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Transport of Calcium and Potassium Ions in Opposite Directions in a Liquid Membrane System

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Abstract. A new type of 18-crown-6 derivative having two carboxylic acids on the side arms transported calcium and potassium ions in opposite directions across a dichloromethane membrane by pH control. Calcium and potassium ions were concentrated in the acidic and the basic phases, respectively. The presence of picrate ion in the acidic phase plays an important role in the transport of potassium ion from the acidic to the basic phase.

Key words. Crown ether, synthetic ionophore, liquid membrane, active transport.

1. 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 [1, 2]. Crown ethers and the related open-chain analogues have been shown to be good carriers, especially for alkali metal and alkaline earth metal cations [3]. In order to realize an active transport system, another structural device is needed for synthetic ionophores [1]. In other words, the carrier must change its complexing ability toward a specific cation between two interfaces. A proton gradient [4–14], oxidation-reduction reaction [15, 16], and light irradiation [17] are mainly used to control the complexing ability. Among them, proton-ionizable host compounds such as carboxylic-type ionophores, which mimic the function of natural ionophores [18], are well documented. On the other hand, we found that crown ethers with an amino group were an alternative type of pH-controlled active carriers [19, 20]. These carriers, however, can transport cations only in one direction.

The enzyme Na^+–K^+ ATPase actively transports Na^+ and K^+ in opposite directions across a biological membrane [21]. Recently we succeeded in mimicking the function of the enzyme in the artificial membrane system using a proton gradient as the driving force [22]. In this case, a bis(crown ether) (**1**) served as an effective carrier. Na^+–Ca^{2+} exchange, which actually occurs in the biological membrane, plays an important role in the maintenance of an organism *in vivo* [11, 23]. Although the system using **1** may be applied to combinations of alkali metal cation pairs, the control between an alkali metal ion (monovalent ion) and an alkaline earth metal ion (divalent ion) would require an alternate device. We describe here an uphill transport of Ca^{2+} and K^+ in opposite directions.

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2. Experimental

^{1}H NMR spectra were taken at 400 MHz on a JEOL JNM-GSX-400 spectrometer using tetramethylsilane as the internal standard. IR and UV spectra were obtained on a Hitachi 260-10 spectrometer and a Shimadzu UV-200 spectrophotometer, respectively. Mass spectra were measured with a Hitachi RMU-600 mass spectrometer. 11,14-Dioxa-7,18-diazatetracosane-9,16-diol (**3**) was prepared by the reaction of ethylene glycol diglycidyl ether with hexylamine according to the literature [24].

2.1. PREPARATION OF 4

N,N'-Dihexyl-1,4,7,10,13,16-hexaoxacyclooctadecane-2,9-dimethanamine (**4**) was prepared by the following sequence [24]. After potassium metal (5.87 g, 0.15 mol) was dissolved in *tert*-butyl alcohol (1000 mL) containing **3** (22.71 g, 0.055 mol), triethylene glycol ditosylate (22.93 g, 0.05 mol) in dioxane (60 mL) was added to the solution over a period of 2 h at 55°C. The mixture was stirred for another 12 h at that temperature. Insoluble matter was removed by filtration and the solvent was evaporated. Water (600 mL) was added to the residue and extracted with dichloromethane (500 mL × 3), dried over $MgSO_4$. The solvent was evaporated. A small portion of sodium carbonate was added to the residue and the mixture was distilled in a Kugelrohr apparatus (195°C/0.05 mm) to give a slightly yellow oil (13.5 g, 55%). ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, CH_3CH_2—), 1.1–1.7 (m, 16H, $CH_3(CH_2)_4CH_2NH$—), 2.1 (bs, 2H, —*NH*—), 2.1–2.9 (m, 8H, —CH_2NHCH_2—), 3.3–4.1 (m, 22H, —OCH_2—, —OCH—); IR (neat) 3310, 2940, 2860, 1450, 1350, 1120 cm^{-1}; MS *m/z* 489 ($M^+ - 1$, 9), 419(34), 202(74), 186(33), 144(56), 142(74), 114(100), 44(49).

Anal. Calcd. for $C_{26}H_{54}N_2O_6 \cdot 0.5\,H_2O$: C, 62.49; H, 11.09; N, 5.61. Found: C, 62.53; H, 10.96; N, 5.24.

