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Abstract. Great efforts have been devoted to the development of sensitive and specific analysis methods for biothiols because of their important roles in biological systems. We present a new detection system for biothiols that is based on the reversible quenching and restoration of fluorescence of terbium chelate caused by Hg^{2+} and thiol species. In the presence of biothiols, a restoration of fluorescence of terbium chelate after quenching by Hg^{2+} was observed due to the interaction of Hg^{2+} with thiol groups, and the restored fluorescence increased with the concentration of biothiols. This method was sensitive and selective for biothiols. The detection limit was 80 nM for glutathione, 100 nM for Hcy, and 400 nM for Cysteine, respectively. The terbium chelate-Hg (II) system was successfully applied to determine the levels of biothiols in cancer cells and urine samples. Further, it was also shown to be comparable to Ellman's assay. Compared to other fluorescence methods, the terbium chelate probe is advantageous because interference from short-lived nonspecific fluorescence can be efficiently eliminated due to the long fluorescence lifetime of terbium chelate, which allows for detection by time-resolved fluorescence. The terbium chelate probe can serve as a diagnostic tool for the detection of abnormal levels of biothiols in disease. *© 2012 Society of Photo-Optical Instrumentation Engineers (SPIE).* [DOI: 10.1117/1.JBO.17.1.017001]

Keywords: biothiols; time-resolved fluorescence; terbium chelate; Hg^{2+}; cancer cells.

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1 Introduction

In organisms, thiol-containing amino acids such as glutathione (GSH), cysteines (Cys), and homocysteine (Hcy) play crucial roles in maintaining normal cellular functions.[1,2] GSH, one of the most abundant intracellular thiols, acts as a redox regulator to maintain the proper cellular ratio of reduced (GSH) to oxidized glutathione (GSSG).[3,4] Alterations to the optimum ratio of GSSG to GSH have been implicated in a variety of diseases, such as heart disease, cancer, stroke, and neurological disorders.[1] Cys and Hcy are essential biological components for the growth of cells and tissues, and the levels of Cys and Hcy are often used as indicators of disease. A deficiency of Cys can cause retarded growth, hair depigmentation, lethargy, liver damage, muscle and fat loss, and skin lesions,[5] whereas high levels of Hcy increase risks for cardiovascular diseases[6] and Alzheimer's disease.[7] Therefore, it is important to measure the levels of biothiols in organisms rapidly and sensitively.

The current methods for the detection of biothiols include fluorescence detection,[8] ultraviolet detection,[9] electrochemical detection,[10] laser-induced fluorescence detection,[11] and mass spectrometry.[12,13] However, all of these methods are time consuming and require expensive and sophisticated instruments because they are often performed in conjunction with separating techniques, such as high-performance liquid chromatography, gas chromatography, and capillary electrophoresis. In the past few years, a number of fluorescent probes that are based on organic dyes,[14,15] quantum dots,[16,17] and gold nanoparticles[18,19] have been developed for the measurement of biothiols, which have increased sensitivity and convenience; however, they still have limitations. The organic dyes have poor aqueous solubility and are prone to photobleaching.[20] The difficult surface conjugation chemistry and possible toxicity *in vivo* of quantum dots are still under investigation.[21] More importantly, all these fluorescent probes suffer from interference by autofluorescence and scattering light from biological tissues and matrices when they are used in biological environments. The autofluorescence of biological samples often largely decreases their sensitivity of detection.[16,22,23] Lanthanide complexes have outstanding fluorescence properties, such as a large Stokes shift (>150 nm), sharp emission (<10 nm full wave at half maximum), and long fluorescence lifetimes (millisecond).[24,25] Time-resolved fluorescence assays (TRF) that are based on lanthanide complexes offer a significant advantage over traditional fluorescence methods in eliminating the background interference and scattering fluorescence through time-delayed detection. The lanthanide complexes have been applied to ultrasensitive analysis of biological molecules, such as DNA and proteins.[26–28] However, very few studies on the determination of metal ions with lanthanide fluorescent probes have been reported.

In this study, we present a new detection method for biothiols that is based on a terbium chelate-Hg (II) system. It is known that Hg^{2+} has a high affinity for thiol-containing biomolecules. The stability of a Hg^{2+}-thiol bond is 10 orders of magnitude greater than the bond of Hg^{2+} with other nucleophiles present in the same environment.[29] We designed a lanthanide fluorescent probe that can be quenched by Hg^{2+} binding to the ligand of the lanthanide probe. In the absence of biothiols, the fluorescence of the lanthanide probe, cs124-DTPA-Tb, was quenched by Hg^{2+} that was in the solution. However, in the presence of biothiols, the lanthanide probe emits a strong fluorescence due to binding of the Hg^{2+} ion to the biothiols (Fig. 1). This is the first

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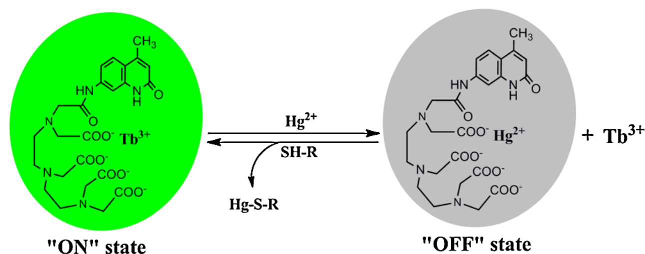

Fig. 1 Schematic illustration of the terbium chelate-Hg (II) system for the detection of biothiols. (Color online only.)

report of a lanthanide probe being successfully used to detect thiol compounds, and we tested this probe for the detection of biothiols in cancer cells and urine. As expected, a high signal-to-noise ratio was obtained, and the background signal was eliminated using time-delayed detection.

