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Readily Available Fluorescence Probes for Zinc Ion in Aqueous Solution of Neutral pH

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Readily Available Fluorescence Probes for Zinc Ion in Aqueous Solution of Neutral pH

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This Paper is Dedicated to Professor Eiichi Kimura on the Occasion of his Retirement.

Zinc ion in aqueous solution of neutral pH was detected by a probe that is readily obtained by simply mixing commercially available cyclen (a Zn(II) receptor) and lumazine or lumichrome (a heterocyclic fluorophore containing an imide moiety as partial structure) in an equal molar ratio. The initially generated cyclen-Zn(II) complex interacts with lumazine to form a cyclen-Zn(II)-lumazine complex whereby the intensity of fluorescence is enhanced. These Zn(II) probes showed excellent selectivity for Zn(II) over other divalent metal ions such as Pb(II), Cu(II), Co(II), Ni(II), and good selectivity over Cd(II). The X-ray crystal structure of the cyclen-Zn(II)-lumazine complex revealed that the cyclen-chelated Zn(II) binds deprotonated lumazine at the N-1.

Keywords: Zinc sensor; Cyclen-Zn(II)-lumazine complex; Cyclen; Lumazine; Lumichrome

INTRODUCTION

Many of transition-metal ions serve as essential trace elements in biological systems. Of these metal ions, Zn(II) is an especially important divalent cation that plays vital roles in many cellular processes including DNA synthesis, microtubule polymerization, apoptosis, gene expression, and protein structure and function [1–4]. The Zn(II) is also implicated in the formation of amyloid plaques during the onset of Alzheimer's disease [5] and activating protein kinase C [6]. Accordingly, Zn(II) has received much attention in recent years and numerous Zn(II) sensors have been developed. Most of the Zn(II) sensors reported are chelation-enhanced fluorescence probes based on fluorophores such as quinoline [7–9], dansyl [10–12], fluorescein [13,14], and anthracene [15]. In these sensors, binding of the Zn(II) to a receptor is coupled to fluorescence emission change of the covalently attached fluorophore [16–19]. We wish to report herein simple probes for the Zn(II) which can be readily obtainable simply by mixing a zinc receptor (cyclen) and a fluorophore (lumazine) in water of neutral pH.

RESULTS AND DISCUSSION

It is well established that Zn(II) forms a complex with 1,4,7,10-tetraazacyclododecane (cyclen), and the cyclen-Zn(II) complex thus formed binds to nitrogen heterocycles containing an imide functionality, such as thymidine, to form a stable ternary complex [20–22]. The acidity of Zn(II) is reinforced by the chelation and displaces the cyclic imide proton to form a coordinative bond between the Zn(II) and the deprotonated imide anion [20–22]. Lumazine is a water-soluble nitrogen heterocyclic compound bearing an imide moiety as partial structure. Its aqueous solution is known to show bluish-green fluorescence at pH 7.0 but it turns to green when the solution becomes alkaline, which is ascribed to the deprotonated lumazine that is generated by base [23]. It was, therefore, thought that lumazine may serve

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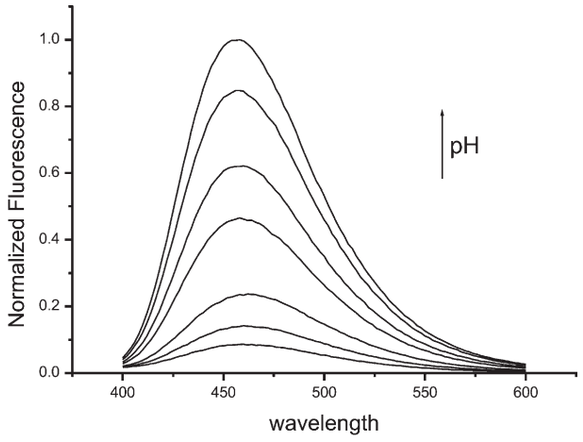


FIGURE 1 Fluorescence emission spectra of lumazine (100 μM) in buffer solution of different apparent pH: 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. Excitation was at 355 nm.

