Communications

Ion Selectivity

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A Highly Active Anion-Selective Aminocyclodextrin Ion Channel**

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Ion-channel proteins are molecular devices found in nature that mediate the transport of charged species across cell membranes. Various functions in the human body, such as nerve and muscle excitation, hormonal secretion, cell proliferation, and homeostasis, are regulated by ion channels.^[1] The efficient functioning of ion-transport machinery in nature has led to considerable interest in understanding the mechanisms by which these proteins mediate selective, regulated transmembrane ion transport.^[2] Furthermore, the ability of ion channels to effect electrical signaling under aqueous saline conditions has inspired their use as biosensors,^[3] therapeutic agents,^[4] and other useful materials.^[5] Despite the potential utility of controlled ion transport, the relative instability of proteins in vitro will likely prohibit their use in commercial applications. Advances in the synthesis of more robust artificial channels provide a viable solution to the problem of ion-channel stability, thus enabling the construction of biomimetic signaling components.

An important feature that dictates the physiological function of ion channels is ion selectivity. Ion channels can be classified as cation or anion selective on the basis of the differential permeability of ions through the channel pore. Ion channels selective for cations over anions have been widely studied, and several synthetic analogues that use β -cyclodextrin,^[6] crown ethers,^[7] peptides,^[8] and calixarenes^[9] are among the molecular scaffolds that have been developed to date. In contrast, there have been far fewer examples of synthetic channels that are selective for anions over cations. Peptidic synthetic channels developed by Gokel^[10] and

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author. Matile^[11] and sterol mimics developed by Regen^[12] have been shown to preferentially transport anions over cations upon self-assembly within phospholipid membranes. Reported herein is a highly active, monomeric cyclodextrinbased ion channel (**1**, Figure 1) that displays not only selectivity for anions over cations but also discriminates among halide anions ($I^- > Br^- > Cl^-$).



Figure 1. Structure of aminocyclodextrin channel 1.

Channel **1** comprises a β -cyclodextrin head group with oligoether chains attached to the primary face of the cyclodextrin through amine linkages. The chains are sufficiently hydrophobic to promote insertion into a phospholipid bilayer membrane^[13] and possess a length that should allow the channel to span the entire membrane.^[14] Channel **1** was synthesized (Scheme 1) from pentabutylene glycol (**2**), which is readily accessible in five steps from commercially available 2,5-dimethoxytetrahydrofuran.^[15,16] Conversion of **2** into the monotosylate **3** was achieved using *p*-toluenesulfonyl chloride (TsCl) and pyridine (pyr). Monotosylate **3** was heated with sodium azide at 80 °C to give the oligoether azide, which was reduced to the corresponding amine **4** using triphenylphosphine and water. N-Alkylation of neat amine **4** with the activated heptaiodocyclodextrin **5**^[17] afforded channel **1**.



Scheme 1. Synthesis of channel 1. DMF = dimethylformamide.

7584

Initial ion-channel activity was assessed by using ²³Na NMR spectroscopy to study Na⁺ transport across vesicle membranes (Figure 2a),^[18] on the basis that other cyclo-



Figure 2. a) Schematic representation of the ²³Na NMR spectroscopic experiment (the italicized Na⁺ ions represent external ions with shifted NMR peaks owing to the presence of the Dy³⁺ shift reagent). b) Stacked NMR plots that indicate line broadening observed with an increase in the channel concentration (mol% relative to phospholipid); as a comparison gramicidin A (gA) was also added. c) Linear correlation between the Na⁺ exchange rates and the concentration of channel added, thus indicating that channel **1** is monomolecular.