2 Materials and Methods

2.1 *Chemicals*

Diethylenetriaminepentaacetic acid dianhydride (98%, DTPA dianhydride); 7-amino-4-methyl-2(1H)-quinolinone (99%, carbostyril 124); anhydrous N,N-dimethylformamide (99.8%, DMF), and homocysteine (Hcy) were purchased from Sigma-Aldrich. Terbium nitrate (99.99%) was purchased from Baotou Ruike Rare Earth Metal Materials Company, Baotou. L-cysteines (Cys), glutathione (GSH, reduced) and 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Beijing Dingguo Biotechnology; N-ethyl maleimide (>98%, NEM) was from J&K Scientific Ltd., Shanghai; mercury nitrate (very toxic, wear protective gloves and eyeglasses) was from Beijing Zhongbiao Huawei Technology Co., Ltd. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Gibco, Shanghai. Trypsin was from Sangon Biotech (Shanghai) Co., Ltd. Distilled and deionized water ($18\ M\Omega \cdot cm^{-1}$) were used for the preparation of all aqueous solutions. Unless otherwise stated, all chemicals were of analytical reagent grade and were used without further purification.

2.2 *Measurements*

Fluorescence spectra were recorded on an LS55 luminescence spectrometer (PerkinElmer, UK). The solution was placed in a quartz micro cuvette with a capacity of 100 μL. The excitation wavelength used was 327 nm for the emission spectra. For the time-resolved fluorescence spectra, a delay time of 50 μs and a gate time of 2 ms were used. UV-vis absorbance was measured with a UV-3150 spectrophotometer (Shimadzu, Japan) at room temperature. The determination of each sample was repeated three times. All error bars represent standard deviations from three repeated experiments.

2.3 *Synthesis of the cs124-DTPA-Tb Complex*

The synthesis of the cs124-DTPA-Tb complex was performed according to previously published methods.[24,26] Typically, 1400 μL of a DMF solution that contained 25 mg of DTPA dianhydride was first prepared, and 100 μL of dry triethylamine was added to the solution while stirring. After the triethylamine was thoroughly mixed, 200 μL of DMF with 12 mg of cs124 was added to the above solution, and the mixture was stirred for 2 h at room temperature. To form the cs124-DTPA-Tb complex, an equal molar quantity of $Tb(NO_3)_3$ was added, and the reaction was kept for 15 min at room temperature.

2.4 *Quenching and Restoration of Fluorescence of cs124-DTPA-Tb*

To a final concentration of 1 μM, 1 μL of Hg^{2+} (100 μM) was added to 99 μL of 0.5 μM cs124-DTPA-Tb aqueous solution, and the solution was thoroughly mixed to quench the fluorescence of cs124-DTPA-Tb. After reacting for 15 min at room temperature, the fluorescence intensity of cs124-DTPA-Tb after being quenched by Hg^{2+} was recorded from 450 nm to 650 nm. A series of Cys, Hcy, and GSH aqueous solutions with different concentrations were prepared for use as the standard analytes, and fresh thiol solutions were added to the above mixture. The mixtures were incubated for 20 min at room temperature, and the fluorescence of cs124-DTPA-Tb that was restored by the thiols was determined. All fluorescence detections were performed under time-resolved mode.

2.5 *Detection of Biothiols in Cancer Cells and Urine*

The experiments were performed with the colorectal carcinoma cell line SW480. The cells were cultured in 75 cm^2 flasks that contained 10 mL of DMEM with 10% fetal bovine serum and 1% antibiotics. The cells were incubated with 5% CO_2 at 37 °C until the seeding density reached 70%. After removing the medium, the cells were digested with trypsin (0.25%) for 30 min and washed with phosphate-buffered saline (PBS; 0.1 M, pH 7.4). The digested cells were collected by centrifugation, washed twice, and resuspended in PBS. The cell lysate was obtained by repeated cycles of freezing and thawing, and the lysate was stored at 4°C until it was required. As a control, the cell lysate was treated with NEM for 2 h at 37°C to block the thiol groups in the cells. For the determination of the total amount of biothiols in the cancer cells, the experimental conditions and steps were followed as mentioned above.

For the detection of biothiols in urine, urine samples were collected from healthy volunteers. To 2 μL of a mixed solution that contained 50 μM cs124-DTPA-Tb and 100 μM Hg^{2+}, 5 μL of urine sample and H_2O were added till the total volume reached 100 μL. The fluorescence assay of urine samples was the same as that described above. The fluorescence intensity of the urine samples at 545 nm was recorded using an excitation wavelength of 327 nm.

2.6 *Measurement of Biothiols by the DTNB Method*

The measurement of biothiols using the DTNB method was performed according to previously published methods.[30] The solution of DTNB was prepared by dissolving DTNB in 0.1 M sodium phosphate (pH 8.0) to a final concentration of 0.4 mg/ml. To prepare a set of standard solutions, cysteine was used as the standard thiol and was dissolved in 0.1 M sodium phosphate (pH 8.0) at an initial concentration of 2 mM. For each standard solution, 50 μL of the DTNB solution was added to 250 μL of each standard. After incubating the solution for 15 min at room temperature, the UV absorbance of each solution was measured at 412 nm. The same procedure was used for the determination of biothiols in the cell lysates and urine.

