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Calix[4]crown in dual sensing functions with FRET

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Abstract—A new calix[4]crown chemosensor based on dual sensing probes reveals Pb^{2+} ion selectivity over other metal ions, which arises from a hypsochromic shift of azo units in UV spectrum as well as a fluorescence enhancement of pyrenyl parts in fluorescence spectrum via a suppressed FRET. © 2005 Published by Elsevier Ltd.

The design of chemosensors able to selectively recognize and sense specific analytes has attracted considerable interests due to their importance in biological and environmental settings.^{1,2} The main issue in designing effective sensor is to easily convert molecular recognition into photochemical changes with a high selectivity and sensitivity. For the chemosensors, it is well known that the photochemical changes in the sensing modules are based on the photo-induced electron transfer (PET), fluorescence resonance energy transfer (FRET), perturbation of optical transitions, and polarizabilities, excimer/exciplex formation, modification of redox potentials in ground or excited states, and photoregulation of binding properties.^{3–7}

Chromogenic and fluorogenic calixarenes have received increasing attention and become promising candidates for sensing probes because they are in a certain preorganized framework to easily accommodate metal ions or neutral molecules, exhibiting a selective change in the UV/vis and fluorescence spectra.^{7–14} It was reported that the absorption band of (azo)calix[4]crown hypsochromically moves by the addition of metal ions due to an electrostatic interaction between the oxygen atom of diazo-phenoxy group and the metal ion.¹⁵ We also recently reported that calix[4]crowns (1) as a PET-utilizing chemosensor, in which the amide groups selectively

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form a complex with \mbox{Pb}^{2+} ion, provides a quenched fluorescence. 16

The FRET is defined as an excited-state energy interaction between two fluorophores in which an excited donor (D) energy is transferred to an acceptor (A) part without any photon-emission.^{4,17} So, the FRET is required to have a certain degree of spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor (quencher).

Taking the dual sensing system (both the above chromogenic and fluoreogenic) into account with the FRET, we have developed calixcrown molecules with a diazogroup giving a visual color change as well as with a pyrenyl group providing a fluorescence change.

Synthetic pathway for **2** is outlined in Scheme 1. Reference materials **1** and **3–6** (Fig. 1 and Scheme 1) were prepared by the adaptation of the published procedures.^{5,15} The reaction of azo-coupled calix[4]monocrown-6 (**6**) with 2.2 equiv of *N*-(1-pyrenylmethyl)chloroacetamide (**5**) in the presence of K_2CO_3 as a base with a catalytic amount of NaI gave **2** in moderate yield with a cone conformation retained.¹⁸

Compound 1 shows a Pb^{2+} ion selectivity over other metal ions in terms of decreasing fluorescence, which is due to a complexation of crown oxygen atoms with the aid of two facing amide oxygen atoms to cause a reverse PET and a heavy metal ion effect.¹⁶ Compound 2 bearing additional diazo-phenyl moieties to 1 also



Scheme 1. Synthetic scheme for 2 and 3. Reagents and conditions: (a) K₂CO₃, NaI, CH₃CN, reflux for 2 days.



Figure 1. Chromo- and fluorogenic compounds.

selectively detects Pb^{2+} ion over other metal ions in the acetonitrile solution. As seen in Figure 2, **2** displays two distinctive UV bands, a pyrenyl part and a diazo-phenyl unit, with maxima at 344 and 365 nm, respectively, which is confirmed by those of reference materials **4** and **5**.¹⁹ Addition of Pb^{2+} ion into the solution of **2**

induced a blue-shifted absorption spectrum of the diazo-phenoxy unit, which is attributable to a phenomenon that the phenolic oxygen atoms of the crown loop are positively polarized when the metal ion is bound, as a result, the excited state in electron transition becomes destabilized (Fig. 3).^{15,20} Enhanced absorption



Figure 2. UV/vis absorbance spectra of 2, 4, and 5 in CH₃CN. Conditions: 2 (0.01 mM)/CH₃CN; 4 (0.02 mM); and 5 (0.02 mM)/CH₃CN.



Figure 3. Wavelength changes of 2 upon the addition of Pb^{2+} ion. Conditions: 2 (0.01 mM)/CH₃CN; Pb^{2+} ion (10 equiv)/CH₃CN.

band in 2-Pb²⁺ (10 equiv) is not due to an electronic transition between two components (pyrene and diazophenyl) but due to a spectral overlap between absorption band of the pyrene units and a blue-shifted band of the azo units, which is also evidenced by the measurement of the spectral changes for 1 and 1-Pb²⁺ (10 equiv) where no change in the absorption band is indicated. So, it is noteworthy that the electronic coupling in the ground state between the diazophenoxy unit and the pyrenyl part in the 2-Pb²⁺ complexation is ignorable.

