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A 'toothpaste tube' model for ion transport through trans-membrane channels

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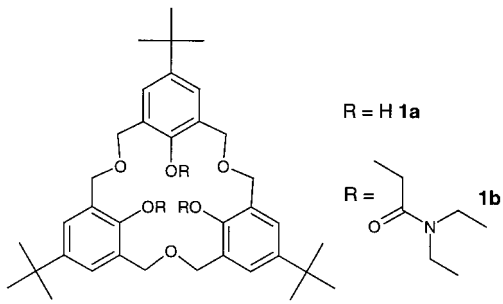
The X-ray crystal structure of the 2 : 1 Na^+ complex of the hexahomooxacalix[3]arene diethylamide derivative 1b shows capsular and nesting inclusion of the two Na^+ cations suggesting a mechanism by which Na^+ and K^+ ions are desolvated and pass through biological ion channels.

The transport of the sodium and potassium cations across biological phospholipid membranes *via* Na^+ and K^+ channel proteins is crucial to many biological processes, notably electrical signalling in the nervous system.[1–3] Key features of the transport of both Na^+ and K^+ are the high degree of selectivity of the discrimination between the two ions (factors of *ca.* 10^4) and the very rapid rate of their diffusion through the channel pore (*ca.* 10^8 ions s^{-1}).[4] Current understanding of this process has advanced markedly this year with the publication of the first X-ray crystal structure of an alkali metal channel protein.[4] This work has demonstrated that alkali metal cations diffuse in solvated form throughout most of the length of the channel before entering a cone-shaped selectivity filter. The selectivity filter discriminates on a size basis, with the cation passing through the neck of the cone with little remaining solvation shell. The high rate of throughput is explained by a 'billiard ball' effect in which incoming cations exert an electrostatic repulsion upon the metal within the selectivity filter, expelling it on to the other size of the membrane.[4] We now report the first model compound to give insight into the mechanism of this 'billiard ball' effect. This work suggests that the incoming cation is robbed of its solvation shell as it enters the cone-like selectivity filter in conjunction with a single solvent molecule in a concerted fashion, as if being squeezed through a tube of toothpaste.

Extensive work over the past fifteen years has centred around the use of the calixarenes as biological models.[5–7] However, the small size of the cone-shaped calix[4]arene does not present a significant aperture for the passage of metal cations through the annulus, although this has been suggested for K^+ recently.[8] The larger, 18-membered ring hexahomooxacalix[3]arenes of type **1**



represent a more appropriate channel mimic. In biological membrane channels the inner surface of the selectivity filter is covered by successive rings of carbonyl oxygen atoms from the main chain amino acid residues.[1,4] This is simulated in the model compound **1b** discussed herein by the phenolic oxygen atoms of the oxacalix[3]arene and the carbonyl groups of the pendant amide functionalities, resulting in a three dimensional cavity comprising a double ring of six oxygen donors. The *p-tert*-butyl aryl moieties provide a hydrophobic, conical entrance to the donor site.

Reaction of pore mimic **1b** with $NaPF_6$ in water–methanol solution results in isolation of a crystalline complex of formula $[Na_2L](PF_6)_2\cdot H_2O\cdot 2MeOH$ **2** (L = **1b**). Ligand **1b** has been shown to exhibit a 1:1 binding constant for $Na^+ > 10^7$ $dm^3\ mol^{-1}$ and the transannular nucleophilic channel is an excellent size match for this cation, while being less readily able to expand sufficiently to encapsulate a K^+ ion.[9] The X-ray crystal structure of **2**† (Fig. 1) shows that the complex has a symmetrical basket, or lobster-pot shape, with one cation, Na(1) firmly encapsulated within the pocket formed by the phenolic and amide oxygen atoms, resulting in a chiral core possessing a pseudo-threefold rotation axis. In addition to the calixarene donor atoms the seven-coordinate Na(1) is also bound to a water molecule situated at the boundary between the hydrophobic cavity formed by the aryl moieties and the binding pocket. While the Na(1)–OH_2 distance is relatively long, 2.437(5) Å, the water molecule is stabilized by hydrogen bonds to the calixarene etheric bridges. Fascinatingly this water molecule also ligates a second Na^+ cation, Na(2), which is apparently in the process of entering the cavity, Na(2)–OH_2 2.277(5) Å. Cation Na(2) exhibits a highly distorted octahedral coordination geometry. In addition to a strong interaction with the intra-cavity water molecule, it is also ligated by two other solvent molecules (methanol) and forms a salt bridge to an extra-cavity PF_6^- anion. In addition to these interactions, Na(2) is strongly bound to one of the oxygen atoms of the calixarene $-CH_2OCH_2-$ bridges. The sixth and final coordination site is filled by an intriguing cation–π interaction with one of the calixarene aryl rings, Na(2)–C 3.026(6) Å. The coordination environment of Na(2) is an excellent illustration of the desolvation process which must occur in biological systems as alkali metal cations enter the conical selectivity filter as they traverse trans-membrane channel pores. The cation interacts with the macrocyclic ligand *via* a mixture of coordination and cation–π interactions, which have been strongly implicated in biochemical systems,[10,11] however, it still retains a partial solvation shell in which all three coordinated solvent molecules are involved in strong hydrogen bonding interactions either to the calixarene itself or to extra-cavity anions. In natural systems these anions are present in the form of anionic carboxylato residues of the channel protein. These second sphere coordination interactions compensate for the enthalpically unfavourable desolvation of the cation as it approaches the narrowing pore aperture. In effect the cation is squeezed into the binding pocket as a consequence of the trans-membrane concentration gradient, with solvent molecules being side-tracked by peripheral stabilising interactions. The feature of the bridging water molecule insulating one cation from another as they progress through the selectivity filter is an experimentally determined property of the K^+ channel of *Streptomyces lividans*, a protein considered to be highly representative of both K^+ and Na^+ channels.[4] Water is a small enough ligand to pass entirely through the pore in an axial coordination site and may well act as a molecular 'staple', dragging the second cation along after it as it follows the first on its passage through the channel,

