

## Transport of metal ions in opposite directions

Yohji Nakatsuji\* and Mitsuo Okahara

*Department of Applied Chemistry, Faculty of Engineering, Osaka University,  
Yamada-oka 2-1, Suita, Osaka 565, JAPAN*

**Abstract** A new type of bis(crown ether)s containing 15-crown-5 and monoaza-18-crown-6 actively transported  $\text{Na}^+$  and  $\text{K}^+$  in opposite directions across a dichloromethane membrane by pH control. On the other hand, some 18-crown-6 derivatives having two carboxylic acids on the side arms successfully transported  $\text{Ca}^{2+}$  and  $\text{K}^+$  in opposite directions. The effect of the structure of the ion carriers on their transport ability was examined. In both cases, the proper choice of transport conditions was found to be essential for the success of the double uphill transport.

### INTRODUCTION

The active transport of a variety of cations by synthetic ionophores across an artificial membrane is interesting for its potential as a model for a biological membrane (ref. 1). Crown ethers and the related open-chain analogues have been shown to be good carriers, especially for alkali metal and alkaline earth metal cations. In order to realize an active transport system, the carrier must change its complexing ability toward a specific cation between two interfaces. Recently, we found that crown ethers with an amino group were pH-controlled active carriers (ref. 2-6). For example, lipophilic monoazacrown ether transports a specific cation against its concentration gradient by pH control in a liquid membrane (ref. 4-6). The selectivity towards alkali metal cations is highly dependent on the ring size of the crown ether. Monoaza-15-crown-5 and -18-crown-6 ethers showed selectivity towards  $\text{Na}^+$  and  $\text{K}^+$ , respectively (ref. 4, 5). The enzyme  $\text{Na}^+$ - $\text{K}^+$  ATPase actively transports  $\text{Na}^+$  and  $\text{K}^+$  in opposite directions across a biological membrane. Simple lipophilic azacrown ethers, however, have no ability to transport a metal ion from an acidic to a basic phase. In order to mimic the function of an enzyme in an artificial transport system, another structural device is needed for the design of the ionophore. In addition,  $\text{Ca}^{2+}$ - $\text{Na}^+$  exchange actually occurs in the biological membrane. The control between an alkali metal ion (monovalent ion) and an alkaline earth metal ion (divalent ion) would require an alternate means. In this study, we describe the molecular design of novel host compounds, which are potentially useful as carriers in an active transport system, and the relation between the structure and the transport ability.

### CATION TRANSPORT STUDIES

Membrane transport experiments were carried out in a U-type cell (tube diameter: 16 mm; arm height: 150 mm) at 25°C (ref. 7, 8). Each cell consisted of a 20 mL membrane phase (dichloromethane containing a carrier, stirred at 500 rpm by a magnetic stirrer) interfaced to two 10 mL of aqueous phases. Both aqueous

phases were sampled after appropriate times and analyzed for cation concentration using a Nippon Jarrel-Ash AA-8500 atomic absorption spectrophotometer. Each experiment was repeated at least three times and the results are reported as the average of the three determinations. In the case of the active transport system, the data for transported cations (%) denote the mean of the increase in cations in one phase and the decrease in cations in the other phase.

### Na<sup>+</sup>-K<sup>+</sup> ATPase MODEL

Lipophilic monoaza-18-crown-6 can selectively transport K<sup>+</sup> from a basic to an acidic phase against its concentration gradient (ref. 4, 5). If a derivative containing monoaza-18-crown-6 possesses complexing ability toward Na<sup>+</sup> under acidic conditions, it has the possibility of transporting K<sup>+</sup> and Na<sup>+</sup> in opposite directions. From this point of view, we synthesized several bis(crown ether)s (1-6) and the corresponding lariat ether (7). The bis(crown ether)s (1-4) were prepared by the reactions of 2-(bromomethyl)-2-methyl-15-crown-5 (ref. 9, 10) and the appropriate azacrown ether derivatives (ref. 11, 12). Compound 5 was prepared by the reaction of hydroxymethyl 15-crown-5 (ref. 13) and the corresponding monoaza-15-crown-5 having a reactive bromomethyl group (ref. 14). Compounds 6 and 7 (lariat ether type) were also derived from 2-(bromomethyl)-2-methyl-15-crown-5 (ref. 15). The proposed structures were consistent with the data obtained from their IR, NMR, and mass spectra and combustion analyses.

