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Photoactive chemosensors 3† : a unique case of fluorescence enhancement with Cu(II)‡

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Received (in Cambridge, UK) 16th September 2002, Accepted 8th October 2002
First published as an Advance Article on the web 30th October 2002

Chemosensor (4a) shows fluorescence enhancement with Cu(II) and can estimate 1–300 μM Cu(II) by using fluorescence (1–20 μM) and UV–Vis (10–300 μM) spectroscopic techniques. Ni(II), Cd(II), Zn(II), Ag(I) and Hg(II) do not interfere in fluorescence studies and only Ag(I) and Hg(II) interfere in UV–Vis studies.

The demand for highly sensitive and selective chemosensors for *in vitro* and *in vivo* studies related to biological metal ions has lead to design and synthesis of numerous chemosensors.[1,2] The synthetic Cu(II) ionophores in general possess, diamide-diamine,[3,4] triamine,[5a] tetraamine,[5b] hydroxamic acid or *O*-acylhydroxylamine[6,7] based motifs and only in one case—tetrathia 14-crown-4[8]—a thio-ether moiety has been used. More recently, a chemosensor based on a tripeptide[9] present in human plasma has been reported. However, type I Cu(II) proteins involve either four (*viz.* two histidine, one cysteine and one methionine-amicyanin, rusticyanin, phytocyanin, plastocyanin *etc.*) or five co-ordination sites (*viz.* two histidine, one methionine along with either two cysteines or one cysteine and one carbonyl-azurin, cytochrome C oxidase *etc.*).[10,11] Conspicuously, in order to achieve Cu(II) selective ionophores, the potential of mixed ligating sites (S, N, O), as prevalent in nature remains more or less untapped.[12,13]

Further, in most of the reported Cu(II) sensors, the fluorescent moiety (usually anthracenyl, dansyl) is placed far away from the cavity and the linker heteroatom of fluorophore does not participate in complexation. As a result electron transfer from hetero atom to fluorophore causes fluorescent quenching (Fig 1A). We envisaged that if the linker heteroatom of the fluorophore efficiently participates in complexation with Cu(II), it must suppress the process of PET from the heteroatom (generally amine nitrogen) to the fluorophore (Fig. 1B). In the case of this effect overweighing the contrary effects of electron transfer quenching by paramagnetic Cu(II), net fluorescence enhancement would be observed.

Based on these features, we report a two thioether and three amine units based chemosensor (**4a**) which shows fluorescence enhancement with Cu(II) and detects 1–20 μM of Cu(II). **4a** also acts as a chromoionophore and detects 10–300 μM Cu(II) by UV–Vis spectroscopy. Ni(II), Cd(II), Zn(II), Ag(I), and Hg(II) (1000 μM) do not interfere in the fluorescence studies and only Ag(I) and Hg(II) interfere in UV–Vis studies.

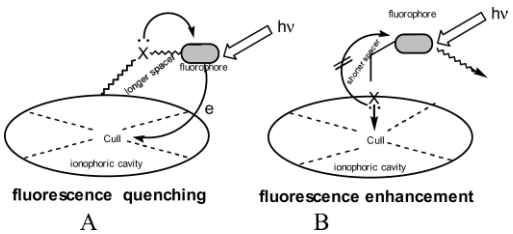


Fig. 1

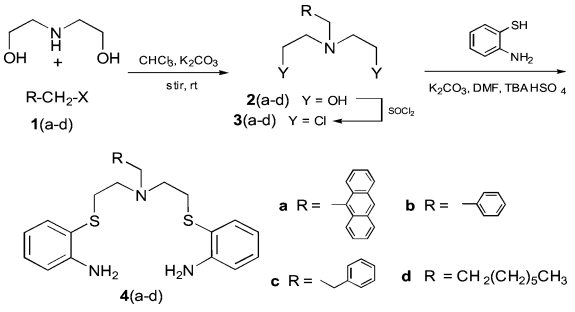
N-(9-Anthracenylmethyl)diethanolamine (**2a**)[14] with thionyl chloride gives *N*-(9-anthracenylmethyl)-bis(2-chloroethyl)-amine hydrochloride **3a** (85%) which undergoes nucleophilic substitution with 2-aminothiophenol under phase transfer catalysed conditions to provide **4a** (70%). Similarly **4b**–**d** have been obtained by reaction sequence as given in Scheme 1.

