the open probabilities and conductances were generated. The uncertainties of the conductances were derived from the uncertainties of the slop of the current–voltage plots.

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