Artificial transmembrane ion channels from commercial surfactants†

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Received (in Cambridge, UK) 11th May 2007, Accepted 13th August 2007
First published as an Advance Article on the web 20th August 2007
DOI: 10.1039/b707194a

Sodium ion transport across a phospholipid bilayer has been demonstrated by a new class of transmembrane ion channel mimetic compounds in which the filtering effect of a calixarene has been coupled to the membrane penetrating qualities of a commercial surfactant.

Transmembrane ion channels are a vital component of every cell. They are able to regulate the transport of cations and anions across the phospholipid bilayers that form the cellular boundary to optimise the intracellular concentrations of specific ions. Analysis of natural transmembrane ion channels shows that they fall into two broad categories: those in which ions move through a pore formed at the confluence of several protein subunits, as in the voltage gated potassium channel, KcsA,[1] and those where they pass through channels formed within a single protein such as the ClC chloride channel.[2]

Ion channel models are of particular interest as they allow different aspects of transmembrane transport to be analysed, for example the structural changes that can alter ion specificity or flux. To perform adequately the artificial channel must span a cellular phospholipid bilayer (implying a concerted length in excess of 4 nm), exhibit specificity for the target ion and a flux between 10^4 and 10^8 ions per second.[3] Synthetic mimicry of ion channel activity has been achieved, to varying degrees of success, through a variety of approaches. A simplified analogue of a natural system may be found in Gokel's chloride transporting SCMTR compounds based on short protein fragments with alkyl or aryl termini.[4] These compounds appear to aggregate and allow chloride to cross cell membranes. Another tactic is to place crown ethers at regular intervals along a rigid (or polypeptide) backbone, as seen in the work of Matile and others.[5] Finally there are numerous approaches based upon macrocycles bearing membrane-spanning substituents.[6] Although the latter are the least similar to natural channels they have received substantial interest due to their modular construction.

In principle an ion-specific channel could be designed by searching the literature for suitable 'filter' molecules and attaching membrane-spanning substituents. When constructing these artificial channel-forming compounds, two other factors must also be considered: ion flux and insertion of the channel in the bilayer. The first of these is often impossible to determine *a priori*. The second requires that the model compound has properties that are complementary to those of the bilayer. Ideally this requires that the termini of the compound are polar to match the phospholipid head groups but the bulk of the molecule is hydrophobic to match the lipid rich core of the bilayer. Incorporation of amphiphilic substituents would therefore seem to be an essential feature in any model, however, many amphiphiles have been shown to disrupt phospholipid bilayers thereby compromising membrane integrity.[7] The commercial Triton® non-ionic surfactants (Fig. 1), widely used to destroy liposome and cell membranes, exemplify such compounds. They are long enough, at 3 to 4 nm, to penetrate lipid bilayers and appear to target areas of osmotic stress but are otherwise non-specific in their activity. The compounds can successfully insert because they contain both amphiphilic and lipophilic regions allowing them to interact favourably with the polar, solvated external and internal bilayer surfaces while passing through the hydrophobic core of the bilayer where lipid groups interdigitate.

As part of our ongoing interest in artificial transmembrane ion channels we wished to harness the membrane penetrating qualities of these surfactants to the ion specific filtering abilities of rigid macrocycles. Thus far the examples in the literature relate predominantly to modified calixarenes[8] and derivatives that pair up within a bilayer to effect transport in a manner reminiscent of the gramicidin class of antibiotics.[9] Herein we report preliminary results for Na^+ transport across a phospholipid bilayer by a new class of transmembrane ion channel mimetic compounds in which the filtering effect of a calixarene has been coupled to the membrane piercing qualities of a commercial surfactant.