3 Results and Discussion

3.1 *Fluorescence Quenching of cs124-DTPA-Tb by* Hg^{2+}

The effect of the Hg^{2+} concentration on the fluorescence of cs124-DTPA-Tb was investigated. As shown in Fig. 2(a), there was a decrease in the fluorescence intensity of cs124-DTPA-Tb after the addition of 400 nM Hg^{2+}. The fluorescence intensity of cs124-DTPA-Tb decreased with an increase in Hg^{2+} concentration, and it was nearly quenched with 2 μM Hg^{2+} [Fig. 2(b)]. Although higher concentrations of Hg^{2+} resulted in more complete fluorescence quenching, increased amounts of thiols are required to bind Hg^{2+} when the fluorescence of cs124-DTPA-Tb was recovered by the thiol compounds. Therefore, a Hg^{2+} concentration of 1 μM was used in the present study to obtain the best sensitivity.

3.2 *Recovery of the Fluorescence of cs124-DTPA-Tb by Biothiols*

Figure 3 shows the fluorescence emission spectra of cs124-DTPA-Tb at different conditions. In the absence of Hg^{2+}, cs124-DTPA-Tb displays the typical emission spectra of Tb^{3+} where intramolecular energy transfer from cs124 to Tb^{3+} generates a strong intensity (curve a). In the presence of Hg^{2+}, the fluorescence intensity of cs124-DTPA-Tb becomes very weak, and only 10% of the intensity of cs124-DTPA-Tb was observed (curve b). However, after the addition of GSH, Cys, or Hcy to the solution, the fluorescence intensity increased to approximately 50% (curve c), 30% (curve d), and 25% (curve e) of cs124-DTPA-Tb, respectively. These results indicate that the presence of thiol compounds can recover the fluorescence of cs124-DTPA-Tb. To ensure that the recovery was a result of the interaction of Hg^{2+} with the thiol species, we investigated the fluorescence of cs124-DTPA-Tb without Hg^{2+} and in the presence of various concentrations of thiol species. As shown in Fig. 4, there were no obvious changes in the fluorescence of cs124-DTPA-Tb after the addition of GSH, Cys, or Hcy, which indicates that these thiol species had no effect on the fluorescence of cs214-DTPA-Tb. Therefore, the fluorescence of cs124-DTPA-Tb was restored by the thiol species, and this was due to the binding of Hg^{2+} to the thiol groups.

3.3 *Sensitivity of cs124-DTPA-Tb-Hg (II) System for the Detection of Biothiols*

To investigate the utility of this system as a quantitative fluorescence method for biothiols detection, a series of GSH, Cys, and Hcy solutions with concentrations from 10 nM to 1 mM were examined. As shown in Fig. 5, increasing of concentrations of biothiols led to an increase in the fluorescence of cs124-DTPA-Tb in the presence of 1 μM Hg^{2+}. The enhanced fluorescence intensity showed a linear relationship with the concentration of GSH, Cys, and Hcy in the range of 0.6 to 10 μM, 0.8 to 10 μM, and 1 to 20 μM, respectively, and the limit of detection was 80 nM for GSH, 100 nM for Hcy, and 400 nM for Cys (insets in Fig. 5). In this system, the restored fluorescence was saturated when the intensity reached approximately 66% of the fluorescence intensity of cs124-DTPA-Tb alone, even if higher concentrations of the biothiols were used. The restoration ability of the three biothiols at the same concentration follows the order: GSH > Hcy > Cys. To return the

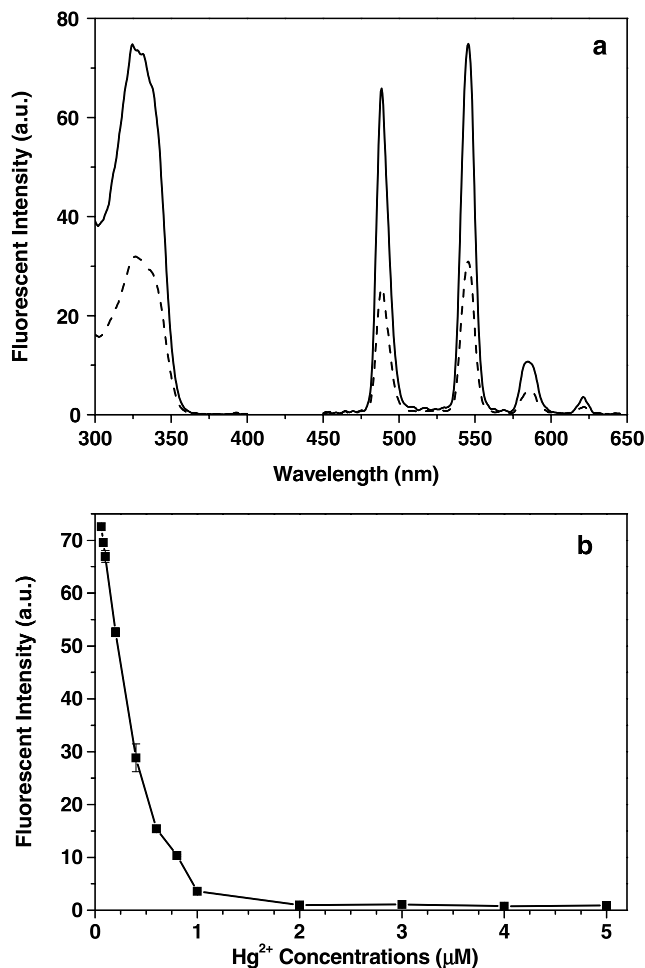

Fig. 2 (a) Excitation and emission spectra of an aqueous solution of 0.5 μM cs124-DTPA-Tb in the absence of Hg^{2+} (solid line) and in the presence of 400 nm Hg^{2+} (dashed line). (b) Fluorescence response of 0.5 μM cs124-DTPA-Tb after the addition of different concentrations of Hg^{2+} ions.

