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A colorimetric and ratiometric fluorescent probe for rapid and sensitive detection of sulfite in sugar

Xiaoming Jiang, Junchao Xu, Youlai Zhang, Hongying Wang, Lintao Zeng, and Yue Zhang

A colorimetric and ratiometric fluorescent probe was developed for the detection of sulfite in sugar. The probe underwent a Michael addition reaction with sulfite, which resulted in significant changes in fluorescence ratios. The probe displayed fast response (within 30 s) and high selectivity towards sulfite over other anions and bio-thiols. The fluorescence intensity ratios were linearly related to the concentrations of SO$_3^{2-}$ ranging from 0 to 15 µM, and the limit of detection was determined to be as low as 12 nM. The probe was employed to determine the concentration of sulfite in sugar with good recoveries.

Introduction

In the food and beverage industry, sulfite (SO$_3^{2-}$) is widely used as preservatives and additives to extend the shelf-life of food and beverage. However, epidemiological studies demonstrate that sulfite is correlated to the cardiovascular diseases such as ischemic heart diseases, myocardial ischemia, spontaneous hypertension and hypoxic pulmonary hypertension. Besides, excess amount of sulfite can cause asthma and allergic reactions in some individuals. Owning to these harmful effects for human, the threshold levels of SO$_3^{2-}$ in food and beverage have been strictly controlled in many countries. To guarantee food safety, it is necessary to monitor the levels of sulfite in food and beverage.

There are some conventional methods for the detection of sulfite, such as titrimetry, chromatography, electrochemistry, capillary electrophoresis and flow injection analysis. Unfortunately, these methods are time-consuming and require complicated pre-treatments, which limits their application. Fluorescent probe is a promising technique due to its non-invasiveness, high sensitivity, high temporal and spatial resolution. Up to date, a number of fluorescence probes for sulfite have been developed based on nucleophilic reaction with aldehyde, the selective deprotection of levulinic acid and the coordination to metal ions. However, these probes have some disadvantages, such as long respond time (5 min—10 h), unsatisfactory limit of detection and interference from biothiols, proteases or esterases, which limit their application. Besides, most of these probes respond to sulfite with changes only in fluorescent intensity, which suffers from errors associated with probe concentration, environment effects and instrumental factors. In contrast, ratiometric fluorescent probes can utilize the ratios of two emissions at different wavelengths as the detecting signal, which are less impressionable to the above mentioned factors and provides more accurate analysis. Therefore, it is highly desired to develop some novel fluorescent probes with colorimetric and ratiometric responses towards sulfite. Recently, some ratiometric fluorescent probes for sulfite have been developed based on Michael addition. However, there is still plenty room for improvement in terms of selectivity and sensitivity.

Herein, we reported a novel colorimetric and ratiometric fluorescent probe for sulfite, as shown in Scheme 1. The probe would undergo Michael addition reaction with SO$_3^{2-}$, and a noticeable fluorescence change from yellow to cyan could be observed. The probe showed high selective response towards SO$_3^{2-}$ over other anions and bio-thiols in PBS buffer (pH = 7.4, 10 mM). Furthermore, the probe was used to detect SO$_3^{2-}$ in sugar.

Results and discussion

Spectral response of the probe PCN toward SO$_3^{2-}$

The UV-vis absorption spectrum of probe PCN (10 µM) was measured in DMSO/PBS solution (v/v = 1/1, pH 7.40), which was the optimized test condition (Fig. S1). As shown in Fig. 1a, PCN displayed a broad absorption band centred at 430 nm...
with yellow colour (Fig. 1a inset). Upon addition of increasing amount of SO$_3^{2-}$ (0–30 μM), the maximum absorption peak at 430 nm gradually decreased and a new absorption band at 345 nm appeared with a well-defined isosbestic point at 358 nm. When the concentration of SO$_3^{2-}$ reached to 30 μM, these changes in the absorption spectra were found to reach a plateau. Meanwhile, the colour of the solution changed from yellow to colourless (Fig. 1a inset), which suggested that the probe PCN could serve as a colorimetric probe for SO$_3^{2-}$. The absorbance ratios ($A_{345}/A_{150}$) was linearly ($R^2 = 0.9907$) related to the concentrations of SO$_3^{2-}$ ranging from 0 to 15 μM (shown in Fig. 2a), which indicated that PCN could be used to quantitatively detect SO$_3^{2-}$.

