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Carbohydrate-Based Switch-On Molecular Sensor for Cu(II) in Buffer: Absorption and Fluorescence Study of the Selective Recognition of Cu(II) Ions by Galactosyl Derivatives in HEPES Buffer

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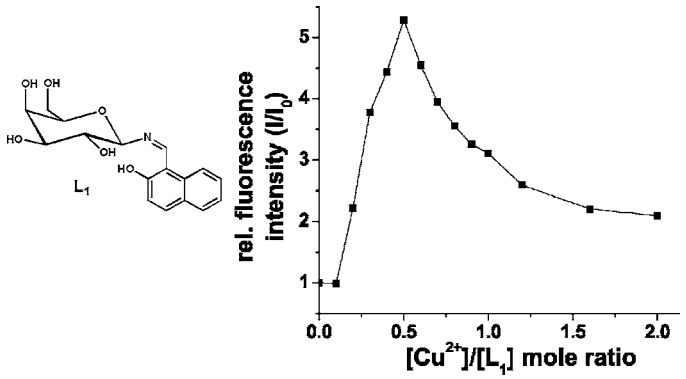
ABSTRACT



1-(β-D-Galactopyranosyl-1′-deoxy-1′-iminomethyl)-2-hydroxynaphthalene (L_1), possessing an ONO binding core, was found to be selective for Cu^{2+} ions in *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] buffer, at concentrations ≤580 ppb, at physiological pH by eliciting switch-on behavior, whereas the other ions, viz., Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+}, caused no significant change in the fluorescence. Whereas the binding characteristics were ascertained by absorption spectroscopy, the species formed were shown by Q-TOF ES MS.

Copper is an essential element of life by being involved in the functional part of a number of enzymes and also being involved in transcriptional events.[1] This necessitates sensing as well as quantifying copper in biological systems, and hence, there is a need to develop biologically compatible sensors. Because carbohydrates are biologically compatible as well as water soluble, it is desirable to have sensors based on such molecular systems. Synthetic carbohydrate-based sensors capable of reporting metal ions, including Cu^{2+}, are scarce in the literature. However, non-carbohydrate-based sensors suitable for biological applications are known in the literature for both copper[2] and zinc.[3] Therefore, the present communication deals with the absorption and fluorescence studies of a simple galactosyl-based naphthyl derivative, 1-(β-D-galactopyranosyl-1′-deoxy-1′-iminomethyl)-2-hydroxynaphthalene (L_1, Scheme 1) (Supporting Information, S1), in the recognition of Cu^{2+} ions in HEPES buffer at pH 7.2−7.4.

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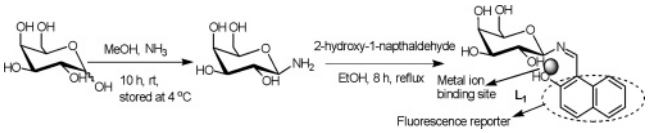
Scheme 1



Titration of L_1 with Cu^{2+} in MeOH resulted in a substantial increase in the fluorescence intensity of a 354 nm band until the $[Cu^{2+}]/[L_1]$ mole ratio is 0.5 (I/I_0 ratio is 18 ± 1) and then a decrease thereafter. Similar titration with Zn^{2+} in MeOH shows a gradual increase in the fluorescence intensity of a 450 nm band until the $[Zn^{2+}]/[L_1]$ mole ratio of 1.0 (I/I_0 ratio of 18 ± 2) and saturation thereafter. The titration of L_1 in MeOH with other biologically relevant divalent ions of Mg, Ca, Mn, Fe, Co, Ni, and Cd exhibited no significant change in the fluorescence intensity of either the 350 or the 450 nm bands. On the basis of the Benesi−Hildebrand equation, K_a's of 50 000 ± 1000 and 50 500 ± 1000 M^{-1} were derived, respectively, for Zn^{2+} and Cu^{2+} (Supporting Information, S2).

The binding of Cu^{2+} and Zn^{2+} to L_1 in MeOH was further ascertained from the absorption spectral changes noticed in the titrations. A plot of absorbance vs the $[M^{2+}]/[L_1]$ mole ratio for various bands clearly indicated the formation of a 1:1 complex in the case of Zn^{2+} and a 1:2 complex in the case of Cu^{2+}, the same as that obtained from the fluorescence studies, and the corresponding logarithmic plots yielded K_a's of 44 500 ± 1500 and 50 500 ± 3500 M^{-1} in the cases of Cu^{2+} and Zn^{2+}, respectively. Similar titrations performed in MeOH with other metal ions did not show any interpretable changes in the spectra (Supporting Information, S3).

