Analytica Chimica Acta 888 (2015) 138–145

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

An effective colorimetric and ratiometric fluorescent probe for bisulfite in aqueous solution

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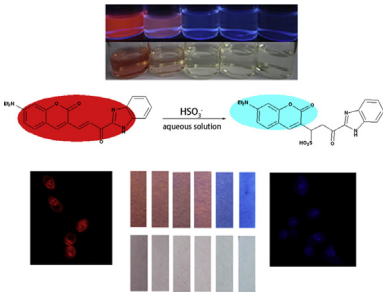
HIGHLIGHTS

- A colorimetric and ratiometric fluorescent probe was developed.
- The probe could detect bisulfite in PBS buffer solution and real samples.
- Bisulfite test paper was made to naked-eye detect bisulfite.
- This probe successfully used to living cell imaging in ratiometric manner.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 April 2015
Received in revised form
10 July 2015
Accepted 11 July 2015
Available online 10 August 2015

Keywords:
Ratiometric fluorescent
Two-photon
Bisulfite
Cell imaging

ABSTRACT

We have developed the first two-photon colorimetric and ratiometric fluorescent probe, **BICO**, for the detection of bisulfite (HSO_3^-) in aqueous solution. The probe contains coumarin and benzimidazole moieties and can detect HSO_3^- based on the Michael addition reaction with a limit of detection 5.3×10^{-8} M in phosphate-buffered saline solution. The probe was used to detect bisulfite in tap water, sugar and dry white wine. Moreover, test strips were made and used easily. We successfully applied the probe to image living cells, using one-photon fluorescence imaging. **BICO** overcomes the limitations in sensitivity of previously reported probes and the solvation effect of bisulfite, which demonstrates its excellent value in practical application.

1. Introduction

Sulfites have been widely applied in food and beverage processing as preservatives because of their ability to inhibit microbial growth and prevent discoloration [1]. However, high levels of sulfites could induce diarrhea, hypotension, allergic reactions and asthma for some individuals [2–4]. Furthermore, a great deal of sulfur dioxide is released in modern industrial processes, which causes serious environment problems. SO_2 can dissolve in water solution and form an equilibrium state between sulfite and bisulfite. In view of the deleterious effects of sulfites, sulfite intake by human body must be limited. Therefore, more analytical techniques should be developed to detect sulfites in practical conditions.

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http://dx.doi.org/10.1016/j.aca.2015.07.026
0003-2670/

To date, the existing methods to detect sulfites include chromatography, spectroscopy, electrochemistry, and capillary electrophoresis [5–9]. Among them, fluorescence spectroscopy has been widely applied in analyte detection because of its high selectivity, low detection limit and suitability for real-time monitoring [10–21]. Previously, a series of small molecule fluorescent probes for sensing sulfites were developed based on the sensing mechanisms as follows: selective de-protection of levulinate [22–27], hydrogen bond formation [28–31] and nucleophilic addition reaction [32–54]. The reported one-photon excited fluorescence probes have excitation wavelength ranges from the ultraviolet (λ_{ex} = 310 nm) [24] to the visible (λ_{ex} = 605 nm) [48]. Low-energy infrared excitation would enable deeper tissue penetration and reduce photodamage to the tissue. Compared to one-photon, the two-photon excitation wavelength is usually in the near-infrared (700–1100 nm), which has broad prospects for biological application. Although some excellent probes emit in NIR [44–52], none of these probes are used by near-infrared (NIR) excitation. Three reported probes with large emission wavelengths (λ_{em} > 640 nm) use dual excited wavelengths and respond to bisulfite and sulfite [45,48,49]. Two bisulfite probes with α,β-unsaturated ketone group exhibit good properties in practical samples, but they lack living cell imaging [46,47]. Therefore, probes with high sensitivity for cell imaging are needed.

Herein, we report a water-soluble colorimetric and ratiometric fluorescent probe **BICO** for the detection of HSO_3^- based on the Michael addition reaction. Also the probe was used to living cell imaging in ratiometric manner. The probe would have more applications after further research because of its potential two-photon fluorescent property.

2. Materials and methods

2.1. Apparatus and chemicals

Thin-layer chromatography (TLC) was conducted on silica gel 60 F_{254} plates (Merck KGaA) and column chromatography was conducted over silica gel (mesh 200–300). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were carried out on a Bruker Avance 400 spectrometer, using DMSO as solvent and tetramethylsilane (TMS) as internal standard. Melting points were determined on an XD-4 digital micro melting point apparatus. IR spectra were performed with an IR spectrophotometer VERTEX 70 FT-IR (Bruker Optics). HRMS spectra were obtained on a Q-TOF6510 spectrograph (Agilent). UV–Vis spectra were measured by using a Hitachi U-4100 spectrophotometer. One-photon fluorescent measurements were recorded on a Perkin–Elmer LS-55 luminescence spectrophotometer. Two-photon fluorescent measurements were taken on a SpectroPro300i and the pump laser beam came from a modelocked Ti:sapphire laser system with a pulse duration of 200 fs and a repetition rate of 76 MHz (Coherent Mira900-D). Quartz cuvettes with a 1 cm path length and 3 mL volume were used for all measurements. The pH measurements were done on a Model PHS-3C pH meter. Unless otherwise stated, all reagents were purchased from J&K, Sinopharm Chemical Reagent Co. and Kermel and used without further purification. Twice-distilled water was used throughout all experiments. The sodium salts were used in stock aqueous solutions. Tap water (Jinan, pH 7.2), sugar (Shandong East Sugar Company, Jinan) and dry white wine (Changyu Pioneer Wine Company Limited, Yantai) were used without any pretreatment.

