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A regenerated "turn on" fluorescent probe for sulfide detection in live cells and read samples based on dihydroxyhemicyanine-Cu^{2+} dye

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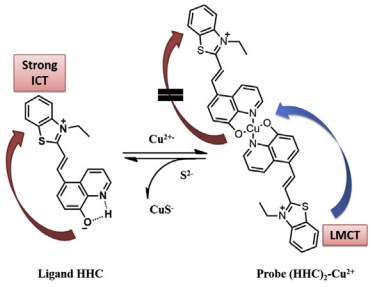
HIGHLIGHTS

- The probe showed excellent selectivity for S^{2-} over various.
- Probe could be regenerated and reversibly detected to S^{2-} and Cu^{2+}.
- Probe can be applied to the quantitative and qualitative determination of S^{2-}.
- Probe has cell permeability and can be used to monitor S^{2-} level in cell imaging.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel "turn-on" fluorescence probe **$(HHC)_2$-Cu^{2+}** for sulfide (S^{2-}) was synthesized and characterized based on dihydroxyhemicyanine-Cu^{2+} complex. The probe displayed high sensitivity and selectivity for S^{2-} over other analytes, including biothiols, NaCN, reactive oxygen species (ROS) and reactive nitrogen species (RNS) in aqueous solution, which was attributed to the large association constant and the fast kinetics of precipitation of CuS. The probe **$(HHC)_2$-Cu^{2+}** can be regenerated and reversibly detected with S^{2-} with a remarkable red-fluorescent change by alternating the addition of S^{2-} and Cu^{2+}. Fluorescent spectra of **$(HHC)_2$-Cu^{2+}** toward S^{2-} showed a high selectivity, a good linearity, a low limit of detection at 0.12 μM, a rapid response time (less than 30 s), and a wide pH range of 7–10. Importantly, the probe was successfully applied to detect the low level of S^{2-} in waste water samples, corrupt blood samples, and living cells.

1. Introduction

Sulfide (S^{2-}) toxicity is well-known and has gained much attention given the strong reductant properties and the direct effects S^{2-} has to human health. Low sulfide concentrations are extremely important for body processes, such as vasorelaxation, hepatic circulation, diminish inflammation, cell angiogenesis, and memory formation. Furthermore, abnormal behavior in the levels of cellular sulfide have been correlated with a number of diseases (e.g., diabetes, liver cirrhosis, Down's syndrome, and Alzheimer's disease [1]). Sulfide is widely used in leather making, battery manufacturing, water treatment, paper making, mineral processing production, organic intermediates, printing and dyeing, and pharmaceuticals [2]. Sulfide anion widely exists in the waste water; once in the water medium, this anion will rapidly hydrolyze to dihydrogen sulfide (H_2S) or hydrogen sulfide (HS^-), which is a more toxic substance [3–5]. Therefore, developing efficient methods for the detection of S^{2-} in complex conditions is important.

Various kinds of testing techniques have been studied for S^{2-};

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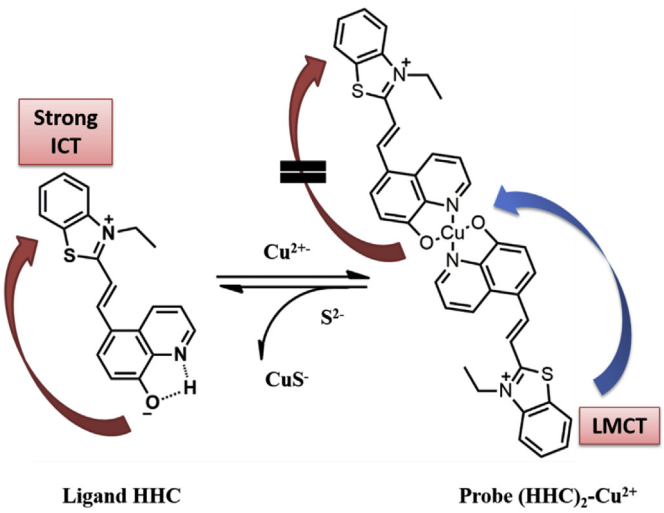
these techniques include titration [6], inductively coupled plasma atomic emission spectroscopy [7,8], hydride generation atomic fluorescence spectrometry [9], electrochemical methods [10], ion chromatography [11], spectrophotometry [12,13], fluorimetry [14–17], and chemosensory methods [18,19]. In the methods of these reports, the chemosensor has gained wide attention because of the simple operation, rapid response, and high sensitivity and selectivity. Recently, the fluorescence probe for Na_2S has been rapidly developed in environmental monitoring and biology. These probes are mainly composed of two sensing mechanisms. On one hand, using the reduction ability and double nucleophilic character of sulfides, we find an irreversible chemical reaction between probe and sulfide as a "reactive probe" [20–38]; on the other hand, we use the CuS affinity between cupric ion and sulfide as "competitive probes" [39–48]. Although the "reactive probes" have been applied to several fields, most of the probes were applied in the organic solvent with slow response, weak selectivity, and low sensitivity. The development of "reactive probe" has been limited [43]. Nevertheless, the "competitive probes," based on the fluorescent probes of the copper complex, has shown obvious characteristics by using the high affinity between Cu^{2+} and S^{2-} (CuS: $K_{sp} = 6.3 \times 10^{-36}$) [49]. Wang et al. have reported a novel peptide-based fluorescence chemosensor L-Cu for hydrogen sulfide, and the L-Cu showed rapid response, high selectivity, remarkable sensitivity, low toxicity, and eminent cell permeation [50]. However, the interference of other analytes, which include biothiols, is not taken into account; Zhao et al. have reported Cu^{2+}-complex-based fluorescent probes for selective detection of S^{2-} with reversible property in acetonitriles [51]. However, this work has done the CH_3CN or the mixed CH_3OH–CH_3CN solution, which limits further development for this probe in the environmentally and biologically. For these problems, this text built a novel "turn-on" fluorescence probe **(HHC)$_2$-Cu^{2+}** for S^{2-} based on Cu^{2+}–hydroxyhemicyanine complex, which has shown a large Stoke's shift (red fluorescence), highly selectivity and anti-interference, remarkable sensing properties and application of the real samples and live cells in the aqueous medium.