However, the fluorescence titrations performed between M^{2+} and L_1 in HEPES buffer, at pH 7.2−7.4, yielded altogether different results. In buffer, only the titration of Cu^{2+} with L_1 showed a gradual enhancement in the fluorescence intensity of the 350 nm band until the $[Cu^{2+}]/[L_1]$ mole ratio was 0.5 and a decrease thereafter with I/I_0 being 5.3 ± 0.3 and a K_a yields of 50 500 ± 700 M^{-1}. Similar titrations performed between L_1 and other divalent ions of Mg, Ca, Mn, Fe, Co, Ni, Zn, and Cd exhibited no significant change in the emission intensities (Figure 1a). At lower and higher pH, viz., 6.0 and 8.0, the fluorescence is further quenched. A change in the anion, viz., $Cu(NO_3)_2$ and $CuCl_2$, indicated no influence of the counteranion on the fluorescence results. Binding of Cu^{2+} with L_1 in HEPES buffer was further confirmed by absorption spectral studies (Figure 1b) that provide a K_a of 42 500 ± 1000 M^{-1}. However, Zn^{2+} does not show any significant binding in buffer (Figure 1c) (Supporting Information, S4).

Titration of a mixture of {L_1 and Cu^{2+}} with M^{2+} (Figure 1d) in HEPES buffer resulted in a decrease of fluorescence intensity by ∼10% in the case of Mg^{2+}, Ca^{2+}, Mn^{2+}, and Zn^{2+}, by ∼30% in the case of Fe^{2+}, Co^{2+}, and Ni^{2+}, and by ∼45% in the case of Cd^{2+}, indicating that there is a net fluorescence increase of L_1 by 290−470% when Cu^{2+} is bound in the presence of other biologically relevant metal ions reported here. This can also be gauged by the fluorescence decay constants obtained during the titrations of {L_1 and Cu^{2+}} with M^{2+}, viz., 37 100, 38 000, 40 000, 23 100, and 29 400 M^{-1}, respectively, for Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+}. Fluorescence studies performed in HEPES buffer by varying the concentration of Cu^{2+} but keeping the $[Cu^{2+}]/[L_1]$ mole ratio at 1:2 clearly indicated that the detection of 580 ppb can be established with an increase in fluorescence by at least 150% (Supporting Information, S4). Thus, Cu^{2+} can be selectively recognized and quantified in HEPES buffer at a physiological pH using L_1 to quite low concentrations even in the presence of other biologically relevant metal ions, by eliciting a switch-on fluorescence behavior (Φ_{L1} = 0.0005, $\Phi_{L1}\epsilon_{L1}$ = 4.7 M^{-1} cm^{-1}; and Φ_{L1+Cu} = 0.0035, $\Phi_{L1+Cu}\epsilon_{L1+Cu}$ = 34.4 M^{-1} cm^{-1}) with a 7.0-fold increase in quantum yield and a ∼7.3-fold increase in brightness ($\Phi_{L1}\epsilon_{L1}$) upon Cu^{2+} addition.

These studies were appropriately compared with those of the synthetic control molecular systems, L_2, L_3, and L_4 (Figure 2, Supporting Information, S1) to establish the role of imine and carbohydrate units in the sensor property of L_1.

Titration of Cu^{2+} with L_2 in HEPES buffer showed no significant change in the fluorescence intensity, suggesting that the −HC═N− moiety in L_1 favors Cu^{2+} binding over that in L_2 owing to the formation of a chelate in the case of L_1. The effect of such a chelation on the recognition of one metal ion over the other has been recently demonstrated in the case of calix[4]arene derivatives.[4] However, the titration with Cu^{2+} resulted in an increase of I/I_0 to only 2.7 in the case of L_3 and to 2.3 in the case of L_4 (Figure 3a). These data suggest that the marginal increase observed in I/I_0 in the case of L_3 and L_4 can be explained on the basis of the presence of imine and naphthylic −OH moieties suitable for forming six-membered chelate and precludes the involvement of the carbohydrate moiety in L_4 in binding at least at the