2.2. PREPARATION OF 2

N,N'-Bis[(2-carboxyphenyl)carbonyl]-*N,N'*-dihexyl-1,4,7,10,13,16-hexaoxacyclooctadecane-2,9-dimethanamine (**2**) was prepared by the following sequence. A solution of **4** (0.98 g, 2 mmol) and phthalic anhydride (1.18 g, 8 mmol) in THF (10 mL) was stirred for 12 h at room temperature. After the solvent was evaporated off, the residue was dissolved in a small portion of dichloromethane. Insoluble matter was removed by filtration. The dichloromethane was evaporated off. The crude product was purified by silica-gel column chromatography ($MeOH/CHCl_3 = 0$–$1/3$) to give a slightly yellow waxy solid (0.82 g, 52%). ^{1}H NMR ($CDCl_3$ saturated with D_2O) δ 0.7–1.8 (m, 22H, $CH_3(CH_2)_4CH_2N$—), 2.8–4.2 (m, 30H, —CH_2NCH_2—, —OCH_2—, —OCH—), and 7.1–8.3 (m, 8H, aromatic); IR 3600–2400, 2920, 2860, 1720, 1610, 1280, 1120, 790, 740; FAB-MS *m/z* 809($(M + Na)^+$, 8), 787 ($M^+ + 1$, 3), 639(33), 491(53), 149(100), 114(74).

Anal. Calcd. for $C_{42}H_{62}N_2O_{12} \cdot H_2O$: C, 62.67; H, 8.01; N, 3.48. Found: C, 62.69; H, 8.38; N, 3.63.

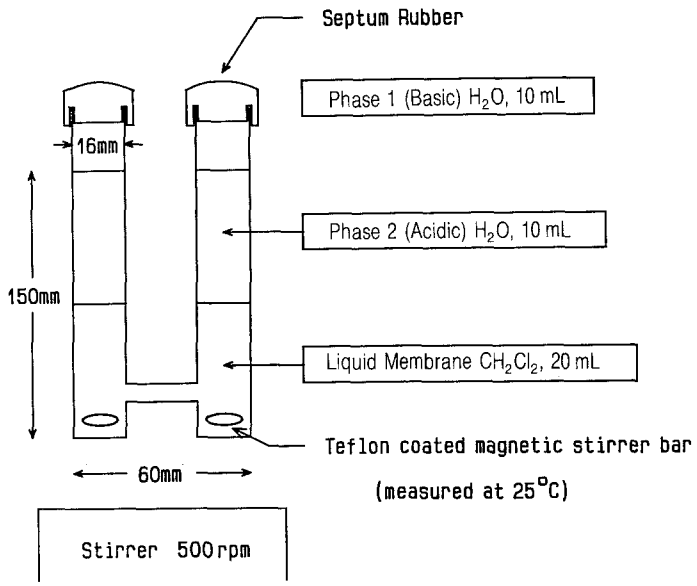


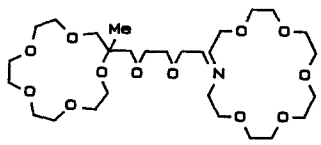
Fig. 1. Liquid membrane cell.

2.3. CATION TRANSPORT STUDIES

Transport experiments were carried out in a U-type cell as shown in Figure 1 at 25°C. A dichloromethane solution (20 mL) containing the ionophore was placed in the bottom of the cell and two portions of aqueous solutions (10 mL) were carefully added on top of them. Both surface areas were 2.0 cm^2. The organic phase was magnetically stirred at 500 rpm. The details of the transport conditions are summarized in the footnotes of Table I. Both aqueous phases were sampled after 48 h and analyzed for cation concentration using a Nippon Jarrel-Ash AA-8500 atomic absorption spectrophotometer. The concentrations of tetramethylammonium ion were determined by ^1H NMR. The concentrations of picrate anion were obtained by calculation based on the absorption at 354 nm in the UV spectrum. 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 ions (%) denote the mean of the increment of ions in one phase and the decrement of the ions in the other phase.

3. Results and Discussion

The structure of the ionophore (**2**) was designed on the basis of findings of Lehn *et al.*, who succeeded in regulating the Ca^{2+}/K^+ selectivity in a passive transport system [11]. The ionophore was prepared by the reaction of 2,9-bis(hexylaminomethyl)-18-crown-6 (**4**) [24] with phthalic anhydride in THF at room temperature. Its structure was confirmed by ^1H NMR and IR spectroscopy, mass spectrometry, and elemental analysis. Although this compound was obtained as a mixture of *syn* and *anti* isomers, the mixture was chemically pure.