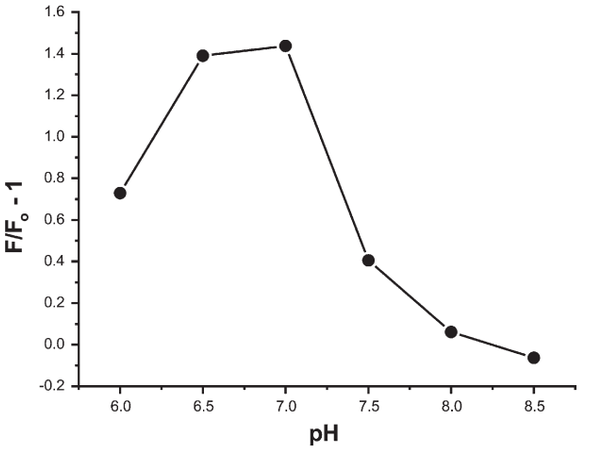
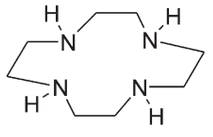


FIGURE 2 Plot of $F/F_0 - 1$ against pH. F and F_0 express fluorescence intensity of the mixture of cyclen (100 μM) and lumazine (100 μM) in the presence and absence of Zn(II) at each pH.

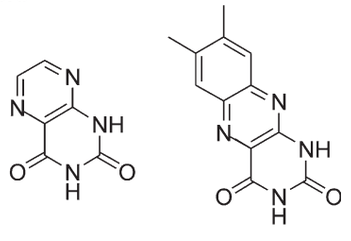
as a viable signaling element for a probe for Zn(II) should it be combined with cyclen.

Receptor



Cyclen

Fluophore



Lumazine Lumichrome

It has been known that lumazine is present predominantly in the form of monoanion in the aqueous solution of pH 10 and the monoanion shows a fluorescence emission maximum at 467 nm with a quantum yield of 0.24 when excited at 370 nm [24]. We have examined pH dependent fluorescence of the aqueous solution of lumazine, the results of which are depicted in Fig. 1. It can be seen in Fig. 1 that the fluorescence intensity at 467 nm is enhanced by 4.1-fold by changing the pH from 7.0 to 9.0, supporting the design rationale that lumazine may be a useful fluorophore for a Zn(II) sensor when combined with cyclen.

As expected, the fluorescence intensity of lumazine remained unchanged when Zn(II) was added to an aqueous solution of lumazine up to three molar equivalent quantity. The emission intensity was, however, increased instantaneously upon addition of cyclen to the neutral aqueous solution containing Zn(II) and lumazine, indicating that in the presence of cyclen, Zn(II) forms a species that is responsible for the increment of fluorescence intensity. Figure 2 shows the effect of pH of the aqueous medium on the fluorescence of the Zn(II) probe, demonstrating that the maximum change of the fluorescence is obtainable at the neutral pH. The fluorescence intensity is decreased drastically as the pH of the medium increases. The fluorescent species is expectedly a ternary complex of cyclen-Zn(II)-lumazine formed with the displacement of one of the acidic protons in lumazine by the cyclen-chelated Zn(II). The maximum fluorescence enhancement was obtained when the ternary mixture was excited at the wavelength of 355 nm (Fig. 3).

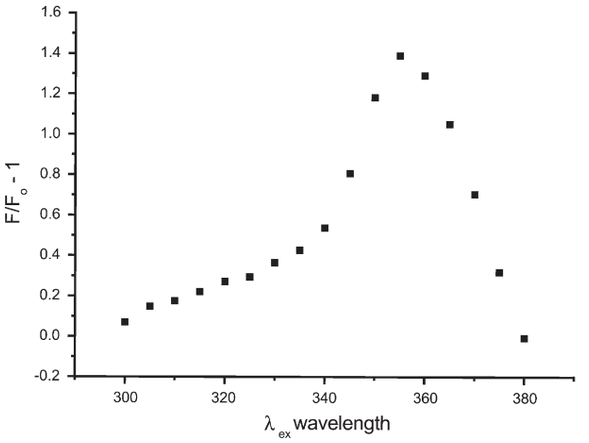


FIGURE 3 Plot of $F/F_0 - 1$ against wavelength of excitation. F and F_0 express fluorescence intensity of the mixture of cyclen (100 μM) and lumazine (100 μM) in the presence and absence of Zn(II) at pH 7.0.