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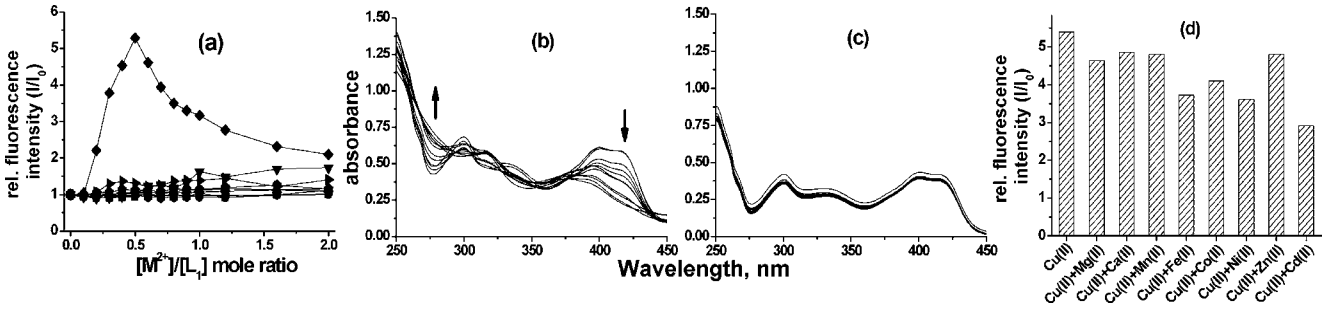


Figure 1. (a) Plot of relative fluorescence intensity (I/I_0) vs $[M^{2+}]/[L_1]$ mole ratio in HEPES buffer. The symbols refer to: ■ (Mg^{2+}), ● (Ca^{2+}), ▲ (Mn^{2+}), ▼ (Fe^{2+}), triangle pointing left (Co^{2+}), triangle pointing right (Ni^{2+}), ◆ (Cu^{2+}), ⬟ (Zn^{2+}), ⬢ (Cd^{2+}). UV−vis absorption spectra measured in the titration of M^{2+} with L_1 in HEPES buffer at different $[M^{2+}]/[L_1]$ mole ratios ranging from 0.0 to 2.0: (b) Cu^{2+}; (c) Zn^{2+}. (d) Histogram showing the relative fluorescence intensity (I/I_0) in the titration of {L_1 + 0.5 equiv of Cu^{2+}} with M^{2+}. The concentration of the competitor metal ions used was 0−2.0 equiv, and at the highest ratio, this comes out to be 4 mol equiv with respect to the copper.

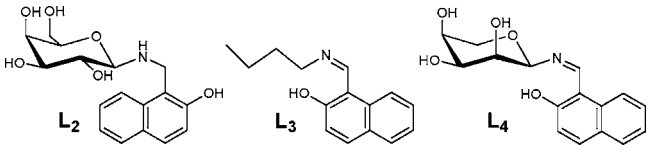


Figure 2. Schematic structures of the control molecules L_2, L_3, and L_4.

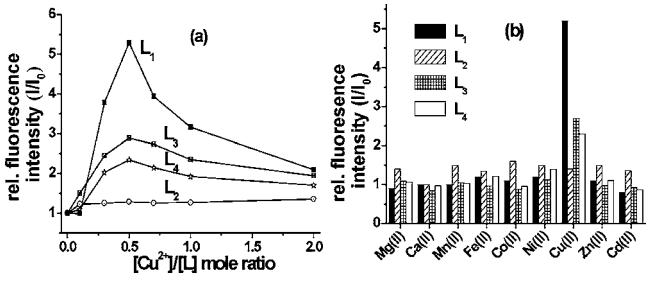


Figure 3. (a) Plot of I/I_0 vs $[Cu^{2+}]/[L]$ mole ratio in HEPES buffer (pH = 7.2). (b) Histogram indicating I/I_0 at the $[M^{2+}]/[L]$ mole ratio of 0.5 in the titration of L_1, L_2, L_3, and L_4 with different M^{2+}.

