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A coordination driven deaggregation approach toward Hg^{2+}-specific chemosensors based on thioether linked squaraine-aniline dyads

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ABSTRACT

Herein, we present an effective strategy based on coordination induced deaggregation signaling to create water-soluble fluorescent probes for the detection of mercury ion by introducing the thioether group linkage into squaraine-aniline dyads. Three unsymmetrical squaraine dyes tethered with thiopodand chains in different length were synthesized accordingly. The deaggregation process of the aggregated squaraine dyes triggered by Hg^{2+} was elucidated in detail. Firstly, the aggregation behavior of squaraine dyes with varying thioether chains in aqueous solutions has been investigated. Secondly, the complexation ability of squaraines with Hg^{2+} was found to be strengthened by increasing the length of the thioether chain. Additionally, an aggregated squaraine solution was designed by adding trace amount of non-ionic surfactant to solubilize the dye in pure water for the detection of Hg^{2+}. Significantly, the aggregated state of squaraine molecules was selectively broken by Hg^{2+} ions despite a wide range of other interfering metals in water media. Under optimum conditions, the fluorescence recovery was linearly proportional to the concentration of Hg^{2+} between 0.03 and 1.8 μM and the detection limit was as low as 6.6 nM (1.3 ppb). Further study of the mechanism by absorption and emission spectroscopy, MS, ^{1}H NMR and IR titration showed that a 1:1 complex was formed between squaraine and Hg^{2+} ion.

1. Introduction

A variety of natural and anthropogenic sources contribute to the widespread presence of mercury contamination in the environment, including oceanic and volcanic emission, solid waste incineration, gold mining, and the combustion of fossil fuels [1]. Due to its bioaccumulative and highly toxic character, mercury can cause serious damage to the central nervous system and constitutes a serious threat to human health [2]. The allowable concentration of inorganic mercury in drinking water stipulated by the U.S. Environmental Protection Agency is 2 ppb [3]. Thus, it is of great importance to develop selective and efficient analytical methods to monitor mercury in aqueous solution. Currently techniques available for mercury detection include fluorescence spectrometry [4], electrochemical methods [5], atomic absorption spectrometry [6], and inductively coupled plasma mass spectrometry (ICPMS) [7]. Due to their intrinsic high sensitivity, good selectivity, fast analysis, and simple design, fluorescence-based methodologies have attracted much attention. Up until now, many fluorescent probes based on DNAzymes [8], small molecules [9], oligonucleotides [10], nanoparticles [11] and polymer protein complexes [12] have been designed for the detection of mercury. However, fluorescence quenching, narrow pH working range, cross-sensitivity toward other metal ions and poor aqueous solubility have limited the satisfactory application of these sensors in practical use. Therefore, it remains a great challenge to design fluorescent probes for mercury detection.

Squaraines are a family of extensively studied zwitterionic dyes possessing sharp and intense absorption and fluorescence in the visible to near-infrared (NIR) region [13]. These dyes have often been applied as NIR probes [14]. The design of fluorescent probes that are water-soluble or can be auxiliarily solubilized without using an organic solvent is crucial to the subsequent applied aspects, and is a common problem that squaraine chemsensors encounter. Other problems such as nucleophilic attack on the electron-deficient squaraine ring and intramolecular photoinduced

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electron transfer (PET) from an aniline side chain (donor) to a squaraine moiety (acceptor) interfere with squaraine molecules being used as fluorescence "turn on" probes [15]. Thus there is still a great need to develop new approaches toward analyte specific chemosensors based on the squaraine chromophore.

