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Visual sensing of Ca^{2+} ion *via* photoreaction of fluorenyl ester-armed cyclen

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Received 10th March 2005, Accepted 21st March 2005
First published as an Advance Article on the web 5th April 2005

Fluorenyl ester-armed cyclen gave fluorenone and related decomposition compounds upon photoirradiation. The reaction was effectively suppressed by the formation of an octacoordinated Ca^{2+} complex while Na$^+$ and other alkali/alkaline earth metal cations had little influence. Since the production efficiency of fluorescent fluorenone related well to the concentration of the Ca^{2+} ion, the photoreaction of this armed cyclen offered the naked-eye detection of Ca^{2+} ion in aqueous samples.

Ca^{2+} ion-selective fluorescent chemosensors with high sensitivity and selectivity have practical applications in rapid analysis,[1–3] because this cation is essential in many biological processes. Chemosensors were usually designed to have Ca^{2+} ion recognition sites and fluorescence signalling functions. Since the Na$^+$ ion is commonly present in biological samples and exhibits similar ligand preferences, the selective chemosensors for Ca^{2+} ion often suffered from the interference of Na$^+$ ion. We report here that photo-reactive armed cyclen **1** offers visual sensing of Ca^{2+} ion in the presence of Na$^+$ ion. This type of armed cyclen is characterized by the parent cyclen ligand and four ester-functionalized sidearms which form octacoordinated complexes with alkali, alkaline earth and lanthanide metal ions.[4–6] Furthermore, the fluorenyl esters and their derivatives were reported to give fluorescent fluorenone and other photo-decomposed products (Fig. 1).[7–10] Several types of armed cyclens have already been used as effective sensing devices.[11–16] Parker *et al.* and Sherry *et al.* presented a series of lanthanide complexes with armed cyclens as luminescent materials, while Kimura *et al.* developed fluorescent probes of the armed cyclen type. We also have synthesized a series of armed cyclens having ester-, amide- and pyridine-functionalized sidearms as octadentate ligands of Na$^+$ ion.[6,17] Although the photochemical reactions of the armed cyclens have rarely been applied in the sensing processes, we attach the photoreactive fluorenyl ester moieties to the octadentate armed cyclen scaffold. Since they coordinate with the cyclen-bound metal cations, their photo-decomposition profiles can be regulated by the natures of the bound metal cations. We demonstrate below that a combination of a photoreactive fluorenyl ester with the octadentate armed cyclen offers a new cation-controlled photoreaction system, which is applicable in visual sensing of Ca^{2+} ion.

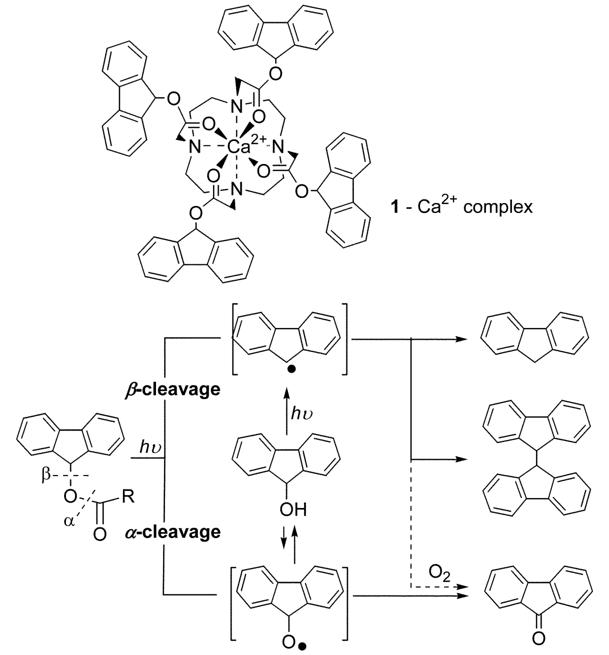

Fig. 1 Armed cyclen **1**–Ca^{2+} complex and its possible photoreaction paths.

