The central 'relay' unit in hydraphile channels as a model for the water-and-ion 'capsule' of channel proteins

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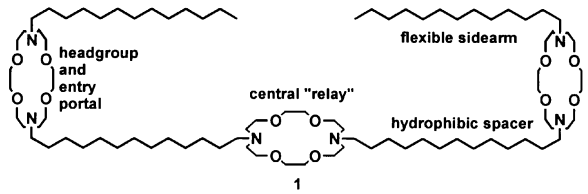
The central relay of hydraphile channels is a model for the central ion-capsule of the KcsA K1 K^+-conducting channel of *Streptomyces lividans*; organization of water and concomitant electrostatic stabilization of a transient K^+ appear to be the functions in both cases.

The synthetic ion channels that we call 'hydraphiles' transport Na^+ in phospholipid bilayers.[1] These compounds use crown ethers, typically 4,13-diaza-18-crown-6, as headgroups[2] to maintain their position at the boundary between the bilayer and the aqueous phase on either side of the membrane. Fluorescence studies[3] have shown that the distal macrocycles of **1** are each 14 Å from the bilayer's midplane and in an environment intermediate in polarity between methanol and ethanol. These data are consistent with the macrocycles being proximal to the glyceryl fragment (midpolar regime) of the phospholipid—at opposite ends of the membrane's insulator slab. The headgroups are connected to a 'central relay unit' by means of hydrophobic dodecyl chains that measure *ca.* 14 Å in the extended conformation. The central relay is a polar residue incorported to provide the transient cation a means of sabilization at the bilayer's midplane—the least polar regime in the membrane. In many of the compounds we have studied, this unit is a third crown ether (*e.g.* **1**)

Recently, the solid state structure of a K^+-selective channel from *Streptomyces lividans* (KcsA K1 channel)[4] was reported and has begun to revolutionize our understanding of channel-forming proteins.[5] In this report, the structure of the natural channel is characterized thus: 'From inside the cell…the pore begins as a tunnel 18 Å in length (the internal pore) and then opens into a wide cavity (~ 10 Å across) near the middle of the membrane.' This description applies almost as well to our hydraphile channels. In the same paper, the following question is posed. 'Why is there a 10 Å diameter cavity in the center of the channel with an ion in it?' In a subsequent theoretical report,[6] it was concluded that the central capsule provides a means for electrostatic stabilization of cations in the non-polar midplane of the phospholipid bilayer. We believe that the cavity is equivalent to our 'central relay unit' and we present evidence to that effect in this report.



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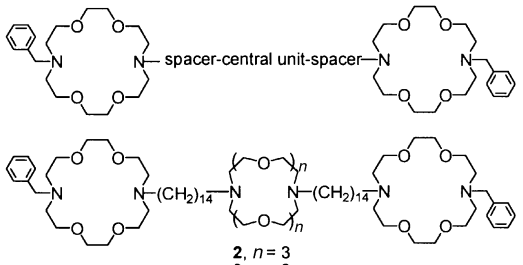
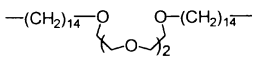
Hydraphiles **1**–**8** were prepared by a three-step sequence. First, monobenzyldiaza-18-crown-6 ($PhCH_2 < N18N > H$) was prepared either by benzylation of diaza-18-crown-6 or by partial hydrogenolysis of dibenzyldiaza-18-crown-6.[7] Alkylation of $PhCH_2 < N18N > H$ with an excess of $Br(CH_2)_nBr$ afforded $PhCH_2 < N18N > (CH_2)_nBr$ which was then allowed to react with $H < N18N > H$. Compound **9** was prepared in an analogous fashion except that $H < N18N > H$ was monoalkylated with dansyl chloride rather than benzyl bromide. Based on studies to determine the optimal length,[8] the hydraphiles were designed to be *ca.* 38–42 Å between the proximal nitrogen atoms of the terminal macrocycles. For small central units, proportionally longer hydrophobic chains were incorporated.



2, $n = 3$
3, $n = 2$
4, $n = 1$



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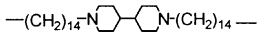

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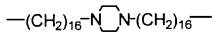

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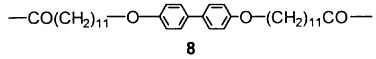

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Sodium cation flux was measured by using the ^{23}Na NMR-based method of Riddell *et al.*[9] This technique permits quantitative detection of the Na^+ transport rate in vesicles prepared[10] from phosphatidylcholine and phosphatidylglycerol (4:1 w/w, pH = 7.3). The NMR experiment is somewhat complex and we have therefore normalized the data relative to a simultaneously determined standard (**9**).[11] Transport rates for **9** have been determined independently more than 10 times and each of the values shown in Table 1 for **1**–**8** represents at least three independent experiments.

The efficacy of a diaza-15-crown-5 central unit (**3**) is half that of the corresponding 18-membered ring (**1**, **2**). A further reduction in transport efficacy is observed when the central macroring is reduced in size to 12 atoms (**4**): transport efficacy falls again (k_{rel} = 69). Transport is about the same (within *ca.* 10% experimental error) when the 4,4′-bipiperidyl central unit (**6**) replaces diaza-12-crown-4 (**4**). The transport rate falls (to k_{rel} = 50) when the central ring of **4** (2N and 2O donors) is 'lysed' (**5**, 4O donors). All transport efficacy is lost when either pyrazine (**7**) or 4,4′-dioxybiphenyl (**8**) is incorporated as the central unit.

