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Ratiometric chemosensing of Mg^{2+} ions by a calix[4]arene diamide derivative

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Abstract—A new chemosensor with a phenanthroimidazole subunit based upon calix[4]arene-diamide has been synthesized, and its Mg^{2+}-selective fluoroionophoric properties were investigated in an aqueous DMSO solution. The compound exhibited a pronounced Mg^{2+}-selective fluoroionophoric behavior over other physiologically relevant metal ions. A significant red shift in fluorescence emission ($\Delta\lambda = 86$ nm) provided the ratiometric determination as well as naked-eye detection of Mg^{2+} ions.

Selective detection and quantification of metal ions is of great importance in chemical, biological, and environmental science. Many intelligent chemosensing systems have been ingeniously devised utilizing the molecular framework of crown ethers, calixarenes, and other unique ionophoric structures.[1,2] However, relatively few studies have reported on the chemosensing of Mg^{2+} ions compared with Ca^{2+} and representative alkali metal ions of Na^+ and K^+. For many applications, the major challenge in the design of Mg^{2+} sensors lies in the discrimination of Mg^{2+} ions from other physiologically abundant ions of Na^+, K^+, and Ca^{2+}.[3] Chemosensors for Mg^{2+} are generally designed to optimally respond to the Mg^{2+} concentrations commonly found in cells, ranging from about 0.1 to 6 mM, and in normal serum, ranging from 0.44 to 1.5 mM.[4] Many of the reported Mg^{2+}-selective chemosensors are based upon the well known molecular motifs of crown ethers and coumarin derivatives.[5,6] For example, coumarin derivative possessing a charged β-diketone structure[7] acts as an efficient optical chemosensor for Mg^{2+} measurements in living cell. More recently, Farruggia et al. have designed 8-hydroxyquinoline derivatives of diaza-18-crown-6 for the fluorescent sensing of Mg^{2+} in living cells.[8]

For the construction of selective chemosensors, calix[4]arenes are highly attractive candidates due to their versatile derivatization possibilities[9] and selective recognition behaviors toward cations, anions, and neutral molecules.[10] Among various derivatives, chromogenic chemosensors having azophenol[11] and merocyanine[12] subunits based on the calix[4]arene-diamide derivative **1** were designed for the sensing of Ca^{2+} ions. In this Letter, we report on selective signaling of Mg^{2+} ions by a calix[4]arene-diamide phenanthroimidazole derivative. The subunit of phenanthroimidazole[13] was introduced into the calix[4]arene-diamide for the purpose of utilizing its chromogenic and fluorogenic responses. The compound exhibited a large Mg^{2+} induced red-shifted emission, which is appropriate for ratiometric chemosensing of Mg^{2+} ions in semi-aqueous solutions.

Chemosensor **3** was prepared from calix[4]arene-diamide following the procedure outlined in Scheme 1. Selective formylation of calix[4]arene-diamide **1** with $SnCl_4/CHCl_2OCH_3$ in $CHCl_3$ afforded dialdehyde **2** (90%).[12,14] Subsequent condensation with 9,10-phenanthrenequinone ($NH_4OAc/HOAc$) generated the desired phenanthroimidazole derivative **3** in moderate yield (65%).[15,16] The phenanthroimidazole moiety is known to be a stable chromophore having high molar absorptivities and fluorophoric properties.[13,15]

The chemosensing behaviors of compound **3** were investigated by UV–vis and fluorescence measurements. Due to the limited solubility of compound **3** in common organic solvents, UV–vis spectra were measured in a

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Keywords: Mg^{2+}-selective chemosensor; Ratiometry; Calix[4]arene; Fluoroionophore.

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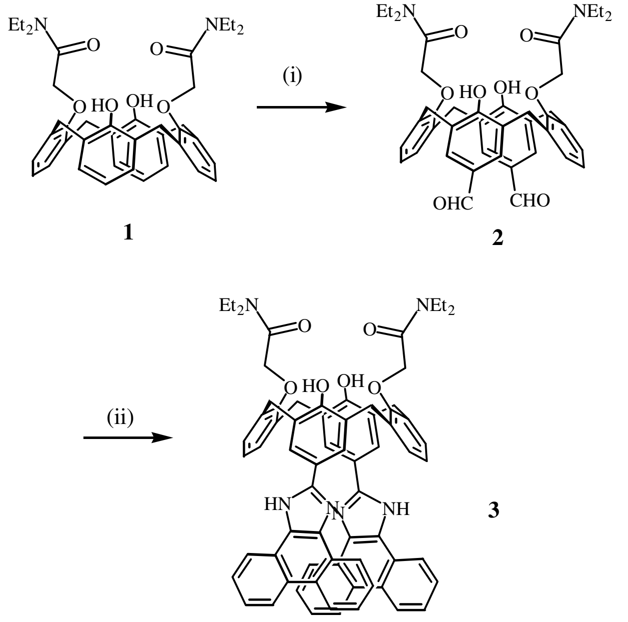

Scheme 1. Reagents and conditions: (i) $SnCl_4/CHCl_2OCH_3$ in $CHCl_3$, rt; (ii) 9,10-phenanthrenequinone, NH_4OAc in HOAc, reflux.