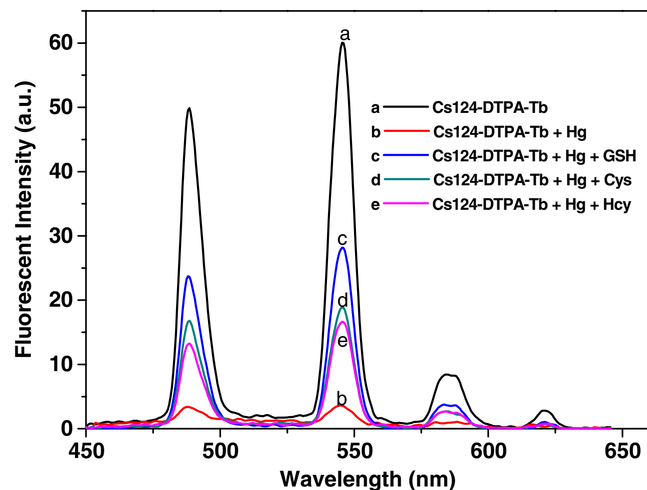

Fig. 3 Fluorescence emission spectra of 0.5 μM cs124-DTPA-Tb in the absence of Hg^{2+} (a), in the presence of 1 μM Hg^{2+} (b), and in the presence of 1 μM Hg^{2+} and 6 μM of GSH (c), Cys (d), and Hcy (e), respectively. (Color online only.)

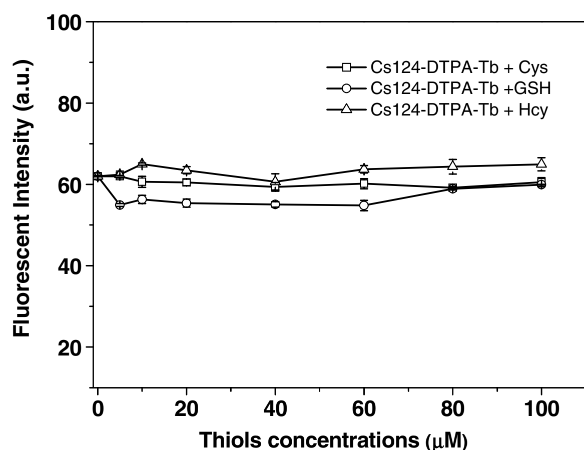

Fig. 4 Fluorescence response of 0.5 μM cs124-DTPA-Tb after the addition of different concentrations of thiols.

fluorescence intensity of cs124-DTPA-Tb to 60% of its initial fluorescence, the required concentration of GSH, Hcy, and Cys was approximately 3, 5, and 16 μM, respectively. The difference in the restoration ability of each compound may be due to differences in the chemical structures of the tested compounds. We speculate that the carboxyl group in these molecules also contributes to the binding of the thiol group to Hg^{2+}.[31] GSH contains two carboxyl groups, and Hcy and Cys contain one carboxyl group each; however, the carboxyl group of Hcy is more flexible due to atom spacing. The total bonding ability of the three biothiols for Hg^{2+} follows the order: GSH > Hcy > Cys. GSH is a major intracellular compound, and its intracellular concentrations are in the millimolar range, whereas its plasma concentrations are in the micromolar range.[32] HPLC analysis shows that the mean plasma concentration of GSH in adults is approximately 7.87 μM.[33] The concentrations of Cys and Hcy in plasma or urine are also at similar levels.[34] The high levels of GSH, Cys, and Hcy in plasma and urine suggest that the cs124-DTPA-Tb-Hg (II) detection system may be suitable for the detection of biothiols in biofluids.

Compared with other fluorescence probes for biothiols (Table 1), our method not only has comparable detection limits, low cost of detection, and an easy preparation of the probe, but also does not involve complex chemical modifications, which most methods have to conduct. More importantly, the background fluorescence, often present in biological samples, can be efficiently eliminated via a detection of time-resolved fluorescence.

3.4 *Selectivity of the cs124-DTPA-Tb-Hg (II) System for the Detection of Biothiols*

To evaluate the selectivity of the cs124-DTPA-Tb-Hg (II) detection system, 12 α-amino acids with different chemical structures were selected to test the specificity for thiol-containing biomolecules. The changes in fluorescence intensity of cs124-DTPA-Tb (0.5 μM) at 545 nm were examined by adding these amino acids under the same experimental conditions. For all amino acids tested, except for the amino acids that contained a thiol group, the change in the fluorescence of cs124-DTPA-Tb after quenching by Hg^{2+} was negligible (<1%), and was at levels similar to those of the blank (Fig. 6). The selective fluorescence restoration of cs124-DTPA-Tb further confirmed that only