The probe PCN itself exhibited an emission band centred at 535 nm in DMSO/PBS solution (v/v = 1/1, pH 7.40), as shown in Fig. 1c. Upon treatment of the probe with increasing amount of SO$_3^{2-}$ (0–30 μM), the maximum fluorescence band at 535 nm remarkably decreased and concomitantly a new emission band appeared at 398 nm, providing a ratiometric manner for the detection of SO$_3^{2-}$. The newly formed emission band centred at 398 nm was increased progressively until the concentration of SO$_3^{2-}$ reached to 30 μM. By plotting the fluorescence intensity ratios ($I_{398}/I_{535}$) versus the concentrations of SO$_3^{2-}$, a good linear relationship was obtained with the concentration ranging from 0 to 15 μM, as shown in Fig. 2b. The limit of detection was calculated to be 12 nM based on signal to noise ratio ($S/N = 3$), which was much lower than the threshold levels of Na$_2$SO$_3$ in food (< 30 mg kg$^{-1}$). To demonstrate practical application of the probe, we tentatively made a paper test strip for the detection of SO$_3^{2-}$. Neutral filter papers were dipped into PCN solution (0.10 mM) for 10 min, and then was dried at ambient temperature. The test paper display yellow emission under UV light (365 nm).

When various concentrations of SO$_3^{2-}$ were dropped onto the test paper, it displayed different colour from yellow to cyan, as shown in Fig. 2c.

**Time-course and pH-dependent fluorescence responses of PCN towards SO$_3^{2-}$**

The time-course fluorescence responses of PCN (10 μM) towards SO$_3^{2-}$ (30 μM) were measured, and the fluorescence intensity at 398 nm and 535 nm was plotted as a function of time (s) for data analysis, as shown in Fig. 1d. Upon the addition of 30 μM SO$_3^{2-}$, the fluorescence intensity of PCN promptly increased and reached to a plateau within 30 s, suggesting that PCN could serve as a “fast response” fluorescent probe for SO$_3^{2-}$.

The pH-dependent fluorescence responses of PCN toward SO$_3^{2-}$ were also investigated, as shown in Fig. 3. The fluorescence intensity ratios of PCN were invariable from pH 4.0 to 9.0, suggesting that this probe was stable at a wide range of pH values. Upon the addition of 30 μM SO$_3^{2-}$, the fluorescence intensity of PCN increased drastically at pH values
The sensing mechanism

$^1$H NMR titration experiments were performed to investigate the sensing mechanism. As shown in Fig. 5, the proton signal at 9.37 ppm was ascribed to the vinyl group of the probe. Upon addition of 3.0 equiv. of SO$_2$$^2$-, the proton signal at 9.37 ppm shifted to 5.08 ppm, implying that the vinyl group of PCN was interrupted by SO$_2$$^2$-. Moreover, the formation of a PCN–Na$_2$SO$_3$ adduct was confirmed by high-resolution mass spectroscopy (Fig. S7), where a dominant peak at an m/z value of 406.0745 (calcd 406.0749) was corresponding to [PCN + HSO$_3$]$.^-$ These results confirmed that SO$_2$$^2$- added to the vinyl group of PCN following Michael addition reaction (shown in Scheme 1).

![Image](image_url)

**Fig. 5** Partial $^1$H NMR spectra of (a) PCN in DMSO-d$_6$ and (b) PCN + Na$_2$SO$_3$ in DMSO-d$_6$–D$_2$O.

**Detection of SO$_2$$^2$- in real samples by the probe**

Finally, we used the probe PCN to detect SO$_2$$^2$- in real samples. Granulated sugar and crystal sugar were purchased from a supermarket, and were used directly in the experiment. To prepare stoke solution, 1.0 g of sugar was dissolved in twice-distilled water and diluted to 10 mL. Aliquots of the sugar solutions were added to the PCN solution (10 µM) in PBS solution (DMSO/PBS = 1/1, pH 7.4), and the emission intensities at 535 and 398 nm were recorded (Fig. S3). Table 1 shows that the probe PCN can determine the concentration of SO$_2$$^2$- in sugar with good recovery. The amount of Na$_2$SO$_3$ in granulated sugar and crystal sugar were determined to be 18.5 and 15.3 mg/kg, respectively.

