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The synthesis of crown ether-appended dichlorofluoresceins and their selective Cu^{2+} chemosensing

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ABSTRACT

Dichlorofluorescein derivatives with two aza-crown ether binding units were prepared by the Mannich reaction and their chemosensing behaviors toward transition metal ions were investigated. An 18-crown-6 ether derivative exhibited pronounced Cu^{2+}-selective fluorescence signaling, with selectivity over other common physiologically important alkali, alkaline earth and transition metal ions. The compound also displayed 1:1 complex formation with Cu^{2+} ion, with a detection limit of 2.9×10^{-6} M in an aqueous 50% DMSO solution at pH 7, showing that it may offer potential as a chemosensor for the detection of submillimolar Cu^{2+} ions in physiological environments. A 15-Crown-5 ether analogue also revealed selective Cu^{2+} signaling, although with somewhat diminished selectivity compared with its 18-crown-6 counterpart.

1. Introduction

The development of chemosensors for the sensing of transition metal ions is one of the most active research fields with great potential for environmental and physiological applications [1]. In particular, Cu^{2+}-sensitive systems have attracted much interest because of their biological and environmental importance [2]. During last decade, various well-functioning Cu^{2+}-selective signaling systems have been investigated utilizing unique molecular frameworks, such as 2-aza-1,3-butadiene [3], bichromophoric azine [4], naphthylimide [5,6], iminoacetate moiety [7,8], redox active thiadiazole [9], cyclam [10], and calix[4]arene [11,12]. In addition to these, Cu^{2+} complex of chromone-dipicolylamine derivative was utilized for the construction of a highly selective turn-on type sensor for pyrophosphate [13] and 2,6-bis(piperazinylmethyl)pyridine derivative was devised as an interesting molecular keyboard for the crossword puzzles and logic memory [14]. On the other hand, molecular visualization systems for imaging Cu^{2+} ions in living cells and smart magnetic resonance contrast agents based on the BODIPY-azathioether [15] and DO3A-iminodiacetate-Gd(III) complex [16] have been reported.

Particularly interesting in the design of Cu^{2+}-selective signaling systems is that a number of valuable compounds have been developed based on the versatile fluorophores of fluorescein and rhodamine. Czarnik reported a unique chemodosimeter system of rhodamine hydrazide which functions by the Cu^{2+}-induced hydrolysis of a lactam moiety [17]. Following this, many attractive fluorogenic and chromogenic probes have been reported with pronounced Cu^{2+} selectivity utilizing the binding or signaling subunits of rhodamine hydrazone and a rhodamine–diacetic acid conjugate [18,19].

A number of well-known binding sites, such as dipicolylamine, 8-hydroxyquinoline, and crown ethers suitably conjugated with fluorescein or rhodamine moieties as signaling units have been prepared [20]. In particular, a series of dipicolylamine-tethered fluoresceins have been designed and their sensitive Zn^{2+} signaling and visualization in cells were systematically investigated [21–24]. More recently, a rhodamine–monoaza-crown ether conjugate utilizing the lactam moiety with dual channel fluorescence for two different metal ions (Fe^{3+} and Hg^{2+}) was reported [25]. In this paper we report new crown ether-appended fluorescein derivatives that exhibit Cu^{2+}-selective chromogenic and fluorogenic signaling behavior over other common physiologically important metal ions.

2. Experimental section

General. 2′,7′-Dichlorofluorescein (DCF), 1-aza-18-crown-6 ether, and 1-aza-15-crown-5 ether were purchased from Aldrich Chemical Co. and used without further purification. ^1H NMR

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(300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Varian Gemini-2000 spectrometer. UV–vis spectra were recorded with a Jasco V-550 spectrophotometer. Fluorescence spectra were measured on an Aminco-Bowman Series 2 Spectrophotometer. Mass spectral data were obtained with a Micromass Autospec mass spectrometer in FAB mode. All solvents used for the measurements of UV–vis and fluorescence spectra were purchased from Aldrich Chemical Co. as 'spectroscopic grade'. Flash chromatography was performed by using silica gel column packages purchased from Biotage.

Synthesis of 1. 1-Aza-18-crown-6 ether (0.98 g, 3.73 mmol) and paraformaldehyde (0.8 g, 26.4 mmol) were dissolved in CH_3CN (5 ml) and refluxed for 30 min. 2′,7′-Dichlorofluorescein (0.5 g, 1.25 mmol) in 30 ml of CH_3CN/H_2O (1:1) was added to the above solution and the mixture was refluxed for 1 day. The organic solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, CH_2Cl_2–CH_3OH) to yield 52% of the pure product. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.04 (d, $J = 7.5$ Hz, 1H), 7.5–7.4 (m, 2H), 7.10 (d, $J = 7.2$ Hz, 1H), 6.84 (s, 2H), 3.76 (s, 4H), 3.6–3.4 (m, 40H), 2.67 (m, 8H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 178.5, 169.9, 154.9, 141.2, 133.7, 130.7, 128.8, 128.1, 127.8, 126.3, 110.9, 109.9, 70.2, 69.1, 68.9, 68.7, 67.2, 53.1, 47.7. MS (FAB, *m*-NBA), calcd for $[M+H]^+$, $C_{46}H_{61}Cl_2N_2O_{15}$, $m/z = 951.3449$. Found, 951.3453.

