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Calix[4]arene-Mediated Transport of Alkali Ions Across Synthetic Black Lipid Membranes (BLM)[1)]

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The calix[4]arene-mediated transport of alkali ions across black lipid membranes (BLM) of synthetic amphiphiles was examined by the charge pulse relaxation technique. Double-chain amphiphiles with cationic, anionic and nonionic head groups formed stable BLM's, and the transport parameters for alkali ions were determined. The calix[4]arene derivative displayed an enhanced transport rate for Na^+ ion across the synthetic BLM's, while the relative adsorption/desorption rate (β) of alkali ions was significantly affected by the surface charge of the BLM's.

The selective transport of ions across bilayer membranes is one of the most essential features of cellular functions. Liposomes and planar bilayer membranes (BLM's) serve as simplified experimental systems for selective transport, and ion transport across these membranes has, in fact, been facilitated in the presence of ionophores[2—4)] or channel-forming molecules.[5)] BLM's are particularly attractive for this purpose, since these systems can provide membrane-transport parameters of ion carriers which are not accessible using liposomes.[2,3a)] Unfortunately, the ionophores employed in these studies were largely restricted to naturally occurring antibiotics, such as valinomycin, and only a few studies have been made for synthetic carriers.[6)] This situation is strange, because synthetic ionophores have been extensively studied for ion transport in bulk- and supported-liquid membranes.[7,8)] The constituents of BLM's have also been limited to a narrow class of amphiphiles, such as monoglycerides or phospholipids. Thus, the interaction between the ionophores and bilayers, and concurrent regulation of the carrier-mediated transport process are largely unexplored. The molecular factors which determine the rate constants in the membrane/aqueous interphase are poory understood. In order to gain insights into the chemistry of membrane transport, a systematic study is undoubtedly required using synthetic ionophores combined with a variety of synthetic BLM's. We have shown that synthetic amphiphiles form stable BLM's, in which the transport of hydrophobic ions is affected by polyion-complex formation[9] as well as by polymerization.[10)] More recently, BLM's comprising amphiphiles having an ether linkage in the alkyl tail portion were found to display selective cation transport, without adding specific transport molecules.[11)]

In this paper, we describe the transport characteristics of alkali ions by a substituted calix[4]arene **1** doped in synthetic BLM's. Calixarenes represent one of the most sophisticated synthetic host molecules, and their molecular design, their receptor properties and construction of related supramolecular systems are topics of current concern.[12)] Hydrophobic branched octyl groups are introduced in **1**, so that **1** is molecularly solubilized and permeable in the hydrophobic interior of BLM's. The *p*-*t*-butylcalix[4]arene-mediated transport of Na^+ ion across BLM's of soybean phospholipids has recently been reported.[6b)] However, the use of such phospholipid mixtures hinders the elucidation of membrane-dependent carrier transport phenomena. In this study, we used double-chained synthetic amphiphiles with cationic, anionic, and nonionic head groups (**2**—**4**) as BLM constituents (Chart 1).

Experimental

The synthesis of **1** has been reported elsewhere.[13)] Amphiphiles were synthesized according to a previously reported procedure.[14)] BLM mesurements were made using a BLM-10A system (USI System Co., Japan).[10,11)] BLM's of **2**—**4** were prepared by brushing a hole (diameter; 0.85 mm) in a Teflon® plate immersed in 0.1 M ($M = mol\,dm^{-3}$) aqueous salts (NaCl, KCl, CsCl, and LiCl) at 25 °C with BLM components dissolved in a mixture of decane and $CHCl_3$ (4 : 1 by volume). Calix[4]arene **1** was dissolved in these lipid solutions at given molar ratios. Ion transport through the BLM's was studied by the charge-pulse technique,[2,3)] as previously described.[9,10)]

Results and Discussion

The permeation of ions across BLM's would proceed via the following four steps:[3,7a)] (1) the formation of a complex between the alkali metal ion M^+ and **1** at one side of the membrane surface; (2) translocation of the complex **1**/M^+ to the opposite side of the membrane; (3) desorption of the ion into the aqueous solution; and (4) back-transport of the free carrier molecule **1**. A schematic illustration of **1**-mediated ion transport across a synthetic BLM is depicted in Fig. 1. In the charge-pulse experiment,[3,9,10)] the membrane is charged up to an initial voltage by a 1-μs electrical pulse to a starting potential (V_m^0). At the end of the pulse, the external circuit is switched to virtually infinite resistance. The redistribution of ions within the membrane and ion transport across the

