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A Selective Fluorometric Sensing System for Guanidinium Ion in the Presence of Primary Ammonium Ions

Michinori TAKESHITA and Seiji SHINKAI*
Chemirecognics Project, ERATO, Research Development Corporation of Japan,
Aikawa 2432-3, Kurume, Fukuoka 830

Pyrene functionalized calix[6]arene selectively recognizes guanidinium ion in the presence of primary ammonium ions, the recognition process being detected by its intramolecular excimer fluorescence change: this is the first selective 'reading-out' system for guanidinium ion.

Guanidinium and their derivatives play important roles in biological systems.1) Therefore there are a few attempts on the development of a sensing system for recognition of guanidinium ion using crown ethers2) and torands.3) However, the recognition methods are hampered by coexistent primary ammonium ions. This flaw particularly becomes serious in *in vivo* sensing of guanidinium ion. Recently, Reinhoudt, Ungaro and their co-workers carried out the selective extraction of guanidinium ion in the presence of alkali metal cations by use of C_3-symmetric calix[6]arenes.4,5) Also Reinhoudt group reported that guanidinium ion can be read-out by CHEMFETs.6) However, selective recognition of guanidinium ion in the presence of primary ammonium ions has not been reported yet. Previously, our and other groups have reported fluorometric 'reading-out' sensing systems for alkali metal cations,7) carboxylic acids8) and ammonium ions9) by pyrene functionalized calixarenes. Since the ion size of the guanidinium group is larger than those of alkali metal cations and primary ammonium ions, the size of the binding site in the receptor for guanidinium ions should be larger than those for alkali metal cations and primary ammonium ions.

Here, we considered that the cavities provided by homotrioxacalix[3]arenes and calix[6]arenes might fit to the size of guanidinium ion because (i) a cone conformer of homotrioxacalix[3]aryl esters favorably binds alkali metal cations larger than a cone conformer of calix[4]aryl esters10) does and (ii) calix[6]aryl 1,3,5-triesters, which are "thermodynamically" immobilized to a cone conformation,11) can bind guanidinium ion but cannot bind K^+,4) the ion size of which is comparable with primary ammonium ions. We thus designed "reading-out"-type guanidinium ion receptors **1** and **2** which carry pyrene groups near the ion-binding $OCH_2C{=}O$ groups on the lower rim of the calixarene platforms. The basic principle for the molecular design is illustrated in Fig. 1. We have found that (i) **1** can bind guanidinium ion and

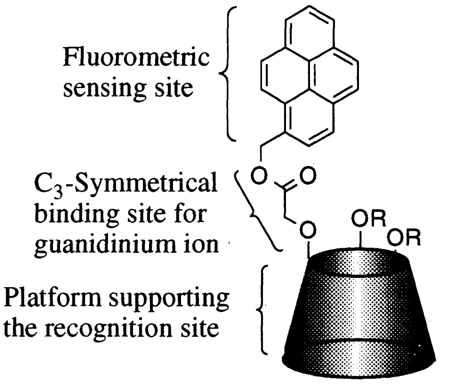
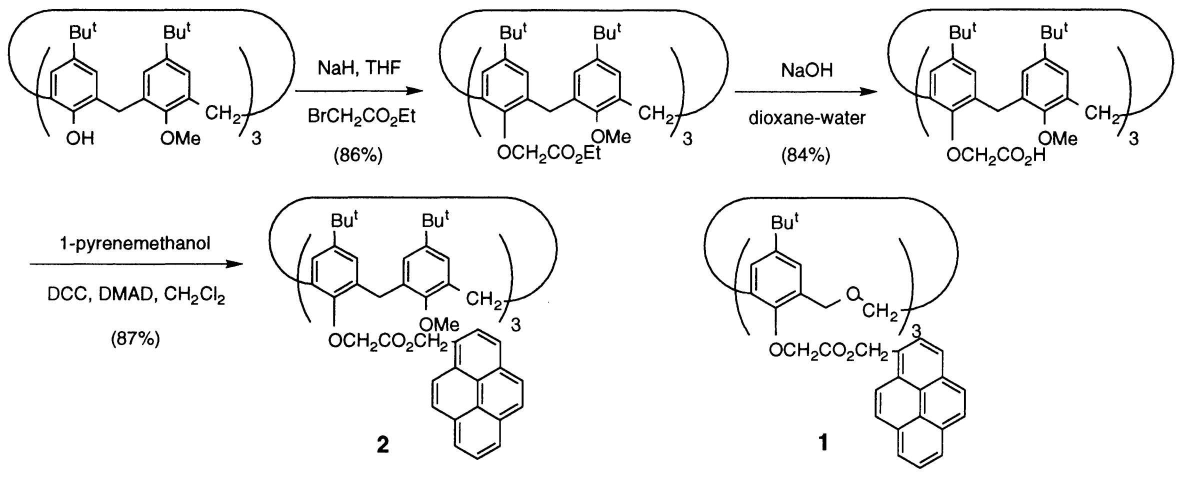


