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Short communication

4-(4,5-Diphenyl-1*H*-imidazole-2-yl)-*N*,*N*-dimethylaniline-Cu(II) complex, a highly selective probe for glutathione sensing in water-acetonitrile mixtures

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ABSTRACT

The imidazole derivative 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-*N*,*N*-dimethylaniline (probe **1**) formed a highly coloured and non-emissive 1:1 stoichiometry complex with Cu(II) in water-acetonitrile 1:1 (v/v) solutions. Among all the amino acids (Lys, Val, Gln, Leu, His, Thr, Trp, Gly, Phe, Arg, Ile, Met, Ser, Ala, Pro, Tyr, Gly, Asn, Asp, Glu, Cys and Hcy) and tripeptides (GSH) tested, only GSH induced the bleaching of the **1**·Cu(II) solution together with a marked emission enhancement at 411 nm (excitation at 320 nm). These chromo-fluorogenic changes were ascribed to a selective GSH-induced demetallation of the **1**·Cu(II) complex that resulted in a recovery of the spectroscopic features of probe **1**. In addition to the remarkable selectivity of **1**·Cu(II) complex toward GSH a competitive limit of detection as low as 2 μM was determined using fluorescence measurements.

1. Introduction

Biothiols, such as glutathione (GSH), cysteine (Cys), and homocysteine (Hcy), are biomolecules containing thiol groups [1]. Cys and Hcy are components of many peptides that have a wide range of cellular biological functions. Besides, the three biothiols play important roles in the body's biochemical defence system because they are involved in reversible redox homeostasis processes which maintain the equilibrium of reduced free thiol and oxidized disulphide forms [2]. As the most abundant reductive biothiol (with concentrations in the millimolar range in living systems), GSH mediates many cellular functions such as maintenance of intracellular redox activities, xenobiotic metabolism, intracellular signal transduction and gene regulation [3]. Moreover, abnormal levels of biothiols affect the normal physiological and pathological functions and are related with a number of diseases such as cancer, AIDS, liver damage, Alzheimer, osteoporosis, inflammatory bowel diseases and cardiovascular diseases [4–9]. In this context, several studies have been devoted to the development of efficient methods for the detection of the concentration of GSH in physiological media. Several strategies such as mass spectrometry [10], high-performance liquid chromatography [11,12], enzymatic methods [13], electrochemical assays [14], surface-enhanced Raman scattering [15–17], and combinatorial library-based sensors [18,19] have been described for the detection and quantification of GSH. However, these methods require expensive equipment, are time-consuming and the selectivity achieved is, in some cases, low.

Bearing in mind the above-mentioned facts, the development of probes able to display colour and/or fluorescence changes in water or mixed aqueous solutions in the presence of target bio-relevant thiols is a timely research area [20]. Within different approaches described for the preparation of chromo-fluorogenic sensors of biothiols, the use of displacement processes involving non-emissive fluorophore-Cu(II) complexes has attracted great attention in the last years [21]. In spite of the fact that most of the reported fluorophore-Cu(II) complexes allowed GSH detection in aqueous environments their response is in general unselective and Cys and Hcy also induced emission modulations [21]. Only three recently published examples, based on the use of a coumarin derivative [22], graphitic carbon nitride [23], and a displacement assay with an iminophenol-Cu(II) complex [24], allowed GSH selective detection in the presence of Cys and Hcy in aqueous environments.

Given our interest in the design of optical chemosensors for the detection of anions and cations of biological and environmental significance

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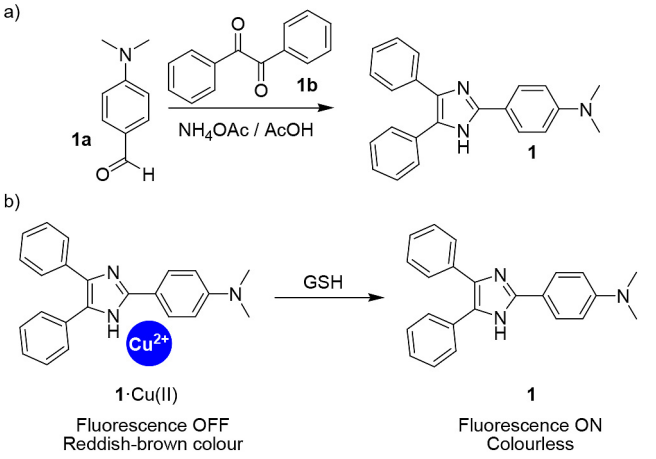

Scheme 1. (a) Synthesis of probe **1** and (b) GSH recognition mechanism using **1**·Cu(II) complex.

