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Fluorescence Sensing and Binding Behavior of Aminobenzenesulfonamido-quinolino-β-cyclodextrin to Zn^{2+}

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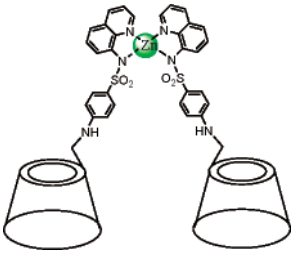
ABSTRACT



A water-soluble fluorescent zinc sensor which binds strongly to Zn^{2+} (log $K = 12.4$) was successfully synthesized under physiological conditions. This sensor exhibits a good fluorescence response to Zn^{2+} over a wide pH range in water. Under the same conditions, several metal ions commonly present in a physiological environment, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, and Co^{2+}, showed little interference to the fluorescence response to Zn^{2+}.

Zinc(II) ion is an abundant component of most living cells and plays an important role in various biological processes such as gene transcription, regulation of metalloenzymes, neural signal transmission, and others.[1−4] Generally, the total concentration of Zn^{2+} in different cells is from the nanomolar range up to about 0.3 mM.[5] Concentrations of Zn^{2+} in the synapse are believed to reach 10−300 μM, and some pathological diseases are closely associated with the concentration of Zn^{2+}.[4,6] Therefore, the detection of Zn^{2+} in vivo has attracted increasing attention. So far, the fluorescence method appears to be the most effective way to detect Zn^{2+}, since most of the common analytical techniques fail to detect Zn^{2+} in biological systems. Due to the $3d^{10}4s^0$ electronic configuration, Zn^{2+} provides no appreciable spectroscopic or magnetic signals required for application of UV−vis spectrometry, Mössbauer spectroscopy, nuclear magnetic resonance (NMR) or electron paramagnetic resonance (EPR) spectroscopy. In the past few years, many fluorescent sensors that can selectively detect Zn^{2+} have been reported.[7−13] However, the majority of these have poor water solubility,

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which inevitably hinders their applications in vivo. Among various fluorescent sensors, 6-methoxy-(8-*p*-toluenesulfonamido) quinoline (TSQ) and its derivatives are the first class of fluorescent probes to be developed for Zn^{2+}.[14] They exhibit high selectivity for Zn^{2+} as compared to Ca^{2+}, Mg^{2+} and other metal ions. In order to improve the water solubility of TSQ, several attempts have been made, such as introducing carboxylic acid groups or ester groups to extend the 6-methoxyl group[15,16] and replacing the methyl group on the benzene ring with a carboxylic acid group.[17] However, an inherent disadvantage of these probes is their sparing solubility in neutral aqueous solution.

Possessing a hydrophobic cavity and numerous hydroxyl groups, cyclodextrins (CDs), cyclic oligosaccharides with 6−8 D-glucose units linked by α-1,4-glucose bonds, are widely used as drug carriers and solubilizers.[18,19] In a preliminary study, we attempted to solubilize TSQ by forming CD/TSQ inclusion complexes, but neither native CDs nor methylated CDs were observed to markedly increase the water solubility of TSQ. Therefore, we covalently linked an analogue of TSQ, i.e., *N*-(8-quinolyl)-*p*-aminobenzenesulfonamide (HQAS), to *β*-CD. The resulting water-soluble HQAS-modified *β*-CD **1** showed satisfactory water solubilty, a high binding affinity and good fluorescence sensing ability to Zn^{2+}. Simultaneously, **1** also possesses the ability to include various organic and biological substrates within its hydrophobic cavity.[20] This property may enable it to adhere to the surface of tissues or cells by including accessible surface molecules in the cavity.[13c−e]

HQAS was prepared according to a procedure similar to that reported by Kojima et al.,[21] where *N*-(8-quinolyl)-*p*-acetylaminobenzenesulfonamide (QAS) was obtained by the reaction of 4-acetamidobenzene-1-sulfonyl chloride with 8-aminoquinoline, followed by hydrolysis in either an acidic or a basic environment to cleave the acetylamino bond. In order to prevent the TSQ framework bound to zinc from being destroyed, the methyl group on the benzene ring was replaced by an amino group. It has been reported that both mono[6-*O*-(*p*-toluenesulfonyl)]-*β*-CD and 6-deoxy-6-formyl-*β*-CD can react with amino group nucleophiles.[22] However, the reaction of mono[6-*O*-(*p*-toluenesulfonyl)]-*β*-CD with HQAS did not give the desired product, presumably because of the relatively weak nucleophilic reactivity of the amino group in HQAS. In contrast, the reaction of 6-deoxy-6-formyl-*β*-CD with HQAS followed by the reduction of the imino group was observed to give the target product in moderate yield. Owing to the good solubilizing properties of the *β*-CD unit, the solubility limit of **1** in water is about 0.6 mM.