Fig. 1. Basic principle for molecular design of a fluorometric sensing system for guanidinium ion.

the fluorescence intensity changes, (ii) the binding event is competitively inhibited by primary ammonium ions whereas the fluorescence intensity in **2** responds only to guanidinium ion even in the presence of excess primary ammonium ions.

The preparation of compound **1** with a cone conformation was reported previously.[9] Compound **2** with a "thermodynamically" stable cone conformation was synthesized from 5,11,17,23,29,35-hexa-*tert*-butyl-37,39,41-trihydroxy-38,40,42-trimethoxycalix[6]arene[4] according to Scheme 1. The products were identified by IR, ^{1}H NMR and mass spectral evidence and elemental analyses.



Scheme 1.

Figure 2 shows a fluorescence spectral change induced by added guanidinium ion. As the guanidinium ion concentration increases, the fluorescence intensity of excimer emission (λ_{max} 478 nm) decreases while that of monomer emission (λ_{max} 377 nm and 396 nm) increases. The change implies that guanidinium ion bound to the ionophoric $OCH_2C{=}O$ cavity suppresses the intramolecular excimer formation among the pyrene groups. As shown in Fig. 3, in contrast, *t*-butylammonium ion scarcely changes the fluorescence intensity of **2**. We also tested L-alanine methyl ester hydrotetraphenylborate and *n*-hexylammonium BPh_4^- but these compounds were also ineffective. The association constants (K_a) for the formation of a 1:1 complex were determined by a Benesi-Hildebrand plot[12] using the fluorescence intensity at 478 nm: K_a = 980 dm^3 mol^{-1} for guanidinium ion and that for *t*-butylammonium ion could not be calculated because of the small change in fluorescence.

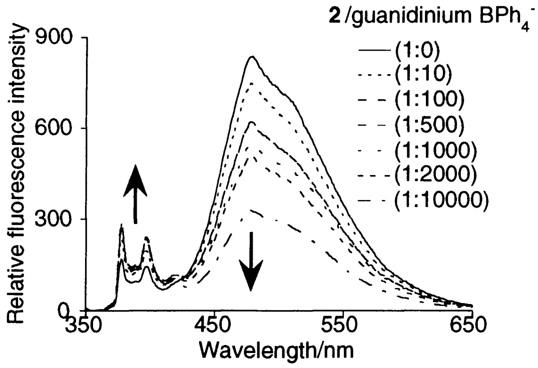


Fig. 2. Fluorescence change in **2** upon addition of guanidinium BPh_4^- ([**2**] = 2x10^{-6} mol dm^{-3}, 20 °C, in $CHCl_3$/THF (4:1 v/v), excitation 346 nm).

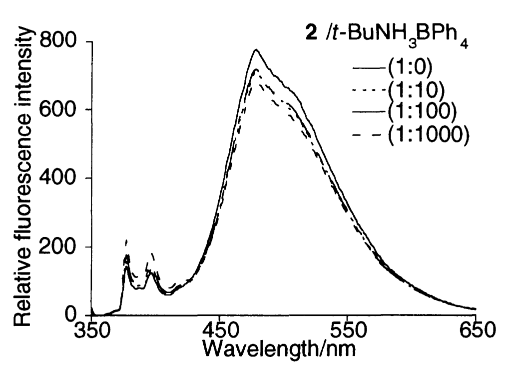


Fig. 3. Fluorescence change in **2** upon addition of *t*-butylammonium BPh_4^- ([**2**] = 2x10^{-6} mol dm^{-3}, 20 °C, in $CHCl_3$/THF (4:1 v/v), excitation 346 nm).

