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L-cysteine recognition triggered by Zn^{2+} complexation with ligand

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ABSTRACT

A new type of benzimidazole-based imine-linked fluorescence chemosensor for Zn^{2+} was synthesized. Coordination of Zn^{2+} with the receptor led to enhanced fluorescence while other metals quenched the receptor fluorescence. The resulting complex had a supramolecular assembly with a 1:1 host-guest ratio with variable size. The $(1.Zn^{2+})_n$ assembly showed high selectivity towards the complexation of L-cysteine even in the presence of other potentially competitive biologically important anionic species.

Research on the interactions of biomolecules with metal ions has grown [1]. Such interactions include the role of metal ions as cofactors that promote the catalytic activities of enzymes. Inspired by these types of interactions, supramolecular chemists have been using these concepts for the construction of various types of supramolecular devices including chemosensors, molecular logic gates, and others [2,3]. Metal ion coordination with organic receptors may provide: a) additional binding sites for the recognition of anions through electrostatic interactions with the metal center allowing recognition of anions in polar solvents and b) better steric orientation for selective binding of anions [4]. In short, metal ion binding with an organic receptor may trigger a positive cooperative effect for anion recognition [5].

The second most important transition metal in human body is zinc. Its deficiency and excess may lead to several disorders like apoptosis, epilepsy, Alzheimer's disease, growth retardation, diarrhea and impotence [6]. Therefore, developing selective and sensitive probes for the detection of Zn^{2+} is a matter of interest.

Sulfur-containing amino acids and peptides have drawn a great deal of attention due to their essential, and significant functions in biological systems [7]. Cysteine comes under the category of an essential amino acid, and a deficiency of cysteine may lead to hair depigmentation, liver damage, skin lesions, and stunted growth [8]. The thiol group of cysteine is involved in many biological oxidation and reduction reactions [9]. High-performance liquid chromatography (HPLC) and electrophoresis are generally used for the detection of cysteine [10–12], methods that are time-consuming and quite expensive. Fluorescence spectroscopy has several advantages to these analytical methods because of its high sensitivity, accuracy, and quick analysis time. Although great efforts have been devoted on fluorescent chemosensors for cysteine [13], the reports on turn-on fluorescent chemosensors remain rare, which are beneficial for bioimaging the distribution of cysteine in cellular processes [14]. Here, we present a new type of benzimidazole-based imine-linked fluorescent chemosensor for Zn^{2+}, which was further utilized in the development of a turn-on fluorescent chemosensor for L-cysteine.

Receptor 1 was prepared according to Scheme 1. Compound 2 [15] was treated with 2-aminobenzimidazole under basic conditions to afford compound 3 that was converted into receptor 1 by reaction with salicylic aldehyde in the presence of a catalytic amount of $Zn(ClO_4)_2$ in MeOH.

The photophysical properties of receptor 1 were investigated by UV–vis absorption profile as well as its fluorescence emissions. The UV–vis absorption spectra recorded at a 10 μM concentration of receptor 1 in DMSO/CH_3CN (1:9; v/v) exhibited a band at 372 nm (Fig. S1). This band appeared to be a consequence of charge transfer between –OH and –CH═N-linkages [16]. Upon excitation at 372 nm, the solution revealed a dual channel emission at 440 and 545 nm. This dual channel emission is the consequence of keto–enol tautomerism involving the –OH and –CH═N-functional groups of the fluorophore [17].

To investigate different analytes, receptor 1 was monitored with a library of 25 anions/dicarboxylic acids/thiols. In this context, a solution of receptor 1 (10 μM) in DMSO/CH_3CN (1:9, v/v) was prepared. Aliquots of anions/dicarboxylic acids/thiols (40 μM) were added, and the respective emission spectra were measured. A sufficient time (approx. 45 min) was allowed for each solution to attain equilibrium before recording the fluorescence profile. Most of the tested species had little effect on the fluorescence intensity of receptor 1. The most probable reason for weak coordination of these species is due to competition between the analyte and solvent for receptor binding sites.

