Pergamon

Tetrahedron Letters 42 (2001) 9143–9146

TETRAHEDRON LETTERS

A new selective fluorescence chemosensor for Cu(II) in water

Marta Beltramello,[a] Maddalena Gatos,[a] Fabrizio Mancin,[a] Paolo Tecilla[b,*] and Umberto Tonellato[a,*]

[a]*Dipartimento di Chimica Organica e Centro CNR Meccanismi di Reazioni Organiche, Università di Padova, Via Marzolo 1, I-35131 Padova, Italy*

[b]*Dipartimento di Scienze Chimiche, Università di Trieste, Via Licio Giorgieri 1, I-34127 Trieste, Italy*

Received 27 September 2001; revised 18 October 2001; accepted 19 October 2001

Abstract—A new chemosensor for the Cu(II) ion has been realized by connecting via an amido bond an anthracenyl residue to the *all cis* 2,4,6-triamino-1,3,5-trihydroxycyclohexane ligand (TACI). This sensor is able to detect micromolar concentrations of Cu(II) ions in water at pH 7 without interference with many other divalent transition metal ions.

The development of fluorescent devices for the sensing and reporting of chemical species is currently of significant importance for both chemistry and biology.[1] More specifically, chemosensors for the detection and measurements of Cu(II) ions are actively investigated as this metal ion is a significant environmental pollutant and an essential trace element in biological systems.[2–17] Essential requisites of such a sensor are high sensitivity and selectivity but, since its main applications are addressed to the analysis of environmental or biological samples, also the ability to operate in water and the low sensitivity to the operative pH are extremely important.[12]

Following our interest in the use of the *all cis* 2,4,6-triamino-1,3,5-trihydroxycyclohexane ligand (TACI)[18] as a platform for the construction of supramolecular devices, we have recently reported a fluorescent chemosensor (ATMCA) obtained by coupling an anthracenyl fluorophore with the trimethoxy derivative of TACI.[19] This sensor was able to detect in water Cu(II), Zn(II) and organic anions, depending on the operative conditions. However, while in the case of Zn(II) and of organic anions the system was effective at neutral pHs, in the case of Cu(II) ions the sensing process was confined to slightly acidic solutions (pH<5) and the selectivity toward other divalent transition metal ions was lower than desired. These limitations are to be ascribed to the structure of the metal ion binding site. As a matter of fact, polyaminic ligands are intrinsically poorly selective[20] and the presence of the anthracenylic amino group makes the fluorescence emission of ATMCA strongly dependent on pH with the maximum intensity at pH 5 or lower.[19]

We speculated that by connecting fluorophore and ligand with an amido group and, therefore, by switching from a purely polyamino to an amino–amido type ligand we could improve the selectivity toward the Cu(II) ion and reduce the influence of the pH on the emission properties of the dye. With this in mind, we synthesized the ligand ATACI (Fig. 1) in which the anthracenyl residue is attached via an amido bond to

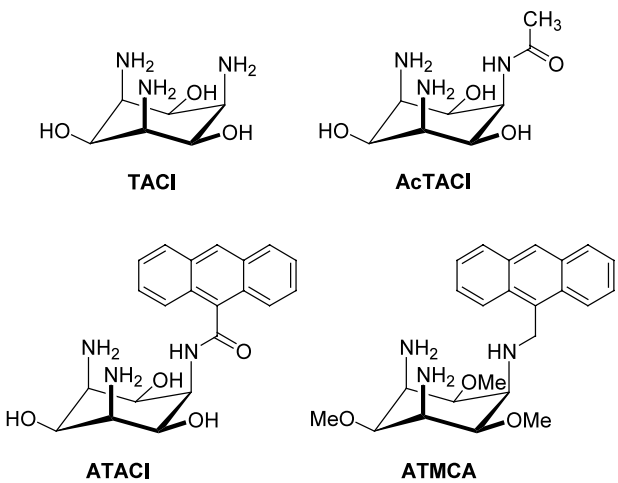


Figure 1. Structures of the ligands.

* Corresponding authors. Tel.: +39-040-6763925; fax: +39-040-6763903 (P.T.); tel.: +39-049-8275269; fax: +39-049-8275239 (U.T.); e-mail: tecilla@dsch.univ.trieste.it; umberto.tonellato@unipd.it

0040-4039/01/$ - see front matter
PII: S0040-4039(01)01965-7

the ligand moiety. Moreover, in order to improve the water solubility of the system, we used TACI as metal ion binding subunit instead of its trimethoxy derivative.