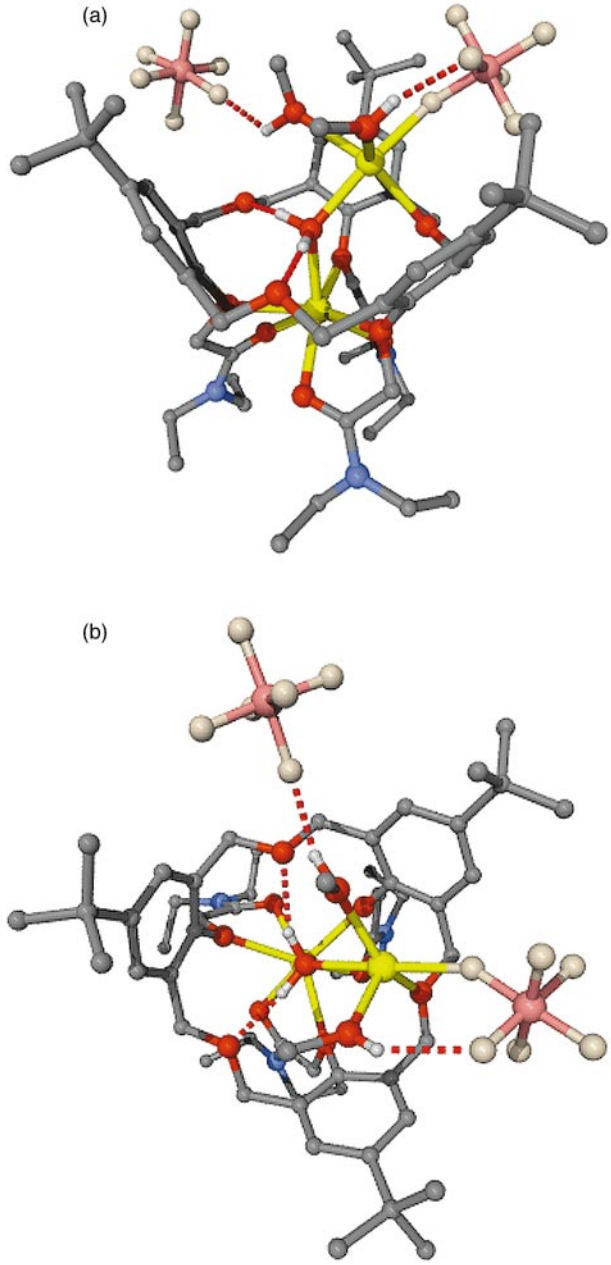


Fig. 1 (a) Side and (b) top views of the conical Na^+ channel mimic **2** showing the encapsulation of one Na^+ ion within the sodium selective cavity, while the second metal ion is beginning the process of desolvation as it enters the conical pore and forms a salt bridge to an extra-cavity hexafluorophosphate anion. The two metal ions are linked by a water molecule situated in the centre of the cavity and able to interact with the etheric oxygen atoms. Colour key: Na, yellow; O, red; N, blue; C, grey; H, small white; P, pink; F, white atoms attached to P. Hydrogen bonding interactions involving the three solvent molecules are represented by red dotted lines. Hydrogen atoms which do not take part in specific hydrogen bonding interactions are omitted for clarity. Selected distances: Na(1)–O(1A) 2.299(4), Na(1)–O(1B) 2.333(5), Na(1)–O(3B) 2.35(2), Na(1)–O(3C) 2.403(12), Na(1)–O(1C) 2.404(5), Na(1)–O(3A) 2.42(2), Na(1)–O(1) 2.437(5), Na(2)–F(1) 2.199(5), Na(2)–O(1) 2.277(5), Na(2)–O(2) 2.292(5), Na(2)–O(3) 2.348(5), Na(2)–O(2A) 2.378(5), Na(2)–C(6A) 3.027(6).

stabilized by hydrogen bonding interactions at every point. This type of mechanism is key to the understanding of the apparently contradictory features of the biological systems; their high selectivity and hence binding ability and their fast kinetics, suggesting only partial desolvation.

