ORGANIC
LETTERS

2009
Vol. 11, No. 7
1655−1658

Highly Sensitive and Selective Fluorescent Sensor for Distinguishing Cadmium from Zinc Ions in Aqueous Media

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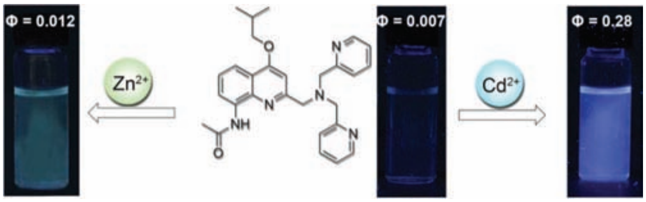
Received February 13, 2009

ABSTRACT



A fluorescent sensor, QA, based on acetamidoquinoline with DPA as receptor, was synthesized. In aqueous buffer solution, QA demonstrates high selectivity for sensing Cd^{2+} with about 40-fold enhancement in fluorescence quantum yield and picomolar sensitivity ($K_d = 0.25 \pm 0.03$ pM). Moreover, sensor QA can distinguish Cd^{2+} from Zn^{2+} via two different sensing mechanisms (PET for Cd^{2+}; ICT for Zn^{2+}), and the discrimination is even possible by "naked eyes".

Cadmium, an important natural element, is widely used in many fields, such as industry, agriculture, military affairs, etc.,[1] but people have come to realize the toxic effects of exposure to excessive cadmium. Cadmium was listed as number 7 on ATSDR's "CERCLA Priority List of Hazardous Substances"[2] and may cause acute and chronic toxicity. It can accumulate in the human body for >10 years,[3] resulting in serious diseases and even certain forms of cancers.[4] Thus, it is desirable to develop some analytical methods for detecting and monitoring cadmium in vitro and in vivo.

In fact, a great number of fluorescent sensors have been actualized to detect and analyze different kinds of heavy toxic metal ions because of their simplicity, high sensitivity, and real-time detection.[5] However, there are only a few cadmium ion fluorescent sensors that have been reported,[6] and even rare examples are available in living cells.[7] So far, the greatest challenge for detecting Cd^{2+} comes from the

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10.1021/ol900315r CCC: $40.75

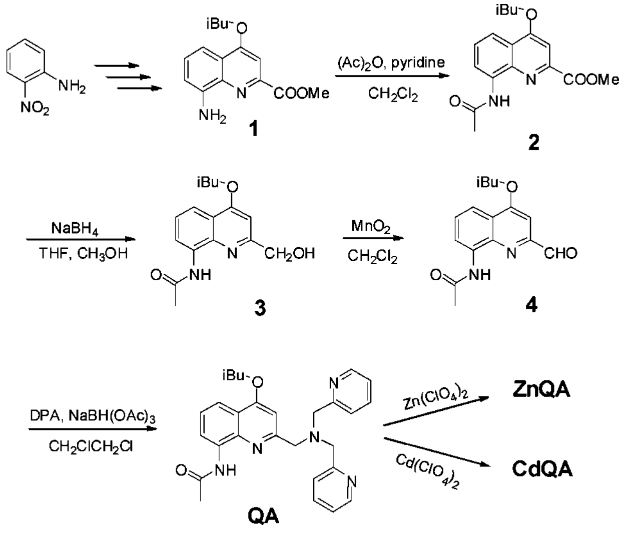
interference of other transition metal ions, in particular Zn^{2+}, which exhibits many properties similar to those of Cd^{2+} because they are in the same group of the periodic table. Therefore, they cause similar spectral changes while coordinated with fluorescent sensors.[8] Thus there is a great need for developing Cd^{2+}-selective sensors that can distinguish Cd^{2+} from Zn^{2+} with high sensitivity and selectivity under physiological conditions.

The most widely used approach in the development of metal ion chemosensors is to incorporate a fluorophore (signaling subunit) with proper ionophore groups that can bind different metal ions. While the ionophore binds the metal ions, the signaling subunit reads out this binding event in such a way that the fluorophore gives rise to variations either in its fluorescence intensity or/and in emission wavelength. Among the available ionophore groups, di-2-picolylamine (DPA) moiety is a good candidate for sensing Zn^{2+}, Cd^{2+}, or other transition metal ions on the basis of either photoinduced electron transfer (PET) or internal charge transfer (ICT) mechanism.[9,10] PET sensors usually exhibit increase or decrease in emission intensity but show little or no spectra shift, whereas ICT sensors exhibit both intensity changes and spectral shifts. Thus, the strategy to design one sensor that can simultaneously respond to different metal ions via different mechanisms should be attractive and powerful for ion sensing.

