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Design of a zinc(II) ion specific fluorescence sensor

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Abstract—We report the synthesis of {[3-(biscarboxymethylamino)-2-methoxy-5-methylphenyl]carboxymethylamino}acetic acid, which functions as a Zn^{2+} selective fluorescence probe (sensor).

In recent years, there has been a growing need for developing highly sensitive and selective probes for the detection of metal ions in biological and environmental samples. A variety of divalent metal ions are known to be involved in the structural, catalytic, and regulatory aspects of the biological system, and some such metal ions serve as prognostics of certain human diseases.[1] For example, Cu^{2+}, Zn^{2+}, and Fe^{2+} have been found to be involved in aggregating β-amyloid peptides during the onset of Alzheimer's disease.[2] However, due to the lack of metal ion specific probes, the relative contribution of one type of metal ion versus the other in causing the disease is not clearly understood. The inability to differentiate among different types of divalent metal ions in biological samples has been one of the major impediments in the area of bio-analytical chemistry.

Although there has been some success in detection of biologically significant metal ions by developing fluorescence probes (e.g., fura-2 for Ca^{2+}), most of the probes exhibit cross-reactivities for other metal ions.[3] This is not surprising since both physical and electronic properties of these metal ions are not too disparate, and they tend to exhibit comparable binding affinities with their cognate chelating agents. Consequently, not only synthetic (organic) probes but also enzymatic probes exhibit cross-reactivities among metal ions.[4] Presently, quinoline–sulfonamide containing compounds (and their derivatives) are regarded to be as the 'gold' standards for detecting fairly low concentrations of Zn^{2+}, albeit such compounds also exhibit weak selectivity for Cu^{2+}.[3d] The origin of such selectivity appears to be encoded by facile changes in the coordination state of Zn^{2+} versus Cu^{2+}.[5]

Due to diversity in functional roles of Zn^{2+} in biological system (viz., DNA synthesis, apoptosis, structural motifs in proteins, enzyme co-factors, etc.), we became interested in designing the Zn^{2+} selective fluorescent probes. In this endeavor, we noted that Cai et al.[6] synthesized *N*,*N*,*N*′,*N*′-tetrakis(carboxylatemethyl)-2,6-diaminocresol as the divalent metal binding probe, and demonstrated that it forms five coordinate trigonal bipyramidal structures with both Co^{2+} and Cu^{2+}, and the ligand–metal conjugate yields a charge-transfer band around 300 nm. The structural data revealed that besides carboxyl and amino groups, the phenolate oxygen of the above ligand was involved in the coordination bond with Co^{2+} and Cu^{2+}, and that the latter exhibited a somewhat distorted configuration. On assumption that the phenolate oxygen might have some role in altering the coordination geometry of divalent metal ions, we synthesized {[3-(biscarboxymethylamino)-2-methoxy-5-methylphenyl]carboxymethylamino}acetic acid (compound **1**) in which the phenolic oxygen was methylated. As elaborated below, the resultant compound emerged out to be a Zn^{2+}-specific fluorescent probe.

Scheme 1 describes the synthesis of compound **1** in three simple steps. Commercially available 2-methoxy-5-methyl-1,3-dinitrobenzene was reduced with tin and concentrated HCl and neutralized with NH_4OH to form the diamine in 70% yield. The diamine was then N-alkylated with bromoethyl acetate in the presence of KI and K_2HPO_4 to form compound **1**′ in 52% yield. Compound

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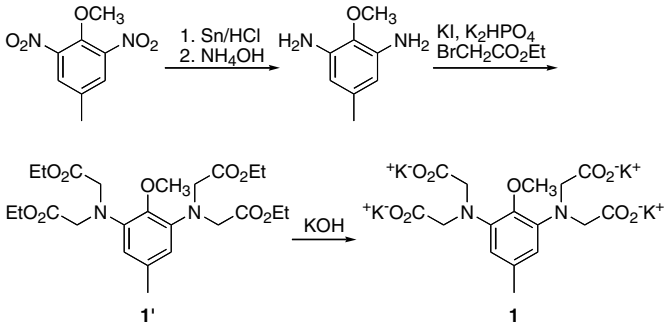

Scheme 1. Synthesis of the sensor.

1′ was hydrolyzed with KOH to form compound **1** (sensor) in 96% yield.

