A fluorescent probe for colorimetric detection of bisulfite and application in sugar and red wine

Haitao Chen¹ · Xiaoming Wu¹ · Jialin Wang¹ · Hao Wang¹ · Feiyan Tao² · Shaoxiang Yang¹ · Hongyu Tian¹ · Yongguo Liu¹ · Baoguo Sun¹

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Abstract A new fluorescent probe made from (*E*)-2-(benzo[d]thiazol-2-yl)-3-(6-hydroxynaphthalen-2-yl) acrylonitrile (Probe 1) was synthesized for the determination of bisulfite concentrations in real food samples (red wine and sugar). Adding bisulfite to a Probe 1 solution caused a marked decrease in fluorescence intensity and a visual color change from yellow to light yellow. This distinct color response indicates that Probe 1 could be used as a visual sensor for bisulfite. Probe 1 can detect bisulfite

quantitatively in the range 0–400 μM with a detection limit of 0.10 μM . This makes Probe 1 a convenient signaling instrument for determining bisulfite levels in sugar and red wine samples.

Keywords Fluorescent probe · Bisulfite · Sugar · Red wine

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✉ Shaoxiang Yang
yangshaoxiang@th.btbu.edu.cn

Haitao Chen
yang123sx@126.com

Xiaoming Wu
wuxiao136ming@qq.com

Jialin Wang
15701577507@163.com

Hao Wang
2461539795@qq.com

Feiyan Tao
75444671@qq.com

Hongyu Tian
tianhy@btbu.edu.cn

Yongguo Liu
liuyg@th.btbu.edu.cn

Baoguo Sun
sunbg@btbu.edu.cn

¹ Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Key laboratory of Flavor Chemistry, Beijing Technology and Business University, No. 11 Fucheng Road, Haidian District, Beijing 100048, People's Republic of China

² Research and Development Centre, China Tobacco Sichuan Industrial Co., Ltd., Chengdu 610066, Sichuan, People's Republic of China

Introduction

Bisulfite (HSO_3^-) is commonly used in the food, drug, and beverage industries because it acts as an antibiotic, antioxidant, and antimicrobial enzyme (McFeeters, 1998; Sun et al., 2014a). However, chronic exposure to excessive levels of HSO_3^- causes harm to biomacromolecules, cells, and tissue, and can cause allergic reactions and asthmatic attacks in some individuals (Wang et al., 2017). Because of its harm to human health, the threshold levels of bisulfite in medicine and food are severely limited in most countries (Yilmaz and Somer, 2007). For example, in China, the level of sulfur (calculated by SO_2) in red wine is strictly controlled at < 0.25 g/kg and at < 0.1 g/kg in white granulated sugar (Wang et al., 2017). Therefore, the detection and limitation of bisulfite in food are essential to human health. Accordingly, there is a need for a fluorescent probe that can selectively and sensitively detect bisulfite in food.

To date, several methods have been developed to determine HSO_3^- content, such as chromatography (McFeeters and Barish, 2003), titration, capillary electrophoresis, electrochemistry (Daunoravicius and Padauskas, 2002; Lu et al., 2006) and spectrophotometry (Hassan et al., 2006). These methods have been used to determine bisulfite content in fruit, wine, starches, beverages, beer, and sugar. However, most of these methods rely on high-cost apparatus and multiple reagents, and their pretreatment processes require substantial amounts of time. Fluorescent probes have become a promising tool for the sensing and monitoring of bisulfite content due to their rapid response, simplicity, real-time detection ability, high sensitivity, and selectivity (Ding et al., 2015; Ding et al., 2016). Over the years, more fluorescent probes for bisulfite have been developed by nucleophilic reaction with aldehyde (Liu et al., 2015; Sun et al., 2012; Yu et al., 2012), C=C double bond (Sun et al., 2017) and levulinate (Chen et al., 2012; Choi et al., 2010; Gu et al., 2011), coordination to metal ions (He et al., 2014; Yuan et al., 2013), complexation with amines (Leontiev and Rudkevich, 2005; Sun et al., 2009), and Michael-type additions (Li, 2015; Peng et al., 2014; Santos-Figueroa et al., 2013; Sun et al., 2013; Sun et al., 2014a; Tan et al., 2014; Tian et al., 2013; Wang et al., 2019; Wu et al., 2013; Wu et al., 2014; Ye et al., 2018). Although many small molecule fluorescent probes have been applied to bisulfite detection, further study is urgently needed to develop efficient and practical sensors that can detect bisulfite in food. In our previous study, a new fluorescent probe was synthesized to detect bisulfite selectively relying on Michael-type additions and was applied as a testing tool to determine bisulfite levels in sugar (Wang et al., 2017). In order to find a more

