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Reversible fluorescent detection for sulfide with quinoline-ligated copper complexes and its application in living cells

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ABSTRACT

To realized highly selective detection for sulfide anion, a simple and hydrophilic fluorescent chemosensor, sodium 2-(2-(1H-benzo[d]imidazol-2-yl)quinolin-8-yloxy)acetate (HL) based on quinoline derivative was designed and synthesized. Once combined with Cu^{2+} in aqueous solution, the forming quinoline-ligated copper complex displayed a rapid response with high selective and sensitive detection for sulfide anion. The fluorescent signal transduction occurs via formation-separation of complex (CuL) and CuS, which achieve reversible "ON–OFF–ON" fluorescence change, and the complex (CuL) through the combination of HL and Cu^{2+} was further confirmed by crystal structure. The detection limits of complex (CuL) to sulfide anion was estimated to be 1.7×10^{-7} M. Furthermore, its potential utility for biological application was explored by fluorescence microscopic imaging in live cells.

1. Introduction

Designing a molecular sensor for selective recognition of different species including cations, anions, amino acids, or small neutral molecules is an important task for scientific researchers [1,2]. In recent years, the detection of anions gains more and more concerns, even though the development of selective chemosensors for the detection of cation has attracted more interest relative to investigated anions [3–7]. As one of the biologically and environmentally important anions, sulfide anions are widely generated as a byproduct in industrial processes, for instance, conversion into sulfur, preparation of sulfuric acid and dyes, cosmetic manufacturing, production of wood pulp, etc. [8–10]. In addition, it is produced by microbial reduction of sulfate by anaerobic bacteria or formed from the sulfur-containing amino acids in meat proteins [11–13]. Therefore, sulfide anions can irritate mucous membranes by ingestion of food or water contaminated with sulfide and even cause unconsciousness and respiratory paralysis [14,15]. What's more, once sulfide anions is protonated, it even becomes H_2S, which is toxic and serves as the third endogenous gaseous transmitter, following nitric oxide (NO) and carbon monoxide (CO) [15,16]. So, it is interesting to design selective method for immediately monitoring sulfide anion in aqueous media and in the biological system.

Compared with the traditionally developed detection techniques for sulfide anion, such as titration [17,18], electrochemical methods [19], fluorimetry [20–22] and inductively coupled plasma-atomic emission spectroscopy [23], fluorescent chemosensors present many advantages, such as high sensitivity and selectivity, low cost, easy operation. The most significant benefit of using the fluorescent probes is the ability to monitor intracellular analytes [23,24]. However, the applications of bioimaging studies or real samples detection of fluorescent chemosensors for sulfide anion have been rarely reported [25], and even the reported methods have problems such as poor solubility in aqueous media [26,27,9], and lack reversibility [28–30]. The design of fluorescent sensors for sulfide anions in aqueous solution is a challenging task owing to the strong hydration of anions, which weakens the interactions of the sensors with the target anions [27,31,32]. But the displacement method can be use to tackle this hurdle [22,33].

According to the displacement method, herein we report a new chemosensor, sodium 2-(2-(1H-benzo[d]imidazol-2-yl)quinolin-8-yloxy)acetate (**HL**). To improve solubility of chemosensor in aqueous media, sodium acetate group was introduced into 8-hydroxy-2-quinoline successfully. **HL** exhibits excellent optical properties and forms complex CuL combine with Cu^{2+}, which displays fluorescence ON–OFF behavior. CuL displays high sensitivity and selectivity for S^{2-} to show fluorescence OFF-ON behavior on

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the basis of forming CuS. Meanwhile, **CuL** displays rapid response (<1 min), well reversibility, lowest detection limit (1.7×10^{-7} M), and high sensitivity for recognizing S^{2-} in 100% aqueous solution. Furthermore, its potential utility for biological applications was confirmed by fluorescence imaging of S^{2-} in live cells.

2. Experimental

2.1. General information and materials

All of the materials for synthesis were purchased from commercial suppliers and used without further purification. All of the solvents used were of analytical reagent grade. ^{1}H NMR (300 MHz), ^{13}C NMR (150 MHz) spectra were measured with TMS as an internal standard. Fourier transform infrared (FTIR) spectra of the materials were conducted within the 4000–400 cm^{-1} wavenumber range using a Nicolet 360 FTIR spectrometer with the KBr pellet technique.Absorption spectra were determined on a Varian UV-Cary100 spectrophotometer. Fluorescence spectra measurements were performed on a Hitachi F-7000 spectrofluorimeter. All pH measurements were made with a pH-10C digital pH meter. Electrospray ionization MS (ESI–MS) spectra were determined on a Bruker Daltonics Esquire 6000 spectrometer.Quantum yields were determined by an absolute method using an integrating sphere on Edinburgh Instrument FLS920.single crystal X ray diffraction measurements were carried out on a Bruker SMART 1000 CCD diffractometer equipped with Mo-Kα radiation (λ = 0.71073 Å).

