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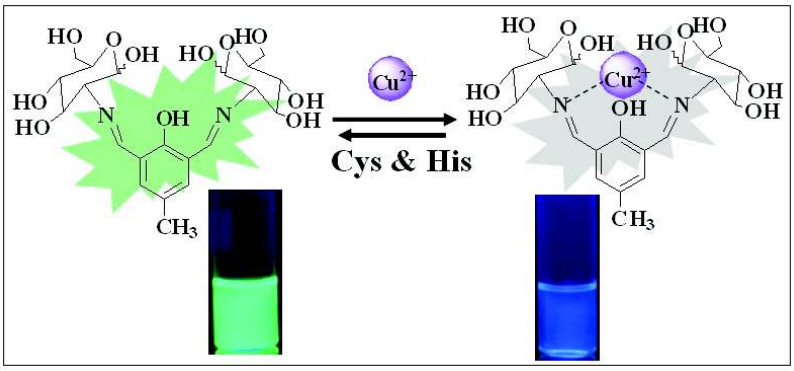
Diimino conjugate of glucosyl-cresol as receptor for Cu^{2+} and its complex for cysteine and histidine#

Atanu Mitra, Sivaiah Areti, Ankit K. Mittal, Snehasis Bhakta and Chebrolu P. Rao*

Bioinorganic Laboratory, Department of Chemistry, Indian Institute of Technology Bombay, Mumbai - 400 076, India.

Abstract

A simple colorimetric approach for Cu^{2+} sensing was developed on a diimino conjugate of glucosyl-cresol **(L).** The **L** is characterized by ^{1}H-NMR and ESI-MS spectra. **L** exhibits intense greenish fluorescent color in ethanol. This highly fluorescing **L** has been used to recognize Cu^{2+} selectively among the ten biologically important metal ions studied. Secondary recognition of **L** as its complex of Cu^{2+} towards cysteine and histidine has been established among twenty naturally occurring amino acids studied. Thus, the sensing of Cu^{2+} by **L**, as well as its Cu^{2+} complex towards amino acids has been demonstrated by absorption and visual color change experiments. Both the absorption and colorimetric titration results suggest that compound **L** is a highly selective sensor for Cu^{2+} and its complex for cysteine and histidine.



Keywords : Diimino conjugate of glucosyl-cresol; Colorimetric detection; Naked-eye sensor for Cu^{2+}; Secondary recognition.

Introduction

Copper is one of the most abundant and important transition metal ions among those present in the biological systems, including humans, owing to its diverse functions exhibited as cofactor in several metalloenzymes.[1-3] Some of these functions are driven solely on the concentration of the copper present in the particular species.[4] Till date, several methods were reported for the detection of copper as its divalent ion, *viz.*, Cu(II),[5,6] best among these is the easy detection of the ion by simple visual colour change and hence is of utmost importance.[7-12] Unfortunately, most of these methods suffer either from non-specific or from non-selective nature since several ions act as competitors.[13-15] Hence, the synthesis of new molecules as naked eye sensor for Cu^{2+} at sub-micromolar concentration with high selectivity is still a challenge to synthetic chemists. Further, if the Cu^{2+} bound receptor can exhibit additional recognition, such as towards amino acids, etc., this will lead to the secondary sensing behavior of the receptor, which will be most welcome. Another challenge that the synthetic chemists often face is the solubility and stability of the receptor molecules in bio-relevant medium to impart its utility to the biological systems. Since the carbohydrate moiety brings in both the water-solubility as well as biocompatibility, C2-glucose based imino-conjugate [2,6-di-(D-glucopyranosyl-2′-deoxy-2′-iminomethyl)-4-methyl-1-hydroxybenzene] has been studied as the receptor molecule towards Cu^{2+} and its complex has been studied towards recognizing naturally occurring amino acids.