The mode of coordination of **1** with Zn^{2+} was investigated by spectrophotometric titration at 25 °C in aqueous buffer solution. Figure 1 illustrated a typical UV−vis titration curve of **1** with Zn^{2+}. As can be seen in Figure 1, the absorption intensity of **1** at 271 nm gradually increased, accompanied by the obvious hypsochromic shift of the absorption peak (from 271 to 259 nm), as the concentration of Zn^{2+} was increased stepwise. Moreover, a new absorption peak appeared at 362 nm in the UV−vis spectrum of **1**/Zn^{2+} system, and its intensity also gradually increased with the addition of Zn^{2+}. This absorption peak is expected to correspond to the coordination of HQAS unit in **1** with Zn^{2+}. That is, two nitrogen atoms in the HQAS unit coordinated with Zn^{2+} to form a five-membered chelate ring, which consequently extended the conjugated system and resulted in the appearance of the new absorption in the long wavelength region. The spectra obtained during the stepwise addition showed the appearance of two isobestic points at ca. 272 and 332 nm. In the control experiment, the UV−vis spectrum of Zn^{2+} within the appropriate concentration range displayed no appreciable absorption between 200 and 450 nm under comparable experimental conditions. Taken together, these phenomena illustrated the transformation from free **1** to the Zn^{2+}-coordinated species. Moreover, the coordination stoichiometry between **1** and Zn^{2+} was obtained by the molar ratio method using UV−vis spectrometry. As

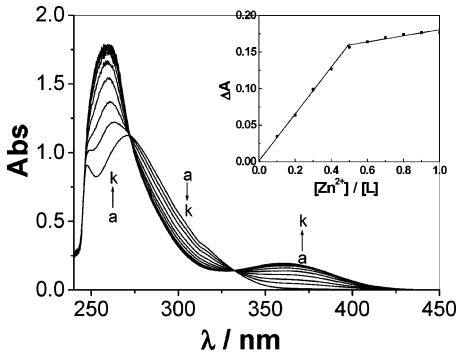

Figure 1. (a) UV−vis spectral changes of **1** upon the addition of Zn^{2+} in buffer solution (pH 7.2, I = 0.1 M $NaNO_3$) at 25 °C ([**1**] = 50 μM, [Zn^{2+}] = 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 μM from a to k). (b) Absorption changes of **1** at 362 nm upon the addition of Zn^{2+}.

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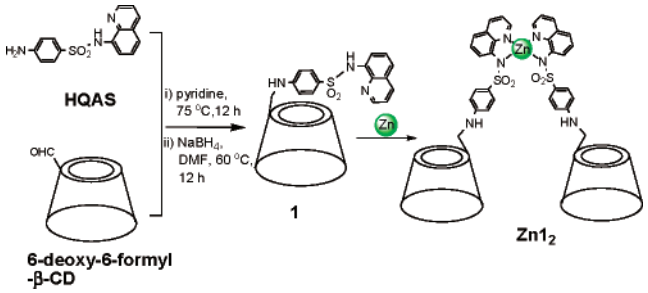
seen in Figure 1 (inset), the curve of $\Delta A_{1/Zn^{2+}}$ ($\Delta A_{1/Zn^{2+}} = \Delta A_{1+Zn^{2+}} - A_1$, A_1 was defined as the absorption intensity of **1** at 362 nm) vs Zn^{2+}/**1** molar ratio showed an inflexion point at a molar ratio of 0.5, which corresponded to a 2:1 coordination stoichiometry between **1** and Zn^{2+}. Therefore, we were able to deduce a possible coordination mode of **1**/Zn^{2+} system as shown in Scheme 1, that is, 2 N atoms

Scheme 1. Synthesis of **1** and $Zn\mathbf{1}_2$



from each of the two units of **1** participate in the four-coordinated environment of Zn^{2+}.

