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A pH-dependent Exchange of Na^+ and Ca^{2+} in a Liquid Membrane System

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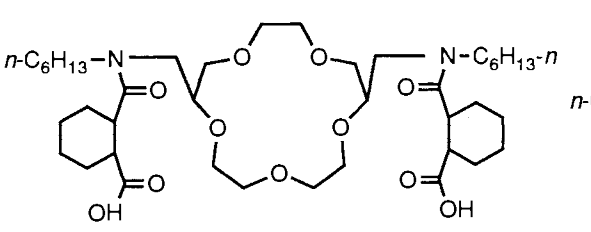
A pH-dependent exchange of Na^+ and Ca^{2+} against each concentration gradient through a bulk liquid membrane was first attained using 15-crown-5 ether dicarboxylic acid derivatives bearing donating ether oxygen atoms on the side arm as the carrier. The structure of the carriers was found to play an important role in realizing the double uphill transport.

The concentrations of a variety of ions in two aqueous phases separated by a biological membrane are strictly regulated to maintain organisms *in vivo*. Each metal ion is transported against its concentration gradient across the biomembrane in response to certain environmental changes. Thus, the active transport of metal cations by synthetic carriers across an artificial membrane is regarded as a model for a biological membrane.1-4) Recently, we realized the cation exchange function of the enzyme Na^+-K^+ ATPase in an artificial membrane system.5) The double uphill transport of K^+ and Ca^{2+} in opposite directions was also attained using an 18-crown-6 ether dicarboxylic acid (**4**).6) We will describe the first example of the Na^+-Ca^{2+} exchange7) by novel synthetic ionophores across an artificial liquid membrane.

The structure of the synthetic ionophore **1** was designed on the basis of the result obtained for the K^+-Ca^{2+} exchange.6, 8) This ionophore, however, disappointedly showed a poor transport ability toward Na^+ as mentioned below. So we also synthesized ionophores **2** and **3** by considering their complexation property toward Na^+. All structures were ascertained by ^1H NMR and IR spectroscopy, mass spectrometry, and elemental analysis.9) Ionophores **1** and **2** were obtained as a mixture of *syn* and *anti* isomers and ionophore **3** was obtained as a mixture of regional isomers.

Transport experiments were carried out in a U-type cell at 25 °C according to the literature.6) 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, respectively. The concentrations of cations and picrate ions were determined by atomic absorption analysis and UV spectroscopy, respectively. The detailed transport conditions and the results in the presence of Na^+ and Ca^{2+} are summarized in Table 1.



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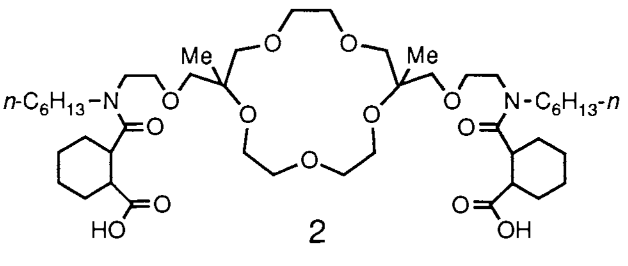

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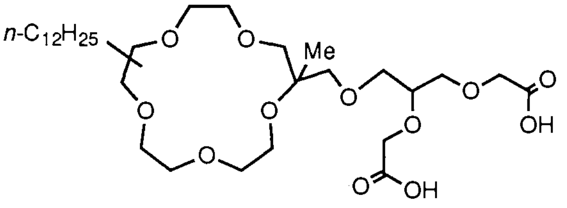

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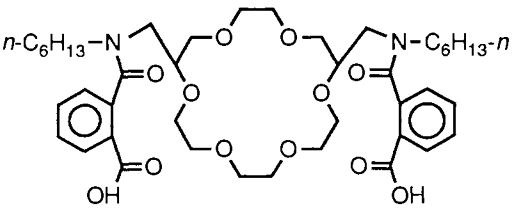

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Table 1. Transport Data for Na^+ and Ca^{2+} with Ionophores **1**-**3**

		Initial Conditions a)						Transported Cations / % d)			
		Phase 1			Phase 2			Phase 1		Phase 2	
Run	Ionophore			pH b)			pH b)	Ca^{2+}	Na^+	Ca^{2+}	Na^+
1	**1**	$CaCl_2$	NaCl	10	-	-	2	-	-	8	2
2	**2**	$CaCl_2$	NaCl	10	-	-	2	-	-	8	7
3	**3**	$CaCl_2$	NaCl	10	-	-	2	-	-	10	2
4	**1**	$CaCl_2$	PicNa	2	-	-	2	-	-	<1	3
5	**2**	$CaCl_2$	PicNa	2	-	-	2	-	-	1	12
6	**3**	$CaCl_2$	PicNa	2	-	-	2	-	-	1	18
7	**1** c)	$CaCl_2$	NaCl	10	$CaCl_2$	PicNa	2	-	2	22	-
8	**2** c)	$CaCl_2$	NaCl	10	$CaCl_2$	PicNa	2	-	7	16	-
9	**3** c)	$CaCl_2$	NaCl	10	$CaCl_2$	PicNa	2	-	8	20	-

a) PicNa denotes sodium picrate. $[CaCl_2]=[NaCl]=[PicNa]=0.01$ M; Phase 1 (H_2O, 10 mL) / Membrane (CH_2Cl_2, 20 mL), [Ionophore]=0.25 mM / Phase 2 (H_2O, 10 mL). b) [Tris]=0.05 M (pH = 10); [HCl]=0.01 M (pH=2). c) $[(CH_3)_4NCl]=0.1$ M was added to Phase 1. d) After 48 h.