ATACI has been synthesized starting from TACI. This was first protected at two of the three amino groups using $(BOC)_2O$ in a $MeOH/CH_2Cl_2$ mixture. The BOC-di-protected derivative was then coupled with 9-anthracenecarboxylic acid using EDC/HOBt as condensing agents and Et_3N in DMF. Deprotection of the amino groups with TFA in CH_2Cl_2 leads to the final product as TFA salt.[21] Using the same procedure we have also prepared the mono-acetylated TACI derivative (AcTACI) which has been used as a reference compound. Both derivatives are soluble in water although to a different extent: up to 0.5 mM for ATACI and 5 mM for AcTACI. UV–vis and fluorescence spectra of ATACI in water solution are typical of the anthracene chromophore.[4] The fluorescence emission is directly proportional to the concentration of the sensor (1–10 μM, at pH 5, 7 and 9), showing that the ligand is not susceptible to self-quenching or to aggregation processes at least in the concentration range explored. As expected, the fluorescence emission of ATACI is little affected by the pH (Fig. 2, ○); less than 40% decrease in emission intensity is observed on going from pH 3 to 12. This decrease follows a sigmoidal curve that spans over a rather broad pH range (from about pH 4.5 to 9.0) and, is apparently related to the subsequent deprotonation of the two amino groups of the ligand. This is confirmed by a potentiometric titration of the AcTACI ligand which gives pK_a values of 6.9 and 8.5 for the two amino groups. Therefore, the deprotonation of AcTACI starts at about pH 5.6 and ends at pH 9.8. Taking into account that the attached anthracenyl residue may decrease the pK_a values,[19,22] the pH effect observed for ATACI (Fig. 2) is to be ascribed to the deprotonation of the amino groups.

Ligands characterized by a mixed amino–amido metal ion binding site, such as ATACI and AcTACI, are usually able to complex strongly and selectively Cu(II)[23] and have been also used in the construction of chemosensors for this metal ion.[3–7] In the usual design, the two donor atoms are in beta position so that the complexation occurs between the amino and the deprotonated amido nitrogen with the formation of a five member chelate ring (see Scheme 1, top). This mode of binding implies deprotonation of the amido group as the key feature for strong binding. This occurs at weakly acidic pH in the case of Cu(II) and at pH values higher than 9 in that of most of the other divalent transition metal ions (Zn(II), Ni(II), Co(II), etc).[23] As a result, in neutral or weakly acidic solutions, this kind of ligand is highly selective for Cu(II) among other metal ions. In our case the amino and the amido nitrogens are more distant (3 carbon atoms) and complexation implies the formation of a six membered ring. However, molecular models indicate that the rigid and tight structure of the ligand helps the formation of the complex positioning the donor atoms with the correct geometry (see Scheme 1, bottom).

A clear indication that the binding of the metal ion occurs as depicted in Scheme 1 comes from the UV–vis titration of AcTACI with Cu(II) (Fig. 3). By adding the metal ion to a solution of the ligand (1.7×10^{-3} M, pH 6.3) a new absorption band centered around 645 nm appears. The absorbance increases linearly with the concentration of Cu(II) up to a ratio ligand/Cu(II) of 1 and then levels off (Fig. 3, inset). This behavior is diagnostic for the formation of a complex with a 1:1 stoichiometry and, more important, the position of the absorption maximum of the complex is typical of an amino/deprotonated-amido nitrogen binding site.[24] Further evidence for this type of binding comes from the potentiometric titration of a 1:1 mixture of AcTACI and Cu(II) which indicates the formation of a very strong complex with a 1:1 stoichiometry. This requires the deprotonation of three acidic functions: the two protonated amines and the amido nitrogen.

The pH dependence of the fluorescence emission of ATACI in the presence of 1 equiv. of metal ion is shown in Fig. 2 (●). The emission intensity remains constant up to pH 5 and then decreases down to a plateau with about 15% of the initial value due to the formation of the complex. At variance with the curve

