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Ratiometric and Water-Soluble Fluorescent Zinc Sensor of Carboxamidoquinoline with an Alkoxyethylamino Chain as Receptor

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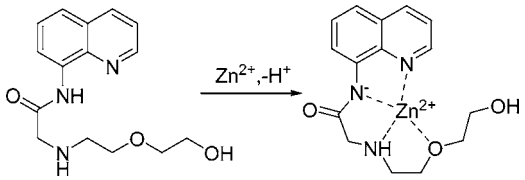
ABSTRACT



A novel "naked-eye" and ratiometric fluorescent zinc sensor (AQZ) of carboxamidoquinoline with an alkoxyethylamino chain as receptor was designed and synthesized. AQZ shows good water solubility and high selectivity for sensing; about an 8-fold increase in fluorescence quantum yield and a 75 nm red-shift of fluorescence emission upon binding Zn^{2+} in buffer aqueous solution are observed. Moreover, AQZ can enter yeast cells and signal the presence of Zn^{2+}.

The design and synthesis of fluorescent sensors with high selectivity and sensitivity is a vibrant field of supramolecular chemistry.[1] Especially of current, significant importance are sensors targeting heavy and transition metal ions, such as zinc cations.[2] Despite having many commercial Zn^{2+} sensors, chemists continue endeavoring to design new ones and to improve their sensitivity, selectivity, and reliability in order to satisfy various needs that are due to the wide existence of Zn^{2+} in organisms and its extensive significance.[3]

As is well-known, a majority of the reported Zn^{2+} sensors have poor water solubility, and recognition of metal ions is accomplished via measuring the metal-induced changes in fluorescence intensity, which may be influenced by many factors. Thus, these sensors are prone to be disturbed in quantitative detection.[4] Ratiometric fluorescent sensors permit signal rationing to detect target molecules by measuring the ratio of fluorescence intensities at two different wavelengths,[5] which are autoemitted by sensors upon binding objects. The autoreferential function of relative changes of two fluorescence intensities may avoid the influences of many nontarget factors in the changes of monofluorescence intensity. Therefore, the design of ratiometric fluorescent sensors is of great current interest.[6−8]

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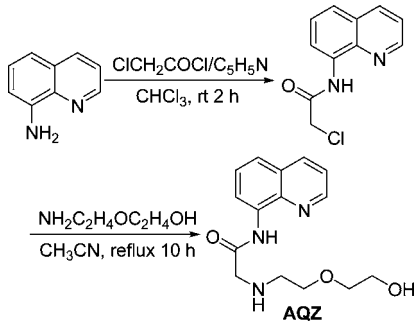
The receptor moiety is the key for the design of ratiometric sensors with good water solubility, except for aromatic fluorophores with hydrophobicity. In the design and synthesis of a ratiometric Zn^{2+} fluoroionophore based on the ICT mechanism, besides the famous di(2-picolyl)amine (DPA) as a specific neutral receptor, well-known sulfonamidoquinoline as a traditional receptor is widely used.[9] We notice that few reports on the Zn^{2+} sensor of carboxamidoquinoline have been published, except for Liu's report on a novel supramolecular system[10] formed by 8-carboxamidoquinolyl-modifed β-cyclodextrin and 1-adamantaneacetic acid as a Zn^{2+} sensor. This system coordinated Zn^{2+} through a cyclodextrin/substrate/metal triple recognition mode. We wondered if a simple, small-molecule, ratiometric, and water-soluble Zn^{2+} sensor could be obtained based on carboxamidoquinoline, that would not need the third component for recognition, would reduce the complexity of measurement, and would delete possible disturbing effects from Cd^{2+}, Cu^{2+}, and Ni^{2+}, etc.

Bearing this in mind, we synthesized a fluorescent sensor of carboxamidoquinoline with an alkoxyethylamino chain as receptor (**AQZ**) starting from 8-aminoquinoline. Here, the introduction of a carboxamido group is of advantage to the deprotonation of the 8-amino group.[11] After binding metal ions, the intramolecular hydrogen bond of 8-aminoquinoline is broken, and the intramolecular electron-transfer process is forbidden,[3a,12] thus enhancing fluorescence emission. Simultaneously, the deprotonation process strengthens the electron-donating ability from the nitrogen atom of the 8-amino group to the quinoline ring. And the electron transfer from the nitrogen atom of the heterocycle to the metal ion further enhances the ICT process. As a result, a red-shift in both emission and absorption wavelength could be observed. Most importantly, the introduction of a 2-(2-hydroxyethoxy)-ethylamino group provides not only another two metal-coordination site (carboxamidoquinoline affords two sites) but also a hydrophilic group.

AQZ was synthesized in a satisfactory yield by conjugating 2-(2-aminoethoxy)ethanol and 2-chloro-*N*-(quinol-8-yl)-acetamide, which was prepared from 8-aminoquinoline and 2-chloroacetyl chloride (Scheme 1).