Like other amino-based fluorescent sensors, **4a** is also pH sensitive. The fluorescence of **4a** remains unaffected between pH 14–6.5, then gradually increases from pH 6.5–1.5, and finally below pH 1.5 no change in fluorescence is observed leading to a sigmoid curve. So, further fluorescence studies are carried out at pH 7 maintained with HEPES buffer (10 mM). The fluorescence emission is directly proportional to the concentration of the **4a** (10–100 μM). Therefore, **4a** is not susceptible to self quenching or to aggregation, at least in the concentration range explored.

4a upon excitation at 365 nm displays λ_{max} at 390, 412 and 440 nm in its fluorescence spectrum. The fluoroionophore **4a** (10 μM) in $CH_3CN:H_2O$ (4:1) at pH 7 (HEPES 10 mM) on addition of Cu(II) (10 μM), leads to significant fluorescence enhancement whereas other metal ions, *viz.* Ni(II), Cd(II), Zn(II), Ag(I) and Hg(II), show no or nominal enhancement even at 1000 μM concentration. The titration of **4a** (10 μM) with Cu(II) nitrate shows gradual enhancement in fluorescence between 1–25 μM of Cu(II) and then achieves a plateau (Fig. 2). The stoichiometry of comlexation is determined through job plot by absorption spectroscopy and is found to be a 1:1 **Cu(II)**–**4a** complex and has log $K = 4.1 \pm 0.1$. For other metal ions log $K < 2$.

Although other metal ions individually do not exihibit any significant fluorescence change over a range of 1–1000 μM, to determine their interference in Cu(II) estimation, the change in fluorescence of **4a** with Cu(II) was evaluated in the presence of a 1000 μM concentration of each of Ni(II), Cd(II), Zn(II), Ag(I), and Hg(II) and no change in fluorescence over that caused by Cu(II) alone was observed (Fig. 3). Thus **4a** displays selective fluorescence enhancement for Cu(II) ions.

The absorption spectrum of **4a** (100 μM) exihibits λ_{max} at 388, 368, 305 and 249 nm, typical for anthracene, and on addition of 1 eq of Cu(II) shows a remarkable increase in



Scheme 1

† For Photoactive Chemosensors 1 : see ref. 13.
For Photoactive Chemosensors 2: Subodh Kumar, Sukhdeep Kaur, Gurpreet Singh, *Supramolecular Chemistry*, 2002, in press.

‡ Electronic supplementary information (ESI) available: 1. synthetic methodology and characterization of compounds. 2. Photophysical information: (a) pH titration, (b) fluorescence measurements, (c) absorption measurements. 3. Stoichiometric determination. Fig. S1: estimation of Cu(II) in the presence of Ni(II), Cd(II) and Zn(II) (10000 μM). See http://www.rsc.org/suppdata/cc/b2/b209053h/

DOI: 10.1039/b209053h

absorption in the 400–550 nm region (see Fig. 4 and 5). A significant change in colour from colourless to greenish yellow is also observed. For quantitative analysis of Cu(II), the absorption is measured at 405 nm.

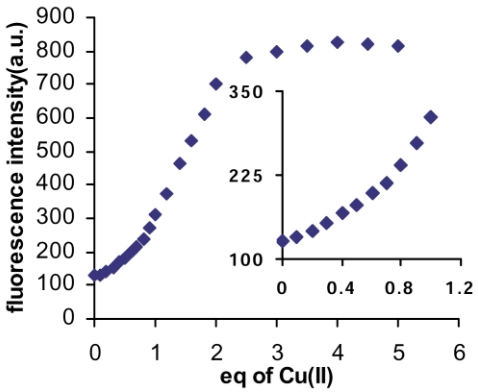


Fig. 2 The fluorescence intensity *vs.* eq. of Cu(II) profile of **4a** at 25 ± 1 °C, pH 7 (HEPES 10 mM) in $CH_3CN:H_2O$ (4:1). [**4a**] = 10 μM, λ_{ex} = 365 nm, λ_{em} = 410 nm.