The fluorescence spectra of 2–5 in CH₃CN (6.0 μ M, $\lambda_{ex} = 344$ nm) are shown in Figure 4. In order to obtain an insight into the *intramolecular* or the *intermolecular* FRET in 2, we newly synthesized 3 as a reference, which consists of both functional groups of chromogenic 4 and fluorogenic 5. As expected, the fluorescence intensity of 3 is weaker than that of 5. Besides, no difference in the fluorescence spectra between the mixture (4 and 5) and 5 only were observed. Those observations strongly indi-



Figure 4. Fluorescence emission spectra of 2-5 (6.0 μ M, excitation at 344 nm) in CH₃CN.

cate that the *intermolecular* FRET quenching effect from pyrenyl group to diazo-phenyl moiety is absolutely excluded. Instead, the *intramolecular* FRET is much more predominant to govern the fluorescence changes.

With those reasons it is quite understandable for 2 to reveal a significant spectral overlap between the fluorescence emission band of pyrene units as a donor and the absorption band of diazo-phenyl units as an acceptor (quencher) to display a notable quenched fluorescence emission. Surprisingly, upon the addition of



Figure 5. Fluorescence emission change of **2** upon the addition of Pb²⁺ ion. Conditions: **2** (6.0 μ M, excitation at 344 nm)/CH₃CN; Pb²⁺ ion (10 equiv)/CH₃CN. Inset: titration curve $I - I_0$ (386 nm) as a function of [Pb²⁺]/[**2**].

Pb²⁺ ion and irradiation at 344 nm, the fluorescence of **2** was observed to revive although the Pb²⁺ ion is known as a quenching metal ion as shown in Figure 5, whereas **3** without ionophoric part for the lead ion did not change in the fluorescence spectrum. It is obviously due to the less overlapped bands between the donor (emission) and the acceptor (absorption) caused by a hypsochromical shift of diazo units by the metal ion complexation, resulting in a diminished FRET effect (Fig. 6). According to the fluorescence emission changes in metal ion titration, we could obtain the association constant²¹ of **2** ($K_a = 4.0 \times 10^6 \text{ M}^{-1}$) for Pb²⁺ ion. Figure 6 shows luminosity changes of **1** (a) and **2** (b) upon Pb²⁺ ion complexation where the luminosity of **2** decreases compared to that of **1** by the FRET, but increases by the addition of Pb²⁺ ion. In addition, the Pb²⁺ ion selectivity was also observed by the selective color change of **2** from pale green to colorless (Fig. 7c).

Consequently, the less fluorescent **2** caused by the FRET revives its fluorescence by the Pb^{2+} ion complexation. Compound **2** might be useful as a selective sensor for Pb^{2+} ion in a dual sensing system, a visual color change as well as a fluorescence change including the FRET concept upon the Pb^{2+} ion complexation.



Figure 6. Changes of spectral overlap for FRET upon Pb^{2+} ion complexation. Conditions: **1** (6.0 μ M, excitation at 344 nm)/CH₃CN; **2** (0.01 mM)/CH₃CN; Pb²⁺ ion (10 equiv)/CH₃CN.



Figure 7. Luminosity changes of (a) 1 ($0.02 \text{ mM/CH}_3\text{CN}$) and (b) 2 ($0.02 \text{ mM/CH}_3\text{CN}$) with Pb²⁺ ion (10 equiv), respectively. Visible color change of (c) 2 ($0.02 \text{ mM/CH}_3\text{CN}$) upon the Pb²⁺ ion addition (10 equiv).

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- 18. 5,17-Bis[(4-nitrophenyl)(azo)phenyl]-26,28-bis[(N-(1-pyrenylmethyl)aminocarbonyl)-methoxy]-25,27-calix[4]monocrown-6 (2). Yield (36%). Mp: 186-188 °C. IR (KBr pellet, cm⁻¹): 3300 (–NH), 1650 (CO), 1521, 1343 (NO₂); ¹H NMR (400 MHz; CDCl₃): δ 8.38 (d, 4H, NO₂Ar-H_{ortho}, J = 8.0 Hz), 8.26–7.83 (m, 24H, pyrene, NO₂Ar– H_{meta}) CONHCH₂pyrene), 7.59 (s, 4H, N₂Ar-H_{ortho}), 6.25 (s, 6H, Ar-H), 5.18 (s, 4H, NHCH2pyrene), 5.10 (s, 4H, ArOCH₂CO), 4.44 (d, 4H, ArCH₂Ar, J = 13.3 Hz), 3.68 (s, 4H, OCH₂CH₂O), 3.36 (s, 4H, OCH₂CH₂O), 3.18 (s, 4H, OCH₂CH₂O), 3.09 (s, 4H, OCH₂CH₂O), 3.04 (d, 4H, ArCH₂Ar, J = 13.4 Hz); ¹³C NMR (CDCl₃): δ 169.6, 160.9, 156.9, 155.7, 149.0, 148.5, 138.0, 133.0, 132.7, 131.9, 131.4, 128.8, 128.7, 128.6, 128.2, 128.0, 126.8, 126.0, 125.9, 125.4, 125.3, 125.0, 124.9, 124.3, 123.8 (pyreneCH, ArCH, CO), 74.2, 73.3, 70.9, 70.8, 70.7, 70.5 (OCH₂CH₂O, ArOCH₂CO), 42.1 (NCH₂pyrene) 32.2 (ArCH₂Ar) ppm. FAB MS *m*/*z* (M⁺): calcd, 1467.57, found, 1467.60. Anal. Calcd for C₈₈H₇₄N₈O₁₄: C, 72.02; H, 5.08. Found: C, 72.08; H, 5.16.

