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Communication

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Novel Chemosensor for the Visual Detection of Copper(II) in Aqueous Solution at the ppm Level

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Supporting Information

ABSTRACT: A new water-soluble, multisite-coordinating ligand LH_7 was prepared by the condensation of tris(hydroxymethyl)aminomethane with 2,6-diformyl-*p*-cresol. LH_7 is a selective chemosensor for Cu^{2+}, under physiological conditions, with visual detection limits of 20 ppm (ambient light conditions) and 4 ppm (UV light conditions). LH_7 can also be used in biological cell lines for the detection of Cu^{2+}.

The recognition of ions and molecules by chemosensors is important for medical diagnostics, chemistry, and biotechnology.[1] In this regard, the development of highly selective and sensitive probes for the detection Cu^{II} is very important because of its ubiquitous presence in environmental and biological systems.[2] Cu^{II} is the third most abundant transition-metal ion in the human body (after Fe^{III} and Zn^{II}) and is essential for many biochemical and physiological functions. Cu^{II} is also an important cofactor in nearly 20 metalloenzymes including tyrosinase, cytochrome *c* oxidase, and Cu/Zn superoxide dismutase.[3] In addition, Cu^{II} is an essential micronutrient for all known life forms.[4] However, Cu^{II} can also be an ecological pollutant and potentially toxic to living cells if present in slightly large concentrations.[5] Thus, Cu^{II} has been implicated in neurodegenerative diseases, such as Menkes, Wilson, and Alzheimer's diseases.[6] In view of the above importance of Cu^{II} in biological and ecological systems, there have been several ongoing efforts to develop accurate and selective detection techniques for Cu^{II}. Most of these techniques are based on chemosensors that undergo a fluorescence intensity enhancement (turn-on)[7a−j,8] or quenching (turn-off)[7k−m,9d] upon interaction with Cu^{II}. Turn-on methods are preferred over the turn-off phenomena because of the possible fluorescence quenching of the ligand by other organic substances in the system. If such interference is absent, the turn-off mechanism is equally viable for detection.[7k−m,9d] In comparison to fluorescence methods, accurate colorimetric techniques are far easier to implement in practical applications, and hence there has also been work in this regard for the detection of Cu^{II}. However, in spite of the impressive number of reports that have appeared in the literature,[8] there appears to be a need to develop better chemosensors that can selectively detect Cu^{II} in low concentrations and, importantly, in aqueous solutions.[9] As mentioned above, a visual detection would be quite attractive from a practical point of view.

Our group has some interest in the synthesis of chemosensors in general and of Cu^{II} in particular.[10] Accordingly, herein, we describe the synthesis and spectroscopic characterization of a novel colorimetric chemosensor that can selectively detect Cu^{II} in ppm levels under physiological pH conditions in an aqueous buffer solution. The multisite-coordinating ligand LH_7 (Figure 1), containing multiple hydroxyl groups, was

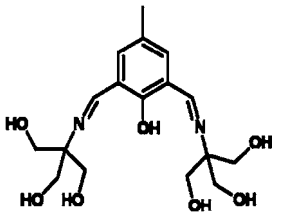

Figure 1. Structure of LH_7.

designed specifically to allow solubility in water. This was synthesized by the condensation of tris(hydroxymethyl)-aminomethane with 2,6-diformyl-*p*-cresol (see the Supporting Information, SI). The absorption spectrum of LH_7 in Tris buffer (pH 7.5) shows a maximum at 434 nm with an extinction coefficient of 10000 M^{-1} cm^{-1}, and the emission spectrum shows an intense peak around 505 nm with a fluorescence quantum yield of 0.37, which does not depend on the excitation wavelength. Upon the addition of 2 equiv of $Cu(NO_3)_2$ in an aqueous buffer solution of LH_7, the yellow color of the ligand-containing solution becomes almost colorless, which is detectable by the naked eye (Figure 2a). The fluorescence of LH_7 is also quenched appreciably, which can be visually detected under UV light (Figure 2b). Upon an increase in the concentration of Cu^{II} in the medium, the absorbance of LH_7 at 434 nm continues to decrease, with concomitant formation of new bands at around 415 and 380 nm (Figure S2 in the SI). The absence of any isosbestic point indicates the presence of multiple equilibria in the complexation event. The fluorescence behavior of the ligand is also very sensitive to the presence of Cu^{II}. The moderate fluorescence intensity of LH_7 continues to decrease upon the addition of Cu^{II} and becomes quenched in the presence of 2 equiv of Cu^{II} (Figure S3 in the SI). At a very high concentration of Cu^{II}, the fluorescence of the ligand is

