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Original article

A squaraine based fluorescent probe for mercury ion *via* coordination induced deaggregation signaling

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ABSTRACT

Due to the high affinity between dithiocarbamate (DTC) and Hg^{2+}, a fluorescent probe based on squaraine chromophore with DTC side arm for Hg^{2+} *via* coordination induced deaggregation signaling has been designed and synthesized. Squaraine has a high tendency to aggregate in aqueous solution, and such self-aggregation usually results in a dramatic absorption spectral broadening with fluorescence emission quenching. The combination of the DTC side arm of the probe with Hg^{2+} induces steric hindrance, leading to the deaggregation of the dye complex, companying with a fluorescence emission restoration. In EtOH–H_2O (20:80, v/v) solution, this "turn on" fluorescent probe has high selectivity and sensitivity toward Hg^{2+} over other metal ions, and the limit of detection for Hg^{2+} was estimated as 2.19×10^{-8} mol/L by $3\sigma/k$.

1. Introduction

Mercury ion is one of the most important metal toxins. The widespread pollution of mercury has resulted in a series of environmental and health issues. Mercury can be accumulated in the human body along the food chain and with age, that consequently leads to damaging the human central nervous system and other organs, further causes severe brain damage, cognitive problems as well as movement disorders, vision and hearing loss and even leading to death [1,2]. Although some quantitative techniques, such as cold vapor atomic absorption spectroscopy (CVAAS) [3] and inductively coupled plasma mass spectroscopy (ICP-MS) [4], are available for detection of mercury, there has been a great need to develop sensitive, real-time and inexpensive methods for the determination of trace levels of mercury. Fluorosensors are attractive choices for this purpose due to their distinct advantages in terms of sensitivity, selectivity, and response time, and their elimination of the need of expensive instruments, highly trained personnel, and tedious maintenance [5–10].

Squaraines are a class of extensively studied zwitterionic dyes possessing sharp and intense absorption and fluorescence in the red and near-infrared (NIR) region [11–14], and have been widely designed as NIR probes for the detection of thiol-containing amino acids [15,16], metal ions [17,18] and proteins [19]. Some analyte-specific chemosensors based on squaraines that act through coordination induced charge transfer [20–22], nucleophilic attack on the electron-deficient central squaraine ring [23–25], or ion driven self-aggregation [26–30] have been reported. However, most of the reported cation sensors provide "turn off" fluorescent signals. Since monitoring a fluorescent increase from a low level is typically more reliable than monitoring a decrease from a high emission level, especially when the fluorescent changes are small, it is an important challenge to design "turn on" fluorescent probes with squaraine chromophore *via* new approaches.

Squaraine dyes have a high tendency to aggregate in aqueous solution, and such self-aggregation usually results in a dramatic absorption spectral broadening with fluorescence emission quenching [31]. The combination of the side arm of the probe with metal ions induces steric hindrance, leading to the deaggregation of the dye aggregates, companying with a fluorescence emission restoration [32]. Inspired by this idea, we have synthesized a new fluorescent probe USQ by introducing DTC group into a squaraine skeleton. USQ exhibits sensitive response only toward Hg^{2+} (Scheme 1).

2. Experimental

Unless stated otherwise, all reagents were acquired from commercial sources and used without further purification. All

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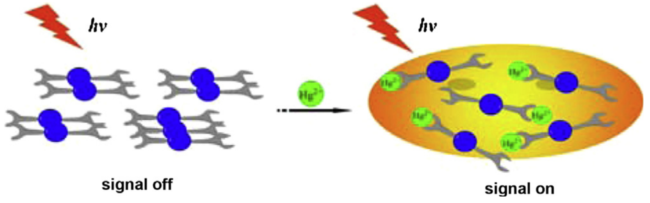


Scheme 1. Rational design of the squaraine based fluorescent probe for Hg^{2+} *via* coordination induced deaggregation signaling.

solvents were purified and redistilled according to standard methods prior to use. Melting points were determined with a SGW X-4 instrument without correction. FTIR were recorded on a Perking Elmer Spectrum 2000 Fourier Transform Infrared Spectrophotometer. The ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured on a Bruker AV-400 spectrometer (TMS as internal standard). Electrospray ionization mass spectra (ESI-MS) were performed on a DECAX-30000 LCQ Deca XP ion trap mass spectrometer. High resolution electrospray ionization mass spectra (HR-ESI-MS) were recorded on an Agilent 6520 Accurate-Mass Q-TOF LC/MS. Fluorescent emission spectra were collected using a Cary Eclipse fluorescence spectrophotometer. Absorption spectra were measured on a Perkin Elmer Lambda 750 UV-vis spectrophotometer.

Synthesis of **3**: *N*-Methyl-*N*-(2-chloroethyl)aniline (0.61 g, 3.6 mmol) and sodium diethyl dithiocarbamate (**2**) (0.90 g, 5.3 mmol) were dissolved in ethanol (50 mL), then a catalytic amount of potassium iodide was added. The stirred mixture was heated to reflux and the reaction was monitored by TLC. After the reaction mixture was filtered and the solvent was removed, the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate, 10:1, v/v) to give **3** as yellow oil (0.85 g), yield 86%. FTIR (KBr, cm^{-1}): ν_{max} 2975, 2931, 2871, 1599, 1505, 1487, 1416, 1351, 1269, 1204, 1141, 985, 915, 748, 692; ^1H NMR (400 MHz, $CDCl_3$): δ 1.28 (t, 6H, J = 7.0 Hz), 3.02 (s, 3H), 3.46–3.49 (m, 2H), 3.60–3.64 (m, 2H), 3.73 (q, 2H, J = 7.1 Hz), 4.04 (q, 2H, J = 6.9 Hz), 6.69 (t, 1H, J = 7.2 Hz), 6.79 (d, 2H, J = 8.0 Hz), 7.21–7.25 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.60, 12.48, 32.96, 38.12, 46.78, 49.61, 51.66, 112.14, 116.37, 129.21, 148.79, 195.03; ESI-MS: m/z 283.4 ($[M+H]^+$).