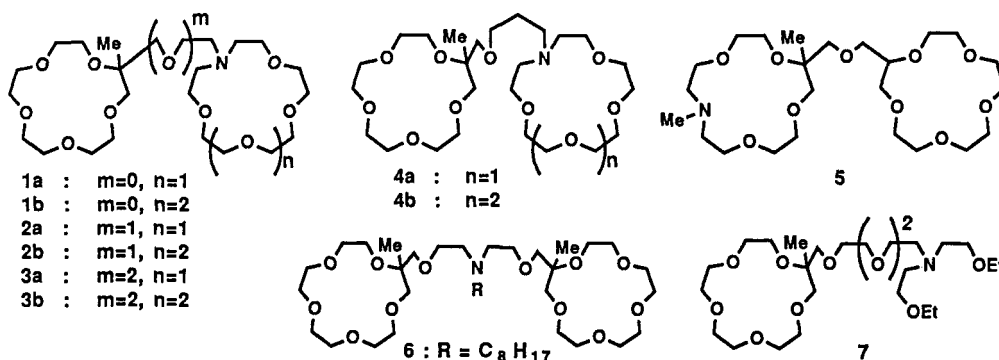


Table 1 shows the quantity of cations transported after 48 h in the active transport system. The bis(crown ether)s containing 15-crown-5 and monoaza-18-

Table 1. Active Transport Data for Na<sup>+</sup> and K<sup>+</sup> a)

Ionophore	Transported Cations (%) <sup>b)</sup>			
	Basic Phase		Acidic Phase	
	Na <sup>+</sup>	K <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>
1a	-	-	2	6
1b	-	-	6	12
2a	5	-	-	19
2b	10	-	-	31
3a	-	-	-	13
3b	13	-	-	25
4a	-	-	-	5
4b	7	-	-	24
5	-	-	5	9
6	5	-	-	-
7	5	-	-	3

a) Transport conditions: aq. phase 1 (10 mL), [PicK] = [PicNa] = 5 × 10<sup>-3</sup> M, [Me<sub>4</sub>NOH] = 0.1 M; org. phase (CH<sub>2</sub>Cl<sub>2</sub>, 20 mL), [Ionophore] = [PicH] = 1.25 × 10<sup>-4</sup> M; aq. phase 2 (10 mL), [PicK] = [PicNa] = [HCl] = 5 × 10<sup>-3</sup> M; 25°C. b) after 48 h.

crown-6 (**2b**, **3b**) actively transported  $\text{Na}^+$  and  $\text{K}^+$  in opposite directions across a dichloromethane membrane by pH control (ref. 8, 16). Ionophores **1a** and **1b** without an oxyethylene chain as the connecting group and **5** concentrated both  $\text{K}^+$  and  $\text{Na}^+$  in the acidic phase.

In the acidic phase, the ionophore is protonated and has to complex with  $\text{Na}^+$  in the form of an ammonium ion. This result clearly shows that an appropriate distance between the protonation site and the coordination site for  $\text{Na}^+$  should be needed. When the base was changed from tetramethylammonium hydroxide to lithium hydroxide in this transport system, the uphill transport in opposite directions disappeared. Both  $\text{K}^+$  and  $\text{Na}^+$  were concentrated in the acidic phase. At this point, the behavior of picrate anion is interesting. In this case, picrate anion was concentrated in the basic phase. On the other hand, when tetramethylammonium ion was used as the base, picrate anion was concentrated in the acidic phase. The control experiment without the ionophore also showed that picrate ion was concentrated in the acidic phase when tetramethylammonium ion was used. Of course, in this case, the transport of  $\text{K}^+$  and  $\text{Na}^+$  was not observed. These results suggest that the tetramethylammonium ion plays an important role in transporting the picrate ion from the basic to the acidic phase. The transfer of the tetramethylammonium ion from the basic to the acidic phase was determined by  $^1\text{H}$  NMR.