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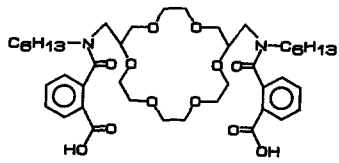

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Table I. Transport data[a] obtained with the ionophore (**2**).

	Initial Conditions				Transported Cations (%)[b]			
	Phase 1		Phase 2		Phase 1		Phase 2	
Run no.	Salt[c]	pH	Salt[c]	pH	Ca^{2+}	K^+	Ca^{2+}	K^+
1	$CaCl_2$, KCl	10.1	–	2.0	–	–	11	2
2	$CaCl_2$, PicK[d]	10.1	–	2.0	–	–	31	15
3	$CaCl_2$, KCl	2.0	–	2.0	–	–	<1	<1
4	$CaCl_2$, PicK	2.0	–	2.0	–	–	<1	7
5	$CaCl_2$, KCl	10.1	$CaCl_2$, PicK	2.0	–	–	41	2
6	$CaCl_2$, KCl, Me_4NCl[e]	10.1	$CaCl_2$, PicK	2.0	–	6	14	–
7	$CaCl_2$, KCl, Me_4NCl[f]	10.1	$CaCl_2$, PicK	2.0	–	8	12	–

[a] Standard transport conditions: Phase 1 (H_2O, 10 mL)/liquid membrane (dichloromethane, 20 mL), [ionophore] = 0.25 mM/Phase 2 (H_2O, 10 mL). The initial pH was adjusted to 10.1 or 2.0 by tris(hydroxymethyl)aminomethane (0.05 M) or HCl (0.01 M), respectively.

[b] After 48 h. The value (%) was the mean of three independent experiments. The deviations from the mean were less than ±15% of the value given.

[c] $[CaCl_2] = [KCl] = [PicK] = 0.01$ M.

[d] Potassium picrate.

[e] Tetramethylammonium chloride ($[Me_4NCl] = 0.1$ M) was added to Phase 1.

[f] Tetramethylammonium chloride ($[Me_4NCl] = 0.3$ M) was added to Phase 1.

Transport experiments were carried out in a U-type cell at 25°C (Figure 1). Dichloromethane was used as the liquid membrane. Both aqueous phases containing metal salts were adjusted to be basic and acidic by tris buffer and hydrochloric acid. The concentrations of cations, picrate ions, and protons were determined by atomic absorption analysis, UV spectroscopy, and pH titration, respectively. The detailed transport conditions and the results in the presence of Ca^{2+} and K^+ are summarized in Table I.

The ionophore (**2**) selectively transported Ca^{2+} in the passive transport from the basic (pH 10.1) to the acidic phase (pH 2.0) when $CaCl_2$ and KCl were used in the source phase (Run 1). Both cations, however, were scarcely transported in the passive transport from the acidic to the other acidic phase (Run 3). When picrate anion was added to the source phase, the K^+/Ca^{2+} selective transport occurred (Run 4). 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 in the transport from the basic to the acidic phase unfortunately increased the transport velocity of both Ca^{2+} and K^+ (Run 2).

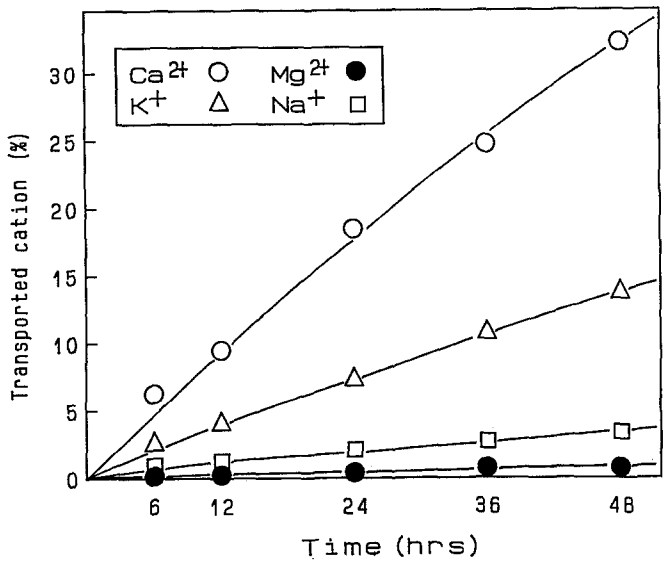

Fig. 2. Competitive passive transport from the basic to the acidic phase in the presence of Ca^{2+}, Mg^{2+}, K^+, and Na^+. Transport conditions: Phase 1 ($[CaCl_2] = [MgCl_2] = [PicK] = [PicNa] = 0.01$ M, [tris(hydroxymethyl)aminomethane] = 0.05 M)/membrane ([ionophore] = 0.25 mM)/Phase 2 ([HCl] = 0.01 M).

