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A Sensitive Fluorescent Probe Based on a HPBI Derivative for Detecting Glutathione

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A novel, sensitive, and highly selective fluorescent probe which contains 2-(2-hydroxyphenyl)benzimidazole receptor groups has been prepared for detecting glutathione (GSH) that operates under aqueous conditions. The complex of the fluorescent compound with copper(II) ion was selectively responsive to GSH and induced a recovery of blue fluorescence.

The design and construction of chemo- and biosensors for recognizing active thiol-containing compounds has received considerable attention in recent years.[1–4] Glutathione reduced (GSH) is known to be the most abundant intracellular nonprotein sulfhydryl compound, which has various cellular functions, mainly due to the thiol group of the cysteine residue.[5–7] Thus, it is important to be able to detect the concentration of GSH in aqueous solution. Although several intriguing strategies have been developed to detect GSH, much effort has been spent to look for new approaches to improve the simplicity and the sensitivity of GSH detection.[8]

2-(2-Hydroxyphenyl)benzimidazole (HPBI) and its derivatives show intense fluorescent emission via excited-state intramolecular proton transfer (ESIPT).[9] They have been used as a new series of fluorescent probes,[10,11] high-energy radiation detectors, plastic scintillators, and polymer ultraviolet stabilizers,[12,13] as well as for their implications in biology and potential applications as molecular switches in logic or memory circuits.[14] Recently, we have synthesized a series of HPBI derivatives, which afforded a hydroxy and the adjacent nitrogen atom binding site cooperatively associating with metal ions.[15–17] They worked as a highly selective probe for copper(II) ions. GSH can also provide additional binding interactions and form complexes with copper(II) ions.[18,19] Thus, we thought that if the new complexes of GSH with copper(II) ions could disrupt the equilibrium between HPBI and copper(II) ions, this three-component competing system of HPBI/Cu^{2+}/GSH would be developed as a rapid and simple spectrofluorimetric method for the analysis of GSH. Herein, we report a water-soluble HPBI derivative that displays fluorescence quenching to copper(II) ions through the hydroxy and the adjacent nitrogen atom cooperative interactions. The introduction of GSH induces a recovery of fluorescence of HPBI/Cu^{2+}, which allows the HPBI/Cu^{2+} to detect GSH. To our best knowledge, this is the first report of a fluorescent probe based on HPBI and copper(II) ions complexes that operate in aqueous solution.

A water-soluble HPBI derivative, **1**, was employed as a fluorescence compound to study the feasibility of this approach because its conformation of hydroxy and nitrogen atom coordinates copper(II) ions to form a chelate ring to induce the fluorescence quenching. Compound **1** showed good water solubility because of the trimethylammonium chloride group. Titration of compound **1** with cupric chloride in water at 20 °C was monitored by absorption spectroscopy. As shown in Figure 1 (left), the maximum absorption of compound **1** in water appears at around 320 nm. Upon adding increasing amounts of Cu^{2+}, significant red shifts of its absorption spectra are observed. Figure 1 (right) shows a correlation between fluorescence emission intensity and the concentrations of Cu^{2+} in water containing compound **1**. The fluorescence intensity of compound **1** decreases linearly upon the conentration of Cu^{2+}.

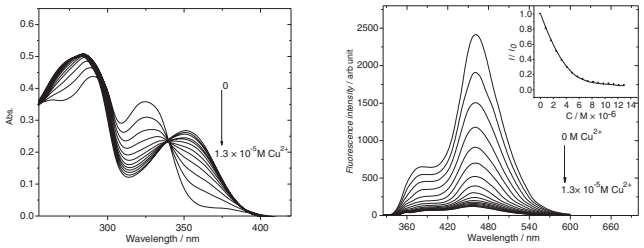


Figure 1. (left) Variation of the absorption spectra of **1** (1×10^{-5} mol dm^{-3}) in water with increasing concentrations of copper(II) ions (0–13 μM). (right) Fluorescence emission spectra of **1** (1×10^{-5} mol dm^{-3}) in water with increasing concentrations of copper(II) ions excited at 320 nm. (inset) Fluorescence emission plotted as a function of concentrations of copper(II) ions (0–13 μM).

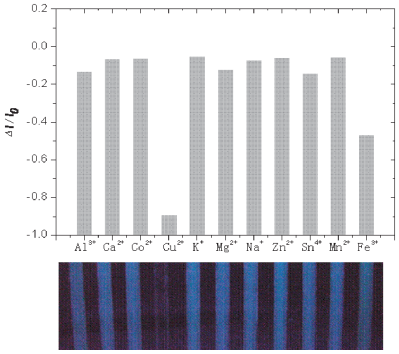


Figure 2. The selectivity of compound **1** to various metal ions. Concentration of compound **1** in water is 1×10^{-5} mol dm^{-3}. Concentrations of all the metal ions are 5×10^{-4} mol dm^{-3}.

The response properties of the fluorescent probe to various metal ions are shown in Figure 2. At the equilibrium states, Cu^{2+} quenches the fluoresence of compound **1** most efficiently, showing the unique response of compound **1** to Cu^{2+} ion. Other metal ions, such as Al^{3+}, Ca^{2+}, Co^{2+}, Na^{+}, and Zn^{2+}, give much less decrease in fluorescence emissions. These results indicate that compound **1** could selectively bind to copper(II) ion.

Next, we examined the fluorescence properties of the probe with Cu^{2+} in the presence of GSH. It was found that the fluorescence emission intensity increased as the concentration of

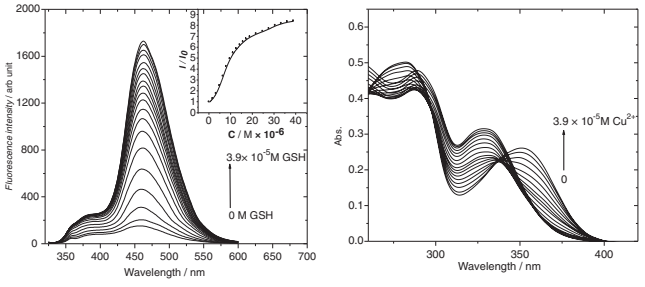


Figure 3. (left) Fluorescence emission spectra of compound **1** (1×10^{-5} mol dm^{-3}) and copper(II) ions (1.3×10^{-5} mol dm^{-3}) in water with increasing concentrations of GSH excited at 320 nm. (Inset) Fluorescence emission plotted vs. concentrations of GSH (0–39 μM). (right) Variation in the absorption spectra of compound **1** (1×10^{-5} mol dm^{-3}) and copper(II) ions (1.3×10^{-5} mol dm^{-3}) in water with increasing concentrations of GSH (0–39 μM) as indicated.

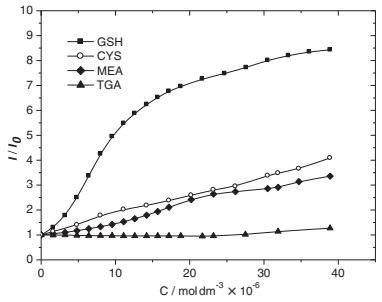


Figure 4. The dependence of the relative fluorescent intensity of compound **1** (1×10^{-5} mol dm^{-3}) and copper(II) ions (1.3×10^{-5} mol dm^{-3}) on the different sulfhydryl compounds at various concentrations in pure water. GSH (■); CYS (○); MEA (□); TGA (▲).

GSH increased from 0 to 3.9×10^{-5} mol dm^{-3} (Figure 3 left). Notably, a pronounced change in the fluorescence signal was observed even when the GSH concentration was as low as 4.8×10^{-6} mol dm^{-3}. At the same time, the maximum absorption of compound **1** in water was blue-shifted from 355 to 320 nm (Figure 3 right). These results indicate that the complex of compound **1** with copper(II) ion could be used as a fluorescent sensor for GSH.

To validate the selectivity of the complex of compound **1** with copper(II) ion to GSH, the changes of emission properties of compound **1** (1×10^{-5} mol dm^{-3}) and Cu^{2+} (1.3×10^{-5} mol dm^{-3}) in water were studied with adding sulfhydryl compounds such as thioglycolic acid (TGA), 2-sulfanylethylamine hydrochloride (MEA), and L-cysteine hydrochloride (CYS). After adding an equimolar amount of these sulfhydryl compounds into aqueous solutions of **1** and Cu^{2+}, the fluorescence emission intensity increased with different intensity (Figure 4). The most striking effects were observed for GSH, and hence we confirmed that the complex of compound **1** with copper(II) ion was selectively responsive to GSH. At the same time, we found that adding Cu^{2+} into a solution of compound **1**, the blue fluorescence quenching was observed. Afterward, the addition of GSH to aqueous solution of compound **1** and Cu^{2+} induced a recovery of blue fluorescence (Figure 5).

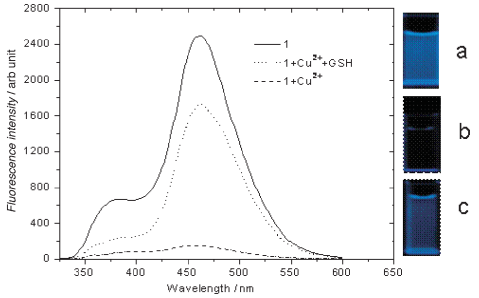
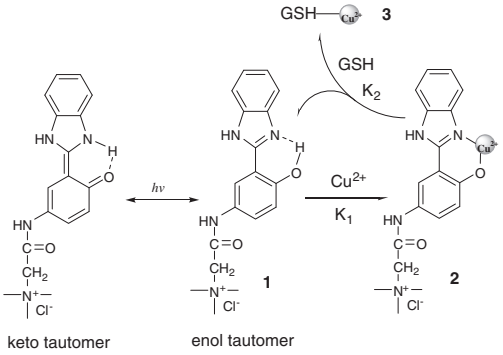


Figure 5. Color change of compound **1** in water with excitation at 365 nm: a) compound **1** (1×10^{-5} mol dm^{-3}); b) compound **1** (1×10^{-5} mol dm^{-3}) and copper(II) ions (1.3×10^{-5} mol dm^{-3}); c) compound **1** (1×10^{-5} mol dm^{-3}) and copper(II) ions (1.3×10^{-5} mol dm^{-3}) with increasing concentrations of GSH to 39 μM. And the relative change of fluorescence emission spectra.



Scheme 1. Schematic routine for competitive binding of compound **1** with Cu^{2+} vs. GSH with Cu^{2+}.

All these above observations can be attributed to the photophysics of the ESIPT mechanism. The HPBI-based functional probes can undergo ESIPT by the structural transformation between the enol and keto tautomers. As shown in Scheme 1, compound **1** exhibits high fluorescence in water without the appearance of copper(II) ions. Complexes **2** were formed when copper(II) ions were added to compound **1** solution. Through the complexation with copper(II) ions, the ESIPT process was efficiently disrupted and a fluorescence quenching was discovered. Afterward, new complexes between GSH and Cu^{2+} formed after the addition of GSH to **2**. The HPBI derivative, compound **1**, was free to lead to a recovery of the ESIPT process, and the blue fluorescence was observed again. Based on this principle, we could develop a rapid and simple spectrofluorimetric method for the analysis of GSH.

In conclusion, we have successfully developed a novel, sensitive, and highly selective fluorescent probe **1** for GSH that operates in aqueous environment. There are two competing equilibria when GSH is added into the system of compound **1** and Cu^{2+}. The first equilibrium, between compound **1** and Cu^{2+} (K_1), can directly be detected through fluorescence changes. The addition of GSH induces a second equilibrium between Cu^{2+} and GSH, to give complex **3**. This perturbs the compound **1**/Cu^{2+} equilibrium, resulting in a change of the fluorescent

intensity of the aqueous system. The present findings will not only extend the application of ESIPT mechanism but also provide a new approach for facile monitoring of GSH.

