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A hemicyanine complex for the detection of thiol biomolecules by fluorescence

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ABSTRACT

Herein, we report the design and preparation of a benzothiazolium hemicyanine derivative that exhibits red emission at 585 nm. Its complex with Hg^{2+} may be used for selective and sensitive sensing of thiol biomolecules through the reversible visual color and florescence changes they induce in the complex. This complex was used to detect three test thiol biomolecules, and exhibited sensitivity in the order cysteine > glutathione > homocysteine. Furthermore, the complex allowed the detection of cysteine at 0.2 μM, lower than the normal human intracellular cysteine concentration (30–200 μM), and was found to be useable over at least five cycles upon alternate addition of cysteine and Hg^{2+}.

1. Introduction

Thiol biomolecules, such as cysteine (Cys), glutathione (GSH), and homocysteine (Hcy), play pivotal roles in many biological processes, and abnormal levels of intracellular thiol biomolecules in the body are related to a number of disorders. For example, Cys deficiency can cause retarded growth, lethargy, liver damage, hair depigmentation, and skin lesions [1]; GSH as a redox regulator is very important in maintaining the redox environment in living cells [2–4]; elevated levels of Hcy in blood serum can cause oxidation of oxygen-containing species, leading to oxidative stress and a high risk of Alzheimer's, cardiovascular, and inflammatory bowel disease [5,6].

Due to the important roles of these thiol biomolecules, considerable research attention has been paid to their determination. Various analytical techniques, such as high-performance liquid chromatography, mass spectrometry [7], gas chromatography [8], electrochemical assay [9,10], and UV–Vis analysis [11,12], have been used for the detection of different thiols. However, fluorescent sensors have recently become widely used for the detection of analytes owing to their high sensitivity, selectivity, and simplicity [13]. A number of fluorescence-based thiol sensors are available. These sensors work on the basis of different reactions, such as Michael addition [14–16], cyclization with aldehydes [17–19], sulfonamide or sulfonate ester cleavage [20–25], and cleavage of disulfides by thiols [26–28]. Although these chemical reactions generally exhibit high selectivity towards the target analytes, they require a long time to complete, which decreases the method sensitivity. These probes also exhibit other drawbacks, such as small Stokes shifts, short excitation/emission wavelengths, problems associated with undesired background fluorescence, and the need for organic solvents. Recently, a particularly attractive strategy has been developed that involves the use of an ensemble

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complex capable of complexation/decomplexation interactions with an analyte [29,30]. The ensemble assay is based on a competitive interaction in which the analyte species and metal ions compete for interaction with the sensor. Compared with reaction-based probes for thiols, complexation-based probes exhibit improved sensitivity. Moreover, the complexation process is reversible and can be repeated over many cycles.

Herein, we report the design and preparation of the benzothiazolium-based derivative **L** (Scheme 1) that exhibits a strong red emission at 585 nm and, since it is a benzothiazolium salt, is soluble in aqueous solution. **L** is a hemicyanine dye with strong intramolecular charge transfer (ICT) character, in which the aniline moiety and benzothiazolium moiety act as electron-donor and electron-acceptor, respectively. The sulfur atom of the benzothiazolium moiety can coordinate with Hg^{2+} to form the ensemble complex **HgL**, which does not fluoresce. Addition of a thiol biomolecule to a solution of the ensemble induces the release of the probe from the complex, resulting in renewed fluorescence (as illustrated in Scheme 1). Furthermore, this fluorescence response is reversible and can be repeated over at least five cycles upon alternate addition of cysteine and Hg^{2+}.

2. Materials and methods

2.1. Instruments

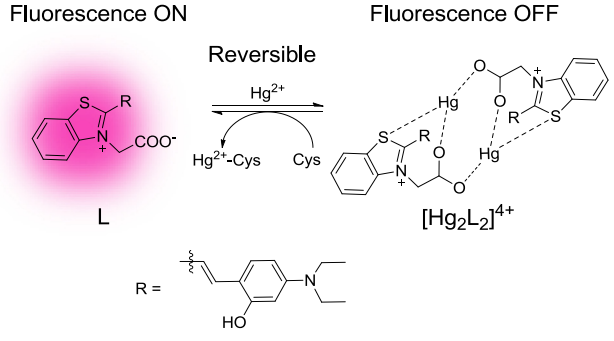
^1H NMR and ^{13}C NMR spectra were obtained using a Bruker 400 MHz NMR spectrometer. Mass spectral analysis was performed on a Waters GCT Premier mass spectrometer. Absorption data were obtained with a Shimadzu UV-1800 UV–Vis spectrophotometer, and fluorescence data were collected using a Shimadzu RF-5301 PC Series fluorescence spectrometer.

