Sodium Cation Transport in Synthetic Channels Obeys the Hammett Relationship in a Phospholipid Bilayer Membrane

Oscar Murillo, Iwao Suzuki, Ernesto Abel, and George W. Gokel*

Bioorganic Chemistry Program and Department of Molecular Biology & Pharmacology Washington University School of Medicine 660 South Euclid Avenue, Campus Box 8103 St. Louis, Missouri 63110

Received March 25, 1996

The study of transmembrane channel structures constitutes one of the most active areas in modern biological chemistry.[1] Despite the enormous amount of information that has accumulated on which proteins are responsible for transport of ionic or molecular species,[2] physical chemical details of transport and selectivity remain largely obscure.[3] Channel model systems (for H^+ or alkali metal transport) include synthetic peptide "bundles"[4] and "half-channel elements," perhaps inspired by gramicidin[5] or amphotericin,[6] prepared by Tabushi,[7] Menger,[8] Kobuke,[9] and Regen,[10] as well as "tunnel-like" structures intended to emulate the structure of bacteriorhodopsin[2] pioneered by Fyles,[11] Lehn,[12] and more recently Ghadiri[13] and Voyer.[14]

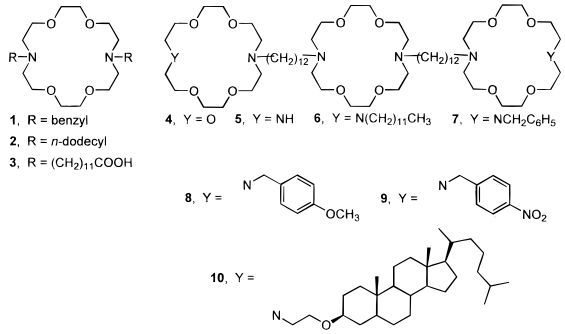
We report here a study involving variants of our original model system that permits us to ask three specific questions about how these synthetic, model compounds function in a phospholipid bilayer vesicle. First, do differences in cation flux merely reflect varied hydrophobicities and levels of penetration into the bilayer? Second, do the channel model systems in reality function as cation carriers? Third, do the distal (terminal) crown residues function as head groups[15] through which the cations must pass on entering and departing the bilayer? We report data below which (1) confirm membrane solubility, (2) discredit the carrier hypothesis, and (3) demonstrate for the first time the application of solution physical chemical analysis which confirms a Hammett relationship for Na^+ cation transport.

Table 1. Octanol−Water Partitioning Coefficients for Crown Ether Derivatives

No.	compound	log P_{oct}(exptl)	log P_{oct}(calcd)[c]
	12-crown-4	0.92[a]	0.57
	15-crown-5	0.33[a]	0.34
	18-crown-6	0.21[a]	0.17
	p-(*tert*-butyl)benzo-27-crown-9	1.89[b]	2.15
	p-(*tert*-butyl)benzo-30-crown-10	1.69[b]	1.65
1	*N*,*N*′-dibenzyldiaza-18-crown-6	4.21	4.10
6	bis(dodecyl) channel		18.48
7	dibenzyl channel		11.23
8	(*p*-methoxy)dibenzyl channel		11.76
9	(*p*-nitro)dibenzyl channel		11.26
10	cholestanyl ether channel		30.08

[a] Reference 19. [b] Reference 20. [c] Calculations used the HINT module of the Sybyl molecular modeling package.[21]

The compounds used in this study include gramicidin, a naturally-occurring channel former, valinomycin, a mitochondrial potassium carrier,[16] dibenzyldiaza-18-crown-6, **1**,[17] derivatives **2** and **3**, and tris(macrocycles) **4**−**10**. Compounds **2**, **7**, **8**, and **9** are described in the supporting information; the others are previously reported.[18]