We recently reported that modulating the 8-position oxygen of the quinoline platform on sensor **HQs**[11] can effectively afford alterable sensing affinities for Zn^{2+}. These results encouraged us to improve sensing properties by introducing a nitrogen atom at the 8-position of quinoline. We expected that the replacement of O by a N atom would generate a novel sensor with high selectivity and sensitivity for zinc or other metal ions. In this context, we present our design and synthesis of sensor **QA**, based on acetamidoquinoline bearing DPA moiety as ionophore, which is able to distinguish cadmium ions from zinc ions in aqueous media. When binding Cd^{2+}, **QA** shows a bright "*Switch-ON*" state with slight emission shift, based on PET mechanism. In contrast, upon binding Zn^{2+}, **QA** shows a distinct emission red shift based on ICT mechanism but with slight emission enhancements. These provide explicit information by color and fluorescence emission changes upon metal complexation; the obvious emission color of cadmium and zinc complex solutions can be discriminated even by "naked eyes".

Sensor **QA** was synthesized from 2-nitroaniline according to the strategy shown in Scheme 1 and characterized by ^{1}H NMR, ^{13}C NMR, and mass spectra analysis (see Supporting Information).

Scheme 1. Synthesis of Sensor **QA**



The spectroscopic properties of **QA** were rated in aqueous solution (10 mM Tris-HCl, 20% DMSO,[12] 0.1 M KNO_3, pH = 7.4) solution. As shown in Figure S1 in Supporting Information, UV spectra of **QA** exhibited a maximum absorption at 247 nm ($\varepsilon = 2.8 \times 10^4$ M^{-1} cm^{-1}). With increasing amounts of Cd^{2+} (0−3.0 equiv), the absorbance at 305 nm decreased gradually, accompanied by a slight blue-shift to 302 nm. Meanwhile, a red-shift peak appeared around 350 nm with three isosbestic points at 333, 264, and 253 nm. Its absorbance at 350 and 305 nm changed linearly with the concentration of Cd^{2+} (0−3.0 equiv) up to a molar ratio (**QA**/Cd^{2+}) of 1:1, and there was saturated. These indicate the 1:1 binding model between **QA** and Cd^{2+}.

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Free **QA** showed weak fluorescence emission at 389 nm upon excitation at the isosbestic point of 333 nm ($\varepsilon = 0.41 \times 10^4\ M^{-1}\ cm^{-1}$, $\Phi_0 = 0.007$, Figure 1) in buffer solution because of the efficient PET quenching from amide and DPA to the quinoline fluorophore. Addition of Mn^{2+}, Fe^{2+}, Mg^{2+}, Ca^{2+}, and Na^+ exerted little or no effect on the emission of **QA**, whereas Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+}, and Pb^{2+} quenched its fluorescence. Remarkable fluorescence enhancements ($\Phi_{Cd} = 0.28$, $\Phi_{Cd}/\Phi_0 = 40$) were detected upon binding Cd^{2+}, showing a typical response of PET sensors. However, upon binding Zn^{2+}, **QA** showed a large spectral red-shift of 87 nm indicating the presence of the ICT process but with slight fluorescence intensity enhancements ($\Phi_{Zn} = 0.019$, $\Phi_{Zn}/\Phi_0 = 1.7$) (see Supporting Information). These significant differences when binding Cd^{2+} versus Zn^{2+} make **QA** highly selective toward Cd^{2+}. The obvious bright-blue and dim-green emission of the **CdQA** and **ZnQA** buffer solutions can easily be distinguished by naked eyes (Figure 1, insert).

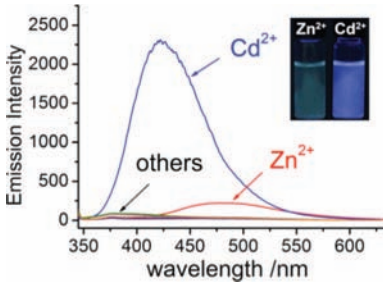

Figure 1. Fluorescence spectra ($\lambda_{ex} = 333$ nm) of **QA** (10 μM) and **QA** in the presence of various metal ions (1 equiv of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} and 100 equiv of Mg^{2+}, Ca^{2+}, and Na^+) in buffer solution (10 mM Tris-HCl, 0.1 M KNO_3, 20% DMSO, pH = 7.4). Insert: visible emission observed in the presence of Cd^{2+} and Zn^{2+}.

