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A selective and sensitive fluorescence probe for imaging endogenous zinc in living cells

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ABSTRACT

A carboxamidoquinoline-based fluorescent Zn^{2+} probe **1** containing *N*/*S*/*S* heteroatoms as a receptor was designed and readily synthesized, which was featured by the Zn^{2+}-induced red-shift of emission (45 nm) based on internal charge transfer (ICT) in an aqueous HEPES buffer ($pH=7.4$). Moreover, spectroscopic studies indicated that the composition of the complex **1**-Zn^{2+} was 1:1, which was also confirmed by X-ray crystallography. In addition, ^{1}H NMR titration experiment suggested that probe **1** transformed from the amide tautomer to imidic acid tautomer after binding with Zn^{2+}. The binding of zinc with **1** was easily reversed by addition of *N*,*N*,*N*′,*N*′-tetrakis(2-picolyl)ethylenediamine (TPEN) or EDTA. Furthermore, probe **1** was successfully applied to image both exogenous and endogenous zinc in living HeLa cells.

1. Introduction

Zinc is the second most abundant *d*-block metal ion in human body[1] and plays a vital role in various biological processes, such as brain function, gene expression, and mammalian reproduction.[2] Disturbance of the zinc homeostasis in living organisms is critically related to a series of disease.[3] Therefore, the detection of zinc has attracted increasing interests. Numerous preceding works have demonstrated that fluorescent sensor is a powerful tool to detect metal ions,[4] anions, and small molecules[5] in vitro and in vivo due to the simplicity and high sensitivity. Fluorescence imaging of zinc is especially suitable because this ion has no facile spectroscopic or magnetic signature.[6a] The past years have witnessed intense research devoted to the development of novel fluorescent probes for recognizing zinc[6] and biological applications.[7] Among the reported zinc sensors, quinoline and its derivatives, due to their unique chemical and physical properties, have aroused enormous attentions.[8,13b] However, most of them suffer from multiple drawbacks, such as selectivity, sensitivity, water solubility, and cell membrane permeability,[8b,c,f,g,10b] which prevent them to satisfy various practical needs. And thus, it still remains a challenge to develop novel fluorescent probes to overcome those disadvantages.

8-Aminoquinoline was commonly employed as fluorophore for the metal ion sensors based on internal charge transfer (ICT), which is one of most important signaling mechanism in designing metal ion sensors. In these sense, the fluorescent probe **1** based on carboxamidoquinoline was designed, which bears **N**/**S**/**S** heteroatoms as a receptor. The spectroscopic studies demonstrated that it is a reversible probe with broad pH stability, highly sensitivity and selectivity for Zn^{2+}. The binding mode was determined by X-ray crystallography and ^{1}H NMR titration experiments. Binding of Zn^{2+} with probe **1** induces deprotonation of carboxamido group and the breakage of intramolecular hydrogen bond of 8-aminoquinoline, which in turn enhance the ICT process as described in the previous report.[8a] As a consequence, the red-shift and enhancement of fluorescence emission were observed. The probe was also successfully used in imaging both exogenous and endogenous zinc in living HeLa cells, demonstrating its practical applications.

2. Results and discussion

2.1. Synthesis

The synthesis of sensor **1** is depicted in Scheme 1 according to the reported procedures with some modifications.[8a,9] The probe **1**, obtained in 2 steps with 62% overall yield starting from readily available 8-aminoquinoline, was fully characterized by ^{1}H NMR, ^{13}C NMR, and MS spectra (Fig. S1–10).

2.2. UV–vis absorption and emission spectra of probe 1

The spectral properties of probe **1** were examined by UV–vis absorbance and fluorescence titrations. Two bands were both red-shifted with addition of Zn^{2+} (0–1.0 equiv) in the absorption

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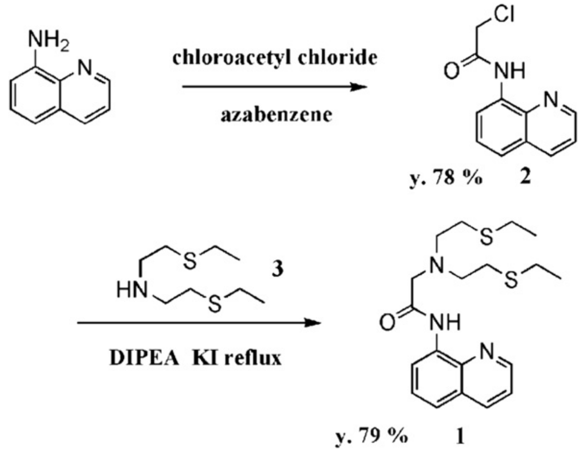

Scheme 1. Synthesis of **1**.

spectra, accompanying three isosbestic points at 245, 279, and 327 nm (Fig. 1). Fluorescence spectra of Zn^{2+} titration were shown in Fig. 2. Probe **1** has a very weak fluorescence emission at about 445 nm with excitation at 345 nm. Upon addition of Zn^{2+}, it displays a 45 nm red-shift of emission to 490 nm and a remarkable enhancement of fluorescence intensity, which is saturated by 1.5 equiv of Zn^{2+} with a ~21-fold increase. It is worth noting that this fluorescence titration experiment was performed in a pure aqueous solution, which is different from other probes determined in organic solvent mixed solutions,[8] and has potential application in biological imaging (vide infra). The solubility of the probe **1** in HEPES buffer was estimated to be more than 500 μM (containing 0.5 % organic solvent) by serial dilution from a concentrated stock solution (100 mM in acetonitrile). The quick changes of the emission wavelength as well as intensity upon zinc addition can be explained by carboxam-idoquinoline's deprotonation process and enhancement of internal charge transfer.