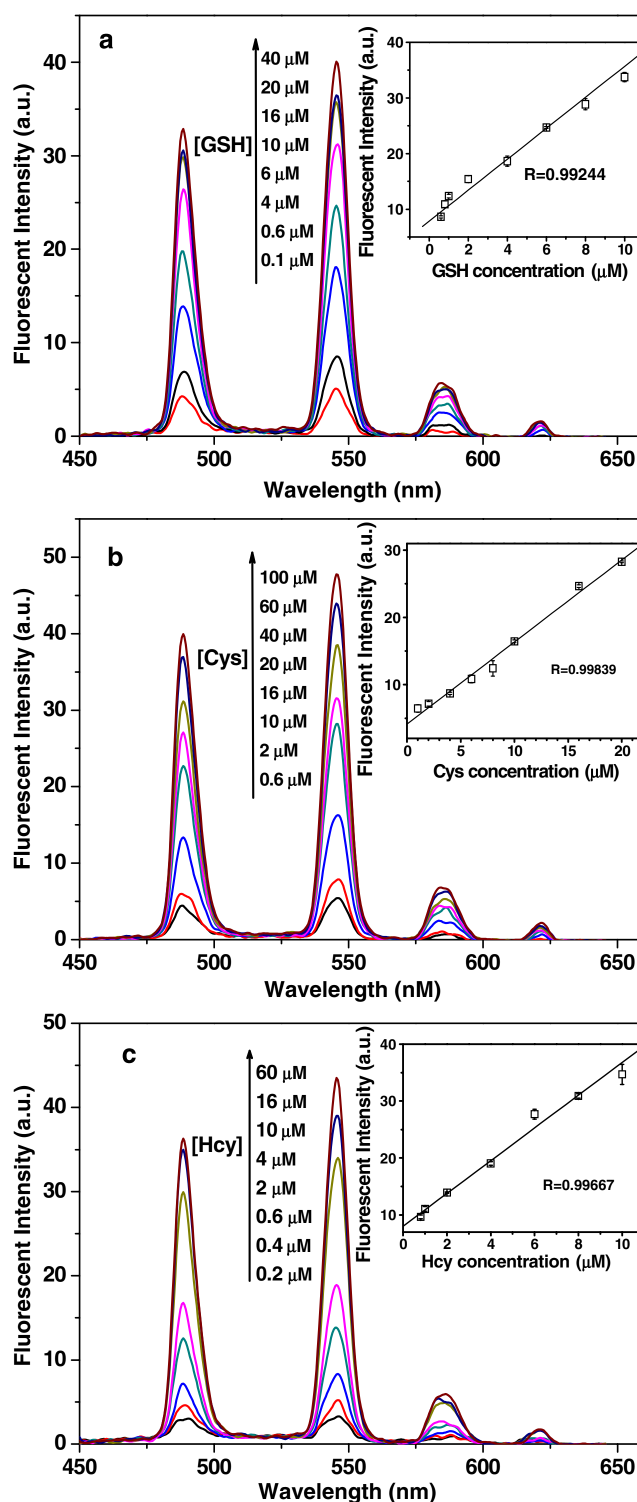

Fig. 5 Effects of the concentrations of GSH (a), Cys (b), and Hcy (c) on the fluorescence intensity of 0.5 μM cs124-DTPA-Tb after quenching with 1 μM Hg^{2+}. Insets: the linear relationship between the fluorescence intensity and the concentration of GSH (a), Cys (b), and Hcy (c). The fluorescence intensity at a wavelength of 545 nm was recorded. (Color online only.)

amino acids that contain a thiol group have a high affinity for Hg^{2+}, and biothiols in an organism may play an important role in avoiding heavy metal poisoning and maintaining the bioactivity of enzymes. Therefore, the cs124-DTPA-Tb-Hg (II)

Table 1 A comparison of fluorescence probes for the detection of biothiols.

Fluorescence probes	Detection limit (nM) Cys	Hcy	GSH	Chemical modification	Real samples	Elimination of background fluorescence	Refs
Ruthenium(II) complex	1400	1190	—	No	No	No	14
Fluorescein derivative	50	100	53	No	No	No	15
CdTe/CdSe quantum dots	131	26	20	Yes	Plasma and urine	Yes	16
CdTe quantum dots	600	—	100	Yes	Plasma	No	17
Nile red-AuNPs	10.2	10.9	—	Yes	No	No	18
Fluorosurfactant-AuNPs	—	180	—	Yes	Urine	No	19
Proposed method	400	100	80	No	Plasma and urine	Yes	

system can be used for the detection of biothiols with high selectivity.

3.5 *Detection of Thiols in Cancer Cells and Urine by the cs124-DTPA-Tb-Hg (II) System*

Although biothiols (GSH, Cys, and Hcy) are present in many forms in biological systems (protein-bound, oxidized, and reduced), a total concentration of biothiols was measured in most clinical tests because thiol groups are easily oxidized. This fluorescent system was applied for the determination of the concentration of biothiols in the cancer cell line SW480. The cells were first treated with lysis solution to release the biothiols in the cytosol and nucleus, and the lysate solution was used for the recovery of fluorescence of cs124-DTPA-Tb, which was quenched by Hg^{2+}. As shown in Fig. 7, the fluorescence of cs124-DTPA-Tb was restored in the presence of the lysate solution from SW480 cells, and the fluorescence intensity of cs124-DTPA-Tb increased with the number of lysed cells. In contrast, only a small residual signal was observed for the lysate solution that was pretreated with the thiol-blocking reagent NEM (curve h in Fig. 7). The residual signal may be because Hg^{2+} can bind with other molecules in the cells, such as thymine,[35] which may have caused the marginal recovery of fluorescence of cs124-DTPA-Tb that was observed. According to the fluorescence intensity that was generated by the biothiols and the calibration curve using Cys as a standard thiol, the lowest detectable concentration of biothiols in SW480 cancer cells was approximately 0.5 μM. This concentration is slightly different when GSH or Hcy was used as the standard thiol for the calibration curve due to the different restoration abilities of GSH, Cys, and Hcy for the fluorescence of cs124-DTPA-Tb. Standard GSH, Cys, and Hcy were added to the cell lysate solution to measure recovery, and the recoveries of GSH, Cys, and Hcy were between 95.3% and 116.2% (Table 2), which indicates that there is hardly system error in the quantitative detection of biothiols with the cs124-DTPA-Tb-Hg (II) system. The cell lysate had a high background fluorescence (curve i in Fig. 7) between 350 to 580 nm under normal fluorescence detection mode; however, under time-resolved fluorescence mode, the