In this study, we report a Cu^{2+}–hydroxyhemicyanine complex-based fluorescence probe **(HHC)$_2$-Cu^{2+}** using "competitive probe" (Scheme 1). The uniqueness and advantage of this work over the previous related work is as followed: (1) We chose 8-hydroxyquinoline unit as the chelating group of **Cu^{2+}**, because the two molecules of 8-hydroxyquinoline for **Cu^{2+}** can form a stable complex, which has a bigger association constant than other kinds of **Cu^{2+}** complexes, and it may eliminate interference from biothiol, NaCN, reactive oxygen species (ROS) and reactive nitrogen species (RNS); (2) the introduction of *N*-ethyl-benzothiazole quaternary ammonium salt was designed to increase the water solubility and cell permeability of the probe; (3) the ligand **HHC** belongs to the D-π-A structure type with connecting 8-hydroxyquinoline unit as an electron donor (D) and *N*-ethyl-benzothiazole quaternary ammonium salt as an electron acceptor (A) that constructs an intramolecular charge transfer (ICT) system with a large Stokes shift. As expected, the probe **(HHC)$_2$-Cu^{2+}** can be used for high-efficiency, rapid response (less than 30 s), and high selectivity over other analytes, including NaCN, biothiols, ROS and RNS, for the detection of sulfides in PBS buffer (10 mM, pH 7.4, 2% DMSO). Furthermore, this probe **(HHC)$_2$-Cu^{2+}** was also successfully applied to real samples (tap water, drinking water, waste water and blood samples) and cell imaging. Comparison with "competitive probes" based on 8-hydroxyquinoline-Cu^{2+} complex, which shown significant performance over previous work (Table S1).



Scheme 1. The structure of probe **(HHC)$_2$-Cu^{2+}** and its corresponding reversible sensing mechanism.

2. Experimental section

2.1. Synthesis of probe *(HHC)$_2$-Cu^{2+}*

The compound **HHC**, (E)-3-ethyl-2-(2-(8-hydroxyquinolin-5-yl) vinyl) benzo [d] thiazol-3-ium iodide was prepared and characterized as the previously reported [52]. The synthesis and characterization of probe **(HHC)$_2$-Cu^{2+}**referred to previous literature [53–59]. A portion of **HHC** (50 mg, 0.15 mmol) and $CuSO_4$ (13 mg, 0.08 mmol) were separately dissolved in methanol and water, which combined together to obtain a purple color solution. The solution was stirred for 30 min at room temperatures; the precipitate was filtered off and washed with methanol-water ($v/v = 1:1$). The crude product was recrystallized from methanol–water ($v/v = 1:1$) to yield the rufous solid of **(HHC)$_2$-Cu^{2+}** complex (45.8 mg, 84%). ESI-Ms: *m/z* (%) of 760.92 [2**HHC** + Cu^{2+}+MeOH-3H]$^+$ and 363.40 [2**HHC** + Cu^{2+}-2H]$^{2+}$/2.

2.2. General optical measurement

Stock solutions of compound **HHC** (5×10^{-4} mol L^{-1}) and **(HHC)$_2$-Cu^{2+}** (5×10^{-4} mol L^{-1}) were prepared in DMSO; the stock solutions of various analytes (1.0×10^{-3} mol L^{-1}) were prepared in water. Test solutions at a concentration of 1.0×10^{-5} mol L^{-1} were prepared by diluting stock solutions in PBS buffer (10 mM, pH 7.4, 2% DMSO). The absorption and fluorescence spectra were scanned from 200 mm to 700 nm and from 550 nm to 800 nm, respectively. For titration experiments, the solutions of S^{2-}were added to the 5 μM host solution of **(HHC)$_2$-Cu^{2+}** (2 mL) in portions (total volumes of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, and 50 μL of 1×10^{-3} mol/L). For fluorescence measurements, the excitation was at 535 nm, and emission was at 592 nm. The slit sizes for excitation and emission were 5 and 10 nm, respectively.

2.3. Cytotoxicity assay

According to a previous methods [60], the cytotoxicity of **(HHC)$_2$-Cu^{2+}** was evaluated at different concentrations (0, 1, 2.5, 5, and 10 μM) by using a standard MTT assay.

2.4. Cell imaging experiment

Cell imaging was observed by using a fluorescence microscope. For fluorescence microscopy experiments, HeLa cells were