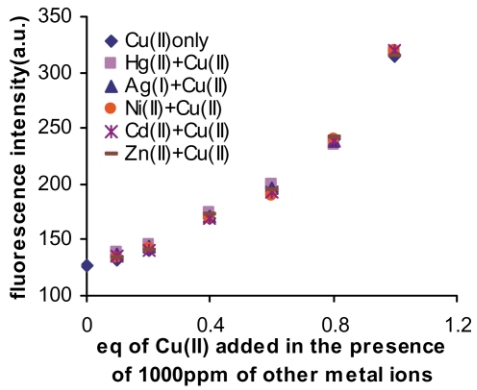


Fig. 3 Estimation of Cu(II) in the presence of Ni(II), Cd(II), Zn(II), Ag(I) and Hg(II) (1000 μM) at pH 7 (HEPES 10 mM) in $CH_3CN:H_2O$ 4:1.

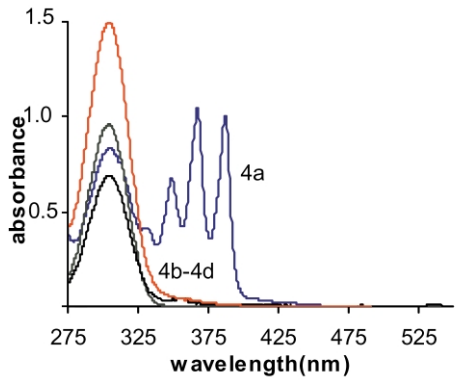


Fig. 4 Absorption spectra of receptors **4a**–**d** (10^{-5} M).

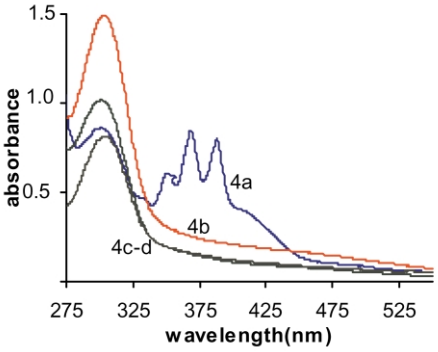


Fig. 5 Absorption spectra of receptors **4a**–**d** on addition of Cu(II) (5 eq).

The titration of **4a** (100 μM) with Cu(II) shows a gradual increase in absorption at 405 nm over a Cu(II) concentration range of 10–300 μM, above which a plateau is achieved . The titration of **4a** (100 μM) with Cu(II) in the presence of 10000 μM Ni(II), Cd(II), Zn(II), Ag(I) and Hg(II) at various concentrations of Cu(II) (20–300 μM) shows that Ni(II), Cd(II) and Zn(II) do not interfere in the estimation of Cu(II) (see ESI†), however Ag(I) and Hg(II) do interfere.

On addition of Cu(II), the increase in absorption at 405 nm is coupled with the decrease in absorption due to anthracene moiety and points towards the anthracene →Cu CT interaction. The comparison of the UV–vis spectra of Cu(II) complexes of **4a**–**4d** also provides an insight into the participation of the anthracenyl appendage in coordination with Cu(II). **4d** which lacks an aryl appendage and **4c** where the aryl appendage is separated from nitrogen by a two carbon spacer, show a small increase (0.1) in the absorption which increases to (0.2) on increasing the proximity of the phenyl ring by one carbon in **4b**. **4a** having an electron rich anthracenyl moiety shows a further increase in absorption to 0.4. These results point towards the participation of the anthracenyl ring in complexation towards Cu(II) but, due to lack of formation of X-ray suitable crystals, conclusive evidence could not be drawn.

Therefore, the strong participation of amine –N– in binding with Cu(II) complexation overweighs fluorescence quenching and helps in the release of fluorescence. Thus **4a** represent a unique case of fluorescence enhancement with Cu(II), a paramagnetic metal ion.

We thank DBT and DST, New Delhi for financial assistance.

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