<table>
<thead>
<tr>
<th>Samples</th>
<th>SO$_3$$^2$- content/(µM)</th>
<th>Added/(µM)</th>
<th>Found/(µM)</th>
<th>Recovery/%</th>
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<td>2.0</td>
<td>4.16</td>
<td>97.5</td>
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<tr>
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<tr>
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<td>5.97</td>
<td>103.5</td>
<td></td>
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</tbody>
</table>

**Experimental**

**Materials and Instrument**

Unless otherwise stated, all chemicals and solvents were of analytical grade and used without further purifications. Pyrene-2-carboxaldehyde and ethyl cyanoacetate were purchased from Aladdin. The $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AV-400 spectrometer with tetramethysilane (TMS) as the internal standard. The chemical shift was recorded in ppm and the following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet. Mass spectra were measured by a HP-1100 LC-MS spectrometer. UV-vis spectra were recorded on a Hitachi UV 3310 spectrometer. Fluorescence spectra were recorded on a Hitachi FL-4700 fluorometer. Fluorescent images were acquired on a Nikon A1 confocal laser-scanning microscope with a 100 objective lens. The solvents used for UV-vis and fluorescence measurements were of HPLC grade.

**Responses of probe toward various analytes**

Stock solution of the probe (1 mM) was prepared in HPLC grade ethanol. Stock solutions of analytes (2.5–5 mM) were...
prepared in twice-distilled water. For optical measurements, probe was diluted to 10 μM in DMSO/PBS solution (v/v = 1/1, pH 7.4), and 3.0 mL of the resulting solution was placed in a quartz cell of 1 cm optical path length each time. The UV-vis or fluorescent spectra titrations were recorded upon addition of analytes. All spectoscopic experiments were carried out at room temperature.

Preparation and characterization of probe

1-Pyrenecarboxaldehyde (230 mg, 1 mmol) and ethyl 2-cyanoacetate (125 mg, 1.1 mmol) were dissolved in 5 mL of dry ethanol, then piperidine (0.1 mL) and acetic acid (0.1 mL) were added. The reaction mixture was heated at 80 °C under nitrogen atmosphere. After the reaction was completed (monitored by TLC), the reaction mixture was cooled, and the product precipitated from the solution. Then, it was filtrated, and the solid was collected. The crude product was recrystallized with ethanol to give the final product as light yellow needles (286 mg, 86%). 1H NMR (400 MHz, DMSO-d6) δ (ppm) 9.37 (s, 1H, =CH2), 8.70 (d, J = 8.0 Hz, 1H, Ar-H), 8.48 – 8.43 (m, 5H, SH, Ar-H), 8.39 (d, J = 8.8 Hz, 1H, Ar-H), 8.28 (d, J = 8.8 Hz, 1H, Ar-H), 8.19 (t, J = 7.6 Hz, 1H, Ar-H), 7.43 (q, J = 7.0 Hz, 2H, -CH2), 1.39 (t, J = 7.0 Hz, 3H, -CH3). 13C NMR (100 MHz, CDCl3) δ (ppm) 162.91, 152.02, 134.76, 131.44, 131.07, 130.39, 130.23, 129.78, 127.34, 127.06, 126.87, 126.65, 126.25, 125.05, 124.67, 124.60, 124.20, 121.83, 116.13, 104.08, 67.29, 14.28. HR-MS (ESI): calculated for [C22H22N2O2 + H]+ 326.1181; Found 326.1138.

Conclusions

In summary, a colorimetric and ratiometric fluorescent probe for SO4 2− was developed based on Michael addition reaction. The probe showed high selectivity and sensitivity toward SO4 2− with fast response and a low limit of detection (12 nM). The fluorescence intensity ratios were linearly related to the concentrations of SO4 2− from 0 to 15 μM, which indicated that PCN could be used to quantitatively detect SO4 2−. A simple test paper was developed for rapid monitoring of SO4 2−, which displayed different colour in the presence of various amounts of sulphite. Moreover, the probe has been successfully used to determine the levels of SO4 2− in sugar samples. Therefore, this probe has great potential application for food safety and quality control.

Acknowledgements

This work was financially supported by NSFC (No. 21203138, 31100301, 21303120), and the Natural Science Foundation of Tianjin (13JCQNJC05300, 13JCJBCJ42100).

Notes and references

A colorimetric and ratiometric fluorescent probe shows fast response and high selectivity towards sulfite with low detection limit (12 nM).