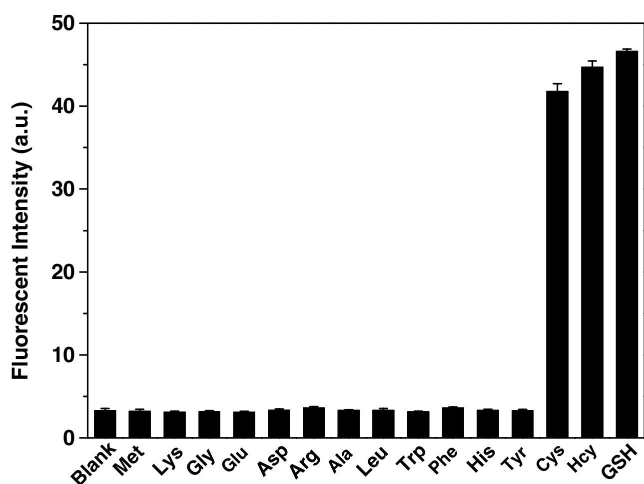

Fig. 6 Fluorescence restoration of 0.5 μM cs124-DTPA-Tb in the presence of various amino acids at a concentrations of 100 μM.

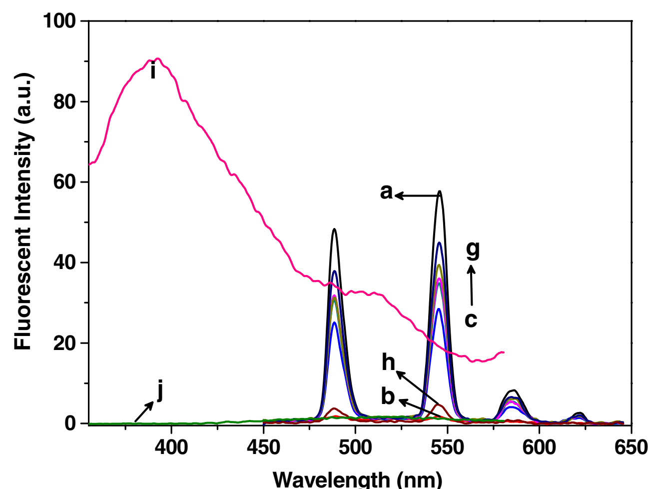

Fig. 7 Fluorescence response of 0.5 μM cs124-DTPA-Tb after the addition of lysis solutions that contained different numbers of SW480 cells. (a) in the absence of Hg^{2+}; (b) in the presence of Hg^{2+}; (c), (d), (e), (f), and (g) added lysis solutions that contained 2×10^5, 4×10^5, 6×10^5, 8×10^5, and 10×10^5 SW480 cells on the basis of (b), respectively; (h): (f) treated with thiol-blocking reagent; (i) and (j): fluorescence emission of lysis solutions measured under fluorescence mode and time-resolved fluorescence mode, respectively. (Color online only.)

Table 2 Recovery of GSH, Cys, and Hcy added to the cell lysate solution.

Sample	Spiked thiol (μM)	Detected thiol (μM)	Recovery (%)	RSD ($n = 3$, %)
GSH	5	4.97 ± 0.01	99.38	0.20
GSH	10	9.53 ± 0.05	95.30	0.52
Cys	5	5.63 ± 0.12	112.61	2.13
Cys	10	11.62 ± 0.02	116.20	0.17
Hcy	5	5.38 ± 0.15	107.58	2.79
Hcy	10	9.51 ± 0.23	95.12	2.42

RSD: Relative standard deviation.

background fluorescence of the lysate was completely eliminated when a time-delay of 50 μs was applied. This showed that the cs124-DTPA-Tb-Hg (II) system could efficiently eliminate interference from autofluorescence.

Biothiols in urine samples from healthy humans were also tested by the cs124-DTPA-Tb-Hg (II) system. The same phenomenon that occured in the determination of thiols in the cell lysate was also observed in the urine samples. The fluorescence intensities of cs124-DTPA-Tb increased with the volume of urine added. The interference from the background fluorescence of the urine sample over the range of 350 to 600 nm was excluded when using the time-resolved fluorescence mode (data not shown). The results of the urine samples are shown in Table 2. To validate the cs124-DTPA-Tb-Hg (II) system, we performed Ellman's assay (DTNB method) for the determination of the total thiols in the cell lysates and urine. DTNB reacts with sulfhydryls under slightly alkaline conditions to release the highly chromogenic compound with a maximum absorbance at 412 nm.[36] Fig. 8 shows that there was a good linear relationship between the absorbance and the concentration of Cys in the range of 6 to 100 μM, and the detection limit was approximately

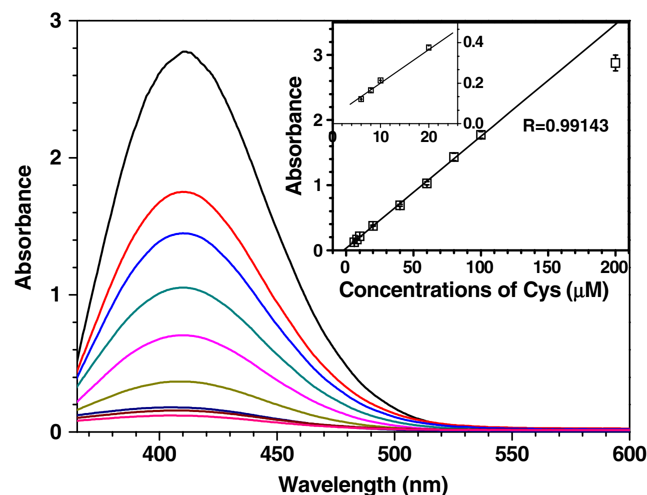

Fig. 8 UV spectra of Ellman's assay for biothiols and the calibration curve (inset). The concentrations of Cys were 6, 8, 10, 20, 40, 60, 80, 100, and 200 μM, respectively. (Color online only.)

Table 3 Measurement of biothiols in a cell lysate solution that contained 2×10^5 SW480 cells and in urine.