As a water-soluble analogue of TSQ, an important and desirable property of **1** is its fluorescence sensing ability to Zn^{2+}. When excited at either 285 nm or 361 nm, **1** exhibited two excitation bands at 285 and 361 nm, and its emission band appeared at 507 nm. In the presence of 0.5 equiv. of Zn^{2+}, the excitation spectrum of **1**/Zn^{2+} system resembled that of **1**, showing two excitation bands at 282 and 362 nm, but the emission band red shifts to 518 nm when excited at either 282 nm or 362 nm, accompanied by an obvious enhancement (ca. 5.7 times) of the emisson intensity (see the Supporting Information). To further investigate the fluorescence sensing ability of **1** for Zn^{2+}, fluorescence titration experiments were also performed. As seen in Figure 2, with the stepwise addition of Zn^{2+} to a solution of **1**, the

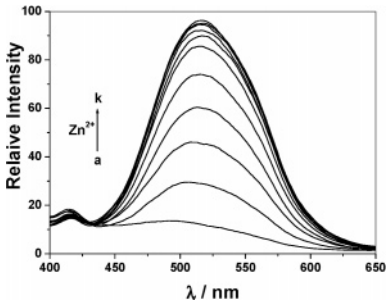


Figure 2. Fluorescence spectral changes of **1** (20 μM) with the addition of Zn^{2+} ([Zn^{2+}] = 0, 2, 4, 6, 8. 10, 12, 14, 16, 18, and 20 μM from a to k) in buffer solution (pH 7.2) at 25 °C (λ_{ex} = 362 nm).

fluorescence emission intensity of **1** at 518 nm increased with successive additions. A possible reason for the enhanced fluorescence may be as follows. Before coordination with Zn^{2+}, two nitrogen atoms of free **1** could form an intramolecular hydrogen bond with hydrogen atoms, which resulted in a photoinduced electron transfer, and the deexcitation of the resulting tautomer occurred mainly via a nonradiative pathway. These processes consequently led to the weak fluorescence of **1**. Once **1** was coordinated with Zn^{2+}, the electron-transfer process was forbidden,[3,23] and an extended π-electron conjugation system was formed synchronously. This conjugation system was involved in an internal charge transfer (ICT) process from the ligand donor to the Zn^{2+} acceptor. Owing to the formation of the extended π-electron conjugation system, the **1**/Zn^{2+} system exhibited an intense greenish fluorescence.

Interestingly, **1** showed good fluorescence sensing ability to Zn^{2+} over a wide pH range. As shown in Figure 3, **1**

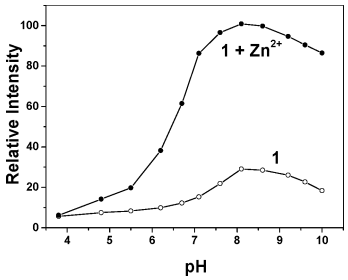


Figure 3. Fluorescence intensities of **1** (20 μM) in the absence (λ_{ex} = 362 nm, λ_{em} = 502 nm) and presence of Zn^{2+} (10 μM, λ_{ex} = 362 nm, λ_{em} = 518 nm) at various pH values at 25 °C.

showed no appreciable sensing ability to Zn^{2+} at a pH value below 3.6, which may be due to the competition of H^+ at low pH values leading to a weak coordination ability of Zn^{2+} with **1**, but exhibited satisfactory Zn^{2+}-sensing abilities when the pH was increased to the 4 to 10 range. At pH ca. 7.2, the $F_{1+Zn^{2+}}/F_1$ value reached its maximum value of 5.7, indicating that **1** possessed the highest sensing ability in an environment similar to serum (pH ca. 7.3).

After validating the strong Zn^{2+}-sensing ability of **1** under physiological conditions, the binding ability of **1** to Zn^{2+} was quantitatively determined by a competitive binding method[8a] using fluorescence titration (see the Supporting Information). The apparent stability constant (logK, $K = [Zn\mathbf{1}_2]/[Zn^{2+}][\mathbf{1}]^2$) of $Zn\mathbf{1}_2$ complex was observed to be equal to 12.4, which is near the reported value of Zinquin acid/Zn^{2+} complex (logK = 13.7).[8a] This result unambiguously demonstrated the strong binding ability of **1** for Zn^{2+}.