2.2. Synthesis of compound *HL*

Sodium 2-(2-(1H-benzo[d]imidazol-2-yl)quinolin-8-yloxy) acetate (**HL**) was prepared from 8-hydroxy-2-methylquinoline according to the literature[35]. The new compound was fully characterized by NMR, ESI–mass spectroscopy. ^{1}H NMR (D_2O, 300 MHz,TMS) δ (ppm) 7.84 (d, J = 8.7 Hz, 1H), 7.48 (d, J = 8.6 Hz, 1H), 7.42–7.32 (m, 2H), 7.22–7.05 (m, 4H), 6.55 (d, J = 6.1 Hz, 1H), 4.28 (s, 2H). ^{13}C NMR (CD_3OD, 150 MHz, TMS): δ (ppm) 175.99, 154.66, 152.17, 147.51, 140.30,138.64, 130.67, 129.00, 124.51, 120.16, 116.37, 111.08, 111.01, 68.65. MS (ESI): m/z = 320.3 ($M-Na^+ + 1$).

2.3. Synthesis of the compound *CuL*

A solution of 0.5 mmol **HL** in water (10 mL) was added dropwise to a solution of 0.5 mmol $Cu(NO_3)_2$ in water (10 mL). Pale yellow precipitate appeared immediately, and then filtered the precipitated solid compound, washed with water, dried in vacuum. Yield: 80%. MS (ESI): m/z = 381.2 ($M-Cu^{2+} + 1$).

2.4. UV–vis and fluorescence spectral measurements

Stock solutions of various ions and HEPES-buffer (10 mM, pH 7.4) were prepared in deionized water. The solutions of metal ions were prepared from their perchlorate salts, and non-metal anions were prepared from their sodium or potassium salts. All the measurements were performed at room temperature unless otherwise stated. **HL** (1.7 mg, 0.05 mmol) and various ions were dissolved in 5 mL deionized water as a stock solution (1 mM) respectively. In titration experiments, each time a 10 μM solution of HL diluted with HEPES-buffer (10 mM, pH 7.4) was filled in a quartz optical cell of 2 cm optical path length, and the ions stock solutions were added into the quartz optical cell gradually by using a micropipet. Spectral data were recorded at 40 s after the addition of the ions. For fluorescence measurements, excitation was provided at 345 nm and emission was collected from 360 to 650 nm.

2.5. Cell culture

SMMC-7721 cells were provided by the Institute of Biochemistry and Cell Biology (China). The cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37 °C humidified air for about 24 h. Then the cells were treated with the relative compounds for fluorescence microscopic imaging. The fluorescence microscopy experiments were performed on a fluorescence microscope (Olympus BX53 fluorescence microscope) with the excitation between 340 and 380 nm.

2.6. Fluorescence microscopic imaging

The cultured cells were incubated with **HL** (10 μM) for 30 min in DMEM supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37 °C. Subsequently, the cells were treated with 15 μM $Cu(ClO_4)_2$. Then the cells were separated into two parts. One part of the cells for control test was washed with phosphate-buffered saline (PBS) three times in order to remove free compound and ions before analysis, then fluorescence microscopic images were acquired. 20 μM Na_2S was added to another part immediately and incubated for another 30 min, then washed with PBS three times for fluorescence microscopic images as well. The fluorescence microscopic imaging was observed with the excitation wavelength between 340 and 380 nm (UV band).

3. Results and discussion

3.1. Fluorescence spectroscopy of *HL* to Cu^{2+}

The fluorescence spectra were obtained by excitation of the fluorophore at 345 nm at room temperature in aqueous solution (HEPES-buffer, pH 7.4). In the absence of metal ions, **HL** exhibits a strong emission at 460 nm with a high quantum yield (Φ_f (1) = 0.32). Upon the addition of Cu^{2+} (2 equiv), the system exhibited significant quenching in the fluorescence intensity (Φ_f (2) = 0.09) in the maximum emission at 460 nm (Fig. 1a). The effective fluorescence quenching of **HL** was attributed to the coordination to a paramagnetic Cu^{2+} center [13]. We also confirmed that $Cu(NO_3)_2$, $Cu(AcO)_2$, $CuCl_2$ $Cu(ClO_4)_2$ and $CuSO_4$ possessed a similar fluorescence behavior (Fig. S1, Supporting information), indicating that these counteranions of Cu^{2+} did not affect the fluorescence changes of **CuL**. The linear relationship of the fluorescence titration and Job's plot showed that the stoichiometric ratio of **HL** with Cu^{2+} appeared to be 1:1 (Fig. S2, Supporting information), which was further conformed by Single-crystal X-ray diffraction data for CuL (Fig. 2). What's more, the association constant of **HL** for Cu^{2+} was 1.3×10^5 M^{-1} (Fig. S3, Supporting information).

3.2. Absorption spectroscopy of *HL* to Cu^{2+}

The coordination of **HL** with Cu^{2+} was also investigated by UV–vis spectrophotometric titration in aqueous solution (HEPES-buffer, pH 7.4) (Fig. 1b). The absorption spectrum of **HL** contains a broad envelope of intensity stretching from 220 to 450 nm. After binding with Cu^{2+}, the main absorption intensity of **CuL** gradually decreased, accompanied by a new red-shift with two well-defined peaks at 298 and 343 nm, implying different coordination sites between quinoline fluorophore and Cu^{2+} [13]. The saturated spectra were readily obtained when 1 equiv of Cu^{2+} was introduced, suggesting that ligand HL has strong affinity for Cu^{2+} and could form 1:1 complexation mode with the Cu^{2+} ion.