Pergamon

Tetrahedron Letters, Vol. 38, No. 15, pp. 2613-2616, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00427-9

Transmembrane Ion Transport Mediated by Amphiphilic Polyamine Dendrimers

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Abstract A series of amphiphilic polyamine dendrimers was efficiently prepared from cholestamine in order to probe the hypothesis that an increasing number of ammonium cations attached to a hydrophobic anchoring group should increasingly facilitate transmembrane ion transport. Results from transport experiments using large unilamellar vesicles are consistent with this new concept.

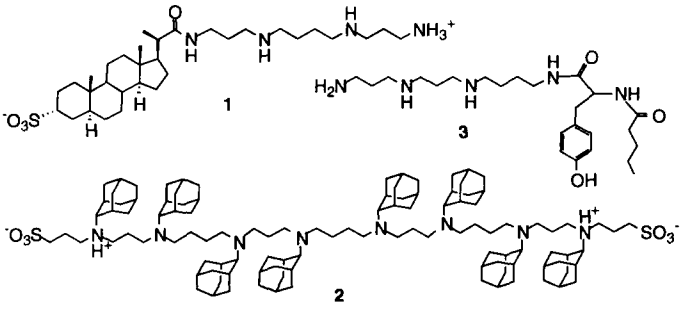


Figure 1

The increasingly refined designs of synthetic, non-peptide ion carriers and channels have lead to a current, vital impact on the molecular description of ion transport across lipid bilayers.[1] Although commonly not considered in the design of artificial pores, the few examples of polyamine-based ion-transporting ionophores appear to act by particularly unique, complex mechanisms.[2-4] For instance, an analog of the naturally occurring antimicrobial squalamine, the cholesterol-based polyamine **1** (Fig. 1) having a C(3)-sulfate and a C(17)-linear polyamine, selectively transports anions through negatively charged lipid bilayers.[2] Based on these findings, Regen and coworkers have proposed a deceptively classical "barrel-stave"-type aggregate containing a highly positively charged interior formed by the polyammonium chains, and zwitterionic head groups formed by the C(3) sulfate and the terminal primary ammonium cation at the membrane/water interface.[2] A completely different mechanism, namely facilitated proton flux along the membrane-spanning, non-aggregated and essentially non-protonated ocataamine **2** was proposed to dissipate transmembrane pH gradients.[3] The unprecedented ion channel formation of the polyamine **3**, a wasp toxin much too short to span a lipid bilayer, must act by a third, distinct and so far uncharacterized mechanism.[4]

Inspired by this apparent diversity and complexity of polyamine-mediated membrane transport, we have hypothesized that multiple ion pair formation of a sufficiently high number of ammonium cations with the

phosphate anions of lipids, initiated and stabilized by a hydrophobic anchoring moiety, may create reversible defects in lipid bilayers. The tentative structure of a pore formed by the dendrimer **4** (Scheme) should illustrate this new concept (Fig. 2). An increasing number of ammonium cations should thus lead to an increasingly facilitated transmembrane ion transport. The dendritic amphiphilic polyamines **4-7** were selected to verify this concept because dendrimer synthesis allows one to increase the number of amines in a controlled manner.[5] Moreover, their steric bulkiness should prevent the polyamine moiety from penetrating the bilayer (as proposed for **1** and **2**)[2,3] or receptor binding sites (as proposed for **3**).[6] Focusing on the function of the polyamine part, a steroid moiety was selected as an easily accessible, hydrophobic anchor with well understood orientation in lipid bilayers.

