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ORIGINAL PAPER

A Chemosensing Ensemble for the Detection of Cysteine Based on the Inner Filter Effect Using a Rhodamine B Spirolactam

Xiao-Feng Yang · Ping Liu · Liping Wang · Minglei Zhao

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Abstract A fluorescent chemosensing ensemble for the detection of cysteine is designed based on the fluorescence inner filter effect. The method employs the coordination of Cu^{2+} ion with salicylaldehyde rhodamine B hydrazone (**I**), a colorless and non-fluorescent rhodamine B spirolactam derivative to form **I**-Cu(II), a pink color but weakly fluorescent complex. When rhodamine B was introduced to the **I**-Cu(II) complex solution, the fluorescence signal of rhodamine B is dramatically decreased because of the fluorescence inner filter effect (IFE). Upon adding cysteine to the above solution, it can complex preferentially to Cu^{2+} compared to **I**, and the **I**-Cu(II) complex dissociates, which thus decreases the fluorescence IFE of the solution, and in turn leading to the fluorescence increase of the chemosensing system. Based on the above mechanism, a fluorescent chemosensing ensemble for cysteine is developed. The fluorescence increase is linearly with cysteine concentration up to 10.0 μ mol L^{-1}, with a detection limit of 1.4×10^{-7} mol L^{-1} (3σ). The optimal conditions of the proposed method were studied and the selectivity of the proposed method was investigated in this paper.

Keywords Cysteine · Rhodamine B spirolactam · Inner filter effect · Fluorescence

Introduction

Cysteine is an important thiol-containing compound and involved in a variety of important cellular functions, such as protein synthesis, detoxification and metabolism, etc. The reversible oxidation–reduction reactions of the thiol group of cysteine are essential process in many biological systems [1]. Furthermore, cysteine is the amino acid that plays a major role in determining three dimensional structure of proteins. Due to its important role in biological system, great attention has been paid to the detection of cysteine. At present, several colorimetric and fluorescent probes for thiol-containing amino acids are reported. The majority of the reported methods are based on the redox chemistry or labeling with chromophores or fluorophores and a combination of separations techniques [2, 3]. Recently, Strongin et al. as well as other researchers have made pioneering advances in developing highly selective probes for cysteine and homocysteine based on the covalent interaction between the probe molecule and the analyte [4–8], causing changes to its absorption or emission properties, which could be used for the direct assay of the amino acid content in body fluids.

The other way which is through coordination interaction between the receptor and the analyte, the so-called "chemosensing ensemble" approach, has been developed [9–11]. In this method, the fluorescent indicator is bound to the receptor through coordination interactions, and the fluorescence of the indicator is either quenched or enhanced by the receptor. When the analyte displaces the indicator, the solution's fluorescence recovers.

In recent years, the chemosensor based on the inner filter effect (IFE) has been reported [12–15]. IFE, *i.e.* the absorption of the exciting light and/or the absorption of the emitted radiation, is a source of errors in fluorimetry. But it can be useful for an optical chemosensor by converting the analytical absorption signals into fluorescence signals. Moreover, since the changes in the absorbance of the absorber translate into exponential changes in fluorescence of fluorophore, an enhanced sensitivity for the analytical method can be obtained with respect to the absorbance value alone [13, 16].

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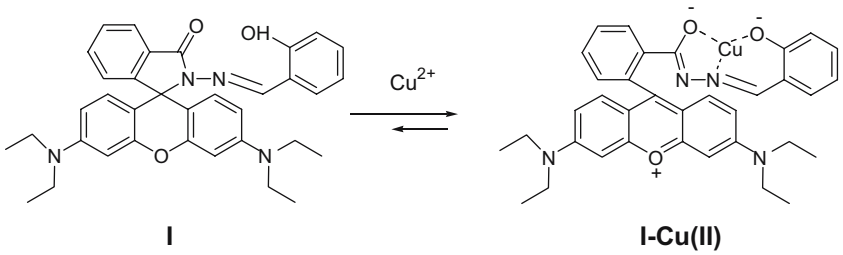
Scheme 1 Proposed ring-opening mechanism of **I** induced by Cu^{2+} ion



The rhodamine framework is an ideal mode to construct "OFF–ON" fluorescent probe due to its excellent spectroscopic properties of large molar extinction coefficient and high fluorescence quantum yields. As is well-known, rhodamine derivatives with spirolactam structure are colorless and non-fluorescent, whereas the ring-opening of the spirolactam gives rise to a strong fluorescence emission [17]. To date, several rhodamine-modified chemosensors and probes based on the spirocyclic ring-opening mechanism haven been developed [18–25].