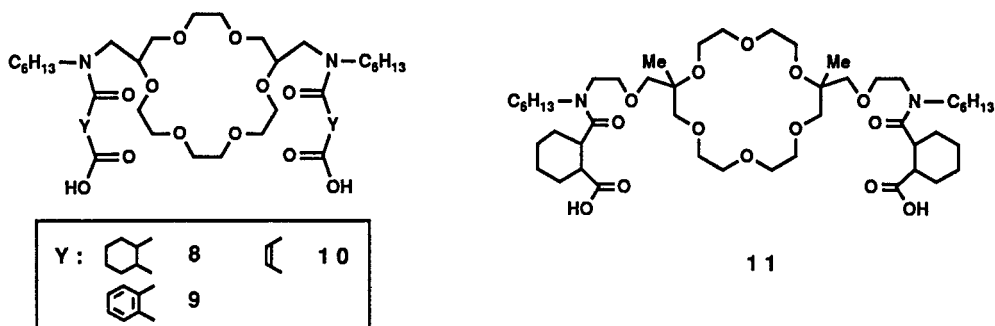
The proton concentrations in the acidic phase also affect the active transport. With an increase in the proton concentration, the transport of  $\text{Na}^+$  from the acidic to the basic phase remarkably decreased. This result suggests that the ionophore tends to transport only  $\text{H}^+$  without  $\text{Na}^+$  from the acidic to the basic phase when the proton concentration is high.

Although the transport mechanism is complicated, the results obtained in this study may be explained as follows. In the basic interface, the ionophore **3b** complexes with  $\text{K}^+$  rather than  $\text{Na}^+$  as expected by the extraction data (ref. 8). The ionophore transports  $\text{K}^+$  with a picrate ion across the liquid membrane to the acidic phase. In the acidic interface, the nitrogen atom of the ionophore is protonated to release  $\text{K}^+$ . After protonation, the ionophore still has the binding site, consisting of the 15-crown-5 ring and an electron-donating side-arm for  $\text{Na}^+$ . Thus, the ionophore can selectively uptake sodium picrate as a form of the ammonium salt from the acidic to the liquid membrane, and the resulting complex moves to the basic phase across the membrane. Consequently,  $\text{K}^+$  and  $\text{Na}^+$  are concentrated in the acidic phase and the basic phase, respectively. When this type of ionophore transports  $\text{Na}^+$  from the acidic to the basic phase, the ionophore demands two picrate ions. According to this cycle, picrate ions should be concentrated in the basic phase. However, tetramethylammonium ion independently transports picrate ion from the basic to the acidic phase. The higher concentration of the picrate ion in the acidic phase is advantageous for the uptake process of  $\text{Na}^+$  in the acidic phase.

#### TRANSPORT OF $\text{Ca}^{2+}$ AND $\text{K}^+$

The function of the enzyme  $\text{Na}^+$ - $\text{K}^+$  ATPase in the artificial system using a proton gradient as the driving force was successfully attained as previously mentioned.

In this case, a bis(crown ether) served as an effective carrier. Although the system using such types of ionophores may be applied to combinations of alkali metal cation pairs, the exchange between an alkali metal ion (monovalent ion) and an alkaline earth metal ion (divalent ion) would need an alternate device. We describe here an uphill transport of  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  in opposite directions (ref. 7, 15).



Compounds 8-10 were prepared by the reaction of 2,9-bis[(hexylamino)methyl]-18-crown-6 (ref. 17) with the corresponding carboxylic anhydride in THF at room temperature. Compound 11 was similarly derived from 2,12-bis(bromomethyl)-2,12-dimethyl-18-crown-6 (ref. 18).

