A Water-soluble fluorescent probe for rapid detection of sulfur dioxide derivatives

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Abstract: The content of sulfur dioxide derivatives in cells is closely related to life and health. Therefore, it is essential to detect the content of sulfur dioxide derivatives in cells under physiological conditions to ensure life and health. In this paper, a novel sulfur dioxide derivative fluorescent water-soluble probe \((\text{E})-1,1,3\text{-trimethyl}-2-(2-(\text{naphthalen}-1\text{-yl})\text{vinyl})-1H-3\lambda^4\text{-benzo}[e]\text{indole}, \text{TNB})\) was synthesized by using naphthalene formaldehyde (1) and 1,1,2,3-tetramethyl-1H-3\lambda^4\text{-benzo}[e]\text{indole} (2) as raw materials. Based on the intramolecular charge transfer (ICT) mechanism of naphthalene ring to benzoindole, TNB exhibits very weak fluorescence emission in PBS buffer (pH=7.4). The olefin unit of TNB can be combined with \(\text{HSO}_3^-/\text{SO}_3^{2-}\) with high selectivity to make \(\pi-\pi\) conjugate interrupt. ICT is blocked, TNB produces a strong fluorescence emission, and the color visible to the naked eye changes from yellow to colorless. The effect of TNB on \(\text{HSO}_3^-/\text{SO}_3^{2-}\) can be completed in 40 s, the fluorescence intensity is increased by 91 times, and the detection limit is as low as 0.089 \(\mu\)M. It is expected to be able to rapidly detect low levels of sulfur dioxide derivatives in cells, which has important application prospects in maintaining life and health.

Keywords: Sulfur dioxide derivatives, Fluorescent, Intramolecular charge transfer

Introduction

With the burning of fossil fuels in human production, a large amount of sulfur dioxide \((\text{SO}_2)\) is produced, which is a main atmospheric pollutant, is easily absorbed by the human body under humid conditions and converted into bisulfite \((\text{HSO}_3^-)\) and sulfite \((\text{SO}_3^{2-})\), and affects human health.\(^1\)-\(^3\) In a physiological environment, sulfite and bisulfite are present in an aqueous solution in a ratio of 3:1.\(^4\)-\(^6\) Sulfite and bisulfite have excellent antioxidant capacity, thereby reducing the growth capacity of microorganisms; therefore, it is widely used in the food preservation industry.\(^7\)-\(^10\) At the same time, endogenous sulfites and bisulfites can also be produced in the human body, playing an important role in life activities. Medical research has shown that high concentrations of bisulfite in humans can disrupt physiological balance, induce cardiovascular disease, nervous system diseases, and a series of allergic reactions;\(^11\)-\(^14\) therefore, the development of a simple and efficient method for the detection of sulfur dioxide derivatives has important practical significance for food safety and life microenvironment detection.

Traditional methods for detecting sulfur dioxide derivatives include titration,\(^15\) electrochemical method,\(^16\) spectrophotometry,\(^17\) high performance liquid chromatography (HPLC),\(^18\) piezoelectric sensors,\(^19\) flow injection analysis, and Capillary electrophoresis\(^20\) and other methods; however, these methods generally have problems such as complex operating system, strong intrusion, and poor sensitivity, which limits their imaging applications.\(^21\) In recent years, fluorescent technology has been widely applied and studied because of its simple operation, high sensitivity, high temporal and spatial resolution, and the ability to detect in vitro and bioimaging.\(^21\)-\(^28\) Although most fluorescent probes currently have high sensitivity and selectivity, these fluorescent probes require an organic
solvent to participate in solubilization, the reaction time with HSO$_3^-$ / SO$_3^{2-}$ is longer (100s), the response environment is limited to acidic conditions (usually pH = 5), which limits their use in physiological detection.\textsuperscript{7,29-31} This paper attempts to design a novel water-soluble colorimetric fluorescent probe based on ICT mechanism for rapid, high sensitivity and specific detection of sulfur dioxide derivatives in physiological environment.

So far, several fluorescent probes have been developed to monitor bisulfite/sulfite. Sun's team designed a novel proportional two-photon fluorescent probe (MBCB) based on a carbazole skeleton for mitochondria. Double detection of SO$_2$ derivatives and viscosity with a response time of 600 s.\textsuperscript{32} Zhao's group constructed a high-efficiency FRET platform proportional fluorescent probe (L-HF3) for the development of a method for detecting sulfur dioxide derivatives, but the disadvantage is that the probe needs to be in an environment containing 40\% EtOH for detection purposes. the reaction time is up to 30 min.\textsuperscript{33} Studies have shown that HSO$_3^-$ / SO$_3^{2-}$ can react with α,β-unsaturated compounds and the reaction rate is fast. Based on this reaction principle, a novel water-soluble fluorescent probe ((E)-1,1,3-trimethyl-2-(2-(naphthalen-1-yl)vinyl)-1H-3λ$^4$-benzo[e]indole, TNB) was designed and synthesized (Figure 1). It is connected to the electron withdrawing group of benzoindole at the 1 position of the naphthalene ring through a double bond, which constitutes the A–π–A’ structure, making the nucleophilic reaction more likely to occur, and selects the benzofluorene unit as the hydrophilic group. It can improve the water solubility of the probe and enhance the application of the probe in physiological detection. Due to the intramolecular charge transfer (ICT) effect in the dipolar dye, the probe exhibits fluorescence quenching in PBS solution, when a small amount of HSO$_3^-$ aqueous solution is added, the fluorescence intensity is significantly enhanced, compared with the previous probes\textsuperscript{29-33}, the probe TNB has excellent selectivity, no organic solvent is involved, high sensitivity and extremely fast response time (40s). The reaction products of TNB and HSO$_3^-$ were studied by $^1$H NMR and HR-MS spectroscopy, and the possible mechanism was proposed. At the same time, the color of the probe changed from yellow to colorless after adding a certain amount of HSO$_3^-$, which indicates that the probe has broad application prospects in the naked eye recognition of HSO$_3^-$.

Materials and Method

All chemicals and solvents were purchased from Macleans Reagent, and no further purification was required unless otherwise stated. The water used in the experiment was deionized water. 1 mM probe stock solution and NaHSO$_3$, Na$_2$S, NaHSO$_4$, Na$_2$SO$_4$, Na$_2$HPO$_4$, Na$_3$PO$_4$, NaNO$_2$, NaH$_2$PO$_4$, NaNO$_3$, NaF, NaBr, Na$_2$CO$_3$, NaHCO$_3$, CH$_3$COONa, NaOH, Hcy, Cys, and GSH solution (10 mM) were prepared using deionized water. The absorption spectrum was measured using an ultraviolet-visible spectrophotometer (Shimadzu, Japan). Fluorescence emission spectra were recorded using an F-7000 fluorescence spectrophotometer (Hitachi, Japan). Different pH standard PBS (10 mM) solutions were prepared using a digital pH meter. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Varian INOVA-400 nuclear magnetic resonance spectrometer using TMS as an internal reference. High resolution spectral analysis (HRMS) was performed on an LTQ Orbitrap XL mass spectrometer. All experiments were carried out at room temperature.

Synthetic route

The synthetic route of the probe TNB is shown in Figure 1.
**Synthesis of probe TNB**

In a 25 ml round bottom flask, compound 1 (3 mmol) and 1-naphthaldehyde (3 mmol) were dissolved in anhydrous methanol (8 ml), then a drop of piperidine was added dropwise to the mixture and heated to 60 °C and after stirring for 2 h, thin-layer-chromatography (TLC) was monitored until the end of the reaction (eluent: methylene chloride / methanol = 10/1). The mixture was cooled to room temperature and separated by column chromatography (eluent: dichloromethane / methanol = 10/1). The dark yellow solid was obtained with a probe TNB yield of 85%. 

**1H NMR** (400MHz, DMSO-d6) δ 9.16 (d, J = 16.0 Hz, 1H), 8.56 (d, J = 4.0 Hz, 1H), 8.50 (d, J = 8.0 Hz, 2H), 8.39 (dd, J = 12.0 Hz, 1H), 8.30-8.14 (m, 4H), 7.92-7.72 (m, 6H), 4.42 (s, 3H), 2.13 (s, 6H).

**13C NMR** (100MHz, DMSO-d6) δ 183.34, 148.00, 140.04, 138.64, 133.86, 133.80, 133.73, 131.73, 131.57, 131.44, 130.53, 129.01, 128.48, 127.99, 127.33, 126.34, 123.84, 123.74, 115.69, 113.99, 54.54, 36.19, 25.50. 

HRMS (ESI Positive) calc. for C$_{27}$H$_{24}$N$_{2}$ $\ [+M]^{+}$ 362.1906. found 362.1903.