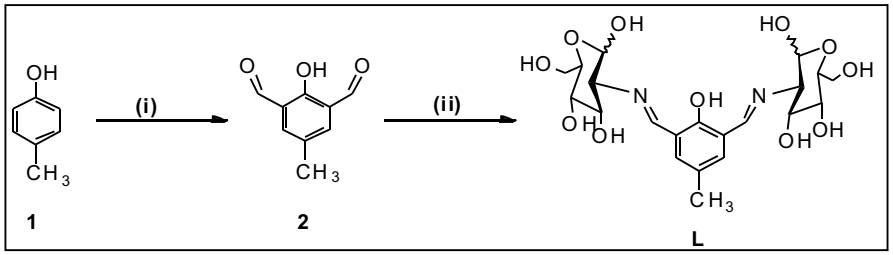
* Corresponding author : C. P. Rao
Tel: +91-22-25767162; Fax: +91-22-25723480
E-mail: cprao@iitb.ac.in

Dedicated to late Prof. D. Loganathan, IIT Madras, Chennai, India

Results and discussion

Development of the receptor molecule

The chemo sensor (**L**) has been synthesized by one step condensation of glucosamine with 2,6-di-oxo-4-methyl-1-hyrdoxybenzene (**2**) as shown in Scheme 1. Initially, **2** was synthesized from 4-methyl-1-hydroxybenzene (**1**, *p*-cresol) using hexamethylenetetramine (HMTA) to introduce formyl group in trifluoroacetic acid medium.[16] The products were characterized by analytical and spectral techniques.



Scheme 1: Synthesis of diimino conjugate of glucosyl-cresol (**L**): (i) CF_3COOH, HMTA, reflux; (ii) glucosamine, ethanol, 80 °C.

Cu^{2+} Recognition by L

The sensitivity of **L** towards different metal ions and their preferential selectivity towards Cu^{2+} has been studied by colorimetric and absorption titrations.

(a) Colorimetric detection of Cu^{2+}: In order to explore the chemo sensor properties of **L**, the receptor was titrated against a number of divalent metal ions, *viz.*, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+} ions in ethanol. The titrations were monitored qualitatively by visually observing the color change. In order to do this, studies were carried out by mixing **L** with metal ions in 1:2 molar ratio in ethanol and the results are shown in Figure 1. Distinct visual color change was noticed only in the presence of Cu^{2+}, while the presence of all other ions exhibited practically no change in the colour of the solution. Thus, in presence of Cu^{2+}, the greenish fluorescent color of **L** was converted to non fluorescent due to the formation of the complex of **L** by Cu^{2+}.

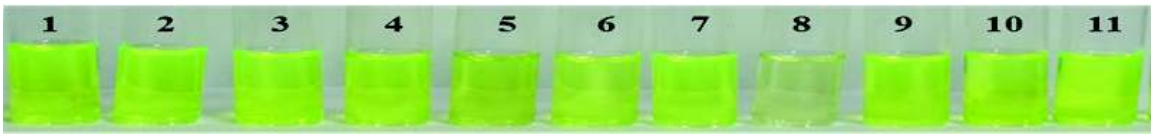


Figure 1: Colour of the ethanolic solutions of **L** in the presence of different metal ions in 1:2 ligand to metal molar ratio. Vial 1 is a simple ligand (control). vials 2 to 11 are in the presence of Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+} ions respectively.

(b) Colorimetric titration in presence of competitive ions: The selectivity of **L** towards Cu^{2+} over the other transition metal ions was further confirmed by challenging the persistence of the final solution color in the presence of other competitive metal ions in the same medium. This was carried out in two different ways. In one, the [**L** + M^{n+}] complex was titrated with Cu^{2+} (Figure 2) and in the other the [Cu**L**] mixture was titrated with the other metal ions (M^{n+}, Figure 3). In both cases, the results are supportive of **L** being selective to only Cu^{2+} as shown in the conclusions. Thus the persistence of color change in the presence of other metal ions indicates selectivity of **L** towards Cu^{2+}.

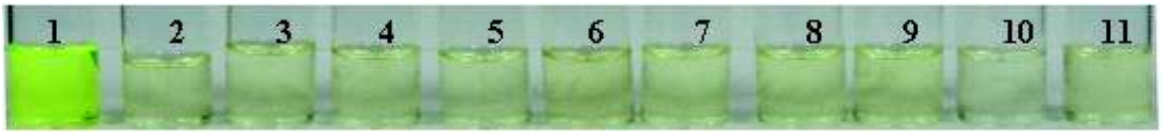


Figure 2: Competitive ion titration. 1 = **L,** 2 = [**L**+Cu^{2+}], 3 - 11 = [(**L** + M^{n+}) + Cu^{2+}] where M^{n+} in 3 = Mg^{2+}, 4 = Ca^{2+}, 5 = Mn^{2+}, 6 = Fe^{2+}, 7 = Co^{2+}, 8 = Ni^{2+}, 9 = Zn^{2+}, 10 = Cd^{2+}, 11 = Hg^{2+}