responsive and visible colorimetric fluorescent probe, a new probe based on (*E*)-2-(benzo[d]thiazol-2-yl)-3-(6-hydroxynaphthalen-2-yl) acrylonitrile (Probe 1) is introduced in this work. The fluorophore of probe 1 is a naphthalene ring moiety and the reaction sites are unsaturated C=C double bonds for bisulfite. The probe shows high sensitivity and selectivity for HSO_3^- and could be used to monitor bisulfite levels in food (red wine and sugar) with high recovery.

Materials and methods

Chemicals

6-Hydroxy-2-naphthaldehyde (99%), homocysteine (Hcy, 99%), piperidine (99%), cysteine (Cys, 99%), glutathione (GSH, 99%), 2-benzothiazoleacetonitrile (98%) were gotten from Bailing Wei (Beijing, P. R. China). Analytes sodium acetate trihydrate (CH_3COONa), sodium chloride (NaCl), sodium thiocyanate (NaSCN), fluoride (NaF), ammonium chloride (NH_4Cl), sodium carbonate (Na_2CO_3), sodium bicarbonate (NaHCO_3), sodium bromide (NaBr), sodium sulfite (Na_2SO_3), potassium iodide (KI), sodium sulfate (Na_2SO_4), sodium nitrate (NaNO_3), sodium nitrite (NaNO_2), sodium hydrogen sulfite (NaHSO_3), calcium chloride (CaCl_2), dimethyl sulfoxide (DMSO), ethanol ($\text{CH}_3\text{CH}_2\text{OH}$) and methanol (CH_3OH) were HPLC grade bought from Banxia Company.

Instruments

Nuclear magnetic resonance (NMR) spectra were gotten on a Bulu Ke AV 600 MHz NMR and TMS was used as internal standard. The high-resolution mass spectrum (HRMS) was carried out at a Bulu Ke Apex IV FTMS. Fluorescence spectra were observed, recorded on a Ri Li F-4600 fluorescence spectrometer. UV-Vis spectra was taken down on a Shimadzu UV-2600 spectrometer.

Preparation of Probe 1

6-Hydroxy-2-naphthaldehyde (**1**, 1.72 g, 0.01 mol) and 2-benzothiazoleacetonitrile (**2**, 1.74 g, 0.01 mol) were dissolved in methanol (25 mL) with piperidine (0.10 g, 0.012 mol). The reaction mixture was refluxed for 3 h. The methanol was distilled off and the product was washed with methanol. The crude product was crystallized again, to give pure (*E*)-2-(benzo[d]thiazol-2-yl)-3-(6-hydroxynaphthalen-2-yl) acrylonitrile (probe 1, 2.53 g).

Preparation of analytes and Probe 1

Probe 1 stock solution (1 mM) was dissolved with DMSO. Analytes Cys, GSH, Hcy, CH_3COONa , NaSCN , NH_4Cl , Na_2CO_3 , NaBr , NaHCO_3 , Na_2SO_3 , NaF , Na_2SO_4 , KI , NaNO_3 , NaNO_2 , NaCl , NaHSO_3 , CaCl_2 were prepared by distilled water to obtain 10 mM aqueous solution (Ye et al., 2018). Distilled water was used to dilute the stock solution and were used at once.

Preparation of sugar and red wine

Sugar and red wine were random purchased from wumei market (Beijing), sugar were dissolved in deionized water (Wang et al., 2017). Crystal sugar (carbohydrate 99.2 g/100 g), granulated sugar (carbohydrate 92.2 g/100 g), soft sugar (carbohydrate 98.9 g/100 g); red wine A (alcohol content, 12%; type, dry), red wine B (alcohol content, 13%; type, dry), red wine C (alcohol content, 12.5%; type, dry). Samples were added different concentrations of NaHSO_3 , the fluorescence signal of all these samples at 525 nm were recorded.