Channel Model Compound Solubility. Octanol−water partition coefficients are reported in Table 1 for six crowns and five channel compounds. Values for five of the crown ethers are reported in the literature;[19,20] the result for dibenzyldiaza-18-crown-6 was determined in this work. Experimentally-determined *P* values were compared with those calculated by using the HINT (solubility) module of the SYBYL molecular

(1) Nicholls, D. G. *Proteins, Transmitters, and Synapses*; Blackwell Science: Oxford, 1994.
(2) (a) Henderson, R.; Baldwin, J. M.; Ceska, T. A.; Zemlin, F.; Beckmann, E.; Downing, K. H. *J. Mol. Biol.* **1990**, *213*, 899−929. (b) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *Nature* **1985**, *318*, 618−624. (c) Baldwin, J. M. *EMBO J.* **1993**, *12*, 1693−1703.
(3) (a) Stein, W. D. *Channels, Carriers, and Pumps*; Academic Press: New York, 1990. (b) Hille, B. *Ionic Channels of Excitable Membranes*; Sinauer Press: Sunderland, MA, 1992.
(4) (a) Mutter, M.; Tuchscherer, G. G.; Miller, C.; Altman, K.-H.; Carey, R. I.; Wyss, D. F.; Labhardt, A. M.; Rivier, J. E. *J. Am. Chem. Soc.* **1992**, *114*, 1463. (b) Ckerfeldt, K. S.; Lear, J. D.; Wasserman, Z. R.; Chung, L. A.; DeGrado, W. F. *Acc. Chem. Res.* **1993**, *26*, 191−197.
(5) (a) Urry, E. W., Goodall, M. C., Glickson, J. D., Meyers, D. F *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1907−1911. (b) Urry, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 672−676.
(6) Bolard, J. *Biochim. Biophys. Acta* **1986**, *864*, 257−304.
(7) Tabushi, I.; Kuroda, Y.; Yokota, K. *Tetrahedron Lett.* **1982**, 4601−4604.
(8) (a) Menger, F. M.; Davis, D. S.; Persichetti, R. A.; Lee, J.-J. *J. Am. Chem. Soc.* **1990**, *112*, 2451−2452. (b) Menger, F. M. *Bol. Soc. Chil. Quim.* **1990**, *35* (1), 33−38.
(9) (a) Kobuke, Y.; Ueda, K.; Sokabe, M.; *J. Am. Chem. Soc.* **1992**, *114*, 7618−1722. (b) Tanaka, Y.; Kobuke, Y.; Sokabe, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *4* (6), 693−694.
(10) Stadler, E.; Dedek, P.; Yamashita, K.; Regen, S. *J. Am. Chem. Soc.* **1994**, *116*, 6677−6682.
(11) (a) Carmichael, V. E.; Dutton, P. J.; Fyles, T. M.; James, T. D.; Swan, J. A.; Zojaji, M. *J. Am. Chem. Soc.* **1989**, *111*, 767−769. (b) Fyles, T. M.; James, T. D.; Kaye, K. C. *J. Am. Chem. Soc.* **1993**, *115*, 12315−12321. (c) Fyles, T.; James, T.; Pryhitka, A.; Zojaji, M. *J. Org. Chem.* **1993**, *58*, 7456−7468. (d) Fyles, T. M.; Heberle, D.; Van Straaten-Nijenhuis, W. F.; Zhou, X. *Supramol. Chem.* **1995**, *6*, 71−78.
(12) (a) Jullien, L.; Lehn, J.-M. *Tetrahedron Lett.* **1988**, 3803−3806. (b) Jullien, L.; Lehn, J.-M. *J. Inclusion Phenom.* **1992**, *12*, 55−74. (c) Pregel, M. J.; Jullien, L.; Lehn, J.-M.; *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1637−1640. (d) Canceill, J.; Jullien, L.; Lacombe, L.; Lehn, J.-M. *Helv. Chim. Acta* **1992**, *75*, 791.
(13) (a) Ghadiri, M. R.; Granja, J. R.; Buehler, L. K. *Natur*e **1994**, *369*, 301−304. (b) Khazanovich, N.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, *116*, 6011−6012.
(14) Voyer, N.; Robitaille, M. *J. Am. Chem. Soc.* **1995**, *117*, 6599−6600.
(15) (a) Gokel, G. W.; Hernandez, J. C.; Viscariello, A. M.; Arnold, K. A.; Campana, C. F.; Echegoyen, L.; Fronczek, F. R.; Gandour, R. D.; Morgan, C. R.; Trafton, J. E.; Minganti, C.; Eiband, D.; Schultz, R. A.; Tamminen, M. *J. Org. Chem.* **1987**, *52*, 2963−2968. (b) Echegoyen, L. E.; Hernandez, J. C.; Kaifer, A.; Gokel, G. W.; Echegoyen, L. *J. Chem. Soc., Chem. Commun.* **1988**, 836−837. (c) Echegoyen, L. E.; Portugal, L.; Miller, S. R.; Hernandez, J. C.; Echegoyen, L.; Gokel, G. W. *Tetrahedron Lett.* **1988**, *29*, 4065−4068. (d) Muñoz, S.; Mallén, J. V.; Nakano, A.; Chen, Z.; Echegoyen, L.; Gay, I.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1992**, 520−522. (e) Muñoz, S.; Mallén, J.; Nakano, A.; Chen, Z.; Gay, I.; Echegoyen, L.; Gokel, G. W. *J. Am. Chem. Soc.* **1993**, *115*, 1705−1711.
(16) Grell, E.; Funck, T.; Eggers, F. In *Membranes*; Eisenman, G., Ed.; Marcel Dekker: New York, 1975; Vol. 3, p 1.
(17) We have developed a shorthand to describe substituted crown compounds: ⟨18⟩ = 18-crown-6, ⟨N15⟩ or ⟨15N⟩ = aza-18-crown-6, and ⟨N18N⟩ = diaza-18-crown-6. *See*: Hernandez, J. C.; Trafton, J. E.; Gokel, G. W. *Tetrahedron Lett.* **1991**, 6269−6272.
(18) Murillo, O.; Watanabe, S.; Nakano, A.; Gokel, G. W. *J. Am. Chem. Soc.* **1995**, *117*, 7665.
(19) Hendrixson, R. R; Mack, M. P; Palmer, R. A.; Ottolenghi, A.; Ghirardelli, R. G. *Toxicol. Appl. Pharmacol.*, **1978**, *44*, 263−268.
(20) Stolwijk, T. B.; Sudholter, E. J. R.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1989**, *111*, 6321.