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References

1 N.-N. Fu, H. Wang, M.-L. Li, G.-J. Zheng, H.-S. Zhang, S.-C. Liang, *Anal. Lett.* **2005**, *38*, 791.
2 Y.-H. Ahn, J.-S. Lee, Y.-T. Chang, *J. Am. Chem. Soc.* **2007**, *129*, 4510.
3 H. Maeda, H. Matsuno, M. Ushida, K. Katayama, K. Saeki, N. Itoh, *Angew. Chem.* **2005**, *117*, 2982.
4 F. Tanaka, N. Mase, C. F. Barbas, III, *Chem. Commun.* **2004**, 1762.
5 C. Hwang, A. J. Sinskey, H. F. Lodish, *Science* **1992**, *257*, 1496.
6 R. Hong, G. Han, J. M. Fernández, B.-J. Kim, N. S. Forbes, V. M. Rotello, *J. Am. Chem. Soc.* **2006**, *128*, 1078.
7 S. S. Hassan, G. A. Rechnitz, *Anal. Chem.* **1982**, *54*, 1972.
8 a) J. Bouffard, Y. Kim, T. M. Swager, R. Weissleder, S. A. Hilderbrand, *Org. Lett.* **2008**, *10*, 37. b) S. Banerjee, S. Kar, J. M. Perez, S. Santra, *J. Phys. Chem. C* **2009**, *113*, 9659. c) B. Tang, Y. Xing, P. Li, N. Zhang, F. Yu, G. Yang, *J. Am. Chem. Soc.* **2007**, *129*, 11666. d) J. H. Lee, C. S. Lim, Y. S. Tian, J. H. Han, B. R. Cho, *J. Am. Chem. Soc.* **2010**, *132*, 1216. e) Z. Yao, X. Feng, C. Li, G. Shi, *Chem. Commun.* **2009**, 5886.
9 a) M. Forés, M. Duran, M. Solà, M. Orozco, F. J. Luque, *J. Phys. Chem. A* **1999**, *103*, 4525. b) K. Das, N. Sarkar, A. K. Ghosh, D. Majumdar, D. N. Nath, K. Bhattacharyya, *J. Phys. Chem.* **1994**, *98*, 9126.
10 A. Sytnik, D. Gormin, M. Kasha, *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11968.
11 A. Sytnik, J. C. Del Valle, *J. Phys. Chem.* **1995**, *99*, 13028.
12 A. Douhal, F. Amat-Guerri, A. U. Acuňa, *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1514.
13 M. A. Ríos, M. C. Ríos, *J. Phys. Chem. A* **1998**, *102*, 1560.
14 D. Haarer, *Jpn. J. Appl. Phys.* **1987**, *26*, 227.
15 J. Ouyang, H. Nishino, K. Kurosawa, *J. Heterocycl. Chem.* **1997**, *34*, 81.
16 M. Sakata, Y. Shirakawa, N. Kamata, Y. Sakaguchi, H. Nishino, J. Ouyang, K. Kurosawa, *J. Heterocycl. Chem.* **2000**, *37*, 269.
17 J. Ouyang, H. Hong, C. Shen, Y. Zhao, C. Ouyang, L. Dong, J. Zhu, Z. Guo, K. Zeng, J. Chen, C. Zhang, J. Zhang, *Free Radical Biol. Med.* **2008**, *45*, 1426.
18 M. Hultberg, A. Isaksson, A. Andersson, B. Hultberg, *Chem. Biol. Interact.* **2007**, *167*, 56.
19 D. N. Kumar, B. K. Singh, B. S. Garg, P. K. Singh, *Spectrochim. Acta, Part A* **2003**, *59*, 1487.