The procedures of HSO_3^- determination

The preparation of test system: Firstly, 0.48 mL DMSO and 0.02 mL probe solution were mixed in cuvette. Secondly, buffer solution was poured into the cuvette and made the capacity of cuvette up to 2 mL. Finally, ion solution was added. After waiting 5 min, it was mixed completely. Fluorescence spectrophotometer conditions: $\lambda_{\text{ex}} = 398 \text{ nm}$, $\lambda_{\text{em}} = 525 \text{ nm}$, temperature: 25°C . Voltage: 700 v. slit width: 10 nm, 10 nm. Sensitivity: 2.

Statistical analysis

All tests were repeated three times. The results were showed as standard deviation. It was performed with origin 8.5 software (OriginLab Corporation, USA). The molecular structures were draw by ChemBioOffice 2008 (CambridgeSoft, UK).

Results and discussion

Probe synthesis

Probe 1 was synthesized through a Knoevenagel condensation reaction of 6-hydroxy-2-naphthaldehyde and 2-benzothiazoleacetonitrile over a piperidine catalyst (Fig. 1). Probe 1 was isolated from simple recrystallization. This synthetic method was facile and the raw materials are common. The probe 1 was characterized by ^1H NMR,

HRMS and ^{13}C NMR (Figures A1-3, in supporting information).

^1H NMR (600 MHz, DMSO), δ (ppm): 10.30(s, OH), 8.49(s, 1H), 8.45(s, 1H), 8.18(d, $J = 7.1 \text{ Hz}$, 1H), 8.17(d, $J = 7.5 \text{ Hz}$, 1H), 8.08(d, $J = 9.0 \text{ Hz}$, 1H), 7.90(d, $J = 8.7 \text{ Hz}$, 1H), 7.86(d, $J = 8.7 \text{ Hz}$, 1H), 7.58(t, $J = 15.3 \text{ Hz}$, 1H), 7.50(t, $J = 15.3 \text{ Hz}$, 1H), 7.21(s, 1H), 7.20(dd, $J = 9.0 \text{ Hz}$, 2.1 Hz, 1H). ^{13}C NMR (125 MHz, DMSO): δ 164.15, 158.81, 153.44, 148.83, 137.08, 134.69, 134.18, 131.72, 127.55, 127.53, 127.48, 127.24, 126.58, 125.28, 123.45, 122.87, 120.43, 117.13, 109.65, 103.47. HRMS: calcd. $[\text{M}-\text{H}]^+$ 327.05976, found 327.05950.

The sensing property of probe 1 for HSO_3^-

The fluorescent spectral responses of Probe 1 (10 μM) and Probe 1- HSO_3^- (300 μM) under various pH conditions (3.0–8.0) were observed at 25°C (Fig. 2A). The fluorescence intensity of free Probe 1 was unchanged over the pH range, while that of Probe 1- HSO_3^- decreased quickly from pH 3.0–6.0, then increased from pH 6.0–8.0. The fluorescence intensity differences between Probe 1 and Probe 1- HSO_3^- were greatest at pH 6.0. Hence, pH 6.0 was chosen for use in further experiments.

Probe 1 (10 μM) had stronger fluorescence in PBS (pH 6.0, PBS:DMSO = 3:1) at 25°C . As HSO_3^- (300 μM) was added, the fluorescence intensity at 525 nm declined by almost four-fold (Fig. 2B) and the color changed from yellow to light yellow (Fig. 1C).

The fluorescence spectral response of Probe 1 (10 μM) for HSO_3^- (300 μM) was observed in 10 mM PBS (pH 6.0, PBS:DMSO = 3:1). As shown in Fig. 3A, the fluorescence intensity was observed each minute and decreased quickly after adding 300 μM HSO_3^- . The fluorescence signal at 525 nm was recorded. Fluorescence and HSO_3^- saturation were reached in 10 min (Fig. 3B). This confirms that Probe 1 took 10 min to react with HSO_3^- completely.

To verify that Probe 1 has the ability to quantitatively detect HSO_3^- in PBS solution, the fluorescence intensity of Probe 1 (10 μM) with different concentrations of HSO_3^- added (0–500 μM) was measured (Fig. 3C). As shown in Fig. 3D, by plotting the data from Probe 1 reacting with different concentration of HSO_3^- , a good calibration curve was acquired. The fluorescence intensity was linearly connected with concentrations of HSO_3^- ranging 0–400 μM , and the detection limit (LOD) was counted as $1 \times 10^{-7} \text{ M}$ based on $\text{S/N} = 3$ according to the definition of a previous report. The fast response and excellent linear relationship provide a real-time quantitative method of HSO_3^- detection.

