

Photocontrol of Na⁺ transport across a phospholipid bilayer containing a bisanthrolycalix[4]arene carrier

Takashi Jin

Section of Intelligent Materials and Devices, Research Institute for Electronic Science, Hokkaido University, Sapporo 060-0812, Japan. E-mail: jin@imd.es.hokudai.ac.jp

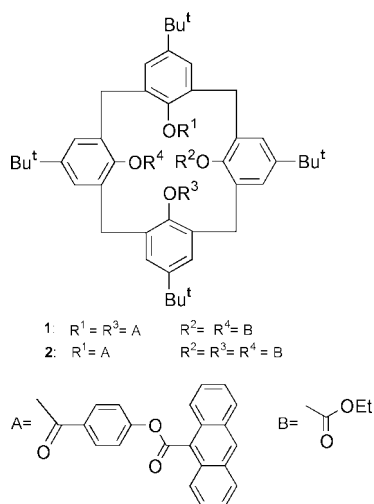
Received (in Cambridge, UK) 13th March 2000, Accepted 13th June 2000

Published on the Web 5th July 2000

Photocontrol of Na⁺ transport across a phospholipid bilayer membrane can be undertaken by using a new calix[4]arene carrier bearing two anthracene moieties.

Photoresponsive compounds are of great interest for the development of photocontrolled transport systems where ion and electron fluxes across membranes can be regulated by light.¹ Since a first report² of photoresponsive crown ethers, a number of the photocontrolled ion transport systems using bulky liquid membranes have been reported.³ However, there are very limited studies of photocontrol of ion fluxes across lipid bilayer membranes.⁴ The photoresponsive ionophores, by which ion fluxes across lipid bilayers can be controlled, are expected to be useful for the study of the response of cellular systems to ion concentrations. Here, we report the first example of photocontrol of Na⁺ transport across a phospholipid bilayer containing a bisanthrolycalix[4]arene carrier **1**.

Calix[4]arene esters are known to have selective complexing abilities⁵ toward Na⁺ ions, and act as selective Na⁺ carriers^{6–8} in lipid bilayer membranes. It has been shown that the rates of Na⁺ transport by a calix[4]arene ester across a phospholipid bilayer membrane are comparable to the rates by an antibiotic ionophore, monensin.⁶ To synthesize a photoresponsive calix[4]arene-based Na⁺ carrier, we introduced two photo-dimerizable anthracenes into the terminal positions (-OCH₂COR) of sodium binding sites in a calix[4]arene ester.⁹ Anthracene



appended calix[4]arenes **1** and **2** were prepared from the reaction of *p*-(9-anthroyloxy)phenacyl bromides (Molecular Prob. Inc.) and bis- or trisethoxycarbonylmethyl ether¹⁰ of *p*-*tert*-butylcalix[4]arene in the presence of K₂CO₃ in THF. The compounds were identified by ¹H NMR and field desorption mass spectroscopy.[†] The signal patterns in ArCH₂Ar protons confirmed that both **1** and **2** adopted cone conformations in CDCl₃. The Na⁺ complexing abilities of **1** and **2** were checked by the ¹H NMR titration experiments.⁵

Photoirradiations were carried out in THF at 25 °C using a 150 W Xe lamp through a band pass filter (λ 360 nm). As shown in Fig. 1(a), the UV irradiation of the solution of the bisanthrolycalix[4]arene **1** caused a decrease in the absorbance of anthracene moieties with two isosbestic points. However, the UV irradiation of the solution of the monoanthroly calix[4]arene **2** did not cause a significant spectral change (Fig. 1(b)). These results indicate that the photodimerization of the two anthracene moieties in **1** readily takes place in THF (Scheme 1), but not in **2**. The slight decrease in the absorption of **2** may be explained by the photobleaching of the anthracene moiety. In the case of **1**, the thermal decomposition of the photodimeric form was hardly observed even after 24 h in the dark, suggesting that the photodimeric form of **1** is very stable compared to the monomeric form of **1**.

The ion transport experiments were conducted by a planar bilayer membrane¹¹ (soybean phospholipid) with the aid of a voltage-clamp technique.[‡] The ion transport selectivity of **1** was evaluated by the measurements of the current–voltage relationships in the unsymmetrical ionic conditions of LiCl (*cis*)–NaCl (*trans*) and KCl (*cis*)–NaCl (*trans*), where the salt concentrations in two chambers were set to 100 mM containing 25 mM HEPES–Tris Buffer (pH 7.2). The reverse potentials were obtained as –80 and –37 mV for the LiCl–NaCl and KCl–NaCl systems at 25 °C. From the Goldman–Hodgkin–Katz equation,¹² the ion permeability ratios (P_{X^+}/P_{Na^+}) across the bilayer membrane were calculated as $P_{Li^+}/P_{Na^+} = 0.044$ and $P_{K^+}/P_{Na^+} = 0.24$, showing that **1** can act as a selective Na⁺ carrier in a phospholipid bilayer membrane.