The titration of Cd^{2+} (Figure 2) showed that the maximum fluorescence emission at 422 nm gradually increased and fluorescence quantum yield was significantly enhanced by about 40-fold under current conditions and by about 30-fold in fully aqueous media (Figure S5, Supporting Information). The fluorescence intensity increased linearly (linear dependency coefficient $R^2 = 0.995$) with the concentration of Cd^{2+} (Figure S2, Supporting Information) up to a molar ratio (**QA**/Cd^{2+}) of 1:1, which was confirmed by Job's plot (Figure S6, Supporting Information). The dissociation constant K_d of **QA** was successfully extracted by titration of **QA** in cadmium-EGTA buffer solution. The K_d value was determined by a nonlinear least-squares analysis of fluorescence intensity at 422 nm versus Cd^{2+} concentration to be 0.25 ± 0.03 pM, demonstrating extremely high affinity of **QA** toward cadmium ions[13] (Figure 2b and Supporting Information).

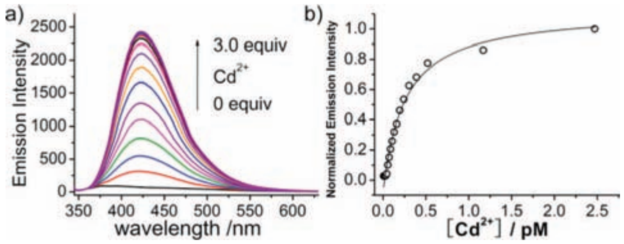

Figure 2. (a) Fluorescence spectra ($\lambda_{ex} = 333$ nm) of 10 μM **QA** upon the titration of Cd^{2+} (0−3.0 equiv) in buffer solution (10 mM Tris-HCl, 0.1 M KNO_3, 20% DMSO, pH = 7.4). (b) Fluorescence intensity as a function of Cd^{2+} concentration.

Moreover, pH effects on the fluorescence of the free **QA** and **CdQA** are also shown in Figures S8 and S9 in Supporting Information. At low pH of 3.56, the fluorescence emission of **QA** was very weak and then increased up to nearly 2-fold at pH 4.58. However, from pH 5 to 6.98, the fluorescence intensity gradually reduced, accompanied by a blue-shift to 377 nm, due to the PET process from amine N atom to quinoline fluorophore. Then the emission intensity remained unaffected up to pH 11. Upon chelating 1 equiv of Cd^{2+}, **CdQA** underwent no obvious spectra shift ($\lambda_{em} = 422$ nm) within a broad pH range of 3.44−9.18, indicating that amide NH was little influenced by pH and coordinated with Cd^{2+} without deprotonation. The similar phenomenon was also observed by Qian's group.[6b] Furthermore, the almost invariable fluorescence intensity from pH 6 to 8 indicated that **QA** is suitable for potential biological applications.

^{1}H NMR studies revealed the different coordination patterns between the binding of Zn^{2+} and Cd^{2+} (Figure 3 and Table S1). Upon interaction with Zn^{2+} and Cd^{2+}, the protons at the ortho position of pyridines downfield shifted from 8.53 to 8.67 and 8.68, respectively ($\Delta\delta_{Zn} = 0.14$; $\Delta\delta_{Cd} = 0.15$ ppm) by N-metal coordination effect.[6b,11a,14] Meanwhile, similar upfield shifts were also observed for the 3-position proton of quinoline, suggesting the direct interaction between fluorophore and metal ions. On the other hand,

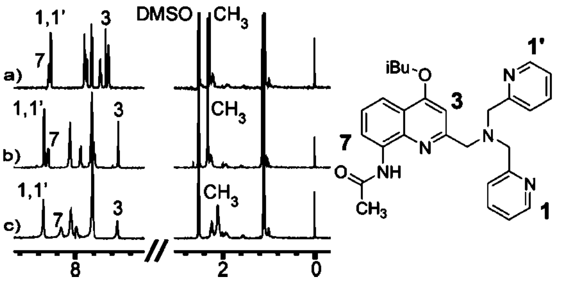

Figure 3. Partial ^{1}H NMR spectra (400 MHz) of **QA** (10 mM) in DMSO-d_6: (a) free **QA**; (b) **QA** + 1 equiv of Zn^{2+}; (c) **QA** + 1 equiv of Cd^{2+}.

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[13] For most Cd^{2+} sensors, the K_d values are in the micromolar range or higher. Rare examples have higher affinities; see refs 7b and 7c.

[14] McDonough, M. J.; Reynolds, A. J.; Lee, W. Y. G.; Jolliffe, K. A. *Chem. Commun.* **2006**, 2971.

upon addition of Cd^{2+}, the signal of the acetyl CH_3 experienced a significant upfield shift up to $\Delta\delta = 0.18$ ppm, in sharp contrast with that (0.04 ppm) in the presence of Zn^{2+}, which can be attributed to the interaction between the carbonyl group and Cd^{2+} but not Zn^{2+}. In addition, the different shifts for the 7-position proton of quinoline were also attributed to the carbonyl group coordination process.