Fluorenyl ester-armed cyclen **1** formed a stable Ca^{2+} complex, in which the Ca^{2+} ion was octacoordinated by four carbonyl oxygen atoms of the ester sidearms and four nitrogen atoms of the cyclen ring (Fig. 1). As demonstrated in the crystal structure of its Na$^+$ complex,[17] the four fluorenyl-residues were bundled above the Ca^{2+}-cyclen ring. In the ^{13}C-NMR spectrum of the Ca^{2+} complex, the signals for two cyclen ring carbons resonated separately at 54.3 and 48.9 ppm at room temperature in CD$_3$CN. ^{1}H-NMR signals for cyclen ring protons were also separately observed at 2.61, 2.81, 3.07 and 3.42 ppm, indicating that armed cyclen **1** formed a Ca^{2+} complex with a quadruple helical structure. When armed cyclen **1** was mixed with two equivalents of Na$^+$ and Ca^{2+} ions, its ESI-MS spectrum exhibited much higher peaks (due to the Ca^{2+} complex) than to that of the Na$^+$ complex; relative peak intensity [cyclen + Ca^{2+}]$^{2+}$: [cyclen + Ca^{2+} + CF$_3$SO$_3^-$]$^+$: [cyclen + Na$^+$]$^+$ = 18 : 61 : 1. ^{1}H-NMR competitive experiments with cryptand[2.2.2] also revealed that the Ca^{2+} complex was more stable than the Na$^+$ complex. Addition of six equivalents of cryptand[2.2.2] to the CH$_3$CN solution of Ca^{2+} complex hardly changed the signals of this complex. In contrast, the signals of free armed cyclen protons appeared in the case of the Na$^+$ complex, indicating that 58% of the Na$^+$ complex and only 0.6% of the Ca^{2+} complex were destroyed. IR and ^{13}C-NMR spectra revealed that the fluorenyl ester moieties coordinated the cyclen-bound Ca^{2+} ion and modified ester bond characters; IR (C=O, KBr), 1683 (Ca^{2+} complex) < 1727 (Na$^+$ complex) < 1731 (no metal cation) cm^{-1}; ^{13}C-NMR (C=O, CDCl$_3$), 177.2 (Ca^{2+} complex) > 175.6 (Na$^+$ complex) > 172.3 (no metal cation) ppm. Therefore, Ca^{2+} coordination with armed cyclen **1** offers the possibility that α-cleavage of the ester bond can be suppressed upon photo-irradiation (see Fig. 1).

Fig. 2 illustrates UV and fluorescence spectral changes of fluorenyl ester-armed cyclen **1**–Na$^+$ complex in an CH$_3$CN solution upon UV irradiation.† Both the absorption centered at 256 nm and fluorescence centered at 505 nm gradually increased with fluorenone production. After 10 min irradiation, the photo-decomposed compounds were characterized, in which 9-fluorenol (2%), fluorenone (39%), and fluorene derivatives (5%) were isolated, together with decomposed cyclen derivatives. When the effects of Na$^+$ and Ca^{2+} complexation on the reaction profiles were compared after 5 min irradiation, there were significant differences in reaction efficiency and product distribution (Table 1).‡ At the initial reaction stage, Ca^{2+}

DOI: 10.1039/b503611a

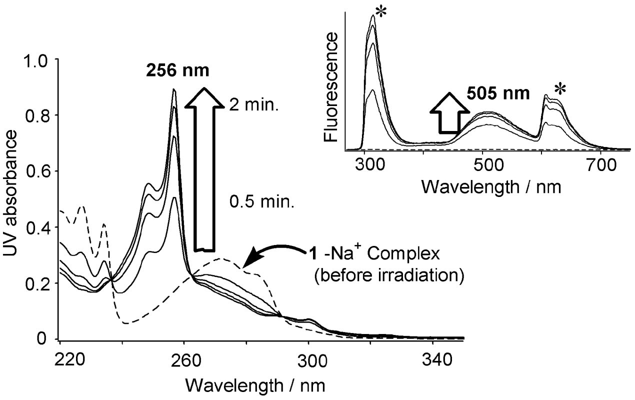

Fig. 2 UV and fluorescence spectral changes of cyclen **1**–Na$^+$ complex upon UV irradiation. Conditions: cyclen **1**–Na$^+$ complex, 1.0×10^{-5}M; in 2 mL of CH_3CN; irradiated by UV lamp (254 nm). An asterisk (*) indicates that this fluorescence is assignable to fluorene derivatives.

Table 1 Photoreaction profiles of armed cyclen **1** in the presence and absence of metal ions

	Fluorene derivatives	Yield (%)^a Fluorenone	9-Fluorenol
No Metal	7	35	< 1
1–Na$^+$	5	39	2
1–Ca^{2+}	7	9	1

^{a} Estimated from HPLC peak area and their UV absorption coefficients.

complexation significantly suppressed fluorenone production, mainly due to α-cleavage, while the Na$^+$ complex showed reactivity and product distribution similar to those observed with cyclen **1** itself. Although the two ions have similar ion-sizes and coordination geometry, the Ca^{2+} ion was confirmed to work as a more effective electron-withdrawing group for the bound fluorenyl ester functions and suppressed the photoreaction more effectively than Na$^+$ ion.

