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A highly selective ratiometric fluorescent and chromogenic probe for sulfite and its applications in imaging of living cells and zebrafish in vivo

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ABSTRACT

Herein we report a new carbazole-benzo[e]indolium dye (CEBI) as a ratiometric fluorescent and chromogenic probe for sulfite (SO_3^{2-}) detection based on intramolecular charge transfer (ICT) mechanism. Upon treatment with SO_3^{2-}, the probe CEBI displayed a remarkable fluorescence ratiometric response (~560-fold fluorescenc enhancement) together with a color change from red to weak red and a large shift (135 nm) in emission wavelength. The experimentally observed changes in the spectral features of the probe upon addition of SO_3^{2-} were systematically studied using high resolution electrospray ionization mass spectrometry (HR-ESI–MS), proton nuclear magnetic resonance (^{1}H NMR), and time-dependent density functional theory (TDDFT) calculations. We found a linear relationship between the ratio of fluorescence intensities at 465 nm and 600 nm (F_{465}/F_{600}) and SO_3^{2-} concentration over the range of 0–40 μM with a detection limit of 0.025 μM. Moreover, the novel probe demonstrated fluorescence imaging of SO_3^{2-} in living cells and zebrafish, suggesting its potential in clinical diagnostics.

1. Introduction

Sulfite (SO_3^{2-}) is an essential preservative widely used as food storage, anti-oxidant and antibacterial agents in the food industry [1,2]. Extensive SO_3^{2-} intake can cause gastrointestinal distress, respiratory illnesses, cardiovascular diseases, and neurological disorders [3,4]. Considering these potential health threats, SO_3^{2-} (or bisulfite, HSO_3^-) content in food products and medicine has been strictly limited in many countries. According to the United States Food and Drug Administration (FDA, USA), the products containing more than 10 mg mL^{-1} of SO_3^{2-} require labeling [5]. Therefore, developing highly selective and sensitive methods for SO_3^{2-} detection is of significant importance.

Various analytical methods, including titration, ion selective electrode, ion chromatography [6–8], voltammetry [9,10] and optical sensors [11–13], have been employed for the determination of SO_3^{2-}. Among the above methods, optical sensors, involving colorimetric and/or fluorescence changes for SO_3^{2-}, are of particular interest because of their high sensitivity, non-invasiveness, ease of operation, real-time detection, and good compatibility for bio-samples [14,15]. Several small-molecule organic fluorescent probes, which have been designed in recent times, can be classified as intensity-based and ratiometric probes [16–18]. However, variation in probe concentration, environment and excitation intensity may influence fluorescence intensity measurement, thus limiting intensity-based probes for further applications. In principle, this problem can be solved by introducing ratiometric fluorescent probe. Ratiometric fluorescent probes have the advantage of measuring fluorescence emission/excitation intensities at two wavelengths, and the ratio of signals is independent of environmental changes [16,19–21]. Ratiometric fluorescent probes function based on different sensing mechanisms, and the most common mechanisms include intramolecular charge transfer (ICT), fluorescence resonance energy transfer (FRET), excited state intramolecular proton transfer (ESIPT), and through-bond energy transfer (TBET) [22–24]. ICT-based fluorescence probes, containing different functional groups, such as, —CHO, C=C, C=N, N=N bond and levulinic acid ester, have been developed for SO_3^{2-} detection in recent years [25–35]. In general, an ICT system includes an electron-donating group and an electron-withdrawing group, and ICT process occurs from donor to acceptor upon photo-excitation.

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This study reports carbazole-benzo[e]indolium dye (CEBI) as the probe for SO_3^{2-} detection based on the nucleophilic attack of SO_3^{2-} on C=C bond of CEBI. The 1-H-benzo[e]indolium and carbazole were used as ICT donor and acceptor, respectively. We studied the mechanism of SO_3^{2-} sensing using absorption and emission spectroscopies, high resolution electrospray ionization mass spectrometry (HR-ESI–MS), proton nuclear magnetic resonance (^{1}H NMR), and time-dependent density functional theory (TDDFT) calculations. The results show that the charge transfer direction was different from the previous reports of carbazole containing probes [36–39]. In addition, we demonstrated the potential of the probe for employing in fluorescence imaging of living cells and zebrafish.

2. Material and methods

2.1. Reagents

1,1,2-Trimethyl-1H-benzo[e]indole was purchased from Ark pharm, Inc., USA. 9-Ethyl-9H-carbazole and salts of different anions, such as n-Bu_4NF, n-Bu_4NCl, n-Bu_4NBr, n-Bu_4NI, n-Bu_4NNO_3, n-Bu_4NOAc, n-Bu_4NHSO_4, n-$Bu_4NH_2PO_4$, n-Bu_4NClO_4, n-Bu_4NSCN, n-Bu_4NCN, $Na_2S{\bullet}9H_2O$ and Na_2SO_3, were purchased from Energy Chemical Ltd., China. $POCl_3$, DMF and DMSO were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Other reagents were of analytical reagent grade and used without further purification or treatment. All aqueous solutions were prepared using ultrapure water obtained from a Milli-Q water purification system (18.2 MΩ cm). The living HeLa (human cervical adenocarcinoma) cells were provided by the Jilin University Hospital of Stomatology, China. Zebrafish samples were obtained from the National Zebrafish Resources of China.

2.2. Instruments

UV–vis and fluorescence spectra were recorded on a Cary 60 spectrometer (Agilent Technologies, USA) and a Cary Eclipse spectrofluorophotometer (Agilent Technologies, USA), respectively. ^{1}H NMR (TMS as internal standard) spectra were recorded on a Mercury 300BB nuclear magnetic resonance spectrometer (Varian Inc., USA). Mass spectra (MS) were obtained using a LC/MS QTOF spectrometer (AB SCIEX Inc., USA). Cells were imaged by an Olympus IX 51 inverted fluorescence microscope (Olympus Corporation, Japan) equipped with integrated color filters. zebrafish larvae were imaged by a LEICA DM IRB inverted fluorescence microscope (Leica Microsystems, Germany) equipped with integrated color filters.