dextrin-based ion channels were cation selective.^[6] In the NMR experiment, a Dy³⁺ complex was used as a shift reagent to differentiate the extravesicular Na⁺ ions from the internal Na⁺ ions.^[19] Ion transport can be visualized and quantified by the line broadening observed for the Na⁺ resonances because of the exchange of the internal and external Na⁺ ions. The rate of exchange is directly proportional to the line broadening observed for the internal Na⁺ signal [Eq. (1)], in which ν and

$$k = 1/\tau = \pi(\nu - \nu_0) \tag{1}$$

 v_0 are the line widths in the presence and absence of the exchange, respectively. For the ²³Na NMR experiment, large unilamellar vesicles of egg-yolk phosphatidylcholine (EYPC) lipids were prepared by multiple extrusions through 0.4-µm polycarbonate membranes.^[16] The vesicles were determined to be approximately 200 nm in diameter using dynamic light scattering. Addition of the DyPPi₃ shift reagent to the vesicle suspension followed by a solution of **1** led to significant line broadening of the internal Na⁺ peak (Figure 2b), thus indicating the channel-mediated exchange of Na⁺ ions across the vesicle membrane.^[20] The dependence of the observed rate of Na⁺ exchange (k_{obs}) on the concentration of **1** can be used to determine the aggregation state of the channel, or the number of **1** monomers that form the active structure [Eq. (2)].^[21] A linear correlation (n = 1) between the

$$k_{\rm obs} \propto [{\rm channel}]^n$$
 (2)

 $\mathrm{Na^{+}}$ exchange rate and the concentration of the channel indicated that the active structure formed from 1 was

monomolecular (Figure 2c). Also, a comparison of the Na⁺ transport rate through **1** with that for the natural ion-channel gramicidin A revealed that **1** was 36% as active as gramicidin A, thus indicating that **1** formed a highly active monomolecular ion channel.^[22] This result is particularly noteworthy in light of the net charge of **1**. The multiple positive charges associated with previously studied hepta-amino, -hydroxyethylamino, and -alkylamino cyclodextrin derivatives at physiological pH values have been widely exploited for binding negatively charged phosphates to the cyclodextrin cavity.^[23] In view of the similar charge densities imparted by the ammonium groups of **1** to the channel pore, it is remarkable that sodium transport is observed through the channel.

To expand the range of ions that could be assessed for transport activity, transmembrane ion-transport properties of **1** were also studied with fluorescence spectroscopy, in which the fluorescence of the pH-sensitive dye 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) was used as the probe.^[24] In the fluorescence assay, the HPTS dye was encapsulated inside small unilamellar vesicles (Figure 3 a) approximately 50–80 nm in diameter, which were prepared by using the detergent dialysis method.^[16] A pH gradient of 0.6 units was introduced by addition of a solution of NaOH (2N) to the vesicle solution, which also introduced a Na⁺ concentration gradient. A solution of **1** was added to the vesicles and the activity of the channel was gauged by monitoring the change in the fluorescence intensity of the deprotonated dye. Finally, gramicidin A was added to the



Figure 3. a) Schematic representation of the fluorescence experiment. b) Change in the fluorescence intensity of the deprotonated dye with a change in the concentration of the channel (mol%). c) Linear correlation between the rate of transport and the concentration of the channel.

Communications

vesicles to ensure complete equilibration of the HPTS dye between the protonated and deprotonated states. Upon addition of a solution of 1 to the vesicles, an increase in the concentration of the deprotonated dye was observed, thus indicating a Na⁺/H⁺ antiport or Na⁺/OH⁻ symport through 1. Moreover, variation in the concentration of 1 induced a corresponding change in the ion-transport rate (Figure 3b). A linear correlation was observed upon plotting the rates for ion transport against the amount of 1 added, thereby confirming that 1 indeed formed a monomolecular ion channel (Figure 3c).^[25]

Having established that channel 1 mediates the rapid transport of Na⁺ ions through a monomolecular pore, the fluorescence assay was used to probe its ion selectivity.^[26] Cation selectivity was tested by repetition of the fluorescence experiment with different alkali hydroxides (Figure 4a). The differences in rate observed for the different cations (Li+, Na⁺, and K⁺) were relatively small, which is characteristic of channels with little or no selectivity among cations. Anion selectivity was investigated by variation of the counteranion (Cl⁻, Br⁻, and I⁻) for the Na⁺ ion. In this case, a consistent difference in the fluorescence profile was observed that was dependant on the nature of the anion added (Figure 4b). In all cases, the fluorescence of the deprotonated dye decreased, which indicated either an X^{-}/H^{+} symport or X^{-}/OH^{-} antiport through the channel. For NaBr and NaI, a rapid decrease in the fluorescence intensity indicative of X⁻ transport was followed by a steady increase to the fully equilibrated state, thus suggesting a slower rate of Na⁺ influx. A gradual