[25] herein we report the selective chromo-fluorogenic detection of GSH using a complex formed by 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-*N*,*N*-dimethylaniline (**1** in Scheme 1) and Cu(II). The emission of probe **1** was selectively quenched in the presence of Cu(II) due to the formation of 1:1 stoichiometry complex. In the presence of GSH the emission of probe **1** was fully restored due to a demetallation reaction.

2. Experimental section

2.1. Chemicals

Commercially available reagents 4-(dimethylamino)benzaldehyde (**1a**), 1,2-diphenylethane-1,2-dione (**1b**), and ammonium acetate were purchased from Sigma-Aldrich and Acros and used as received. TLC analyses were carried out on 0.25 mm thick pre-coated silica plates (Merck Fertigplatten Kieselgel 60F_{254}) and spots were visualized under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh).

2.2. Materials and methods

All melting points were measured on a Stuart SMP3 melting point apparatus. IR spectra were determined on a BOMEM MB 104 spectrophotometer using KBr discs. NMR spectra were obtained on a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C using the solvent peak as internal reference at 25 °C. All chemical shifts are given in ppm using $^{\delta}$H Me_4Si = 0 ppm as reference. Assignments were supported by spin decoupling-double resonance and bi-dimensional heteronuclear correlation techniques. UV/visible titration profiles were carried out with JASCO V-650 spectrophotometer (Easton, MD, USA). Fluorescence measurements were recorded with a JASCO FP-8500 spectrophotometer.

2.3. Synthesis of probe *1*

4-(Dimethylamino) benzaldehyde (**1a**) (0.15 g, 1 mmol), 1,2-diphenylethane-1,2-dione (**1b**) (0.33 g, 1 mmol) and NH_4OAc (1.54 g, 20 mmol) were dissolved in glacial acetic acid (5 mL), followed by stirring and heating at reflux for 8 h. The reaction mixture was cooled to room temperature, ethyl acetate was added (15 mL) and the mixture was washed with water (3 × 10 mL). After, the organic phase was dried with anhydrous $MgSO_4$, the solution was filtered and the solvent was evaporated to dryness. The resulting crude product was purified by column chromatography (silica gel, DCM/MeOH (100:01)) and was obtained as yellow oil (11 mg, 10%).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.04 (s, 6H, NMe_2), 6.84 (dd, J = 6.8 and 2.0 Hz, 2H, H2 and H6 aniline), 7.27–7.32 (m, 2H, 2 × H4 Ph), 7.34–7.39 (m, 4H, 2 × H2 and H6 Ph), 7.61 (d, J = 8.4 Hz, 4H, 2 × H3 and H5 Ph), 8.03 (dd, J = 6.8 and 2.0 Hz, 2H, H3 and H5 aniline) ppm.

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 40.36, 112.83, 119.39, 127.31, 127.70, 128.00, 128.20, 128.70, 129.10, 134.62, 147.71, 151.70 ppm.

IR (liquid film): ν = 3420, 2926, 2856, 1692, 1646, 1615, 1549, 1509, 1495, 1446, 1405, 1363, 1250, 1202, 1172, 1124, 1071, 1026, 1002, 966, 945, 822, 766, 696 cm^{-1}.

3. Results

The synthesis of 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-*N*,*N*-dimethylaniline (probe **1**) was published elsewhere (using different catalysts such as oxalic acid, $SnCl_4$–SiO_2, H_3BO_3-ultrasounds) [26]. In this study we used a one-step Radziszewski reaction between 4-dimethylamino benzaldehyde (**1a**) and 1,2-diphenylethane-1,2-dione (**1b**) in the presence of ammonium acetate in acidic media which directly yielded probe **1** in 10 % yield (or in 88 % yield inethanol in presence of I_2) (see Scheme 1).