In our recent studies, we synthesized salicylaldehyde rhodamine B hydrazone (**I**), which is a colorless, non-fluorescent compound and exhibits no absorption in the visible region. Upon mixing with Cu^{2+} ion, the spirolactam ring of **I** was opened and a **I**-Cu(II) complex was formed (Scheme 1). During this process, both a pink color and the absorption characteristics of rhodamine B appeared, and the mixture solution exhibits significant absorption at 558 nm. But at the same time, the **I**-Cu(II) complex is weakly fluorescent, which might be due to the paramagnetic nature of Cu^{2+} ion (3 d^9) and the fluorescence of the ring-opened amide form of **I** is dramatically quenched.

Furthermore, we observed the UV–vis absorption of **I**-Cu(II) complex can be modulated by cysteine. The **I**-Cu(II) shows significant absorption at 558 nm. However, upon mixing with cysteine, the absorption of **I**-Cu(II) solution decreases. On the other hand, we observed that the absorption spectrum of rhodamine B overlaps well with that of **I**-Cu(II) complex. Thus, in a "**I**-Cu(II) + rhodamine B" solution, the effective intensity of the excitation light of rhodamine B would be increased with decreasing absorbance of **I**-Cu(II) complex.

Based on the above mechanism, we present a new chemosensing ensemble for the detection of cysteine based on the fluorescence IFE between **I**-Cu(II) complex and rhodamine B. **I**-Cu(II) complex is colorful but weakly fluorescent, when rhodamine B was added to the **I**-Cu(II) complex solution, the fluorescence signal of rhodamine B is dramatically decreased because of the fluorescence IFE. Upon adding cysteine to the above solution, it can complex preferentially to Cu^{2+} compared to **I**, and the **I**-Cu(II) complex dissociates, which thus decreases the fluorescence IFE of the solution, and in turn leading to the fluorescence increase of the chemosensing system. Based on the above mechanism, a fluorescent chemosensing ensemble for cysteine was developed. The results showed the proposed method is selective and sensitive.

Experimental

Apparatus

The fluorescence spectra and relative fluorescence intensity were measured with a Sanco CRT-970 spectrofluorimeter (Shanghai, China) with a 10 mm quartz cuvette. The excitation and emission wavelength bandpasses were both set at 10 nm. The absorption spectra were recorded with a Shimadzu UV-1700 spectrophotometer. Mass spectra were obtained with AXIMA-CFR plus MALDI-TOF Mass Spectrometer. IR spectrum was taken in KBr disks on a Bruker Tensor FTIR spectrophotometer. The pH was measured with a Model pHs-3B meter (Shanghai, China). All the measurements were operated at room temperature about 298 K.

Reagents

A stock cysteine solution (2.0 mmol L^{-1}) was prepared by dissolving 12.1 mg of L-cysteine with 50 mL of 2.0 mmol L^{-1} HCl. Compound **I** solution (1.0 mmol L^{-1}) was prepared by dissolving 28.0 mg of **I** in 50 mL ethyl

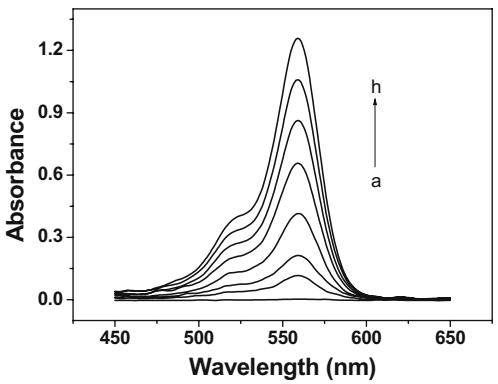


Fig. 1 The UV–vis absorption spectra of **I** (20.0 μmol L^{-1}) in the presence of different concentration of Cu^{2+}. The absorbance spectra were measured in 40% ethanol aqueous solution at pH 7.1 of Tris–HCl buffer. Cu (II) concentration: (a), 0.0; (b), 1.0; (c), 2.0; (d), 4.0; (e),6.0; (f), 8.0; (g), 10.0; (h), 12.0 μmol L^{-1}