**Determination of UV-Vis absorption and fluorescence spectra**

The probe TNB was dissolved in deionized water to obtain a probe solution (1 mM). NaH$_2$SO$_3$, Na$_2$S, NaH$_2$SO$_4$, Na$_2$SO$_4$, Na$_2$HPO$_4$, Na$_2$PO$_4$, NaNO$_2$, Na$_2$HPO$_4$, NaNO$_3$, NaF, NaBr, Na$_2$CO$_3$, NaHCO$_3$, CH$_3$COONa, NaOH, Hcy, Cys, and GSH solution (10 mM) were prepared using deionized water. Add the probe stock solution (40 μL) and the appropriate analyte to the ep tube (4.0 mL) and dilute to the mark with different ratios of DMSO:PBS buffer (pH 7.4) to prepare a 4.0 mL solution for spectrometry. The UV-Vis absorption spectrum data was measured using a UV-Vis spectrophotometer (model). Fluorescence emission spectra were obtained using an F-7000 fluorescence spectrophotometer (Hitachi, Japan). The excitation spectrum of the fluorescence spectrum was 400 nm. The excitation slit was 5 nm. The emission slit was 5 nm, and the photomultiplier tube voltage was 700 V. All data were measured in parallel three times.

**Results and discussion**

At present, HSO$_3^-$ fluorescent probes have been reported to be sensitive, have a wide detection range, do not damage samples, and have little damage to cells under physiological conditions. However, they all need to introduce organic solvents to achieve optimal detection conditions, and it has the problems of shorter excitation wavelength, larger autofluorescence, and higher signal-to-noise ratio. Fluorescent probes of ruthenium derivatives containing a π-π structure for the detection of HSO$_3^-$ and SO$_3^{2-}$ have been used as nucleophilic
reagents and alkenyl bridge additions in the molecule.

Based on this, the water-soluble group of 2,3,3-trimethylbenzoindolemethane was synthesized into the naphthaldehyde at the 1st position by addition-elimination reaction to obtain the probe TNB. The structure of the probe TNB and the product after reaction with HSO$_3^-$ was confirmed by NMR (Fig. S5-S6, ESI†) and HRMS (Fig. S7-S8, ESI†). The fluorescence response of the probe to HSO$_3^-$ in a solution of different organic solvents was investigated. As shown in the Fig.S1 A and B, when using solvents with different DMSO contents as the test environment, the fluorescence response of the probe to HSO$_3^-$ is basically the same. When the DMSO content is 0, the probe shows a very weak background fluorescence intensity, which indicates that the probe does not need to introduce an organic solvent to achieve the best detection effect.

UV-Vis absorption spectra of probes interacting with HSO$_3^-$

UV-Vis titration spectroscopy experiments were carried out using a solution containing different HSO$_3^-$ concentrations in a PBS solution having a probe concentration of 10 $\mu$M (PBS buffer: 10 mM, pH 7.4). As shown in Fig. 2A, as the concentration of bisulfite increases, the absorbance at 436 nm gradually decreases. The maximum absorption wavelength shifts blue to 400 nm, and a new ultraviolet absorption peak appears at 310 nm, indicating that there is a new one. The substance is produced, which may be an addition reaction of HSO$_3^-$ with a double bond (Fig. 2B). This process leads to intramolecular conjugation, and the ultraviolet absorption is blue-shifted. When the concentration of HSO$_3^-$ reached 100 $\mu$M, $\Delta$ Abs reached a plateau. In addition, as the concentration of HSO$_3^-$ increases, the yellow solution gradually becomes colorless, allowing the purpose of qualitative detection of HSO$_3^-$ by naked eye colorimetry.

Fluorescence Spectral Response and Selectivity of Probe to HSO$_3^-$

Fluorescence spectroscopy experiments showed that TNB exhibited fluorescence quenching in PBS buffer. In PBS buffer solution at pH 7.4, the fluorescence intensity of TNB at 470 nm ($\lambda_{ex}$ = 400 nm) increased significantly with increasing HSO$_3^-$ concentration (Fig. 3A). The increase in fluorescence intensity was attributed to Interruption of the ICT process. When the HSO$_3^-$ concentration is higher than 100 $\mu$M, the fluorescence intensity reaches a plateau. The TNB fluorescence intensity showed a good linear relationship in the range of HSO$_3^-$ concentration from 0 to 60 $\mu$M (Fig. 3B). The fluorescence detection limit (LOD = 3$\sigma$/k) was calculated according to the literature to be 0.089 $\mu$M, this indicates that the probe can be used to detect trace amounts of endogenous...
sulfur dioxide derivatives. In this paper, the intra- and inter-day precision verification experiments were carried out (Fig. S9, ESI†). The research shows that the probe does not affect the measurement time within the allowable range of HSO₃⁻, which provides a basis for the accuracy of probe detection.