The photoreaction of fluorenyl ester-armed cyclen **1** was applicable to the detection of Ca^{2+} ion by the naked eye. Fig. 3 lists pictures of photoreaction mixtures, in which the cyclen **1**–NaCl complex and Ca^{2+} ion were mixed in various mole ratios. After an aqueous solution of $CaCl_2$ (2.0×10^{-3} mol L^{-1}, 0.05 mL) was typically added to a series of CH_3CN solutions containing the cyclen **1**–NaCl complex (1.0, 2.5, 5.0 and 7.5×10^{-6} mol L^{-1}, 10.00 mL), UV irradiation was carried out for 1 min (see 4th column in Fig. 3). The observed fluorescence intensity at 505 nm was modestly recorded in the presence of a large excess of Ca^{2+} ion, but significantly increased as the mole ratio of cyclen **1** to Ca^{2+} ion approached 1: relative fluorescence intensity (mole ratio) = 5.1 (1/10) < 11 (2.5/10) < 27 (5.0/10) << 100 (7.5/10). The intense fluorescence was only observed by the naked-eye at the mole ratio of 7.5 : 10, though its image was overexposed due to the excess emission (Fig. 3). Thus, the naked-eye observation offered an approximate estimate of the Ca^{2+} ion concentration. This visual sensing system operated well in the presence of equimolar Li$^+$, Na$^+$, K$^+$, Mg^{2+} and Zn^{2+} cations, though addition of 5 equivalents of Na$^+$ cation caused significantly disturbance. It provides an effective basis for the *in situ* determination of biologically important metal cations.

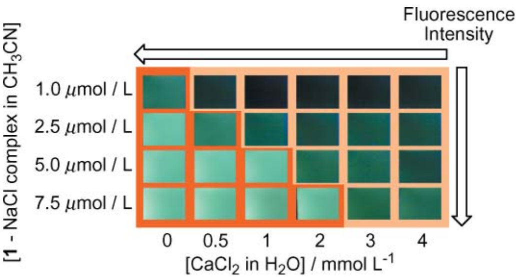

Fig. 3 Visual sensing of Ca^{2+} ion concentration: pictures of photoreaction mixtures. Cyclen **1**–NaCl complex and Ca^{2+} ion were mixed in various mole ratios and UV-irradiated for 30 s: CH_3CN solution containing cyclen **1**–NaCl complex (1.0, 2.5, 5.0, 7.5×10^{-6} mol L^{-1}, 10.00 mL); aqueous $CaCl_2$ sample solution (0, 0.5, 1.0, 2.0, 3.0, 4.0×10^{-3} mol L^{-1}, 0.05 mL).

Acknowledgements

The authors thank Professor Yasuo Kubo of Shimane University for his valuable comments on photochemistry. This research was supported in part by the Hayashi Memorial Foundation for Female Natural Scientists.

Notes and references

† The armed cyclen **1**–NaCl complex was employed in Figs. 2 and 3, because the free ligand was oily and unstable. Photo-irradiation was carried out in a CH_3CN solution (1.0×10^{-5} mol L^{-1}) by UV lamp (model UVGL-25; UVP Inc., 254 nm, 4 W). The reaction mixture was analyzed by an HPLC method: Mightysil Si 60 (4.6 × 150 mm) (Kanto Chemical Co. Inc.); ethyl acetate : hexane = 1 : 4. The obtained fluorene, 9-fluorenol and fluorenone were identified by comparison with authentic samples.

‡ Metal free cyclen ligand **1** was prepared *in situ* by the addition of four equivalents of cryptand[2.2.1] to a CH_3CN solution of cyclen **1**–NaCl complex (see ref. 17). Its Ca^{2+} complex was prepared by addition of 1.5 equivalents of $Ca(CF_3SO_3)_2$ in CH_3CN. After removal of solvent, the CH_2Cl_2-soluble fraction was separated. The precipitation from CH_2Cl_2–hexane gave a white powder of armed cyclen **1**–Ca^{2+} complex (yield, 31%): mp 209–211 °C (decomposition); υ_{max}(neat)/cm^{-1} 1683 (CO); *m/z* (ESI, CH_3CN) 550 (M^{2+}) and 1250 ($M + CF_3SO_3^+$); δ_H(400 MHz, CD_3CN) 2.61 (4H, br d), 2.81 (4H, br d), 3.07 (4H, br s), 3.42 (4H, br s), 3.68 (4H, br d), 4.27 (4H, br d), 6.27 (4H, br s), 7.06 (4H, s), 7.11 (4H, br s), 7.34 (4H, br s), 7.49 (4H, br s), 7.64 (4H, br s), 7.73 (8H, br s) and 8.05 (4H, br s); δ_C(100 MHz, CD_3CN) 48.86, 54.29, 57.39, 79.37, 121.15 (q, $J_{C-F} = 319$ Hz), 121.61, 126.75, 129.20, 131.33, 140.06, 141.30 and 181.14; Found: C, 58.15; H, 4.25; N, 3.98. Calc. for $C_{68}H_{60}N_4O_8$–$Ca(CF_3SO_3)_2 \cdot 2.5H_2O$: C, 58.20; H, 4.54; N, 3.88.

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