To explore the sensitivity of Probe 1 for HSO_3^- detection, various compounds, including AcO^- , Br^- , Cl^- , I^- , SCN^- , NO_3^- , NO_2^- , SO_3^{2-} , SO_4^{2-} , HCO_3^- , CO_3^{2-} , Cys,

Fig. 1 Synthesis of probe 1

Fig. 2 (A) Fluorescent intensity of probe 1 (10 μM) in the absence and presence of HSO_3^- (300 μM) in different pH buffer solution; (B) fluorescence spectra of probe 1 (10 μM) and probe 1 (10 μM) with HSO_3^- (300 μM) in PBS (pH 6.0) with DMSO (v/v, 3:1) at 25 $^\circ\text{C}$; (C) the color changed of probe 1 (10 μM) in the absence and presence of HSO_3^- (300 μM). “PBS” is phosphate buffer solution

Hcy, GSH, F^- , HSO_3^- , Na^+ , K^+ , NH_4^+ , and Ca^{2+} were tested. As shown in Fig. 4A, these competitors resulted in limited fluorescence responses, having nearly no interference for HSO_3^- detection. This suggests that Probe 1 is highly selective for HSO_3^- and is not interfered with by

other competing anions. Only HSO_3^- caused the color of the Probe 1 solution to change from yellow to light yellow (Fig. 4B). Therefore, Probe 1 has the specificity and ability to detect HSO_3^- under complicated conditions.

Fig. 3 (A) Time-dependent fluorescence spectra of probe 1 (10 μM) in the presence of HSO₃⁻ (300 μM); (B) time-dependent fluorescence intensity changes of probe 1 (10 μM) in the presence of HSO₃⁻ (300 μM); (C) fluorescence spectra of probe 1 (10 μM) with HSO₃⁻

(0–500 μM); (D) the plot of fluorescence intensity difference with HSO₃⁻ from 0 to 400 μM in PBS (10 mM, pH 6.0). The test was repeated 3 times

Reaction mechanism

To demonstrate the identification mechanism of Probe 1 for HSO₃⁻, HRMS and ¹H NMR titration were carried out. As shown in supplementary Figure S6, the proton signal of the bridge double bond at 8.49 ppm (H8) disappeared and a new peak appeared at 6.94 ppm (H8'). To gain some insights into this mechanism, measurement of the UV–Vis absorption of Probe 1 and Probe 1-SO₃H were necessary (Figure A4, supporting information). The addition of HSO₃⁻ led to a decreasing trend in the UV–Vis spectral intensity, suggesting that the C=C bonds are interrupted by nucleophilic addition (Figure A5, supporting information). The mechanism was further confirmed by HRMS, in which the peak appeared at *m/z* 407.01767 (calcd. 407.01646), corresponding to [M – 2H]⁻ (Figure A6, supporting information). These results suggest that the reaction mechanism by which Probe 1 reacts with HSO₃⁻ is via nucleophilic addition with carbon–carbon double bonding (Fig. 5).

Detection of HSO₃⁻ in sugar and red wine

Bisulfite is used widely in food as a preservative and antioxidant but excess amounts have negative health implications. We applied Probe 1 to detect HSO₃⁻ in real samples to verify the practical applicability of Probe 1 in food. Crystal sugar, soft sugar, granulated sugar, and three different red wines were obtained from Wumei Market, Beijing. Deionized water was used to dissolve sugar samples (Sun et al., 2014b; Wang et al., 2017). The wines and sugar solutions were added to PBS (pH 6.0, 10 mM), including Probe 1, and the fluorescence signals at 525 nm were recorded for data analysis. As shown in Table 1, the HSO₃⁻ levels in the three kinds of red wine were 0.7, 1.2, and 0.5 μM. In crystal sugar it was 0.6 μM, in granulated sugar it was 1.4 μM, and in soft sugar it was 0.6 μM. Then, different concentrations of HSO₃⁻ were added to the red wine and sugar samples. Probe 1 determined HSO₃⁻ concentrations with high recovery rates ranging from 94.34 to 105.10%. To prove that Probe 1 can detect HSO₃⁻ accurately, the titration method was used to analyze the