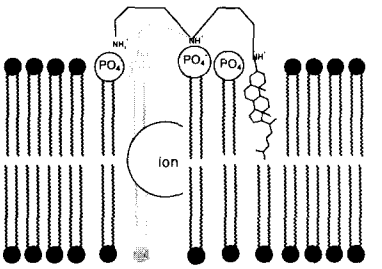
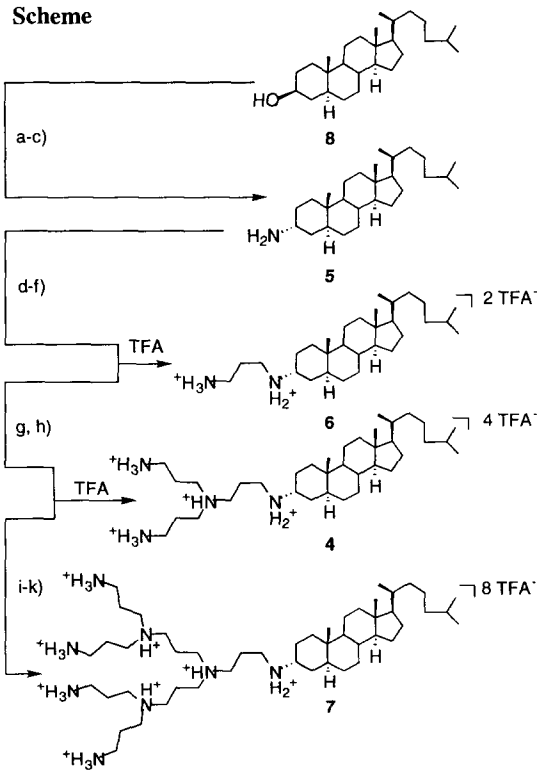


Figure 2

Scheme



a) TsCl, Py (81%), b) NaN_3, DMF (87%), c) H_2, Pd-C (89%), d) acrylonitrile, DMF (93%), e) BOC_2O, Et_3N (63%), f) $NaBH_4$, $CoCl_2$ (69%), g) acrylonitrile, AcOH (56%), h) H_2 (73 psi), PtO_2, EtOH-$CHCl_3$ (53%), i) acrylonitrile, AcOH (89%), j) 1. H_2 (60 psi), PtO_2, EtOH-$CHCl_3$, 2. BOC_2O, Et_3N, CH_2Cl_2 (18%), k) TFA, CH_2Cl_2 (quant).

Cholestamine **5** was prepared by tosylation of cholestanol (**8**), substitution by azide and subsequent reduction. Michael addition of amine **5** to acrylonitrile, BOC-protection of the secondary amine at C(3), reduction[7] and deprotection afforded the diammonium salt **6**. Another two cycles of Michael addition, catalytic reduction[8] and final deprotection gave polyammonium salts **4** and **7**, respectively. Work-up and purification of the polar polyamine **7** required the intermediate introduction of BOC protecting groups, which were subsequently removed.[9]

Membrane insertabilities and transport properties of (poly)amines **4-7** were assessed by a standard spectroscopic assay.[10] The pH-sensitive fluorescent dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, Fluorescent Probes) was trapped in uniformly sized, small unilamellar vesicles (SUVs) prepared from fresh egg yolk phosphatidyl choline (PC, Avanti) by the dialytic detergent removal technique using sodium cholate as a detergent and a

HEPES buffer (100 mM HEPES buffer, pH 7.4, 0.1 mM HPTS).[11] Extravesicular HPTS was removed by a second dialysis against 100 mM HEPES buffer, pH 7.4, without HPTS, followed by size exclusion chromatography (Sephadex G50). The final lipid concentration was 10 mM.

In the experiments summarized in Figure 3, 1.9 ml buffer solution (100 mM HEPES, pH 7.4) was placed in a thermostated fluorescence cell (20° C), and 100 µl lipid suspension was added through a house-made injector port. Fluorescence emission at 510 nm (excitation 460 nm) was recorded during an entire experiment period. The following addition of 20 µl of 0.1 mM methanolic solutions of amine **4-7** to the gently stirred mixture gave negligible changes in fluorescence emission, suggesting that neither lysis nor membrane fusion were induced. Then, a base-pulse, namely 40 µl of 2 M NaOH, was applied resulting in an extravesicular pH of 7.8. The subsequent increase of the intravesicular pH was monitored by the increase of fluorescence emission. Finally, the vesicles were lysed by adding 50 µl of 1.2% Triton-X100.

