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Calixarene-based photoresponsive ion carrier for the control of Na^+ flux across a lipid bilayer membrane by visible light

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Abstract

A photoresponsive ion carrier based on calix[4]arene was synthesized for the control of Na^+ flux across lipid bilayer membranes by visible light. Calix[4]arene was chosen as a basic skeleton of a photoresponsive ion carrier because its ether derivatives are known to act as Na^+ ion carriers in lipid bilayer membranes. For the synthesis of a photoresponsive carrier, dimethylaminoazobenzene was introduced as a photochromic moiety to an ether derivative of *p-tert*-butylcalix[4]arene. The ion transport ability of the dimethylaminoazobenzene appended calix[4]arene was examined by using a voltage-clamp method. When the calix[4]arene derivative was incorporated into a planar lipid bilayer membrane which separated two chambers filled with 100 mM of NaCl solutions, membrane currents resulting from Na^+ flux were observed under applying external voltages between two chambers. The concentration dependence of the calix[4]arene derivative on the membrane currents indicated the formation of a 1:1 complex with Na^+ ions for the calix[4]arene-mediated ion transport across the lipid bilayer membrane. By using visible light (> 400 nm), Na^+ flux across lipid bilayer membranes containing the dimethylaminoazobenzene appended calix[4]arene derivative could be controlled.

Keywords: Azobenzene; Calixarene; Carrier; Ion flux; Lipid bilayer membrane; Photocontrol

1. Introduction

Lipid bilayer membranes that respond to light are of great interest for the development of the artificial visual systems for use in optelectronic devices and optical transducers [1]. There are many studies of the photocontrol of ion transport across lipid bilayer membranes using photoresponsive compounds such as amphiphilic azobenzene [1-3] and spirobenzopyran derivatives [4, 5]. Most of the studies have used changes in membrane capacitance due to the disruption of lipid bilayer structures, which results from the photoisomerization of the compounds incorporated into the lipid bilayers [1-5]. Recently, several groups have reported photocontrollable ion transport systems, where channel [6, 7] or carrier molecules [8, 9] attaching photochromic compounds were incorporated into lipid bilayer membranes. In these systems, photochromic reaction has been conducted by irradiation of UV light (300-380 nm). However, the prolonged UV irradiation usually results in photodamage to photochromic compounds and lipid molecules [10]. In this paper, we report a visible light-driven ion transport system using a calix[4]arene-based photoresponsive ion carrier, where Na^+ flux across a lipid bilayer can be controlled by irradiation of light (> 400 nm).

Lipid bilayer membranes are impermeable to small ions. In addition, the membrane-forming materials (natural and synthetic lipids) are usually insensitive to light by themselves. Therefore, to control ion flux across lipid bilayer membranes, photoresponsive ionophoric compounds should be incorporated into the

membranes. In this study, calix[4]arene was chosen as a basic skeleton of a photoresponsive ion carrier because its ether derivatives are known to act as Na^+ ion carriers in lipid bilayer membranes [11, 12]. For the synthesis of a photoresponsive carrier, dimethylaminoazobenzene was introduced as a photochromic compound to an ether derivative of *p-tert*-butylcalix[4]arene [13]. Among azobenzene derivatives, dimethylaminoazobenzene has an intense absorption in the visible region, which is attributed to the □-□* transition of the *trans* isomer [14-17]. Hence, the photochemical *trans* to *cis* isomerization of dimethylaminoazobenzene can be undertaken by irradiation of visible light (Scheme 1). We expected that the dimethylaminoazobenzene appended calix[4]arene derivative mediates Na^+ ion transport across lipid bilayer membranes, and the ion transport ability can be controlled by irradiation of visible light. To assess the ion flux across lipid bilayer membranes, we measured membrane currents across a planar bilayer using a voltage-clamp method.

2. Experimental details

2.1 Materials

Tetrakis(ethoxycarbonylmethyl) ether of *p-tert*-butylcalix[4]arene (**1**) was purchased form Tokyo Organic Chemicals. 4-Dimethylaminoazobenzene-4'-sulfonyl chloride was purchased from Molecular probe Inc. Soybean phospholipids (Nakarai Tesque, Kyoto, Japan) were used for the formation of planar bilayer membranes.