The synthesis of the modified calixarenes is shown in Scheme 1. Compound **1** was prepared through tosylation of commercially available Triton X-100®. Unlike similar surfactants (*e.g.* Triton X-45®), which have variable compositions, NMR indicated that commercial Triton X-100® was over 97% pure with $n = 10$. Reaction of **1** with 4-*tert*-butylcalix[4]arene in a 2 : 1 ratio, according to literature procedures for 1,3-dialkylation of calixarenes,[10] yielded compound **2**. Characteristic doublets in the ^1H NMR at 4.38 and 3.30 ppm indicated that **2** remained in the cone conformer. Initial planar bilayer experiments failed to detect reproducible channel activity, presumably because once a sodium cation approached the annulus of the calixarene it interacted strongly with the 2,4-phenolic moieties and blocked further ion movement. Mass spectrometric evidence for the Na^+ complex supports this explanation. To avoid Na^+ complex formation, and to determine if cavity size also influenced cation conductance, we

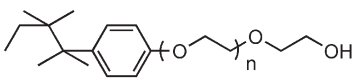

Fig. 1 Commercial membrane-disrupting surfactants: Triton X-45® (n = 3–4), Triton X-100® (n = 9).

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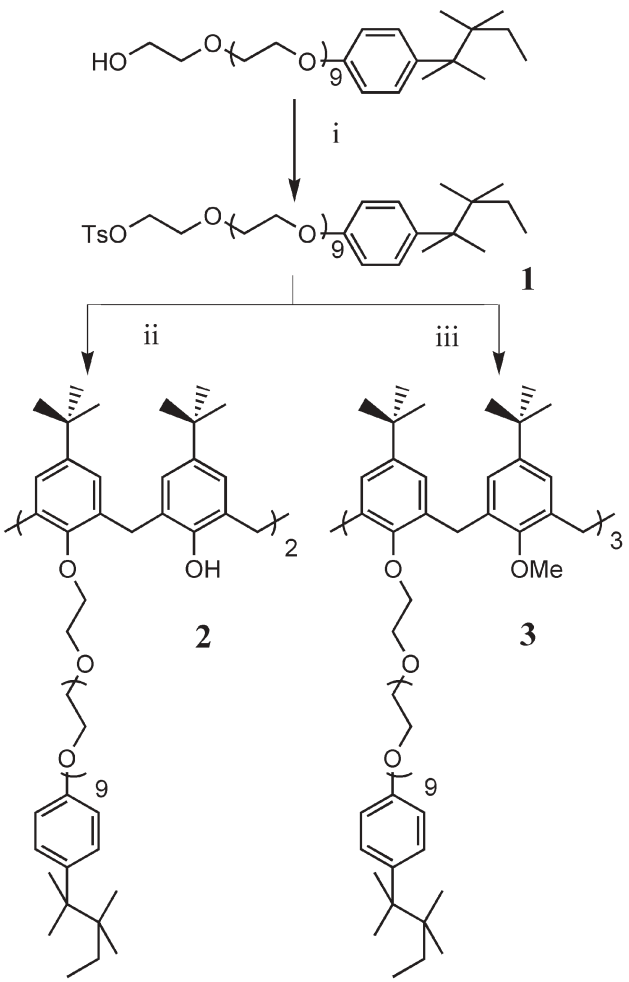
† Electronic supplementary information (ESI) available: Synthetic details, characterisation data and computational methods. See DOI: 10.1039/b707194a



Scheme 1 Synthesis of Tritonylcalixarenes. (i) *para*-tosyl chloride, $NaOH_{(aq)}$, H_2O–THF, 0 °C, (ii) 4-*tert*-butylcalix[4]arene, K_2CO_3, acetonitrile, reflux, (iii) 4-*tert*-butylcalix[6]arene trimethylether, K_2CO_3, acetonitrile, reflux.

prepared a trisubstituted derivative with no free phenolic groups, **3**, from the larger homologue, 4-*tert*-butylcalix[6]arene trimethylether.[11]‡ In solution, and in metal-free crystal structures, the methyl groups occlude the lower entrance to the central calixarene cavity,[12] however, in every example to date where a metal is held by lower rim phenolic ethers they are found *exo* to the cavity. This rearrangement also extends to the ethylether homologues. We therefore did not expect the methyl groups to prevent metal ion conductance through the central channel formed by the Triton X-100® substituents.