Synthesis of 2. Compound **2** was prepared by the same procedure for the compound **1** by using 1-aza-15-crown-5 ether and purified by the column chromatography. Yield = 48%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.05 (d, $J = 7.4$ Hz, 1H), 7.5–7.45 (m, 2H), 7.08 (d, $J = 7.3$ Hz, 1H), 6.84 (s, 2H), 3.76 (s, 4H), 3.6–3.45 (m, 32H), 2.68 (m, 8H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.1, 168.4, 155.0, 141.9, 133.5, 130.6, 128.9, 128.6, 128.0, 127.0, 111.3, 109.1, 69.9, 68.8, 68.3, 67.3, 54.1, 49.3. MS (FAB, *m*-NBA), calcd for $[M+H]^+$, $C_{42}H_{53}Cl_2N_2O_{13}$, $m/z = 863.2925$. Found, 863.2928.

Measurements of UV–vis and fluorescence spectra. The UV–vis and fluorescence spectra were obtained in solvent systems of 50% aqueous DMSO solution. For UV–vis measurements, sample solutions were obtained by mixing appropriate amount of stock solution of **1** or **2** (1.0×10^{-3} M in DMSO) with stock solution containing metal perchlorate and buffer (20 mM) in water and finally diluted with DMSO to make the solution having desired concentrations of chemosensor, metal ions, and buffer in 50% aqueous DMSO solution. Fluorescence measurements were carried out with a slit width of 10 nm. Detection limit was calculated from a plot of the fluorescence changes as a function of $\log[Cu^{2+}]$. A linear regression curve was fitted to the intermediate values of the sigmoidal plot. The point at which this line crossed the ordinate axis was taken as the detection limit [26]. For the Job plot analyses, a 1.0×10^{-3} M solution of Cu^{2+} in 20 mM hepes and 1.0×10^{-3} M in DMSO of **1** was prepared, and a titration was performed under the condition of $[\mathbf{1}] + [Cu^{2+}] = 1.0 \times 10^{-5}$ M in final solution (3 ml). Fluorescence intensity was measured for each solution and the values of $(I_o - I) \times X_\mathbf{1}$ (mole fraction of **1**) were calculated and plotted against the $X_\mathbf{1}$ in final solution [27].

3. Results and discussion

DCF fluorophores have been readily derivatized via the Mannich reaction to yield various supramolecular systems having unique functions [28,29]. Mannich reaction of DCF [30] with monoazacrown ethers afforded bis-crown derivatives **1** (52%) and **2** (48%) in moderate yields (Fig. 1). The chemosensing behaviors of the DCF-crown derivatives **1** and **2** were investigated by UV–vis and fluorescence measurements. The optimization for the selective signaling of targeting transition metal ions in common organic solvents suggested that a significant chromogenic and fluorescence signaling toward Cu^{2+} ions could be realized in 50% aqueous DMSO solution.

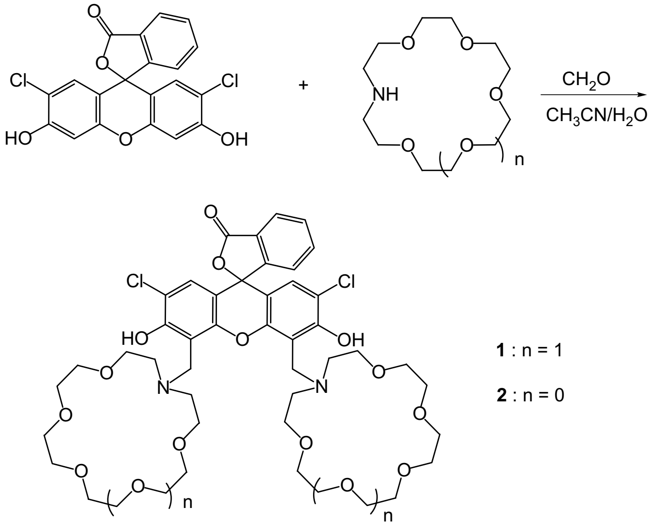

Fig. 1. Synthesis of crown appended dichlorofluoresceins **1** and **2**.

Compound **1** exhibited an intense absorption band at 509 nm with a shoulder around 480 nm in an aqueous 50% DMSO solution. Upon interaction with Cu^{2+} ions, **1** exhibited a significantly diminished absorption around 501 nm (Fig. 2). The ratio A/A_o of absorbance in the presence (A) and in the absence (A_o) of metal ions measured at 509 nm was 0.17 for Cu^{2+} ions. The decreased absorption and slight blue-shift from 509 to 501 nm resulted in a change of solution color from yellow to almost colorless. With Pb^{2+} ions, the absorption also decreased significantly ($A/A_o = 0.37$), and the solution color changed from yellow to light pink. The other metal ions tested including physiologically relevant metal ions did not induce meaningful changes in the absorption spectrum: A/A_o at 509 nm ranged between 0.94 (Mg^{2+}) and 1.06 (Ba^{2+}). These chromogenic behaviors of **1** allowed for convenient naked-eye detection of Cu^{2+} and Pb^{2+} ions.

