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Communication

Unimolecular artificial transmembrane channel with terminal dihydrogen phosphate groups showing transport selectivity for ammonium

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ABSTRACT

A new artificial transmembrane channel molecule bearing dihydrogen phosphate groups has been synthesized. The terminal dihydrogen phosphate groups enable the channel to be highly negatively charged at both ends of the channel structures. The artificial channel could incorporate into the lipid bilayer efficiently under low concentration. The channel displays high NH_4^+/K^+ selectivity due to the electrostatic interaction and hydrogen bonding between NH_4^+ and the terminal dihydrogen phosphate groups.

Natural ion channels are a kind of membrane proteins that are able to mediate the flux of ions to generate resting membrane potentials and physiological signals, and regulate cell environments [1]. The channels are able to transport ions in a selective manner, which are achieved under the aid of specific chemical groups precisely located within the channel structures. For example, the selectivity filter decorated with carbonyl oxygen atoms in the natural KcsA K^+ channel allows to conduct K^+ ions while excluding Na^+ ions [2]. Inspired by the important function of channel proteins, chemists have made significant progresses in the construction of artificial ion channels to mimic the ion transport of natural channels [3–11] and develop advanced materials [12–16]. During the past decades, significant efforts have been devoted to achieve the construction of artificial channels with high transport selectivity by using supramolecular strategy [17,18]. Comparing to the dynamic structure of supramolecular channels, unimolecular channels possess more stable structures, which allow for accurate manipulation of the structure [19–24]. Recently, we have developed a new strategy to build unimolecualr artificial transmembrane channels with confined tubular structures based on pillararene backbones [25]. We demonstrated that the introduction of negative charged groups at both ends of the channels not only enhanced the membrane-incorporation ability of the channels but also provided filters for achieving cation transport selectivity [26–28]. It was envisioned that the introduction of negative charged groups with more density would lead to higher cation transport selectivity. However, this has not been achieved yet due to the decreased hydrophobicity of the channel molecules by introducing negative charged groups, which lead to the weak membrane-incorporation ability of the channel molecules. Herein we demonstrated that the unimolecular artificial transmembrane channel with terminal phosphate groups exhibited not only high membrane-incorporation ability but also high NH_4^+/K^+ transport selectivity.

Under physiological conditions, the dihydrogen phosphate group can ionized to produce two negative charges. Thus, the channel **1** containing terminal the dihydrogen phosphate groups were designed (Fig. 1), which should be highly negatively charged at both ends of the channel molecules under neutral pH. The channel **1** was prepared by using pillar [5]arene decaacid **2** as starting material [29–41]. Firstly, the channel precursor **3** was prepared by coupling the peptide composed of D-Phe and L-Trp with **2** in the presence of EDCI. The compound **3** was then deprotected by TFA to yield channel **1**. The alternated D- and L-amino acids were designed to ensure the nonpolar side chains of the amino acid residues outward, which were expected to increase the membrane-insertion ability of the channel molecules. The indole group might further enhance the membrane-insertion ability of the channel due to the formation of hydrogen bonding between its amide and lipid head-group oxygen atoms [42–44].

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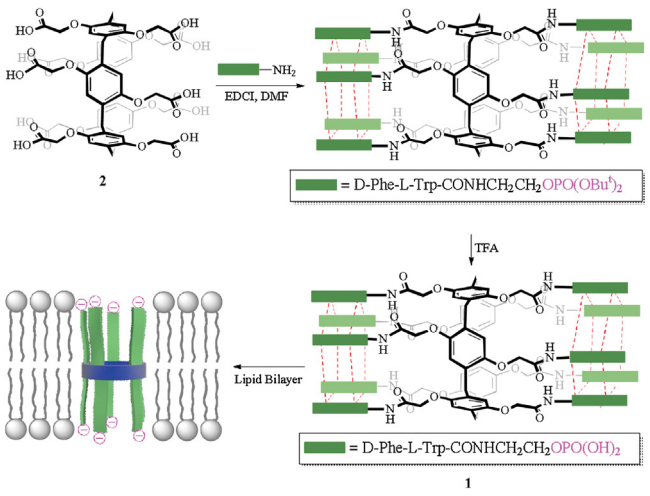

Fig. 1. Chemical structure and synthetic procedures of the channel **1** and its schematic representation for the formation of unimolecular transmembrane channel in lipid bilayers.

The length of the channel molecule was determined to be 3.5 nm, which matches well with the thickness of the hydrophobic area of the lipid bilayer.