The ionophores 8-11 selectively transported  $\text{Ca}^{2+}$  in the passive transport from the basic (pH 10.1) to the acidic phase (pH 2.0) when  $\text{CaCl}_2$  and  $\text{KCl}$  were used in the source phase (Runs 1-4). The transport velocity of  $\text{Ca}^{2+}$  was markedly

Table 2. Transport Data for  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  with Ionophores 8-11

Run	Ionophore	Initial Conditions <sup>a)</sup>						Transported Cations(%) <sup>d)</sup>			
		Phase 1		Phase 2		pH <sup>b)</sup>	Phase 1		Phase 2		
			pH <sup>b)</sup>					$\text{Ca}^{2+}$	$\text{K}^{+}$	$\text{Ca}^{2+}$	$\text{K}^{+}$
1	8	$\text{CaCl}_2$	$\text{KCl}$	10.1	-	-	2.0	-	-	30	4
2	9	$\text{CaCl}_2$	$\text{KCl}$	10.1	-	-	2.0	-	-	11	2
3	10	$\text{CaCl}_2$	$\text{KCl}$	10.1	-	-	2.0	-	-	3	1
4	11	$\text{CaCl}_2$	$\text{KCl}$	10.1	-	-	2.0	-	-	25	11
5	8	$\text{CaCl}_2$	$\text{PicK}$	10.1	-	-	2.0	-	-	41	13
6	11	$\text{CaCl}_2$	$\text{PicK}$	10.1	-	-	2.0	-	-	34	24
7	8	$\text{CaCl}_2$	$\text{KCl}$	2.0	-	-	2.0	-	-	<1	<1
8	11	$\text{CaCl}_2$	$\text{KCl}$	2.0	-	-	2.0	-	-	<1	<1
9	8	$\text{CaCl}_2$	$\text{PicK}$	2.0	-	-	2.0	-	-	<1	8
10	11	$\text{CaCl}_2$	$\text{PicK}$	2.0	-	-	2.0	-	-	<1	17
11	8	$\text{CaCl}_2$	$\text{PicK}$	6.7	-	-	2.0	-	-	4	29
12	11	$\text{CaCl}_2$	$\text{PicK}$	6.7	-	-	2.0	-	-	1	35
13	8	$\text{CaCl}_2$	$\text{PicK}$	10.1	-	-	6.7	-	-	21	4
14	11	$\text{CaCl}_2$	$\text{PicK}$	10.1	-	-	6.7	-	-	20	11
15	8	$\text{CaCl}_2$	$\text{KCl}$	10.1	$\text{CaCl}_2$	$\text{PicK}$	2.0	-	-	40	-
16	11	$\text{CaCl}_2$	$\text{KCl}$	10.1	$\text{CaCl}_2$	$\text{PicK}$	2.0	-	-	26	-
17 <sup>c)</sup>	8	$\text{CaCl}_2$	$\text{KCl}$	10.1	$\text{CaCl}_2$	$\text{PicK}$	2.0	-	6	22	-
18 <sup>c)</sup>	11	$\text{CaCl}_2$	$\text{KCl}$	10.1	$\text{CaCl}_2$	$\text{PicK}$	2.0	-	8	16	-
19	8	$\text{CaCl}_2$	$\text{KCl}$	10.1	$\text{CaCl}_2$	$\text{PicK}$	6.7	-	5	24	-
20	11	$\text{CaCl}_2$	$\text{KCl}$	10.1	$\text{CaCl}_2$	$\text{PicK}$	6.7	-	11	27	-

a)  $[\text{CaCl}_2]=[\text{KCl}]=[\text{PicK}]=0.01$  M; Phase 1 ( $\text{H}_2\text{O}$  10 mL) / Membrane ( $\text{CH}_2\text{Cl}_2$  20 mL),  $[\text{Ionophore}]=0.25$  mM / Phase 2 ( $\text{H}_2\text{O}$  10 mL) b)  $[\text{Tris}]=0.05$  M (pH = 10.1);  $[\text{HCl}]=0.01$  M (pH=2.0);  $[\text{Tris}]=0.05$  M,  $[\text{HCl}]=0.045$  M (pH=6.7) c)  $[(\text{CH}_3)_4\text{NCl}]=0.1$  M was added to Phase 1 d) after 48 h.