Sample	Concentration of thiols[a] (μM)		F-test[b]
	DTNB method	cs124-DTPA-Tb method	
Lysate	18.63 ± 0.03	16.65 ± 0.13	18.78
Urine	7.01 ± 0.05	6.71 ± 0.21	17.64

[a]Mean values ($n = 3$) $\pm$ standard deviation.
[b]The F-test value is 19.00 at a 95% confidence level.

6 μM (signal/noise >3). The detection limit of the cs124-DTPA-Tb-Hg (II) system for Cys (400 nM) is at least 10 times lower than the DTNB method. From the insets in Figs. 5(b) and 8, the slopes from the calibration curves for these two methods were 1.22 a.u./µm and 0.01 a.u./µm, respectively, which indicates that the cs124-DTPA-Tb-Hg (II) system is much more sensitive than the DTNB method (>100 times). The total thiol concentration in the cell lysate and urine samples is shown in Table 3. The F-test shows that there were no significant differences between these two methods.

Ellman's assay is based on the reaction of thiol-disulfide exchange. A number of thiols have been observed to give an incomplete reaction with DTNB even during prolonged assay times,[37,38] which is due to the reaction equilibrium and steric or electrostatic constraints of the bulky DTNB molecule.[39–41] In addition, the absorbance of the yellow-colored product (TNB^{2-}) that is used to quantify the thiol slowly changes due to hydrolysis and autoxidation.[36] In the cs124-DTPA-Tb-Hg (II) system, cs124-DTPA-Hg^{2+} is a very stable complex (binding constant $K = 10^{26.4}$ M^{-1}); the fluorescence of cs124-DTPA-Tb is not dependent on time (stable for years, data not shown). The binding of thiol compounds with cs124-DTPA-Hg^{2+} is reversible. When an excess of simple thiol is presented, thiol groups in thiol compounds can be regenerated, which is useful for thiol groups in proteins, especially in enzymes.

4 Conclusions

In summary, we have developed a simple, sensitive, and selective detection system for biothiols. This detection system is based on the reversible switch of the fluorescence of terbium chelate cs124-DTPA-Tb caused by Hg^{2+} and thiol species. This detection system shows high sensitivity and selectivity for biothiols. The detection limit for GSH, Hcy, and Cys was 80, 100, and 400 nM, respectively. Other amino acids tested showed no interference in the detection of thiol species. Compared with the reported fluorescent methods for biothiols detection, the main advantage of the cs124-DTPA-Tb-Hg (II) system is that the lanthanide fluorescent probe can eliminate interference from various nonspecific fluorescent sources, especially background fluorescence from biological matrices. The detection system was successfully applied to the detection of biothiols in cancer cells and urine. There were no significant differences between this method and the classical DTNB method. The method based on the terbium chelate probes provides an alternative method for monitoring the levels of biothiols. We believe that this method has the potential to serve as a diagnostic tool in diseases with abnormal levels of biothiols.

