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A water-soluble, low-cytotoxic and sensitive fluorescent probe based on poly(ethylene glycol) for detecting sulfide anion in aqueous media and imaging inside live cells

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ABSTRACT

Sulfide anions are generated not only as a byproduct from industrial processes but also in biosystems. Hence, fluorescent probes for detecting sulfide anion which are water soluble, sensitive, selective and biocompatible are highly sought-after. In this study, we report a water-soluble, low-cytotoxic and sensitive fluorescent sensor for detecting sulfide anion. In this probe, the strong electron-withdrawing dinitrobenzenesulfonate ester group is incorporated onto fluorescein fluorophore, and correspondingly the fluorescence of fluorescein is efficiently quenched; while when the dinitrobenzenesulfonate ester is cleaved by the nucleophilic sulfide anion, the substantial fluorescence enhancement can be observed. Furthermore, poly(ethylene glycol) is coupled onto the fluorophore to impart the probe water-soluble and low cytotoxicity. The probe is capable of permeating the cell membrane and realizing sulfide anion monitoring and imaging in live cells and real sample. This technically-simple modification strategy may be suitable for fabricating some other fluorescent probes with enhanced biocompatibility and water solubility.

1. Introduction

To design and synthesize selective and sensitive sensing systems to detect various chemically and biologically pertinent species has attained a significant interest [1–3]. Among these species, sulfide anion is a very important member. Sulfide is a toxic anion, generated not only as a byproduct in industrial processes but also in biosystems due to microbial reduction of sulfate by anaerobic bacteria and sulfide generation from the sulfur-containing amino acids in meat proteins [4]. In addition, sulfide is employed in the production of sulfur and sulfuric acid, dyes and cosmetic manufacturing, production of wood pulp, etc. [5,6]. Most of the sulfide present in raw waters is derived from natural sources and industrial processes. It is particularly noticeable in some groundwaters, depending on source rock mineralogy and microorganisms present [7,8]. Exposure to a high level of sulfide can lead to irritation in mucous membranes, unconsciousness, and respiratory paralysis. Once protonated, sulfide anion turns into HS^- (under acidic pH, HS^- converts to H_2S) and becomes even more toxic and caustic [8]. Recent studies have shown that protonated sulfide is involved in multiple physiological processes in central nervous system, respiratory system, gastrointestinal system and endocrine system [9,10]; it is also related to diseases like Alzheimer's disease, Down's syndrome, diabetes, and liver cirrhosis [11,12], Therefore, the detection of sulfide anions is of high importance from industrial, environmental and biological points of view.

Many approaches, such as colorimetric, electrochemical analysis and gas chromatography, have been employed to measure and trace sulfide anion [13–17]. However, relatively high cost, time-consuming processes, destruction of tissues or cell lysates and lack of temporal and spatial resolution prohibit them from having applications in many biological studies [18,19]. On the other hand, fluorescent techniques are extremely attractive in this regard due to their simplicity, high sensitivity and real-time detection, as well as the capability for nondestructive detection of biological events in live cells or tissues [20–55].

Until now, several elegant fluorescent probes for sulfide anions have been reported [18,19,56–70]. In general, these probes were

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designed based on: (a) Reduction reaction between an azide group and S^{2-} [19,56–59]; (b) Formation of a copper-centered coordination complex in which Cu^{2+} can be released by binding with S^{2-} [18,60–67]; (c) The nucleophilic nature of S^{2-} [68–70]. However, much improvement is still needed for S^{2-} probes for possible environmental and bio-related applications in terms of water solubility, low toxicity and usability in biological matrixes.

On the other hand, previous researches demonstrated that, electron-withdrawing groups such as dinitrophenyl ether or dinitrobenzenesulfonyl, when coupled onto fluorophores, can efficiently quench the fluorescence of the fluorophores [71–73]; while the cleavage of these groups will restore the fluorescence. By using this protocol, a couple of elegant thiol probes have been designed based on the thiol-mediated cleavage of dinitrophenyl ether or dinitrobenzenesulfonyl group due to the nucleophilicity of thiols [71–73]. However, these probes are not water soluble and only usable in water/solvent mixture (e.g. ethanol or dimethylformamide); in addition, the response time is usually relatively long (dozens of minutes). Compared to thiols, sulfide anion is a much more effective nucleophile and reacts readily with sulfonate esters. This fact along with the above research results concerning thiol probes provided us with a clue for the design of novel fluorescent probes for sulfide anion. We thus anticipated that, incorporating the strong electron-withdrawing group dinitrobenzenesulfonate ester onto fluorescein fluorophore could significantly diminish the fluorescence; while when the dinitrobenzenesulfonate ester is cleaved by the more nucleophilic sulfide anion, we should observe a substantial fluorescence turn-on response. Furthermore, if the well-known hydrophilic polymer poly(ethylene oxide) (PEO) [29], which has been used for a wide range of biomedical applications because of its biocompatible, non-toxic, non-antigenic and non-immunogenic properties, is coupled onto the fluorophore, we could ensure water solubility, cell membrane permeability and low-cytotoxicity for the probe as well. With these ideas in mind, in this study, we demonstrate a fluorescent turn-on probe for S^{2-}, which is based on the nucleophilicity of sulfide anion and features good water solubility, low cytotoxicity and high sensitivity. The schematic illustration for the selective sensing for sulfide anion by the probe is shown in Fig. 1. The salient features of this probe include: First, the preparation of the probe is technically simple, and it can be easily obtained through a two-step-reaction synthetic route. In addition, the probe is able to selectively detect sulfide anion in totally aqueous media (100% water); and it is also capable of permeating the cell membrane and tracing sulfide anion in live cells. Moreover, with a relatively short response time, this probe is also very sensitive with a low detection limit of 150 nM.