Compound **1** exhibited a strong emission around 530 nm in an aqueous 50% DMSO solution at pH 7.0. The fluorescence spectra of **1** in the presence of representative physiologically important metal ions are shown in Fig. 3. As can be seen in Fig. 3, compound **1** revealed a marked Cu^{2+}-selective fluorescence quenching as

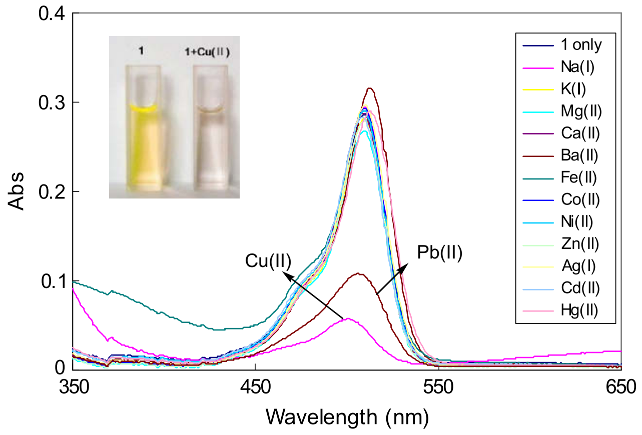

Fig. 2. UV–vis spectra of **1** in the presence of various metal ions. $[\mathbf{1}] = 5.0 \times 10^{-6}$ M. $[M^{n+}] = 5.0 \times 10^{-4}$ M. In 1:1 mixture of DMSO and hepes-buffered H_2O (pH 7.0, 20 mM).

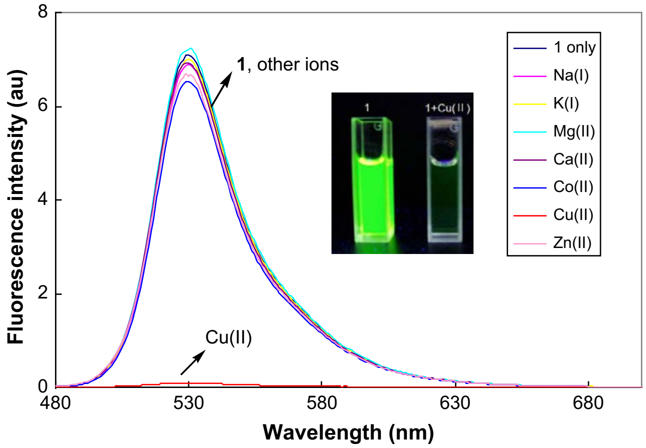

Fig. 3. Fluorescence spectra of **1** in the presence of representative physiologically important metal ions. $[\mathbf{1}] = 5.0 \times 10^{-6}$ M. $[M^{n+}] = 5.0 \times 10^{-4}$ M. In 1:1 mixture of DMSO and hepes-buffered H_2O (pH 7.0, 20 mM). $\lambda_{ex} = 470$ nm.

compared with other surveyed metal ions. The prominent fluorescence was almost completely quenched by Cu^{2+} ions. Binding of paramagnetic Cu^{2+} ions in many chemosensing systems is known to commonly result in fluorescence quenching [31]. On the other hand, the fluorescence emission profile did not significantly change in terms of maximum intensity or general peak shape in the presence of other physiologically important metal ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, and Zn^{2+}). The efficiency of the quenching of **1** as expressed by the ratio (I_o/I) of fluorescence intensity in the absence (I_o) and presence (I) of metal ions at 530 nm was 77.9 for Cu^{2+}, and the ratios for common physiologically relevant metal ions varied in a relatively limited range from 0.94 (Cd^{2+}) to 1.08 (Co^{2+}). In fact, in view of selective Cu^{2+} sensing with **1** in real samples, considerable interferences were observed with transition metal ions of Ni^{2+} (1.24), Hg^{2+} (1.64), and Pb^{2+} (2.58), as well as with alkaline-earth Ba^{2+} ions (1.24) (Fig. 4). However, these ions have no significant distribution in physiological environments [32] and selective Cu^{2+} signaling using **1** in physiologically relevant samples is possible. To have an insight into the effects of pH on the chemosensing behavior of **1**, we measured signaling behaviors of **1** in other representative pH-buffered solutions. Contrary to the pronounced Cu^{2+} selectivity in hepes-buffered solution at pH 7.0 (Fig. 4), compound **1** exhibited somewhat reduced selectivity toward Cu^{2+} ions. For example, the I_o/I values for the representative transition metal ions are 15.1 (Cu^{2+}), 0.96 (Zn^{2+}), 0.94 (Cd^{2+}), and 6.25 (Hg^{2+}) for acetate buffered solution at pH 4.8 and 2.75 (Cu^{2+}), 0.93 (Zn^{2+}), 1.01 (Cd^{2+}), and 1.03 (Hg^{2+}) for tris buffered solution at pH 8.1, which are much inferior to the results obtained in hepes-buffered solution.