2.2 Synthesis of a calix[4]arene-based photoresponsive carrier

Mono-hydrazide calix[4]arene derivative (**2**) was prepared from the reaction of hydrazine and calix[4]arene (**1**) at room temperature. The calix[4]arene (**3**) was synthesized through the reaction of the calix[4]arene (**2**) and 4-dimethylaminoazobenzene-4'-sulfonyl chloride in the presence of triethylamine in THF (Scheme 2). The calix[4]arene (**3**) was isolated by using a silica-gel column chromatography ($CHCl_3$/MeOH) and identified by ^1H NMR and MS spectra [18].

2.3 Ion transport across lipid bilayer membranes

A simplified diagram of an ion transport apparatus is shown in Fig. 1. Planar lipid bilayer membranes (soybean phospholipids) were formed at an aperture (0.2 mm diameter) in a Teflon film (12. 5 □m thickness) which separated two Teflon chambers (each volume is 1.5 mL with surface area of 1 cm^2) by the folding method [19]. An amplifier (CEZ-2300; Nihon Kohden, Ltd., Tokyo, Japan) was used in a voltage clamp mode to amplify the membrane currents and to control the voltages across the bilayer membrane. The command voltage (-100 ~+100 mV) was fed to one side of the chamber via an Ag/AgCl electrode through an agar bridge and the other side of chamber grounded via an Ag/AgCl electrode through an agar bridge. The visible light was irradiated to the □-□* transition band (around 440 nm) of the *trans* form of dimethylaminoazobenzene using a150 W Xe lamp through glass fibers (□ 450 nm or > 400 nm). All measurements were performed at 25 ℃.

2.4 Incorporation of photoresponsie calix[4]arene derivatives into planar bilayer membranes

After a planar lipid bilayer membrane was formed, an aliquot of DMSO solution (100 □M) of the calix[4]arene (**3**) was added to the left side of the chamber grounded under stirring. The membrane currents were measured after the currents reached at a stationary level (5-10 min later).

3. Results and discussion

Dimethylaminoazobenzene derivatives are unique photochromic compounds that respond to visible light. It has been shown that dimethylaminoazobenzene can undergo *trans-cis* isomerization as shown in Scheme 1 [14-17]. The *trans* form is thermodynamically more stable than the *cis* form. The *trans* form is converted into the *cis* form by visible light irradiation, and the resultant *cis* isomer can return to the original *trans* form thermally in the dark. In this study, dimethylaminoazobenzene was introduced as a photoresponsive moiety to an ether derivative of *p-tert*-butylcalix[4]arene as shown in Scheme 2. ^{1}H NMR spectra of aromatic protons of the ether derivative of calix[4]arene (**1**) and the dimethylaminoazobenzene appended calix[4]arene (**3**) are shown in Fig. 2. The calix[4]arene (**1**) gives only a single ^{1}H signal, while the calix[4]arene (**3**) gives four ^{1}H signals arising from the aromatic protons of its phenolic moieties. This signal pattern indicates the decrease in the molecular symmetry of the calix[4]arene from AAAA to AAAB or ABBB (A, B: phenolic unit). From the integrated intensity of the proton signals, the ratio of the aromatic protons between the dimethylaminoazobenzene and phenolic moiety of the calix[4]arene (**3**) is found to be 1:1(8H: 8H). This ^{1}H NMR data clearly supports mono-dimethylaminoazobenzene appended structure for the calix[4]arene (**3**) as shown in Scheme 2. Electron ionization mass spectrum of the calix[4]arene (**3**) gives a peak of 1222 (m/z) ascribed to $(M\text{-}N(CH_3)_2)^+$ and also supports the structure of the calix[4]arene (**3**) (Inset in Fig. 2).