Scheme 1. Synthesis of **AQZ**



As expected, **AQZ** showed a very weak fluorescence ($\Phi_0 = 0.008$, $\lambda_{max(em)} = 440$ nm) in tris-HCl (0.01 M) solution, and its fluorescence was slightly influenced by the addition of Cd^{2+}, Ag^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Na^+, K^+, Fe^{3+}, and Hg^{2+} (Figure 1). A fluorescence quenching was detected upon the addition of Cu^{2+} ($\Phi/\Phi_0 = 0.32$, $\lambda_{max(em)} = 442$ nm), Co^{2+} ($\Phi/\Phi_0 = 0.22$, $\lambda_{max(em)} = 442$ nm), and Ni^{2+} ($\Phi/\Phi_0 = 0.22$,

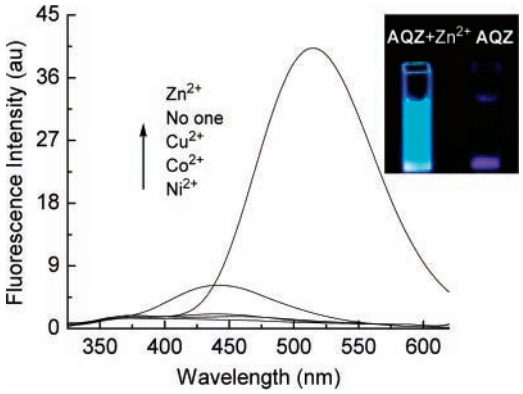


Figure 1. Fluorescence spectra of **AQZ** (10 μM) in tris-HCl (0.01 M) solution (methanol/water = 1:9, v/v, pH = 7.22) in the presence of different metal ions (5 equiv), and nearly no response to some other metal ions (Cd^{2+}, Ag^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Na^+, K^+, Fe^{3+}, Hg^{2+}). (Inset) Visible emission observed from **AQZ** in the absence and presence of Zn^{2+} (5 equiv).

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$\lambda_{max(em)} = 442$ nm). However, a fluorescence enhancement ($\Phi/\Phi_0 = 7.96$, $\lambda_{max(em)} = 515$ nm) and a remarkable red-shift of 75 nm were observed for **AQZ** upon binding Zn^{2+} by comparison with that of only **AQZ** in the solution. Actually, an obviously blue-green emission of the solution can easily be observed by the naked eye.

The signal response of **AQZ** toward Zn^{2+} was recorded in aqueous buffer solution in both emission and absorption spectra. Upon the addition of increasing amounts of Zn^{2+}, about an 8-fold increase in fluorescence quantum yield and a 75 nm red-shift from 440 to 515 nm of fluorescence emission were observed (Figure 2). Its intensity ratio at 515

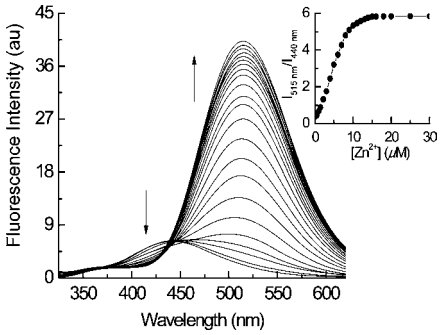

Figure 2. Fluorescence spectra of **AQZ** (10 μM) in tris-HCl (0.01 M) solution (methanol/water = 1:9, v/v, pH = 7.22) in the presence of different concentrations of Zn^{2+} (0−3 equiv). (Inset) Ratiometric calibration curve $I_{515\ nm}/I_{440\ nm}$ as a function of Zn^{2+} concentration.

and 440 nm ($I_{515\ nm}/I_{440\ nm}$) increased linearly with the concentration of Zn^{2+} (0−1 equiv, linearly dependent coefficient: $R^2 = 0.9887$) up to a mole ratio (**AQZ**/Zn^{2+}) of 1:1, and there it remained. Meanwhile, there was a 39 nm red-shift from 305 to 344 nm of absorption wavelength with three isosbestic points at 242, 280, and 324 nm, respectively (Figure 3). Its absorbance ratio at 344 and 305 nm ($A_{344\ nm}/A_{305\ nm}$) also increased linearly with the concentration of Zn^{2+} (0−1 equiv, linearly dependent coefficient: $R^2 = 0.9896$) up to a mole ratio (**AQZ**/Zn^{2+}) of 1:1, and there it remained. These imply the formation of a complex with 1:1 stoichiometry of **AQZ** and Zn^{2+}, and the association constant is 6.7×10^6 M^{-1}.[13] Moreover, a Job's plot, which exhibits a maximum at 0.5 M fraction of Zn^{2+}, further indicates that only a 1:1 complex is formed (Figure S1, Supporting Information).

