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An aza-coumarin-hemicyanine based near-infrared fluorescent probe for rapid, colorimetric and ratiometric detection of bisulfite in food and living cells

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ABSTRACT

A novel aza-coumarin-hemicyanine conjugated hybrid was prepared, which was found to be a promising colorimetric and ratiometric near-infrared (NIR) fluorescent probe for detection of bisulfite/sulfite in aqueous solution. This probe shows a rapid response (within 30 s) and high selectivity and sensitivity for bisulfite/sulfite, giving distinct colorimetric and ratiometric fluorescence changes at 717 and 560 nm. The detection limit for bisulfite was estimated to be 87 nM. In addition, detection of bisulfite in food samples and bioimaging of both exogenous and endogenous bisulfite in living cells with this probe were successfully applied, which indicates that this probe holds great potential for bisulfite detection both in real samples and in living cells.

1. Introduction

Recently, much effort has been given to the development of fluorescent probes for chemically and/or biologically important anions due to the simplicity, high sensitivity and non-invasiveness of fluorescence detection [1,2]. Among them, ratiometric fluorescent probes allow the measurement of emission intensities at two different wavelengths, which could overcome the limitations of intensity-based probes and provide a self-calibration correction [3–5], while near-infrared (NIR, 650–900 nm) fluorescent probes have the merits of high sensitivity, minimum autofluorescence interference, deep tissue penetration and minimum photo-damage to biological samples, and hence are more suitable for bioimaging applications [6–10]. Therefore, ratiometric NIR fluorescent probes are highly desirable in practical applications.

Among various anions, bisulfite/sulfite (HSO_3^-/SO_3^{2-}) anions have received considerable attention in recent years [11]. These anions have been widely used in our daily life as food preservative because of their antimicrobial, bacteriostasis and antioxidant properties [12,13]. However, studies have shown that extensive intake of these anions would induce harmful effects to cells and tissues, causing asthmatic attacks and allergic reactions in some individuals, and some people are extremely sensitive even to low levels of them [14]. It is also well known that HSO_3^-/SO_3^{2-} anions are the major derivatives of sulfur dioxide (SO_2) in aqueous media. Although SO_2 has been known as a toxic air pollutant for a long time [15], recent studies revealed that SO_2 can be endogenously generated in cells during oxidation of hydrogen sulfide or sulfur containing amino acids [16–18]. Moreover, studies also showed that endogenous SO_2 exhibits unique bioactivities such as regulating blood insulin levels, maintaining the biological sulfur balance in the body as well as relaxing blood vessels, suggesting endogenous SO_2 is a biological gasotransmitter [19–21]. Despite these important findings, the underlying molecular events of SO_2 in living systems remain largely unknown. Thus, the development of fluorescent probes for HSO_3^-/SO_3^{2-} detection in food samples and living cells is of great significance.

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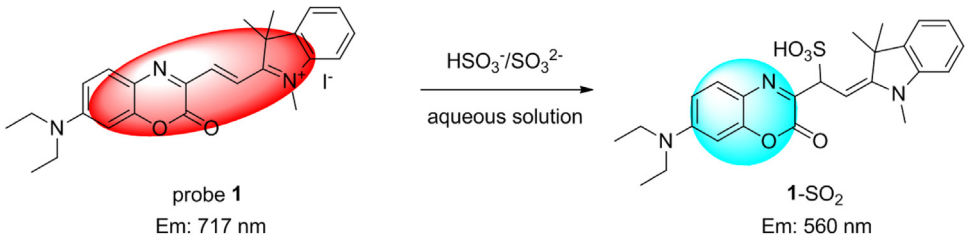

Scheme 1. Probe **1** (an aza-coumarin-hemicyanine hybrid) as a ratiometric NIR fluorescent probe for sensing of bisulfite/sulfite.

In the past few years, a number of fluorescent probes have been developed for detection of HSO_3^-/SO_3^{2-} with high selectivity [22–49]. However, some of them are intensity-based probes [22–26] and most of them showed fluorescent signal changes only in the visible region [22–46], which limited their biological applications. So far, ratiometric NIR fluorescent probes for HSO_3^-/SO_3^{2-} are still very rare [47–50]. Continuing our interest in developing fluorescent probes for HSO_3^-/SO_3^{2-} [51–53], herein, we report a new ratiometric probe with significant NIR fluorescence changes for HSO_3^-/SO_3^{2-} (probe **1** in Scheme 1). This probe is based on a novel aza-coumarin-hemicyanine hybrid. Importantly, it is not only easy to prepare, but also shows rapid, highly selective and sensitive colorimetric and ratiometric NIR fluorescent signal changes for HSO_3^-/SO_3^{2-} in aqueous solution under mild conditions. Moreover, it can be used to detect HSO_3^-/SO_3^{2-} in real food samples and image both exogenous and endogenously generated HSO_3^-/SO_3^{2-} in living cells, indicating that this probe is excellent and promising.