2.2. Reagents

2-Methylbenzothiazole, bromopropionic acid, and 4-diethylamino-2-hydroxybenzaldehyde were purchased from Aldrich and used without further purification. Mercuric perchlorate and piperidine were purchased from Sigma and used as received. Anhydrous solvents, such as tetrahydrofuran, ethanol, and DMSO, were of HPLC-grade and confirmed free form fluorescent impurity. All amino acids used in this study were purchased from Aldrich and were used without further purification.

2.3. Computational methodology

Geometry optimization and single point energy calculation for the monomer and complexes formed were carried out using density functional theory (DFT) with the Gaussian 09 program [31–33]. The B3LYP method in conjunction with the LanL2DZ basis set was applied to investigate system examined. The variation of enthalpy (ΔH) and variation of Gibbs free energy (ΔG), are derived as the difference between the total energy of the reaction products and the energy of the reactants. The excited state and time-dependent factors are investigated using time-dependent density functional theory (TD-DFT) at the same level of theory [34]. Topological properties of electron density was probed using the AIM 2000 software for the atom-in-molecule (AIM) theory. The following characteristics of each bond critical point (BCP) and bond ring critical point (RCP) are considered in this study: the electron density ($\rho(r)$), its Laplacian ($\nabla^2(\rho(r))$ [34–36].



Scheme 1. Schematic illustration of the interconversion between **L** and Hg_2L_2 upon alternate addition of Hg^{2+} and thiol biomolecules.

3. Results and discussion

3.1. The characterization of sensor *L* and its complex with Hg^{2+}

The probe **L** was prepared via a condensation reaction between the 2-methylbenzothiazole salt and 4-diethylamino-2-hydroxybenzaldehyde in ca. 60% yield, and was characterized by ^1H NMR and mass analyses (see the supplementary data). The global minimum structure of **L** with the atom numbering scheme is displayed in Fig. 1. The Cartesian coordinates for the structure of **L** are shown in Table S1.

Since **L** is a strong intramolecular charge transfer compound, its electronic properties are strongly affected when it coordinates with mercuric ions, resulting in changes in the UV–Vis and fluorescent emission spectra. **L** contains mercury-coordinating species to increase its affinity to Hg^{2+} ions.

As shown in Fig. 2a, free **L** exhibits a characteristic absorption band centered at 540 nm in an ethanol/4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) solution, and has a high molar extinction coefficient (ca. 10^4 M^{-1} cm^{-1} at 540 nm). When Hg^{2+} ions are added to the solution of **L**, a new absorption band at 460 nm emerges with increasing intensity, and the signal at 540 nm decreases in intensity. Moreover, an obvious isosbestic point at 490 nm is observed, indicating the formation of a 1:1 complex between Hg^{2+} and **L**. On the basis of 1:1 stoichiometry, the dissociation constant ($\mathbf{K_d}$) and the association constant ($\mathbf{K_a}$) were calculated through a nonlinear curve fitting [37] to be 3.57×10^{-18} M^3 and 0.28×10^{18} M^{-3}, respectively. The method for determining these values and the experimental data are given in SI. In contrast, **L** displays a characteristic emission wavelength at 585 nm with a fluorescence quantum yield (Φ) of 0.175 (calculated using rhodamine B ($\Phi = 1$) as a reference). Fig. 2b shows the fluorescence changes exhibited by **L** in the presence of Hg^{2+}. Addition of Hg^{2+} to the solution of **L** induces progressive fluorescence quenching. When one equivalent of Hg^{2+} is added, the emission intensity is quenched by over 95%. Fig. 2c shows the

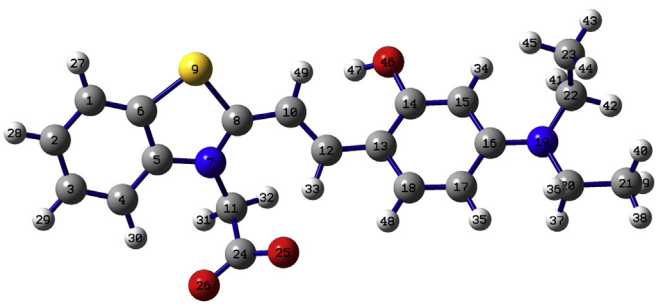


Fig. 1. The optimized geometry of **L** at the B3LYP/LanL2DZ level of theory.