Figure 4. a) Fluorescence change of deprotonated HPTS for different cations (Li⁺, Na⁺, and K⁺) upon addition of 1.1 mol% channel 1. b) Fluorescence change of deprotonated HPTS for different anions (Cl⁻, Br⁻, and I⁻) upon addition of 0.4 mol% channel 1.

decrease in fluorescence intensity was observed for NaCl, thereby indicating comparable rates for Na⁺ and Cl⁻ transport. The curves obtained for the NaX salts were fit to exponential equations to corroborate this hypothesis. The curves obtained with NaBr and NaCl were fit to Equation (3),

$$Rate = A + Be^{-k_1 t} - Ce^{-k_2 t}$$
(3)

in which k_1 and k_2 represent the competing rates for ion transport.^[16] However, the curve for NaI was fit to Equation (4), in which an extra term was required because of the

$$Rate = A + Be^{-k_1 t} - Ce^{-k_2 t} + De^{-k_3 t}$$
(4)

significant rate of I⁻ transport through the lipid membrane (k_3) .^[27] In all cases, the second exponential terms bore negative coefficients consistent with an increase in fluorescence intensity, and the k_2 values were within the same range as the rates determined for the addition of NaOH. These data are indicative of a slower increase in fluorescence intensity because of Na⁺ transport. The rates (k_1) that correspond to the rapid decrease in fluorescence intensity because of X⁻ transport were found to vary considerably depending on the nature of the anion. The k_1 value obtained for NaCl (0.0097 s^{-1}) was comparable with the rate of Na⁺ transport $(k_2 = 0.0097 \text{ s}^{-1})$, the k_1 value obtained for NaBr (0.018 s⁻¹) was approximately 2.7 times faster than the rate of Na⁺ transport ($k_2 = 0.0066 \text{ s}^{-1}$), and the k_1 value obtained for NaI (0.071 s^{-1}) was found to be significantly higher than that obtained for Br⁻ transport (ca. 9.5 times faster than k_2 (0.0075 s^{-1})). In addition, the k_3 value obtained for NaI (0.002 s^{-1}) matched well with the rate observed for the influx of I^- ions into vesicles without channels. $\ensuremath{^{[28]}}$ The necessity of including a third term for membrane-permeable I⁻ ions corroborates the assertion that anions do indeed travel through the pore formed by 1. Only two terms would be necessary to fit the data if anion transport only occurred through the membrane in response to Na⁺ transport through the channel.

The role of electrostatics in ion selectivity has been observed with natural ion channels, wherein the charges of the amino acid residues at the pore dictate the preference of the channel for different ions.^[29] Anion selective channels have a net positive charge owing to the presence of positively charged amino acids that line the pore of the channel. Channel 1 possesses a positive charge at the pore opening because of the basic amine groups at the chain-functionalized rim of the cyclodextrin. This positive charge likely leads to electrostatic interactions that favor the transport of anions over cations. The selectivity among the anions $(I^- > Br^- >$ Cl⁻) follows the Hofmeister series, in which various phenomena, such as protein precipitation, enzymatic activity, and polymer conformation, are influenced by the polarizability of the environmental anions.^[30] The more polarizable I⁻ ion should face a lower activation barrier to entry into the hydrophobic interior of 1, thus resulting in a faster rate of transport.[31]

In conclusion, the ability of aminocyclodextrin ion channel $\mathbf{1}$ to mediate transport of anions in preference to

7586 www.angewandte.org



cations at rates that compare with, or even exceed, those of natural channel proteins is reported. The ability of this robust synthetic channel to effect conductance across a hydrophobic barrier is an important first step toward devices that take advantage of biomimetic electrical signaling under aqueous saline conditions. Investigations into the basis of anion selectivity, the range of ions that are permeable, and pH effects on ion-channel activity are currently underway.

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Angew. Chem. Int. Ed. 2005, 44, 7584-7587

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