Conductance measurements were undertaken on a planar bilayer composed of a mixture of palmitoyl triglycerides with an electrolytic solution containing Na^+ at physiological levels (150 mM) and buffered to pH 7.2. Aliquots of calixarenes **2** or **3** were added and the current at −50 mV was recorded. Where calixarene **2** had given no response, **3** elicited classic ion channel activity. At low concentrations of calixarene **3** small stepwise changes of about 26 pS were observed in the current, corresponding to a flux of 7×10^6 ions s^{-1}, with channel conductance typically lasting from 0.5 to 20 seconds (Fig. 2a). At high concentrations of **3** stepwise insertion of several molecules was

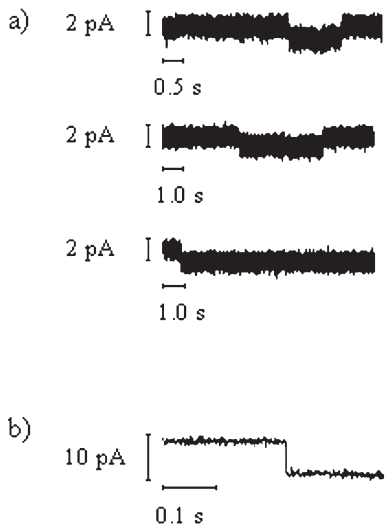


Fig. 2 Typical results from planar bilayer experiments in the presence of 150 mM Na^+ with varying concentrations of **3**: (a) 4 μM, (b) 64 μM.

detected and, intriguingly, much larger step changes in current were also observed (Fig. 2b). When Triton X-100® was added at the same concentration it caused the bilayer to burst whereas it had remained intact in the presence of **3**. The large change in conductance indicated that several calixarenes had inserted into the bilayer simultaneously as sequential insertions give a characteristic step pattern. Many synthetic and natural transmembrane channels are formed by the confluence of several intertwining molecules[1,4] but our observations suggest an additive effect through the

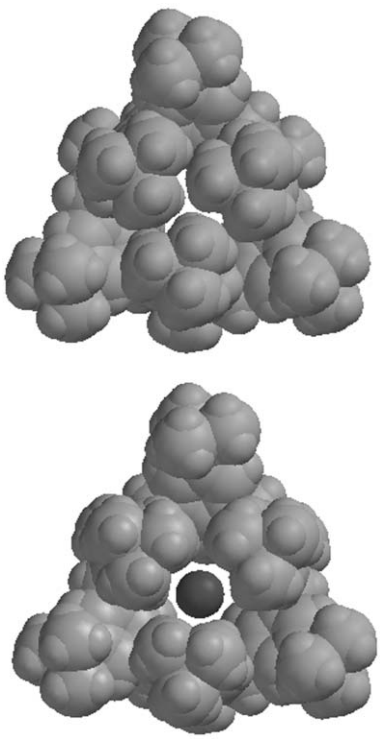


Fig. 3 Calixarene conformers: closed (top) and open (bottom, with Na^+ to scale).

concerted action of three or four molecules of **3** as the currents detected were exact multiples of the single channel events. Had interstitial channels formed between the calixarenes we would have observed transmembrane leakage and non-quantized changes in current. Experiments with K^+ in place of Na^+ have so far shown no conductance even though the calix[6]arene cavity should be able to accommodate a hydrated K^+ ion.[6] The reason for this lies in the preferred geometry of 4-*tert*-butylcalix[6]arene trimethylether derivatives. Crystallographic data for cone conformers show three of the *tert*-butylphenol groups meeting over the central cavity while alternating, methoxy substituted rings adopt open, or winged, positions (Fig. 3, top). The *tert*-butyl groups must rotate to allow metal ions to pass through the upper rim (Fig. 3, bottom). Computer simulations show that such a mechanism is possible with the 'open' form some 8 kJ mol^{-1} more stable than the 'closed' form.† However, while Na^+ can traverse the cavity, K^+ is too large to pass through.

In conclusion, preliminary results indicate that calix[6]arenes incorporating surfactant moieties can operate as single channel Na^+ channel mimics at low concentrations but aggregate to form multiple channels at higher concentrations. Work is ongoing to confirm the expected current–voltage relationships for Na^+ transport and selectivity over K^+ together with any anion dependence. These results will be reported in due course.

We wish to acknowledge the use of the EPSRC's Chemical Database Service at Daresbury.

Notes and references

‡ All compounds were characterised by ^1H and ^{13}C NMR and high resolution mass spectrometry.

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