The *trans-cis* isomerization in the dimethylaminoazobenzene appended calix[4]arene (**3**) was characterized by monitoring the absorption spectra. Fig. 3 shows the spectral change for the photochemical *trans* to *cis* isomerization and the thermal back reaction of the azobenzene moiety of calix[4]arene (**3**) in benzene. Upon irradiation of visible light (450 nm), the □-□* absorbance (λ_{max} = 440 nm) of the *trans* form decreased due to the photoisomerization of the *trans* to *cis* form, while the □-□* absorbance (λ_{max} = 380 nm) of the *cis* form increased. The absorption intensity (440 nm) of the *trans* form was decreased to be 60 % by the irradiation of visible light for 5 min. The absorption spectrum of the *trans* form completely recovered within 10 min in the dark. These results indicate that the *tran-cis* isomerization in calix[4]arene (**3**) can be controlled by switching on-off the visible light.

To examine the ion transport ability, the photoresponsive calix[4]arene derivative (**3**) was incorporated into a planar bilayer membrane and the membrane currents resulting form ion flux across the bilayer were measured using a voltage-clamp method [11,12, 20]. Fig. 4 shows the generation of membrane current resulting from Na^+ transport mediated by calix[4]arene (**3**), where two chambers are filled with 100 mM of NaCl (pH 7.2 adjusted by 25 mM HEPES-TRIS buffer). When an aliquot (20 □l) of DMSO solution (100 □M) of calix[4]arene (**3**) was added to the chamber under applying an external voltage of 100 mV, the membrane current gradually increased and reached at a stationary level after ca. 5 min. With increasing the concentration of calix[4]arene (**3**) in the chamber, the membrane current linearly increased (inset). The linear relationship between the membrane current and the concentration of calix[4]arene (**3**) indicates that the calix[4]arene (**3**) forms a 1:1 complex with Na^+ ions, and the Na^+ ions are transported as carrier complexes across the planar bilayer membrane [11].

The ion transporting selectivity of calix[4]arene (**3**) was evaluated by the measurements of reversal potentials under asymmetrical ionic conditions (100 mM salts) in two chambers. Reversal potentials (V_m) were obtained as -79 and -37 mV for the NaCl/LiCl and NaCl/KCl ionic conditions at 25 ℃, where 100 mM NaCl solution was filled in the grounded site of the chamber. The ratio of ion permeability (P_i) across

the lipid bilayer were calculated to be $P_{Li^+}/P_{Na^+} = 0.046$ and $P_{K^+}/P_{Na^+} = 0.24$ using the Goldman-Hodgkin-Katz equation [11,12]:

$$V_m = \frac{RT}{F} \ln \frac{P_{X^+}[X^+]}{P_{Na^+}[Na^+]} \quad (1)$$

where R, T and F express the gas constant, absolute temperature, and the Faraday constant, respectively. The values of the permeability ratio show that the flux of Na^+ ions is larger than that of Li^+ and K^+ ions by a factor of 20 and 4 fold, respectively. Thus the dimethylaminoazobenzene appended calix[4]arene (**3**) has a capacity of selective Na^+ transport across lipid bilayer membranes.

Fig.5 shows the effect of visible-light irradiation (> 400 nm) on the membrane currents at 50 mV across a planar bilayer membrane in the absence and in the presence of calix[4]arene (**3**). In the absence of calix[4]arene (**3**), the membrane current resulting from ion flux was not generated, and changes in the membrane current were not observed by irradiation of the visible light. When a 10 μl of DMSO solution (100 μM) of calix[4]arene (**3**) was added to the chamber, a 30 pA of the membrane current due to Na^+ flux was generated. The membrane current was significantly increased by a factor of ca. 30 % after irradiation of the visible light. When the light was turned off, the membrane current decreased to be the current level before light irradiation within a few minutes. It should be noted that the time-response of the changes in the membrane currents is the similar order to that of the changes in the absorption spectra of calix[4]arene (**3**) by visible-light irradiation as shown in Fig. 3. This finding suggests that the photoisomerization of calix[4]arene (**3**) in the planar lipid membrane causes the change in the membrane currents. Thus the increase in the membrane currents induced by visible-light irradiation can be attributed to the increase in the concentration of the *cis* form of calix[4]arene (**3**) in the lipid bilayer membrane. Since the molecular size of the *cis* form of calix[4]arene (**3**) is smaller than that of the *trans* form, the diffusion of sodium complex of the *cis* form through the lipid bilayer should be faster than that of the *trans* form. The difference in the diffusion rates between the sodium complexes of two isomers may result in a change in the Na^+ flux across the lipid bilayer.