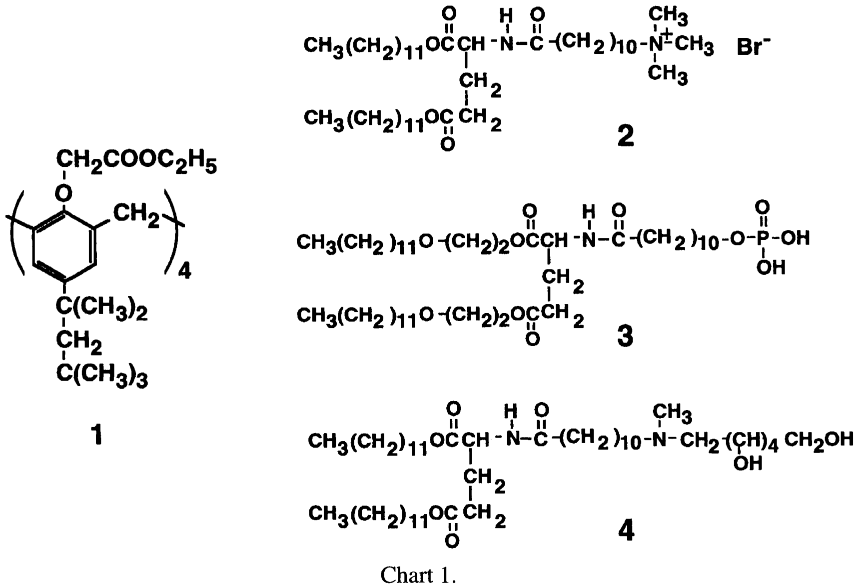

Chart 1.

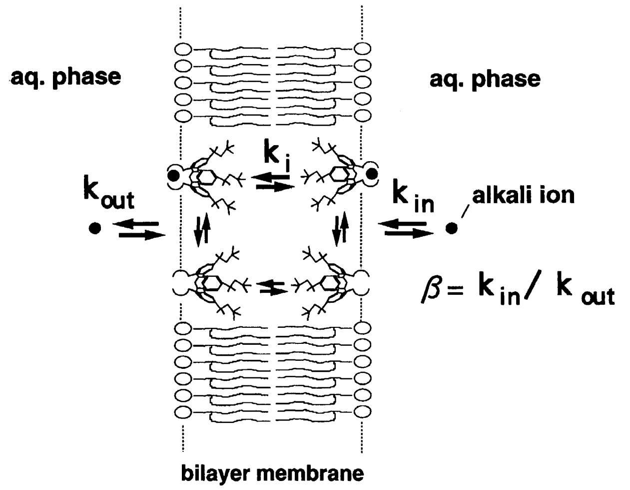

Fig. 1. Schematic illustration of alkali ion transport mediated by calix-[4]arene **1**.

membrane then lead to a decay of the voltage (V_m). The experimental set-up and an equivalent circuit diagram of the charge-pulse technique are given elsewhere.[3a,9,10] The decay ($V_m(t)$) is analysed by fitting it into following two relaxation processes: ($\tau_1 < \tau_2$),[3b]

$$V_m(t) = V_m^0\{a_1\exp(-t/\tau_1) + a_2\exp(-t/\tau_2)\}, \tag{1}$$

$$a_1 + a_2 = 1, \tag{2}$$

where τ_i and a_i are the relaxation times and amplitudes, respectively. The decay rate of V_m is related to the time course of the concentrations (N' and N'') of the permeable ion in the left-hand and right-hand potential minimum (N' and N'' are expressed in mol cm^{-2}). The rate change of N' and N'' is determined by the absorption rate from the aqueous phase to the membrane (rate constants k_{in}), the desorption rate (rate constant k_{out}), as well as by the rate of translocation across the central barrier (rate constant k_i). At equilibrium (V_m=0), the interfacial concentrations are equal and are given by the partition coefficient γ:[3b]

$$N' = N'' = N_t/2 \quad (V_m = 0) \tag{3}$$

$$N_t/2c = k_{in}/k_{out} = \gamma \cdot d/2 = \beta \tag{4}$$

N_t is the total equilibrium concentration of the permeable ion in the membrane and d is the membrane thickness; c is the concentration of the permeable ion in the aqueous phase (c=0.1 M). The rate constants (k_i) as well as N_t are calculated by:[3b]

$$\tau_1 = 1/2k_i(1 + bN_t), \tag{5}$$

$$b = z^2F^2/(4RTC_M), \tag{6}$$

$$a_1 = bN_t/(1 + bN_t), \tag{7}$$

Here, $1/b$ is the surface density of elementary charges that are needed to charge the membrane capacitance (C_M) to a voltage of $4RT/F$ ($1/b \approx 4\times10^{-13}$ mol cm^{-2} for $|z|=1$). z is the valency of the permeable ion, F the Faraday constant, R the gas constant, and T the absolute temperature.