The selectivities between two alkali metal cations or between two alkaline earth metal cations are also interesting to estimate the ability of the ionophore. The passive transport data in the presence of Ca^{2+}, Mg^{2+}, K^+ and Na^+ from the basic to the acidic phase are shown in Figure 2. The quantity of metal cations transported increases in the order: $Mg^{2+} < Na^+ < K^+ < Ca^{2+}$. Ca^{2+} was selectively transported using ionophore **2** and Ca^{2+}/Mg^{2+} selectivity was clearly observed. The passive transport data in the presence of Ca^{2+}, Mg^{2+}, K^+, and Na^+ from the acidic to acidic phase are shown in Figure 3. In this case, K^+ was transported in preference to Na^+ as expected by considering the structure of the ionophore; alkaline earth metal ions were scarcely transported.

In the case of a simple combination of the transport conditions of Runs 1 and 4 (Table I), the uphill transport of Ca^{2+} and K^+ in opposite directions did not occur (Run 5). After 48 h, 39% of the picrate anion was transported from the acidic to the basic phase. In order to clarify a remarkable transfer of the picrate ion from the acidic to the basic phase without the increase of K^+ in the basic phase (Phase 2) in Run 5, the control experiment (Phase 1: $[CaCl_2] = [KCl] = 0.01$ M, [tris(hydroxymethyl)aminomethane] = 0.05 M; Phase 2: $[CaCl_2] = [PicK] = [HCl] = 0.01$ M) containing no ionophores was carried out. About 50% of the picrate ion moved from the acidic to the basic phase without the transfer of both Ca^{2+} and K^+ after 48 h. The amount of the picrate ion transported was almost coincident with the amount of protons consumed. These results show that the free picric acid is mainly responsible for the behavior of the picrate ion in the control experiment. To attain the uphill transport of Ca^{2+} and K^+ in opposite directions, another device is necessary because a gradual increase of the picrate anion in the basic phase should

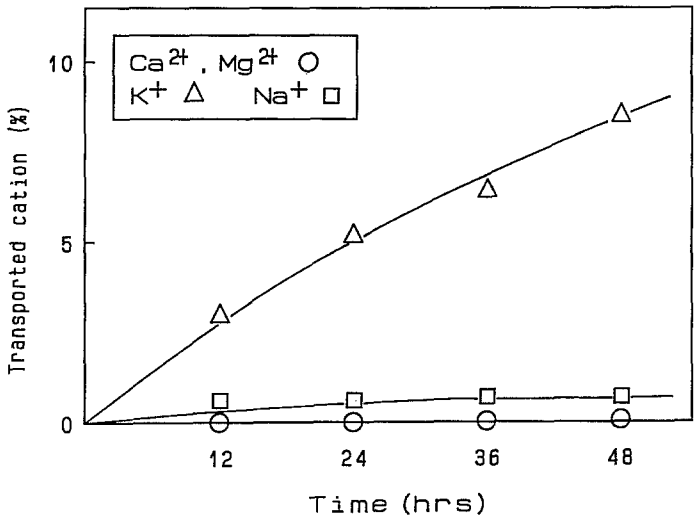

Fig. 3. Competitive passive transport from the acidic to the acidic phase in the presence of Ca^{2+}, Mg^{2+}, K^+, and Na^+. Transport conditions: Phase 1 ($[CaCl_2] = [MgCl_2] = [PicK] = [PicNa] = [HCl] = 0.01$ M)/membrane ([ionophore] = 0.25 mM)/Phase 2 ([HCl] = 0.01 M).