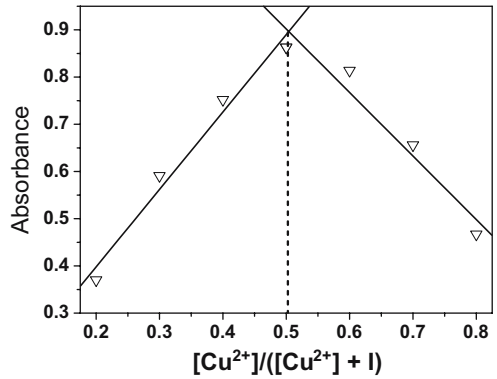


Fig. 2 Job's plots of the complexation Between **I** and Cu^{2+}. The total concentration of **I** and Cu^{2+} was 20.0 μmol L^{-1}. The absorbance was measured at 558 nm in 40% (v/v) ethanol aqueous solution

acetate. Cu^{2+} solution (0.01 mol L^{-1}) was prepared by dissolving 0.2497 g of $CuSO_4 \cdot 5H_2O$ in 100 mL water. Rhodamine B solution (1.0 mmol L^{-1}) was prepared by dissolving 23.5 mg of rhodamine B in 50 mL water. A 0.05 mol L^{-1} Tris – HCl buffer solution (pH 7.1) was employed. The sources of reagents were as follows: L-cysteine and $CuSO_4 \cdot 5H_2O$ were obtained from Shanghai Chemical Reagent Co.; rhodamine B was obtained from Beijing Chemical Reagent Plant.

All the reagents were of analytical-reagent grade, and doubly distilled water was used throughout.

Synthesis of I

I was synthesized and purified according to the method reported in the literature [21]. Briefly, **I** was readily obtained through conversion the carboxylic group of rhodamine B to the corresponding hydrazide followed by the reaction with appropriate salicylaldehyde. The desired product was identified by MS and ^{1}HNMR.

Preparation of I-Cu(II) complex

To a 50 mL flask, 43.5 mg of $Cu(NO_3)_2 \cdot 3H_2O$ (obtained from Shanghai Chemical Reagent Co.) in 25 mL anhydrous ethanol was stirred, and **I** (0.1008 g, 1.8×10^{-4} mol) was added slowly. The reaction solution was refluxed for 2 h, cooled and evaporated in vacuo to give **I**-Cu(II) complex, which was not purified but was confirmed by MS (calculated for $[\mathbf{I} + Cu]^{2+}$ 623.8, found 623.3).

Procedure

Typically, to a 10-mL volumetric tube containing 4.0 mL of ethanol, 0.4 mL pH 7.1 Tris–HCl buffer, 0.2 mL of 10.0 μmol L^{-1} rhodamine B, 0.2 mL of 1.0 mmol L^{-1} **I** and 1.2 mL of 0.1 mmol L^{-1} Cu^{2+}, different concentration of cysteine was added and the reaction mixture was diluted to 10 mL with water. Then, the fluorescence intensity was recorded at λ_{ex} / λ_{em}=560 / 583 nm. Meanwhile, a blank solution containing no cysteine was prepared and measured under the same conditions for comparison. The fluorescence increase of the proposed chemosensing system by cysteine was represented as F / F_0, where F and F_0 are the fluorescence intensity of the system with and without cysteine, respectively.