(c) Absorption titration of L by Cu^{2+}: In order to quantify the visual colour change occurred upon mixing **L** with M^{n+} and also to establish the complex formation with Cu^{2+}, titrations were carried out by measuring the UV-visible absorption spectra (Figure 4). The absorption spectrum of **L** in ethanol exhibited a band at ~455 and 250 nm. Upon addition of increasing concentrations of Cu^{2+}, the spectra showed increase in the absorbance at 250 nm, and the 455 nm band shifted by 75 nm to give its new absorbance at 380 nm. However Fe^{2+}, Ni^{2+} and Zn^{2+} showed only marginal changes in their absorbance at 455 nm and band shifted by 35 nm only instead of 75 nm to give its new absorbance at 420 nm.

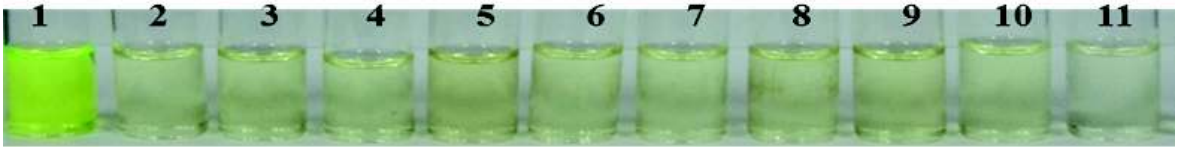


Figure 3: Competitive metal ion titration of [**L**+Cu^{2+}] by M^{n+} in ethanol with: 1 = **L**, 2 = (**L** + Cu^{2+}), 3 - 11 = [(**L** + Cu^{2+}) + M^{n+}] where M^{n+} in 3 = Mg^{2+}, 4 = Ca^{2+}, 5 = Mn^{2+}, 6 = Fe^{2+}, 7 = Co^{2+}, 8 = Ni^{2+}, 9 = Zn^{2+}, 10 = Cd^{2+}, 11 = Hg^{2+}

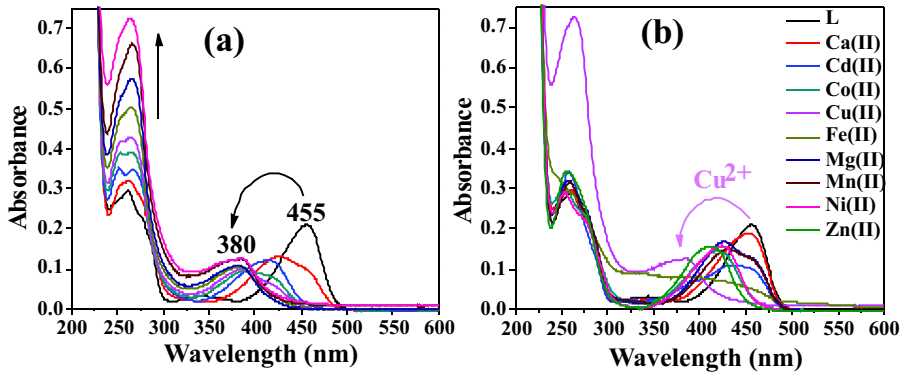


Figure 4: UV-visible absorption spectra for the titration of **L** with M^{2+} in ethanol: Spectral traces in case of (a) **L** against Cu^{2+} {0 (black) to 10 (pink) equivalents}; and (b) **L** with M^{2+} at 10 equivalents of concentration.

Recognition of cysteine and histidine by [CuL**] Complex**

(a) Colorimetric titration: The Cu^{2+} complex [Cu**L**] exhibited weak fluorescent color in ethanol. The utility of [Cu**L**] in recognizing a particular amino acid has been investigated using all the twenty naturally occurring amino acids. In order to study the amino acid recognition, the receptor **L** and Cu^{2+} were taken in 1:2 molar ratio, and the *in situ* generated complex [Cu**L**] was titrated with different amino acids and the visual color changes observed are shown in Figure 5. Among the twenty naturally occurring amino acids studied, only cysteine and histidine brings back the original color of the receptor **L**, suggesting the displacement of Cu^{2+} from its complex. Hence, the *in situ* generated Cu^{2+} complex of **L** can be used as a secondary sensor towards cysteine and histidine.