2.2. Synthesis of (E)-3-(3-(1H-benzo[d]imidazol-2-yl)-3-oxoprop-1-en-1-yl)-7-(diethylamino)-2H-chromen-2-one (probe **BICO**)

7-(Diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (**1**) and 1-(1H-benzo[d]imidazol-2-yl)ethanone (**2**) were synthesized according to literature methods [55]. Compound **1** (5 mmol), **2** (7.5 mmol) and piperidine (catalyst, 1 mL) were dissolved in absolute ethanol (100 mL). The mixture was refluxed for 9 h under nitrogen. After cooling to room temperature, the red solid was filtered. Then, the crude solid was recrystallized from ethanol to obtain red product (**BICO**) in 61% yield. R_f = 0.23 (petroleum ether/ethyl acetate 2:1); mp. 287–292 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.09 (t, J = 7.0 Hz, 6H, CH_3), 3.47 (q, J = 7.0 Hz, 4H, CH_2), 6.60 (d, J = 2.0 Hz, 1H, ArH), 6.78 (dd, J = 2.0 and 9.0 Hz, 1H, ArH), 7.35–7.37 (m, 2H, ArH), 7.54 (d, J = 9.0 Hz, 1H, ArH), 7.59 (s, 1H, ArH), 7.83 (d, J = 15.8 Hz, 1H, −CO−CH=), 7.88 (s, 1H, ArH), 8.40 (d, J = 15.8 Hz, 1H, =CH−Ar), 8.45 (s, 1H, 4-H of coumarin), 13.39 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 12.83, 44.85, 96.67, 108.99, 110.43, 113.46, 121.27, 131.43, 140.31, 147.25, 149.83, 152.62, 175.04, 160.15, 181.51. IR (KBr) ν: 3250 (NH), 3063 (ArH), 2969 (CH_2CH_3), 1721 (C=O), 1563 (C=C). HRMS: m/z $[M+H]^+$ calcd for $[C_{23}H_{21}N_3O_3 + H]^+$: 388.1661, found 388.1670.

2.3. Cell culture and cell imaging

A549 cells were cultured in RPMI-1640 with 10% CBS at 2 × 10^4 cells per well. The probe was dissolved in DMSO at a storage concentration of 10 mM. Cells were adherent-cultured in 24-well culture plates for 12 h. After washing away the culture medium with phosphate-buffered saline solution (PBS), A549 cells of control group were loaded with 1.0 μM probe solution at 37 °C for 1 h. Another two groups were pretreated with 1.0 μM probe solution at 37 °C for 1 h, followed by incubation with $NaHSO_3$ for 0.5 h. Then washed 2 times with PBS and underwent imaging measurement by ultraviolet light with a confocal microscope (LSM700). The exciting light was 405 nm. The emission range of the blue channel was 400–490 nm and the red channel was 490–700 nm.

3. Results and discussion

3.1. Synthesis of probe **BICO**

Fluorophores are usually constructed by enlarging the conjugated system and aromatic heterocycles are often linked to fluorophores by a C=C bond as Michael addition receptor. However, besides sulfites, other nucleophilic ions also attack C=C double bond based on Michael addition mechanisms [56–63]. Therefore, to increase selectivity of Michael addition toward bisulfite, we designed a probe with a benzimidazole ketone and a coumarin fluorophore linked by a C=C double bond. Probe BICO was readily synthesized by Claisen–Schmidt condensation reaction from coumarin-aldehyde (**1**) and benzothiazole-ethanone (**2**) (Scheme 1) [55,64]. The structure of **BICO** was confirmed by NMR, HRMS, IR and X-ray single crystal diffraction (Fig. S1, CCDC NO. 1035510).

3.2. Property studies of probe **BICO**

All the samples were tested in PBS buffer (pH 7.4, 1 mM CTAB) without toxic organic solvents, which is efficient and environment-friendly. Cationic surfactant CTAB could build a micellar system to absorb anions to accelerate the solubility and sensitivity of the probe [65,66]. Probe **BICO** alone was red (in the web version) under visible light and exhibited an absorption maximum at 492 nm (Fig. 1A). When various analytes ($CH_3CO_2^-$, ClO_3^-, CN^-, CO_3^{2-}, $H_2PO_4^-$, HCO_3^-, HPO_4^{2-}, F^-, Cl^-, Br^-, I^-, IO^{3-}, NO_2^-, NO_3^-, S^{2-}, $S_2O_3^{2-}$, SO_3^{2-},