2. Materials and methods

2.1. Materials and instrumentation

All chemicals and solvents were purchased from commercial suppliers and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 or 600 spectrometer. Data of high-resolution mass spectrometry (HR-MS) was obtained with an LC/Q-TOF MS spectrometer (Agilent). The pH was measured using a PB-10 digital pH-meter (Sartorius). UV–vis and fluorescence spectra were recorded on an Agilent Cary-100 UV–vis spectrophotometer and an Agilent Cary Eclipse fluorescence spectrophotometer, respectively. Standard quartz cuvettes with a 10 mm lightpath were used for all optical spectra measurements. Cell imaging was performed in an inverted fluorescence microscopy with a 20 × objective lens.

2.2. Synthesis of probe *1*

Compound **2** was first synthesized by the literature methods [54–56], which can be found in the Supplementary data.

To a mixture of compound **2** (100 mg, 0.4 mmol) and **3** (122.3 mg, 0.4 mmol) was added 10 mL of absolute ethanol. Piperidine (10 μL) was then added to the solution. The mixture was then heated to reflux for 6 h. After the resultant mixture was cooled to room temperature, the solvent was removed under reduced pressure. The residue was then purified by column chromatography (dichloromethane/methanol, 30:1, v/v) on silica gel to give probe **1** as a red brown powder (120 mg, yield: 56%). M.p. >300 °C. TLC (silica plate): R_f ~0.26 (dichloromethane/methanol, 10:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, J = 15.5 Hz, 1H), 7.99 (d, J = 15.5 Hz, 1H), 7.79–7.72 (m, 2H), 7.61–7.51 (m, 3H), 6.87 (dd, J = 9.3, 2.6 Hz, 1H), 6.45 (d, J = 2.4 Hz, 1H), 4.36 (s, 3H), 3.58 (dd, J = 13.9, 6.8 Hz, 5H), 1.86 (s, 6H), 1.33 (t, J = 7.1 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 180.54, 154.07, 151.15, 145.62, 143.05, 141.68, 133.82, 130.05, 129.89, 127.18, 122.57, 115.52, 113.17, 96.95, 52.32, 46.26, 44.52, 36.38, 27.13, 22.10, 21.80, 12.87. HR–MS Calcd for $C_{25}H_{28}N_3O_2^+$ $(M-I^-)^+$ 402.2176, found 402.2179.

2.3. Sample preparation and optical measurements

Stock solutions of probe **1** (1 mM) were prepared in DMF (HPLC grade). Stock solutions (10–100 mM) of the analytes (NaF, NaCl, NaBr, NaOAc, NaN_3, $NaHCO_3$, Na_3PO_4, NaSCN, Na_2SO_4, $NaNO_3$, NaClO, NaCN, $Na_2S_2O_3$, NaHS, $Na_2S_2O_7$, phenylalanine (Phe), glycine (Gly), cysteine (Cys), homocysteine (Hcy), glutathione (GSH), $H_2NCH_2CH_2NH_2$, $HOCH_2CH_2NH_2$, $C_6H_5CH_2NH_2$, and $C_6H_5NH_2$) were prepared in ultrapure water. The stock solutions were used freshly and were diluted to desired concentrations with water when needed. For a typical optical measurement, a solution of probe **1** (10 μM) was prepared in PBS buffer solution (10 mM, pH 7.4, with 10% DMF, v/v). Then 3.0 mL of the probe solution was placed in a quartz cell. After the temperature reached at 25 °C (controlled by a temperature controller), the UV–vis or fluorescent spectra were recorded upon addition of an analyte (at indicated time).

2.4. Measurements of bisulfite in food samples

Crystal sugar and granulated sugar were purchased from a local supermarket and used in the real sample analysis. Sugar sample solutions were prepared by dissolving 2.5 g of sugars in deionized water and diluting to 10 mL. Aliquots of the sugar sample solution were added directly to the probe **1** (10 μM) in PBS buffer (10 mM, pH 7.4, with 10% DMF, v/v), and after incubation for 30 s at 25 °C, the emission intensities of the solution at 560 and 717 nm were recorded. Aliquots of the food samples were also spiked with $NaHSO_3$ (1.0 or 2.0 μM) that had been accurately prepared. The resulting samples were then treated with probe **1** (10 μM) for 30 s and the emission intensities at 560 and 717 nm were recorded. The results shown in Tables 1 and 2 were reported as the average values from three experiments.

2.5. Cell imaging experiments

HeLa cells were cultured according to our previously published procedures [51–53,57,58] and were seeded in a 12-well culture plate for one night before cell imaging experiments. For imaging exogenous HSO_3^- in living cells, part of cells were incubated with 5 μM of probe **1** for 30 min at 37 °C and washed three times with prewarmed PBS buffer, and then imaged. Meanwhile, another part of cells were treated with $NaHSO_3$ (20 μM) for 30 min at 37 °C, washed three times with prewarmed PBS buffer, and then incubated with probe **1** (5 μM) for 30 min. After washing cells with prewarmed PBS buffer, cells were imaged. For imaging endogenously generated HSO_3^- in living cells, the same procedure was applied. After the cells were treated with a SO_2 donor (*N*-benzyl-2,4-dinitrobenzenesulfonamide, 100 μM) instead of $NaHSO_3$ for 30 min at 37 °C, the cells were then incubated with probe **1** (5 μM)