ORGANIC LETTERS

2007
Vol. 9, No. 24
4995−4998

A Water-Soluble, Small Molecular Fluorescent Sensor with Femtomolar Sensitivity for Zinc Ion

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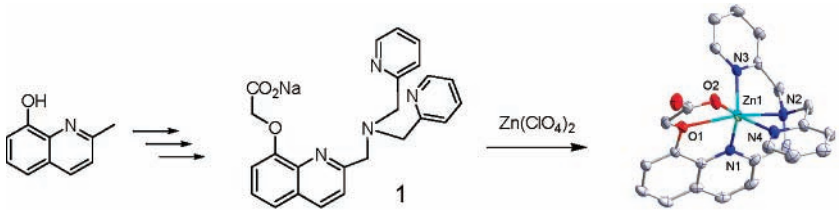
Received September 6, 2007

ABSTRACT



A water-soluble fluorescent sensor, 1, based on the quinoline platform, demonstrates femtomolar sensitivity for zinc ion with a 14-fold enhanced quantum yield upon chelation to zinc ion and also exhibits high selectivity to zinc ion over other physiological relevant divalent metals in the presence of EDTA. X-ray crystal structure of zinc complex reveals that an acetic carboxylic group participates in coordination, which significantly enhances the affinity of 1 for zinc ion.

Recent advances in biology have shed light on the biological roles of zinc, particularly, on its functions related to neurobiology.[1] It is believed that disorder of zinc homeostasis is implicated in a number of diseases,[2] such as Alzheimer's disease. Therefore, developing methods for selectively visualizing free zinc in living systems, where its concentration is in the range of 1 fM in bacterial cells to 0.1 mM in some vesicles,[3] can allow one to understand the physiological and pathological roles of zinc in nature.[1b] For this purpose, a number of fluorescent sensors for zinc with apparent dissociation constants in the nanomolar range or higher,[4] based on quinoline,[5] fluorescein,[6] and peptides as well,[7] have been reported. Yet, there are few small molecular fluorescent sensors with dissociation constants for zinc ions at the level of picomolarity[4,8] or femtomolarity except for biosensors.[9,10]

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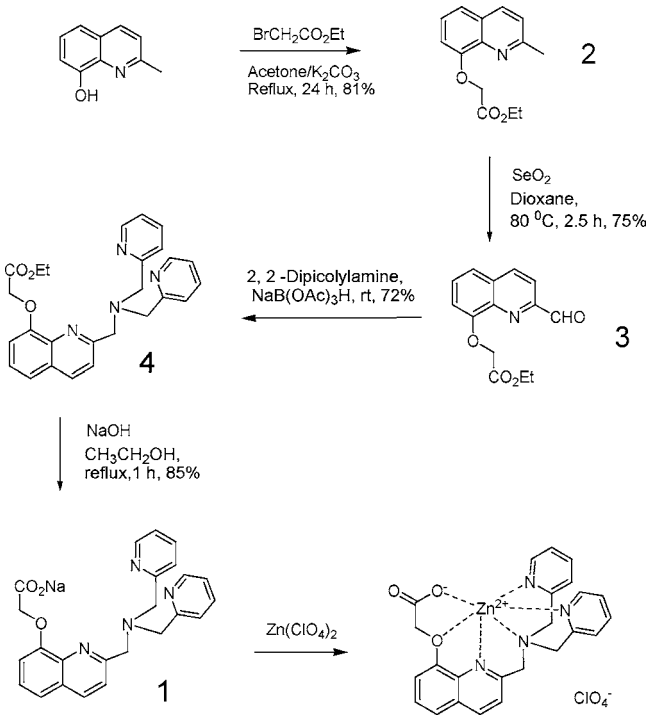
10.1021/ol702192m CCC: $37.00
Published on Web 10/23/2007

Thus, it is desirable to develop new small molecular fluorescent sensors with extremely high affinity for zinc ions and good selectivity over other physiological relevant divalent metals.

