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Evidence for multiple alkali metal cation complexation in membrane-spanning ion transporters

Hossein Shabany,[a] Clare L. Murray,[a] Charles A. Gloeckner,[b] Michael A. Grayson,[b] Michael L. Gross[b] and George W. Gokel*[a]

[a] *Bioorganic Chemistry Program and Dept. of Molecular Biology & Pharmacology, Washington University School of Medicine, 660 South Euclid Ave., Campus Box 8103, St. Louis, MO 63110, USA. E-mail: ggokel@molecool.wustl.edu*

[b] *Department of Chemistry, Washington University, St. Louis, MO 63130, USA*

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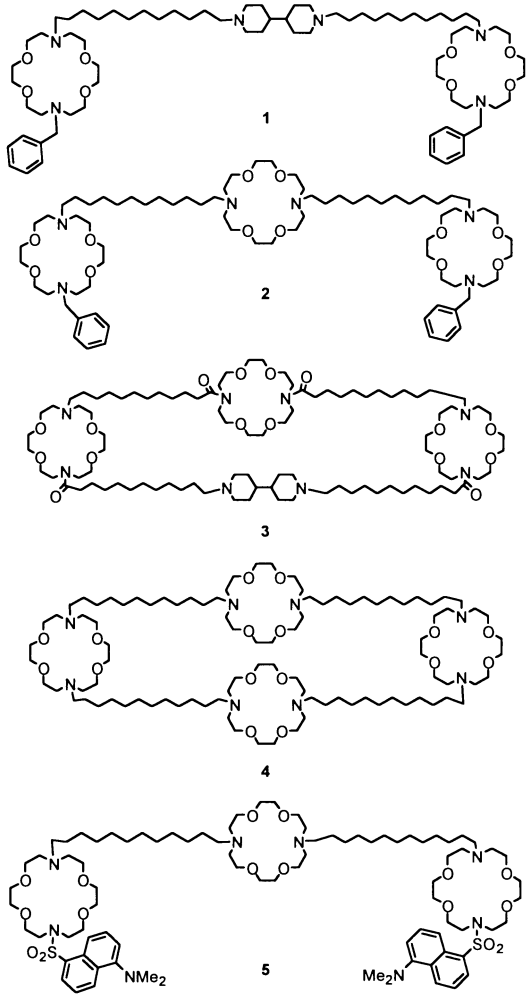
The polynitrogen-containing cation transporters 1–4 are shown by electrospray mass spectrometry to form multicationic species. In the absence of Na^+, protonated species dominate but increasing the sodium concentration leads to all binding sites being occupied.

The study of cation-conducting channels currently presents an enormous challenge to both chemists and biologists.[1] An important goal for the former is to prepare compounds that are inherently simpler than protein, or even peptide, channels[2] that transport cations at substantial rates across phospholipid bilayer membranes. With functionality achieved, experimental study of the systems should offer insight into the details of such phenomena as ion transport, selectivity, and gating kinetics. A major question concerns the chemical details of cation transport within the pore. A postulate that K^+ transport and selectivity were influenced by cation–π interactions within the pore[3] was disproved by site-directed mutagenesis.[4] The chemical roles of polyarenes in synthetic channels[5] and of the central ion capsule[6] in the KcsA K1 channel of *Streptomyces lividans*[7] beg further exploration of alkali metal cation interactions in low polarity media. In a previous study, electrospray mass spectrometric results demonstrated that all three macrocyclic rings of a hydraphile channel-former could simultaneously bind cations.[8] We now report that the novel tetramacrocyclic[9] and nitrogen-heterocycle-containing[10] channel-formers reported in the preceding two communications can bind a full complement of alkali metal ions by using crown ethers but not piperazine or bipiperidyl donors.