As shown in Figure 4, we infer that **QA** adopts two coordination conformations in sensing Cd^{2+} and Zn^{2+}. In sensing Cd^{2+}, the DPA group would grasp the Cd^{2+} in a suitable conformation,[11] and the 8-position amide group coordinates Cd^{2+} as auxiliary interactions without deprotonation, which promotes the stabilization of Cd-DPA moiety, resulting in the efficient inhibition for PET process of the tertiary amine. On the other hand, Zn^{2+} bound the 8-position N atom more tightly as a result of the deprotonation of amide[6b,10] but did not effectively bind with the tertiary amine, which hinted at the feasibility of the PET process of the tertiary amine being not fully shut down. Subsequently, a distinct emission red-shift with slight enhancements was observed, indicating an ICT process for Zn^{2+} binding. The similar phenomenon was also observed in sensor **HQ2**,[11a] in which the deprotonation of the phenol group enhanced the interaction between Zn^{2+} and oxygen and consequently weakened the interaction between Zn^{2+} and tertiary amine of DPA. Furthermore, the HRMS analysis revealed the singly charged [**QA** + Zn − H]$^+$ complex, indicating that the deprotonation process occurred. Unfortunately, an attempt to detect the doubly charged [**QA** + Cd]$^{2+}$ species failed (Figure S10, Supporting Information).

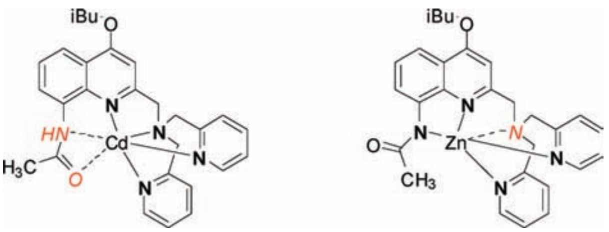

Figure 4. Proposed coordination conformation for **QA** with Cd^{2+} and Zn^{2+}.

The titration of sensor **QA** with Cd^{2+} in the presence of potential competing metal ions was further determined (Figures 1 and 5). With the addition of 1 equiv of Cd^{2+}, **QA** gives rise to a remarkable fluorescence enhancement (vide supra). The competition experiments indicate that Mn^{2+}, Fe^{2+}, Mg^{2+}, Ca^{2+}, and Na^+ have negligible effect on Cd^{2+} sensing. Since Co^{2+}, Ni^{2+}, and Cu^{2+} also appear to bind DPA-based sensors, they quench the fluorescence of the **CdQA** complex ($F/F_0 < 6$). It should be highlight that the fluorescence emission is blue-shifted to 444 nm from 476 nm on addition of Cd^{2+} to a solution of **ZnQA** with enhanced fluorescence intensity ($F/F_0 = 1.3$ to 6.1; F measured at 422 nm), indicative of excellent selectivity for Cd^{2+} over Zn^{2+} (Figure S11, Supporting Information). In brief, sensor **QA** has excellent selectivity over most competing metal ions and capability of distinguishing Cd^{2+} from Zn^{2+}.

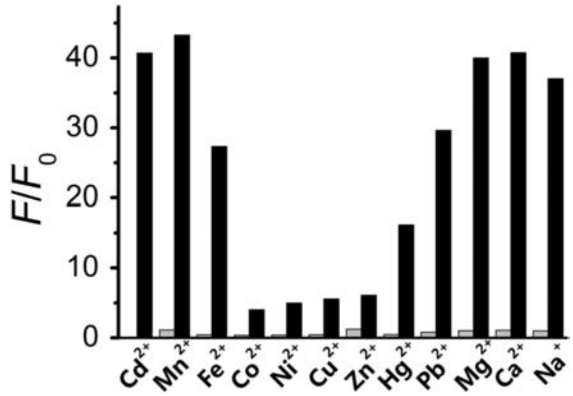

Figure 5. Metal ion selectivity profiles of **QA** (10 μM**)** in the presence of various metal ions in buffer solution: (gray bars) fluorescence intensity at 422 nm in the presence of 1 equiv of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, and Pb^{2+} and 100 equiv Mg^{2+}, Ca^{2+}, and Na^+; (black bars) fluorescence intensity in the presence of various metal ions, followed by 1 equiv of Cd^{2+}.

In conclusion, we have developed a new highly sensitive and selective florescent sensor for Cd^{2+}, **QA**. It can selectively detect Cd^{2+} over alkali and alkaline metal ions and can also distinguish Cd^{2+} from Zn^{2+}. The picomolar dissociation constant for Cd^{2+} indicates this sensor is capable of imaging trace cadmium ions in aqueous media.

Acknowledgment. We thank the Chinese Academy of Sciences “Hundred talents program”, the National Natural Science Foundation of China (90813002), and the State Key Laboratory of Fine Chemicals, Department of Chemical Engineering, Dalian University of Technology for financial support.

Supporting Information Available: Synthetic procedures, characterization of **QA**, and additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL900315R