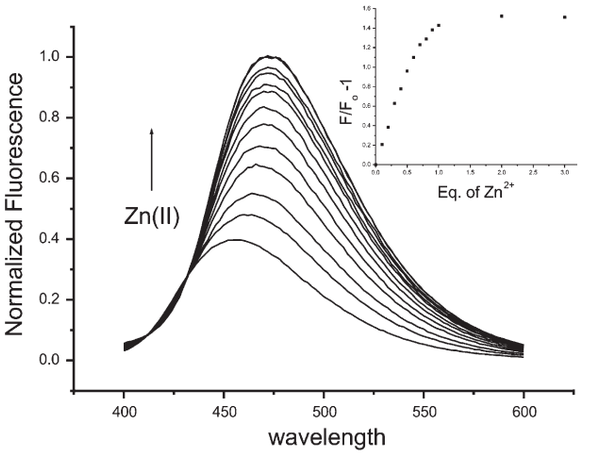


FIGURE 4 Fluorescence emission spectra obtained by addition of Zn(II) (0–300 μM) to pH 7.0 buffer solution containing cyclen (100 μM) and lumazine (100 μM). Excitation was made at 355 nm. Inset: Plot of $F/F_0 - 1$ against concentration of Zn(II) expressed by number of equivalency to the concentration of lumazine, F and F_0 represent fluorescence intensity at λ_{max} in the presence and absence of Zn(II).

Fluorescence emission spectra of the cyclen–lumazine probe at pH 7.0 in the presence of varied concentrations of Zn(II) are shown in Fig. 4. The emission maximum at 450 nm experiences a gradual red shift upon its intensity increase. As can be seen from the inset of Fig. 4, the fluorescence intensity increases linearly with the concentration of Zn(II) up to the point where an equimolar amount of Zn(II) is added and remains at a plateau, supporting the proposition that a 1:1:1 ternary complex of cyclen-Zn(II)-lumazine is formed. It is worth mentioning that a similar fluorescence enhancement effect was observed when an equimolar powdery mixture of cyclen and lumazine was added to an aqueous Zn(II) solution of neutral pH. The maximum enhancement of fluorescence intensity was achieved in several minutes after the powdery mixture had been added. This experiment demonstrates that Zn(II) in aqueous solution may be detected readily by the addition of the powdery mixture. We have determined the association constant (K_{ass}) as well as thermodynamic parameters for the formation of the ternary complex from Zn(II)-cyclen and lumazine in the aqueous medium at 30°C by isothermal titration calorimetry (ITC) [24]. Figure 5 shows the ITC plot of the titration of Zn(II)-cyclen with lumazine, from which the molar enthalpy (ΔH^0) and free energy (ΔG^0) were obtained to be -5.94 and $-5.29\,\text{kcal mol}^{-1}$, respectively. The entropy (ΔS^0) was calculated to be -2.11 eu from the Gibbs–Helmholtz equation and the K_{ass} value of $6556 \pm 129\,\text{M}^{-1}$ was obtained from the equation, $\Delta G^0 = -RT \ln K_{ass}$ in which R and T represent the gas constant and absolute temperature, respectively. On the basis of the thermodynamic parameters, it may be concluded that the ternary