Synthesis of USQ: A mixture of semisquaric acid **4** (30 mg, 0.10 mmol) and **3** (28 mg, 0.10 mmol) were dissolved in 30 mL benzene/*n*-BuOH (1:1, v/v) in a 100 mL round bottom flask equipped with a Dean-Stark trap. The solution was refluxed under the protection of nitrogen for 12 h. After cooling, most of the solvent was first removed under reduced pressure, and then the blue crude product was purified by column chromatography over silica gel. The elution of the column with a mixture of methylene dichloride and ethyl acetate (40:1, v/v) was used to afford the desired green squaraine dye USQ (15 mg), yield 27%. Melting point: 196–200 °C. FTIR (KBr, cm^{-1}): ν_{max} 2957, 2930, 1608, 1585, 1396, 1384, 1332, 1172, 843, 780; ^1H NMR (400 MHz, $CDCl_3$): δ 0.99 (t, 6H, J = 7.2 Hz), 1.25–1.32 (m, 6H), 1.36–1.45 (m, 4H), 1.61–1.69 (m, 4H), 3.24 (s, 3H), 3.44 (t, 4H, J = 8.0 Hz), 3.51–3.56 (m, 2H), 3.72 (q, 2H, J = 7.2 Hz), 3.79–3.83 (m, 2H), 4.05 (q, 2H, J = 7.2 Hz), 6.74 (d, 2H, J = 9.2 Hz), 6.87 (d, 2H, J = 9.2 Hz), 8.39 (dd, 4H, J = 1.6, 9.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.58, 12.50, 13.86, 20.24, 29.62, 32.93, 38.61, 46.92, 49.84, 51.28, 112.40, 112.43, 119.56, 120.33, 132.94, 133.65, 153.84, 153.95, 183.41, 187.66, 189.26, 194.07; ESI-MS: m/z 566.4 ($[M+H]^+$); HR-ESI-MS: Calcd. for $C_{32}H_{44}N_3O_2S_2$ ($[M+H]^+$): 566.2875, Found: 566.2898.

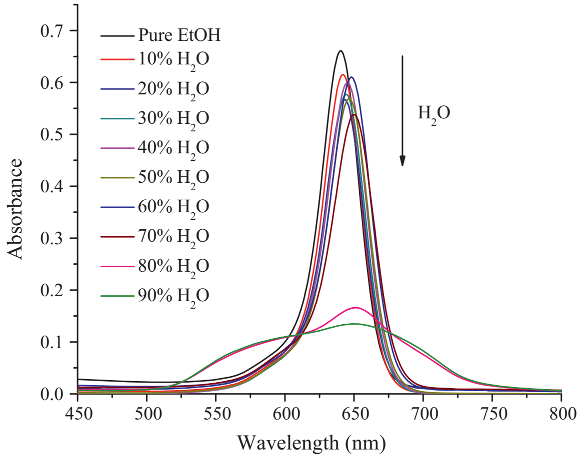
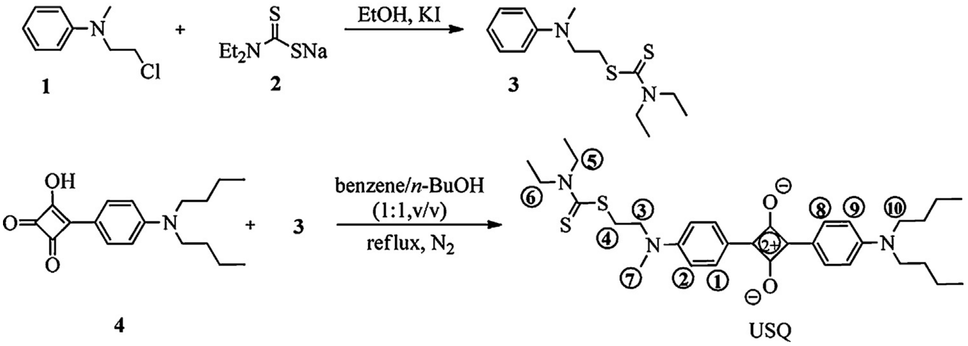


Fig. 1. Changes in absorbance of USQ (4.0 μmol/L) with increase of H_2O percentage (0–90%) in $EtOH–H_2O$ solutions.

3. Results and discussion

USQ was prepared through a two-step process as shown in Scheme 2. *N*-Methyl-*N*-(2-chloroethyl)aniline (1) and semisquaric acid **4** were obtained according to the literature [33,34]. The aniline derivative **3** was obtained by the treatment of sodium diethyl dithiocarbamate (2) with 1. Then a condensation reaction between compound **4** and **3** afforded the target product USQ. The new compounds were fully characterized by FTIR, ^1H NMR, ^{13}C NMR and MS (Figs. S1–S10 in Supporting information).

Squaraine dyes exhibit a high tendency to form aggregates in aqueous medium, which can significantly alter photophysical properties including a dramatic absorption change and fluorescence quenching. As shown in Fig. 1, USQ possess a strong absorption band around 640 nm in the aqueous EtOH solution with the water concentration from 0% to 70%. As the percentage of water increased to 80%, the monomer peak at around 640 nm almost disappeared and a broad band belonging to aggregates emerged.



Scheme 2. Synthesis of USQ sensor.