Unusually long open times, determined by planar bilayer conductances studies, for a synthetic tris(macrocycle) that functions as a transmembrane channel in a phospholipid bilayer

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Two tris(macrocycle)s are shown by bilayer clamp studies to function as cation channels in a phospholipid bilayer; one of the pair of closely related compounds shows unusually long open times compared either to the other tris(macrocycle) or to natural channel-forming proteins.

The transport of sodium, potassium, and calcium cations *in vivo* is accomplished almost exclusively with the aid of proteins believed to have multiple transmembrane spans. Many of the structural features of natural cation-conducting channels have been inferred from biophysical and molecular biological studies.[1] The critical importance to biology of protein channel compounds and the fact that the chemical details of channel function remain obscure has fostered the design, synthesis, and study of several model channel systems. We have recently reviewed this area.[2] Our own cation channel model system was designed to possess macrocyclic polyethers that function both as head groups and as entry portals for cations. The original design used flexible *n*-dodecyl chains to connect the head-groups to a third macrocycle that was intended to function as a 'relay' unit, midway between the two.[3] The headgroups were attached on each distal side to a second 12-carbon chain that was expected to interact with the phospholipid bilayer in which it was embedded and to stabilize the extended conformation. The fact that alkyl-substituted crowns can serve as head groups for the formation of micelles[4] and stable vesicles[5] comports with our design. Based on the accumulated experimental evidence,[3] we believe that the benzyl channel (**1**, shown) adopts the conformation illustrated in Fig. 1.

We have prepared several channel model compounds in this class in which the distal macrocycles are substituted by aralkyl residues. Such residues include benzyl (**1**), methoxybenzyl[6] (**2**) and indolylmethyl.[7] We have previously shown that these structures conduct Na^+ through a phospholipid bilayer using a dynamic NMR method previously described.[8] We now show that these compounds exhibit cation transport behaviour typical of, if somewhat less effective than, that of transmembrane protein channels. These results were obtained by using a patch clamp amplifier and a bilayer headstage (planar bilayer conductance or pbc method). The black lipid bilayer membrane† is formed over a pinhole in a Teflon disk separating two compartments containing salt solutions. Bilayer formation is detected by a sudden drop in conductance. A continuous series of 10 mV, square-wave pulses (train pulse) is applied to the membrane to monitor its capacitance. A 10^{-6} M channel solution (in CF_3CH_2OH) was added and the solution was stirred for 1–10 min. A holding potential (see Figs. 2 and 3)was then applied and the channel responses were recorded.‡ The data shown in Fig. 2 (applied potential +100 mV) are typical for compound **2** but they represent only a small fraction of the data collected.

The recordings show the ion flux, usually for a single channel. The baseline corresponds to periods in which there is no observed cation flux. When a flat-topped peak is observed, it indicates that ions (presumably Na^+) are flowing. If each channel passes ions by the same mechanism, then a fixed peak height will be observed corresponding to whether the system is open or closed. When more than one channel is open simultaneously, similar flat-topped peaks will be observed but these will have an amplitude multiplied by an integer corresponding to the number of channels open. Well behaved, one- and two-channel opening behaviour is apparent in the traces shown in Fig. 2.

Similar, albeit somewhat noisier, data were obtained for **1** and are shown in Fig. 3(*a*). The traces shown are selected from and typical of approximately 100× the amount of data shown for **1**. Plotting current amplitude of single transitions *vs*. holding voltage for this system gives a straight line with $R > 0.98$ for 11

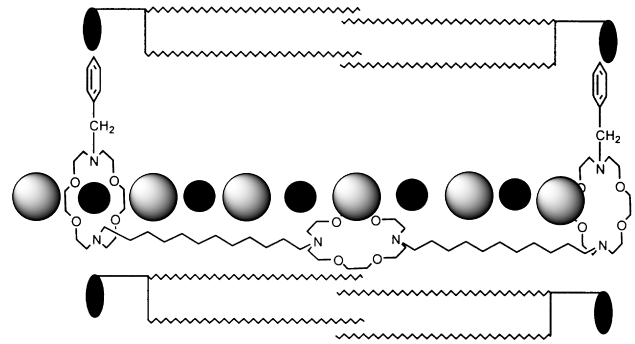
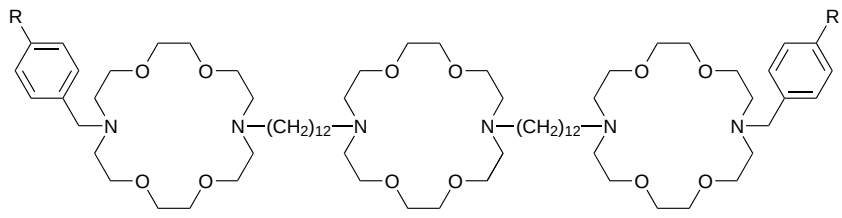


Fig. 1 Conformation of **1** in a phospholipid bilayer inferred from experimental evidence. Sodium (larger spheres) and water are shown schematically only to indicate their presumed presence.