Probe **1** was not fully water soluble and, for this reason, we carried out the spectroscopic characterization in water-acetonitrile mixtures. In this respect, water (pH 7.4)-acetonitrile 1:1 (v/v) solutions of probe **1** (5.0×10^{-5} mol L^{-1}) presented an absorption band, of charge-transfer nature (due to the presence of a donor *N*,*N*-dimethylaniline moiety and an electron-deficient imidazole heterocycle as acceptor group), centred at ca. 320 nm. In a first step, UV–visible changes of probe **1** solutions were studied in the presence of 10 eq. of selected metal cations (i.e. Cu (II), Pb(II), Mg(II), Ge(II), Ca(II), Zn(II), Co(II), Ni(II), Ba(II), Cd(II), Hg (II), Fe(III), In(III), As(III), Al(III), Cr(III), Ga(III), K(I), Li(I) and Na(I)). The obtained results are shown in Fig. 1. As could be seen, among all cations tested, only Cu(II) was able to induce a remarkable appearance of a new red-shifted band centred at ca. 490 nm. These facts were reflected in a marked colour change from colourless to reddish-brown (Fig. 1). In more detail, addition of increasing quantities of Cu(II) induced a progressive decrease of the band centred at 320 nm with a growth in absorbance at 490 nm (see Supporting Information for the UV–visible titration profile of probe **1** with Cu(II)). The appearance of a red-shifted band upon addition of Cu(II) is tentatively attributed to an interaction of this cation with the acceptor part of the probe **1**, i.e the imidazole ring.

Changes in the UV–visible bands (reflected in marked colour changes) of probe **1** upon addition of Cu(II) were ascribed to the formation of a 1:1 stoichiometry complex as was assessed from the Job's

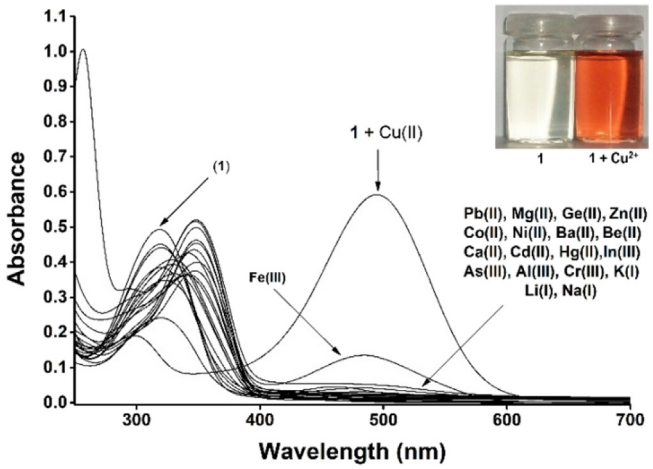

Fig. 1. UV–visible spectra of **1** in water (pH 7.4)-acetonitrile 1:1 (v/v) (5.0×10^{-5} mol L^{-1}) alone and in the presence of 10 eq. of selected metal cations. The inset shows the change in colour of **1** in the presence of Cu(II).

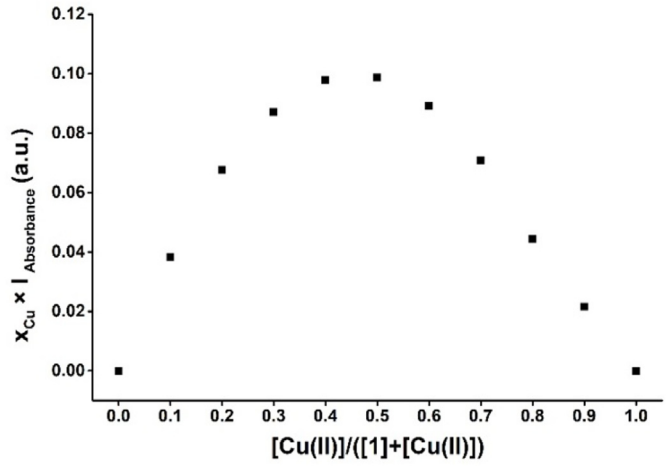

Fig. 2. Job's plot of **1** and Cu(II) in water (pH 7.4)-acetonitrile 1:1 (v/v). Total concentration of **1** and Cu(II) of 2.0×10^{-5} mol L^{-1}.

plot shown in Fig. 2. From the UV–visible titration profile a logarithm of the stability constant for the formation of the **1**·Cu(II) complex of 5.0 ± 0.1 was determined.

Furthermore, probe **1** was also emissive and, upon excitation of water (pH 7.4)-acetonitrile 1:1 (v/v) solution of the probe at 320 nm, a marked fluorescence at 445 nm appeared (see Fig. 3). The emission behaviour of probe **1** in the presence of selected cations was also tested. As in the UV–visible studies, the unique cation able to induce changes in the emission band of probe **1** was Cu(II). As could be seen in Fig. 3, addition of increasing amounts of Cu(II) to water (pH 7.4)-acetonitrile 1:1 (v/v) solution of probe **1** induced a progressive quenching of the 445 nm emission together with a moderate blue shift of the band. From the emission titration profile obtained, a limit of detection of 3.2 μM for Cu(II) was determined.