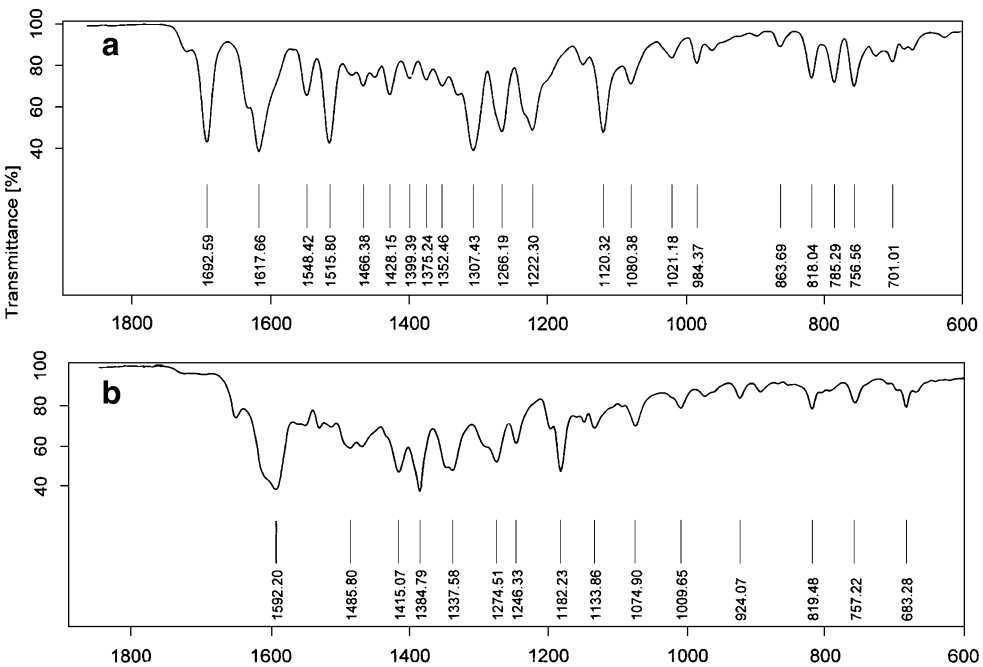


Fig. 3 IR spectra of compound **I** (a) and **I**-Cu(II) complex (b)

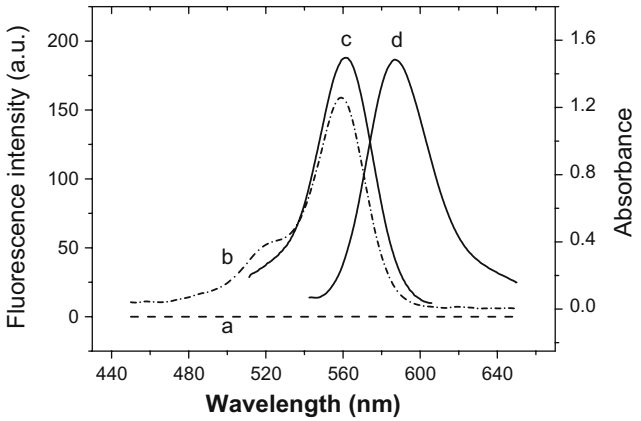


Fig. 4 UV–vis absorption spectra of **I** (20.0 μmol L^{-1}) in the absence (a) and presence of 12.0 μmol L^{-1} Cu(II) (b), and fluorescence excitation (c) and emission spectra of 0.2 μmol L^{-1} rhodamine B (d)

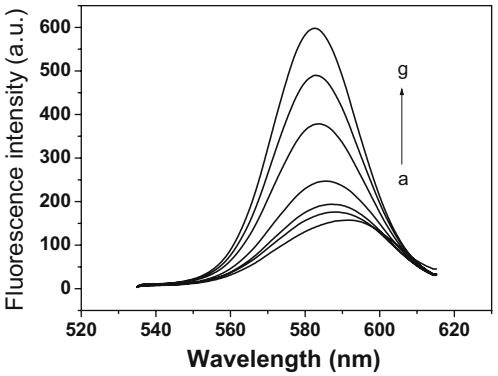


Fig. 6 Synchronous fluorescence spectra of **I**-Cu(II) + rhodamine B system upon adding different concentrations of cysteine. Cysteine concentration: (a) 0.0; (b) 0.4; (c) 0.6; (d)1.0; (e) 2.0; (f) 4.0; (g) 6.0 μmol L^{-1}. Other conditions were the same as those described in the Experimental section. λ_{ex}: 515–595 nm, λ_{em}:535–615 nm, $\Delta\lambda$=20 nm

Results and discussion

Binding interaction of I and Cu^{2+} ion

Prior to application in chemosensing of cysteine, the binding interaction of **I** with Cu^{2+} was studied. **I** is a colorless, non-fluorescent compound showing no absorption at visible region. Upon incubation with Cu^{2+} ion, a pink color was observed instantaneously. Figure 1 shows the UV–vis spectra of **I** in the presence of different concentration of Cu^{2+}, and it can be observed that a characteristic absorption at about 558 nm was appeared. To gain insight into the fluorescent signaling properties of **I** toward Cu^{2+} ions, the above resulting colored solution was subsequently treated with 1.0 mmol L^{-1} EDTA, and the pink color of the solution disappeared, proving that the coordination of **I** with Cu^{2+} is chemically reversible, which is the fundamental for the design the chemosensing ensemble. Moreover, Job's method for the absorbance was applied to study the binding stoichiometry of **I** and Cu^{2+} [19, 21, 23], and Fig. 2 indicates that a 1: 1 stoichiometry is most possible for the binding mode of **I** and Cu^{2+}.