affected by the lipophilicity of the ionophores. Both cations, however, were scarcely transported during the passive transport from the acidic to the other acidic phase (Runs 7, 8). When picrate anion was added to the source phase,  $K^+/Ca^{2+}$  selective transport occurred (Runs 9, 10). This is reasonably explained by considering that the ionophore needs the presence of a lipophilic anion in the uptake process under acidic conditions. The addition of the picrate anion to the source phase during the transport from the basic to the acidic phase unfortunately increased the transport velocity of both  $Ca^{2+}$  and  $K^+$  (Runs 5, 6). In the case of a simple combination of the transport conditions of Runs 1 and 9, the uphill transport of  $Ca^{2+}$  and  $K^+$  in opposite directions did not occur (Run 15). Accordingly, to attain the uphill transport of  $Ca^{2+}$  and  $K^+$  in opposite directions, the concentration of the picrate ion in the basic phase should be lowered using another device. Since tetramethylammonium ion can transport the picrate ion as the salt across the dichloromethane membrane, tetramethylammonium chloride was added to the basic phase. The decrease in the amount of the picrate ion in the basic phase enabled the double uphill transport (Runs 17, 18). When two aqueous phases were arranged to be basic and neutral, the double uphill transport was also observed (Runs 19, 20). This is the reason why the passive transport velocity for  $K^+$  with ionophore 8 was remarkably raised by changing the uptake phase from acidic to neutral (compare Runs 9 and 11). To the best of our knowledge, this is the first example of the uphill transport of  $Ca^{2+}$  and  $K^+$  in opposite directions in a liquid membrane system.

#### Acknowledgment

The Ministry of Education, Science, and Culture of Japan is acknowledged for the support of the NMR (JEOL JNM-GX-400) facilities used in the present work at the Faculty of Engineering, Osaka University as well as for the partial support of this work through a Grant in Aid for Scientific Research on Priority Areas.

#### REFERENCES

1. M. Okahara and Y. Nakatsuji, *Top. Curr. Chem.*, **128**, 37 (1985).
2. Y. Nakatsuji, H. Kobayashi, and M. Okahara, *J. Chem. Soc., Chem. Commun.*, **1983**, 800.
3. Y. Nakatsuji, H. Kobayashi, and M. Okahara, *J. Org. Chem.*, **51**, 3789 (1986).
4. Y. Nakatsuji, M. Sakamoto, M. Okahara, and K. Matsushima, *Chem Express*, **1**, 431 (1986).
5. Y. Nakatsuji, M. Sakamoto, M. Okahara, and K. Matsushima, *Nippon Kagaku Kaishi*, **1987**, 430.
6. Y. Nakatsuji, R. Wakita, Y. Harada, and M. Okahara, *J. Org. Chem.*, **54**, 2988 (1989).
7. Y. Nakatsuji, T. Inoue, M. Wada, and M. Okahara, *J. Inclusion Phenomena*, **10**, 379 (1991).
8. M. Okahara, Y. Nakatsuji, M. Sakamoto, and M. Watanabe, *J. Inclusion Phenomena*, **12**, 199 (1992).
9. Y. Nakatsuji, T. Nakamura, M. Okahara, D.M. Dishong, and G.W. Gokel, *J. Org. Chem.*, **48**, 1237 (1983).
10. Y. Nakatsuji, T. Nakamura, T. Yonetani, H. Yuya, and M. Okahara, *J. Am. Chem. Soc.*, **110**, 531 (1988).
11. H. Maeda, S. Furuyoshi, Y. Nakatsuji, and M. Okahara, *Bull. Chem. Soc. Jpn.*, **56**, 212 (1983).
12. A. Masuyama, Y. Nakatsuji, I. Ikeda, and M. Okahara, *Tetrahedron Lett.*, **22**, 4665 (1981).
13. I. Ikeda, H. Emura, and M. Okahara, *Synthesis*, **1984**, 73.
14. R. Wakita, M. Tsubakihara, Y. Nakatsuji, and M. Okahara, *Synthesis*, **1990**, 1011.
15. Y. Nakatsuji, M. Wada, A. Ogawa, and M. Okahara, unpublished results.
16. Y. Nakatsuji, M. Sakamoto, and M. Okahara, *J. Chem. Soc., Chem. Commun.*, **1988**, 1101.
17. T. Kikui, H. Maeda, Y. Nakatsuji, and M. Okahara, *Synthesis*, **1983**, 185.
18. Y. Nakatsuji, T. Mori, and M. Okahara, *Tetrahedron Lett.*, **25**, 2171 (1984).