The possibility of forming transmembrane channel in lipid bilayers of **1** was firstly investigated by assessing their proton transport activity with large unilamellar vesicles (LUVs) [45–47]. LUVs were made from egg yolk phosphatidyl choline (EYPC) containing pH sensitive 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) as a fluorescence probe (pH 7.2). A suspension of LUV entrapped with HPTS was added to a buffer (pH 6.0) to produce a higher H^+ concentration outside the vesicles. The flux of H^+ into the vesicles was assessed by monitoring the fluorescence intensity of HPTS. With addition of pure DMSO to the vesicle suspension, little fluorescence intensity change was observed, suggesting that the bilayer is un-permeable for H^+. However, upon addition of the solution of **1** in DMSO to the vesicle suspension under the same conditions, the fluorescence intensity of HPTS increased significantly during 5 min (Fig. 2a). These results demonstrated that the compound **1** was able to insert into bilayers and form transmembrane channels to mediate the transport of H^+. It was found that the transport activity, as indicated by the final fluorescence intensity reached, was strongly dependent on the concentration of **1** [represented by the molar ratio relative to lipid (x)]. As x increased from 0 to 0.75%, the fluorescence intensity increased significantly (from 13% to 75%) (Fig. 2b). Further increasing x caused only a slight increase in fluorescence intensity. The effective concentration required for 50% transport activity (EC_{50}) and the Hill coefficient (n) were calculated to be 0.17% and 0.6 by fitting the plot with Hill equation [48]. The low EC_{50} indicates that the new channel with terminal negative charged phosphate groups is very effective in incorporation into the lipid bilayers, while the small n value shows that the channel works in a unimolecular manner [49].

To further confirm the formation of transmembrane channels in the lipid bilayers, the conductance measurements on a planar lipid bilayer were also performed [50]. For the experiments, two compartments containing KCl solution (1.0 mol/L) were separated by a planar lipid bilayer composed of diphytanoyl-phosphatidylcholine (diPhyPC). The solution of **1** in DMSO was added to the *cis* compartment, which was grounded. A clamped voltage of +100 mV was applied across the bilayer, and the conductance traces were recorded. It was observed that the traces displayed regular square-like signals (Fig. 3a). The presence of the conductance signals strongly supports the formation of transmembrane channel of **1** in

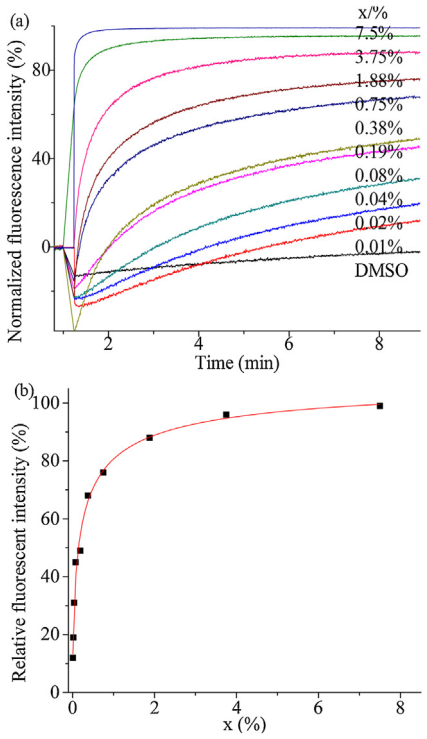

Fig. 2. The proton transport experiments of **1** on LUV by using HPTS assay. (a) Transport activity under different concentrations of **1**. (b) The relationship of the transport activity *vs.* the concentration of **1**. The solid red line is the fitting plot by using Hill equation.