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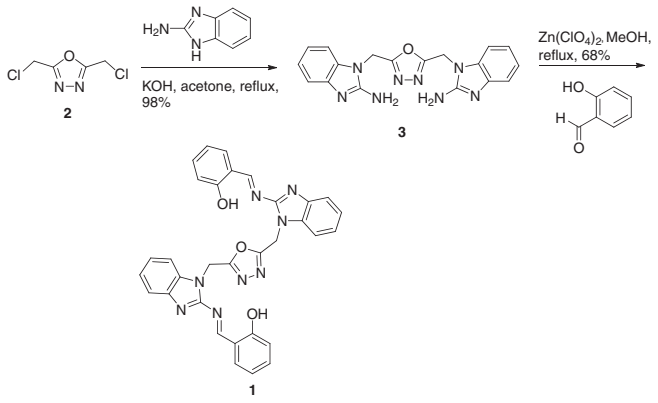

Scheme 1. Synthesis of compound **1**.

In another set of experiments, the impact of different cations on the fluorescence profile of receptor 1 was tested. These typical experiments involved the metal recognition properties of receptor 1 by mixing a 10 μM solution of receptor 1 with a metal nitrate salt (40 μM) in DMSO/CH_3CN (1:9, v/v) solvent. The fluorescence spectrum of each solution was measured with excitation at 372 nm. Although most of the tested metal ions quenched the emission intensity of receptor 1, Zn^{2+} coordination led to enhanced fluorescence (Figs. 1A and S2). This enhancement in intensity is due to the greater molecular rigidity of the complex, which makes non-radiative decay less probable from the excited state, and coordination from imine linkages, which otherwise prevails due to *cis-trans* isomerism.

To gain further insight into the sensor activity of receptor 1 for Zn^{2+}, a titration was performed with 10 μM of receptor 1 and sequential addition of Zn^{2+} (0 –120μM) in DMSO/CH_3CN (1:9, v/v) solvent (Fig. 1B). Successive additions of Zn^{2+} to receptor 1 solution led to quenching of the fluorescence intensity at 545 nm that originates from the keto form of receptor 1 and enhancement at 412 nm, which was assigned to the enol form of the receptor. These results indicate that the keto and enol forms of pure receptor 1 are in equilibrium. Zn^{2+} coordination led to binding with the enol form and thus, removal of the keto form from the equilibrium. A comparison of binding of receptor 1 with Zn^{2+} (Fig. S2) and titration of receptor 1 with Zn^{2+} (Fig. 1B) showed differences in their spectra. The addition of 40 μM of Zn^{2+} in a single portion to 10 μM of receptor 1 led to enhanced fluorescence intensity of receptor 1 at only 440 nm (Fig. S2), while the stepwise addition of Zn^{2+} to a solution of receptor 1 had affected the shape and position of the emission originally at 440 nm. We previously reported a similar effect highlighting the "tuning of the fluorescence profile of a sensor" through the addition of the same analyte but varying its time of addition [18]. The binding constant for the $1.Zn^{2+}$complex was calculated to be 7.9 (± 0.9) × 10 M^{-1} using the Benesi–Hildebrand method (Fig. S3) [19].