Fig. 4 (A) Fluorescence intensity change of probe 1 (10 μM) upon addition of various species (300 μM for each. 1, blank; 2, AcO^- ; 3, Br^- ; 4, Cl^- ; 5, I^- ; 6, SCN^- ; 7, NO_3^- ; 8, NO_2^- ; 9, SO_3^{2-} ; 10, SO_4^{2-} ; 11, HCO_3^- ; 12, CO_3^{2-} ; 13, Cys; 14, Hcy; 15, GSH; 16, F^- ; 17, Na^+ ; 18, K^+ ; 19, NH_4^+ ; 20, Ca^{2+} . 300 μM for HSO_3^-). The test was repeated 3 times; (B) the solution color of probe 1 + various compounds and probe 1 + various compounds + HSO_3^- (300 μM for each. 1, AcO^- ; 2, Br^- ; 3, Cl^- ; 4, I^- ; 5, SCN^- ; 6, NO_3^- ; 7, NO_2^- ; 8, SO_3^{2-} ; 9, SO_4^{2-} ; 10, HCO_3^- ; 11, CO_3^{2-} ; 12, Cys; 13, Hcy; 14, GSH; 15, F^- ; 16, Na^+ ; 17, K^+ ; 18, NH_4^+ ; 19, HSO_3^- ; 20, Ca^{2+} . 300 μM for HSO_3^-)

Fig. 5 The mechanism for probe 1 with HSO_3^-

concentrations of HSO_3^- in red wine and sugar. The relative deviation ranged from 3.22 to 8.69% (Table A1, supporting information). The results demonstrate that Probe 1 performs well as a probe for the quantitative detection of HSO_3^- in food.

A comparison of the chemical structures, detection limits, and response times of the proposed probe and some previously reported HSO_3^- fluorescent probes is listed in supplementary Table A2. These detection limit and response time of Probe 1 are comparable to those of other reported probes. In our previous study (Wang et al., 2017), a new fluorescent probe was synthesized to detect HSO_3^- in sugar, but the visible colorimetry was not good enough.

The present study shows that Probe 1 has a novel structure and is very easy to synthesize. Upon the addition of HSO_3^- , Probe 1 has an obvious color change from yellow to light yellow. Probe 1 was successfully used to detect HSO_3^- concentrations in sugar and red wine. Furthermore, this experimental method is a friendly environment, with a reduction in the use of toxic reagents and risks to the analyst and the generation of residues. Probe 1 is applicable only to liquid phase analysis, solid samples must first be dissolved in solution.

In summary, we have developed a responsive and visible color-changing fluorescent probe for the detection of HSO_3^- . The function of Probe 1 relies on nucleophilic

Table 1 Determination of HSO_3^- concentrations in sugar and red wine samples

Sample	HSO_3^- detected (μmol)	HSO_3^- added (μmol)	HSO_3^- detected (μmol)	Recovery (%)	RSD (%; $n = 3$)
Crystal sugar	0.60 ± 0.01	10.00	11.00	103.78	0.18
		30.00	32.00	104.58	0.13
Granulated sugar	1.40 ± 0.03	10.00	11.00	96.50	0.09
		30.00	33.00	105.10	0.12
Soft sugar	0.60 ± 0.02	10.00	10.00	94.34	0.30
		30.00	30.00	98.04	0.13
Wine A	0.70 ± 0.01	10.00	10.40	97.20	0.28
		30.00	30.50	99.35	0.10
Wine B	1.20 ± 0.02	10.00	11.40	101.79	0.24
		30.00	30.90	99.04	0.13
Wine C	0.50 ± 0.01	10.00	10.70	101.91	0.27
		30.00	30.60	100.33	0.10

The test was repeated 3 times

addition to C=C double bonds, which was verified by HRMS and ^1H NMR studies. Importantly, once reacted with HSO_3^- , the color of Probe 1 solution visibly alters from yellow to light yellow. The color change is so obvious that Probe 1 can be used in practical applications for HSO_3^- detection. Finally, we have shown that this probe can be used to measure HSO_3^- quantitatively in sugar and red wine samples. Therefore, this probe has practical significance for the detection of HSO_3^- in a wide range of food sampling conditions. Further studies are underway to develop a more rapid and selective fluorescent probe with a more obvious color response.

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Compliance with ethical standards

Conflict interest All authors declare no conflict of interest.

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