Acknowledgments

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References

1. S. Deneke, "Thiol-based antioxidants," *Curr. Top. Cell. Regul.* **36**, 151–180 (2001).
2. Z. Wood, E. Schröder, J. Robin Harris, and L. Poole, "Structure, mechanism and regulation of peroxiredoxins," *Trends Biochem. Sci.* **28**(1), 32–40 (2003).
3. I. Rahman and W. MacNee, "Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches," *Free Radic. Biol. Med.* **28**(9), 1405–1420 (2000).
4. J. Schulz et al., "Glutathione, oxidative stress and neurodegeneration," *Eur. J. Biochem.* **267**(16), 4904–4911 (2000).
5. S. Shahrokhian, "Lead phthalocyanine as a selective carrier for preparation of a cysteine-selective electrode," *Anal. Chem.* **73**(24), 5972–5978 (2001).
6. H. Refsum et al., "Homocysteine and cardiovascular disease," *Annu. Rev. Med.* **49**(1), 31–62 (1998).
7. S. Seshadri et al., "Plasma homocysteine as a risk factor for dementia and Alzheimer's disease," *New Engl. J. Med.* **346**(7), 476–483 (2002).
8. A. Ivanov, I. Nazimov, and L. Baratova, "Qualitative and quantitative determination of biologically active low-molecular-mass thiols in human blood by reversed-phase high-performance liquid chromatography with photometry and fluorescence detection," *J. Chromatogr. A* **870**(1–2), 433–442 (2000).
9. E. Kaniowska et al., "Urinary excretion measurement of cysteine and homocysteine in the form of their S-pyridinium derivatives by high-performance liquid chromatography with ultraviolet detection," *J. Chromatogr. A* **798**(1–2), 27–35 (1998).
10. D. Cole, D. Lehotay, and J. Evrovski, "Simplified simultaneous assay of total plasma homocysteine and methionine by HPLC and pulsed integrated amperometry," *Clin. Chem.* **44**(1), 188–190 (1998).
11. C. Chassaing et al., "Determination of reduced and oxidized homocysteine and related thiols in plasma by thiol-specific pre-column derivatization and capillary electrophoresis with laser-induced fluorescence detection," *J. Chromatogr. B* **735**(2), 219–227 (1999).
12. V. Ducros et al., "Gas chromatographic-mass spectrometric determination of total homocysteine in human plasma by stable isotope dilution: method and clinical applications," *J. Chromatog. B* **729**(1–2), 333–339 (1999).
13. M. Rafii et al., "High-throughput and simultaneous measurement of homocysteine and cysteine in human plasma and urine by liquid chromatography-electrospray tandem mass spectrometry," *Anal. Biochem.* **371**(1), 71–81 (2007).
14. R. Zhang et al., "Turn-on luminescent probe for cysteine/homocysteine based on a ruthenium(II) complex," *Inorg. Chem.* **49**(17), 7898–7903 (2010).
15. X. Chen et al., "A thiol-specific fluorescent probe and its application for bioimaging," *Chem. Commun.* **46**(16), 2751–2753 (2010).
16. Y. Zhang, Y. Li, and X. Yan, "Photoactivated CdTe/CdSe quantum dots as a near infrared fluorescent probe for detecting biothiols in biological fluids," *Anal. Chem.* **81**(12), 5001–5007 (2009).
17. B. Han, J. Yuan, and E. Wang, "Sensitive and selective sensor for biothiols in the cell based on the recovered fluorescence of the CdTe quantum dots-Hg (II) system," *Anal. Chem.* **81**(13), 5569–5573 (2009).
18. Y. Huang and H. Chang, "Nile red-adsorbed gold nanoparticle matrixes for determining aminothiols through surface-assisted laser desorption/ionization mass spectrometry," *Anal. Chem.* **78**(5), 1485–1493 (2006).
19. J. Lin, C. Chang, and W. Tseng, "Fluorescent sensing of homocysteine in urine: Using fluorosurfactant-capped gold nanoparticles and o-Phthaldialdehyde," *Analyst* **135**(1), 104–110 (2010).
20. M. Bruchez, Jr. et al., "Semiconductor nanocrystals as fluorescent biological labels," *Science* **281**(5385), 2013–2016 (1998).
21. U. Resch-Genger et al., "Quantum dots versus organic dyes as fluorescent labels," *Nat. Methods* **5**(9), 763–775 (2008).
22. E. Diamandis, "Immunoassays with time-resolved fluorescence spectroscopy: principles and applications," *Clin. Biochem.* **21**(3), 139–150 (1988).
23. Y. Wu, W. Zheng, and J. Qu, "Sensing cell metabolism by time-resolved autofluorescence," *Opt. Lett.* **31**(21), 3122–3124 (2006).
24. M. Li and P. Selvin, "Luminescent polyaminocarboxylate chelates of terbium and europium: the effect of chelate structure," *J. Am. Chem. Soc.* **117**(31), 8132–8138 (1995).
25. M. Montalti et al., "A luminescent anion sensor based on a europium hybrid complex," *J. Am. Chem. Soc.* **123**(50), 12694–12695 (2001).
26. Y. Chen et al., "Sensitized luminescent terbium nanoparticles: Preparation and time-resolved fluorescence assay for DNA," *Anal. Chem.* **79**(3), 960–965 (2007).
27. M. Schferling and O. Wolfbeis, "Europium tetracycline as a luminescent probe for nucleoside phosphates and its application to the determination of kinase activity," *Chem. Eur. J.* **13**(15), 4342–4349 (2007).
28. K. Sokolov, G. Chumanov, and T. M. Cotton, "Enhancement of molecular fluorescence near the surface of colloidal metal films," *Anal. Chem.* **70**(18), 3898–3905 (1998).
29. C. Bridges and R. Zalups, "Molecular and ionic mimicry and the transport of toxic metals," *Toxicol. Appl. Pharmacol.* **204**(3), 274–308 (2005).
30. G. T. Hermanson, *Bioconjugate Techniques*, 2nd ed., Academic Press, San Diego, CA (2008).
31. C.-C. Huang and H.-T. Chang, "Selective gold-nanoparticle-based "turn-on" fluorescent sensors for detection of mercury(II) in aqueous solution," *Anal. Chem.* **78**(24), 8332–8338 (2006).
32. J. Richie, Jr. et al., "Blood glutathione concentrations in a large-scale human study," *Clin. Chem.* **42**(1), 64–70 (1996).
33. R. Paroni et al., "HPLC with o-phthalaldehyde precolumn derivatization to measure total, oxidized, and protein-bound glutathione in blood, plasma, and tissue," *Clin. Chem.* **41**(3), 448–454 (1995).
34. A. Pastore et al., "Fully automated assay for total homocysteine, cysteine, cysteinylglycine, glutathione, cysteamine, and 2-mercaptopropionyl-
glycine in plasma and urine," *Clin. Chem.* **44**(4), 825–832 (1998).
35. Y. Wang, F. Yang, and X. Yang, "Colorimetric biosensing of mercury(II) ion using unmodified gold nanoparticle probes and thrombin-binding aptamer," *Biosens. Bioelectron.* **25**(8), 1994–1998 (2010).
36. P. C. Jocelyn, "Spectrophotometric assay of thiols," *Methods Enzymol.* **143**, 44–67 (1987).
37. H. Faulstich, P. Tews, and D. Heintz, "Determination and derivatization of protein thiols by n-octyldithionitrobenzoic acid," *Anal. Biochem.* **208**(2), 357–362 (1993).
38. S. K. Wright and R. E. Viola, "Evaluation of methods for the quantitation of cysteines in proteins," *Anal. Biochem.* **265**(1), 8–14 (1998).
39. C. Riener, G. Kada, and H. Gruber, "Quick measurement of protein sulfhydryls with Ellman's reagent and with 4,4'-dithiodipyridine," *Anal. Bioanal. Chem.* **373**(4), 266–276 (2002).
40. M. K. Han, S. Roseman, and L. Brand, "Sugar transport by the bacterial phosphotransferase system. Characterization of the sulfhydryl groups and site-specific labeling of enzyme I," *J. Biol. Chem.* **265**(4), 1985–1995 (1990).
41. J. M. Wilson et al., "A spectrophotometric method for studying the rates of reaction of disulfides with protein thiol groups applied to bovine serum albumin," *J. Am. Chem. Soc.* **102**(1), 359–363 (1980).