The membrane capacitance C_M is measured by the voltage-jump method, and is given by[3a,9]

$$C_M = I_0T/V_0A, \tag{8}$$

where I_0 is the current extrapolated to zero time, T is the exponential decay time, V_0 is the applied voltage, and A is the effective membrane area.[15] Thus, the rate constant of translocation (K_i) and the relative rate constant of adsorption and desorption ($\beta = k_{in}/k_{out}$) can be evaluated from the time course of $V_m(t)$.[3b,9,10]

Stable BLM's are obtained from double-chained amphiphiles **2**—**4**. The membrane capacitance (C_M) obtained for the BLM's in the presence of various alkali ions are not affected by the alkali-ion species present (Li^+, Na^+, K^+, Cs^+):[11] **2**; 1.1 μF cm^{-2}, **3**; 0.3 μF cm^{-2}, **4**; 0.7 μF cm^{-2}. These C_M values are in the range of those reported for synthetic BLM's,[2,9—11] and are unaltered in the presence of **1**, at least up to a content of 2 mol %.[16] Since the C_M value is inversely proportional to the bilayer thickness,[2,3] it is apparent that the bilayer structures in these BLM's are not affected by the addition of **1**.

Figure 2 displays the dependence of the β and k_i values on the molar ratio [**1**]/[**2**] ([Na$^+$]=0.1 M, at 25 °C). As the molar composition of **1** in BLM **2** increased, the β value slightly increased from 2.0×10^{-8} to 2.9×10^{-8} cm. In contrast, k_i showed remarkable increases with a tendency to saturate above a [**1**]/[**2**] ratio of ca. 0.8×10^{-2}. At higher concentrations of **1**, its adsorption at the membrane surface probably increases the microviscosity of BLM **2**, resulting in the observed saturation behavior. Figure 3 summarizes the dependence of the β values on the kind of alkali ions in the BLM's **2**—**4**. Without calixarene **1** (open bars), the β values for amphiphiles **2** and **4** show rather small variations with cationic species. Phosphate BLM **3** gives smaller β values for the Li$^+$, K$^+$, Cs$^+$ ions. The larger β value (i.e., higher affinity) for Na$^+$ is a characteristic of the ether linkage-containing "ionophilic" BLM's (as described previously[11]). In the presence of **1** (filled bars, **1**: 1.25 mol%), BLM **3** and **4** showed remarkably enhanced β values for Na$^+$ ion, in accordance with the ion selectivity of calix[4]arenes.[12,13] Apparently, the observed accumuration of the Na$^+$ ion in these BLM's reflect their complexation with **1** at the membrane-solution interface, and this is facilitated when the matrix bilayer possesses anionic and nonionic surfaces. The increase in β for the Na$^+$ ion is less pronounced in the case of cationic BLM **2**, which points to less accumlation of cations as a result of an electrostatic repulsion with the cationic bilayer surface.

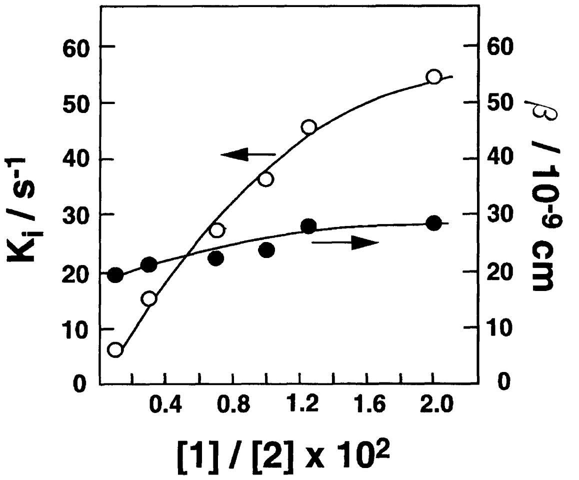

Fig. 2. Dependence of K_i and β values on the molar ratio of calix[4]arene **1** and BLM **2**.

Figure 4 displays the influence of the amphiphilic structures on the translocation rate constants (k_i). Without **1**, the k_i values of BLM **2** (k_i: ca. 6 s^{-1}) and **4** (k_i: 13—15 s^{-1}) are not dependent on the kind of alkali metal ions (open bars). Anionic BLM **3** alone showed enhanced k_i values for the Na$^+$ and K$^+$ ions, as reported previously.[11] On the other hand, in the presence of **1**, a remarkable enhancement in k_i is observed, especially for Na$^+$ in all of the BLM's **2**—**4** (filled bars, k_i: 46—50 s^{-1}). These similar k_i values observed for the Na$^+$ ion indicate that the translocation rate is limited by the microviscosity of these BLM's, which are comparable with each other. The k_i values are enhanced in the order Na$^+$>Cs$^+$≈K$^+$>Li$^+$. This tendency is also observed for the β value (Fig. 3), and is in good agreement with those reported for the solvent extraction of alkali ions from the aqueous to organic phases by calix[4]arenes.[8,13]