4-[(4-Nitrophenyl)(azo)]-[N-(1-pyrenylmethyl)aminocarbonyl]methoxybenzene (3). Yield (82%). Mp: 154– 156 °C. IR (KBr pellet, cm⁻¹): 3300 (–NH), 1650 (CO), 1521, 1343 (NO₂); ¹H NMR (200 MHz; CDCl₃): δ 8.35 (d, 2H, NO₂Ar–H_{ortho}, J = 7.8 Hz), 8.27–7.81 (m, 13H, pyrene, NO₂Ar–H_{meta}, N₂Ar–H_{ortho}), 6.96 (d, 2H, N₂Ar– H_{meta}, J = 7.6 Hz) 6.92 (s, 1H, CONHCH₂pyrene), 5.28 (d, 2H, NHCH₂pyrene, J = 5.6 Hz), 4.67 (s, 2H, ArOCH₂-CO) ppm; ¹³C NMR (CDCl₃): 167.1, 160.1, 155.7, 148.4, 147.6, 131.2, 130.7, 130.2, 127.1, 128.4, 127.7, 127.3, 127.2, 126.2, 125.5, 125.4, 125.1, 124.8, 124.7, 124.5, 123.2, 122.5, 115.1, 67.5, 41.6 ppm. FAB MS m/z (M⁺): calcd, 514.53, found, 514.02. Anal. Calcd for C₃₁H₂₂N₄O₄: C, 72.36; H, 4.31. Found: C, 72.31; H, 4.36.

- 4-[(4-Nitrophenyl)(azo)]phenol (4). A solution of phenol (2.00 g, 21.3 mmole) in tetrahydrofuran (300 mL) was treated with 4-nitrobenzenediazonium tetrafluoroborate (5.54 g, 23.4 mmole). The reaction mixture was stirred for 30 min at 0 °C and pyridine was added dropwise. The reaction mixture was stirred for the additional 48 h at 0 °C and treated with 10% aqueous HCl solution (300 mL) and extracted with CH₂Cl₂ (300 mL). The organic layer was washed with 10% aqueous HCl solution $(2 \times 300 \text{ mL})$ and dried over MgSO₄. Removal of the organic solvent in vacuo afforded a reddish solid. Column chromatography using CHCl₃/acetone (4:1) as eluent ($R_{\rm f} = 0.45$) on silica gel gave 4. Yield (35%). Mp: 168-170 °C. IR (KBr pellet, cm⁻¹): 3428 (OH), 1505, 1336 (NO₂); ¹H NMR (400 MHz; CDCl₃): δ 9.43 (s, 1H, ArOH), 8.44 (d, 2H, NO₂Ar-H_{ortho}, J = 8.8 Hz), 8.08 (d, 2H, NO₂Ar- H_{meta} , J = 8.8 Hz), 7.96 $(s, 2H, N_2Ar-H_{ortho}, J = 8.8 \text{ Hz}), 7.08 (s, 2H, N_2Ar-H_{meta})$ J = 8.7 Hz); ¹³C NMR (CDCl₃): δ 161.7, 155.9, 148.2, 146.1, 125.6, 124.6, 122.8, 115.8 (ArCH) ppm. FAB MS m/z (M⁺): calcd, 243.22, found, 243.00. Anal. Calcd for C₁₂H₉N₃O₃: C, 59.26; H, 3.73. Found: C, 59.21; H, 3.74
- The perchlorate salts of Li⁺, Na⁺, K⁺, Rb⁺, Cs⁺, Ag⁺, Ca²⁺, Mg²⁺, Pb²⁺, Zn²⁺, Al³⁺, and In³⁺ ions (stock solutions = 1.0 mM in CH₃CN) were tested to evaluate the metal ion binding properties of 2 (stock solution = 0.6 mM in CH₃CN). For all fluorescence measurements, the excitation was made at 344 nm to give fluorescence intensity at room temperature.
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