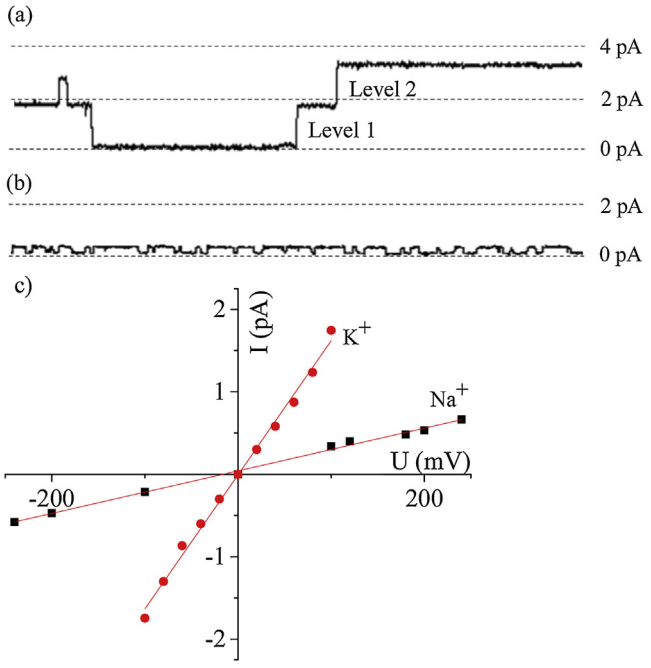

Fig. 3. Conductance measurements of **1** on planar lipid bilayers. Current traces (20 s) at 100 mV in (a) KCl (1.0 mol/L) and (b) NaCl (1.0 mol/L); (c) *I–V* plots in KCl and NaCl solutions (1.0 mol/L).

the bilayer. For the conductance measurements, the number of the current levels in the trace reflects the amount of the channel molecules in the bilayer. The variation in the channel conformations would lead to different currents of the levels. In our experiments, two main levels of currents were observed in

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conductance trace (Fig. 3a), indicating that two channels formed in the bilayer under the experimental conditions. By further analyzing the conductance trace, it was found that the current values at each level were identical. This result clearly demonstrated that the channels had only one stable channel conformation in the bilayer [51]. The current-voltage (*I-V*) plot of the channel was further measured by performing the experiments at different voltages (Fig. 3c). It was found that the plot exhibited a linear *I–V* relationship in the range of -100 mV to +100 mV. The corresponding conductance (γ_{K^+}) of the channel for K^+ was calculated to be 16 ± 0.5 pS. The conductance measurements were also performed in NaCl (1.0 mol/L) solution by using the same method. It was found that the single channel current was obvious weaker than that in KCl solution (Fig. 3b). The corresponding conductance (γ_{Na^+}) for Na^+ was determined from the *I–V* plot to be 2.6 ± 0.3 pS (Fig. 3c). This small conductance value suggested the weak transport activity for Na^+, which might be result of strong coordination of the phosphate groups with Na^+.

The transport selectivity of **1** for cations and chloride anion were measured by using unsymmetrical salt solution on the two sides of the bilayers. The reversal potential (ε_{rev}), a voltage potential required for blocking ion current, was determined from the corresponding *I–V* plots (Fig. S17 in Supporting information). Using the obtained ε_{rev} value, the permeability (*P*) ratios of two respective ions was calculated from the Goldman-Hodgkin-Katz equation [52] and the results were shown in Fig. 4. Because of the weak transport activity for Na^+, the permeability ratio of K^+ to Na^+ could not obtained by using this method (Fig. S17). Thus, the selectivity between these two ions was determined from the ratio of their conductance, which was also shown in Fig. 4. It was found that the transport selectivity of the channel was in the order of $NH_4^+ > Cs^+ > Rb^+ > K^+ > Cl^- > Na^+$. The channels exhibited an Eisenman I selectivity toward the alkaline cations [53,54]. This selectivity sequence was caused by the increased dehydration penalty of the cations. The channel **1** displayed highest NH_4^+/K^+ selectivity, which could be rationalized by considering that, in addition to the electrostatic interactions, NH_4^+ can form intermolecular hydrogen bonds with the oxygen atoms of the phosphate groups [27,55].

In conclusion, we have prepared a new kind of artificial transmembrane channel by attaching the peptides containing dihydrogen phosphate groups onto the pillar[5]arene backbone. The terminal dihydrogen phosphate groups enable the channel to be highly negatively charged at both ends of the channel structures. The artificial channel could incorporate into the lipid bilayer efficiently under low concentration. The channel displays high NH_4^+/K^+ selectivity due to the electrostatic interaction and hydrogen bonding between NH_4^+ and the terminal dihydrogen phosphate groups. It has been well established that the dihydrogen phosphate group could be hydrolyzed by alkaline phosphatase. The transport activity of channel developed herein may be regulated by the enzyme, which is currently under investigations.

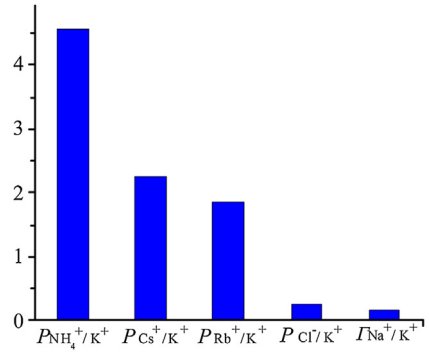

Fig. 4. The transport selectivity of **1** for different ions.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2019.05.009.

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