2.3. Synthesis of the probe CEBI

Fig. 1 describes the synthetic route to prepare 2,2′-(9-ethyl-9H-carbazole-3,6-diyl)-bis(ethene-2,1-diyl)-bis(3-ethyl-1,1-dimethyl-1H-benzo[e]indol-3-ium) iodide (CEBI).

2.3.1. Synthesis of 9-ethyl-9H-carbazole-3,6-dicarbaldehyde (**1**)

In a 100 mL round bottomed flask, 0.98 g of 9-ethyl-9H-carbazole (5 mmol) in 20 mL of DMF was taken, and then 0.5 mL of $POCl_3$ (5.4 mmol) liquid was gradually added to the flask. After stirring the resulting mixture at 100 °C for 24 h, it was cooled to room temperature, then poured into ice-water (100 mL), and neutralized with 4 mol L^{-1} NaOH solution. The precipitate was filtered, washed with water, and purified by column-chromatography on a silica gel column with a mixture of petroleum ether (PE) and ethyl acetate (EtOAc) (PE:EtOAc, 5:1, *v:v*) as the eluent. Finally, 0.44 g of **1** as a white solid (yield: 35%) was obtained. ESI–HR–MS, *m/z*: 274.2471 [**1** + H]$^+$ (calcd. 274.2472 for $C_{16}H_{13}NNaO_2^+$). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.10 (s, 2H), 8.87 (s, 2H), 8.07 (d, 2H), 7.87 (d, 2H), 4.55 (t, 2H), 1.37 (t, 3H).

2.3.2. Synthesis of 3-ethyl-1,1,2-trimethyl-1H-benzo[e]indol-3-ium (**2**)

In a 100 mL round bottomed flask, 1.05 g of 1,1,2-trimethyl-1H-benzo[e]indole (5 mmol) in 20 mL of MeCN was taken, and 0.48 mL of iodoethane (6 mmol) was added dropwise under stirring. The solution was refluxed for 12 h and then cooled to room temperature. The mixture was concentrated under reduced pressure, and then ethyl ether was added and stirred. The precipitate was filtered, washed with ethyl ether, and then purified by column-chromatography on a silica gel column with a mixture of CH_2Cl_2 and MeOH (20:1, *v:v*) as the eluent. Finally, 1.40 g of **2** as a white solid (yield: 77%) was obtained. ESI–HR–MS, *m/z*: 238.1592 [**2-I**$^-$]$^+$ (calcd. 238.1590 for $C_{17}H_{20}N^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17-8.00 (m, 3H), 7.87 (d, 1H), 7.80-7.60 (m, 2H), 4.86 (q, 2H), 3.23 (s, 3H), 1.87 (s, 6H), 1.68 (t, 3H).

2.3.3. Synthesis of CEBI

To synthesize the probe CEBI, 0.15 mg of **1** (0.6 mmol), 0.55 mg of **2** (1.2 mmol) and 0.15 mL of piperidine (1.5 mmol) were mixed in 15 mL of MeOH and then stirred at 80 °C for 24 h. The precipitate produced was filtered, washed with MeOH, and then purified by column-chromatography on a silica gel column with a mixture of CH_2Cl_2 and MeOH (100:1, *v:v*) as the eluent. Finally, 0.25 g of CEBI as a red solid (yield: 44%) was obtained. ESI–HR–MS, *m/z*: 345.6957 [CEBI-2I$^-$]$^{2+}$ (calcd. 345.6958 for $C_{50}H_{49}N_3^{2+}$). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.55 (s, 2H), 8.79 (d, 2H), 8.49 (dd, 4H), 8.34-8.13 (m, 6H), 8.03-7.91 (m, 3H), 7.91-7.80 (m, 3H), 7.78-7.70 (m, 2H), 5.00 (d, 4H), 4.67 (d, 2H), 2.12 (s, 12H), 1.59 (t, 6H), 1.44 (t, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.63, 154.10, 143.69, 138.20, 133.10, 131.07, 130.35, 130.06, 128.40, 127.19, 127.04, 126.85, 125.13, 123.44, 123.06, 113.06, 111.10, 109.29, 53.63, 42.39, 38.12, 25.81, 14.12, 13.96.

2.4. Spectroscopic experiments

The probe CEBI was dissolved in DMSO to obtain a 1 mM stock solution. This standard solution (10 μL) was added to 1 mL DMSO/H_2O mixture (2:8, *v/v*; HEPES 10 mM, pH = 7.4) containing testing anions. Blank solution without anions was prepared following the same procedure. The resulting solutions contained 10 μM of CEBI. The UV–vis absorption spectra were recorded in the wavelength range of 250–650 nm using a 1 cm quartz cell. The fluorescence emission spectra were recorded in the wavelength range of 400–700 nm at the excitation wavelength (λ_{ex}) of 360 nm. Both the excitation and emission slits were set at 5 nm.

2.5. Preparation of cells and zebrafish

The HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin and incubated under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h. Cells were seeded on a dish for fluorescence microscopic imaging by inversion fluorescence microscope.

Zebrafish (adult and embryos) samples were maintained at 28.5 °C on a 14 h light/10 h dark cycle. Embryos were grown in E3 embryo media (5 mM NaCl, 0.17 mM KCl, 0.33 mM $MgSO_4$, and 0.33 mM $CaCl_2$). The five-day-old zebrafish larvae were used for fluorescence microscopic imaging experiments, performed by an inversion fluorescence microscope.