Taking into account the high affinity of Cu(II) toward thiol moieties [27] we envisioned that **1**·Cu(II) complex could be a promising ensemble for fluorescence "off-on" detection of certain biothiols via a Cu (II) displacement approach. For this purpose, water (pH 7.4)-acetonitrile 1:1 (v/v) solution of **1**·Cu(II) complex (6.2×10^{-6} mol L^{-1}) were prepared and the UV-absorption behaviour in the presence of GSH (2.0 eq.) and selected amino acids (2.0 eq. of Lys, Val, Gln, Leu, His, Thr, Trp, Gly, Phe, Arg, Ile, Met, Ser, Ala, Pro, Tyr, Gly, Asn, Asp, Glu, Cys and Hcy) was tested. The obtained results are shown in Fig. 4.

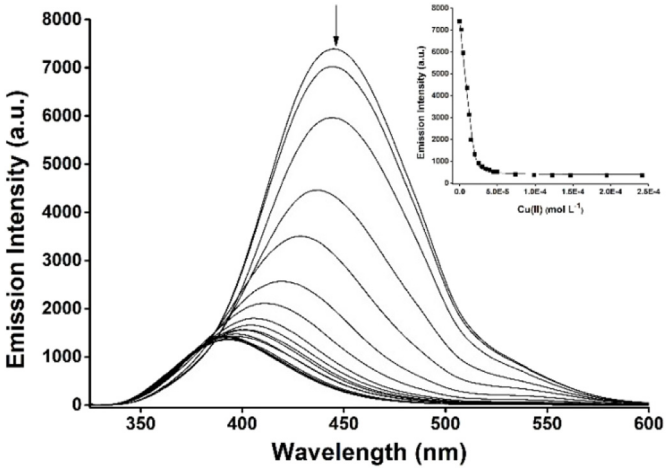

Fig. 3. Fluorescence titration profile of **1** in water (pH 7.4)-acetonitrile 1:1 (v/v) (5.0×10^{-5} mol L^{-1}) upon addition of increasing amounts of Cu(II) (from 0 to 10 eq.). Inset: plot of the emission intensity at 445 nm *vs* Cu(II) concentration.

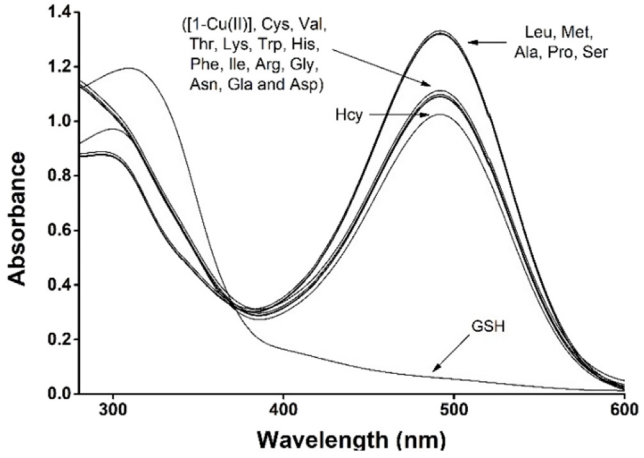

Fig. 4. UV–visible changes of water (pH 7.4)-acetonitrile 1:1 (v/v) solution of **1**·Cu(II) complex (6.2×10^{-6} mol L^{-1}) in the presence of GSH (2.0 eq.) and selected amino acids (also 2.0 eq.).

As could be seen in Fig. 4, only GSH was able to induce a complete disappearance of the 490 nm band ascribed to the **1**·Cu(II) complex, which was reflected in a marked colour change from reddish-brown to colourless. In contrast, none of the amino acids tested induced remarkable changes in the visible band. Addition of increasing quantities of GSH induced a progressive decrease of the absorbance at 490 nm (see Supporting Information). From the titration profile a limit of detection for GSH of 3 μM was determined.

Additionally, the fluorescence response of water (pH 7.4)-acetonitrile 1:1 (v/v) solution of **1**·Cu(II) complex in the presence of GSH and selected amino acids was also tested. Upon excitation at 320 nm, water (pH 7.4)-acetonitrile 1:1 (v/v) solution of **1**·Cu(II) complex presented a weak emission band centred at 404 nm (see Fig. 5). Addition of amino acids induced negligible changes in the emission profile (data not shown) whereas in the presence of increasing quantities of GSH a marked emission enhancement together with a moderate red shift (to 411 nm) was observed (see Fig. 5). From the emission titration profile, obtained upon addition of increasing quantities of GSH, a limit of detection of 2 μM for GSH was calculated. The obtained limit of detection is similar to that found by Jiang et al. using a coumarin-Cu(II) complex (0.36 μM) [22] and by Kim and co-workers using and iminophenol-Cu(II) complex (5.86 μM) [24]. However, Yan and co-workers measured a limit of detection of 0.02 μM for GSH using graphitic carbon nitride [23].

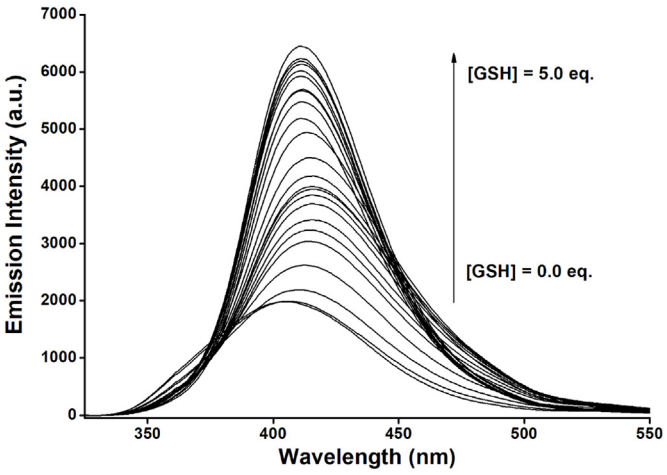

Fig. 5. Fluorescence titration profile of **1**·Cu(II) complex in water (pH 7.4)-acetonitrile 1:1 (v/v) (6.2×10^{-6} mol L^{-1}) upon addition of increasing amounts of GSH (excitation at 320 nm).

The UV–visible and emission changes obtained when GSH was added to aqueous solutions of **1**·Cu(II) complex pointed to a demetallation process as mechanism of the optical response observed. GSH is able to displace Cu(II) from the **1**·Cu(II) complex restoring the UV–visible and emission spectra of probe **1**. The absence of optical response in the presence of thiol-containing amino acids Cys and Hcy and the remarkable selectivity of the **1**·Cu(II) complex toward GSH could be ascribed to a preferential coordination of the tripeptide with Cu(II). The structure of GSH presented several potential coordinating sites (amino, sulfhydryl and carboxylates) in a flexible backbone and could coordinate Cu(II) more effectively than Cys and Hcy which presented the same functional groups but in a more rigid skeleton [28]. This **1**·Cu(II) demetallation process regenerates the optical features of the free probe.

Besides, the selectivity of **1**·Cu(II) for GSH detection in the presence of other competitive biothiols (such as Cys and Hcy) was also tested (see Supporting Information). For this purpose, the emission intensity of **1**·Cu(II) (at 411 nm upon excitation at 320 nm) alone, in the presence of GSH (2.0 eq.) and with a mixture of GSH + Cys + Hcy (2.0 eq. of each biothiol) was measured. The emission intensity measured in the presence of GSH and with the three biothiols are nearly the same. This fact pointed to a selective response of **1**·Cu(II) toward GSH (this biothiol is the only able to demetallate **1**·Cu(II)) and opens the possible use of this complex for the detection of this tripeptide in real samples.

4. Conclusions

In summary, we described herein the use of probe **1** complexed with Cu(II) as selective and sensitive chromo-fluorogenic sensor for GSH. Probe **1** forms a coloured and weakly-emissive complex with Cu(II) in water (pH 7.4)-acetonitrile 1:1 **(v/v)** solution. Moreover, **1**·Cu(II) complex exhibits unique selectivity and sensitivity for GSH detection in aqueous environments. Addition of GSH to water (pH 7.4)-acetonitrile 1:1 **(v/v)** solutions of **1**·Cu(II) complex induced a marked bleaching of the colour and the appearance of an intense emission band. The optical changes where ascribed to a GSH-induced demetallation of **1**·Cu(II) complex which regenerated the free probe. The response to GSH was quite selective because other biothiols tested (Cys and Hcy) were unable to induce any colour or emission changes. Besides, the system presented a competitive limit of detection for GSH (2 μM using emission measurements). Moreover, the **1**·Cu(II) complex is one of the few examples of chromo-fluorogenic probes which selectively detect GSH in the presence of Cys and Hcy in aqueous environments.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dyepig.2018.05.069.

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