S0002-7863(96)00950-X CCC: $12.00

Table 2. Sodium Cation Transport by Ionophores

	ionophore	rel. rate ($CHCl_3$)	rel. rate (bilayer)
	valinomycin	1.0	0.14
	gramicidin	0.02	1.00
1	$PhCH_2\langle N18N\rangle CH_2Ph$	0.53	0.01
2	$C_{12}\langle N18N\rangle C_{12}$	0.48	0.01
3	$HOOC(CH_2)_{11}\langle N18N\rangle(CH_2)_{11}COOH$	0	0.01
4	$\langle 18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18\rangle$	0.58	0.02
5	$H\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle H$	0.27	0.28
6	$C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}$	0.26	0.28
7	$PhCH_2\langle N18N\rangle C_{12}\langle N18N\rangle C_{12}\langle N18N\rangle CH_2Ph$	0.46	0.38
8	$[p\text{-}MeOC_6H_4CH_2\langle N18N\rangle C_{12}]_2\langle N18N\rangle$	0.43	0.43
9	$[p\text{-}O_2NC_6H_4CH_2\langle N18N\rangle C_{12}]_2\langle N18N\rangle$	0.44	0.30
10	chol-O-$(CH_2)_2[\langle N18N\rangle C_{12}\langle N18N\rangle]_2$	0.62	0.02

modeling package.[21] The conclusion from these data is that structures **1** and **6**−**10** partition completely from water into a hydrophobic solvent.

