Talanta 143 (2015) 294–301

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# A reversible fluorescence chemosensor for sequentially quantitative monitoring copper and sulfide in living cells

Qingtao Meng [a,*], Run Zhang [b], Hongmin Jia [a], Xue Gao [a], Cuiping Wang [a], Yu Shi [b], Arun V. Everest-Dass [b], Zhiqiang Zhang [a,*]

[a] School of Chemical Engineering, University of Science and Technology Liaoning, Anshan 114044, China
[b] Department of Chemistry and Biomolecular Sciences, Faculty of Science and Engineering, Macquarie University, Sydney, NSW 2109, Australia

ARTICLE INFO

*Article history:*
Received 13 January 2015
Received in revised form
21 April 2015
Accepted 23 April 2015
Available online 2 May 2015

*Keywords:*
Fluorescein
Dual-functional chemosensor
Detection
Fluorescence imaging
Quantification

ABSTRACT

We report a novel, selective and sensitive strategy for the sequentially "ON–OFF–ON" fluorescent detection of $Cu^{2+}$ and $S^{2-}$ based on a fluorescein derivative, FL. The specific binding of FL towards $Cu^{2+}$ in aqueous and biological media led to the intensive green fluorescence quenching and a notable increase of the absorbance maximum at 480 nm. In the presence of $S^{2-}$, the intensity and overall pattern of the fluorescence emission and UV–vis spectra of FL–$Cu^{2+}$ ensemble were recovered since the abolishment of paramagnetic $Cu^{2+}$. This displacement approach exhibited highly specificity, and sensitivity with detection limits of 3 nM for $Cu^{2+}$ and 150 nM for $S^{2-}$. The fluorescence "ON–OFF–ON" circle can be repeated to a minimum of 5 times by the alternative addition of $Cu^{2+}$ and $S^{2-}$, implying that FL is a renewable dual-functional chemosensor. The biocompatibility of FL toward breast carcinoma cells, MDA-MB-231 was confirmed by MTT assay. The reversible "ON–OFF–ON" fluorescent response of FL to $Cu^{2+}$ and $S^{2-}$ in living system was further confirmed by confocal fluorescence imaging of living cells. The quantification of $Cu^{2+}$ and $S^{2-}$ in single intact cell was realized by the flow cytometry analysis.



## 1. Introduction

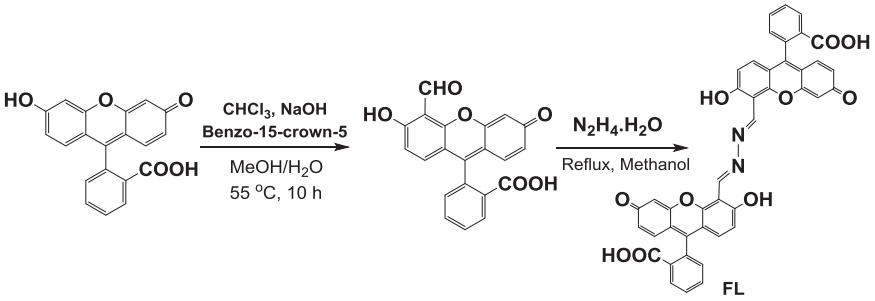
Bioactive ions as well as molecules play fundamental roles in a wide spectrum of biological processes, such as intra/extracellular communications, enzyme-involved redox reaction, apoptosis, *etc.* [1–7]. Keeping the integrity and balance of these species would be of vital importance for physiological and pathological processes. Among these species, acts as a component of cuproenzymes, copper (Cu) is an essential trace element which ranks the third in abundance in the human body [8]. Disturbance in the cellular homeostasis of copper ions may cause cell death and neurodegenerative disease, such as Wilson's disease, Alzheimer's disease, Parkinson's disease, *etc.* [9–18], probably since its involvement in the production of reactive oxygen species. Sulfide ($S^{2-}$) has long been known as a toxic species generated not only as a byproduct in industrial processes but also in biosystems due to microbial reduction of sulfate by anaerobic bacteria and formation of sulfur-containing amino acids in meat proteins [19–24]. Exposure to high level of $S^{2-}$ can produce various physiological and biochemical problems including irritation in mucous membranes, unconsciousness and respiratory paralysis. Furthermore, the protonated forms $HS^-$ or $H_2S$ are more toxic than the $S^{2-}$ itself [19,23,25]. Therefore, a rapid and sensitive method for immediate copper and sulfide monitoring in aqueous media and in biological systems is highly essential.