When the hydraphiles were designed, the central unit was intended to be a 'relay' for transient cations. It was presumed that the cations would be partially solvated as is thought to be the case in protein channels. Crown ethers often bind cations that, in turn, are bound to water. In the present case, we postulate that the central unit helps to organize water and that the cation 'passes over' it rather than necessarily being exchanged for water bound in the cavity. 18-Membered crown ethers are well known to bind water. Indeed, we reported the

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Table 1 Sodium cation transport by hydraphile ionophores[a]

Compound	Structure	d^a/Å	$k_{rel}{}^b$ (%)
1	$PhCH_2 < N18N > C_{14} < N18N > C_{14} < N18N > CH_2Ph$	43	211
2	$PhCH_2 < N18N > C_{12} < N18N > C_{12} < N18N > CH_2Ph$	38	200
3	$PhCH_2 < N18N > C_{14} < N15N > C_{14} < N18N > CH_2Ph$	42	100
4	$PhCH_2 < N18N > C_{14} < N12N > C_{14} < N18N > CH_2Ph$	41	69
5	$PhCH_2 < N18N > C_{12}O(CH_2CH_2O)_3C_{12} < N18N > CH_2Ph$	42	50
6	$PhCH_2 < N18N > C_{14} < NC_5 > — < C_5N > C_{14} < N18N > CH_2Ph$	42	80
7	$PhCH_2 < N18N > C_{16} < NC_4N > C_{16} < N_{18}N > CH_2Ph$	42	< 2
8	$PhCH_2 < N18N > COC_{11} < OC_6H_4–C_6H_4O > C_{11}CO < N18N > CH_2Ph$	38	< 2
9	$Dn < N18N > C_{12} < N18N > C_{12} < N18N > Dn$	38	100

[a] Distance measured on CPK molecular models between the two proximal nitrogen atoms on the distal macrocycles. [b] Rate relative to dansyl channel, arbitrarily set at 100. Comparative rates are recorded for 10 μM ionophore concentration.

first case of a protonated azacrown complexing a water molecule in 1977.[12] Other examples abound.[13] The solid state structures show a water molecule bridging alternate heteroatoms in the 18-membered ring macrocycles. Such bridging is also possible for the 15- and 12-membered ring compounds but the number of symmetry equivalent bridges is reduced. Thus, on a single surface of 18-crown-6, there are five possible 1,3-heteroatom arrangements that could be bridged by water. In 15-crown-5, there are only three and in 12-crown-4, only two are possible. The Na^+ transport rates observed for **2** (18-crown), **3** (15-crown) and **4** (12-crown) are 211, 100 and 69. This is a ratio (for **2**:**3**:**4**) of 2.1:1:0.7. The ratio of symmetry equivalent water bridges is 1.7:1:0.7. Opened-chained compound **5** can also form water bridges but it is not even as well organized as is **4**. The data presented here for the change from 18- to 15-membered rings and open chains differ some from an earlier,[14] related study.[15] The trend is similar, however.

The 4,4′-bipiperidyl unit of **6** was included because it is a fairly rigid molecule[16] and the N↔N separation is almost identical to the transannular N,N-distance in 4,13-diaza-18-crown-6. When the 4,4′-ring junction is *anti*, the cyclohexane-like units are coplanar. Molecular models show that the overall aspect is concave when the ring junction is *syn*, the nitrogen atoms are focused to a point above the ring junction, and the N↔N separation is barely 6 Å. We speculate that the bipiperidyl unit adds rigidity to the overall structure while providing H-bonding donor or acceptor sites either in the form of neutral nitrogen, protonated nitrogen, or the carbonyl groups. The argument is bolstered by the ESI-MS study that shows **6** to be incapable of binding three cations whereas **1** can easily do so (see following communication).[17]

When a macrocycle comprises the central unit, water may be organized by complexation and appropriately situated either to solvate a transient cation or to exchange with the water present in the partially hydrated cation. Such an arrangement is tantamount to the now well known second-sphere coordination of metal–ammonia complexes.[18] Such a coordinative arrangement is also possible, although probably less effective for the *syn*-oriented bipiperidyl units. In the latter case, however, a partially solvated cation of the form $M(OH_2)_2{}^+$ could bridge the N↔N space as illustrated in Fig. 1. The hydraphiles containing either piperidine in which the nitrogens are clearly very close together (*ca.* 3 Å) or biphenylene, which lacks significant donor groups, fail to transport cations despite the presence of all of the other essential elements.

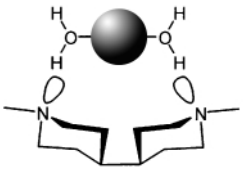

Fig. 1 Presumed coordination of a dihydrated alkali metal cation by 4,4′-dipiperidine.

We believe that simple, structural models such as the hydraphiles offer an effective means to probe complex biological phenomena. Clearly, these central units lack the focused, α-helices that afford electrostatic stabilization in the KcsA K1 channel.[1,4] Still, variations in the structure of the central relay are possible in this model system which permits functional subunits to be transformed—clearly an advantage over single site mutagenesis. Additional structural alterations are in progress and the results will be reported in due course.

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