We determined the influence of divalent metal ions on the fluorescence spectral profile of compound **1**. Figure 1 shows the fluorescence emission spectra of 200 μM compound **1** ($\lambda_{ex} = 300$ nm) with nearly stoichiometric concentrations of selected divalent metal ions (present in the biological system). Note that compound **1** has a weak fluorescence emission peak around 386–390 nm, which is differently affected by different metal ions. For example, whereas the emission intensity of compound **1** (at 390 nm) is barely affected in the presence of Mg^{2+}, it is slightly increased in the presence of Ca^{2+}. On the contrary, the fluorescence intensity of compound **1** decreases in the presence of Cu^{2+}, Ni^{2+}, and Co^{2+}. The most dramatic effect of fluorescence profile of compound **1** is observed in the presence of Zn^{2+}. As noteworthy from the data of Figure 1, the presence of stoichiometric concentration of Zn^{2+}, the fluorescence emission intensity of compound **1** is increased by about 10-fold. Such an enhancement in fluorescence intensity is not kinetically controlled as the time dependent incubation of Zn^{2+} with compound **1** does not alter the emission intensity. This deduction is equally valid for the interaction of other metal ions with compound **1**, irrespective of their spectral modulating features. We further observed that the Zn^{2+}-induced fluorescence

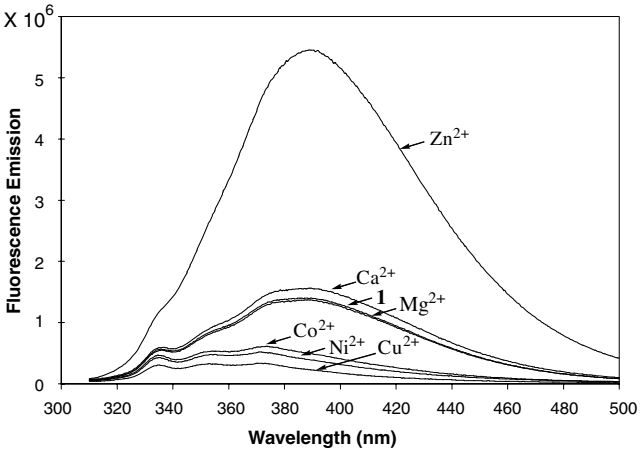

Figure 1. Fluorescence emission spectra of compound **1** in 20 mM HEPES buffer (pH 7.0) in the presence of different metal ions. The concentrations of compound **1** and the metal ions were maintained at 200 μM.

enhancement of compound **1** was maintained even in the presence of 100-fold excess of Ca^{2+} (data not shown). Hence, compound **1** can be utilized to detect Zn^{2+} in the physiological milieu containing the high concentrations of Ca^{2+}. A cumulative account of these results leads us to propose that compound **1** functions as a Zn^{2+} specific fluorescent probe (sensor).

To determine the magnitude of Zn^{2+} induced fluorescence spectral changes of compound **1** as well as its binding affinity, we performed a detailed spectrofluorometric titration studies. Figure 2 (right panel) shows the fluorescence emission spectra of compound **1** (corrected for the buffer) as a function of increasing concentrations of $ZnCl_2$. The fluorescence emission intensity at 386 nm ($\lambda_{ex} = 300$ nm) shows a saturating profile as a function of $ZnCl_2$ (Fig. 2, left panel). Since the concentration of compound **1** was comparable to the initial concentrations of Zn^{2+}, the binding constant of compound **1**–Zn^{2+} complex was calculated by a complete solution of the quadratic equation, describing their interaction.[7] The solid line is the best fit of the experimental data for the K_d value of 3.9 ± 1.8 μM and the stoichiometry of 1:1 (i.e., 1 mol of bound Zn^{2+} per mol of compound **1**). Of these parameters, the stoichiometry of the complex suggested that only one of the two metal chelating group was involved in the binding of Zn^{2+}, the feature contrary to that observed for the binding of Co^{2+}/Cu^{2+} with *N*,*N*,*N*′,*N*′-tetrakis(carboxylatemethyl)-2,6-diaminocresol.[6] We surmise that the origin of the unusual stoichiometry of compound **1**–Zn^{2+} conjugate lies in the methylation of phenolate oxygen of compound **1.**

The question arises why compound **1** (vis a vis analogous compounds reported in the literature) functions as Zn^{2+} selective fluorescent sensor. Although a definitive answer to this question must await structural determinations of different metal ions conjugated to compound **1**, circumstantial evidence leads us to conjecture the following. It has been well established that unlike Cu^{2+}, Co^{2+}, and Ni^{2+} (which predominate either as the square planar or tetrahedral coordination state), Zn^{2+} preferentially exists in the octahedral state, and in this state, it can interact with all six groups contributed by the two iminodiacetate moieties of compound **1**. This hypothesis is supported by the stoichiometry of compound **1**–Zn^{2+} complex being equal to 1:1 (Fig. 2). It is possible that compound **1**–Zn^{2+} complex has limited nonradiative decay pathways available to it (leading