2. Experimental

2.1. Materials and reagents

Methoxypolyethylene glycol amine (average molecular weight around 2000, PEG2000), fluorescein isothiocyanate, N,N-diisopropylethylamine, 2,4-dinitrobenzenesulfonyl chloride, sodium salts of anion (S^{2-}, F^-, Cl^-, Br^-, I^-, CO_3^{2-}, NO_3^-, $S_2O_3^{2-}$, SO_3^{2-}, HSO_3^-, SO_4^{2-}), and 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Aldrich. The purified water used in this study was the triple-distilled water which was further treated by ion exchange columns and then by a Milli-Q water purification system. N, N-Dimethyl-formamide (DMF) was dried with CaH_2 and vacuum distilled. Methanol and dichloromethane were analytically pure solvents and distilled before use.

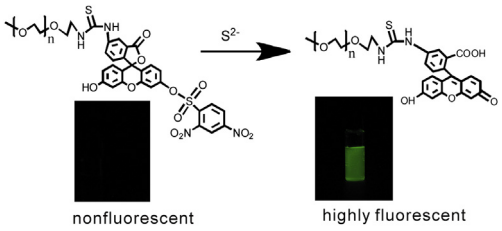

Fig. 1. Schematic illustration for the structure of the probe and its selective detection of sulfide anion. (The probe: 10^{-2} mg/mL in pH 7.4 HEPES buffered water, sulfide anion: 8×10^{-5} M).

2.2. Synthesis of the probe

The probe was synthesized through a two-step reaction. First, methoxypolyethylene glycol amine (average molecular weight 2000) (0.4 g, 0.2 mmol) and fluorescein isothiocyanate (79 mg, 0.2 mmol) were dissolved in 5 mL DMF in a flask. The reaction mixture was stirred under N_2 at room temperature overnight, afterwards the solvent was removed under reduced pressure. The residue was dissolved in 3 mL CH_2Cl_2, and then 30 mL diethyl ether was added to precipitate the product. The mixture solution was centrifuged (10,000 r/s, 8 min). And the intermediate product **1** was obtained as a solid (442 mg, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.37 (methoxy protons of PEG), 3.63 (methylene protons of PEG backbone), 6.5–8.2 (fluorescein aromatic protons).

Second, **1** (430 mg, 0.18 mmol) and N,N-diisopropylethylamine (65 mg, 0.5 mmol) were dissolved in 5 mL CH_2Cl_2 in a flask, the mixture was stirred under N_2. Then 2,4-dinitrobenzenesulfonyl chloride (134 mg, 0.5 mmol) was added into the above solution dropwise. The solution was stirred at room temperature overnight. After that, the solution was filtered, 30 mL of CH_2Cl_2 was added into the filtrate, and the filtrate was then washed with deionized water. The combined organic phase was dried over anhydrous $MgSO_4$. Then the solvent was evaporated under vacuum and the residue was dissolved in 3 mL CH_2Cl_2, and then 30 mL diethyl ether was added to precipitate the product. The mixture solution was centrifuged (10,000 r/s, 8 min). And the product (the probe) was obtained as a yellow solid (452 mg, 96%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.37 (methoxy protons of PEG), 3.63 (methylene protons of PEG backbone), 6.5–8.2 (fluorescein aromatic protons), 8.3–8.7 (dinitrobenzene protons).

2.3. Cytotoxicity

The cell line, L929 (murine aneuploid fibro-sarcoma cell) was incubated in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) at 37 °C with 5% CO_2. The cytotoxicity of the probe against L929 cells was assessed by MTT assay according to ISO 10993-5.

2.4. Cell incubation and imaging

Two cell lines, Hela (human cervical cancer cell) and L929 (murine aneuploid fibrosarcoma cell), were incubated in RPMI1640 medium supplemented with 10% Fetal Bovine Serum (FBS, Invitrogen). One day before imaging, cells were passed and plated on 30-mm glass culture dishes. For the experiments, cells were washed with RPMI1640, incubated in RPMI1640 medium containing N-methylmaleimide (which is a trapping reagent of thiol species) for 20 min and then the probe at 37 °C under 5% CO_2 for 2 h, and then treated with Na_2S (2 μM and 8 μM respectively) for 30 min. After that, the cells were washed with PBS for three times and then imaged on an Olympus IX71 inverted fluorescence microscope equipped with a DP72 color CCD (blue light excitation).

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