4.Conclusion

A photoresponsive ion carrier based on calix[4]arene (**3**) has been synthesized for the control of Na^+ flux across lipid bilayer membranes by visible light. The photocontrollability of Na^+ flux using calix[4]arene (**3**) has been demonstrated by measurements of the membrane currents across planar lipid bilayer membranes. The photoresponsive ion carrier (**3**) would be also useful for the regulation of Na^+ concentrations in other lipid membrane systems such as liposomes. To the best of our knowledge, this is the first example of visible-light control of Na^+ flux across lipid bilayer membranes. The photoresponsive lipid bilayer membrane prepared in this study may be of interest as a simple artificial model of naturally occurring photo-excitable membranes containing photoreceptors.

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The author thanks Mr. E. Yamada (Faculty of Engineering, Hokkaido Univ.) for measurements of ^1H NMR spectra. This work was partly supported by the Ministry of Education, Science, Sport and Culture of Japan (Grant-in-Aid for Scientific Research c, No15550142).

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[18] ^{1}H NMR data for **3**: δ_H (400 MHz, $CDCl_3$), 0.82, 1.33 and 1.34 (s each, 18H, 9H and 9H, CMe_3), 1.20 (t, 9H, CH_2CH_3), 3.12 (s, 6H, NMe_2), 3.22 and 3.30 (d each, 4H, $ArCH_2Ar$, *J* 13.0 Hz), 4.14 (m, 6H, CH_2CH_3), 4.39 (dd, 4H, $COCH_2$, *J* 12.0 Hz), 4.48 (s, 2H, $COCH_2$), 4.54 and 4.55 (d each, 4H, $ArCH_2Ar$, *J* 13.0 Hz), 4.92 (s, 2H, $COCH_2$), 6.58 and 6.65 (d each, 4H, Ar-H, *J* 2.5 Hz), 7.17 and 7.21 (s each, 4H, Ar-H), 6.76, 7.91, 8.02 and 8.38 (d each, 8H, Ar-H in azobenzene moiety, *J* 8.8 Hz), 10.22 and 11.32 (s each, 2H, NH); EI-MS spectrum, *m/z* 1222 $(M\text{-}N(CH_3)_2)^+$.