Furthermore, IR spectra of **I** and **I**-Cu(II) were taken in KBr disks, and the results were shown in Fig. 3. The peak at 1692.59 cm^{-1}, which corresponds to the characteristic amide carbonyl absorption of **I**, was shifted to 1592.20 cm^{-1} upon chelating with Cu^{2+}. These results show that a strong binding participation of the carbonyl group occurs with Cu^{2+}.

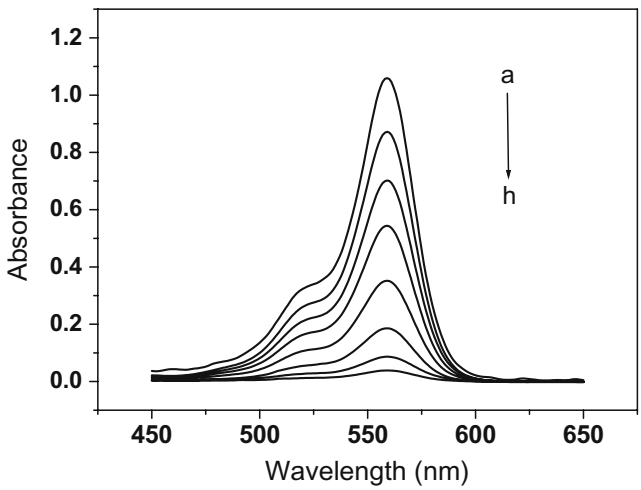


Fig. 5 UV–vis absorption spectra of **I**-Cu(II) complex (10.0 μmol L^{-1}) upon adding different concentration of cysteine. The absorbance was measured in 40% ethanol aqueous solution at pH 7.1 of Tris–HCl buffer. Cysteine concentration: (a), 0; (b), 4.0; (c), 8.0; (d), 12.0; (e), 16.0; (f), 20.0; (g), 24.0; (h), 28.0 μmol L^{-1}

Choice of absorber and fluorophore

As is well-known, the performance of IFE requires the presence of two dyes in the chemosensing ensemble, one acting as the analyte-sensitive absorber and the other as the analyte-independent fluorophore whose excitation or emission intensity is modulated by varying the absorption of the absorber.

To exploit the IFE for fluorescence response to the absorption changes of **I**-Cu(II) complex, the select of

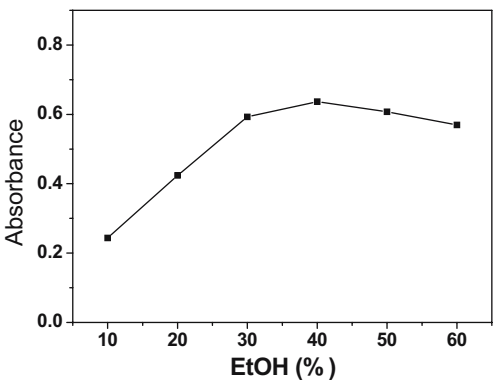


Fig. 7 Effect of ethanol content on absorbance of **I**-Cu(II) complex. The mixture solution was kept at room temperature in pH 7.1 of Tris–HCl buffer. **I,** 10.0 μmol L^{-1}; Cu(II), 6.0 μmol L^{-1}

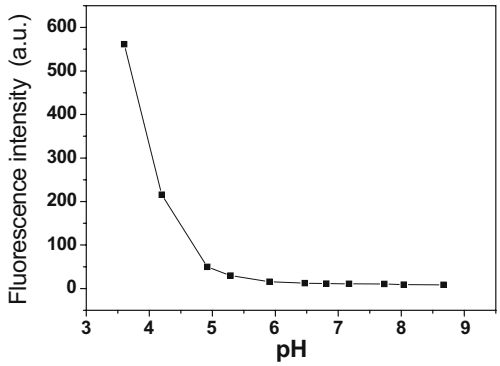


Fig. 8 Effect of pH on the fluorescence background of **I** (10.0 μmol L^{-1}). The fluorescence intensity was measured in 40% ethanol aqueous solution at different pH buffer

fluorophore is important. In the present study, rhodamine B is selected as it exhibits an excellent excitation or emission spectrum overlapping with the absorption maximum of **I**-Cu(II) complex (Fig. 4). Further, we select rhodamine B as fluorophore because of its fluorescence feature free from the influence of external factors such as pH and metal ions.