It is noteworthy that cationic BLM **2** showed a large k_i value for the Na$^+$ ion in the presence of **1**, in spite of its less enhanced β value (Fig. 3). As cationic species present at a high cation concentration (0.1 M) in the aqueous phase, their adsorption to one of the cationic bilayer surfaces after an applied electric pulse would not be an inefficient process. It is thus possible that the release of the Na$^+$ ion from translocated **1** at the cationic bilayer surface is more facilitated (larger k_{out} value) compared to at the anionic and nonionic bilayer surfaces; this situation leads to the smaller enhancement observed for β (=k_{in}/k_{out}). In addition, the relative transport rate, $k_i^{rel}=k_i(\mathrm{Na}^+)/k_i(\mathrm{K}^+)$, is larger for BLM **2** (k_i^{rel}=2.7) than those of BLM **3** and **4** (k_i^{rel}=ca. 1.7). This is due to the smaller

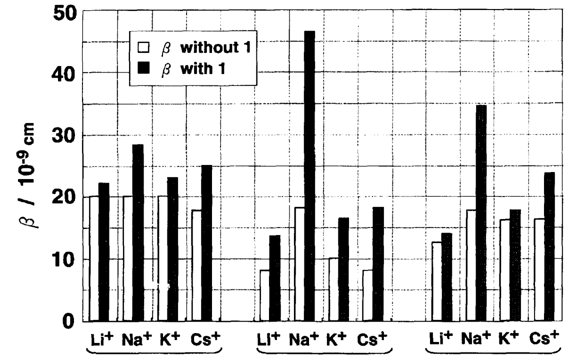

Fig. 3. Relative adsorption/desorption rate (β) for varied ion species. Dependence on amphiphilic structure and calixarene **1**. Open bar : without **1**, filled bar : in the presence of **1** (1.25 mol%).

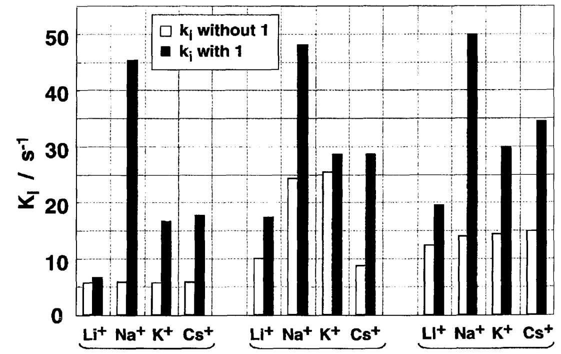

Fig. 4. Rate constant of translocation (k_i) for varied ion species. Dependence on amphiphilic structure and calixarene **1**. Open bar : without **1**, filled bar : in the presence of **1** (1.25 mol%).

$k_i(K^+)$ value obtained for BLM **2**. Similarly, the transport rates for Li^+ and Cs^+ ions across BLM **2** in the presence of **1** are smaller than those observed for BLM's **3** and **4**. We may assume that cation transport across the cationic BLM is a basically inefficient process; however in the presence of a suitable carrier molecule, transport can be facilitated. It is important to note that the elementary transport steps of a synthetic carrier molecule are significantly modified by the amphiphilic structure of the BLM's. The present results cleary indicate that synthetic BLM's constitute moleculary organized interfaces with varied surface charges, and that this characteristics is distinct from continuous bulk membranes or supported membranes in conventional synthetic-carrier-mediated ion transport.

Conclusion. We demonstrated that calix[4]arene derivative **1** displayed selective ion transport in synthetic BLM's. The surface charges of BLM significantly influence the relative adsorption/desorption rate constant (β) as well as the Na^+ to K^+ transport rate (k_i^{rel}). Regulation of the carrier function by using synthetic BLM has not been reported. It is clear that synthetic BLM's not only provide highly organized molecular matrices for carrier-translocation, but also affect the ion-complexation/release processes at the membrane interface. A suitable combination of synthetic carrier molecules and synthetic BLM's with varied surface structures would provide moleculary controlled supramolecular transport systems.

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15) In a typical measurement (BLM **2**, [CsCl]=0.1 M), parameters of $a_1=0.83$, $a_2=0.17$, $\tau_1=5.4\times10^{-3}$ s, $\tau_2=2.1\times10^{-2}$ s, $N_t=5.3\times10^{-12}$ mol cm^{-2} were obtained.

16) When **1** was added at the molar content of 5 mol %, stable BLM was not obtained.