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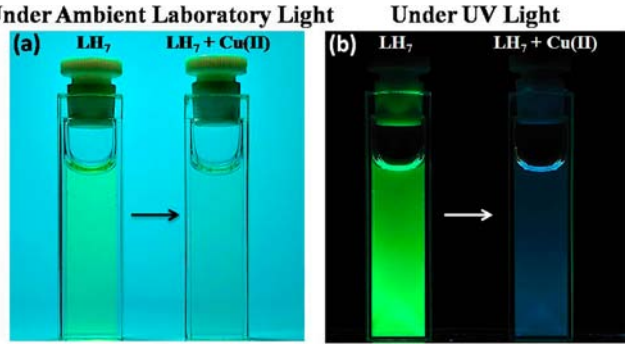

Figure 2. Photograph of a 10 μM LH_7 solution in Tris buffer (pH 7.5) in the absence and presence of Cu^{II} under (a) ambient laboratory light and (b) UV light.

completely quenched. This clearly indicates that the complex is nonfluorescent in nature.

The binding stoichiometry between LH_7 and Cu^{II} has been measured using the Job's plot method using a change in absorption at 375 nm (Figure S4 in the SI), where the concentrations of LH_7 and Cu^{II} were varied, keeping the total concentration constant. Analysis of the experimental result with a multipeak fitting method indicates that two different types of stoichiometric complexes, 1:2 and 2:1, for LH_7 and Cu^{II}, are present in the solution. For the binding constant measurement, we have kept the concentration of LH_7 constant and measured the change in absorbance with an increase in the Cu^{II} concentration. For 1:2 and 2:1 complexation between LH_7 and Cu^{II}, the overall equilibrium can be written as

$$2L + M \overset{K_1}{\rightarrow} L_2M \quad (1)$$

$$L + 2M \overset{K_2}{\rightarrow} LM_2 \quad (2)$$

where L stands for ligand and M stands for Cu^{II}. K_1 and K_2 are the binding constants for the $(LH_7)_2Cu^{II}$ and $(LH_7)Cu^{II}_2$ complexes, respectively. Although we could not isolate crystals of the copper complexes, we were able to obtain crystals of a dimeric zinc(II) complex, $[\{LH_6Zn_2(OAc)_2\}\{OAc\}]$ (Figure S1 in the SI), which indicates the binding modes of the ligand. Upon the addition of Cu^{II}, the absorbance of LH_7 at 430 nm continues to decrease and new bands start to develop on the higher energy side. Under the assumption $[M]_T \gg [L_2M]$, the change in absorbance at 430 nm due to complex formation can be written as

$$A_{430} = \varepsilon^{L}_{430}\left([L]_T - \frac{(1 + 4K_1[L]_T M_T - \sqrt{1 + 8K_1[L]_T[M]_T})}{4K_1[M]_T} - \frac{K_2[L]_T[M]_T^2}{1 + K_2[M]_T^2}\right) \quad (3)$$

where A_{430} and ε^{L}_{430} are the absorbance and molar extinction coefficient of the LH_7 at 430 nm, respectively. $[L]_T$ and $[M]_T$ stand for the initial total concentrations of LH_7 and Cu^{II}, respectively. Figure S5 in the SI shows the change in the absorbance of LH_7 at 430 nm upon an increase in the concentration of Cu^{II}. This was fitted by using eq 3, and the binding constants for the two complexes $[(LH_7)_2Cu^{II}]$ and $[(LH_7)Cu^{II}_2]$ are estimated as 1×10^2 and 4×10^4 M^{-2}, respectively. The spectroscopic behavior of LH_7 upon the addition of Cu^{II} salts having different counteranions (e.g., sulfate, nitrate, acetate, chloride, perchlorate) in Tris buffer remain the same as in the case of $Cu(NO_3)_2$, indicating that there is no role for counteranions on the complex formation (see Figure S6 in the SI). The change of color of LH_7 from yellow to colorless by the addition of different copper salts has also been seen.

To illustrate the use of LH_7 as a specific Cu^{II} sensor, we have studied the complexation behavior of LH_7 with Cu^{II} in the presence of different metal ions like K^+, Ca^{2+}, Hg^{2+}, Mg^{2+}, Na^+, Cd^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}. A competitive experiment has been done for 5 μM LH_7 with 15 μM (3 equiv) of Cu^{II} in the presence of 50 μM (10 equiv) of other metal salts. As shown in Figure 3, there is no detectable change in the