Cation transport through a phospholipid bilayer for the hydraphile family has been demonstrated by using fluorescence (H^+),[11] ^{23}Na NMR (Na^+),[12] and planar bilayer conductance methods (Na^+).[13] The ability of hydraphile channels to span the bilayer[14] has been confirmed and the optimum length for this family of channels has been established.[15] The compounds reported here are elaborations of the previously known systems and all exhibit effective Na^+ transport in a phospholipid bilayer (NMR method). Thus, under comparable conditions, the transport rates in mixed phosphatidyl choline–phosphatidyl glycerol bilayer liposomes, the following Na^+ transport rates were observed at room temperature: **1**, 80%; **2**, 210%; **3**, 50%; and **4**, 340%. In all cases, the values are expressed as a percent of the rate observed for the dansyl-sidearmed channel, **5** (100%).

Compounds **2**[11] and **5**[14] were prepared as previously reported and the synthesis of **4** is described in the preceding communication.[9] Compound **1** was prepared by treatment of $PhCH_2{<}N18N{>}H$ with $Br(CH_2)_{12}Br$ (Na_2CO_3, KI, 2 h) in boiling PrCN to give $PhCH_2{<}N18N{>}(CH_2)_{12}Br$ as a yellow oil (39%). Further reaction of the above with 4,4′-bipiperidine (procedure as above, 16 h) gave **1** as a colorless solid (8%, mp 55–56 °C). The preparation of **3** required a more elaborate approach. Benzyldiaza-18-crown-6 was alkylated with $Br(CH_2)_{11}CO_2Me$ (Na_2CO_3, cat. KI, BuCN, 20 mL, 24 h, 50%)



to give $PhCH_2{<}N18N{>}(CH_2)_{11}CO_2Me$. Hydrolysis (2 M NaOH, 93%) followed by treatment with $(COCl)_2$ (2 M solution in CH_2Cl_2, 2 h) gave $PhCH_2{<}N18N{>}(CH_2)_{11}COCl$, which was allowed to react directly in CH_2Cl_2 with $H{<}N18N{>}H$ (Et_3N, 6 equiv., cat. DMAP, to give the tris(crown) diamide as a yellow oil (65%). The tris(crown) diamide was debenzylated (H_2–Pd/C, 60 psi, abs. EtOH) to give $[H{<}N18N{>}(CH_2)_{11}CO]_2{<}N18N{>}$ (yellow oil, 95%). A similar strategy was used to prepare $ClCO(CH_2)_{11}NC_5H_9C_5H_9N(CH_2)_{11}COCl$, beginning with the alkylation of 4,4′-bipiperidine by $Br(CH_2)_{11}CO_2Me/Na_2CO_3$ (cat. KI, BuCN, 20 mL, 24 h) to give $MeO_2C(CH_2)_{11}NC_5H_9C_5H_9N(CH_2)_{11}CO_2Me$ (71%). This, in turn, was hydrolyzed (2 M NaOH, 94%, colorless solid, mp 260 °C, decomposed) and treated with ClCOCOCl (excess, 2 M

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Table 1 Electrospray mass spectrometric analysis of compounds **1**–**4**

Ion	*m/z*	Rel. int. (%)
1	1204.98	n/a
[**1**·1Na]$^{+}$	1227.8	30
[**1**·2Na]$^{2+}$	625.5	100
[**1**·3Na]$^{3+}$	424.7	0
[**1**·4Na]$^{4+}$	324.2	0
2	1299.01	n/a
[**2**·1Na]$^{+}$	1322.5	72
[**2**·2Na]$^{2+}$	672.8	100
[**2**·3Na]$^{3+}$	456.2	20
[**2**·4Na]$^{4+}$	347.9	0
3	1675.3	n/a
[**3**·1Na]$^{+}$	1698.7	30
[**3**·2Na]$^{2+}$	861.1	100
[**3**·3Na]$^{3+}$	581.8	45
[**3**·4Na]$^{4+}$	442.1	0
4	1713.5	n/a
[**4**·1Na]$^{+}$	1737.7	38
[**4**·2Na]$^{2+}$	880.2	100
[**4**·3Na]$^{3+}$	594.4	87
[**4**·4Na]$^{4+}$	451.3	47

solution in CH_2Cl_2). The latter was treated with [H < N18N > $(CH_2)_{11}CO]_2$ < N18N > (Et_3N, 6 equiv., cat. DMAP, CH_2Cl_2, 48 h) to afford tetraamide **3** as a yellow oil (30%).