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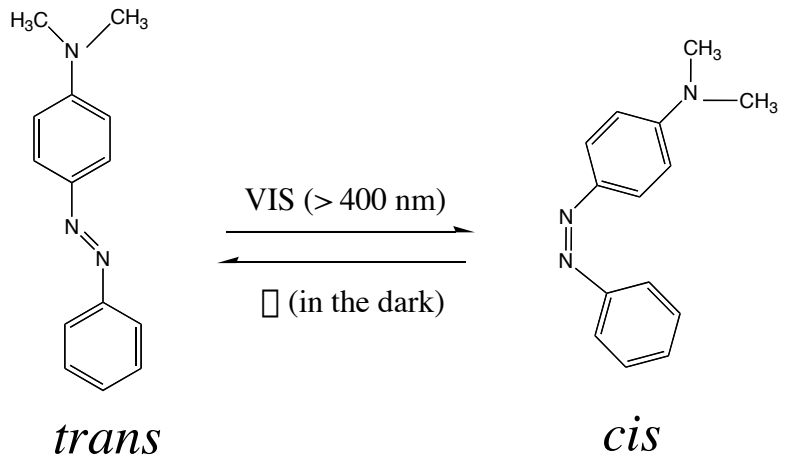
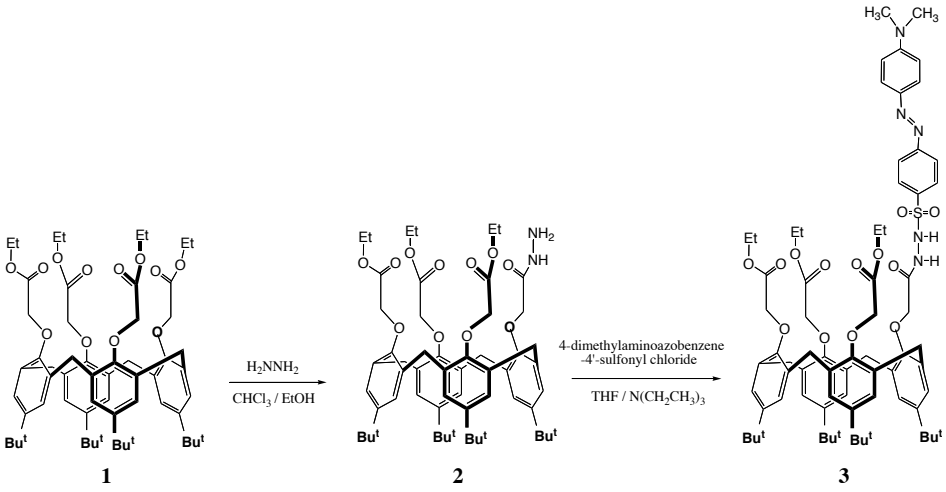
Schemes



Scheme 1. Photoisomerization of dimethylaminoazobenzene.



Scheme 2. Synthesis of a photoresponsive ion carrier based on calix[4]arene.

Figures

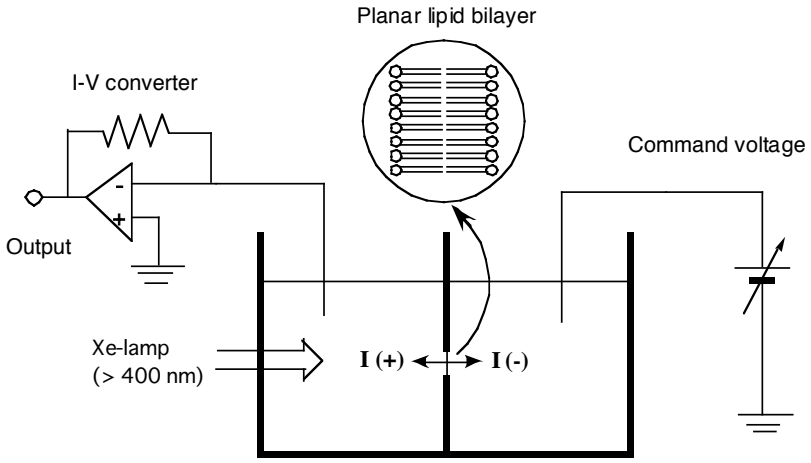


Fig. 1. A schematic diagram of an apparatus for ion transport experiments.