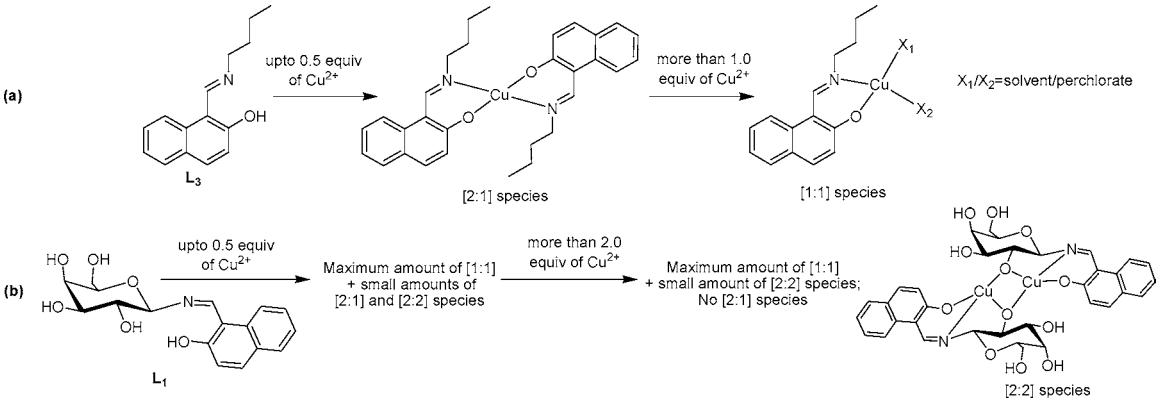
$[Cu^{2+}]/[L_4]$ mole ratio of 0.5. The sharp contrast observed in I/I_0 between L_1 and L_4 is attributable to the axial orientation of C_2−OH in the case of ribosyl-based L_4. However, at the mole ratio of $[Cu^{2+}]/[L]$ beyond 0.5, a dinuclear copper species is favored in which the carbohydrate-C_2−OH is involved in bridging. Thus, all these results obtained in the titrations of aqueous HEPES buffer support the involvement of the imine and the carbohydrate moieties present in L_1 in Cu^{2+} recognition. Further, the titration of Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} with the sensor molecule, L_1, and the control molecules, L_2, L_3, and L_4, under similar conditions in HEPES buffer did not alter the emission intensities to any significant extent (Figure 3b).

Because Cu^{1+} is possible to be present in association with Cu^{2+} in biological systems, the interaction of Cu^{1+} with L_1 was studied. Titration of L_1 with Cu^{1+} in HEPES buffer shows an increase in the fluorescence of the 356 nm band to an I/I_0 value of 3.0 ± 0.5, which is comparable with that observed with Cu^{2+}. The results of the Cu^{1+} titration were confirmed by repeating the experiment four times. Further, in the titration of Cu^{1+}, the 440 nm band also exhibited similar behavior, but the peak I/I_0 value was only 1.5 ± 0.1, indicating a rather less significant change in this band. Thus, the fluorescence behavior of Cu^{1+} with L_1 is almost the same as that of the Cu^{2+} titration. To further confirm this, two additional sets of experiments were carried out. This includes titration of [L_1 + 0.5 equiv of Cu^{2+}] with Cu^{1+} and a reverse experiment, viz., titration of [L_1 + 1.0 equiv of Cu^{1+}] with Cu^{2+}, where the results indicate that both of the ions were behaving similarly. This means that L_1 can be used to detect and/or measure the copper ions either as 1+ or 2+ or as a mixture. By combining these results, it is possible to conclude that the carbohydrate-based ligand (L_1) reported here can detect both Cu^{2+} and Cu^{1+} and hence can be very well suited for application in biological systems.

Titration of L_3 and L_1 with Cu^{2+} was also carried out using Q-TOF ES MS. In the titration of L_3 with Cu^{2+}, besides the ligand molecular ion peak (m/z = 228), only a 2:1 (ligand/Cu^{2+} ratio, m/z = 516) species was present at the 0.2 mole ratio of Cu^{2+}. At 0.5 mol equiv of Cu^{2+}, the 2:1 species further increased with the appearance of a small portion of 1:1 (m/z = 289). However, further addition of Cu^{2+} in the titration exhibits a large increase in the 1:1 species whereas the 2:1 species does not increase any further as shown (Supporting Information, S6); the corresponding species formed are shown in Scheme 2a. Comparison of this with the fluorescence behavior indicates clearly that the fluorescing behavior of these species follows a trend, 1:1 > 2:1. In the titration of L_3 with Cu^{2+}, no traces of 2:2 species were formed.