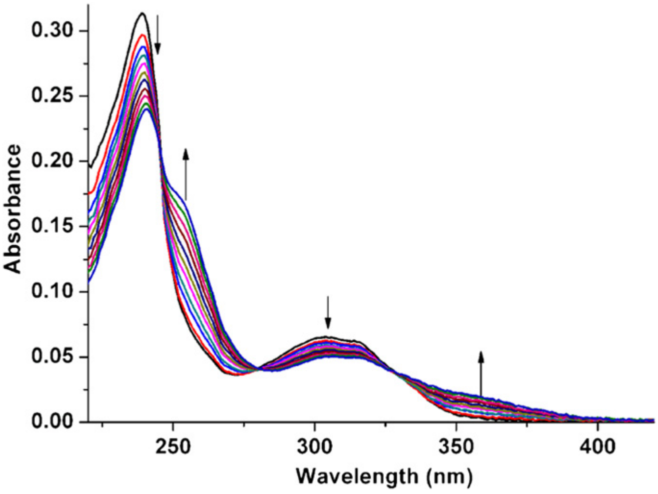

Fig. 1. UV–vis absorption spectra of probe **1** in HEPES buffer (10 mM, pH=7.4) in the presence of different concentrations of Zn^{2+}. [**1**]=10 μM, [Zn^{2+}]=0–10 μM.

2.3. Job's plot and ^1H NMR titration

To determine the binding stoichiometry of **1**-Zn^{2+} complex, we adapted the Job's plot based on the fluorescence titration. The maximal fluorescence intensity appears at about 0.5 mol fraction (Fig. 3), indicating that **1**-Zn^{2+} complex has 1:1 stoichiometry. The most convincing evidence was provided by the crystal structure of **1** complexed with zinc (Fig. 4a), which can better explain the fluorescence sensing mechanism. The crystal structure unambiguously demonstrates that the probe **1**, acting as a quinquidentate ligand via its N and S atoms, forms a 1:1 complex with Zn^{2+}. The stronger coordination of Cu^{2+} by **1** (Fig. 4b), reflected by the shorter bond lengths in the complex (Tables S1 and S2), supported that Cu^{2+} is a potent competitive quencher against Zn^{2+} (vide infra). The bond formation between **1** and Zn^{2+} is further supported by ^1H NMR titration. In Fig. 5, as addition of 1 equiv $Zn(ClO_4)_2$, the NH proton shifted to upfield from 11.34 ppm and diminished gradually, and a new peak formed at 10.01 ppm, which results from the existence of the OH proton. This suggested that probe **1** complexed with Zn^{2+} in the imidic acid tautomer in CH_3CN predominantly (Scheme S1). The probe **1** is also a sensitive sensor for other zinc metal sources demonstrated in Fig. 6.

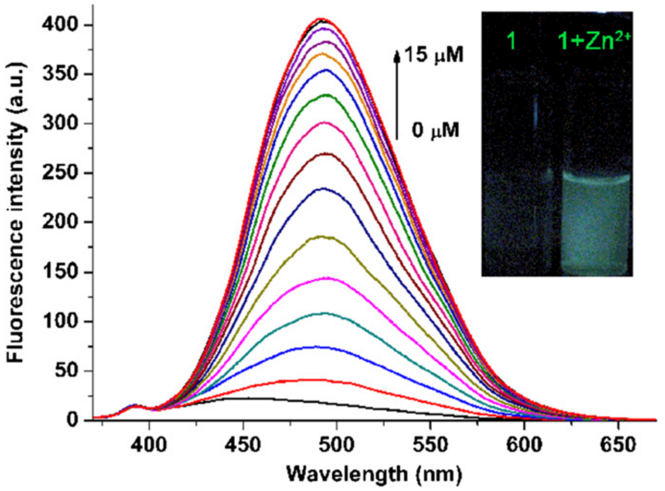

Fig. 2. Fluorescence spectra of **1** (10 μM) in the presence of an increasing Zn^{2+} concentration (0–15 μM) in HEPES buffer (10 mM, pH=7.4) excited at 345 nm. Inset: fluorescence changes of **1** (10 μM) in absence and presence of Zn^{2+} (10 μM).

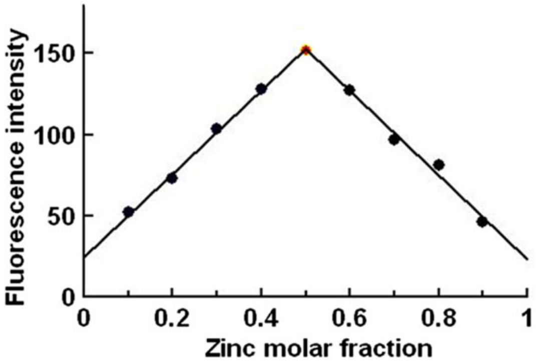

Fig. 3. Job's plot of **1**-Zn^{2+} in 10 mM HEPES buffer (pH=7.4). The total molar concentration of **1** and Zn^{2+} is 10 μM.

2.4. Determination of dissociation constant and the detection limit

Determination of the dissociation constant, K_d, of **1**-zinc complex was performed by the Eq. 1 proposed by Kowalczy et al.[10a] from direct fluorometric titration (Fig. 7a). In the Eq. 1, F stands