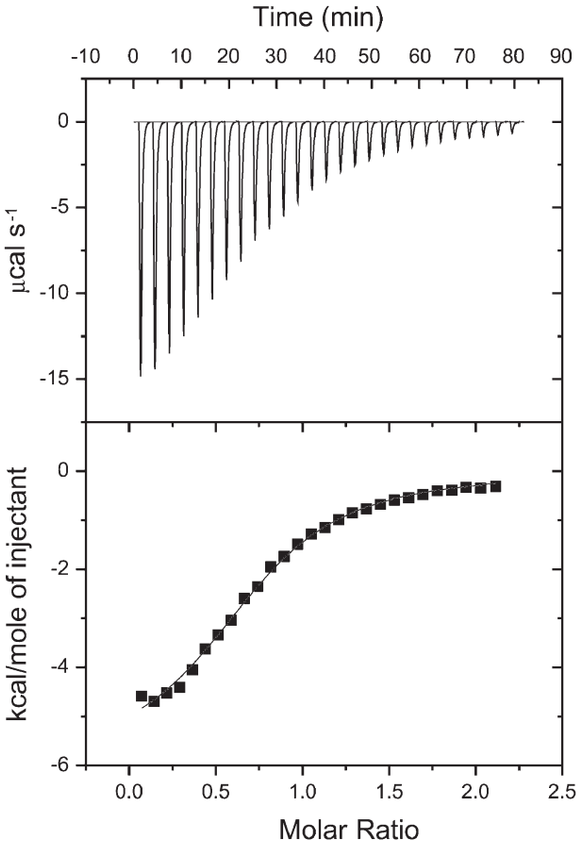


FIGURE 5 Plot of the ITC titration of Zn(II)-cyclen (1.0 mM) with lumazine (10 mM) in the buffer pH 7.0 at 30°C.

complex formation is enthalpy driven, suggesting that strong electrostatic interactions are in effect between the Zn(II) in Zn(II)-cyclen and the lumazine.

The cyclen–lumazine combination showed a good selectivity for Zn(II) over the other divalent metal ions (Fig. 6 and Table I). No significant fluorescence intensity increase was observed upon addition of Ni(II), Pb(II), Cu(II), or Co(II), which may be due to either the fluorescence quenching properties of the metal ions or failure of these metal ions to form

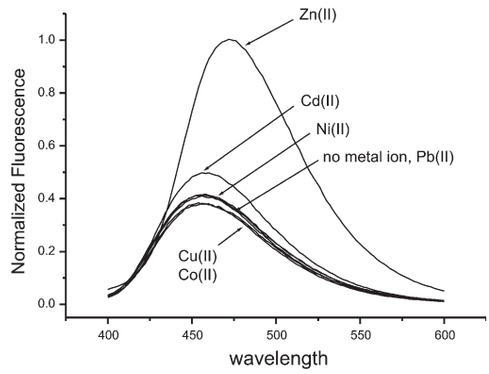


FIGURE 6 Fluorescence spectra obtained at $\lambda_{ex} = 355$ nm.

TABLE I Selectivity for metal ions

Ternary complex for metal ions	Zn(II)	Cd(II)	Ni(II)	Pb(II)	Cu(II)	Co(II)
Cyclen + Lumazine*	1.43	0.20	0.01	0.01	−0.07	−0.07
Cyclen + Lumichrome†	0.57	0.09	−0.03	−0.03	−0.10	−0.12

*Each metal ion (100 μM) was added to the HEPES buffer (pH = 7.0) solution containing cyclen (100 μM) and lumazine (100 μM) and fluorescence emission was monitored. The change of fluorescence intensity was expressed as $F/F_0 - 1$ in which F and F_0 represent the fluorescence intensity at λ_{max} in the presence and absence of metal ion, respectively. †These data were obtained in the similar fashion except that lumichrome was used instead of lumazine as the fluorophore and pH of the buffer was 7.5 rather than 7.0.

the fluorescent ternary complexes. Although addition of Cd(II) increased the fluorescence intensity, the increase corresponds to only 14% of that caused by Zn(II).

Since UV light is known to cause damage to cells, we were interested in finding fluorophore whose ternary complex with Zn(II) and cyclen can be excited by visible light. We have examined lumichrome as an alternative fluorophore to lumazine for the Zn(II) probe. Lumichrome also bears an imide moiety as partial structure, and thus is expected to form a ternary complex with cyclen-Zn(II). The fluorescence emission enhancement of the aqueous solution of cyclen and lumichrome resulting from addition of Zn(II) was most profound when the solution of the ternary complex was excited at 420 nm, which falls in the visible region. Figure 7 shows changes of the fluorescence emission of the cyclen-Zn(II)-lumichrome mixture resulting from the increased concentration of Zn(II) at pH 7.5. The plot of the intensity increase against concentration of Zn(II) presented in the inset of Fig. 7 indicates that the fluorescent ternary complex consists of lumichrome, Zn(II), and cyclen in a 1:1:1 stoichiometry.

