Active Transport of Sodium and Potassium Ions in Opposite Directions by a Novel Bis(crown ether)

Yohji Nakatsuji, Masayuki Sakamoto, and Mitsuo Okahara*

Department of Applied Chemistry, Faculty of Engineering, Osaka University, Yamada-oka, Suita, Osaka 565, Japan

Sodium and potassium cation were actively transported in opposite directions across a dichloromethane membrane by a novel bis(crown ether), which has a structure consisting of 15-crown-5 and monoaza-18-crown-6 linked by two oxyethylene units.

The enzyme (Na^+, K^+)-ATPase actively transports Na^+ and K^+ in opposite directions across a biological membrane.[1] Although active and selective transport in the same direction has been realized by using a variety of synthetic ionophores,[2] the function of (Na^+, K^+)-ATPase has never been simulated in an artificial system with a synthetic ionophore. Recently, Dugas *et al.*[3] attempted to mimic the function of the ATPase by using a crown ether with a primary amino group in the side chain, which is a modification of our transport system.[4] They succeeded in changing the transport selectivity towards Na^+ and K^+ in response to proton concentrations in the passive transport system but not in the active transport system.[3]

We now describe the first successful double active transport in a liquid membrane.

In order to realize the function of (Na^+, K^+)-ATPase, it is necessary to design an ionophore with different selectivity towards Na^+ and K^+ in both aqueous phases. Lipophilic monoaza-18-crown-6 seemed to be suitable as an active transport agent for K^+ because its complexing ability is decreased by protonation on the ring nitrogen atom under acidic conditions.[5] If a compound based on monoaza-18-crown-6 were to possess an additional co-ordination site for Na^+ in the acidic region, such an ionophore might well show double active transport. Methyl-substituted lariat ethers based on 15-crown-5 were considered to be promising candidates because of their Na^+/K^+ selectivity.[6] According to this consideration, the ionophore (**1**) was prepared by reaction of 2-(bromomethyl)-2-methyl-15-crown-5 [7] with *N*-[2-(2-hydroxyethoxy)ethyl]-monoaza-18-crown-6.[8] The structure was confirmed by ^1H n.m.r. and i.r. spectroscopy, mass spectrometry, and elemental analysis.†

The stability constants of (**1**), measured by ion-selective electrodes in methanol at 25 °C [log $K'(K^+)$ = 5.59; log $K'(Na^+)$ = 4.29], showed good K^+/Na^+ selectivity [$K'(K^+)/K'(Na^+)$ = 20].

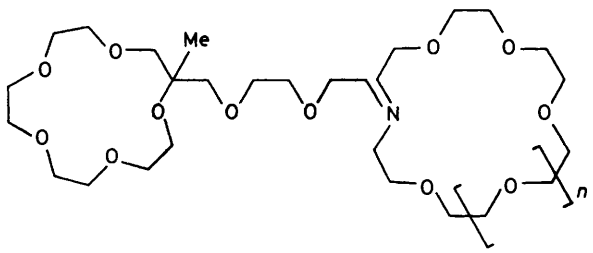
Transport data are summarized in Table 1. Proton concentrations in the acidic phase exert a marked effect on Na^+ transport from the acidic to the basic phase (runs 1—3). When

† *Selected spectroscopic data* for ionophore (**1**): ^1H n.m.r. ($CDCl_3$) δ 1.20 (s, 3H), 2.62—3.00 (m, 6H), and 3.40—3.92 (m, 46H); mass spec. *m/z* 597 (M^+, 0.8%), 277(16), 276(100), 232(9), 101(15), 99(8), 57(4), 45(18), and 43(7); i.r. (neat) 2770, 1460, 1300, 1250, and 1120 cm^{-1}; satisfactory elemental analyses were obtained.

Table 1. Transported data[a] obtained with the ionophore (**1**).