Another important property of **1** is its sensing selectivity to various metal ions, especially to possible competing ions when **1** is used as a Zn^{2+} sensor in the physiological environment. We determined the fluorescence intensities of **1** at 518 nm in the presence of various metal ions and compared the results with that observed for free **1** at 518

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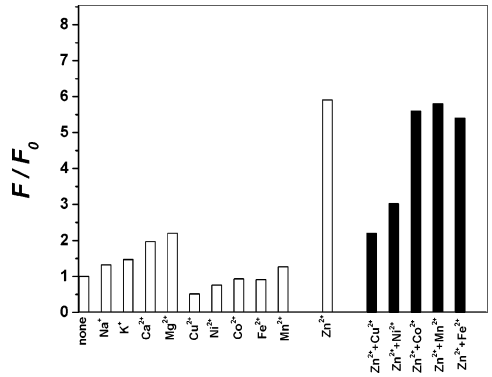

Figure 4. Fluorescence intensities of **1** (20 μM) in the prescence of various metal ions and ion mixtures. $[Na^+] = [K^+] = [Ca^{2+}] = [Mg^{2+}] = 5$ mM, $[Zn^{2+}] = [Cu^{2+}] = [Ni^{2+}] = [Co^{2+}] = [Fe^{2+}] = [Mn^{2+}] = 10$ μM. These data were measured in buffer solution (pH 7.2) at 25 °C ($\lambda_{ex} = 362$ nm, $\lambda_{em} = 518$ nm).

nm. As seen in Figure 4, the addition of a large excess (250 equiv.) of Na^+, K^+, Ca^{2+}, and Mg^{2+}, which always exist at high concentrations in living cells, resulted in only slight fluorescence enhancement ($F/F_0 < 2.3$) presumably due to the poor coordination ability of alkaline metal ions or alkaline earth metal ions with the HQAS unit in **1**. On the other hand, the addition of various transition metal ions led to quite different results. The addition of Co^{2+}, Fe^{2+}, and Mn^{2+} gave appreciable changes ($F/F_0 < 1.2$) but in no case approached that observed with Zn^{2+}. Recall that Zn^{2+} significantly enhanced the fluorescence intensity ($F/F_0 = 5.7$) of **1**. Moreover, a comparison of fluorescence intensities of **1** in the presence of Zn^{2+}-containing ion mixtures was also useful to reveal the influence of coexisting ions in the physiological environment on the Zn^{2+} sensing ability of **1**. As seen in Figure 4, **1** showed satisfactory fluorescence enhancement factors ($F/F_0 > 5$) to most Zn^{2+}-containing ion mixtures except Zn^{2+}/Cu^{2+} and Zn^{2+}/Ni^{2+}mixture. These results unambiguously demonstrate the high sensing selectivity of **1** to Zn^{2+} even in the presence of other ions. Although Cu^{2+} was observed to be unfavorable to the Zn^{2+}-sensing of **1**, its interference in the fluorescence response may be masked with a copper binding protein such as bovine serum albumin.[8a]

A preliminary study on the Zn^{2+}-sensing behaviors of **1** in the biological system was carried out by fluorescence microscopy using yeast (saccharomyces cerevisiae)[24] as model cells. The results showed that **1**-stained yeast cells exhibited good fluorescence responses for Zn^{2+}. After incubation at 35 °C for 1 h in the presence **1**, the originally non-luminescent yeast cell presented a very weak background fluorescence without the addition of Zn^{2+} (see the Supporting Information), but exhibited a strong green fluorescence, as seen by fluorescence microscope, upon the addition of Zn^{2+} (Figure 5). These results indicated that **1** may be used as a possible sensor to detect Zn^{2+} released from stimulated cells.[13c,d,e]

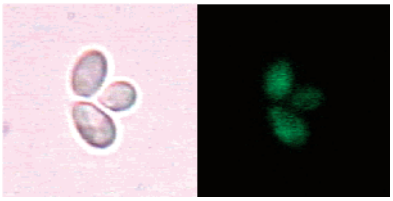

Figure 5. Optical microscopic (left) and fluorescence microscopic (right) images of **1**-stained yeast cells with the addition of Zn^{2+} (25 μM).

In summary, we successfully prepared a water-soluble analogue of TSQ, which showed strong binding affinity as well as a good sensing ability and selectivity for Zn^{2+} in aqueous solution. Significantly, its sensing ability was not obviously affected by other biologically important cations such as Na^+, K^+, Mg^{2+}, and Ca^{2+} under physiological conditions. Considering its convenience in preparation and high sensing ability for Zn^{2+}, it is expected to be useful as an imaging reagent of Zn^{2+} in living tissue or in cells.

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Supporting Information Available: Experimental details, syntheses of HQAS and **1**, competitive binding spectra used to calculate log K, as well as exciation and emission spectra of **1** in the presence and absence of Zn^{2+}. This material is available free of charge via the Internet at http://pubs.acs.org.

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