Compounds **1**–**4** present an opportunity to directly assess the interactions of the hydraphile's modular elements and Na^+. Assuming that a crown macroring will bind a single Na^+ ion, we anticipate that we will observe ions corresponding to [**1**·2Na]$^{2+}$, [**2**·3Na]$^{3+}$, [**3**·3Na]$^{3+}$, and [**4**·4Na]$^{4+}$. The projected maximum complexation by **1** or **3** is based on the expectation that 4,4′-bipiperidyl will not complex Na^+. This is particularly important considering the conclusions of the preceding communication that the bipiperidyl unit can organize water but its nitrogen donor groups are too far apart to effectively coordinate with Na^+.[10]

The mass spectrometric analyses were conducted using an electrospray ion source (ESI-MS).[16] The inlet temperature was *ca.* 55 °C. Typically, 1.5 mg of channel was dissolved in 1 mL of $CHCl_3$ and the spray solution was prepared by adding 1 mL of MeOH–$CHCl_3$ 1 : 1 (v/v) and 40 μL of 100 μM NaOH to 60 μL of the sample solution. After mixing, 20 μL of the sample solution was loop injected by continuous infusion [10 μL min^{-1} MeOH–$CHCl_3$ (1 : 1, v/v)]. The instrument continuously scanned (magnetic) at 20 s decade^{-1} of mass over the range from 2000 to 400 Da. The data thus obtained are shown in Table 1.

For each structure, we have calculated the anticipated molecular weights for adducts of **1**–**4** with 1, 2, 3 and 4 Na^+ ions. In each case, the most abundant ion (base peak) observed was the disodium adduct. In all cases, the maximum number of Na^+ ions complexed was equal to the number of macro-rings and no higher order ions were detected. It is also interesting to observe that the ion currents for the complexes containing either three or four Na^+ ions (**2**, **3** or **4**) were significantly larger than observed for either cation complex of **1**.

The most significant finding of the present study is that in all cases, the number of Na^+ ions bound by each host molecule corresponded to the number of macrocycles. In no case did the number of Na^+ ions exceed the number of macro-rings indicating a generalized affinity of the cation for any of these structures. In principle, if 4,4′-bipiperidyl could support cation complexation directly, the complexes [**1**·3Na]$^{3+}$ and [**3**·4Na]$^{4+}$ would have been observed at *m/z* values of 424.7 and 442.1, respectively. Peaks corresponding to such complexes were sought but not observed.

An interesting observation is that the higher organization of **3** relative to **2** appears to result in a more stable tris(Na^+) complex. Thus, the [host·2Na]$^{2+}$: [host·3Na]$^{3+}$ ion abundance ratios for **2** and **3** are 100 : 20 and 100 : 45. The 'central' macrocycle is bis(amidated) in **3** and is expected to be a weaker donor for Na^+. It appears that the diamine opposite can help to stabilize this complex relative to the situation in which the additional structural element is absent.

The critical inference we draw from these data is that direct interaction between the bipiperidyl unit of **1** and a cation is not detectable, even in the low dielectric medium of electrospray mass spectrometry. Extending this to the low polarity, insulating regime of a bilayer, we infer that bipiperidyl cannot directly support cation complexation in that situation either. This does not prove, but strongly supports, the notion that the function of **1** as a channel is due, in part, to the interaction of bipiperidyl with intrapore water or waters of hydration rather than directly with Na^+.[10]

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16 Only sectors one and two, a reversed geometry BE, extended mass range configuration (Vacuum Generators ZAB-T), were used for the mass analysis. The electrospray ion source operated as follows: spray needle voltage, 8940 V; counter electrode, 5255 V; sampling cone, 4200 V; accelerating voltage, 4120 V; source temperature, 75% power (*ca.* 55 °C). Sample preparation was as follows: 1.5 mg of sample was dissolved in 1 mL chloroform.