The model structure reported herein demonstrates conclusively, in a well-characterised system, the ability of conical channels to stack multiple cations by means of a wide variety of stabilizing interactions. Clearly identifiable features, namely inter-cation insulation by a single solvent molecule, strong cation binding and stabilising interactions conducive to desolvation, mimic closely those implicated in natural systems. Preliminary electrophysiological results‡ indicate an inward current at a potential consistent with sodium crossing the cell membrane, together with attenuation of the trans-membrane potassium current, when ligand **1b** is allowed to superfuse the cells. These initial data suggest that ligand **1b** inhibits transport of potassium and allows an influx of sodium thus performing the function of a selectivity filter on the surface of a cell. As a whole, the structure of complex **2** provides a well resolved 'snap-shot' of the cation transport process.

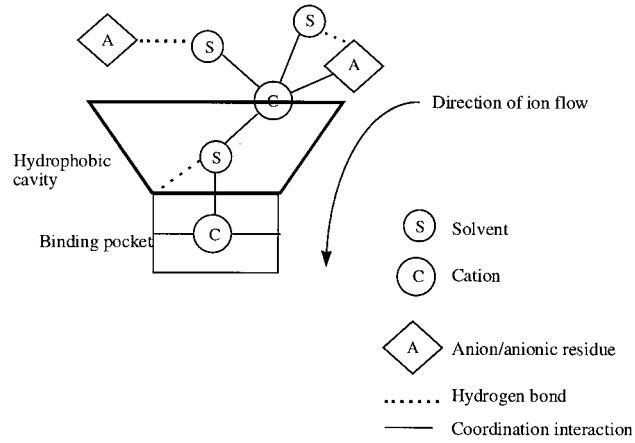


Fig. 2 Diagrammatic representation of the key structural features of **2**.

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Notes and references

† *Crystal data* for **2**: $C_{56}H_{95}F_{12}N_3Na_2O_{12}P_2$, M = 1338.27, monoclinic, space group $C2/c$, a = 43.506(3), b = 13.8660(11) c = 24.2283(16) Å, β = 111.811(2)°. U = 13569.4(17) Å^3, Z = 8, μ = 1.66 cm^{-1}, T = 120 K, Reflections measured: 45 436, unique data: 11 181 (R_{int} = 0.118), parameters: 820, $R1$ [$F^2 > 2\sigma(F^2)$] 0.1095, $wR2$ (all data) 0.2683. CCDC reference number 182/1166. See http://www.rsc.org/suppdata/cc/1999/553 for crystallographic files in .cif format.

‡ Whole-cell patch clamp data from mouse neuroblastoma × rat glioma hybrid cells, NG108-15, with trans-membrane ion currents stimulated by ramping from a holding potential of −80 mV to between −180 and +60 mV.

1 B. Hille, *Ionic Channels of Excitable Membranes*, Sinauer, Sunderland, 1992.
2 Y. Kobuke, in *Advances in Supramolecular Chemistry, vol. 4*, ed. G. W. Gokel, JAI Press, Greenwich, 1997, pp. 163–210.
3 W. Kaim and B. Schwederski, *Bioinorganic Chemistry*: *Inorganic Elements in the Chemistry of Life*, Wiley, Chichester, 1994.
4 D. A. Doyle, J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait and R. MacKinnon, *Science*, 1998, **280**, 69.
5 C. D. Gutsche, *Calixarenes*, ed. J. F. Stoddart, Royal Society of Chemistry, Cambridge, 1989.
6 A. Ikeda and S. Shinkai, *Chem. Rev.*, 1997, **97**, 1713.
7 L. H. Yuan, S. H. Chen, H. M. Zhao and Y. C. Ning, *Acta Chim. Sinica*, 1994, **52**, 1035.
8 P. Schmitt, P. D. Beer, M. G. B. Drew and P. D. Sheen, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1840.
9 H. Matsumoto, S. Nishio, M. Takeshita and S. Shinkai, *Tetrahedron*, 1995, **51**, 4647.
10 J. C. Ma and D. A. Dougherty, *Chem. Rev.*, 1997, **97**, 1303.
11 J. L. Atwood, F. Hamada, K. D. Robinson, G. W. Orr and R. L. Vincent, *Nature*, 1991, **349**, 683.

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