Unlike N-1 substituted imide-bearing heterocycles, lumazine bears two acidic protons that can be deprotonated, i.e. protons at the N-1 and N-3 positions. Accordingly, the deprotonation of lumazine by the cyclen-chelated Zn(II) may take place at either the N-1 or N-3 position. We have established by the X-ray crystallographic structural analysis of the crystal of cyclen-Zn(II)-lumazine complex that the binding of the Zn(II) to lumazine occurs at the N-1 position. The crystal of the ternary cyclen-Zn(II) complex with lumazine was obtained in the form of perchlorate salt from the aqueous solution of an equimolar mixture of lumazine, cyclen-Zn(II) complex, and LiOH. An ORTEP drawing of the cyclen-Zn(II)-lumazine complex with the atom-numbering system is shown in Fig. 8. Crystallographic data and diffraction data collection parameters are collected in Table II, and selected bond distances and bond angles are listed in Table III.

Zn(II) is coordinated in a square pyramidal fashion by four ring nitrogen atoms of cyclen and the N-1 nitrogen atom of lumazine. The Zn(II)–N(1)

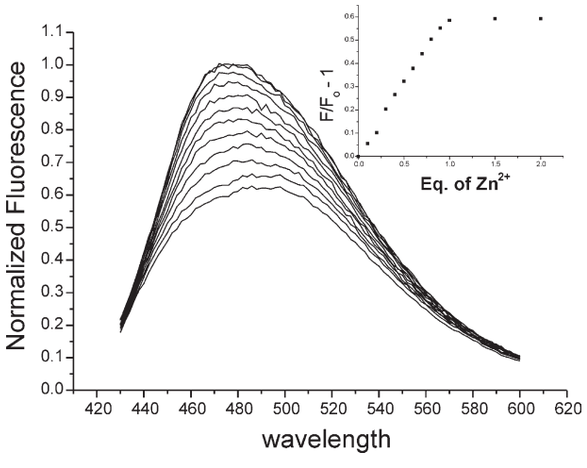


FIGURE 7 Fluorescence emission spectra obtained by addition of Zn(II) (0–200 μM) to a buffer solution containing cyclen, pH 7.0 (100 μM) and lumichrome (100 μM). Excitation was made at 420 nm. Inset: Plot of $F/F_0 - 1$ against concentration of Zn(II) expressed by number of equivalency to the concentration of lumichrome, F and F_0 represent fluorescence intensity at λ_{max} in the presence and absence of Zn(II).

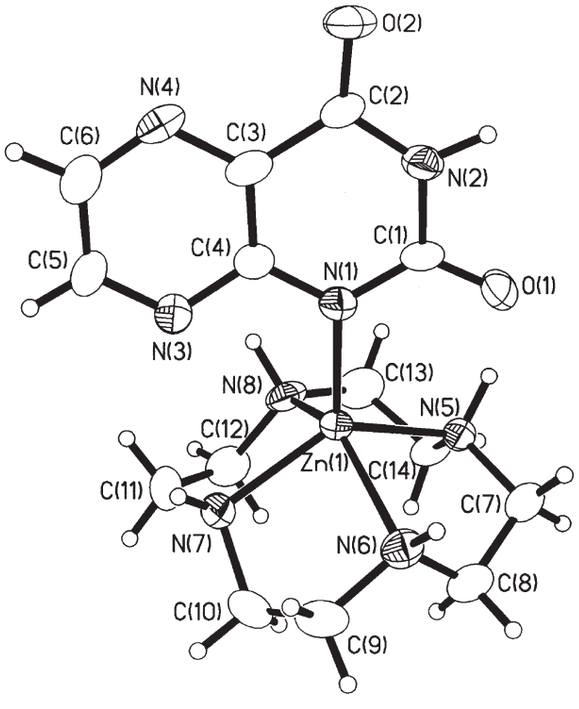


FIGURE 8 ORTEP drawing of the X-ray crystal structure of cyclen-Zn(II)-lumazine complex. Thermal ellipsoids are shown at the 50% probability level. Perchlorate counter ion is omitted for clarity.