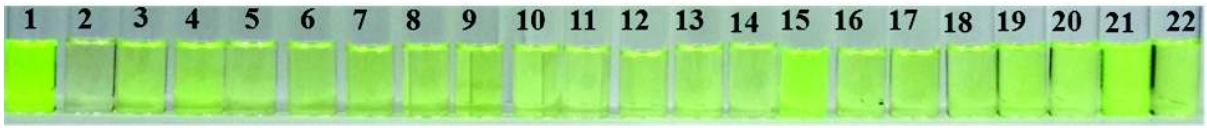


Figure 5: Color change under visible light; 1=**L**, 2=(**L**+Cu^{2+}), 3 - 22=[(**L** + Cu^{2+}) + AA] where AA (Amino acids) in 3=Asp, 4=Asn, 5=Glu, 6=Gln, 7=Gly, 8=Ala, 9=Val, 10=Leu, 11=Ile, 12=Tyr, 13=Trp, 14=Phe, 15=His, 16=Lys, 17=Arg, 18=Pro, 19=Ser, 20=Thr, 21=Cys, 22=Met.

(b) Absorption studies of the selective recognition of cysteine and histidine by [CuL] Complex: The recognition of cysteine and histidine by the [CuL] complex was further supported by carrying out the absorption studies with all the naturally occurring amino acids, where the complex was *in situ* generated (Figure 6). These results clearly show that the 380 nm band that is characteristic of the Cu^{2+} complex, shifted back to 455 nm representing the release of **L** from its complex [Cu**L**] only in the presence of cysteine and histidine and not with other amino acids. Thus the [Cu**L**] acts as secondary sensor for cysteine and histidine selectively among the twenty naturally occurring amino acids studied.

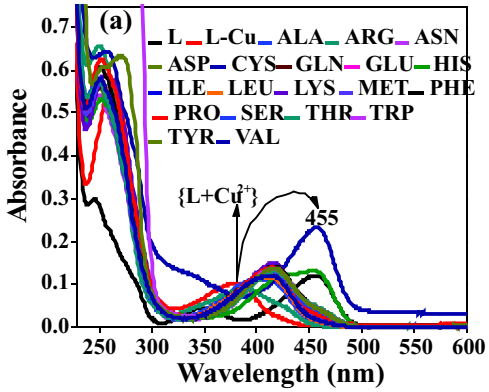


Figure 6: UV-visible absorption spectral data for the titration of [**L**+Cu^{2+}] with amino acid (10 equivalents) in ethanol.

Experimental

Materials and methods:

All the solvents used were of HPLC grade. All metal salts, *viz*.; $Ca(ClO_4)_2.4H_2O$, $Mg(ClO_4)_2.6H_2O$, $Mn(ClO_4)_2.6H_2O$, $Fe(ClO_4)_2.xH_2O$, $Co(ClO_4)_2.6H_2O$, $Ni(ClO_4)_2.6H_2O$, $Cu(ClO_4)_2.6H_2O$, $Hg(ClO_4)_2.4H_2O$, $Zn(ClO_4)_2.6H_2O$, $Cd(ClO_4)_2.H_2O$ were purchased from Sigma Chem. Co., USA. ^1H and ^{13}C NMR spectra were measured on a Varian Mercury 400 MHz NMR spectrometer. The mass spectra were recorded on Q-TOF micromass (YA-105) using electrospray ionization method. The absorption spectra were measured on Varian Cary 100 Bio instrument. The visual colour detection experiments were carried out by using 10^{-2} M solutions. Ethanolic solutions of the ligand and the metal ions were mixed to have 1:1 molar ratio wherein the final concentration was $5x10^{-3}$ M. The ligand was initially dissolved in a minimum volume (100 µL) of DMSO and was diluted using ethanol to give a stock solution of 10^{-3} M. Other metal ion stock solutions were prepared to have the same concentration in order to carry out the titrations. The requisite volume of the metal ion solution was added to a 50 µL ligand solution to get the corresponding ligand to metal ion molar ratio by making the total volume to 3 mL using ethanol.