95% aqueous DMSO solution (DMSO:H_2O = 95:5, v/v). Compound **3** revealed a strong absorption band at 332 nm. Treatment with 100 equiv of Mg^{2+} ions resulted in a slight red-shift of the absorption band to 347 nm (Fig. 1). The resulting solution changed color from slight yellow to a deep orange. Other surveyed metal ions including representative alkali (Li^+, Na^+, K^+), alkaline earth (Ca^{2+}, Ba^{2+}), and transition metal ions (Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}) revealed no significant changes in the absorption spectra under the same conditions. In this case, the changes in absorbance at 332 nm of **3** alone and **3** in the presence of Mg^{2+} ions follow the Beer's law, which suggests that the system does not self-aggregate under the experimental conditions.[17]

The fluoroionophoric properties of compound **3** were investigated in a 95% aqueous DMSO solution where most pronounced Mg^{2+}-selectivity was observed. In this medium, **3** exhibited a broad emission band at 409 nm (λ_{ex} = 325 nm). Upon interaction with various metal ions, the fluorescence intensity of **3** was affected in varying degrees (Fig. 2). In particular, Mg^{2+} ions exclusively induced dramatic changes in fluorescence spectra by revealing new broad emission bands around 495 and 521 nm with significantly diminished band at 409 nm. The color of the emissions illuminated with a UV lamp changed considerably from blue to green (Graphical abstract). The effects of potential interfering metal ions of physiological relevance, namely, Na^+, K^+, and especially Ca^{2+} ions, were not very significant in the 95% aqueous DMSO solution. The selective fluorescence signaling might be due to the Mg^{2+} induced triggering of an internal charge transfer from the phenanthroimidazole subunit to the phenolic moiety of the binding site under the employed conditions.[6b] We have tried to obtain more evidence for the Mg^{2+}-selective signaling, such as the possibilities of metal ion induced conformational changes and aggregation–deaggregation, by measuring NMR spectra of compound **3**, but failed due to the limited solubility in aqueous 95% DMSO solution.

Compound **3** exhibited some responses toward surveyed metal ions when observed at 409 nm as can be seen in Figure 2. At first glance, compound **3** did not appear to be a selective chemosensor for the specific target metal ion of Mg^{2+}; however, the selective signaling of Mg^{2+} ions over other possibly interfering metal ions could be realized by ratiometric analysis of the responses utilizing the fluorescence intensities at 493 and 409 nm (Fig. 3). The ratios of the intensity (I_{493}/I_{409}) were ranged over a small variation between 0.08 and 0.10 for the surveyed metal ions, except for the Mg^{2+} ions. Indeed, Mg^{2+} ions induced an approximately 16-fold change in this ratio, suggesting that efficient Mg^{2+}-selective signaling could be realized with **3**.

The quantitative behaviors of **3** in the sensing of Mg^{2+} ions were further characterized by fluorescence titration with $Mg(ClO_4)_2$ in a 95% aqueous DMSO solution. Upon addition of Mg^{2+} ions, the fluorescence intensity

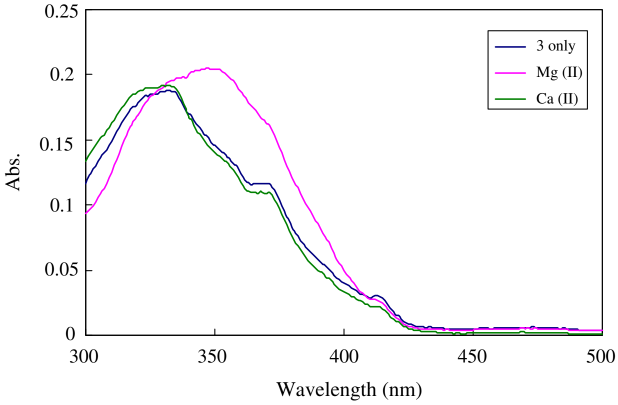

Figure 1. UV–vis absorption spectra of **3** in the absence and presence of Mg^{2+} and Ca^{2+} ions. [**3**] = 5.0×10^{-6} M, [M^{2+}] = 5.0×10^{-4} M. In Tris buffered (10 mM, pH 8.1) DMSO:H_2O = 95:5, v/v. The reported pH value refers to the H_2O portion before mixing with DMSO.