TABLE II Crystallographic parameters

Formula	$C_{14}H_{23}ClN_8O_6Zn$
Fw	500.22
Crystal color	Pale yellow
Crystal dimensions (mm)	0.30 × 0.30 × 0.20
Crystal system	Triclinic
Space group	*P*-1
Lattice parameters	$a = 11.6842(5)$ Å $\alpha = 97.2530(10)°$ $b = 11.8525(5)$ Å $\beta = 92.5730(10)°$ $c = 14.3821(6)$ Å $\gamma = 94.7500(10)°$ $V = 1965.92(14)$ Å^3
Z	4
r_c (g cm^{-3})	1.690
Radiation	Mo Kα($\lambda = 0.71073$ Å)
m (cm^{-1})	14.38
q range for data collection (deg)	2.10–24.15°
Refinement method	Full-matrix least-squares on F^2
No. of indep reflnc ($\|I_0\| > 3s(I_0)$)	5898
R	0.0869
R_w	0.2573

bond distance of 1.977 Å is considerably shorter than the Zn(II)–N bond distances between the Zn(II) and the four nitrogen atoms in the cyclen, suggesting that the Zn(II) prefers anionic nitrogen to neutral amino groups in forming the coordinative bond. The average bond distance between the Zn(II) and the nitrogen atoms in the cyclen is 2.152 (± 0.003) Å. The hydrogen atoms of the NH and CH_2 groups were located by geometrical calculations. The distance between the carbonyl oxygen atom at the 2-position of lumazine and the hydrogen atom at the N(5) of cyclen in the ternary complex was estimated to be 2.20 Å when the NH bond was assumed to be 0.98 Å, suggesting that an intramolecular hydrogen bond is formed between the N(5) and O(1). Klein and Tatischeff reported that the fluorescence of lumazine in aqueous solution of pH 10 is mainly due to the N-1 deprotonated anion that is present in about 75% [24]. The X-ray crystallographic data of the cyclen-Zn(II)-lumazine ternary complex are in accord with the report of Klein and Tatischeff [25]. The present observation that the cyclen-chelated Zn(II) coordinates lumazine at its N-1 position is consistent with the finding reported recently by Kimura *et al.* that the proton at the N-1 position of 6-((1,4,7,10-tetraazacyclododecyl)methyl)uracil is selectively deprotonated upon its cyclen forming a complex with Zn(II) [26]. Lumazine can be considered as uracil that is fused to pyrazine.

TABLE III Selected bond distances (Å) and bond angles (deg) of cyclen-Zn(II)-lumazine ClO_4^-

Zn(1)–N(1)*	1.977(5)	Zn(1)–N(5)	2.142(5)
Zn(1)–N(6)	2.200(6)	Zn(1)–N(7)	2.103(6)
Zn(1)–N(8)	2.163(6)	N(1)–C(1)	1.354(9)
Zn(1)–C(4)	1.366(9)	N(1)–C(3)	1.355(9)
Zn(1)–C(5)	1.320(9)	O(1)–C(1)	1.245(9)
Zn(1)–C(2)	1.220(8)		
O(1)···N(5)	2.836(8)		
O(1)···H(5)	2.20		
N(1)–Zn(1)–N(7)	119.7(2)	N(1)–Zn(1)–N(5)	108.1(2)
N(7)–Zn(1)–N(5)	132.1(2)	N(1)–Zn(1)–N(8)	107.2(2)
N(7)–Zn(1)–N(8)	82.7(3)	N(5)–Zn(1)–N(8)	80.8(2)
N(1)–Zn(1)–N(6)	115.6(2)	N(7)–Zn(1)–N(6)	81.5(2)
N(5)–Zn(1)–N(6)	80.5(2)	N(8)–Zn(1)–N(6)	136.7(2)

*ESD in parentheses. †Value from the calculated H position (N–H = 0.98 Å).

In conclusion, whereas most of the Zn(II) sensors reported so far are macromolecules in which a fluorophore is conjugated covalently to a receptor for Zn(II), requiring laborious chemical synthesis for their preparations, the present probes are readily generated by simply mixing an equivalent amount of cyclen (a receptor for Zn(II)) and lumazine (fluorophore) in water. No organic cosolvent is required. For more practical purposes, the powdery mixture of cyclen and lumazine may be added to the aqueous solution of the analyte. These probes showed good selectivity for Zn(II) over Cd(II), and excellent selectivity over other transition metal divalent ions in neutral aqueous solution. Finally, the probe consisting of cyclen and lumichrome and its ternary complex with Zn(II) can be excited at 420 nm, a wavelength relatively harmless to cells.