Owing to the advantages of highly sensitivity, specificity, simplicity, and the potential application in clinical biochemistry and environment, considerable efforts have been devoted to develop $Cu^{2+}$ or $S^{2-}$ chemosensors in the past few years [9,26–37]. Nevertheless, the development of fluorescent chemosensor capable of sequential recognition and sensing both of $Cu^{2+}$ and $S^{2-}$ is still one of most challenge [38,39], especially quantification in living systems [38]. Recently, metal-based receptors have received considerable attention in the area of anion recognition [40–42], since they show a greater enhancement in anion-binding affinity than purely organic receptors. These types of anion sensors usually employ the displacement method [21,35,37,32]. It is well known that $Cu^{2+}$ is a fluorescence quencher due to its notorious paramagnetic nature [26,39]. Interestingly, the resultant fluorescence sluggish $Cu^{2+}$–fluorophore complex have potential applicability as a promising $S^{2-}$-selective fluorescence turn-on sensor *via* $S^{2-}$ induced $Cu^{2+}$ displacement approach forming very stable CuS ($K_{sp}=6.3\times10^{-36}$) species [28,37].

With this in mind, we propose a novel fluorescein–aldazine based fluorescent chemosensor FL for the reversible detection $Cu^{2+}$ and

* Corresponding author.
*E-mail addresses:* qtmeng@ustl.edu.cn (Q. Meng), zhangzhiqiang@ustl.edu.cn (Z. Zhang).

http://dx.doi.org/10.1016/j.talanta.2015.04.072
0039-9140/




**Scheme 1.** The synthetic route of FL.

$S^{2-}$ both in aqueous solution and living cells in this contribution (Scheme 1). It uses a fluorescein dyes as the fluorophore and aldazine salicylaldehyde-based as the $Cu^{2+}$ binding site [43–47]. FL exhibited obvious fluorescent quenching and adsorption increasing after binding to $Cu^{2+}$ to form FL–$Cu^{2+}$ ensemble. The new complex can specifically interact with $S^{2-}$ in aqueous to liberate the fluorophore, FL, accompanied by a remarkable recovery of fluorescence and adsorption. The photophysical properties and sequential recognition behaviors of FL to $Cu^{2+}$ and FL–$Cu^{2+}$ to $S^{2-}$ were investigated in detail through fluorescence spectrum and UV–vis absorption spectrum. The cytotoxicity of FL toward breast carcinoma cells, MDA-MB-231 was examined using a MTT assay. The extraordinary analytical performance of the proposed chemosensor, including high sensitivity, specificity, good biocompatibility enable the investigation of reversible fluorescent response of FL to $Cu^{2+}$ and $S^{2-}$ in living cells by confocal microscope. Quantitative monitoring of intracellular $Cu^{2+}$ and $S^{2-}$ was achieved by the flow cytometry analysis.

## 2. Experiment

### 2.1. Reagents and instruments

All reagents and solvents were of analytical reagent grade and used without further purification unless otherwise noted. Fluorescein, and hydrazine hydrate were purchased from Sinopharm Chemical Reagent Co., Ltd. (China); benzo-15-crown-5 was purchased from Aladdin. Fresh stock solution of metal ions (nitrate salts, 20 mM) and anions (sodium salts, 20 mM) in $H_2O$ were prepared for further experiments. $^1$H NMR and $^{13}$C NMR spectra were recorded with an AVANCE500MHZ spectrometer (BRUKER) with chemical shifts reported as ppm (in DMSO, TMS as internal standard). ESI mass spectra (ESI-MS) were recorded on an Agilent 1100 MSD mass spectrometer. The elemental analyses of C, H, N and O were performed on a Vario EL III elemental analyzer. Fluorescence spectra were determined with LS 55 luminescence spectrometer (Perkin Elmer, USA). The absorption spectra were measured with a Lambda 900 UV/VIS/NIR spectrophotometer (Perkin Elmer, USA). Fluorescent live cell images were acquired on a Olympus Fluoview FV 1000 IX81 inverted confocal laser-scanning microscope with an objective lens (40 ×). The excitation wavelength was 473 nm for imaging of FL, and 405 nm for imaging DAPI. The relative fluorescence intensities of images were analyzed by using Image J software version 1.44p. Flow cytometric analysis was recorded on a BD FACSAria II flow cytometer with a 488 nm laser. The data were analyzed with flowing software.