Fig. 2 shows the effect of UV (> 310 nm) irradiation on the membrane currents resulting from Na⁺ transport by **1** and **2**. Upon UV irradiation of the bilayer membrane containing **1**, the Na⁺ currents (at 100 mV) immediately decreased (Fig. 2(a)), while UV irradiation of the membrane containing **2** did not cause any change (Fig. 2(b)). This behavior is consistent with the results of the spectral change of **1** and **2** in THF by UV irradiation. In the case of **1**, it should be noted that the Na⁺ currents are going to recover after the light-off, which can be explained by the increase in the concentration of the monomeric

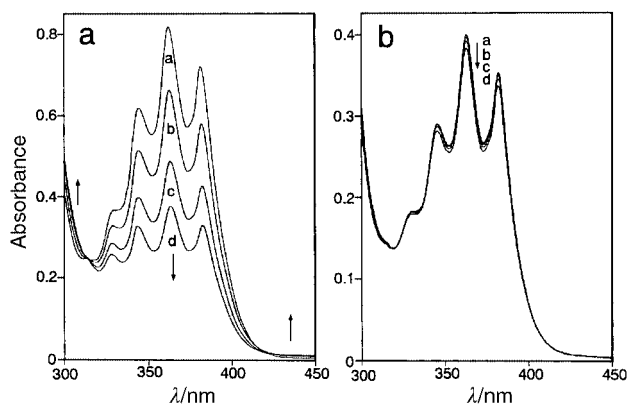
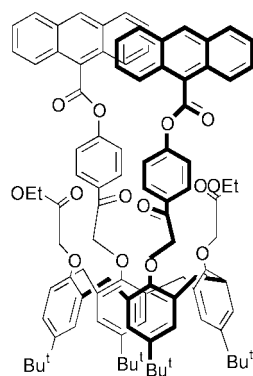
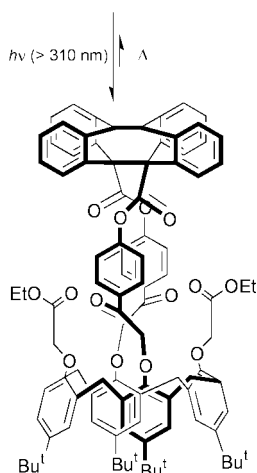


Fig. 1 The absorption spectra of **1** (a) and **2** (b) in THF before and after UV irradiation (360 nm): (a) 0 s; (b) 30 s; (c) 1 min; (d) 5 min.



Monomeric form



Photodimeric form

Scheme 1

form of **1** in the membrane region at the aperture in a Teflon film. This may be due to the lateral diffusion of the monomeric form of **1** from the membrane which is not irradiated by light. In fact, after a long irradiation time (*ca.* 30 min), the membrane current decreased to be the current (0.5 pA) of a control level. The increase in Na⁺ currents, which result from the thermal

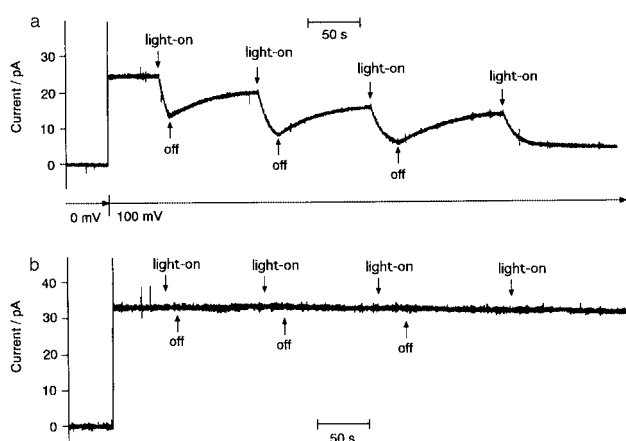


Fig. 2 The changes in Na⁺ currents across the lipid bilayer membrane containing **1** (a) or **2** (b) at 100 mV by UV irradiation (> 310 nm). Two chambers were filled with 100 mM NaCl aq. solutions (pH 7.2, 25 mM HEPES–Tris buffer). The bilayer membranes were prepared by the lipid–calix[4]arene mixtures (lipid: **1** or **2** = 100: 1 w/w). The UV irradiation (3.2 mW mm⁻²) was performed through a glass fiber, where the spot of the light was *ca.* 5 mm diameter.

decomposition of the dimeric form of **1**, was not observed within 1 h. These results indicate that the photodimerization of two anthracene moieties in **1** takes place in the lipid membrane and the Na⁺ transport activity diminishes in the photodimeric form of **1**.

In conclusion, we have demonstrated that photocontrol of Na⁺ fluxes across a bilayer membrane can be undertaken by using a bisanthrocalix[4]arene **1**. This is the first report of a photoresponsive Na⁺ carrier which is active in a phospholipid bilayer. We believe that the new calix[4]arene **1** has potential for use as a photoresponsive Na⁺ carrier for the regulation of Na⁺ concentrations in biological membrane systems.

The author thanks Dr G. Nishimura and Mr T. Ohta for technical support. The author also thanks Mr E. Yamada for the measurements of ¹H NMR spectra.