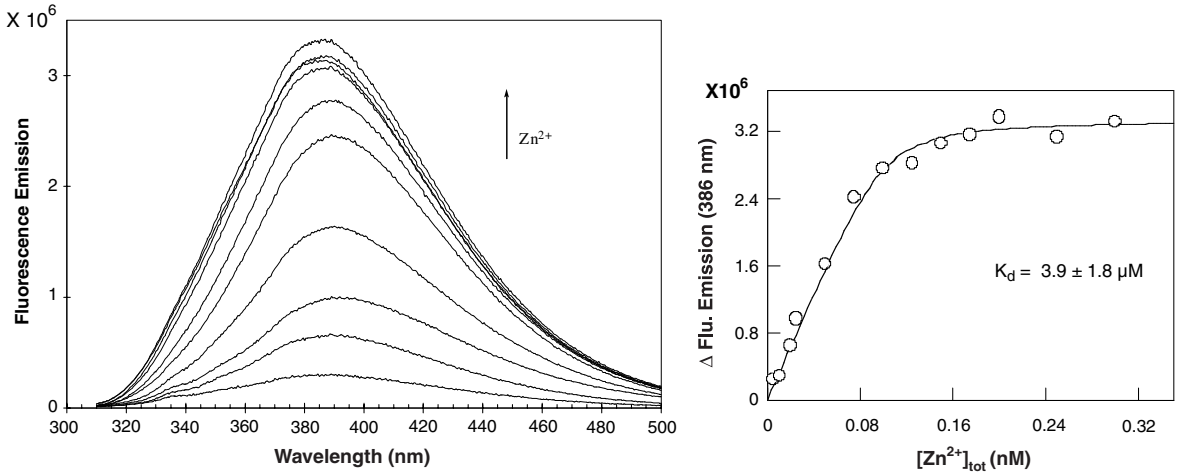

Figure 2. Fluorescence emission spectra of 100 μM of compound **1** in HEPES buffer in the presence of different concentrations of Zn^{2+}. The concentration of Zn^{2+} was increased from 10 to 300 μM (left panel). The right panel shows the increase in fluorescence intensity (at 386 nm) as a function of Zn^{2+} concentration. The solid smooth line is the best fit of the data for the K_d value of 3.9 ± 1.8 μM and the stoichiometry of 1:1 for compound **1**–Zn^{2+} complex.

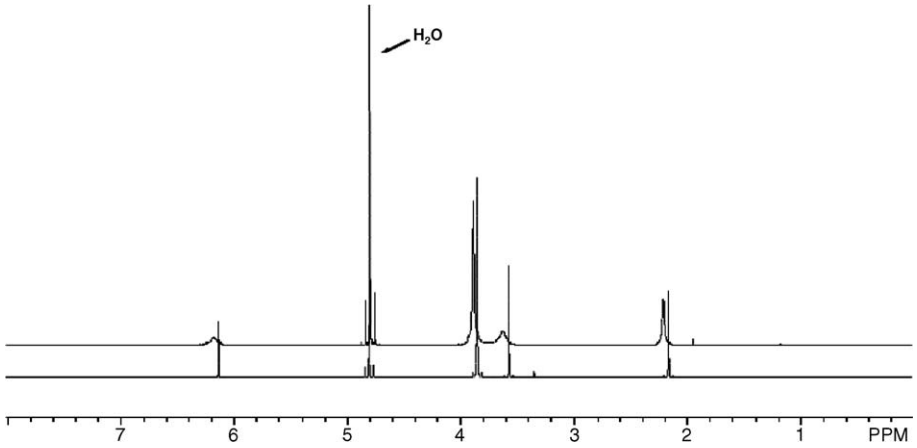

Figure 3. ^1H NMR trace of compound **1** (bottom trace) and the 1:1 mixture of **1** and Zn(II) (top trace) in D_2O.

to enhanced fluorescence intensity) compared to compound **1** itself, while the tetracoordinated metal complexes with unfilled d-orbitals (Cu^{2+}, Co^{2+}, and Ni^{2+}) quench the excited singlet state of the sensor thereby reducing its fluorescence intensity.

Compound **1**–Zn complex has low solubility in water (the solubility was not a factor in the concentrations used in fluorescence experiments) and methanol. All attempts at recrystallization of the complex so far led to the formation of a white powder. Saturated solution of the complex in D_2O was subjected to ^1H NMR analysis in an attempt to elucidate the structure of the complex. Comparison of the ^1H NMR of compound **1** and its 1:1 mixture with Zn(II) (Fig. 3) indicated that the zinc binding induces a deshielding effect on all the protons accompanied by substantial peak broadening. The broadening of the peaks imply that the complex is fluxional, which may explain the poor recrystallization properties.

It should be emphasized that compound **1** can be easily synthesized in three steps from readily available starting materials. This is a major advantage over the synthetic protocols of known divalent metal ions sensors in the literature. Due to its high solubility in the aqueous medium, it has the potential to serve as a selective fluorescence probe (sensor) for detection of Zn^{2+} in biological samples. Moreover, low solubility of the complex could be useful in extracting Zn^{2+} ions from environmental matrix, when high concentrations of Zn are present. We are currently in the process of modifying compound **1** to shift its excitation and emission spectra in the visible region, as well as to enhance the binding affinity for Zn^{2+}, and we will report these findings subsequently.

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Supplementary data

Experimental procedures and spectral data for all the materials are available as supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.02.029.

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