The Cu^{2+}-selective signaling of **1** was not affected by the background presence of other physiologically important metal ions (Fig. 5). The effects of coexisting metal ions on the fluorescence change of the **1**-Cu^{2+} system were assessed using the ratio $(I_o - I)/(I_o - I_{Cu(II)})$, where I_o, $I_{Cu(II)}$, and I represent the fluorescence intensity of **1** in the absence of any metal ions, in the presence of Cu^{2+} ions, and the fluorescence intensity of **1** in the presence of Cu^{2+} and coexisting metal ions, respectively. Ratios were within a relatively limited range of 0.96 (Fe^{2+}) to 1.02 (Co^{2+}) for common physiologically coexisting metal ions. Interferences from Ba^{2+} (0.53), Hg^{2+} (0.75), and Pb^{2+} (0.72) were significant; however, possibility of interference from these metal ions might be negligible in common physiological samples.

The quantitative nature of **1** in Cu^{2+} sensing was investigated by fluorescence titration. The titration resulted in a smooth decrease in fluorescence intensity of **1** as the concentration of Cu^{2+} increased (Fig. 6). The stoichiometry of the complex formation of **1** with Cu^{2+} was determined to be 1:1 using a Job's plot (Fig. 7) [27]. The association constant, K_{assoc}, estimated by nonlinear curve fitting of the titration data, was 1.9×10^5 M^{-1} [33].

In the present case, Cu^{2+} sensing by **1** is reversible. For example, the fluorescence of **1** was significantly quenched in the presence of 10 equiv of Cu^{2+} ions. Subsequent addition of EDTA (50 equiv) resulted in almost full recovery of fluorescence. Upon further addition of Cu^{2+} ions to this solution, the recovered fluorescence was effectively quenched again.

The signaling seems to be due to selective complexation of inherent fluorescence quenching Cu^{2+} ions with the crown moieties of **1**. Generally, the functional ionophores derived from aza-crown ethers have been designed for the alkali and alkaline-earth cations. However, the presence of one or two nitrogen atoms in the binding site of aza-crown ethers as in compounds **1** and **2** allowed possibility for the recognition of transition metal cations of Ni^{2+}, Cu^{2+}, or Zn^{2+}. In fact, complexing properties of an aza-crown ether derivative having coumarin moiety toward Cu^{2+} ions were reported [34]. The compound seems to form a sandwich type complex consisting of two crown ether moieties and a Cu^{2+} ion as has been reported in a supramolecular sandwich system consisting of two

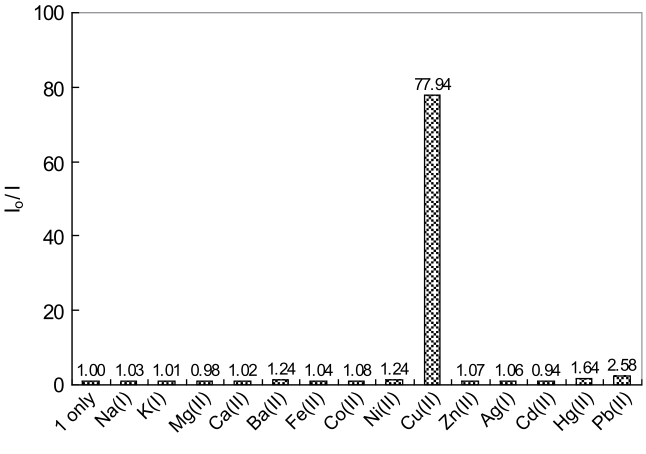

Fig. 4. Changes in fluorescence intensity ratio (I_o/I) at 530 nm of **1** in the presence of various metal ions. $[\mathbf{1}] = 5.0 \times 10^{-6}$ M. $[M^{n+}] = 5.0 \times 10^{-4}$ M. In 1:1 mixture of DMSO and hepes-buffered H_2O (pH 7.0, 20 mM). $\lambda_{ex} = 470$ nm.

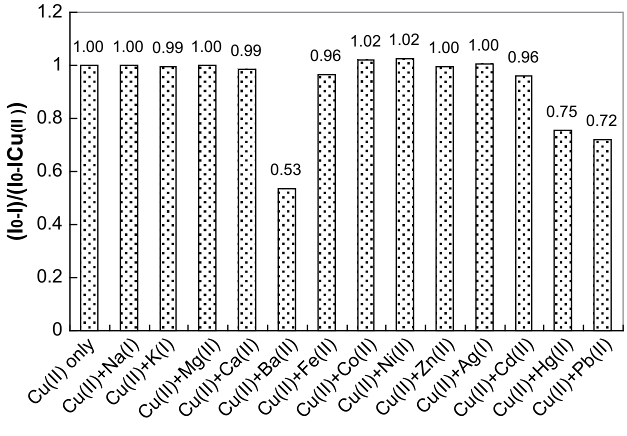

Fig. 5. Selective signaling of Cu^{2+} by **1** in the presence of various metal ions as background. $[\mathbf{1}] = 5.0 \times 10^{-6}$ M, $[Cu^{2+}] = 5.0 \times 10^{-5}$ M, $[M^{n+}] = 5.0 \times 10^{-4}$ M. In 1:1 mixture of DMSO and hepes-buffered H_2O (pH 7.0, 20 mM). $\lambda_{ex} = 470$ nm.

