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A fluorescent turn-on probe based on benzo[*e*]indolium for bisulfite through 1,4-addition reaction

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ABSTRACT

A new fluorescent turn-on probe for HSO_3^- bearing a benzo[*e*]indolium moiety was developed. The HSO_3^- undergoes 1,4-addition reaction with the C-4 atom in the ethylene group, resulting in a prominent fluorescence enhancement and a fluorescent color change. The reaction could be completed in 1 min in PBS buffer, and displayed a high selectivity and sensitivity for HSO_3^- against other anions, including CN^-. Moreover, the practical value of the probe was confirmed by application to detect the level of HSO_3^- in sugar samples.

1. Introduction

There has been a growing interest in the development of new anion sensors and probes that selectively recognize specific anions because of anions play an important role in a wide range of chemical industry and biological processes [1]. Among these anions, bisulfite (HSO_3^-) has been widely used as antimicrobial agent, enzyme inhibitor and antioxidant for foods, beverages and pharmaceutical products [2]. However, it has been discovered that certain concentration level causes asthmatic attacks and allergic reactions, such as difficulty in breathing, wheezing, hives, and gastrointestinal distress [3]. Due to the mentioned harmful effects toward people, the threshold levels of HSO_3^- in food and medicine have been controlled rigorously in many countries. Therefore, the development of rapid, facile, and reliable detection techniques for HSO_3^- is of great importance for food quality assurance and quality control. Many conventional analytical techniques have been reported for HSO_3^- detection, such as spectrophotometry [4], spectrofluorimetry [5], chemiluminescence measurements [6], phosphorimetry [7], chromatography [8], electrochemical [9], and enzymatic techniques [10]. However, these methods for HSO_3^- detection require troublesome sample pretreatment, reagent preparation, time-consuming, and complicated instruments. In addition, some of them are not sensitive enough to determine low concentration of HSO_3^-.

Fluorescence spectroscopy has become a powerful tool for sensing and imaging trace amounts of samples because of its simplicity and sensitivity [11]. Some fluorescent probes have been exploited for detecting HSO_3^- by their specific reactions with an aldehyde [12] or levulinated group [13] *in vitro* assay. However, the aldehyde-based probes can only be operated in acidic conditions; and the labile ester linkage in levulinate-type probes may induce a high background signal in biological imaging, as it can potentially be cleaved by proteases and esterases to produce active fluorophores. Guo has developed a ratiometric fluorescent probe based on diethylaminocoumarin-hemicyanine dye for sensing HSO_3^- both *in vitro* and *vivo* [14]. Unfortunately, all the probes that mention above displayed a response time more than 5 min. Thereby, searching for quick reactions that are sensitive and selective toward HSO_3^- has always been attractive and challenging.

Herein, we report a new fluorescent turn-on probe **1**, which was connected by a benzo[*e*]indolium fluorophore and a benzene moiety through an ethylene group (Scheme 1). The probe has shown a selective response to HSO_3^- over other anions in PBS buffer (pH = 7.4, 10 mM), and a distinguished fluorescent color change from colorless to cyan could be observed. HSO_3^- is expected to be undergone 1,4-addition reactions with the C-4 atom in the ethylene group, which is activated by the electron-withdrawing feature of the positively-charged benzo[*e*]indolium fragment. The bulkiness of HSO_3^- prevented it to attack toward C-2 atom, although C-2 atom was an effective target for HS^- [15] and CN^- [16].

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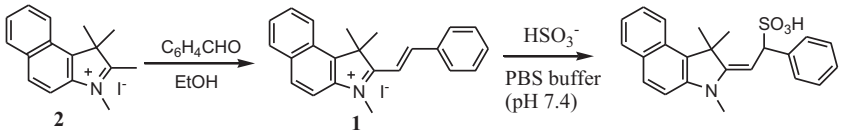

Scheme 1. Synthesis of **1** and its sensing mechanism.

2. Experimental

2.1. General

All reagents and solvent were purchased from commercial source and used without further purification, if not stated. All reactions were carried out on the magnetic stirrers and their reaction process was monitored on thin layer chromatography (TLC). Absorption and fluorescence spectra were taken on a Shimadzu UV-1800 spectrophotometer and a Hitachi F-2700 fluorescence spectrometer, respectively. ^{1}H NMR and ^{13}C NMR measurements were recorded at 600 and 150 MHz on a Brucker Avance 600-MHz spectrometer. Dimethyl sulfoxide (DMSO-d_6) was solvent, and tetramethylsilane (TMS) was used as internal standard. A pH meter (Mettler Toledo, Switzerland) was used to determine the pH values. HRMS (ESI) were taken on a Fourier transform ion cyclotron resonance mass spectrometry (Varian 7.0T).