8-Hydroxy-2-methylquinoline (Oxn) is a well-known building block for constructing chelation-enhanced fluorescent sensors for transition metals.[11] For example, Oxn derivatives exhibited remarkable fluorescence properties for sensing zinc ions upon the grafting auxochromic groups to the Oxn platform.[12] A recent report demonstrated that peptide sensors comprising Oxn units exhibited various affinity for zinc ions in the nanomolar to micromolar range by modulating peptide scaffolds.[7] Intrigued by the remarkable fluorescent properties of Oxn, we designed small molecular fluorescent sensor **4**, based on the quinoline platform (Scheme 1). To achieve high affinity and selectivity for zinc

Scheme 1. Synthesis of Fluorescent Sensor **1**



ions, a strong chelator, such as the di-2-picolylamine (DPA) moiety, was incorporated into Oxn at the 2 position. On the other hand, an ester moiety was attached at the 8 position and could be further saponified to a carboxylic moiety, which will not only enhance affinity of **1** for zinc but also increase its solubility in aqueous buffer. Compound **1** was synthesized from Oxn according to the strategy shown in Scheme 1 and characterized by ^{1}H NMR, ^{13}C NMR, elemental analysis, and X-ray (see Supporting Information).

As shown in Figure 1, UV−vis spectra of **1** exhibited a

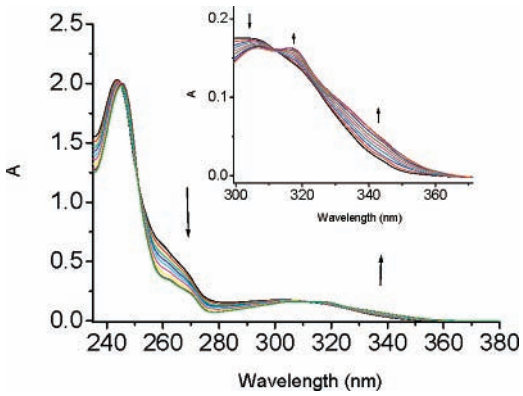


Figure 1. UV−vis spectra of **1** (50 μM) was titrated with Zn^{2+} (0−1 equiv) in buffer (25 mM HEPES, 0.1 M $NaClO_4$, pH 7.4, 25 °C). Inset: enlarged spectra of **1** between 370 and 300 nm.

maximal absorption at 244 nm and two broad bands around 270 and 315 nm before titrations in an aqueous buffer (25 mM HEPES, 0.1 M $NaClO_4$, pH 7.4, 25 °C). Upon addition of Zn^{2+} (0−1 equiv), the absorbance at 244 nm decreased slightly, accompanied by the little bathochromic shift, whereas an obvious reduction in the absorbance of the broad band around 270 nm was observed. Meanwhile, a new absorption peak appeared at 320 nm and tailed out to 370 nm with some changes in pattern (Figure 1, inset), which could be attributed to the interaction between the Oxn moiety and zinc.[13] Moreover, there are three isosbestic points at 245, 250, and 312 nm, which indicate the formation of only one UV active zinc complex.

Sensor **1** showed weak fluorescent emission at 425 nm upon excitation at 315 nm (ϵ = 3020 M^{-1} cm^{-1}, Figures S1 and S2). Its quantum yield is 0.03 in reference to quinine sulfate[14] in the presence of EDTA, which is thought to scavenge adventitious metals. The fluorescent intensity of **1** was dramatically enhanced upon titration of 1 equiv of Zn^{2+} in buffer, accompanied by a red-shifted maximal emission peak appearing at 438 nm (Figure S2). The quantum yield of zinc complex was measured to be 0.43. Both UV−vis and fluorescent titrations showed that saturated spectroscopic absorptions could be easily reached while 1 equiv of zinc ions was introduced, indicative of very tight binding and 1:1 stoichiometry for zinc ion and sensor **1**. The latter was further confirmed by Job's plot (Figure S3) and the X-ray structure of the zinc complex (Figure 3).

Due to high affinity of **1** for zinc ions, it is unfeasible to directly calculate the binding constant based on UV−vis or

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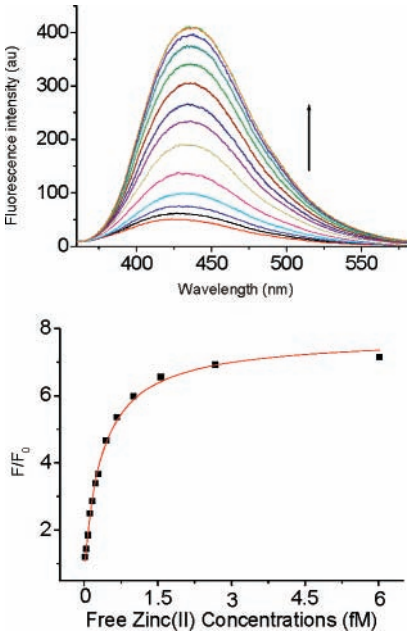