In the titration of L_1 with Cu^{2+}, as the mole ratio of Cu^{2+} increases from 0 to 0.2 to 0.5, the formation of 2:1 (ligand/Cu^{2+} ratio, m/z = 728) species is increased considerably and

Scheme 2[a]



[a] Species formed based on the Q-TOF ES MS experiment: titration of (a) L_3 and (b) L_1 with Cu^{2+}.

decreases thereafter, and the species is completely absent at higher Cu^{2+} mole ratios. On the other hand, the formation of 1:1 ($m/z = 395$) species increases as Cu^{2+} is added and a steep increase is observed beyond the Cu^{2+} mole ratio of 2.0. As the concentration of Cu^{2+} increases, the formation of 2:2 ($m/z = 789$) species also increases, whereas the molecular ion peak of the ligand ($m/z = 334$) decreases progressively and disappears beyond 2 equiv of Cu^{2+} addition. The isotopic pattern of the peaks resulting from the copper-bound species was consistent with the isotopes of copper and their abundance ratio. The extent of all these species can be clearly seen from the Supporting Information (S6), and the species formed are shown in Scheme 2b. Comparison of this with the fluorescence titration indicates clearly that the fluorescing behavior of these species follows a trend, 2:1 ≫ 2:2 > 1:1. The nature of the 1:1 species formed in the case of L_1 and L_3 could differ in the fact that the coordination in L_3 is simply bidentate, whereas L_1 can extend a pseudocoordination from the carbohydrate C_2-OH additionally. An optimized structure using hybrid density functional theory (B3LYP) with a basis set of LANL2DZ for Cu^{2+} resulted in a $Cu^{2+}\cdots O_{carb}$ distance of 3.26 Å. The Cu^{2+} is coordinatively unsaturated in both the 1:1 species, and to fill the coordination either the anion or the solvent, or both, will be used up. Although L_1 can give rise to 2:2 species, L_3 does not exhibit any such species. This contrasting behavior observed between L_1 and L_3 is explainable based on the fact that L_1 can form dinuclear species using its carbohydrate C_2-OH moiety, whereas L_3 cannot, owing to the presence of a butyl moiety instead of the carbohydrate moiety.

Synthetic reactions carried out between $M(OAc)_2 \cdot nH_2O$ and L_1 in MeOH resulted in the formation of $\{Cu(L_1)(OAc)(H_2O)_2\}_2$, **1**, and $\{Zn(L_1)(OAc)(MeOH)_2\}$, **2**, and these exhibited fluorescence characteristics similar to those obtained in the solution titration studies reported here. The dinuclear Cu(II) complex, **1**, exhibits a spectrum that is in agreement with that obtained from the titration studies at the $[Cu^{2+}]/[L_1]$ mole ratio >1.0. Similarly, the absorption spectra of **1** and **2** agree well with those obtained at the $[M^{2+}]/[L_1]$ mole ratio >1.0 (Supporting Information, S5). The 2:2 dinuclear complex proposed for **1** was supported by crystal structure determinations of 4,6-di-*O*-protected-D-glucopyranosylamine derivatives where the Cu_2O_2 core was stabilized by two intramolecular H-bonds utilizing equatorial C_2-OH of the carbohydrate.[5] The presence of equatorial C_2-OH is still retained in L_1 (galactosyl based) but not in L_4 (ribosyl based), and hence, L_1 stands as a better molecule for sensing Cu^{2+}.

Although the carbohydrate-based metal ion sensor molecules are scarce in the literature, L_1 stands as a unique molecular system that works well (by exhibiting a 7-fold increase in the quantum yield) in detecting very low concentrations of Cu^{2+} even in the presence of other biological metal ions under physiological pH. Also demonstrated were the merits of L_1 against three control molecules, the involvement of the carbohydrate moiety in metal ion recognition, the binding characteristics and the detection of copper-bound species, recognition of Cu^{1+}, and the switch-on behavior in the presence of Cu^{2+}. Thus, the galactosyl-based L_1 may offer its utility for studying Cu^{2+} in biological samples even in association with other metal ions as well as Cu^{1+}, as no such synthetic glyco-conjugate sensor is known to date for transition metal ions.

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Supporting Information Available: Synthesis, characterization, and experimental details (S1); fluorescence and absorption in MeOH (S2 and S3); fluorescence in HEPES buffer (S4); **1** and **2** (S5); and mass spectral data for Cu^{2+} titration (S6). This material is available free of charge via the Internet at http://pubs.acs.org.

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