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VOLTAGE-DEPENDENT ION CHANNEL FORMATION BY RIGID ROD-SHAPED POLYOLS IN PLANAR LIPID BILAYERS

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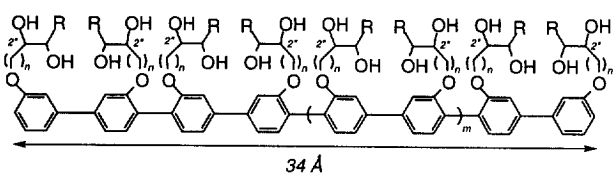
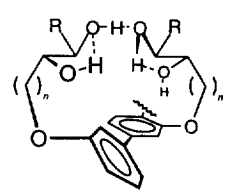
Abstract In this *Letter*, we describe the appearance of large, voltage-dependent currents in BLM induced by rigid rod-shaped polyols that function without charge and permanent dipole moment. The capacity of these symmetrical, nonpeptide models to form either short-living nanopores or small ion channels is shown to depend critically on the length of rigid-rod scaffold as well as the nature of the lateral side chains.

The molecular organization of biological ion channels is a topic of current concern. To elucidate the structural prerequisites for ion channel formation, the use of synthetic models with structural versatility beyond the limits of peptide chemistry has received increasing attention.[1-7] Although this approach has already afforded substantial insights into the molecular mechanism of ion channel formation, further investigation of regulatory mechanisms such as ion selectivity, ligand-[4] and voltage-gating[5-7] with nonpeptide ion channel models proved difficult. Additional incentive to investigate voltage-gating in particular emerged recently from the potential applicability of functional models to the development of biomimetic antimicrobials with low toxicity and high resistance to enzymatic degradation and microbial resistance.[8,9]

The present study focuses on unexpected, voltage-dependent large channels with unusually short lifetime formed by rigid rod-shaped polyol[10-12] **1** (Fig. 1) in black lipid membranes (BLM). Voltage-gating has been



1: n = 1, m = 1, R = H, 2"*R*
2: n = 3, m = 1, R = H, 2"*S*
3: n = 1, m = 1, R = $CH_2CH_2CH_3$, diastereomeric mixture
4: n = 1, m = 0, R = H, diastereomeric mixture



1a: n = 1, R = H
2a: n = 3, R = H
3a: n = 1, R = CH_2CH_2Cl

Figure 1

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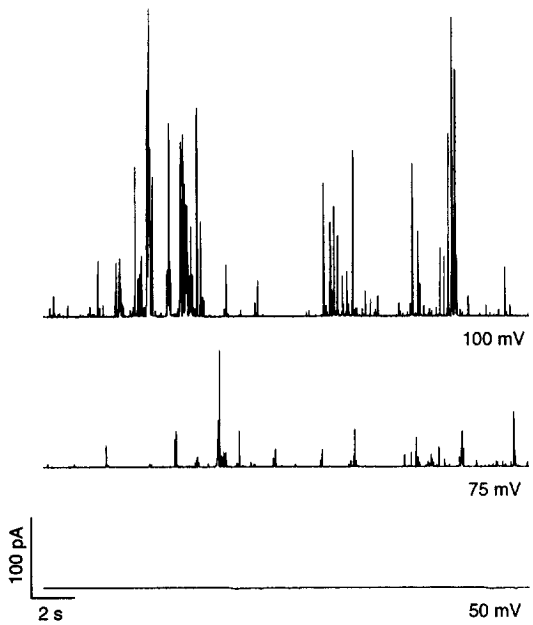

Figure 2

proposed to originate from a permanent macrodipole moment of the channel forming molecule and/or from an asymmetric distribution of charges along the membrane spanning part of ion channels.[8,9] Studies with derivatives of the "classical" model peptides gramicidin[13-15] and mellitin[9] as well as with synthetic peptides[16] have indicated that either structural prerequisites can indeed yield voltage-gating. A few voltage-dependent nonpeptide models were devised recently on the basis of an asymmetric distribution of charges.[5-7]

The effect of polyols **1-4** on the conductance of BLMs, spanning the apertures in nonpolar Teflon septi that separate two symmetric 2 M KCl solutions, was studied using a 3:1 mixture of brain phosphatidyl serine (PS) and cholesterol.[11,17] Figure 2 shows a representative record of currents after 1 hour incubation of 5 μM polyol **1** from both sides of the bilayer. The application of voltages higher than 50 mV resulted in consecutive appearances of large currents of unusually short duration. It is seen that the single channel events are not resolved. However, even when measured with a 10 kHz filter after 40 min incubation, the stationary currents of an open single channel could not be measured. Our conclusion is that the lifetime of the active structure of **1** is below 0.1 ms. At 75 mV and 100 Hz filtering by a low-pass Bessel filter, the largest observed current reached 176 pA, at 100 mV 453 pA (Fig. 2). The probability to observe an open channel, P_o, increased rapidly with the applied voltage. Figure 3 shows results in comparison to an exponential dependence with the characteristic voltage of e-fold increase of 27 mV.