Moreover, the Zn^{2+}-sensing ability of **AQZ** at different pH values was also investigated. As shown in Figure 4, **AQZ** had no fluorescence response to Zn^{2+} in the acidic environment due to the protonation of the amino group of **AQZ** leading to a weak coordination ability of Zn^{2+};[9h] however, satisfactory Zn^{2+}-sensing abilities were exhibited when the pH was increased from 6.1 to 11.8. At pH = 7.22, the $I_{\mathbf{AQZ}+Zn(II)}/I_{\mathbf{AQZ}}$ value reached its maximum value of 5.7, indicat-

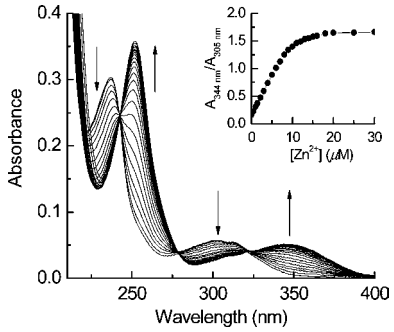

Figure 3. Absorption spectra of **AQZ** (10 μM) in tris-HCl (0.01 M) solution (methanol/water = 1:9, v/v, pH = 7.22) in the presence of different concentrations of Zn^{2+} (0−3 equiv). (Inset:) Ratiometric calibration curve $A_{344\ nm}/A_{305\ nm}$ as a function of Zn^{2+} concentration.

ing that **AQZ** possessed the highest sensing ability under the physiological pH window.

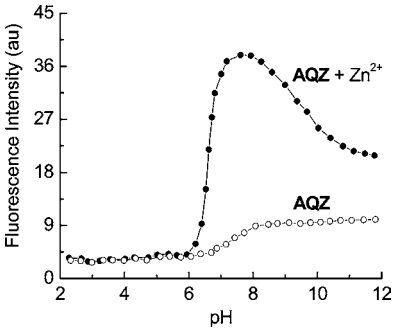

Figure 4. Fluorescence intensity of **AQZ** (10 μM) at various pH values in methanol/water (1:9, v/v) solution in the absence and presence of Zn^{2+} (1 equiv).

To further explore the selectivity of **AQZ** for Zn^{2+}, the competition experiments were conducted in the presence of Zn^{2+} mixed with Cd^{2+}, Ag^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Na^+, K^+, Fe^{3+}, Hg^{2+}, Ni^{2+}, Co^{2+}, and Cu^{2+}, respectively. While a range of metal ions bound to the sensor, the addition of 1 equiv of Zn^{2+} outcompeted most (Figure S2, Supporting Information). The metal ions Co^{2+} and Cu^{2+}, especially Cu^{2+}, remained bound, and thus fluorescence emission of **AQZ** had a small enhancement. In addition, these free cations would have little influence in vivo since they exist at a very low concentration.[14]

A preliminary study on the Zn^{2+}-sensing behaviors of **AQZ** in living yeast cells (*Saccharomyces cerevisiae*)[15] was carried out by fluorescence microscopy. After incubation with **AQZ** at 37 °C for 1 h, the cells displayed very weak

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intracellular straining, demonstrating that **AQZ** is cell permeable (Figure 5). The cells also exhibited strong blue-green fluorescence with the addition of Zn^{2+}. These results indicate that **AQZ** may be used as a possible sensor to detect Zn^{2+} released from stimulated cells.[16]

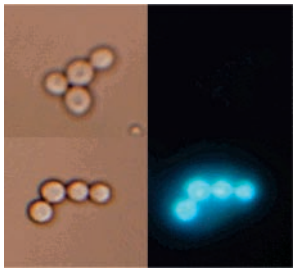

Figure 5. Phase contrast (left) and fluorescence (right) microscopy images of yeast cells incubated for 1 h with **AQZ** (40 μM), without (top) and with (bottom) the addition of Zn^{2+}(1 equiv).

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In conclusion, we have developed a new class of "naked-eye" and ratiometric fluorescent sensor **AQZ** for Zn^{2+} in aqueous solution. A highly Zn^{2+}-selective fluorescence-enhancing property in conjunction with a remarkable red-shift of fluorescence emission was observed. Moreover, **AQZ** can be used as an imaging reagent of Zn^{2+} in living tissue or in cells. It might be the first simple, small-molecule, ratiometric, and water-soluble Zn^{2+} fluorescent sensor derived from carboxamidoquinoline, and we hope that carboxamidoquinoline could become a popular moiety for the design of Zn^{2+} sensors in the future, just as toluenesulfonamidoquinoline has done.

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Supporting Information Available: Synthetic details, characteristics, and spectroscopic data of **AQZ**. This material is available free of charge via the Internet at http://pubs.acs.org.

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