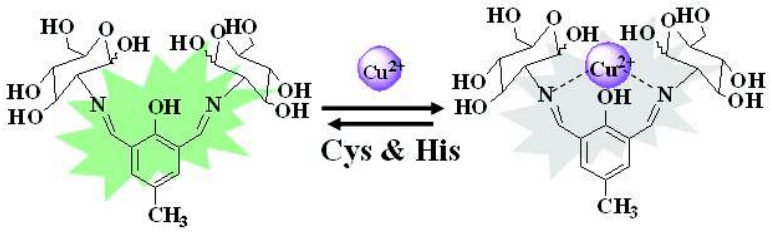
Synthesis and characterization of di-formyl-p-cresol (2): p-Cresol (4.03 g, 37.3 mmol) and HMTA (10.6 g, 75 mmol) were taken in a flask and trifluoroacetic acid (TFA, 50 mL) was added to it under N_2 atmosphere. The resulting solution was refluxed for 24 h and quenched by adding 4M HCl (200 mL). The mixture was further stirred for 10 minutes and extracted with DCM. The organic phase was subsequently washed with 4M HCl, water and then with brine solution. The organic layer was then dried over Na_2SO_4 and evaporated under vacuum. Finally the product **2** was purified by column chromatography using ethyl acetate and petroleum ether (2:8) to get the desired solid product (4.53 g, 74 %). ^1H-NMR ($CDCl_3$): 11.449 (s, broad, 1H, Ar-OH), 10.204 (s, 2H, -CHO), 7.762 (s, 2H, Ar-H), 2.380 (s, 3H, $-CH_3$). ESI MS: m/z 165 ($[M+H]^+$, 65 %), 328 ($[2M]^+$, 100 %).

Synthesis and characterization of L: Glucosamine hydrochloride (4.3 g, 20 mmol) was suspended in ethanol. Etherial trietylamine solution was added drop wise to attain pH of 7 to 8. The supernatant was then decanted and a freshly distilled ethanol (~20 mL) was added. Diformyl-p-cresol, **2** (1.64 g, 10 mmol) was added to it. The mixture was then refluxed for 12 h at ~70ºC. The solid formed was filtered and washed with ice cold ethanol several times, followed by diethylether.

The compound was dried under vacuum and stored at 4 ºC. Yield (2.77 g, 57 %, m.p ~193 °C). ^{1}H-NMR (DMSO-d_6): 3.12-3.75 (m, 5H, C2-H, C3-H, C-4H, C5-H & C6-H), 4.46-5.38 (m, 4H, C1-OH, C3-OH, C-4OH, C6-OH), 5.62 (d, 1H, C1-H, $J_{C1\text{-}H\text{-}C2\text{-}H}$=5.2 Hz), 6.60-8.12 (6H, Ar-H), 8.90 (d, 1H, CH=N), 13.60 (t, 1H, Aromatic-OH). ESI MS m/z = 334 ([M+H]$^+$, 100 %). Anal. calcd. for [$C_{21}H_{30}N_2O_{11}$]: C 51.85, H 6.22, N 5.76 Found C 51.49, H 6.26, N 5.98.

Conclusions:

A new diimino conjugate of glucosyl-cresol **L** was synthesized and characterized. **L** showed greenish fluorescence which forms complex selectively with Cu^{2+} to become non fluorescent. Thus, the conjugate **L** acts as a primary naked eye sensor for Cu^{2+} among the ten different metal ions studied. Binding of Cu^{2+} to **L** was also supported by absorption titration. In presence of Cu^{2+}, the absorption spectra of **L** exhibited a blue shift of 75 nm of the 455 nm band and appeared at 380 nm with increased absorbance. Owing to this unique characteristic of [Cu**L**], its use has been extended to selectively recognize cysteine and histidine which is in line with the metal-to-amino acid preference among the twenty naturally occurring amino acids. The selectivity is due to the displacement of Cu^{2+} from [Cu**L**] by cysteine and histidine. The sensing of cysteine and histidine was well demonstrated using absorption spectra and visual color changes during the titration. Thus, **L** acts as a highly fluorescent primary naked eye sensor for Cu^{2+} and the complex [Cu**L**] acts as secondary sensor towards cysteine and histidine (Scheme 2).



Scheme 2: Plausible binding mode of **L** with Cu^{2+}.

Acknowledgements

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