Figure 2. Fluorescence emission spectra (excitation 315 nm) of **1** (2.5 μM) in zinc(II)−TPEN buffer solutions. Top: the spectra were measured at 25 °C, pH 7.4 in buffered zinc(II) solution comprising 25 mM HEPES, 0.1 M $NaClO_4$, 0.25 mM total TPEN, and 0−0.24 mM $Zn(ClO_4)_2$. Bottom: ratio of integrated fluorescence intensity of **1**+ zinc to that of apoligand as a function of the concentration of free zinc(II). The data were fitted to be a 1:1 binding model according to ref 6a to generate log $K_d = -15.35 \pm 0.03$ ($R^2 = 0.997$).

fluorescence titrations.[5b] An alternative approach is to use known metal−ligand buffer solutions, which are able to generate accurate concentrations of free zinc ions. Initially, a zinc−TNA (log $K = 10.66$) buffer or zinc−HEDTA (log $K = 14.6$) buffer was employed to determine the binding affinity of **1**. To our surprise, neither of them worked. Realizing that the dissociation binding constant of **1** for zinc is extremely low, we used a zinc−TPEN (*N*,*N*,*N*′,*N*′-tetrakis-(2-pyridylmethyl)ethylenediamine, log $K = 15.4$, $pK_{a1} = 7.12$, $pK_{a2} = 4.81$, $pK_{a3} = 3.30$, $pK_{a4} = 2.88$) buffer, as described by O'Halloran,[3a,10] with free zinc concentrations ranging between 0.05 and 8 fM under the present conditions. A typical fluorescent titration profile of **1** in the presence of 0.25 mM TPEN is shown in Figure 2. The apparent dissociation constant of **1** was extracted to be 0.45 ± 0.02 fM by a nonlinear curve fit.[6a] To our knowledge, a small fluorescent molecule with femtomolar sensitivity for zinc ions is rare. This K_d value is significantly smaller than that of a DPA-substituted quinoline sensor ($K_d = 59$ nM) as reported by Nagano.[13a] We speculated that this was due to the fact that oxygen at the 8 position and the carboxylic group participated in the chelation to zinc ion. This speculation was corroborated by NMR titrations. NMR data showed that the chemical shifts of the CH_2CO_2Na group dramatically shifted downfield to $\Delta\delta = 0.5$ ppm upon titration of 1 equiv of zinc. Meanwhile, the chemical shifts of $N(CH_2)_3$ and aromatic protons also experienced the similar trend (Figure S5). Furthermore, X-ray crystal structure of the zinc complex of **1** also indicated that the carboxylic group participated in the chelation to zinc ion.

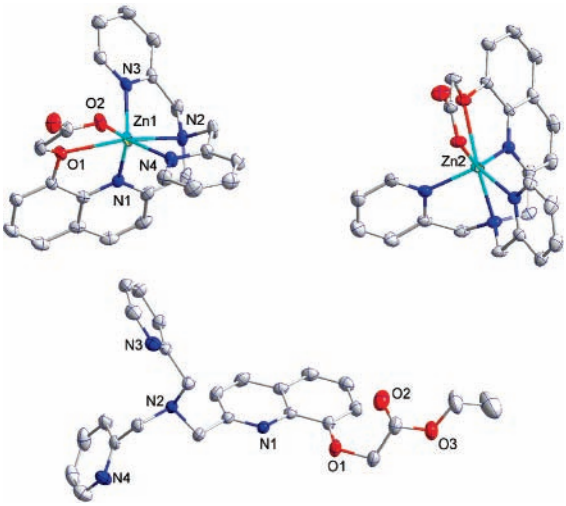

Figure 3. X-ray crystal structures of the zinc complex of **1** (top) and **4** (bottom). All hydrogen atoms, disordered perchlorate, and solvent molecules were deleted for clarity.