Notes and references

† Selected data for **1**: δ_H(400 MHz, CDCl₃), 1.06 and 1.24 (s, 18H, CMe₃), 1.30 (t, 6H, CH₂CH₃), 3.32 (d, 4H, ArCH₂Ar, *J* 13.0), 4.19 (q, 4H, CH₂CH₃), 4.87 (s, 4H, OCH₂CO₂), 5.09 (d, 4H, ArCH₂Ar, *J* 13.0), 5.83 (s, 4H, OCH₂COPh), 6.76 and 7.00 (s, 4H, ArH), 7.36–8.49 (26H, COPh and anthracene); field desorption mass spectrum, *m/z* 1497 (M⁺).

‡ Planar bilayer membranes were formed at an aperture (diameter, 0.2 mm) in a Teflon film (thickness, 12.5 μm) which separated two Teflon chambers (internal volume of each chamber is 1.7 ml with surface area of 1 cm²). The side to which compounds were added was defined as the 'cis' chamber and the opposite side was the 'trans' chamber. A patch clamp amplifier (CEZ-2300; Nihon Kohden, Ltd., Tokyo, Japan) was used in a voltage clamp mode to amplify the currents and to control the voltages across the bilayer membranes. The command voltage was fed to the trans chamber via an Ag/AgCl electrode through an agar bridge and the cis chamber was grounded via an Ag/AgCl electrode through an agar bridge. The voltage was referenced to the cis side with respect to the trans side.

- 1 J.-M. Lehn, *Supramolecular Chemistry—Concepts and Perspectives*, VCH, Weinheim, 1995.
- 2 S. Shinkai, T. Nakaji, Y. Nishida, T. Ogawa and O. Manabe, *J. Am. Chem. Soc.*, 1980, **102**, 5860.
- 3 See for example: S. Shinaki, T. Nakaji, T. Ogawa, K. Shigematsu and O. Manabe, *J. Am. Chem. Soc.*, 1981, **103**, 111; M. Irie and M. Kato, *J. Am. Chem. Soc.*, 1985, **107**, 1024; S. Shinkai, T. Ogawa, K. Shigematsu and O. Manabe, *J. Chem. Soc., Perkin Trans. 1*, 1987, 449; M. Sato, T. Kinoshita, A. Takizawa and Y. Tsujita, *Polym. J. (Tokyo)*, 1989, **21**, 369; M. G. Kodzwa and D. G. Rethwisch, *Polym. Mater. Sci. Engl.*, 1997, **76**, 279.
- 4 K. Kano, Y. Tanaka, T. Ogawa, M. Shimomura, Y. Okahata and T. Kunitake, *Chem. Lett.*, 1980, 421; J. Sunamoto, K. Iwamoto, Y. Mohri and K. Kominato, *J. Am. Chem. Soc.*, 1982, **104**, 5502; H. Nakanishi and H. Yamaguchi, *Kagaku Kogyo*, 1992, **43**, 198; M. Tanaka and Y. Yonezawa, *J. Phys. Chem.*, 1996, **100**, 5160; Y. Lei and J. K. Hurst, *Langmuir*, 1999, **15**, 3424.
- 5 F. Arnaud-Neu, E. M. Collins, M. Deasy, G. Ferguson, S. J. Harris, B. Kaitner, A. J. Lough, M. A. McKervey, E. Marques, B. L. Ruhl, M. J. Schwing-Weill and E. M. Seward, *J. Am. Chem. Soc.*, 1989, **111**, 8681; T. Jin and K. Ichikawa, *J. Phys. Chem.*, 1991, **95**, 2601; T. Jin, *Phys. Chem. Chem. Phys.*, 2000, **2**, 1401.
- 6 T. Jin, K. Kinjo, T. Koyama, Y. Kobayashi and H. Hirata, *Langmuir*, 1996, **12**, 2684.
- 7 N. Kimizuka, T. Wakiyama, A. Yanai, S. Shinkai and T. Kunitake, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 3681.
- 8 T. Jin, M. Kinjo, Y. Kobayashi and H. Hirata, *J. Chem. Soc., Faraday Trans.*, 1998, **94**, 3135.
- 9 A similar design of ionophoric calix[4]arenes for the photocontrol of metal-binding activity has been reported: G. Gang, T. Sasaki, K. Nakashima and S. Shinkai, *Chem. Lett.*, 1992, 1287; G. Gang, T. Sasaki, Y. Kawahara and S. Shinkai, *Tetrahedron. Lett.*, 1992, **33**, 2163; T. Tsudera, A. Ikeda and S. Shinkai, *Tetrahedron*, 1997, **53**, 13609.
- 10 K. Iwamoto and S. Shinkai, *J. Org. Chem.*, 1992, **57**, 7066.
- 11 M. Montal and P. Mueller, *Proc. Natl. Acad. Sci. USA*, 1972, **69**, 3561.
- 12 *Membrane Transport*, ed. S. L. Bonting and J. J. H. M. de Pont, Elsevier, New York, 1981.