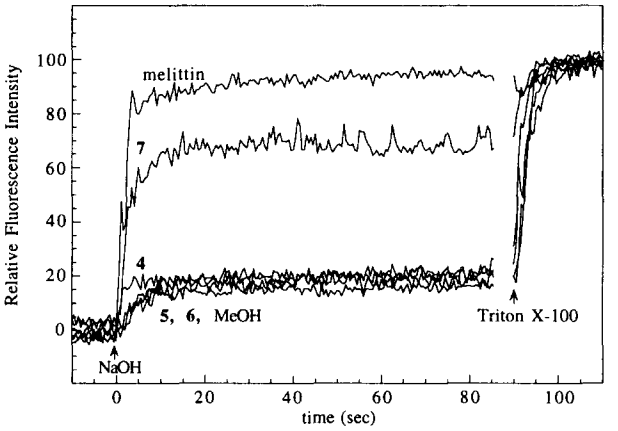


Figure 3

This assay quantitatively measures the movement of protons and/or hydroxide anions across lipid bilayers mediated by melittin, gramicidin, and (poly)amines **4-7**. At the final concentration of 1 µM, amine **5** and polyamines **4** and **6** do not exhibit activity over the negative control (MeOH, as shown in Fig. 3). Dendrimer **7**, however, is almost as potent as ion-channel forming polypeptides melittin (Fig. 3) or gramicidin (not shown). Further tests have demonstrated that dendrimer **7** is still active at 125 nM concentrations, and that tetraamine **4** is significantly active at 10 µM, while diamine **6** and amine **5** remain almost inactive at this concentration.

These results demonstrate that an increasing number of amine groups attached to a hydrophobic anchoring moiety in fact leads to an increasingly facilitated transmembrane ion transport. The proposed new concept thus holds, and structural variations based on these findings may open routes towards new classes of antimicrobials and drug delivery systems.

Preliminary results further indicated that the ion transport mediated by the amphiphilic polyamine dendrimer **7** might be sensitive with respect to composition and surface charge of the lipid bilayer, ion charge,

membrane potential and pH. A detailed study of the (super)structure, activity and transport mechanism of membrane-bound polyamine dendrimers is ongoing and will be published in due course.

Acknowledgment: We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, and Georgetown University for support of this work. We also thank Dr. Karel Ubik from the Institute of Organic Chemistry and Biochemistry, Academy of Science of the Czech Republic for measuring the mass spectra.

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9. Selected spectroscopic data:. **4:** ^{1}H NMR (270 MHz, $CDCl_3$-CD_3OD) δ 3.18 (m, 6H), ~3.0 (m, 1H), 3.01 (t, 6H, *J* = 7 Hz), 2.2 (m, 6H), 2.1 ~ 0.8 (m, 31H), 0.91 (d, 3H, *J* = 6.5 Hz), 0.87 (d, 6H, *J* = 6.5 Hz), 0.85 (s, 3H), 0.67 (s, 3H). FAB-MS: 559.4 ($M + H^+$). **5:** ^{1}H NMR ($CDCl_3$) δ 3.57 (br.s, 1H), 2.0 ~ 0.8 (m, 31H), 0.88 (d, 3H, *J* = 6.5 Hz), 0.86 (d, 3H, *J* = 6.5 Hz), 0.85 (d, 3H, *J* = 6.5 Hz), 0.78 (s, 3H), 0.64 (s, 3H). EI-MS: 387.5 (M^+). **6:** ^{1}H NMR ($CDCl_3$-CD_3OD) δ 3.1 (m, 5H), 2.1 (m, 2H), 2.1 ~ 0.8 (m, 31H), 0.91 (d, 3H, *J* = 6.5 Hz), 0.87 (d, 6H, *J* = 6.5 Hz), 0.84 (s, 3H), 0.67 (s, 3H). FAB-MS: 445.4 ($M + H^+$). **7:** ^{1}H NMR ($CDCl_3$-CD_3OD) δ 3.1 (m, 28H), 2.1 (m, 14H), 2.1 ~ 0.8 (m, 31H), 0.91 (d, 3H, *J* = 6.5 Hz), 0.87 (d, 6H, *J* = 6.5 Hz), 0.84 (s, 3H), 0.67 (s, 3H). FAB-MS: 787.8 ($M + H^+$).
10. See ref 2. and references therein.
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(*Received in USA* 28 *January* 1997; *accepted* 21 *February* 1997)