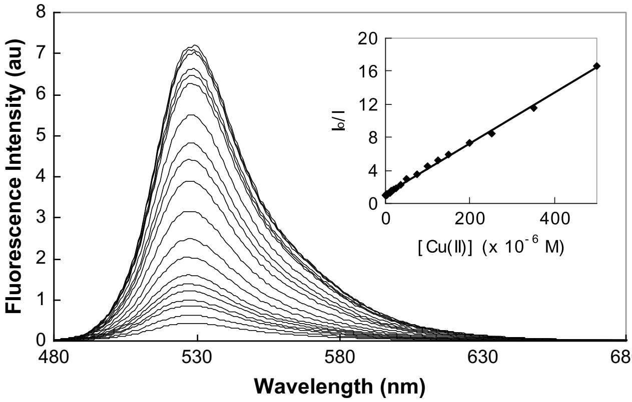

Fig. 6. Fluorescence titration of **1** with Cu^{2+}. Inset shows the Stern–Volmer plot of **1**-Cu^{2+} system. [**1**] = 5.0×10^{-6} M. In 1:1 mixture of DMSO and hepes-buffered H_2O (pH 7.0, 20 mM). $\lambda_{ex} = 470$ nm.

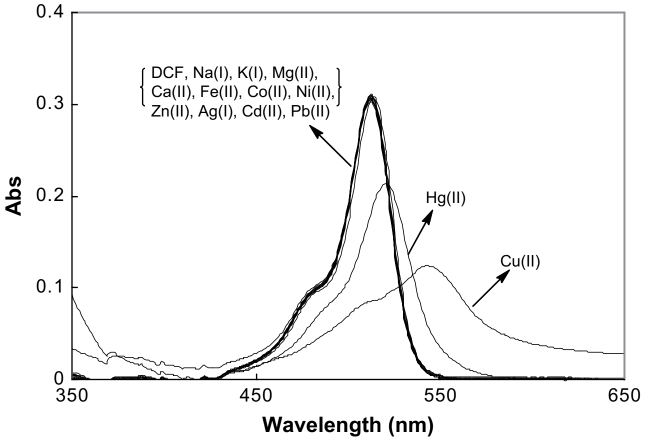

Fig. 8. UV–vis spectra of DCF in the presence of various metal ions. [DCF] = 5.0×10^{-6} M. [M^{n+}] = 5.0×10^{-4} M. In 1:1 mixture of DMSO and hepes-buffered H_2O (pH 7.0, 20 mM).

crown ether molecules and a trigonal–bipyramidal $[Cu(H_2O)_5]^{2+}$ complex [35].

The role of crown moieties in selective signaling of Cu^{2+} ions of **1** was evidenced by the responses toward various metal ions of DCF itself under the same condition. Treatment with 100 equiv of various metal ions resulted in significant changes in absorption behavior of DCF exclusively with Cu^{2+} and Hg^{2+} ions (Fig. 8), and concomitantly the solution color changed from fluorescent yellow to pink with Cu^{2+} ions. Similarly, the fluorescence emission of DCF in the presence of various metal ions resulted in significant quenching with Cu^{2+} and Hg^{2+} ions (left bar, Fig. 9). In fact, the fluorescence emission at 533 nm of DCF was almost completely quenched with Cu^{2+} ions, which is comparable to the behavior of the crown appended DCF **1**. These observations seem to be feasible for DCF as a Cu^{2+}-selective signaling system, however, DCF itself could not tolerate the interference from other commonly existing metal ions. In competition experiments of **1** in the presence of 100 equiv of various coexisting metal ions as background, treatment of Cu^{2+} ions (10 equiv) induced rather insignificant responses (right bar, Fig. 9), that is unfavorable for DCF to be act as a Cu^{2+}-selective sensor in real system.

The quenching nature of the complexed Cu^{2+} ions resulted in efficient reduction of the fluorescence. A remarkable fluorescence quenching of crowned fluorescein was observed in the case of Cu^{2+} ion, which was caused by the specific interaction between the chemosensor and Cu^{2+} ion. We tentatively ascribed this quenching process as a static quenching mechanism that involves the interaction between the fluorescence molecule and the metal ion to form a nonfluorescent complex. The linear Stern–Volmer plot also suggested the static quenching process of **1**-Cu^{2+} system (inset of Fig. 6) [36]. Complexation of **1** with Cu^{2+} ions was further confirmed by mass spectral evidence. A characteristic peak for $[\mathbf{1} + Cu + H]^+$ at *m*/*z* 1014.0 was observed in FAB-MS. Attempts to obtain NMR evidences of the complex formation were not successful due to the presence of paramagnetic Cu^{2+} ions in the samples.