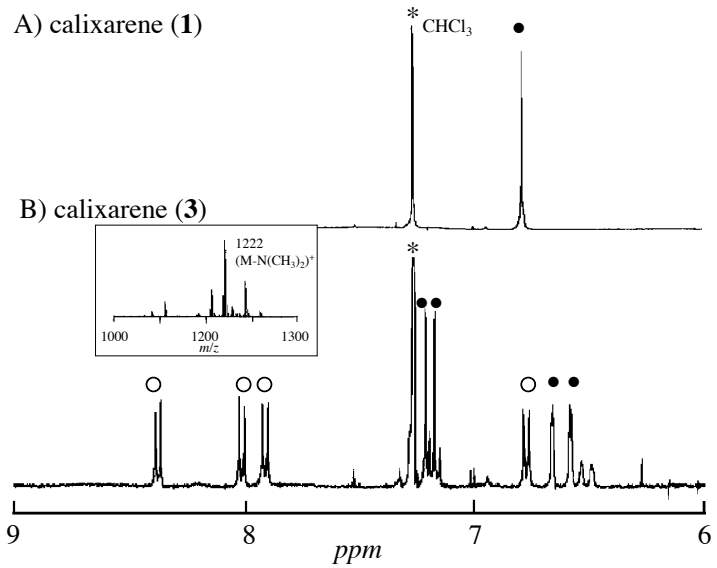


Fig. 2. ^{1}H NMR spectra of calix[4]arene (**1**) and calix[4]arene (**3**) in $CDCl_3$. Closed and open circles indicate the proton signals from phenolic and azobenzene moieties of the calix[4]arenes, respectively. Inset shows MS spectrum of the calix[4]arene (**3**).

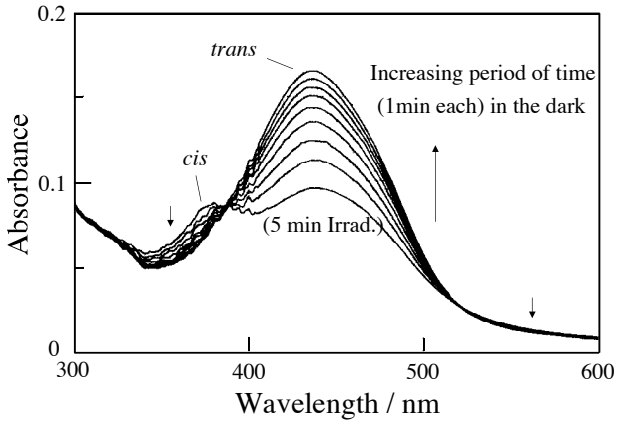


Fig. 3. Photochemical *trans* to *cis* isomerization induced by irradiation of visible light (450 nm) and the thermal back reaction in the dark.

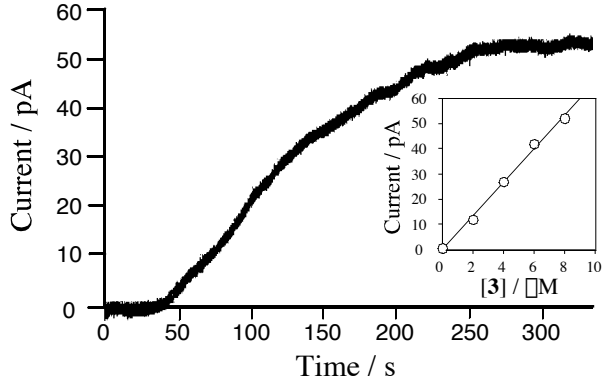


Fig. 4. Generation of membrane currents upon addition of calix[4]arene (**3**) under applying an external voltage of 100 mV, where two chambers were filled with 100 mM of NaCl aqueous solutions. Inset shows dependence of the membrane currents on the concentration of calix[4]arene (**3**) in the chamber.

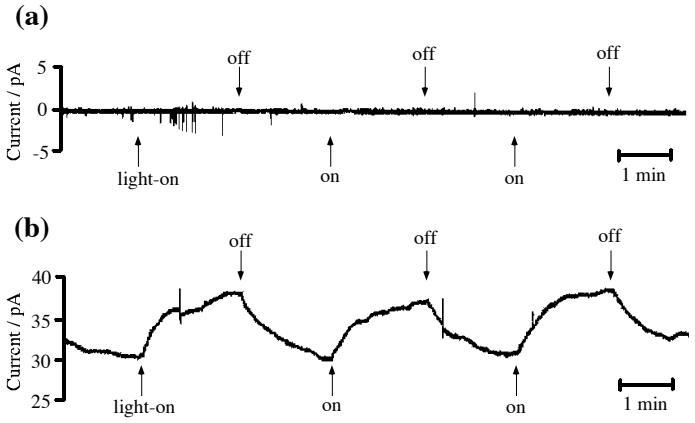


Fig. 5. Effects of visible light irradiation (> 400 nm) on the membrane currents at 50 mV in the absence (a) and in the presence (b) of calix[4]arene (**3**). Two chambers were filled with 100 mM of NaCl aqueous solutions.