The fluorescence increase of **I**-Cu(II) + rhodamine B to cysteine is strongly dependent on the relative molar ratio of the absorber and fluorophore. As a compromise of high sensitivity and wide dynamic working range, the molar ratio of **I**-Cu(II) to rhodamine B was selected at 60: 1.

Chemosensing of cysteine

The chemosensing of cysteine was first studied by adding different concentration of cysteine to **I**-Cu(II) complex solution. Figure 5 shows the absorption spectra upon adding different concentration of cysteine, and it can be seen that the absorption at 558 nm decreased with increasing cysteine concentration, which indicates that **I**-Cu(II) complex is a cysteine-sensitive absorber. Moreover, the fluorescence signal of the **I**-Cu(II) complex solution in the presence of 40.0 μmol L^{-1} cysteine was measured, and the fluorescence signal increased about 10%, indicating that the fluorescence increase caused by the redox chemistry between thiols and Cu^{2+} is unimportant. This excludes the possibility that the fluorescence increase of the solution caused by the conversion of the paramagnetic (quenched) Cu(II) complex to a diamagnetic (fluorescent) Cu(I) state.

The fluorescence response of "**I**-Cu(II) complex + rhodamine B" system toward different concentration of cysteine was studied, and the results were shown in Fig. 6. It can be seen that the fluorescence intensity of the system increased with increasing the concentration of cysteine. It was also observed that with increasing the concentration of cysteine, the fluorescence emission maximum of the system blue-shifted about 8 nm, which might be explained as follows: in the absence of cysteine, the **I**-Cu(II) complex reabsorbs most of the rhodamine B's emission at the wavelengths where there is significant absorption. Therefore, rhodamine B's emission is especially reduced for $\lambda<580$ nm. When the high concentration of cysteine was present in the system, the IFE was removed and the fluorescence emission of rhodamine B was measured, causing the blue-shift of the fluorescence spectroscopy of the present system.

Effect of ethanol content on the absorbance of I-Cu(II)

In pure aqueous solution, the absorbance of **I** with the addition of Cu^{2+} ions were very low, which might be due to the poor solubility of **I** in water. On the other hand, when the absorbance of **I**-Cu(II) was measured in ethanol–water solution, a strong absorbance signal was obtained. To obtain a more optimal condition for the chemosensing of cysteine, the effect of ethanol content on the absorbance signal of **I**-Cu(II) was studied in the range 10–60% (*v*/*v*), and the results was shown in Fig. 7. From Fig. 7, it can be seen that the absorbance increases with increasing the ethanol content and reaches a stable signal when ethanol

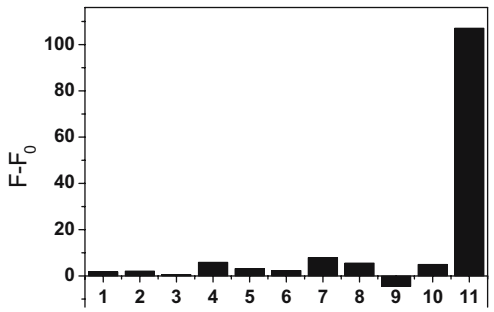


Fig. 9 Fluorescence intensity of **I**-Cu(II) + rhodamineB system upon adding different amino acids. 1, L-threonine; 2, L-arginine; 3, L-serine; 4, DL-tyrosine; 5, DL-tryptophan; 6, L-methionine; 7, L-glutamic acid; 8, glycine; 9, L-norvaline; 10, L-aspartic acid; 11, L-cysteine. Other conditions were the same as those described in the Experimental section. The concentrations of all amino acids were 4.0 μmol L^{-1}

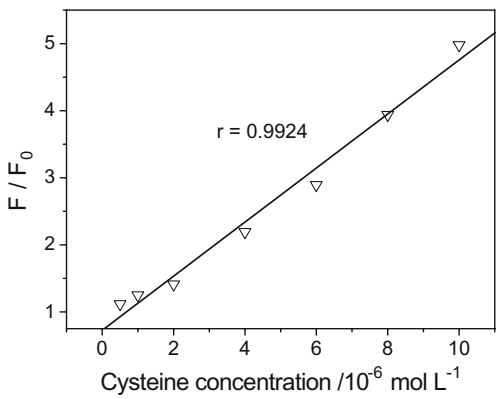


Fig. 10 Linear plots of F/F_0 *vs* cysteine concentration over the entire dynamic concentration range. Where F and F_0 are the fluorescence intensity of **I**-Cu(II) + rhodamine B system in the presence and absence of cysteine, respectively. Other conditions were the same as those described in the Experimental section

content is about 40% (v/v). Based on the above results, the "**I**-Cu(II) + rhodamine B" chemosensing ensemble were performed in 40% aqueous ethanol solution.