![Figure 3](image)

Figure 3 Fluorescence emission A Spectrum of probe TNB (10 μM) with 10 equivalents of bisulfite (100 μM) added to PBS buffer (pH = 7.4, 10 mM), slit: 5 / 5 nm. B Linearly fit the fluorescence spectrum and calculate the minimum detection limit.

It is important that the probe has good selectivity for a particular analyte. In this paper, HCO₃⁻, CO₃²⁻, Br⁻, S²⁻ and other anions were selected as interfering ions. Figure S2 (ESI†) shows the specific fluorescence response of TNB to HSO₃⁻ in PBS buffer. Various analytes were added to the PBS buffer solution of the probe TNB, and then the fluorescence intensity at 470 nm was recorded. Figure S2 A (ESI†) shows the fluorescence intensity of the probe TNB. When HSO₃⁻ is added in the presence of other interfering ions, the fluorescence of the probe TNB is greatly enhanced, which indicates that in the presence of other interfering ions, it does not affect the probe TNB to HSO₃⁻ specific selection.

The specific effect of the probe TNB on HSO₃⁻ can be conveniently determined by colorimetry. Various analytes were separately added to the probe TNB in PBS buffer solution, and the color change was observed under natural light. As shown in Figure S2 B (ESI†), only the probe solution containing HSO₃⁻ was colorless, and the rest were yellow. The fluorescent color of the probe TNB changes in the presence of various analytes. The blue fluorescent color was found to occur only in the presence of HSO₃⁻ (Figure S2 C ESI†). This result confirmed that the probe TNB can be used as a specific fluorescent probe for HSO₃⁻ detection.

Effect of probe TNB on response time and pH of HSO₃⁻

Response time is a key factor in evaluating the properties of fluorescent probes. To investigate the reaction performance of probes TNB and HSO₃⁻, we used fluorescence spectroscopy to investigate the response time of probes with 10 times the amount of HSO₃⁻ reaction. Time is plotted as a function of the peak at 470 nm maximum fluorescence intensity. As shown in Fig. S3 A, the fluorescence intensity increases rapidly within 10s, which indicates that the probe TNB can respond very rapidly to HSO₃⁻, and the fluorescence intensity at 475 nm increases with the reaction time. This reaction reached equilibrium within 35 s.

Further, we investigated the effect of pH on the fluorescence response of TNB to HSO₃⁻. As shown, the probe exhibited weak fluorescence emission in the range of pH 1.0 to 10.0. When 10 equivalents of HSO₃⁻ were added, the pH showed an increase in fluorescence in the range of 7 to 10, and there was no significant change in 1 to 6 (Figure S3 B ESI†), indicating that the probe can detect HSO₃⁻ sensitively under physiological conditions.

Research on sensing mechanism
In order to verify the mechanism of the bisulfite probe TNB, the mass spectrum (MS) and $^1$H NMR spectra of the probe after HSO$_3^-$ reaction were shown in Fig. S4 (ESI†), and the mass spectrum peak of the probe TNB was at m/z = 362.1906. At the same time, after adding an equivalent amount of HSO$_3^-$, the mass spectrum peak of the product after the reaction was converted to m/z = 466.1449. As shown in the figure, the chemical shifts of Ha and Hb in the probe TNB were changed to 4.32 ppm and 5.94 ppm, respectively. This means that the double bond of the compound is broken by HSO$_3^-$, and since the π-conjugation is interrupted by HSO$_3^-$, the ICT process is blocked, causing the molecule to emit fluorescence at the naphthalene ring.

Conclusions

In summary, we have developed a new water-soluble fluorescent probe TNB based on benzoindole, which can specifically detect sulfur dioxide derivatives in aqueous solutions. Benzoindole is linked to the 1 position of naphthalene by a double bond to form an ICT structure from naphthalene to hemi-cyanine. The probe has some excellent properties, such as rapid qualitative detection of HSO$_3^-$ by colorimetry. By fluorescence experiment, the effect on bisulfite can be completed within 40 s, the fluorescence intensity is increased by 91 times, and the detection limit is as low as 0.089 μM. More importantly, the probe can achieve optimal test conditions without the aid of organic solvents, and exhibits excellent fluorescence response in a weak alkaline environment, which provides the necessary conditions for detecting sulfur dioxide derivatives content in a living environment.

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