The appearance of these voltage-gated large currents depends on the length of rigid-rod molecule as well as the nature of diol-containing lateral substituents. Consistent with our results using SUVs which showed hexamer **4** located at membrane/water interface,[11] no changes in conductance of the BLM were observed after two-sided addition of up to 5 μM of **4**. Instead of voltage-dependent large currents, small currents with an on/off-pattern reminiscent of discrete single channels were observed with **2** and **3** at concentrations above 0.5 μM and 5 μM, respectively (50 Hz filter, Fig. 4). These

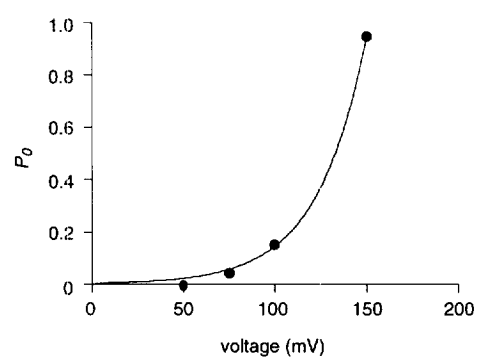

Figure 3

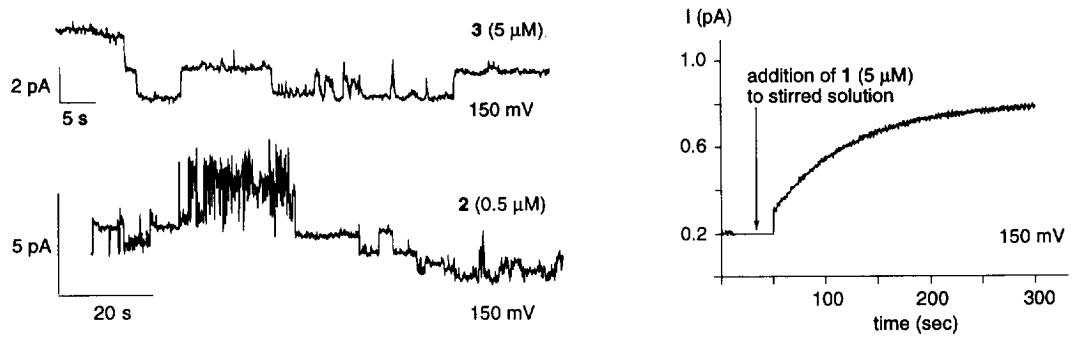

Figure 4

small currents and a reversal potential indicating cation selectivity (i.e., 40 - 50 mV for **2**, negative at the side of the higher salt concentration in 2 M KCl/0.2 M KCl) are consistent with the monomeric structures **2a** and **3a** previously proposed to rationalize the selective proton transport of **1-3** in small unilamellar vesicles (Fig. 1).[11,12] Namely, the formation of an intramolecular hydrogen-bonded chain (HBC)[11,18] in **2a/3a** creates four pseudo-macrocycles aligned along one rigid-rod scaffold. With six oxygens per ring, **2a/3a** bear structural similarity to ion channel forming oligomeric crown ethers.[1-3] The increased (0.5 μM) activity of the "23-crown-6" **2a** compared to **3a** is consistent with a larger, more stable pseudo-macrocycle. Molecular models further indicate that a hydrophobic cover of the hydrophilic HBC by the terminal propyls may stabilize "19-crown-6" **3a**. Similar small channels that could originate from monomeric **1a** were not found in addition to the voltage-dependent large currents. However, an exponentially increased macroscopic current within 5 min after the addition of **1** may contain single channel currents comparable to those observed for **2** and **3** but unresolved under the experimental conditions (1 Hz filter, Fig. 4). Such initial increases have been observed before for the ion channel forming polyene nystatin.[19]

The unexpectedly large conductance and voltage dependence observed with the uncharged, highly symmetrical polyol **1** is intriguing. Voltage-induced, rapid relocations of **1** in the lipid bilayer that temporarily disturb the suprastructure of the membrane could account for the formation of such nanopores. However, voltage-directed self-assembly of membrane-spanning **1** is more likely because the clustered appearance and the short life-time of the large currents implies involvement of suprastructures of **1**. Thus, a diameter of $\geq$ 11.0 Å was calculated for a cylindrical pore of 34 Å length' to produce the observed maximal currents,[20] and computer models for barrel stave-type self-assemblies with increasing numbers of monomers per supramolecule were prepared (Cerius2, Molecular Simulations, Inc., Fig. 5). The rod-rod distance along

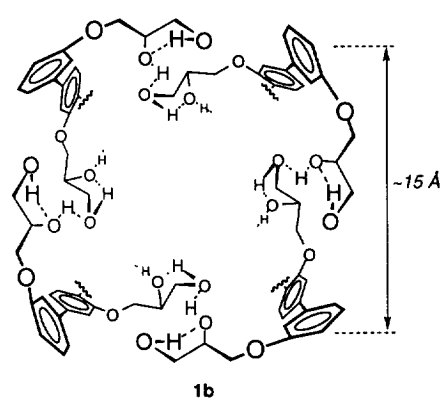

Figure 5

interdigitating side chains was in all cases 15 ± 1 Å. The pore diameter closest to the estimated experimental value of d ≥ 11.0 Å was 12 Å for the tetrameric self-assembly **1b**. Voltage-gating by the hypothetical active structure **1b** could originate from an induced macrodipole of polarized, *inter*molecular HBCs. However, further studies will be needed to clarify the molecular mechanism of the formation of the short-living nanopores formed by **1**. We are currently most interested in the design and synthesis of lateral side chains that permanently stabilize self-assembled rigid-rod nanopores similar to **1b**.

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