The applicability of **1** in the analysis of Cu^{2+} ions in physiological samples was investigated by fluorescence titration in the presence of physiologically abundant metal ions as background ([Na^+] = 138 mM, [K^+] = 4 mM, [Mg^{2+}] = 1 mM, [Ca^{2+}] = 3 mM, [Fe^{2+}] = 0.02 mM, [Zn^{2+}] = 0.02 mM, and [Co^{2+}] = 0.002 mM) [32]. The titration demonstrated a clear Cu^{2+} concentration-dependent behavior (Fig. 10). From this plot, the detection limit of **1** for Cu^{2+} ions in a physiological environment was calculated to be 2.9×10^{-6} M [26]. Although there were somewhat undesirable interferences from Ba^{2+}, Pb^{2+} and Hg^{2+}, these observations imply that crown-appended derivative **1** could be used as a selective chemosensor for the analysis of submillimolar Cu^{2+} ions in physiological environments.

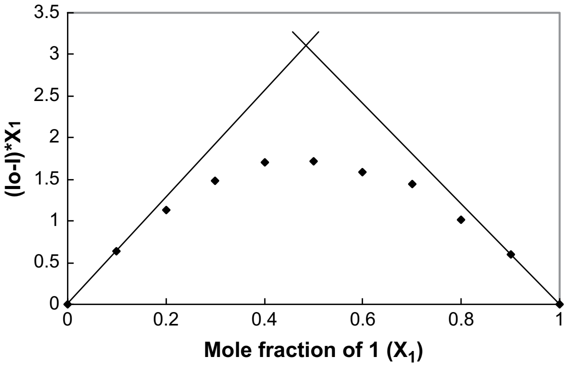

Fig. 7. Job's plot of **1**-Cu^{2+} system in an aqueous 50% DMSO solution.

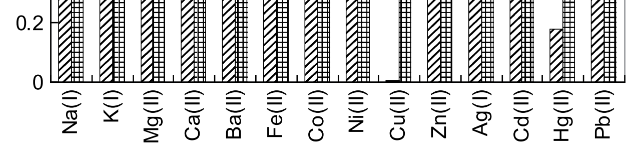

Fig. 9. Changes in fluorescence intensity ratio (I/I_o) at 533 nm of DCF in the presence of various metal ions. [DCF] = 5.0×10^{-6} M. For each of the metal ions, left bar: [M^{n+}] = 5.0×10^{-4} M. Right bar: [M^{n+}] = 5.0×10^{-4} M, [Cu^{2+}] = 5.0×10^{-5} M. In 1:1 mixture of DMSO and hepes-buffered H_2O (pH 7.0, 20 mM). $\lambda_{ex} = 470$ nm.

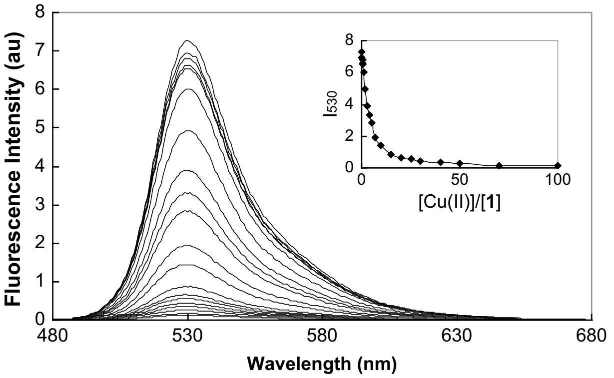

Fig. 10. Fluorescence titration of **1** with Cu^{2+} ions in the presence of physiologically important metal ions as background in an aqueous 50% DMSO solution ($[Na^+] = 138$ mM, $[K^+] = 4$ mM, $[Mg^{2+}] = 1$ mM, $[Ca^{2+}] = 3$ mM, $[Fe^{2+}] = 0.02$ mM, $[Zn^{2+}] = 0.02$ mM, and $[Co^{2+}] = 0.002$ mM).

The chemosensing behaviors of compound **2**, which has 15-crown ether moieties, were also investigated. Generally, the fluorescence behavior of **2** toward various metal ions was similar to that of **1**. Compound **2** exhibited effective fluorescence quenching down to the baseline in the presence of Cu^{2+} ions ($I_o/I = 145.5$), while other physiologically important metal ions did not induce notable responses (Fig. 11). However, the Cu^{2+} selectivity of **2** was less pronounced compared with **1**. Although the changes in the fluorescence intensity ratio I_o/I for most metal ions were minor (0.87 for Na^+ - 1.06 for Mg^{2+}), in addition to Ag^+ (0.76), Cd^{2+} (0.83), Pb^{2+} (2.82), Hg^{2+} (3.03), and especially Co^{2+} (3.49) also significantly interfered the selective sensing of Cu^{2+} ions. The binding stoichiometry was found to be 1:1 and K_{assoc} of **2**-Cu^{2+} system was $1.2 \times 10^5\ M^{-1}$.