The effective discrimination between these two ions was further corroborated by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of a mixture of **2** (4.0×10^{-3} mol dm^{-3}) and guanidinium BPh_4^- (0.05 mmol, suspension) in 0.5 ml of $CDCl_3$ (20 °C) shows two kinds of methylene bridge protons of calix[6]arene at 3.42 and 4.38 ppm (6 H each, doublet) which are shifted by 0.10 ppm to lower magnetic field and by 0.09 ppm to higher magnetic field, respectively. These shifts mean that the phenyl units of calix[6]arene were flattened by formation of the complex with guanidinium ion.[4,5] In contrast, no change was observed for the ^{1}H NMR spectrum of a mixture of **2** and *t*-butylammonium BPh_4^- in the same condition. These results suggest that **2** can bind guanidinium ion but can not bind *t*-butylammonium ion.

Figure 4 shows the influence of coexistent *t*-butylammonium ion on the guanidinium induced fluorescence change. It is seen from Fig. 4 that the fluorometric sensing of guanidinium ion at 10^{-3} mol dm^{-3} is scarcely affected by coexistent *t*-butylammonium ion in the homogeneous solution.

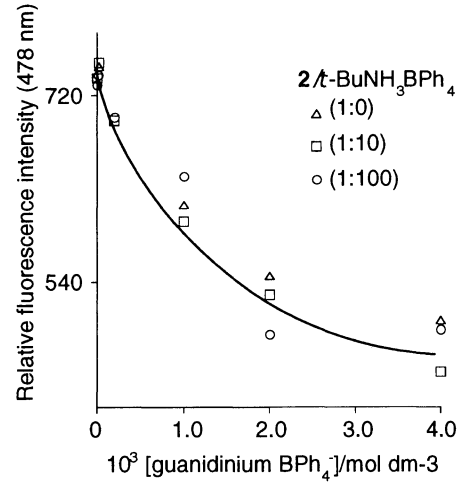


Fig. 4. Fluorescence intensity (478 nm) change in **2** upon addition of guanidinium BPh_4^- in the presence of *t*-butylammonium BPh_4^- ([**2**] = 2×10^{-6} mol dm^{-3}, 20 °C, in $CHCl_3$/ THF (4:1 v/v), excitation 346 nm).

Compound **1** shows spectral changes similar to Figs. 2 and 3 upon addition of guanidinium and *t*-butylammonium ions, respectively. The careful examination of the fluorescence intensity *vs*. concentration plots disclosed, however, that the K_a for *t*-butylammonium ion (*ca*. 7×10^4 dm^3 mol^{-1}) is much greater than that for guanidinium ion (3.6×10^3 dm^3 mol^{-1}). This means that *t*-butylammonium ion is effectively bound to the ionophoric cavity in **1** but the ion size is too small to suppress the intramolecular excimer formation among the pyrene groups. In fact, the plot of the fluorescence intensity *vs*. guanidinium ion concentration was seriously perturbed by coexistent *t*-butylammonium ion or L-alanine methyl ester hydrotetraphenylborate.

Preliminary two-phase extraction and detection of guanidinium ion from aqueous phase was carried out. The conditions were as follows: An aqueous solution of guanidinium chloride (2×10^{-5} to 2×10^{-2} mol dm^{-3}), $NaBPh_4$ powder (5×10^{-2} mol dm^{-3}), and a chloroform solution of **2** (2×10^{-6} mol dm^{-3}) were mixed. The mixture was shaken for 1 min and the fluorescence spectrum of organic phase was measured. The results are shown in Fig. 5. The fluorescence intensity was decreased with increasing guanidinium concentration but saturated at [guanidinium Cl] = 6×10^{-4} mol dm^{-3}.

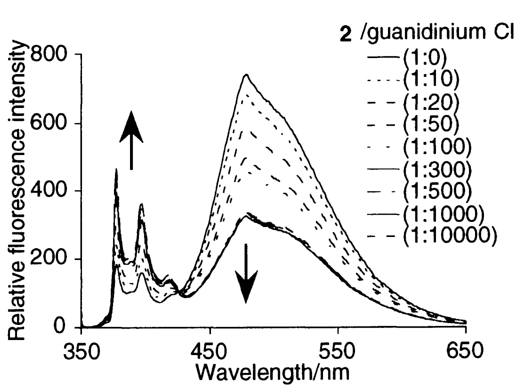


Fig. 5. Fluorescence change in **2** (in $CHCl_3$) by two-phase extraction of guanidinium chloride in water phase ([**2**] = 2×10^{-6} mol dm^{-3}, 20 °C, excitation 346 nm).