Aggregation is a general phenomenon often observed in the solution state of squaraines, and absorption spectrometry is a common method for understanding the aggregation behavior of these dyes [16]. H-aggregates are considered to be arranged in parallel stacks or card packs, exhibiting blue shifts of the major long wavelength transitions in the absorption spectrum compared to monomers [17]. J-aggregates with a "head-to-tail" or in-line dipole arrangement have also been reported in squaraines in solution [18], and show a narrow red-shifted band relative to the monomers. There may also exist transient states including both H- and J-aggregates, with the absorption spectra of such aggregates being broadened with inconspicuous wavelength transitions [19]. Different aggregation patterns may be associated with different molecular structures. In the present work, based on a coordination driven deaggregation strategy, squaraine chemsensors were designed for Hg^{2+}-specific fluorescence "turn on" detection in aqueous solution, and in recent years, such deaggregation induced fluorescence approaches based on squaraine dyes have also been applied to the detection of protein and ATP [20].

Due to the high affinity between thioether and Hg^{2+}, a series of fluorescent probes **1a–c** based on thioether linked squaraine-aniline dyads for Hg^{2+} via coordination induced deaggregation signaling have been designed and synthesized. Previously the use of podand linked squaraine-aniline dyads permitted observation of a proton controlled intramolecular photoinduced electron transfer process [21]. Squaraines have a high tendency to aggregate in aqueous solution, and such self-aggregation usually results in a dramatic absorption spectral broadening with fluorescent emission quenching. Complexation of the thioether linkage of the probe with Hg^{2+} induces steric hindrance, leading to the deaggregation of the dye complex, coupled with a fluorescent emission restoration. Using this coordination induced deaggregation signaling based strategy, we have recently reported the synthesis of squaraine based chromophores with dithiocarbamate (DTC) side arms [19,22]. Our continuing interest is in designing squaraine based probes with higher water solubility for their potential application in environmental conditions. To our surprise, compared to **1a**, squaraine **1b** is highly sensitive to Hg^{2+}, and when tethered to a thiopodand with three sulfur atoms, **1c** shows remarkable selective response toward Hg^{2+}.

2. Experimental

2.1. Instrumental analysis

All solvents were purified and redistilled according to standard methods prior to use. Unless stated otherwise, all reagents were obtained from commercial sources. Melting points were determined with a SGW X-4 instrument without correction. FTIR spectra were recorded on a Perking Elmer Spectrum 2000 Fourier Transform Infrared Specrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AV-400 spectrometer (TMS as internal standard). Electrospray ionization mass spectra (ESI-MS) were recorded 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. Absorption spectra were measured on a Perkin Elmer Lambda 750 UV–vis spectrophotometer. The pH-measurements were carried out with a PHS-3C instrument. Fluorescent emission spectra were measured using a Cary Eclipse fluorescence spectrophotometer.

2.2. Reagents and general methods

The solutions of metal ions (Na^+, Li^+, K^+, Mg^{2+}, Ba^{2+}, Ca^{2+}, Al^{3+}, Pb^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Hg^{2+}) were prepared in twice distilled water. In metal-ion titrations, 2.0 mL solution of **1** was taken in a quartz cuvette of 1 cm path length, and small volumes (0.1–1.0 μL) of the metal solutions were added to the cuvette. Phosphate buffers (PBs) were prepared by mixing 0.01 M NaOH, 0.01 M H_3PO_4, 0.01 M NaH_2PO_4, and 0.01 M Na_2HPO_4 in proper ratios to gain the desired pH. The stock solutions of squaraine dye **1** (2.5 μM) were prepared by dissolving compound **1** in AcOH. In metal-ion titrations, 2.0 mL solution was taken in a quartz cuvette of 1 cm path length, and small volumes (0.5–1.0 μL) of **1** and the metal solutions were added to the cuvette.

2.3. Synthetic procedures

2.3.1. Synthesis of *1a–c*

A mixture of thioether linked dianiline **2** (0.10 mmol) and semisquaric acid **3** (30 mg, 0.10 mmol) was dissolved in 60 mL *n*-heptanol in a 100 mL round bottom flask equipped with a Dean-Stark trap. The solution was refluxed under reduced pressure (76 mmHg, 133 °C) for 10 h. After cooling down, most of the solvent was first removed under reduced pressure, and then the crude product was purified by column chromatography over silica gel. Elution of the column with a mixture of methylene dichloride and ethyl acetate (1:1, v/v) afforded the desired green squaraine dyes **1a–c**.