EXPERIMENTAL

All reagents used in this work were obtained from Sigma–Aldrich Chemical Co. and were used without further purification. All buffer solutions were prepared using deionized water and compositions of the buffer solutions are as the following: pH 6.0 (MES 10 mM, $NaNO_3$ 100 mM); pH 6.5 (MES 10 mM, $NaNO_3$ 100 mM); pH 7.0 (HEPES 10 mM, $NaNO_3$ 100 mM); pH 7.5 (HEPES 10 mM, $NaNO_3$ 100 mM); pH 8.0 (HEPES 10 mM, $NaNO_3$ 100 mM); pH 8.5 (CHES 10 mM, $NaNO_3$ 100 mM); pH 9.0 (CHES 10 mM, $NaNO_3$ 100 mM). All metal ion solutions were prepared from the respective metal perchlorate and deionized water. Melting point was determined on a Thomas–Hoover capillary melting point apparatus and was not corrected. IR spectrum was measured on a Bruker Equinox 55 FT-IR spectrometer. Elemental analysis was performed at Pohang University of Science and Technology, Pohang, Korea. Fluorescence measurements were carried out with PTI model D-104 microscope photometer. Isothermal titration calorimetric experiments were performed with an isothermal titration calorimeter purchased from Microcal Inc.

Fluorimetric Study

A 3.0 ml solution containing 100 μM of cyclen and lumazine in the HEPES buffer (pH 7.0) was prepared, and an initial fluorescence measurement was made. Aliquots of Zn(II) from standardized stock solution were titrated into the solution to give

final concentrations of 10–100 (10 μM increments), 200 and 300 μM, and the fluorescence spectra were recorded. The fluorescence spectra were acquired by exciting at 355 nm.

Synthesis of Cyclen-Zn(II)-Lumazine Perchlorate

Cyclen-Zn(II) complex was prepared by stirring at room temperature for 2 h the mixture obtained by adding slowly a solution of Zn(II) $(ClO_4^-)_2 \cdot 6H_2O$ (108 mg, 0.29 mmol) in CH_3OH (10 ml) to a solution of cyclen (50 mg, 0.29 mmol) dissolved in CH_3OH (10 ml), then evaporation of the solvent *in vacuo* to give cyclen-Zn(II) $(ClO_4^-)_2$. Lumazine (18 mg, 0.11 mmol) was added to an aqueous solution of the cyclen-Zn(II) complex (48.5 mg, 0.11 mmol) and $LiOH \cdot H_2O$ (4.5 mg, 0.11 mmol) obtained by dissolving them in 1 ml of distilled water, and the resulting mixture was heated at 40–50°C until a clear solution was obtained. The solution was then allowed to stand in a refrigerator for a day, whereby crystals precipitated. The precipitate was collected and recrystallized from distilled water to give pale yellow triclinic crystals (29 mg, 52% yield). Mp. >300°C. IR (KBr): 3433, 3294, 3237, 1685, 1635 cm^{-1}. ^1H NMR (DMSO-d_6) 2.45 (1H, s), 2.61 (1H, s), 2.65–2.79 (16H, m), 3.29 (2H, d, $J = 7.5$ Hz), 8.29 (1H, s), 8.50 (1H,s). Anal. Calcd for $C_{14}H_{23}ClN_8O_6Zn$: C, 33.61; H, 4.63; N, 22.40. Found: C, 33.35; H, 4.79; N, 22.15.

X-ray Crystallographic Study

A triclinic crystal of cyclen-Zn(II)-lumazine ClO_4^- with dimensions of 0.30 mm × 0.30 mm × 0.20 mm was used for the X-ray diffraction data collection. The lattice parameters and intensity data were obtained with Siemens SMART CCD diffractometer having Mo Kα radiation ($\lambda = 0.71073$ Å). The structure was solved by Patterson methods (SHELXS-86), and the non-hydrogen atoms were refined anisotropically (SHELXL-86). Final full-matrix least square refinements on F^2 was based on 5898 observed reflections to give $R = 0.0869$ and $R_W = 0.2573$. Two crystallographically independent cyclen-Zn(II)-lumazine ClO_4^- are present in a unit cell.