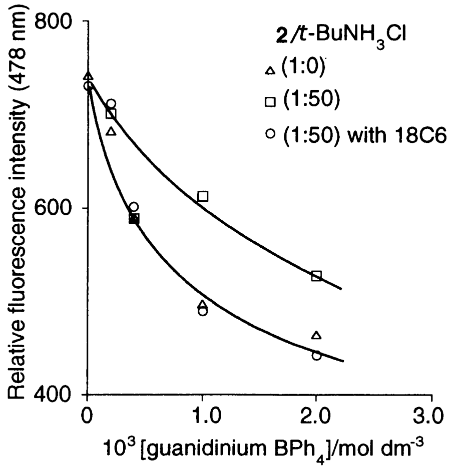


Fig. 6. Fluorescence change (478 nm) in **2** by two-phase extraction of guanidinium ion in the presence of *t*-butylammonium ion (Excitation 364 nm, [**2**] = 2×10^{-6} mol dm^{-3}, 20 °C, in $CHCl_3$, 18C6: dicyclohexano-18-crown-6).

Two-phase extraction and detection of guanidinium ion in the presence of *t*-butylammonium ion was also carried out. Figure 6 shows a fluorescence (478 nm) change in **2** upon addition of guanidinium chloride into aqueous phase in the presence of *t*-butylammonium chloride. The coexistent *t*-butylammonium ion interfered with the fluorometric detection of guanidinium ion. Addition of dicyclohexano-18-crown-6 which shows the high affinity with primary ammonium ions removed the effect of coexistent *t*-butylammonium ion, and thus a fluorometric "reading-out" of guanidinium ion in the two-phase extraction system became possible even in the presence of *t*-butylammonium ion.

In conclusion, the present study has established a selective sensing system for biologically important guanidinium ion in the presence of primary ammonium ion. In the present experiments we used BPh_4^- salts to enhance the solubility in organic media. The preliminary experiments indicate that the concentration of guanidinium ion in the presence of primary ammonium ions in water can be determined by the combination of two-phase ion-pair extraction of guanidinium ion with BPh_4^- with the fluorometric sensing with **2**.

References

1) Reviews: C. L. Hannon and E. V. Anslyn, "Bioorganic Chemistry Frontiers 3," ed by H. Dugas, Springer-Verlag, Berlin (1993).
2) E. P. Kyba, R. C. Helgeson, K. Madan, G. W. Gokel, T. L. Tarnowski, S. S. Moore, and D. J. Cram, *J. Am. Chem. Soc.*, **99**, 2564 (1977); J.-M. Lehn, P. Vierling, and R. C. Hayward, *J. Chem. Soc., Chem. Commun.*, **1979**, 296; T. B. Stolwijk, P. D. J. Grootenhuis, P. D. van der Wal, E. J. R. Sudhölter, D. N. Reinhoudt, S. Harkema, J. W. H. M. Uiterwijk, and L. Kruise, *J. Org. Chem.*, **51**, 4891 (1986).
3) T. W. Bell and J. Liu, *Angew. Chem., Int. Ed. Engl.*, **29**, 923 (1990).
4) A. Casnati, P. Minari, A. Pochini, and R. Ungaro, *J. Chem. Soc., Chem. Commun.*, **1991**, 1413.
5) A. Casnati, P. Minari, R. Ungaro, W. F. Nijenhuis, F. de Jong, and D. N. Reinhoudt, *Isr. J. Chem.*, **32**, 79 (1992).
6) F. J. B. Kremer, G. Chiosis, J. F. J. Engbersen, and D. N. Reinhoudt, *J. Chem. Soc. Perkin Trans. 2* **1994**, 677.
7) I. Aoki, H. Kawabata, K. Nakashima, and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, **1991**, 1771; T. Jin, K. Ichikawa, and T. Koyama, *ibid.*, **1992**, 499; C. Pérez-Jiménez, S. J. Harris and D. Diamond, *ibid.*, **1993**, 480.
8) I. Aoki, T. Sakaki, S. Tsutsui, and S. Shinkai, *Tetrahedron Lett.*, **33**, 89 (1991).
9) M. Takeshita and S. Shinkai, *Chem. Lett.*, **1994**, 125.
10) K. Araki, N. Hashimoto, H. Otsuka, and S. Shinkai, *J. Org. Chem.*, **58**, 5958 (1993).
11) H. Otsuka, K. Araki, T. Sakaki, K. Nakashima, and S. Shinkai, *Tetrahedron Lett.*, **34**, 7275 (1993).
12) H. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).

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