Transport Studies. The cation conduction ability of compounds **1**−**10** was assessed by using two different techniques (Table 2). Sodium cation transport through a phosphatidylcholine/phosphatidylglycerol bilayer was assessed by dynamic ^{23}Na-NMR as previously described.[22] Bulk liquid membrane transport rates were measured using a concentric tube apparatus[23] ($CHCl_3$ membrane).[24] In all cases, gramicidin or valinomycin was run concurrent with the compound under study so that rate constants obtained could be normalized to a value of 1.0 (gramicidin exchange rate $\sim 175\ s^{-1}$, valinomycin transport rate $\sim 4.17 \times 10^{-8}$ mol·h^{-1}).

We correctly anticipated that among the compounds studied, valinomycin would be the best carrier in $CHCl_3$ and gramicidin would be the most effective transporter in a bilayer. Carriers **1** and **2** transported sodium picrate through $CHCl_3$ (picrate concentration measurements) with rates of 2.22×10^{-8} and 2.01×10^{-8} mol·h^{-1}, respectively. Under identical conditions, transport by gramicidin was less than twice the diffusion rate (0.08×10^{-8} mol·h^{-1}; blank value: 0.013×10^{-8} mol·h^{-1}). Compound **6** was found to transport Na^+, but its rate (1.1×10^{-8}) was about half that for either **1** or **2**. Compounds **4** and **5**, which constitute major fragments of the channel structure, gave rates of 2.4×10^{-8} and 1.13×10^{-8} mol·h^{-1}, respectively. It is noteworthy that although **5** and **6** function similarly as carriers and are both inferior to **4**, exactly the opposite relationship is observed in the bilayer (see column 2 of Table 2). Gramicidin transports cations in a bilayer by dimerizing to form a membrane-spanning, ∼30 Å long, tunnel-like structure. Dimerization is possible in bulk $CHCl_3$, but the shortest transport path is ∼1 cm (10^8 Å), an impossible distance for "tunnel" formation. Indeed, the efficacy of gramicidin in $CHCl_3$ was only ∼8% of that of valinomycin.

A comparison of compounds **4** and **5** is particularly interesting. Transport by **5** ($k_{rel} = 0.28$) in a bilayer is identical to that of **6**. On the other hand, **4** ($k_{rel} = 0.02$) does not transport cations in the bilayer system. The order of efficacy in the bilayer is **5** ≈ **6** ≫ **4**. In contrast, in the $CHCl_3$ membrane, **5** and **6** have similar transport rates (0.27, 0.26), but the rate for **4** is more than twice as large (0.58; order: **4** > **5** ≈ **6**). Compound **7** shows transport activity in the bilayer ∼40% of that of gramicidin and ∼35% better than for **6**. In the bulk membrane system, its rate is ∼46% of that of valinomycin, nearly 6-fold that of gramicidin, and ∼75% better than for **6**. These differences in relative efficacy in the various membrane systems strongly suggest that different mechanisms operate although the above is admittedly not a direct mechanistic test.

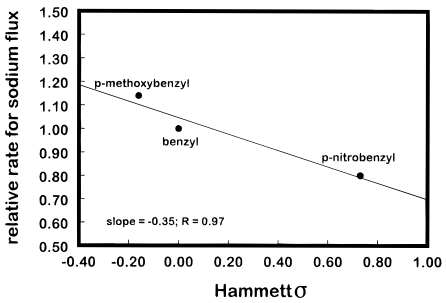


Figure 1. Hammett-type plot for **7**−**9**.