The ionophore **1** selectively transported Ca^{2+} in the passive transport from the basic (pH 10) to the acidic phase (pH 2) when $CaCl_2$ and NaCl 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 in the absence of lipophilic anions such as picrate ions as observed in the case of the Ca^{2+}-K^+ exchange.[6)] When picrate anion was added to the source phase, the selective transport of Na^+ occurred (Run 4), but the extent of Na^+ ions transported (Na^+: 3%) was relatively small in comparison with the case of the Ca^{2+}-K^+ exchange (K^+: 7%).[6)] As a result, the transport of Na^+ from the acidic to the basic phase was rather difficult under active transport conditions (Run 7). These findings strongly suggest that the carrier demands a much higher complexing ability in this transport system. In order to strengthen the uptake property of the carrier towards Na^+ ion under acidic conditions, we introduced the concept of the "lariat ether" developed by Gokel *et al.*[10)] to the molecular design of new ionophores. The ether oxygen atoms of the side chain of the 15-crown-5 ether derivative bearing a methyl group at the pivot carbon was known to increase the complexing ability toward Na^+.[11)] As expected, ionophores **2** and **3** having donating ether oxygen atoms on the side arm, effectively transported Na^+ from the acidic to the other acidic phase in the passive transport experiment (Runs 5 and 6). These two ionophores clearly transported Na^+ and Ca^{2+} in opposite directions across the liquid membrane (Runs 8 and 9). During this experiment, Ca^{2+} was transported from the basic to the acidic phase by pH control and Na^+ was transported from the acidic to the basic phase according to the concentration gradient of picrate ions. The more than 5% of Ca^{2+}(Na^+) transported after 48 h clearly demonstrates that the ionophore repeatedly carried the cations since the initial amount of cations is 20 times that of the ionophore.

Table 2. Passive Transport Data for Na^+, K^+, Mg^{2+}, and Ca^{2+} with Ionophore **3**

Run	Initial Conditions[a)] Source Phase / pH[b)]	Initial Conditions[a)] Receiving Phase / pH[b)]	Transported Cations / %[c)] Ca^{2+}	Mg^{2+}	K^+	Na^+
10	10	2	47	1	7	9
11	2	2	1	<1	4	14

a) PicK and PicNa denote potassium picrate and sodium picrate, respectively. $[CaCl_2]=[MgCl_2]=[PicK]=[PicNa]=0.01$ M; Source Phase (H_2O, 10 mL) / Membrane (CH_2Cl_2, 20 mL), [Ionophore]=0.25 mM / Receiving Phase (H_2O, 10 mL). b) [Tris]=0.05 M (pH = 10); [HCl]=0.01 M (pH=2). c) After 48 h.

The selectivities between two alkali metal cations or between two alkaline earth metal cations are also interesting in order to estimate the ability of the ionophore. The passive transport data by ionophore **3** in the presence of Ca^{2+}, Mg^{2+}, K^+, and Na^+ are summarized in Table 2. The transport rate from the basic to the acidic phase showed that the quantity of metal cations transported increases in the order: $Mg^{2+} < K^+ < Na^+ < Ca^{2+}$ (Run 10). Ca^{2+} was selectively transported using ionophore **3** and Ca^{2+}/Mg^{2+} selectivity was clearly observed. In the passive transport in the presence of Ca^{2+}, Mg^{2+}, K^+, and Na^+ from the one acidic phase to the other, Na^+ was transported in preference to K^+ as expected by considering the 15-crown-5 ring structure; alkaline earth metal ions were scarcely transported (Run 11).

Further modification of transport conditions and the structure of ionophores to improve the transport efficiency are now in progress.

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9) Synthetic procedures for these new compounds will be published elsewhere. All compounds were purified by column chromatography on silica gel. Analytical data are as follows. Ionophore **1**: a slightly yellow liquid, ^1H NMR($CDCl_3+D_2O$) 0.88 (t, 6H), 1.1-2.5 (m, 32H), 2.6-2.9 (m, 4H), 2.95-3.95 (m, 26H). Anal. Found: C, 61.03; H, 9.31; N, 3.49%. Calcd for $C_{40}H_{70}O_{11}N_2 \cdot 2H_2O$; C, 60.73; H, 9.43; N, 3.54%. Ionophore **2**: a slightly yellow liquid, ^1H NMR($CDCl_3+D_2O$) 0.88 (t, 6H), 1.16 (s, 6H), 1.2-2.3 (m, 32H), 2.7-2.9 (m, 4H), 3.1-4.0 (m, 32H). Anal. Found: C, 61.89; H, 9.67; N, 3.21%. Calcd for $C_{46}H_{82}O_{13}N_2 \cdot 1H_2O$; C, 62.13; H, 9.52; N, 3.15%. Ionophore **3**: a colorless liquid, ^1H NMR($CDCl_3+D_2O$) 0.88 (t, 3H), 1.15 (s, 3H), 1.2-1.5 (m, 22H), 3.41-3.82 (m, 24H), 4.15 (s, 2H), 4.33 (s, 2H). A satisfactory elemental analysis was obtained as the form of dimethyl ester of **3**. Anal. Found: C, 60.88; H, 9.70%. Calcd for $C_{33}H_{62}O_{12}$; C, 60.90; H, 9.60%.
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