	Initial conditions						Transported cations (%)[b]			
	Phase 1 (basic)			Phase 2 (acidic)			Phase 1		Phase 2	
Run. no.	PicK[c]	PicNa[c]	$[Me_4NOH]$/M	PicK	PicNa	[HCl]/M	Na^+	K^+	Na^+	K^+
1	P[d]	A[d]	0.1	A	P	0.1	0			39
2	P	A	0.1	A	P	0.005	16			35
3	P	A	0.025	A	P	0.005	21			30
4	P	P	0.1	A	A	0.005			10	29
5	P	A	0.1	P	P	0.005	10	2		
6	P	P	0.1	P	P	0.1			28	41
7	P	P	0.1	P	P	0.005	8			25
8[e]	P	P	0.1	P	P	0.005			1	12

[a] Standard transport conditions: Phase 1 (H_2O, 10 ml)/liquid membrane (dichloromethane, 20 ml), [ionophore] = [picric acid] = 0.000125 M/Phase 2 (H_2O, 10 ml). [b] After 24 h. The value (%) was the mean of three independent experiments. The deviations from the mean were less than ±15% of the value given. [c] PicK and PicNa denote potassium picrate and sodium picrate, respectively. [PicK] = [PicNa] = 0.005 M. [d] P and A denote the presence and the absence of potassium picrate or sodium picrate (shown as the heading of the column). [e] Ionophore (**2**) used in place of ionophore (**1**).



(**1**) $n = 1$
(**2**) $n = 0$

the proton concentration is too high in the acidic phase, the transport of Na^+ is not detected in the basic phase (run 1). Passive transport from the basic to the acidic phase shows K^+ selectivity (run 4). On the other hand, passive transport in the opposite direction displays Na^+ selectivity, as expected (run 5). The total of cations transported from the basic to the acidic phase (run 4) is more than that from the acidic to the basic phase (run 5). This reflects the decrease in complexing ability of the ionophore on protonation of the ring nitrogen atom in the acidic phase. The proton concentration in the acidic phase is of importance again in active transport systems (runs 6 and 7). When the proton concentration is high (run 6), both Na^+ and K^+ are actively transported from the basic to the acidic phase. This result agrees with the result of run 1. Active transport in both directions is attained under the conditions of run 7.

Apart from the transport conditions, the structure of the ionophore is also important. The ionophore (**2**) does not concentrate Na^+ and K^+ in the different phases (run 8). Since the K^+/Na^+ selectivity of the ionophore (**2**), as indicated by stability constants [log $K'(K^+)$ = 4.33; log $K'(Na^+)$ = 4.06; $K'(K^+)/K'(Na^+)$ = 1.9], is lower than that of the ionophore (**1**), the transport selectivity of the former towards K^+ from Phase 1 to Phase 2 should be lower than that of the latter. This suggests that the transport of Na^+ from the acidic to the basic phase by the ionophore (**2**) does not compensate for the transport of Na^+ in the opposite direction.

Although the transport mechanism is complicated, the phenomenon observed in run 7 may be explained as follows. In the basic interface, the ionophore (**1**) complexes with K^+ rather than Na^+ as indicated by the stability constants. The ionophore (**1**) transports K^+ with a lipophilic anion (picrate) across the membrane to the acidic phase. In the acidic interface, the nitrogen atom of the ionophore is protonated to release K^+. After protonation, the ionophore still has the binding site, consisting of the 15-crown-5 ring and an electron-donating side-arm for Na^+. Two types of complex are considered as candidate for the main carrier of Na^+ from the acidic to the basic phase. One is the 1:1 complex of the ionophore (**1**) and sodium picrate. The other is the complex of the ammonium picrate of the ionophore (**1**) and sodium picrate. However, since a higher hydrogen ion concentration, which must increase the amount of the ammonium picrate of the ionophore (**1**), is not advantageous to Na^+ transport (runs 1 and 2), the contribution of the latter is presumably low. Of course, chloride may act as a counter anion for the complexes. Though the nature of the main carrier of Na^+ is uncertain, the protonation process must play an important role when the ionophore recognizes the cation. In any case, the result demonstrates the active transport of Na^+ from the acidic to the basic phase.

Studies of the modification of the transport conditions and the structure of the ionophore are now in progress, to clarify the transport mechanism.

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