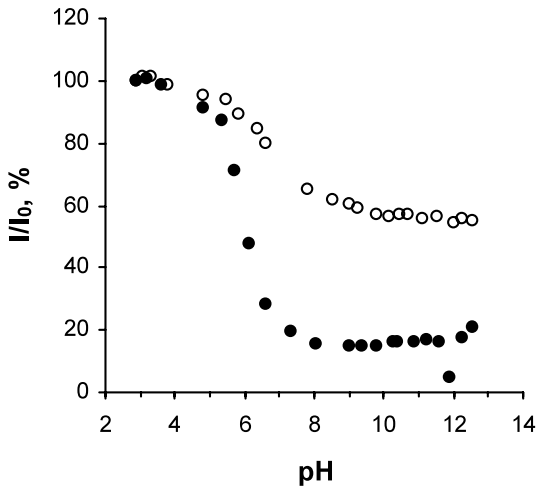
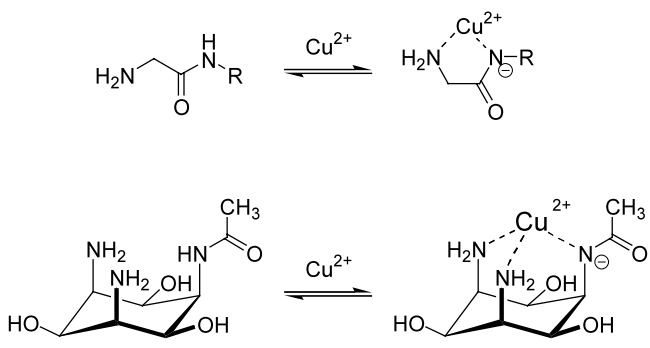


Figure 2. pH Dependence of fluorescence intensity ($\lambda_{exc}=330$ nm, $\lambda_{em}=415$ nm) for ATACI (○, 5 μM) and ATACI in the presence of 1 equiv. of Cu(II) ion (●, 5 μM).



Scheme 1. Mode of binding of 'classical' amino-amido type ligands (top) and the proposed mode of binding for ATACI and AcTACI (bottom).

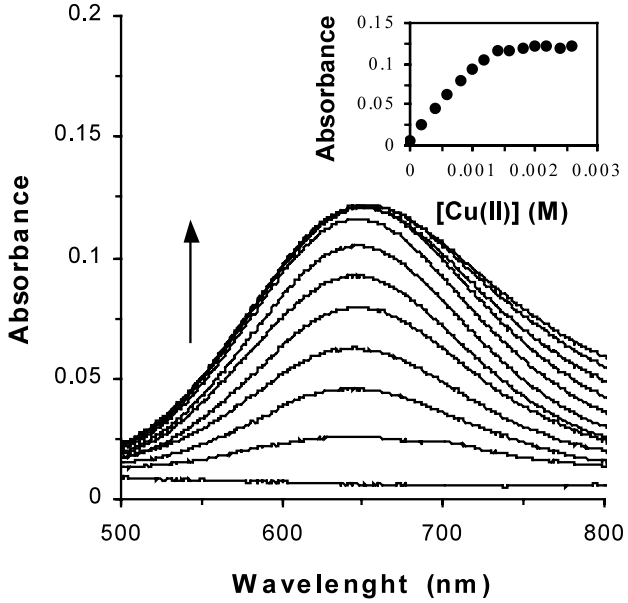


Figure 3. UV–vis titration of AcTACI (1.7×10^{-3} M) at pH 6.3 (MES buffer 0.05 M) with Cu(II). The inset shows the binding isotherm at 645 nm.

obtained in the absence of the metal ion (○), the sigmoid is much sharper and the quenching process spans only two pH units. At pH 7 or higher the complex is totally formed and the complexation of the metal ion switches off the fluorescence emission of the sensor, usually by an energy or electron transfer mechanism.[1b,4b] Moreover, at pH 7 the difference in the fluorescence emission of the free and complexed ligand is appreciably large and, therefore, under these conditions, the system displays the highest sensitivity as Cu(II) sensor.

The titration with Cu(II) of a 5 μM solution of ATACI at pH 7 is shown in Fig. 4 (●). The emission intensity decreases smoothly, almost linearly, pointing to a plateau value of about 15% of the initial value. On the other hand, upon addition of Cu(II), the ATACI absorption spectra do not change appreciably at the excitation wavelength used (330 nm). Interpolation of the emission intensity versus Cu(II) concentration, assuming a 1:1 model, gives a good fit and allows to estimate a log K_{app} value of 6.2±0.2. Therefore, under these conditions, concentrations of Cu(II) down to the micromolar range can be easily detected and evaluated.

To test the sensor specificity, which is the ability to sense only the target metal ion, we titrated the ATACI solutions with other divalent metal ions (Fig. 4). Ni(II), Co(II), Zn(II), Pb(II), Cd(II), Mn(II) have no effect on the emission intensity of the sensor. On the contrary, addition of Fe(II) and Hg(II) causes relatively minor changes in the emission. As shown in Fig. 4 a decrease of about 20% was observed after the addition of 2 equiv. of these metal ions.