X-ray diffraction study of the zinc complex of **1** reveals that each asymmetric unit contains two similar zinc coordination sets, where zinc ion was chelated by quinoline and a carboxylic moiety and was also wrapped by two pyridyl moieties in an extended wing shape (Figure 3, top). The bond lengths between zinc and nitrogens are similar to those in the X-ray crystal structures of other zinc−DPA complexes.[8b,15] The X-ray crystal structure also demonstrates that zinc ion coordinates with oxygen atoms. However, the bond length of O1−Zn1 (2.425 Å) is obviously longer than that of O2−Zn1 (2.034 Å), which was supposed to be caused by the rigid structure of Oxn. These data are indicative of weak chelation of zinc to O1. Crystal structure of **4** shows that the molecule is in an extended state (Figure 3, bottom).

The selectivities of **1** to various metal ions were examined in buffer. As shown in Figure 4 (top), fluorescence intensity of **1** was slightly quenched upon titration of 1 equiv of transition metals except for Cd^{2+}. The interference of Cd^{2+} was also observed for other zinc fluorescent sensors.[8,13a,15b] The addition of 1 equiv of Ca^{2+} and Mg^{2+} slightly enhanced fluorescence ($F/F_0 < 1.5$, gray bar in Figure 4, top). To further gauge selectivity to transition metal ions, we also examined transition metal/zinc coexisted systems. The data showed that fluorescence was slightly enhanced for Mn^{2+}, Co^{2+}, Fe^{2+}, Ni^{2+}, and Cu^{2+} in the presence of 1 equiv of zinc, whereas a satisfactory fluorescence enhancement was achieved for Ca^{2+}, Mg^{2+}/Zn^{2+} systems (black bar in Figure 4, top). Moreover, the dissociation constant of **1** for zinc in

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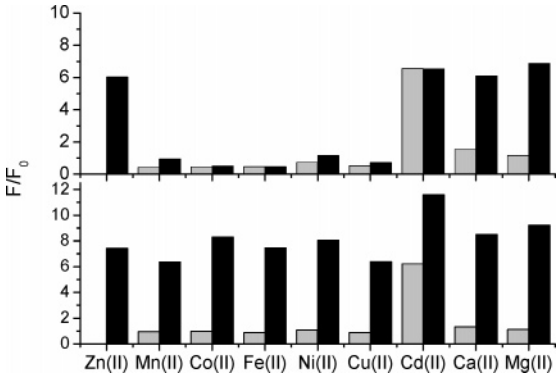

Figure 4. Metal ion selectivity profiles of **1** (5 μM) in the absence (top) and presence of 2 equiv of EDTA (bottom): ratio of relative integrated emission between 360 and 580 nm of **1** + 1 equiv indicated metal ions to that of apoligand (gray bar); ratio of relative integrated emission of **1** + 1 equiv indicated metal ions, followed by 1 equiv of Zn^{2+} to that of apoligand (black bar); 25 mM HEPES buffer containing 0.1 M $NaClO_4$, pH 7.4, 25 °C.

the presence of 1 equiv of Ca^{2+} is very close to that of **1** in the absence of Ca^{2+} (Figure S4). These results are indicative of high selectivity for zinc over Ca^{2+} under the present conditions.

Considering that affinity of EDTA for zinc is weaker than that of **1**, we rationalized that interference of unfavorable transition metals could be suppressed by addition of EDTA. Therefore, selectivity experiments were repeated in the presence of 2 equiv of EDTA. Not surprisingly, fluorescence profiles were similar to these in the absence of EDTA (gray bar in Figure 4, bottom), but while zinc ions were introduced, all fluorescence intensities were restored to the level of $F/F_0 > 6$ (black bar in Figure 4, bottom). These findings indicate that EDTA is able to scavenge unfavorable transition metals without interfering with the ability of **1** to sense zinc ions.

In conclusion, we have successfully prepared a water-soluble and small molecular fluorescent sensor. We found that sensor **1** binds extremely tight to zinc ions with an apparent dissociation constant in the femtomolar range. Moreover, sensor **1** shows good selectivity for zinc over other physiologically relevant metal ions in the presence of EDTA. The findings indicate that our powerful sensor is likely to image free zinc ions in extremely low range in biological systems.

Acknowledgment. We thank the Chinese Academy of Sciences "Hundred talents program" for financial support. We are grateful to Professor Jinshi Ma at CAS for his helpful discussion.

Supporting Information Available: Synthetic procedures, characterizations of **1**, and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

OL702192M