Determinations of Thermodynamic Parameters for the Binding of Lumazine to Cyclen-Zn(II) with a Isothermal Titration Calorimeter

An aqueous solution (1.4 ml, pH 7.0) of cyclen-Zn(II) (1.0 mM) was added to the calorimetry cell. To this solution was injected a 10 μl portion of aqueous lumazine solution (10 mM) 27 times. The mixture was continuously stirred and was kept at an operating temperature of 30°C. The data were analyzed and fitted using the software Origin.

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References

[1] Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543.
[2] Zalewski, P. D.; Forbes, lan J.; Betts, W. H. *Biochem. J.* **1993**, *296*, 403.
[3] Berg, J. M. *J. Biol. Chem.* **1990**, *265*, 6513.
[4] Coyle, P.; Zalewski, P. D.; Philcox, J. C.; Forbes, lan J.; Ward, A. D.; Lincon, S. F.; Mahadevan, I.; Rofe, A. M. *Biochem. J.* **1994**, *303*, 781.
[5] Bush, A. I.; Pettingel, W. H.; Multhaup, G.; Paradis, M. D.; Vonsattel, J.-P.; Gusella, J. F.; Beyreuther, K.; Masters, C. L.; Tanzi, R. E. *Science* **1994**, *265*, 1464.
[6] Csermely, P.; Szamel, M.; Resch, K.; Somogyi, J. *J. Biol. Chem.* **1988**, *263*, 6487.
[7] Savage, D. D.; Montano, C. Y.; Kasarskis, E. *J. Brain Res.* **1989**, *496*, 257.
[8] Budde, T.; Minta, A.; White, J. A.; Kay, A. R. *Neuroscience* **1997**, *79*, 347.
[9] Nasir, M. S.; Fahrni, C. J.; Suhy, D. A.; Kolodsick, K. J.; Singer, C. P.; O'Halloran, T. V. *J. Biol. Inorg. Chem.* **1999**, *4*, 775.
[10] Koike, T.; Watanabe, T.; Aoki, S.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 12696.
[11] Prodi, L.; Bolletta, F.; Montalti, M.; Zaccheroni, N. *Eur. J. Inorg. Chem.* **1999**, 455.
[12] Thompson, R. B.; Maliwal, B. P.; Feliccia, V. L.; Fierke, C. A.; McCall, K. *Anal. Chem.* **1998**, *70*, 4717.
[13] Hirano, T.; Kikuchi, K.; Urano, Y.; Higuchi, T.; Nagano, T. *J. Am. Chem. Soc.* **2000**, *122*, 12399.
[14] Walkup, G. K.; Burdette, S. C.; Lippard, S. J.; Tsien, R. Y. *J. Am. Chem. Soc.* **2000**, *122*, 5644.
[15] Akkaya, E. U.; Huston, M. E.; Czarnik, A. W. *J. Am. Chem. Soc.* **1990**, *112*, 3590.
[16] de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
[17] Bargossi, C.; Fiorini, M. C.; Montalti, M.; Prodi, L.; Zaccheroni, N. *Coord. Chem. Rev.* **2000**, *208*, 17.
[18] Kimura, E.; Koike, T. *Chem. Soc. Rev.* **1998**, *27*, 179.
[19] Kimura, E.; Aoki, S. *Biometals* **2001**, *14*, 191.
[20] Shionoya, M.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1993**, *115*, 6730.
[21] Shionoya, M.; Ikeda, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *116*, 3848.
[22] Koike, T.; Gotoh, T.; Aoki, S.; Kimura, E.; Shiro, M. *Inorg. Chim. Acta* **1998**, *270*, 424.
[23] Budavari, S. *The Merck Index*; 11th Ed., Merck and Co.: Rahway, 1989; p 880.
[24] Jelesarov, I.; Bosshard, H. R. *J. Mol. Recog.* **1999**, *12*, 3.
[25] Klein, R.; Tatischeff, I. *Photochem. Photobiol.* **1987**, *45*, 55.
[26] Kimura, E.; Kitamura, H.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1997**, *119*, 10909.