1a: 42 mg, yield 72%; m.p. 173–174 °C; FTIR (KBr): ν_{max} 2956, 2925, 2870, 1584, 1433, 1410, 1387, 1362, 1339, 1292, 1172, 1147, 1129, 1109, 939, 921, 835, 787, 763 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (t, J = 8.6 Hz, 4H), 7.26–7.23 (m, 2H), 6.76–6.70 (m, 7H), 3.67 (t, J = 7.4 Hz, 2H), 3.55 (t, J = 7.4 Hz, 2H), 3.44 (t, J = 7.8 Hz, 4H), 3.13 (s, 3H), 2.96 (s, 3H), 2.82–2.74 (m, 4H), 1.69–1.61 (m, 4H), 1.45–1.36 (m, 4H), 0.99 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.00, 187.47, 183.33, 154.05, 153.22, 148.50, 133.82, 132.86, 129.41, 120.46, 119.56, 116.93, 112.49, 112.31, 112.16, 53.15, 52.75, 51.30, 39.20, 38.74, 29.63, 29.54, 29.39, 20.23, 13.85; ESI-MS: m/z 584.3 ([M+H]$^+$); HR-ESI-MS: Calcd for $C_{36}H_{46}N_3O_2S$ ([M+H]$^+$): 584.3311, Found: 584.3331.

1b: 32 mg, yield 50%; m.p. 158–159 °C; FTIR (KBr): ν_{max} 2956, 2925, 1585, 1435, 1385, 1360, 1287, 1172, 1131, 1108, 923, 837, 787, 748 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39–8.37 (m, 4H), 7.26–7.20 (m, 2H), 6.74–6.69 (m, 7H), 3.66 (t, J = 6.4 Hz, 2H), 3.54 (2H), 3.44 (t, J = 7.6 Hz, 4H), 3.16 (s, 3H), 2.96 (s, 3H), 2.77–2.71 (m, 8H), 1.69–1.61 (m, 4H), 1.45–1.36 (m, 4H), 0.99 (t, J = 7.1 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.93, 187.31, 183.30, 154.04, 153.16, 148.52, 133.80, 132.80, 129.32, 120.44, 119.51, 116.68, 112.49, 112.21, 112.17, 52.94, 52.48, 51.29, 39.29, 38.65, 32.83, 32.58, 29.61, 29.56, 29.15, 20.22, 13.86; HR-ESI-MS: Calcd for $C_{38}H_{50}N_3O_2S_2$ ([M+H]$^+$): 644.3344, Found: 644.3345.

1c: 46 mg, yield 65%; m.p. 92–93 °C; FTIR (KBr): ν_{max} 2955, 2923, 2855, 1727, 1588, 1462, 1390, 1344, 1290, 1177, 785 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (dd, J = 9.1, 1.9 Hz, 4H), 7.26–7.21 (m, 2H), 6.76 (dd, J = 19.4, 9.7 Hz, 7H), 3.69 (t, J = 7.3 Hz, 2H), 3.54 (t, J = 7.6 Hz, 2H), 3.44 (t, J = 7.8 Hz, 4H), 3.17 (s, 3H), 2.96 (s, 3H), 2.80–2.71 (m, 12H), 1.69–1.61 (m, 4H), 1.46–1.36 (m, 4H), 0.99 (t, J = 7.3 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.93, 187.23, 183.30, 154.09, 153.20, 148.59, 133.83, 132.83, 129.32, 120.47, 119.53, 116.68, 112.53, 112.24, 112.21, 52.96, 52.56, 51.31, 39.29, 38.57, 32.77, 32.60, 32.55, 32.49, 29.63, 29.58, 29.08, 20.23, 13.85; ESI-MS: