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Colorimetric and fluorescent detection of biological thiols in aqueous solution

Yin-Hui Li [a], Jin-Feng Yang [a,b], Chang-Hui Liu [a], Ji-Shan Li [a], Rong-Hua Yang [a,*]

[a] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China
[b] Anesthesia Department, Affilicated Cancer Hospital, Xiangya School of Medicine, Central South University, Changsha 410013, China

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ABSTRACT

A new colorimetric and fluorescent probe, 2-(2,4-dinitrostyryl)-1,3,3-trimethyl-3*H*-indolium iodide (DTI), for selective and sensitive detection of biological thiols is reported. In aqueous solution at physiological pH 7.4, biological thiols react with DTI *via* Michael addition to give the brownish red adduct concomitant with fluorescence emission decrease.

1. Introduction

Biological thiols such as glutathione (GSH), cysteine (Cys), and homocysteine (Hcy) play crucial roles in the cellular antioxidant defense system. It has been suggested that alternations in the level of cellular thiols are linked to a number of diseases, such as leukocyte loss, psoriasis, liver damage, cancer, and AIDS. Additionally, several forms of drug resistance have been correlated with elevated levels of GSH. Thus, it is important to conveniently monitor the concentration of biological thiols in physiological media. A wide variety of detection techniques are applied, such as high-performance liquid chromatography (HPLC) [1], electrochemical assays [2], mass spectrometry [3], surface-enhanced Raman scattering (SERS) [4], localized surface plasmon resonance [5], interplasmon coupling in Au nanorods [6], combinatorial library-based sensors [7], enzymatic methods [8], fluorescent detection based on synthetic receptors [9] and quantum dots [10]. Among them, optical detection methods have proven most convenient. By far, various colorimetric and fluorescent probes for thiol-containing amino acids and peptides are desirable. The majority of the reported methods are based on redox chemistry or labeling with chromophores or fluorophores and a combination of separation techniques, and there is still plenty of room for improvement in terms of selectivity, sensitivity, and performance with a different interaction mechanism.

Herein, we have designed and synthesized a new hemicyanine probe 2-(2,4-dinitrostyryl)-1,3,3-trimethyl-3*H*-indolium (DTI), where two unites of indole quinoline and dinitrobenzene are covalently linked by a double bond. By taking advantage of the strong electron-withdrawing capacity of the 2,4-dinitrobenzene moiety, the double bond of DTI could be susceptible to the nucleophilic biological thiols *via* Michael addition reaction (Scheme 1).

2. Experimental

All the solvents and chemicals were of analytical reagent grade and were supplied by Alfa aesar or Sigma–Aldrich. The DTI probe was synthesized by one step. 2,4-Dinitrobenzaldehyde (0.392 g, 2 mmol) was dissolved in 10 mL of acetic anhydride, then 1,2,3,3-tetramethyl-3*H*-indolium iodide (0.903 g, 3 mmol) and sodium acetate (0.246 g, 3 mmol) were added. The solution was stirred at room temperature for 12 h followed by the addition of 20 mL of diethyl ether. Brown precipitate was obtained. The precipitate was filtered and washed with cold ethyl acetate three times. Drying the precipitate in vacuum afforded DTI as a reddish-brown product (0.62 g, 65%).

The stock solution of 1.0×10^{-3} mol/L DTI was obtained by dissolving the compounds in CH_3CN. Working solutions were prepared by sequential dilution of the stock solution with Tris–HCl solution (50 mmol/L, pH 7.4). All stock solutions of amino acids were diluted in double distilled water. UV–vis spectra were recorded in 1 cm path length quartz cuvettes on a Hitachi U-4100 UV/vis spectrophotometer (Kyoto, Japan). Fluorescence emission

* Corresponding author.
E-mail address: yangrh@pku.edu.cn (R.-H. Yang).

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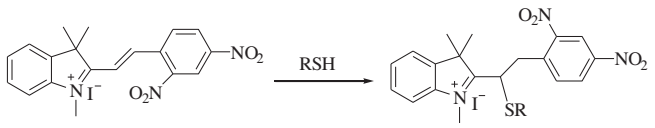

Scheme 1. Reaction of probe DTI with thiols.

spectra were measured on Hitachi F-7000 Fluorescence spectrophotometer (Kyoto, Japan). pH value was measured by model 868 pH meter (Orion). Data processing was performed with Sigmaplot software.

3. Results and discussion

During the addition reaction, the conjugated system of DTI was broken, resulting in dramatic changes in both the UV–vis and fluorescence spectra when probe DTI was treated with Cys as the model compound of biothiols (Fig. 1A). Free DTI displayed two absorption bands at 276 nm and 370 nm in aqueous solution (Tris–HCl buffer, 50 mmol/L, pH 7.4), which were responsible for the yellow color of the solution. In the presence of Cys, both the absorbances at 276 nm and 370 nm decreased dramatically while two new bands at 306 nm and 465 nm appeared with increasing the concentration of Cys. Meanwhile, in the titration traces, four clear isosbestic points at 266, 297, 320, 432 nm were observed. Such shift in absorption behavior changes the color of the resultant solution from yellow into brownish red, allowing "naked-eye" detection. Similar experimental phenomena were also observed when we added Hcy or GSH into the solution of DTI. The profound shift indicates that the conjugation between indole quinoline and dinitrobenzene was broken due to the plausible Michael addition of Cys to DTI.

We also noticed that the reaction of DTI with Cys produced an "on–off" type of fluorescence emission at 510 nm with maximum excitation at 370 nm. In the absence of Cys, DTI showed very strong fluorescence intensity at 510 nm. Upon addition of Cys to the solution of DTI, a dramatic turn-off fluorescence response was observed (Fig. 1B). There was a good linearity between the fluorescence decrease and Cys concentrations in the range of 1.0×10^{-7} mol/L to 9.0×10^{-7} mol/L. The regression equation was $F_0/F = 1970.866 - 2144.161$ [Cys] (μmol/L) with a linear coefficient of 0.989. The detection limit that was taken to be 3 times the standard derivation of a blank solution was estimated to be 2.6×10^{-8} mol/L.

To investigate the effect of pH on the fluorescence response of DTI to Cys, the fluorescence intensity changes of DTI induced by Cys were measured at various pHs. In the absence of Cys, DTI showed little change in fluorescence intensity at lower pH (Fig. 2). But the intensity decreased in alkaline conditions. When Cys was added to DTI in buffers at various pHs, significant fluorescence change was detected at pH range of 6.0–8.0. The curves indicated that DTI responded to biothiols under physiological conditions.

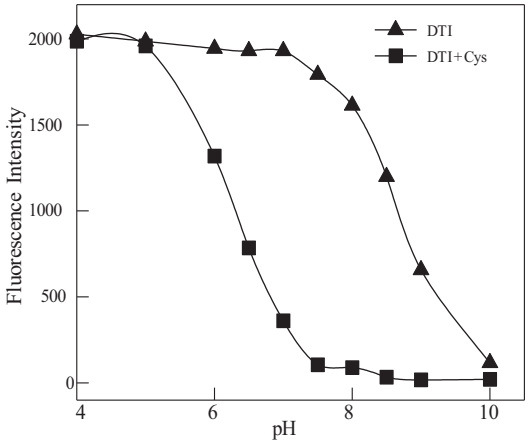

Fig. 2. The fluorescence intensity of DTI at 510 nm in the presence and absence of Cys under different pH system, λ_{ex} = 370 nm.

The reaction mechanism was confirmed by using ^{1}H NMR spectroscopy and electrospray ionization mass spectrometry (ESI-MS) (data not shown). Upon addition of Cys, a vinyl proton of DTI around δ 8.4 dramatically disappear with the concomitant appearance of a new peak at δ 7.1 and δ 6.7. And the aromatic protons produced corresponding chemical shifts. In addition, the m/z formula $[M+H]^+$ of the free DTI was found to be 352.0 (calcd. 479.0) in its ESI-MS. After addition of Cys to the DTI solution, a new peak at m/z 494.9 (DTI+Cys+Na^+) was clearly observed, which is assigned to the 1:1 complex between DTI and Cys.

To examine the selectivity of DTI toward thiol-containing amino acids, changes in the fluorescence spectra of DTI by addition of various amino acids were measured in the Tris–HCl buffer solution (Fig. 3). Significant decrease at 510 nm has been found upon addition of Hcy or Cys to DTI solution, with an evident color change from yellow to brownish red. On the other hand, treatment of up to 50 equiv. of other 19 amino acids (Tyr, Gly, Phe, Met, Leu, Arg, Pro, Lys, Glu, Gln, Asp, Iso, Ile, Val, His, Ser, Ala, Thr, Try) with DTI did not induce any obvious change in the fluorescence spectra,

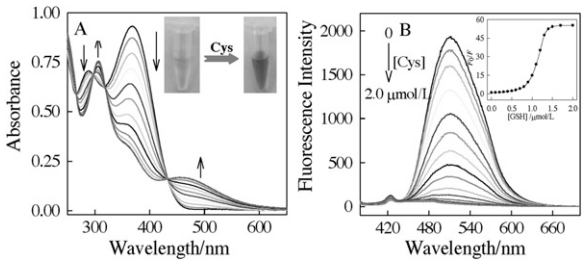

Fig. 1. Absorption (A) and fluorescence spectra (B) of DTI in the presence of increasing amounts of Cys in Tris–HCl solution (50 mmol/L, pH 7.4). The arrow indicates the signal changes as increases in Cys concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.7, 2.0 μmol/L). Inset: the ratio of F_0/F of DTI as a function of the concentrations of Cys.

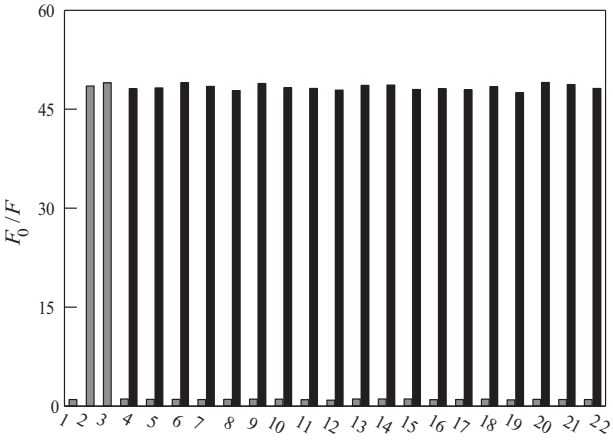

Fig. 3. The selectivity of DTI toward various amino acids. Gray bars: the ratio of F_0/F of DTI in the presence of various amino acids; Black bars: the ratio of F_0/F of DTI in the presence of the mixture of each amino acid and GSH, (1) Free DTI. (2) Cys. (3) Hcy. (4) Tyr. (5) Gly. (6) Phe. (7) Met. (8) Leu. (9) Arg. (10) Pro. (11) Lys. (12) Glu. (13) Gln. (14) Asp. (15) Iso. (16) Ile. (17) Val. (18) His. (19) Ser. (20) Ala. (21) Thr. (22) Try.

even high concentrations. The competitive experiments have also shown that the fluorescence intensities of DTI were quenched to be as big as that of Cys upon addition of Cys to the mixtures of DTI and other amino acids. These results clearly indicated that the approach is selective toward thiol-containing amino acids in the presence of other amino acids.

4. Conclusion

In conclusion, we have developed a rapid and simple method with high selectivity and sensitivity for detection of biothiols in aqueous solution. The recognition of biothiols gave obvious color changes from yellow to brownish red, which was clearly visible to the naked eye, while it also showed a distinct fluorescence response.

Acknowledgments

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