increase the amount of K^+ transported from the basic to the acidic phase, as shown in the passive transport experiment (Run 2). Since tetramethylammonium ion can transport the picrate ion as the salt across the dichloromethane membrane, tetramethylammonium chloride was added to the basic phase in order to decrease the concentration of the picrate ion (Runs 6 and 7). The amounts of the picrate ion concentrated in the basic phase in Runs 6 and 7 were 10% and 4%, respectively. Judging from the difference in the amounts of the picrate ion in the basic phase (Phase 1) between Runs 5 and 6 (or 7), at least 2.9×10^{-5} mol (Run 6) or 3.5×10^{-5} mol (Run 7) of the picrate ion should be transported in reverse from the basic to the acidic phase after 48 h. On the other hand, the amounts of tetramethylammonium ion transported from the basic to the acidic phase were determined with ^1H NMR to be 7.0×10^{-5} mol (Run 6) and 8.6×10^{-5} mol (Run 7). This result is reasonably explained by considering that the picrate ion was independently transported from the basic to the acidic phase by forming a lipophilic salt with the tetramethylammonium ion. The decrease in the amount of the picrate ion in the basic phase enabled the double uphill transport (Runs 6 and 7). Since the initial amount of Ca^{2+} (K^+) is 20 times that of the ionophore, more than 5% of the cations transported after 48 h strongly demonstrates that the ionophore repeatedly carried the cations. 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.

Although the mechanism is complicated, the phenomena observed in Runs 6 and 7 may be explained as follows (Figure 4). In the basic interface, the ionophore takes up Ca^{2+} rather than K^+ as the salt. The ionophore transports Ca^{2+} across the membrane to the acidic phase. In the acidic interface, the Ca^{2+} salt of the ionophore is protonated to give the free acid, which releases Ca^{2+}. When the ionophore is present as the free acid, the 18-crown-6 ring of the ionophore can

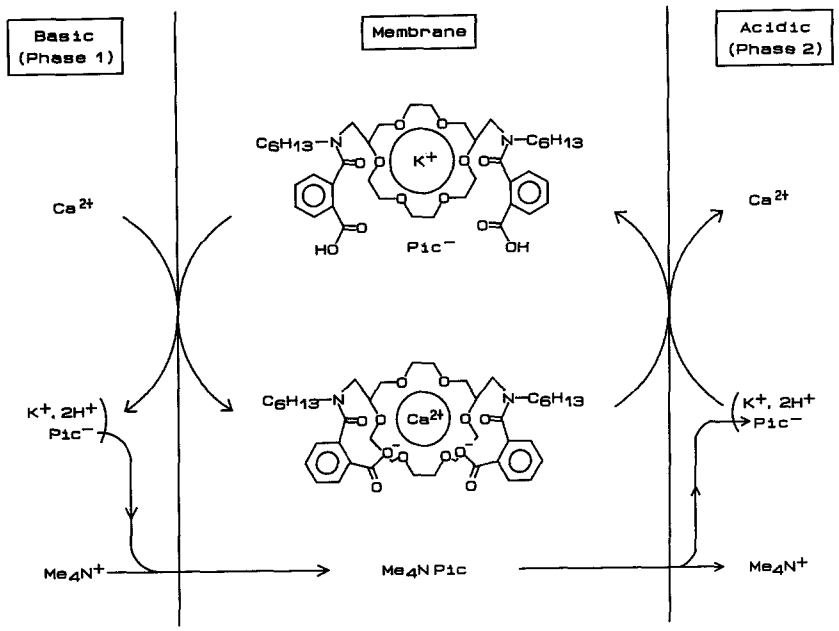

Fig. 4. Probable transport mechanism.

complex with K^+. In this case, the ionophore needs the assistance of the lipophilic anion (picrate ion) to transfer the cation from the aqueous phase to the organic phase. The ionophore transports potassium picrate from the acidic to the basic phase. The picrate anion should be gradually concentrated in the basic phase without the need for any devices. The accumulation of the picrate anion in the basic phase is considered to be a drawback because of the increase in the transport velocity of K^+ from the basic to the acidic phase. Conveniently, the tetramethylammonium ion can transport picrate ion as the salt from the basic to the acidic phase according to its concentration gradient, as mentioned above. Consequently, Ca^{2+} and K^+ are concentrated in the acidic phase and the basic phase, respectively.

4. Conclusion

The transport of Ca^{2+} and K^+ in opposite directions against their concentration gradients has been demonstrated for the first time in an artificial membrane system. A proper choice of transport conditions was clearly shown to be important, along with the molecular design of the ionophore for the success of the double uphill transport.

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