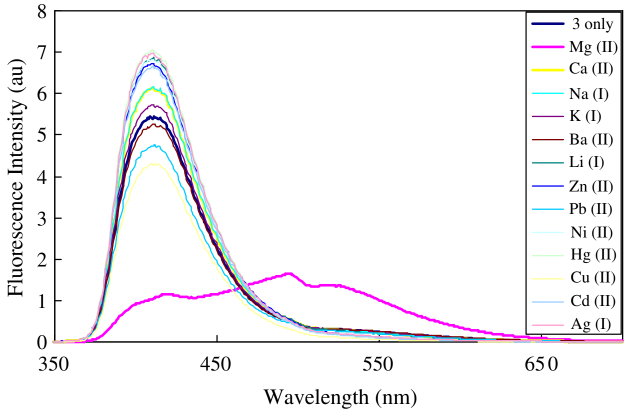

Figure 2. Fluorescence spectral changes of **3** in the presence of various metal ions. [**3**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M in Tris buffered (10 mM, pH 8.1) DMSO:H_2O = 95:5, v/v. λ_{ex} = 325 nm.

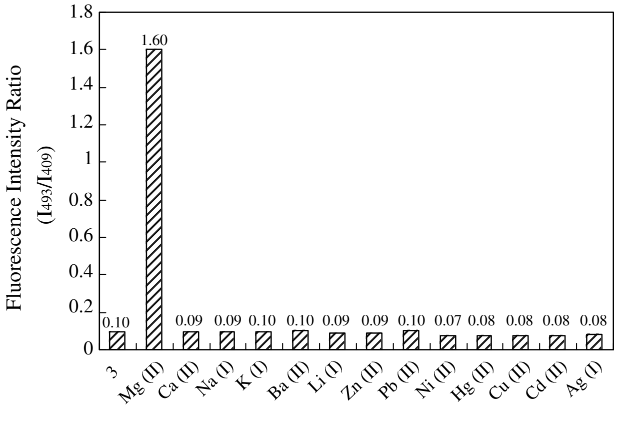

Figure 3. Selective ratiometric behavior of **3** expressed in I_{493}/I_{409} toward various metal ions. [**3**] = 5.0×10^{-6} M, [M^{n+}] = 5.0×10^{-4} M, in Tris buffered (10 mM, pH = 8.1) DMSO:H_2O = 95:5, v/v.

of **3** at 409 nm decreased steadily while new bands around 495 and 521 nm appeared, resulting in fluorescence color changes from blue to green, as described earlier. The titration results were analyzed by a nonlinear curve-fitting procedure[18] for the complex formation of the **3**–Mg^{2+} having 1:1 stoichiometry, and the K_{assoc} value was estimated as 2.97×10^4 M^{-1}. Based on the concentration-dependent fluorescence changes, the detection limit[5b] of **3** for the sensing of Mg^{2+} ions was determined to be 1.38×10^{-5} M, implying that the compound could be used for the analysis of Mg^{2+} ions at the sub-millimolar concentrations frequently found in extracellular fluids.

Compound **3** exhibited a selective signaling behavior toward Mg^{2+} ions in the presence of other physiologically relevant metal ions. For example, the fluorescence intensity ratio (I_{409}/I_{493}) of a **3**–Mg^{2+} system obtained by treating **3** (5.0×10^{-6} M) with 100 equiv of Mg^{2+} ions was not significantly affected by the presence of alkali (500 equiv), alkaline earth (500 equiv), and transition metal ions (20 equiv) (Fig. 4). The selective signaling of Mg^{2+} ions was also ascertained by fluorescence titration experiments performed in the presence of common interfering metal ions as background (Fig. 5). The competition experiments and titration results confirmed that compound **3** can be used as a selective chemosensor for the analysis of Mg^{2+} ions in the presence of physiologically important metal ions in semi-aqueous solutions.

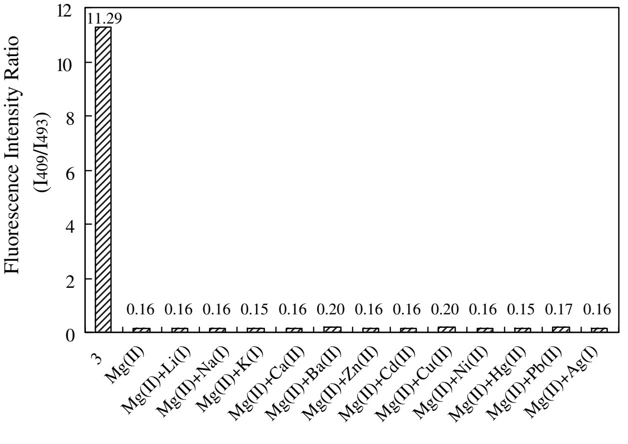

Figure 4. Mg^{2+}-selective ratiometric behavior of **3** in the presence of varying competitive metal ions in DMSO–water (95:5, v/v). [**3**] = 5.0×10^{-6} M in Tris buffer (10 mM, pH = 8.1). λ_{ex} = 325 nm.