### 2.2. Synthesis and characterization the fluorescent chemosensor (FL)

To a solution of 4-fluoresceincarboxaldehyde [48] (0.360 g, 1 mmol) in 15 mL methanol, hydrazine (0.5 equiv.) in 15 mL methanol was added slowly at room temperature [49]. The stirred reaction mixture was heated to reflux for 7 h. The solution color changed from pale to yellow. The reaction mixture was cooled to room temperature. The precipitate was washed with ethanol and dried under vacuum to obtain FL in 90% yield. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$(ppm): 12.376 (1H, s), 10.239 (1H, s), 9.721 (1H, s), 8.035 (*d*, J=7.5 Hz, 1H), 7.829 (*t*, *J*=7.25 Hz, 1H), 7.750 (*t*, *J*=7.5 Hz, 1H), 7.356 (*d*, *J*=6.5 Hz, 1H), 6.990 (1H, s), 6.790–6.847 (2H, dd), 6.600–6.640 (2H, dd), $^{13}$C NMR (DMSO-$d_6$, 125 MHz) $\delta$(ppm): 169.97, 162.59, 161.61, 161.01, 153.64, 153.62, 152,65, 152.12, 137.14. 134.45, 131.68, 130.27, 127.44, 126.15, 125.48, 125.45, 114.74, 114.67, 111.21, 110.74, 106.89, 104.27, 83.72, and 57.42. ESI-mass spectra (positive mode, *m*/*z*): Calcd for $C_{42}H_{24}N_2O_{10}$: 716.1 [FL]; Found: 717.3 $[FL+H^+]^+$. Elem Anal: Calcd for FL: C 70.390, H 3.376, N 3.909, O 22.325. Found: C 70.135, H 3.437, N 3.892, and O 22.429.

### 2.3. General procedures of spectra detection

Stock solutions of FL was prepared in HEPES aqueous buffer (DMSO: $H_2O$=3:7, 20 mM, pH=7.4). Excitation wavelength for FL was 470 nm. Before spectroscopic measurements, the solution was freshly prepared by diluting the high concentration stock solution to corresponding solution (10 μM). Each time a 3 mL solution of chenosensor was filled in a quartz cell of 1 cm optical path length, and different stock solutions of cations were added into the quartz cell gradually by using a micro-syringe. FL–$Cu^{2+}$ solution for $S^{2-}$ detection was prepared by the addition of 3.0 equiv. of $Cu^{2+}$ to FL (10 μM) solution in HEPES buffer (DMSO: $H_2O$=3:7, 20 mM, pH=7.4).

### 2.4. Quantum yield measurement

Fluorescence quantum yield was determined using optically matching solutions of fluorescein ($\Phi_f$=0.85 in 0.1 N NaOH aqueous solutions) as standard at an excitation wavelength of 470 nm and the quantum yield is calculated using the following equation [50].

$$\Phi_{unk} = \Phi_{std} \frac{\left(F_{unk}/A_{unk}\right)}{\left(F_{std}/A_{std}\right)} \left(\frac{\eta_{unk}}{\eta_{std}}\right)^2 \quad (1)$$

where $\Phi_{unk}$ and $\Phi_{std}$ are the radiative quantum yields of the sample and standard, $F_{unk}$ and $F_{std}$ are the integrated emission intensities of the corrected spectra for the sample and standard, $A_{unk}$ and $A_{std}$ are the absorbances of the sample and standard at the excitation wavelength, and $\eta_{unk}$ and $\eta_{std}$ are the indices of refraction of the sample and standard solutions, respectively. Excitation and emission slit widths were modified to adjust the luminescent intensity in a suitable range. All the spectroscopic measurements were performed in triplicate and averaged.

### 2.5. Association constant calculation

Generally, for the formation of 1:1 complexation species formed by the chemosensor compound and the guest cations, the