Finally, we tested the selectivity of the sensor, which is the ability to sense the Cu(II) ion in the presence of other metal ions. Fig. 5 shows the titration with Cu(II) of a solution containing ATACI at pH 7 and all the other metal cations (each 10 μM). The curve thus obtained (○) is very close to the one in the presence of Cu(II) alone (●) and the similarity is even better when the titration is performed in the absence of Hg(II) and Fe(II) (□). The results demonstrate that, on one hand, the ATMCA sensor does not respond to a large selection of divalent metal ions other than Cu(II) and, on the other hand, the presence of these metal ions does not interfere with the Cu(II) determination.

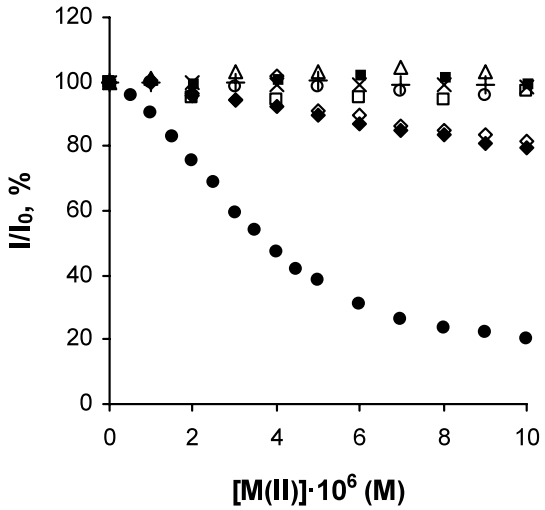


Figure 4. Spectrofluorimetric titrations of ATACI (5×10^{-6} M) at pH 7.0 with different metal ions: Cu(II) (●), Ni(II) (○), Co(II) (+), Zn(II) (△), Pb(II) (□), Hg(II) (◇), Cd(II) (■), Mn(II) (X), Fe(II) (◆). [Hepes buffer] = 0.01 M, λ_{exc} = 330 nm, λ_{em} = 415 nm, 25°C.

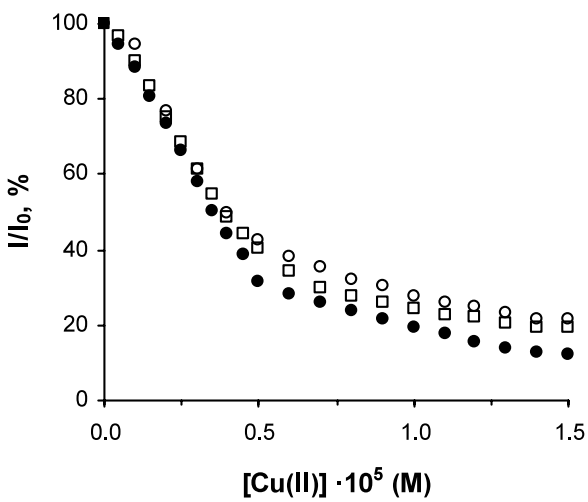


Figure 5. Spectrofluorimetric titrations of ATACI (5×10^{-6} M) at pH 7.0 with Cu(II) alone (●), in the presence of Ni(II), Co(II), Zn(II), Pb(II), Cd(II), Mn(II) each 10 μM (□), and in the presence of all the above metal ions and Hg(II) and Fe(II) (○). [Hepes buffer] = 0.01 M, λ_{exc} = 330 nm, λ_{em} = 415 nm, 25°C.

In conclusion, the ATACI ligand has many of the features required for a Cu(II) chemosensor to be used in practical applications. It is very sensitive and selective and operates in neutral aqueous solutions with little dependence on small variations of the pH value. The results obtained also show that the TACI ligand is a really versatile platform for the construction of such type of devices and studies are in progress to extend the use of this ligand in the design of chemosensors for other metal ions and organic substrates.

Acknowledgements

Financial support for this research has been partly provided by the Ministry of Instruction University Research (MIUR) under the framework of the 'Supramolecular Devices' project and by Regione Friuli Venezia Giulia 'fondo 2000'.