All these observations manifest that the DCF derivatives having two aza-crown ether moieties exhibited a pronounced Cu^{2+}-selective chemosensing behavior in aqueous environments. The chemosensing behavior was not significantly affected by the presence of common possibly interfering metal ions as background. Although the prepared crown-dyes have limitations in sensor characteristics including selectivity, detection limit, and especially turn-off type nature compared with other leading Cu^{2+}-signaling systems, the fluorescein-crown conjugate could be used as a new Cu^{2+}-selective chemosensing or basic signaling platform for the construction of other supramolecular systems.

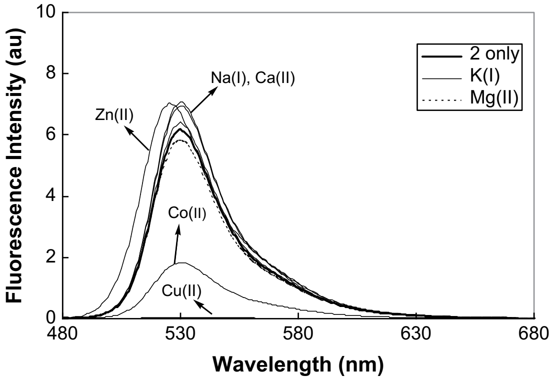

Fig. 11. Changes in fluorescence intensity of **2** in the presence of representative physiologically important metal ions. $[\mathbf{2}] = 5.0 \times 10^{-6}$ M. $[M^{n+}] = 5.0 \times 10^{-4}$ M. In 1:1 mixture of DMSO and hepes-buffered H_2O (pH 7.0, 20 mM). $\lambda_{ex} = 470$ nm.

4. Conclusion

Two new crown-appended fluorescein derivatives were prepared and their transition metal ion-selective chemosensing behaviors were investigated by UV–vis and fluorescence measurements. The 18-crown-6 ether derivative showed a prominent Cu^{2+}-selectivity in samples containing other common physiologically abundant metal ions. Crown appended compound exhibited fluorogenic and chromogenic responses that facilitate Cu^{2+} sensing. The 15-crown-5 ether derivative also revealed similar Cu^{2+}-selectivity, however, with somewhat inferior signaling selectivity. The 18-crown-6 ether-appended dichlorofluorescein might be able to be used as a new Cu^{2+}-sensing probe in aqueous media containing common physiologically important metal ions.

References

[1] de Silva AP, Gunaratne HQN, Gunnlaugsson T, Huxley AJM, McCoy CP, Rademacher JT, et al. Signaling recognition events with fluorescent sensors and switches. Chemical Reviews 1997;97:1515.
[2] Krämer R. Fluorescent chemosensors for Cu^{2+} ions: fast, selective, and highly sensitive. Angewandte Chemie International Edition 1998;37:772.
[3] Martinez R, Zapata F, Caballero A, Espinosa A, Tarraga A, Molina P. 2-Aza-1,3-butadiene derivatives featuring an anthracene or pyrene unit: highly selective colorimetric and fluorescent signaling of Cu^{2+} cation. Organic Letters 2006;8:3235.
[4] Martinez R, Espinosa A, Tarraga A, Molina P. New Hg^{2+} and Cu^{2+} selective chromo- and fluoroionophore based on a bichromophoric azine. Organic Letters 2005;7:5869.
[5] Xu Z, Qian X, Cui J. Colorimetric and ratiometric fluorescent chemosensor with a large red-shift in emission: Cu(II)-only sensing by deprotonation of secondary amines as receptor conjugated to naphthalimide fluorophore. Organic Letters 2005;7:3029.
[6] Bojinov VB, Panov IP. Novel 4-(2,2,6,6-tetramethylpiperidin-4-ylamino)-1,8-naphthalimide based yellow-green emitting fluorescence sensors for transition metal ions and protons. Dyes and Pigments 2009;80:61.
[7] Kim SH, Han SK, Park SH, Lee SM, Lee SM, Koh KN, et al. Use of squarylium dyes as a sensing molecule in optical sensors for the detection of metal ions. Dyes and Pigments 1999;41:221.
[8] Gunnlaugsson T, Leonard JP, Murray NS. Highly selective colorimetric naked-eye Cu(II) detection using an azobenzene chemosensor. Organic Letters 2004;6:1557.
[9] Hennrich G, Walther W, Resch-Genger U, Sonnenschein H. Cu(II)- and Hg(II)-induced modulation of the fluorescence behavior of a redox-active sensor molecule. Inorganic Chemistry 2001;40:641.
[10] Kim SH, Kim JS, Park SM, Chang SK. Hg^{2+}-selective OFF–ON and Cu^{2+}-selective ON–OFF type fluoroionophore based upon cyclam. Organic Letters 2006;8:371.
[11] Bodenant B, Weil T, Businelli-Pourcel M, Fages F, Barbe B, Pianet I, et al. Synthesis and solution structure analysis of a bispyrenyl bishydroxamate calix[4]arene-based receptor, a fluorescent chemosensor for Cu^{2+} and Ni^{2+} metal ions. The Journal of Organic Chemistry 1999;64:7034.
[12] Liang Z, Liu Z, Jiang L, Gao Y. A new fluorescent chemosensor for copper(II) and molecular switch controlled by light. Tetrahedron Letters 2007;48:1629.
[13] Huang X, Guo Z, Zhu W, Xie Y, Tian H. A colorimetric and fluorescent turn-on sensor for pyrophosphate anion based on a dicyanomethylene-4*H*-chromene framework. Chemical Communications 2008:5143.
[14] Guo Z, Zhu W, Shen L, Tian H. A fluorophore capable of crossword puzzles and logic memory. Angewandte Chemie International Edition 2007;46:5549.
[15] Zeng L, Miller EW, Pralle A, Isacoff EY, Chang CJ. A selective turn-on fluorescent sensor for imaging copper in living cells. Journal of the American Chemical Society 2006;128:10.
[16] Que EL, Chang CJ. A smart magnetic resonance contrast agent for selective copper sensing. Journal of the American Chemical Society 2006;128:15942.
[17] Dujols V, Ford F, Czarnik AW. A long-wavelength fluorescent chemodosimeter selective for Cu(II) ion in water. Journal of the American Chemical Society 1997;119:7386.
[18] Xiang Y, Tong A, Jin P, Yong J. New fluorescent rhodamine hydrazone chemosensor for Cu(II) with high selectivity and sensitivity. Organic Letters 2006;8:2863.
[19] Zhang X, Shiraishi Y, Hirai T. Cu(II)-selective green fluorescence of a rhodamine–diacetic acid conjugate. Organic Letters 2007;9:5039.
[20] Nolan EM, Jaworski J, Okamoto KI, Hayashi Y, Sheng M, Lippard SJ. QZ1 and QZ2: rapid, reversible quinoline-derivatized fluoresceins for sensing biological Zn(II). Journal of the American Chemical Society 2005;127:16812.
[21] Walkup GK, Burdette SC, Lippard SJ, Tsien RY. A new cell-permeable fluorescent probe for Zn^{2+}. Journal of the American Chemical Society 2000;122:5644.
[22] Burdette SC, Walkup GK, Spingler B, Tsien RY, Lippard SJ. Fluorescent sensors for Zn^{2+} based on a fluorescein platform: synthesis, properties and intracellular distribution. Journal of the American Chemical Society 2001;123:7831.