The Hammett Relationship. When the dodecyl side chains of **6** were exchanged for benzyl groups (**7**), a significant increase (∼1.4-fold) in Na^+ transport in the bilayer was observed. We have previously shown[18] that the central crown ring is not required to be in a tunnel conformation, and we have speculated that the crown serves as a head group stabilized by the attached benzyl group.[25] If the distal crowns serve as orifices in the membrane through which Na^+ passes, the transport rate should be altered by a change in electron density on the benzyl group. We thus prepared 4-methoxyphenyl (**8**) and 4-nitrophenyl (**9**) analogs of **7**. Their relative transport rates in the bulk $CHCl_3$ membrane were identical within experimental error. When studied in the phospholipid bilayer, however, the rates varied significantly and regularly as shown by the data in Table 2 which are graphed in Figure 1 *vs* the Hammett σ°.[26]

Benzyl channel **7** was, prior to this experiment, the most active of the compounds synthesized. Addition of a methoxy group to **7** increased activity (**8**) even further. The straight line apparent in Figure 1 ($r = 0.97$, slope $= -0.3$) shows that the variation in activity correlates strongly to the electronic effect. Whether this correlation relates to the ability of the distal crowns to function as head groups, relays, or both is currently unknown.

Conclusions. We report here the first series of physical organic chemical studies of a functioning cation channel model. The channel model compounds have a partition coefficient that favors the phospholipid bilayer over water by $\geq 10^{10}$ as judged from calculated log *P* values. Differences in transport rate therefore do not arise merely from differences in solubility. Second, the channel model does not function as a "supercarrier" whereby the cation is transported stoichiometrically as a host−guest complex. This conclusion is supported by a complete lack of coincidence between Na^+ transport rates in a phospholipid bilayer and in a bulk organic membrane. Finally, three channel model structures which differ only by the substituent attached to the terminal benzyl groups show an excellent correlation to Hammett parameters indicating clearly that the cation passes through the crown ether head group.

Acknowledgment. We thank the NIH for a grant (GM 36262) that supported this work.

Supporting Information Available: Experimental procedures and data (6 pages). See any current masthead page for ordering and Internet access instructions.

JA960950D

(21) The calculations take into account all atoms (including H). The "solvent condition" parameter was set to neutral and the "polar proximity" was calculated using a through space function $me^{(-nr)}$ where $m = 5$ and $n = 1$.

(22) (a) Riddell, F. G.; Hayer, M. K. *Biochim. Biophys. Acta* **1985**, *817*, 313−317. (b) Buster, D. C.; Hinton, J. F.; Millett, F. S.; Shungu, D. C. *Biophys. J.* **1988**, *53*, 145−152. (c) Riddell, F. G.; Arumugam, S.; Brophy, P. J.; Cox, B. G.; Payne, M. C. H.; Southon, T. E. *J. Am. Chem. Soc.* **1988**, *110*, 734−738. (d) Riddell, F. G.; Arumugam, S. *Biochim. Biophys. Acta* **1989**, *984*, 6−10. (e) Riddell, F. G.; Tompsett, S. J. *Biochim. Biophys. Acta* **1990**, *1024*, 193−197.

(23) Lamb, J. D.; Christensen, J. J.; Izatt, S. R.; Bedke, K.; Astin, J. S.; Izatt, R. M. *J. Am. Chem. Soc.* **1980**, *102* (10), 3399. (b) Lamb, J. D.; Izatt, R. M.; Garrick, D. G.; Bradshaw, J. S.; Christensen, J. J. *J. Membr. Sci.* **1981**, *9*, 83−107.

(24) Chloroform (6 mL) was used as the bulk membrane membrane. The source phase was aqueous sodium picrate (1 mM) in the presence of excess NaOH (100 mM). The receiving phase was initially distilled, deionized water. Aliquots were withdrawn at the specified intervals and analyzed by UV−visible spectroscopy.

(25) (a) Kumpf, R. A.; Dougherty, D. A. *Science* **1993**, *261*, 1708. (b) Dougherty, D. A. *Science* **1996**, *271*, 163−167.

(26) Taft, R. W. *J. Phys. Chem.* **1960**, *64*, 1805.