1 R = H, **2** R = OMe

points. The linear relationship between current amplitude and applied voltage for benzyl channel **1** shows that it obeys Ohm's law and that there are no voltage-gated phenomena. The slope of the curve gives the channel's conductance. For **1**, it was determined to be 12.8 pS for a 0.1 M NaCl solution. We note that although the overall behaviour of **1** and **2** are similar to that observed for a typical, natural sodium channel, the magnitude of ion flux [12.8 pS for **2** (100 mM Na^+)] is less than the *ca*. 18 pS typically observed for the transmembrane proteins.[9] This channel is thus remarkably active for such a low molecular mass system. The methoxybenzyl channel (**2**) appears by ^{23}Na NMR studies to be about half as effective as the gramicidin channel,[10] a dimeric peptide, which also exhibits a lower flux than do proteins.

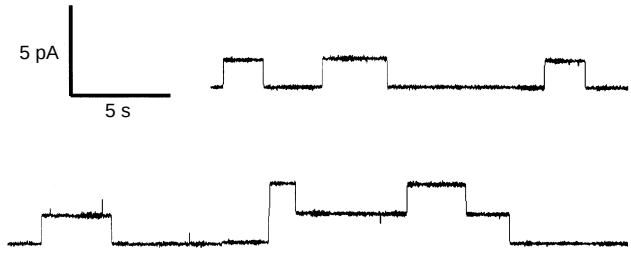


Fig. 2 Bilayer clamp traces for methoxybenzyl channel (**2**) recorded at +100 mV

Traces are shown in Fig. 3 for **1** and **2** (additional data) on the same scale as each other and as in Fig. 2. Data were recorded at 120 mV for **1** and at 80 mV for **2**. These differences in holding voltage are expected to change the amplitude but not to have a significant effect on channel open times. Benzyl channel, **1**, opens and closes on a timescale of a few seconds typical of many proteins [Fig. 3(*a*)]. As in the methoxybenzyl case, integral amplitudes are observed for the square peaks. Compound **1** shows peaks indicating both longer and shorter opening times but none of the traces thus far obtained indicate that any channel remains open for more than *ca*. 5 s. As apparent in Fig. 2, **2** also shows 'typical' opening and closing behaviour much of the time. During the course of recording, however, several of the data segments showed behaviour that stood in striking contrast to this 'typical' behaviour. Specifically, **2** showed peaks indicating that single channels remained open for more than 30 s [Fig. 3(*b*)]. These remarkably long open times for **2** have been observed at several different applied potentials

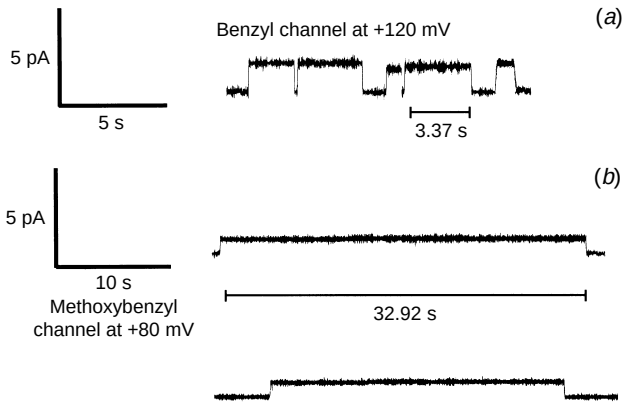


Fig. 3 Comparison of observed open times for the benzyl channel (**1**) and methoxybenzyl channel (**2**)

in the range ±80–120 mV. Thus, this unusual behaviour does not appear to correlate with potential differences or polarity.

Channel structures **1** and **2** have a molecular mass of *ca*. 1300 and an extended length of > 30 Å. It seems remarkable that changing a hydrogen to a methoxy group at the distal ends of this system could so dramatically alter the cation transport behaviour. We are currently attempting to clarify the cause of this behaviour and to determine if other terminal groups can lifewise affect cation transport so dramatically.

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Footnotes

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† Bilayers were prepared by the 'painting method' using L-α-lecithin (a soybean lipid mixture containing 20% phosphatidylcholine, remainder unspecified). The bilayers (capacitance ≥100 pF) were allowed to stabilize for ≥1 h prior to adding the transporter (2–40 pmol). After addition of the ionophore, the mixture was stirred for *ca*. 10 min and then the membrane was allowed to equilibrate for *ca*. 5 min. Recordings were obtained during *ca*. 9 h for the benzyl channel (membrane stable) and *ca*. 3 h for the methoxybenzyl channel (> 100-fold the amount shown). Each experiment was repeated a minimum of three times.

‡ Single channel signals were detected and recorded using a Warner Instruments PC-505 Patch Clamp Amplifier equipped with a bilayer headstage (50 GΩ), a Digidata 1200A A/D converter, and the computer based data acquisition software Axoscope (v. 1.1.1). The holding voltage was changed manually to obtain channel responses at different voltages. Single-channel current amplitude and standard deviations were determined using Axoscope and/or pCLAMP6.

§ 'Unmodified Na^+ channels activated by batrachotoxin have a dominant conductance of 18 ± 2 pS in 100 mM Na^+.[9]

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