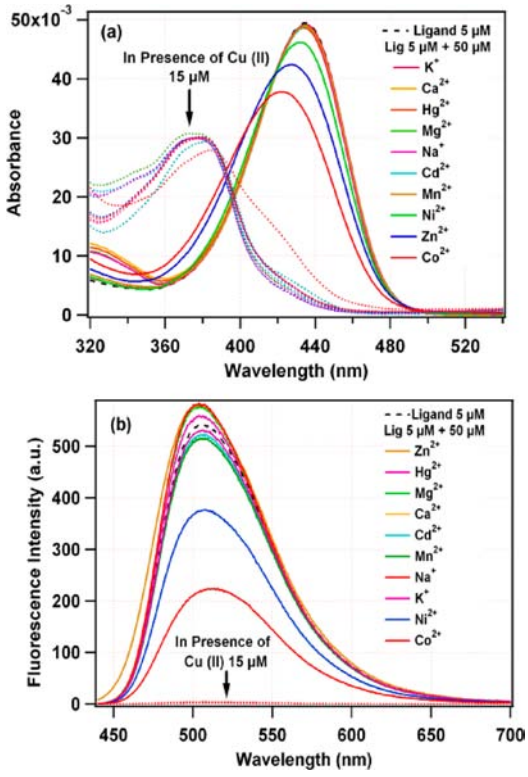

Figure 3. UV−vis absorption (a) and emission (b) spectra of LH_7 (5 μM) in Tris buffer (pH 7.5) in the absence and presence of 50 μM of various metal ions (e.g., K^+, Ca^{2+}, Hg^{2+}, Mg^{2+}, Na^+, Cd^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}) (solid lines). In both figures, dotted lines are for 5 μM LH_7 and 50 μM of various metal ions in the presence of 15 μM Cu^{2+}.

absorption spectrum of LH_7 even in the presence of 10 equiv of metal ions except for Cu^{II}. However, upon the addition of 3 equiv of Cu^{II} to the mixture, the absorption intensity around 430 nm is reduced and a new band appears at 380 nm (similar to the result obtained upon treatment of LH_7 with only Cu^{II}). The behavior of the fluorescence spectra also indicates that LH_7 can be solely used as a Cu^{II} sensor.

To test this sensor in a biological system, we have used the mammalian kidney cell line COS-7. The cells were grown in standard conditions[11] and treated with the ligand (200 μM; we ascertained, by preliminary cytoxicity experiments, that cell death occurs at a ligand concentration of 250 μM), and at the end of 30 min, Cu^{II} was added (2 mM) and the cells were incubated further for a period of 30 min. The cells were washed twice with 1× phosphate-buffered saline, and the fluorescence from the living cell was evaluated under a fluorescence

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microscope, both before and after the addition of Cu^{II}. As shown in Figure 4, cells treated with the ligand exhibited intense green emission and the same disappeared when suchcells were treated with Cu^{II}. Untreated cells do not show any fluorescence.

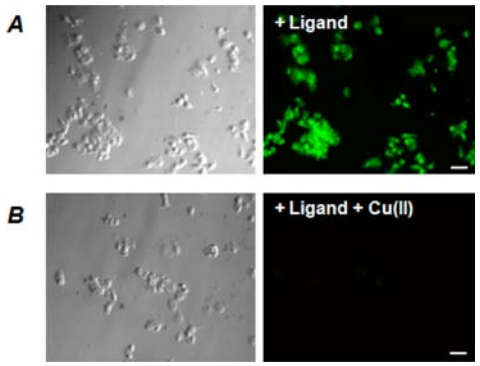

Figure 4. Live-cell imaging of COS-7 cells treated with LH_7 before (A) and after (B) incubation with Cu^{II}. The right panel represents the bright-field transmission images, and the left panel represents the fluorescence images. Fluorescence imaging was done with the same exposure time. Scale, 20 μm.

In conclusion, we have reported a new sensor for Cu^{II}, which changes its color from yellow to colorless in the presence of 2 equiv of Cu^{II}. The effect of other metal ions has almost no effect on the sensing of Cu^{II} by the designed sensor. The yellow color of the sensor solution in aqueous buffer is prominently visible when its concentration is 10 μM, and the addition of 20 μM Cu^{II} completely decolorizes the sensor solution, revealing that the detection level is up to 20 ppm of Cu^{II} by the naked eye by this sensor. However, under UV light illumination, one can visually detect even 4 ppm Cu^{II} in aqueous buffer solution without the aid of any sophisticated instruments. Nevertheless, if one uses a spectrophotometer or spectrofluorimeter, the lower limit of detection can be as low as 1 ppm. This sensor can also be used for the quantitative estimation of Cu^{II} because the binding constants and fluorescence quantum yields of the complexes formed are known. Moreover, we have successfully shown that the sensor has no detrimental effect in a biological system, which indicates its potential for in vivo applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

X-ray crystallographic data in CIF format, experimental details, and additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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