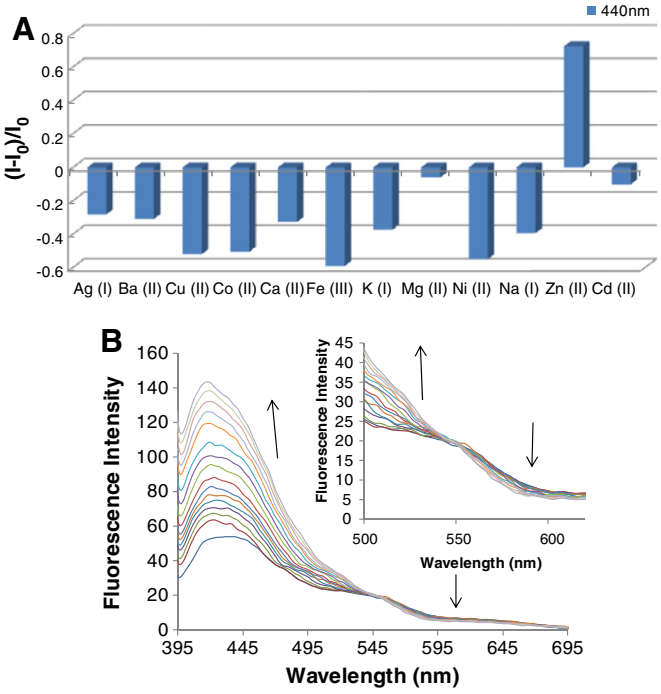

Fig. 1. (A) Fluorescence ratio ($(I - I_0)/I_0$) of receptor 1 (10 μM) upon addition of metal nitrate salts (40 μM) in a DMSO/CH_3CN (1:9, v/v) solvent system excited at 372 nm; (B) Changes in fluorescence spectra of receptor 1 (10 μM) in a DMSO/CH_3CN (1:9, v/v) solvent system excited at 372 nm upon consecutive additions of Zn^{2+} ions (0–120 μM) (inset represents the decrease in fluorescence intensity around 545 nm).

In order to evaluate potential interference from other metal ions for Zn^{2+} estimation, a competitive binding test was performed, which showed that other metal ions did not affect Zn^{2+} recognition with receptor 1, as shown in Fig. 2. Although Fig. 1A shows the influence of several metal ion on the fluorescence profile of 1; however, Fig. 2 advocates no influence of any of these metals ions on the fluorescence profile of 1 for Zn^{2+}. This highlights the much stronger binding affinity of 1 for Zn^{2+} as compare to other metal ions.

To confirm the stoichiometry of the $1.Zn^{2+}$ complex, a Job's plot analysis was performed, which showed a 1:1 stoichiometry for the complex (Fig. S4) [20]. CHN analysis also supported the formation of a 1:1 complex. A comparison of IR spectra of receptor 1 and $1.Zn^{2+}$ showed that there were shifts of the imine linkages, –CH═N–, as well as the –OH band upon complexation with Zn^{2+} (Fig. S5). It was concluded that the nitrogen atom of the –CH═N– group and the oxygen atom of the –OH group were involved in metal coordination. Surprisingly, the mass spectrum of $1.Zn^{2+}$ did not indicate formation of a 1:1 metal complex as the mass obtained was substantially higher than expected. This contemplates us to evaluate the metal complex with transmission electron microscopy (TEM) and results suggested the formation of nano-aggregates of the metal complex (32–50 nm) (Fig. 3). These types of metal complex could be expected since receptor 1 has two well-defined pseudocavities, which are able to form a supramolecular assembly similar to the one adopted by bi-functional groups [21].

This leads us to conclude that the formation of the $(1.Zn^{2+})_n$ supramolecular assembly occurred as shown in Scheme 2, where the size of the assembly was dependent on the mixing time of the precursors (Fig. S6). The supramolecular assembly of $(1.Zn^{2+})_n$ was thought to be an excellent candidate for recognition of anions/dicarboxylic acids/thiols, since metal coordination could provide better steric orientation and additional binding sites through electrostatic interactions between the metal center and an anionic analyte. For the recognition of anionic

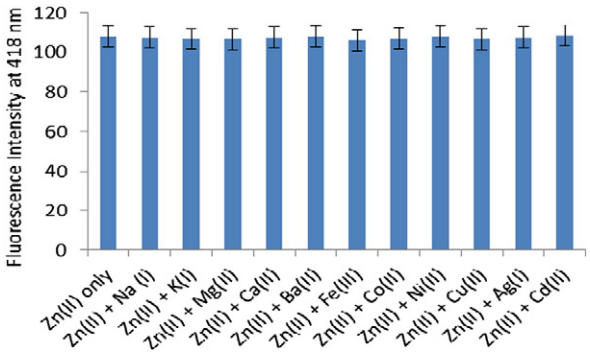

Fig. 2. Detection of Zn^{2+} with receptor 1 in the presence of other metal ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Ag^+, Cd^{2+}) in DMSO/CH_3CN (1:9, v/v).