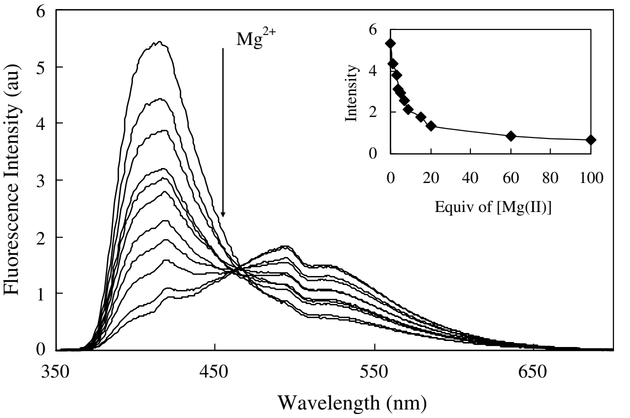

Figure 5. Fluorescence titration of **3** with Mg^{2+} ions in the presence of physiologically important metal ions in Tris buffered (10 mM, pH = 8.1) DMSO–water (95:5, v/v). [**3**] = 5.0×10^{-6} M. λ_{ex} = 325 nm. [Na^+] = 138 mM, [K^+] = 4 mM, [Ca^{2+}] = 3 mM, [Zn^{2+}] = 0.02 mM, [Cu^{2+}] = 0.015 mM, and [Co^{2+}] = 0.002 mM.

The interaction of **3** with Mg^{2+} ions was further evidenced by FAB-MS and IR measurements. Upon treatment with 5 equiv of $Mg(ClO_4)_2$, **3** exhibited a pronounced peak at $m/z = 1104.2$ for [**3**–2H + Mg]$^+$. In its IR spectra **3** also experienced a considerable shift in the carbonyl stretching band from 1655 to 1601 cm^{-1} upon treatment with $Mg(ClO_4)_2$, which might be due to the complex formation with Mg^{2+} ions.

In summary, a new fluoroionophore based upon calix[4]-arene-diamide was prepared, and its Mg^{2+}-selective chemosensing behavior was investigated. Fine-tuning of the solvent system resulted in an exclusively selective ratiometric signaling of Mg^{2+} ions over other common, physiologically important metal ions. The selective discriminating behavior of **3** for Mg^{2+} ions suggests that this new chemosensor can be applied to the analysis of Mg^{2+} ions in semi-aqueous environments.

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16. *Preparation of* **3**: To a solution of compound **2** (500 mg, 0.71 mmol) in CH_3CO_2H (100 mL) were added 9,10-phenanthrenequinone (590 mg, 2.80 mmol) and ammonium acetate (4.36 g, 56.5 mmol). The reaction mixture was heated at 120 °C for 14 h and then the precipitate formed was filtered to give a pale-brown solid. The solid was washed several times with acetic acid and water and dried in a vacuum oven to yield **3** in pale-brown powder. The product was purified by crystallization from DMF (65% yield). ^{1}H NMR (300 MHz, DMSO-d_6 + CD_3OD) δ 8.84 (d, $J = 8.1$ Hz, 4H), 8.57 (br d, 4H), 8.09 (s, 4H), 7.74 (t, $J = 7.4$ Hz, 4H), 7.63 (t, $J = 6.9$ Hz, 4H), 7.22 (d, $J = 7.8$ Hz, 4H), 6.90 (t, $J = 7.1$ Hz, 2H), 4.92 (s, 4H), 4.60 (d, $J = 12.9$ Hz, 4H), 3.62 (d, $J = 13.2$ Hz, 4H), 3.4–3.5 (m, 8H), 1.15–1.27 (m, 12H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.3, 154.7, 154.0, 149.8, 133.7, 129.3, 128.4, 127.6, 127.1, 127.0, 125.4, 125.2, 124.1, 122.1, 120.7, 73.6, 40.5, 31.0, 14.0, 12.8. HR-Mass (FAB, *m*-NBA) Calcd for $C_{70}H_{63}N_6O_6$ $[M+H]^+$ 1083.481. Found: 1083.442.
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