References

1. (a) *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; ACS Symposium Series 538, American Chemical Society: Washington DC, 1993; (b) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Parodi, L.; Taglietti, A. In *Transition Metals in Supramolecular Chemistry*; Sauvage, J.-P., Ed.; Wiley: Chichester, 1999; pp. 93–134; (c) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
2. Kramer, R. *Angew. Chem., Int. Ed.* **1998**, *37*, 772.
3. Grandini, P.; Mancin, F.; Tecilla, P.; Scrimin, P.; Tonellato, U. *Angew. Chem., Int. Ed.* **1999**, *38*, 3061.
4. (a) De Santis, G.; Fabbrizzi, L.; Lichelli, M.; Mangano, C.; Sacchi, D.; Sardone, N. *Inorg. Chim. Acta* **1997**, *257*, 69; (b) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Perotti, A.; Taglietti, A.; Sacchi, D. *Chem. Eur. J.* **1996**, *2*, 75.
5. Torrado, A.; Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1998**, *120*, 609.
6. Corradini, R.; Dossena, A.; Galaverna, G.; Marchelli, R.; Panagia, A.; Sartori, G. *J. Org. Chem.* **1997**, *62*, 6283.
7. Prodi, L.; Bolletta, F.; Montalti, M.; Zaccheroni, N. *Eur. J. Inorg. Chem.* **1999**, 455.
8. Sasaki, D. Y.; Shnek, D. R.; Pack, D. W.; Arnold, F. H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 905.
9. Yoon, J.; Ohler, N. E.; Vance, D. H.; Aumiller, W. D.; Czarnik, A. W. *Tetrahedron Lett.* **1997**, *38*, 3845.
10. Bhattacharya, S.; Thomas, M. *Tetrahedron Lett.* **2000**, *41*, 10313.
11. Bodenant, B.; Weil, T.; Businelli-Pourcel, M.; Fages, F.; Barbe, B.; Pianet, I.; Languerre, M. *J. Org. Chem.* **1999**, *64*, 7034.
12. Klein, G.; Kaufmann, D.; Schürch, S.; Reymond, J.-L. *Chem. Commun.* **2001**, 561.
13. Ghosh, P.; Bharadwaj, P. K.; Roy, J.; Ghosh, S. *J. Am. Chem. Soc.* **1997**, *119*, 11903.
14. Yang, J.-S.; Lin, C.-S.; Hwang, C.-Y. *Org. Lett.* **2001**, *3*, 889.
15. Rurark, K.; Kollmannsberger, M.; Resch-Genger, U.; Daub, J. *J. Am. Chem. Soc.* **2000**, *122*, 968.
16. Ramachandram, B.; Samanta, A. *Chem. Commun.* **1997**, 1037.
17. Unterreitmaier, E.; Schuster, M. *Anal. Chim. Acta* **1995**, *309*, 339.
18. Hegetschweiler, K. *Chem. Soc. Rev.* **1999**, *28*, 239 and references cited therein.
19. Fabbrizzi, L.; Licchelli, M.; Mancin, F.; Pizzeghello, M.; Rabaioli, G.; Taglietti, A.; Tecilla, P.; Tonellato, U. *Chem. Eur. J.* in press.
20. Pina, F.; Bernardo, M. A.; Garcia-España, E. *Eur. J. Inorg. Chem.* **2000**, 2143.
21. Selected analytical data for ATACI·2TFA (hygroscopic solid): ^{1}H NMR (CD_3OD) δ 3.80 (m, 2H, *CH*OH), 4.40 (m, 1H, *CH*OH), 4.58 (m, 2H, *CH*NH_3^+), 4.70 (m, 1H, CHNHAr), 7.59 (m, 4H, Ar); 8.16 (dd, 4H, Ar); 8.69 (s, 1H, Ar). ^{13}C NMR (CD_3OD) δ 53.0, 53.2, 70.3, 126.2, 127.0, 128.3, 129.6, 130.1, 132.5, 132.9, 135.7, 172.3. Anal. calcd. for $C_{21}H_{23}N_3O_4$+2CF_3COOH+2H_2O: C, 46.52; H, 4.53; N, 6.51. Found: C, 46.25; H, 4.44; N, 6.42.
22. Shionoya, M.; Ikeda, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *116*, 3848.
23. Siegel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385.
24. (a) Cleij, M. C.; Tecilla, P.; Tonellato, U.; Scrimin, P. *Gazz. Chim. Ital.* **1996**, *126*, 827; (b) Dallavalle, F.; Folesani, G.; Marchelli, R.; Galaverna, G. *Helv. Chim. Acta* **1994**, *77*, 1623; (c) Dallavalle, F.; Folesani, G.; Bertuzzi, R.; Marchelli, R.; Galaverna, G. *Helv. Chim. Acta* **1995**, *78*, 1785.