[23] Woodroofe CC, Lippard SJ. A novel two-fluorophore approach to ratiometric sensing of Zn^{2+}. Journal of the American Chemical Society 2003; 125:11458.

[24] Komatsu K, Kikuchi K, Kojima H, Urano Y, Nagano T. Selective zinc sensor molecules with various affinities for Zn^{2+}, revealing dynamics and regional distribution of synaptically released Zn^{2+} in hippocampal slices. Journal of the American Chemical Society 2005;127:10197.

[25] Zhang X, Shiraishi Y, Hirai T. Fe(III)- and Hg(II)-selective dual channel fluorescence of a rhodamine–azacrown ether conjugate. Tetrahedron Letters 2008;49:4178.

[26] Shortreed M, Kopelman R, Kuhn M, Hoyland B. Fluorescent fiber-optic calcium sensor for physiological measurements. Analytical Chemistry 1996;68:1414.

[27] Connors KA. Binding constants. New York: Wiley; 1987.

[28] Mokhir A, Kiel A, Herten DP, Kraemer R. Fluorescent sensor for Cu^{2+} with a tunable emission wavelength. Inorganic Chemistry 2005;44:5661.

[29] Swamy KMK, Lee YJ, Lee HN, Chun J, Kim Y, Kim SJ, et al. A new fluorescein derivative bearing a boronic acid group as a fluorescent chemosensor for fluoride ion. The Journal of Organic Chemistry 2006;71:8626.

[30] Sparano BA, Shahi SP, Koide K. Effect of binding and conformation on fluorescence quenching in new 2′,7′-dichlorofluorescein derivatives. Organic Letters 2004;6:1947.

[31] Pina F, Bernardo MA, Garcia-Espana E. Fluorescent chemosensors containing polyamine receptors. European Journal of Inorganic Chemistry 2000;10:2143.

[32] Hay RW. Bio-inorganic chemistry. Chichester: Ellis Horwood; 1984. p. 10.

[33] Kuzmic P. Program DYNAFIT for the analysis of enzyme kinetic data: application to HIV proteinase. Analytical Biochemistry 1996;237:260.

[34] Badaoui FZ, Bourson J. Chromoionophores derived from a coumarin linked to monoaza- and diaza-crown ethers: formation of divalent 3d metal complexes. Analytica Chimica Acta 1995;302:341.

[35] Shivaiah V, Das SK. Fivefold coordination of a Cu(II)-aqua ion: a supramolecular sandwich consisting of two crown ether molecules and a trigonal–bipyramidal $[Cu(H_2O)_5]^{2+}$ complex. Angewandte Chemie International Edition 2006;45:245.

[36] Gao L, Wang JQ, Huang L, Fan XX, Zhu JH, Wang Y, et al. Novel inorganic–organic hybrid fluorescence chemosensor derived from SBA-15 for copper cation. Inorganic Chemistry 2007;46:10287.