Effect of pH

The effect of pH on the fluorescence signal of "**I**-Cu(II) + rhodamine B" chemosensing ensemble was studied. Initially, the effect of pH on the fluorescence background of **I** was examined, and the results were shown in Fig. 8. As can be seen from Fig. 8, the minimum fluorescence background of the system was obtained when the pH of the system is above 5.3. On the other hand, the fluorescence intensity of rhodamine B is stable above pH 4.0 [26]. Therefore, to obtain a low fluorescence background and an optimal condition for fluorescence measurement of rhodamine B in the reaction mixture at the same time, pH 7.1 of Tris–HCl buffer was selected in the following experiment.

Selectivity of the method

The selectivity of the "**I**-Cu(II) + rhodamine B" chemosensing ensemble were studied and Fig. 9 shows the changes of fluorescence intensity of the system caused in the presence of different amino acids. It can be seen that only cysteine induced fluorescence recovery of the solution significantly, while other amino acids, such as L-threonine, L-arginine, L-serine, DL-tyrosine, DL-tryptophan, L-methionine, L-glutamic acid, glycine and L-aspartic acid slightly enhanced the fluorescence intensity of the solution. The excellent selectivity of the method is due to the strong banding affinity of thiol groups with Cu^{2+}. As the displacement of the Cu^{2+} ion in **I**-Cu(II) complex is not an exclusive feature of cysteine, the present system can also shows similar response to other thiol-containing compounds, such as homocysteine and glutathione.

Analytical figures of merit

Under the selected experimental conditions, the F / F_0 value is directly proportional to the cysteine concentration up to 10.0 μmol L^{-1}(as shown in Fig. 10). According to IUPAC, the detection limit was determined from three times the standard deviation of the blank signal (3σ) as 1.4×10^{-7} mol L^{-1}. The relative standard deviation (n=8) was 4.7% for 4.0 μmol L^{-1} of cysteine.

Conclusions

In summary, a simple fluorescent chemosensing ensemble for cysteine was designed based on the fluorescence inner filter effect between **I**-Cu(II) complex and rhodamine B. Compared with classical fluorescent chemosensor for cysteine in which the recognition moiety is covalent linking to a fluorophore, the spatial separation of signaling group and fluorophore offers considerable flexibility in chemosensor design. The sensitivity and dynamic working range of the fluorescence response is dependent on the extinction coefficient of the **I**-Cu(II) complex of the absorber as well as the molar ratio of the absorber and fluorophore. Moreover, because the changes in the absorbance of the absorber translate into exponential changes in fluorescence of fluorophore, an enhanced sensitivity for the analytical method can be obtained with respect to the absorbance value alone.