2.2. Preparation of probe *1*

1,2,3,3-Tetramethylbenz[*e*]indolium iodide (**2**) was prepared according to the method we reported previously [16c]. Then, probe **1** was prepared by a simple one-step of **2** with benzaldehyde in ethanol solution. **2** (1.05 g, 3 mmol) was dissolved in ethanol (5 mL), then benzaldehyde (0.32 g, 3 mmol) was added and the reaction mixture heated at reflux for 12 h. Then reaction was cooled to room temperature and the precipitate was collected by filtration, washed with cold ethanol, and dried *in vacuo*. Probe **1** was obtained as an orange solid in 80% yield (1.0 g). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.31 (d, 2H), 8.23 (m, 1H), 8.13 (d, 3H), 7.82 (t, 1H), 7.76 (d, 1H), 7.74 (t, 2H), 7.63 (m, 3H), 4.30 (s, 3H), 2.02 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 186.2, 155.1, 142.8, 141.6, 137.9, 136.7, 134.3, 133.6, 133.4, 132.6, 131.8, 130.6, 130.0, 126.6, 116.8, 116.3, 57.3, 38.7, 28.4. HRMS (ESI) calcd. for $[M]^+$ 312.1752, found 312.1739 (Figs. S1–S3).

2.3. ^{1}H NMR analysis experiments of *1*

The solution of probe **1** (3×10^{-3} M) in DMSO-d_6 (450 μL) and D_2O (50 μL) was placed in the NMR tube, and sodium bisulfite powder was added. All the sodium bisulfite was soluble.

2.4. Titration experiments of *1*

Deionized water was used as the solvent for titration experiment. The titrations were carried out in 10-mm quartz cuvettes at room temperature. Probe **1** was dissolved in DMF (spectroscopic grade) to afford a concentration of 10 mM stock solution, and then diluted with PBS buffer (pH = 7.4, 10 mM). Anions (as their Na^+ salt, 10 mM and 100 mM) in deionized water were added to the diluted probe solution and used for the sensing behavior experiment. The excitation wavelength was 400 nm, and the PMT voltage was 700 V. The excitation and emission slit width were 5 nm and 5 nm, respectively.

2.5. Sample test

Granulated sugar, soft sugar and crystal sugar purchased from supermarket were used in the sample analysis. Sample solution was prepared by dissolving 5.0 g of sugar in deionized water and diluting to 10 mL. Aliquots of the sugar solution were added directly to the PBS buffer (pH = 7.4, 10 mM) containing **1** (10 μM), and the emission intensity at 465 nm were recorded.

3. Results and discussion

3.1. Optimization of experimental conditions

Firstly, the pH value and response time were examined to optimize the detection conditions. Probe **1** possesses fine water solubility in low concentration, and a series of buffer solutions were employed for the sensing systems. Fig. 1 shows the fluorescence intensities of probe **1** at 465 nm in the absence and presence of HSO_3^- in various buffer solutions. The probe is stable in a pH range of 1–10, and displays the best response in the physiological pH region. Hence, PBS buffer (pH = 7.4, 10 mM) was chosen as the sensing system. Probe **1** showed a very quick response to HSO_3^- in PBS buffer (pH = 7.4, 10 mM). Time-dependent modulations in the fluorescence of the probe (10 μM) in the presence of HSO_3^- (10 μM) suggested that the reaction could be completed within 1 min under the experimental condition. If the HSO_3^- concentration was above 50 μM, the reaction could be completed within 30 s (Fig. S4). In fact, the unprecedented response speed of probe **1** to HSO_3^- is more rapidly than the reported HSO_3^- probes. Thus, the measurements were delayed 1 min after HSO_3^- added in the titration experiment.

3.2. Spectral studies of *1*

The sensing potential of **1** toward HSO_3^- was investigated carefully in PBS buffer (pH = 7.4, 10 mM). Fig. 2 shows the fluorescence response of probe **1** (10 μM) with different amounts of HSO_3^-. The free probe **1** exhibited very weak fluorescence emission at 560 nm which was induced by the π–π conjugation of probe **1**. Upon addition of HSO_3^-, the emission at 465 nm increased remarkably, which could be attributed to the new formed 2-methylene benzo[*e*]indoline moiety. The fluorescence intensities became constant when the amount of HSO_3^- reached 5 equiv., led to a *ca.* 90-fold fluorescence enhancement. Meanwhile, an obvious fluorescence color change from dark to cyan was also observed (inset

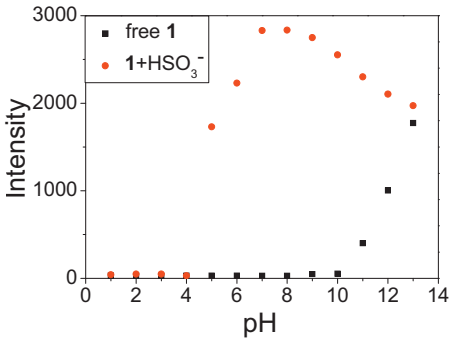

Fig. 1. Fluorescence intensities of **1** (10 μM) at 465 nm in the absence and present of HSO_3^- (50 μM) under different pH conditions.