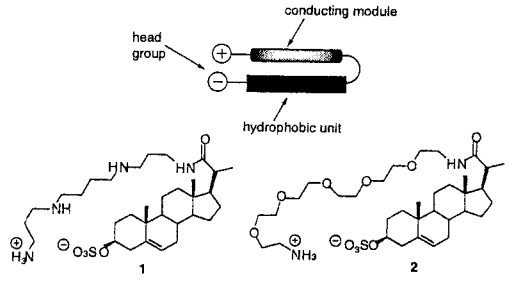
Modular Control over the Selectivity of Self-Assembling and Membrane-Spanning Ion Conductors

Sijbren Otto, Manette Osifchin, and Steven L. Regen*

Department of Chemistry and Zettlemoyer Center for Surface Studies Lehigh University Bethlehem, Pennsylvania 18015

Received February 17, 1999
Revised Manuscript Received June 22, 1999

We have recently described a new design principle for the construction of synthetic ionophores.[1,2] In essence, a rigid hydrophobic unit is used to drag a covalently attached array of relay elements (i.e., a "conducting module") into a lipid membrane. When two or more of these modules span the bilayer, a contiguous pathway (i.e., an "ion conductor") is produced that permits a flow of ions.[3] In previous work, we reported that one such ionophore (**1**), having 23,24-bisnor-5-cholenic acid as the hydrophobic unit and spermine as the conducting module, was active for H^+/OH^- transport but not for Na^+ ion transport.[1,4] In this paper, we show that replacement of the polyamine moiety by a polyether chain produces an analogous ionophore having *exactly the opposite properties*; i.e., **2** is active for Na^+ but not for H^+/OH^- transport. We also provide kinetic evidence for membrane-spanning dimers as being the active species in both cases. The likely origin for this difference in ion selectivity and the implications of these findings for the rational design of new synthetic ionophores having enhanced activity as well as membrane-selectivity are briefly discussed.



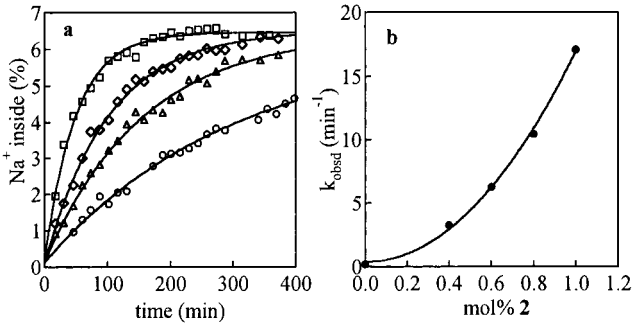


Figure 1. (a) Entry of Na^+ into (C14:1) vesicles containing 0.4, 0.6, 0.8, and 1.0 mol % of **2** (increasing rates, respectively) as a function of time at 35 °C. (b) Plot of k_{obsd} versus mol % **2**; the solid line represents a nonlinear least-squares fit of the data according to eq 4, where $n = 2.0 \pm 0.1$.

The synthesis of **2**, starting from 23,24-bisnor-5-cholenic acid, has previously been described.[4] To characterize its ionophoric properties, we first measured its ability to promote the transport of Na^+ across bilayers made from 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine (C14:1) PC. For this purpose, $^{23}Na^+$ NMR spectroscopy was used as an analytical tool because it can directly monitor the flow of ions into the interior of vesicles.[5] In brief, a membrane-impermeable paramagnetic shift reagent is added to the external aqueous solution and the percentage of Na^+ that enters the vesicles is quantified by integration of internal and external $^{23}Na^+$ NMR absorbances. By using procedures similar to those previously described, varying mole percentages of **2** were incorporated into 200-nm unilamellar vesicles that were made from (C14:1) PC.[1] Thus, thin films composed of (C14:1) PC plus **2** (deposited from homogeneous solutions) were dispersed in aqueous LiCl and subjected to freeze−thawing and extrusion to produce the requisite vesicles.[1,6] Since the introduction of an ionophore under these conditions leads to its incorporation in both halves of the bilayer, such methods are generallly referred to as a "double-sided" addition. Dilution with aqueous NaCl, addition of a shift reagent, and analysis of internal Na^+ as a function of time yielded kinetic profiles that are shown in Figure 1a. Gel filtration and analysis of the vesicle fractions established that more than 90% of the ionophore was bound to these membranes. Observed pseudo-first-order rate constants (k_{obsd}) that were calculated from these data were found to have a second-order dependency on the mol % of **2** (Figure 1b). In the absence of ionophore, Na^+ permeation was very slow. In separate experiments, we have found that in sharp contrast to **1**, which readily discharges pH gradients across egg PG bilayers, **2** exhibited negligible activity for such discharge.[7]

The second-order dependency of k_{obsd} on the mol % of **2** lends strong support for a model in which (i) monomers of **2** are in equilibrium with dimers, (ii) monomers are thermodynamically favored and their concentration can be approximated by the total concentration of **2** that is present, and (iii) dimers are responsible for ion transport. Thus, for the general case of transport-active aggregates, the rate of ion flow is expected to obey eq 1, where k_{obsd} is a pseudo-first-order rate constant that is the product of the aggregate concentration and a rate constant, k_2 (eq 2). If the

(1) Merritt, M.; Lanier, M.; Deng, G.; Regen, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 8494.

(2) For a review of other strategies to synthetic ionophores, see: Gokel, G. W.; Murillo, O. *Acc. Chem. Res.* **1996**, *29*, 425. For selected examples, see: (a) Weiss, L. A.; Sakai, N.; Ghebremariam, B.; Ni, C.; Matile, S. *J. Am. Chem. Soc.* **1997**, *119*, 12141. (b) Murillo, O.; Suzuki, I.; Abel, E.; Murray, C. L.; Meadows, E. S.; Jin, T.; Gokel, G. W. *J. Am. Chem. Soc.* **1997**, *119*, 5540. (c) Meillon, J.-C.; Voyer, N. *Angew. Chem., Int. Ed., Engl.* **1997**, *36*, 967. (d) Fyles, T. M.; Loock, D.; van Straaten-Nijenhuis, W. F.; Zhou, X. *J. Org. Chem.* **1996**, *61*, 8866. (e) Tanaka, Y.; Kobuke, Y.; Sokabe, M. *Angew. Chem., Int. Ed., Engl.* **1996**, *34*, 693. (f) Menger, F. M.; Davis, D. S.; Persichetti, R. A.; Lee, J.-J. *J. Am. Chem. Soc.* **1990**, *112*, 2451. (g) Fuhrop, J.-H.; Liman, U.; Koesling, V. *J. Am. Chem. Soc.* **1988**, *110*, 6840. (h) Fyles, T. M.; Loock, D.; Zhou, X. *J. Am. Chem. Soc.* **1998**, *120*, 2997. (i) Pechulis, A. D.; Thompson, R. J.; Fojtik, J. P.; Schwartz, H. M.; Lisek, C. A.; Frye, L. L. *Biorg. Med. Chem.* **1997**, *5*, 1893. (j) Abel, E.; Maguire, G. E. M.; Meadows, E. S.; Murillo, O.; Jin, T.; Gokel, G. W. *J. Am. Chem. Soc.* **1997**, *119*, 9061. (k) Clark, T. A.; Buehler, L. K.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 651. (l) Murray, C. L.; Gokel, G. W. *Chem. Commun.* **1998**, 2477. (m) Ni, C.; Matile, S. *Chem. Commun.* **1998**, 755.

(3) In this paper, we refer to assemblies of these membrane-spanning *synthetic* ionophores as "ion conductors" as opposed to naturally occurring "channel formers", which produce trans-membrane channels or pores through which selected ions can diffuse: Voet, D.; Voet, J. G. *Biochemistry*; John Wiley & Sons: New York, 1990; Chapter 18.

(4) Sadownik, A.; Deng, G.; Janout, V.; Regen, S. L.; Bernard, E. M.; Kikuchi, K.; Armstrong, D. *J. Am. Chem. Soc.* **1995**, *117*, 6138.

(5) (a) Degani, H.; Elgavish, G. A. *FEBS Lett.* **1978**, *90*, 357. (b) Gupta, R. K.; Gupta, P. *J. Magn. Reson.* **1982**, *47*, 344. (c) Pike, M. M.; Simon, S. R.; Balschi, J. A.; Springer, C. S., Jr. *Proc. Natl Acad. Sci. U.S.A.* **1982**, *79*, 810. (d) Pregel, M. J.; Jullien, L.; Lehn, J. M. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1637.

(6) Stadler, E.; Dedek, P.; Yamashita, K.; Regen, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 6677.

(7) Specific procedures and conditions that were used were similar to those previously described.[1] The maximum concentration of **2** that was tested was 1 mol %.

10.1021/ja990505t CCC: $18.00
Published on Web 07/27/1999

aggregate concentration is expressed in terms of the monomer concentration and the dissociation constant (K) that defines the aggregate-monomer equilibrium (eq 3), then k_{obsd} will vary with the monomer concentration to the nth power (eq 4). Thus, the second-order dependency of k_{obsd} on the mole percentage of **2** in bilayers of (C14:1) PC supports the existence of transport-active dimers, similar to what has been observed for **1** in pH discharge experiments.[1]

$$\text{Rate} = k_{obsd}[Na^+] \quad (1)$$

$$k_{obsd} = k_2\,[\text{aggregate}] \quad (2)$$

$$K = [\text{monomer}]^n/[\text{aggregate}] \quad (3)$$

$$k_{obsd} = k_2[\text{monomer}]^n/K \quad (4)$$

To gain insight into whether dimers of **2** function as ion carriers or as membrane-spanning agents, we examined their activity in PC bilayers of varying thickness. In principle, the activity of a membrane-spanning agent should be very senstive to the thickness of its host membrane.[8] Specifically, when the bilayer thickness begins to exceed the length of the agent, ionophoric activity should be sharply reduced or eliminated due to its inability to provide a contiguous pathway for ion flow. For a carrier mechanism, however, ionophoric activity is expected to show only a modest dependence on membrane thickness; e.g., kinetic studies involving valinomycin (a well-established ion carrier) have shown that the transport rate of valinomycin/Rb^+ across lipid membranes made from α-monoglycerides decrease by a factor of only 7 when the fatty acid chain length is increased from 16 carbons (palmitoleoyl) to 20 carbons (11-eicosenoyl).[9] With this rationale in mind, **2** was incorporated into three fluid phospholipid membranes having acyl chains that differ by two methylene units; i.e., vesicles made from (C14:1) PC, 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine (C16:1) PC, and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (C18:1) PC, which bear monounsaturated fatty acids having 14, 16, and 18 carbons, respectively. Examination of **2** by CPK molecular models indicates an overall length that is slightly shorter than (C14:1) PC, when the conjugate is placed in a macrocyclic conformation, when the headgroup of the conjugate and that of the phospholipid are aligned, and when the alkyl chains are fully extended. If **2** functioned as an ion conductor, one might expect to observe a strong decrease in activity on going from (C14:1) PC to (C16:1) PC to (C18:1) PC.

Transport experiments that were carried out with the above three PC membranes yielded results that are shown in Figure 2a. The linear correlation that was found between k_{obsd} and (mol % **2**)^2 for the C16:1 bilayers lends further support for the existence of transport-active dimers. When **2** was incorporated into C18:1 bilayers, its transport activity was found to be very low. For this reason, only one data point is given where 2 mol % of the ionophore was used. According to our kinetic model, the slopes of these lines yield values of k_2/K that decrease by a factor of >300 on going from C14:1 to C18:1. Although we are presently unable to separate these kinetic and thermodynamic terms, the fact that k_2/K varies by *more than 2 orders of magnitude* provides compelling evidence that a membrane-spanning agent is involved.[10,11] Analogous pH discharge experiments that were carried out with **1**, in bilayers made from PG forms of C14:1, C16:1 and C18:1, gave very similar results (Figure 2b). In this case, values of k_2/K decrease by a factor of ca. 3700. Thus, **1** and **2** both function as membrane-spanning ion conductors.

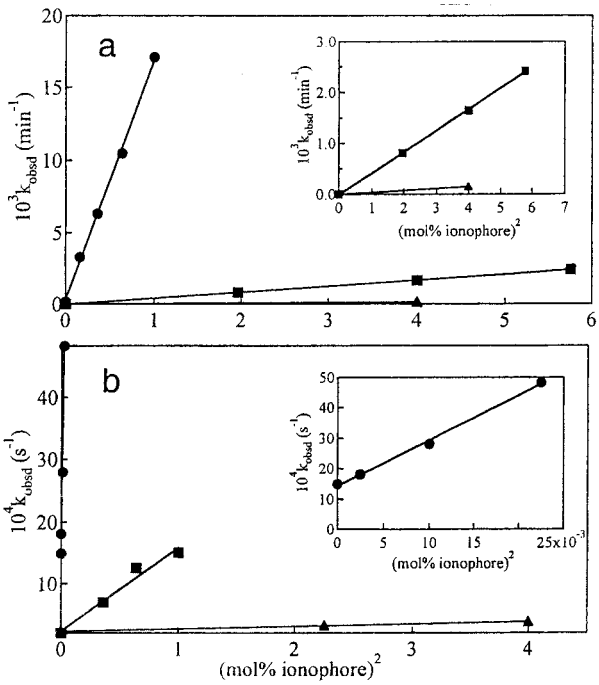


Figure 2. (a) Plot of k_{obsd} versus (mol % **2**)^2 for (a) vesicles made from (●) C14:1, (■) C16:1, and (▲) C18:1 PC at 35 °C. (b) Plot of k_{obsd} versus (mol % **1**)^2 for vesicles made from PG forms of (●) C14:1, (■) C16:1, and (▲) C18:1 at 23 °C. Insets show expanded *X*- or *Y*-axis.

The striking differences in selectivity between **1** and **2**, we believe, is a likely consequence of **1** being able to undergo protonation. Specifically, whereas a "string" of protonated nitrogens will repel oncoming Na^+ ions, a string of ethyleneoxy units can bind and "pass along" such ions through a lipid bilayer. The effectiveness of **1** in discharging pH gradients can also be accounted for in terms of a protonated polyamine module; i.e., such a module would be ideally suited for a Cl^-/OH^- antiport pathway, as we have previously suggested.[1] Since a polyether module cannot support such a pathway, one would not expect **2** to have the ability to discharge pH gradients, which is, in fact, the case.

The modular control over the ion selectivity that is described herein demonstrates significant flexibility in our design principle. At the same time, it provides further evidence that **1** and **2** function as true ionophores. Finally, our kinetic results, which point toward membrane-spanning dimers as being the active species, highlight two new avenues of research that warrant detailed exploration; i.e., the design and synthesis of second-generation, sterol-based ionophores of varying length and ones that can be prepared in the form of dimers. In particular, the strong dependency of the activity of **1** and **2** on membrane thickness suggests a fundamentally new approach toward drug design; i.e., the creation of ionophores that can recognize and exploit small differences in membrane thickness. Synthetic efforts are now being directed along both of these avenues.

Acknowledgment. We are grateful to the National Science Foundation (Grant CHE-9612702) for support of this research.

Supporting Information Available: Procedures for vesicle formation, synthesis of **2**, Na^+ transport measurements, analysis of ionophore content and curve-fitting to determine n values (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA990505T

(8) Stein, W. D. *Channels, Carriers and Pumps: An Introduction To Membrane Transport*; Academic Press: New York, 1990.

(9) Benz, R.; Frohlich, O.; Lauger, P. *Biochim. Biophys. Acta* **1977**, *464*, 465.

(10) An attractive model for membrane-spanning dimers that we currently favor is one that is analogous to gramicidin-based dimers, where two hairpin-like conformations lie in the two leaflets of the bilayer and form end-to-end dimers.

(11) In preliminary studies, we have found that **2** leads to a large conductance in membranes formed from diphytanoyl PC. However, the observed conductance was found to be quiet, indicating that any channels, if present, are quite small.