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References

1. Wood ZA, Schröder E, Harris JR, Poole LB (2003) Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci 28:32–40
2. Nekrassova O, Lawrence NS, Compton RG (2003) Analytical determination of homocysteine: a review. Talanta 60:1085–1095
3. Haugland RP (2002) Handbook of fluorescent probes and research products, 9th ed. Molecular Probes, Eugene, OR, pp 79–98
4. Rusin O, Luce NNSt, Agbaria RA, Escobedo JO, Jiang S, Warner IM, Dawan FB, Lian K, Strongin RM (2004) Visual detection of cysteine and homocysteine. J Am Chem Soc 126:438–439
5. Wang W, Rusin O, Xu X, Kim KK, Escobedo JO, Fakayode SO, Fletcher KA, Lowry M, Schowalter CM, Lawrence CM, Fronczek FR, Warner IM, Strongin RM (2005) Detection of homocysteine and cysteine. J Am Chem Soc 127:15949–15958
6. Zhang DQ, Zhang M, Liu ZQ, Yu MX, Li FY, Yi T, Huang CH (2006) Highly selective colorimetric sensor for cysteine and homocysteine based on azo derivatives. Tetrahedron Lett 47:7093–7096
7. Zhang M, Yu MX, Li FY, Zhu MW, Li MY, Gao YH, Li L, Liu ZQ, Zhang JP, Zhang DQ, Yi T, Huang CH (2007) A highly selective fluorescence turn-on sensor for cysteine/homocysteine and its application in bioimaging. J Am Chem Soc 129:10322–10323
8. Tang B, Xing YL, Li P, Zhang N, Yu FB, Yang GW (2007) A rhodamine-based fluorescent probe containing a Se-N bond for detecting thiols and its application in living cells. J Am Chem Soc 129:11666–11667
9. Fu YY, Li HX, Hu WP, Zhu DB (2005) Fluorescence probes for thiol-containing amino acids and peptides in aqueous solution. Chem Commun, pp 3189–3191
10. Shao N, Jin JY, Cheung SM, Yang RH, Chan WH, Mo T (2006) A spiropyran-based ensemble for visual recognition and quantification of cysteine and homocysteine at physiological levels. Angew Chem Int Ed 45:4944–4948
11. Han MS, Kim DH (2004) Rationally designed chromogenic chemosensor that detects cysteine in aqueous solution with remarkable selectivity. Tetrahedron 60:11251–11257
12. Shao N, Zhang Y, Cheung SM, Yang RH, Chan WH, Mo T, Li KA, Liu F (2005) Copper ion-selective fluorescent sensor based on the inner filter effect using a spiropyran derivative. Anal Chem 77:7294–7303
13. Gabor G, Walt DR (1991) Sensitivity enhancement of fluorescent pH indicators by inner filter effects. Anal Chem 63:793–796

14. He HR, Li H, Mohr G, Kova´cs B, Werner T, Wolfbeis OS (1993) Novel type of ion-selective fluorosensor based on the inner filter effect—an optrode for potassium. Anal Chem 65:123–127
15. Tohda K, Lu HW, Umezawa Y, Gratzl M (2001) Optical detection in microscopic domains. 2. Inner filter effects for monitoring nonfluorescent molecules with fluorescence. Anal Chem 73:2070–2077
16. Yuan P, Walt DR (1987) Calculation for fluorescence modulation by absorbing species and its application to measurements using optical fibers. Anal Chem 59:2391–2394
17. Dujols V, Ford F, Czarnik AW (1997) A long-wavelength fluorescent chemodosimeter selective for Cu(II) ion in water. J Am Chem Soc 119:7386–7387
18. Kwon JY, Jang YJ, Lee YJ, Kim KM, Seo MS, Nam W, Yoon J (2005) A highly selective fluorescent chemosensor for Pb^{2+}. J Am Chem Soc 127:10107–10111
19. Xiang Y, Tong AJ (2006) A new rhodamine-based chemosensor exhibiting selective Fe(III)-amplified fluorescence. Org Lett 8:1549–1552
20. Wu D, Huang W, Duan C, Lin Z, Meng Q (2007) Highly sensitive fluorescent probe for selective detection of Hg^{2+} in DMF aqueous media. Inorg Chem 46:1538–1540
21. Xiang Y, Tong AJ, Jin PY, Ju Y (2006) New fluorescent rhodamine hydrazone chemosensor for Cu(II) with high selectivity and sensitivity. Org Lett 8:2863–2866
22. Xiang Y, Mei L, Li N, Tong AJ (2007) Sensitive and selective spectrofluorimetric determination of chromium(VI) in water by fluorescence enhancement. Anal Chim Acta 581:132–136
23. Zheng H, Qian ZH, Xu L, Yuan FF, Lan LD, Xu JG (2006) Switching the recognition preference of rhodamine B spirolactam by replacing one atom: design of rhodamine B thiohydrazide for recognition of Hg(II) in aqueous solution. Org Lett 8:859–861
24. Lee MH, Wu JS, Lee JW, Jung JH, Kim JS (2007) Highly sensitive and selective chemosensor for Hg^{2+} based on the rhodamine fluorophore. Org Lett 9:2501–2504
25. Zhang M, Gao YH, Li MY, Yu MX, Li FY, Li L, Zhu MW, Zhang JP, Yi T, Huang CH (2007) A selective turn-on fluorescent sensor for Fe(III) and application to bioimaging. Tetrahedron Lett 48:3709–3712
26. Kojima H, Hirotani M, Urano Y, Kikuchi K, Higuchi T, Nagano T (2000) Fluorescent indicators for nitric oxide based on the rhodamine chromophore. Tetrahedron Lett 41:69–72