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A ratiometric strategy to detect hydrogen sulfide with a gold nanoclusters based fluorescent probe

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ABSTRACT

The emergence of ratiometric fluorescent probes have offered more convincing results to the bioanalytical field of research. In particular, using nanoparticles as scaffolds for the construction of ratiometric systems has received increasing attention. In this work, a novel design strategy was implemented for ratiometric sensing of hydrogen sulfide (H_2S), in which bovine serum albumin templated gold nanoclusters (BSA-AuNCs) was served as the internal reference fluorophore and HSip-1, a azamacrocyclic Cu^{2+} complex based fluorescent probe toward H_2S, acted as both the signal indicator and specific recognition element. Under single wavelength excitation, the nanohybrid probe HSip-1@AuNC emitted dual fluorescence at 519 and 632 nm, coming from HSip-1 and AuNCs respectively. The effective fluorescence response of organic dye to H_2S and constant fluorescence of AuNCs enabled the proposed HSip-1@AuNC to achieve the ratiometric measurement with a dynamic linear range of 7–100 μM and a detection limit of 0.73 μM. This probe also possesses high selectivity, stability against pH change and continuously light illumination. In addition, we provided HSip-1@AuNC as a valuable tool to analyze sulfides in serum samples and perfect recoveries verified its potential in biological applications.

1. Introduction

Fluorescence techniques have provided critical information for early diagnosis and therapeutics through visualization and monitoring physiological parameters and related events at the cellular, even molecular scale [1]. Fluorescent probes with ratiometric measurement, different from the past single wavelength intensity modulation type (turn-on or turn-off) sensors, employ the ratio of two emission intensities at different wavelengths. The way of self-calibration of fluorescence intensity through monitoring the two distinct signals before or after reacting with analyte could efficiently eliminate the background interferences and the fluctuation of detection conditions arising from dye localization or instrumental factors, resulting in a more reliable data [2,3]. Currently, most of the reported ratiometric fluorescent probes are developed by using dual-emission organic fluorescent materials [4,5], however, such fluorescent molecular sensors are difficult to design and still suffer from photobleaching and interferences. These problems, to some extent, can be solved through taking nanoparticles with versatile composition and architecture as candidates for creating nanosensors [6–8]. Considering the attractive features of gold nanoclusters, including unique fluorescent characteristics, ultra-small size, excellent optical/colloidal stability, biocompatibility, and facile synthesis, they are supposed to be perfectly suitable platforms for constructing ratiometric nanosensors aimed at biological application [9–11]. Among various reported gold nanoclusters synthesized by different approaches [12,13], bovine serum albumin templated gold nanoclusters (BSA-AuNCs) is believed to be ideal because of the presence of functional groups (e.g., carboxyl and amine groups) in the protein scaffold, which allow them to be further modified with biological function related molecular [14–16]. Moreover, the intense and stable fluorescent signal of BSA-AuNCs render them competent as the excellent optical reference moiety. Along with various target-responsive molecular dye, a class of perfect design scheme of ratio type probe for different analyte of biological interest is thus formed.

Hydrogen sulfide (H_2S), a newly identified gaseous signaling molecule, has recently became a research focus in biological field due to its multiple functions in the physiological and pathological processes [17,18]. Previous studies have demonstrated that endogenous H_2S can mediate a wide range of physiological effect in cardiovascular, neuronal, immune, endocrine, gastrointestinal systems [19], and its antioxidant, anti-apoptotic signaling effects also show therapeutic benefit for the treatment of ischemia-induced heart failure [20–22]. In living organism, enzymatically generated H_2S distributes in many organs and tissues [23–25] with

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the physiological concentration range of 10–100 μM or much lower to nanomolar concentration [26,27]. Recent studies reveal mismanagement of the H_2S level has a close relationship with numerous diseases, such as Alzheimer, Down syndrome and cancer [28–30]. Therefore, sensitive and real-time detection of H_2S under physiological conditions become in high demand, which could offer more detailed understanding of the key roles H_2S playing in physiological activities. To meet this challenge, tremendous efforts have been employed to seek suitable analytical methods. Among them, fluorescent probes have enjoyed the most success in tracking and quantifying H_2S [31,32]. Over the past few years, a large number of fluorescent probes have been successfully developed by utilizing some specific reactions, such as nucleophilic reaction [33,34], reduction of azide, nitro or hydroxyl amine groups [35], metal sulfide precipitation [36,37] etc. These probes have been applied for detecting exogenous or endogenous hydrogen sulfide in living cells, blood and tissue samples, and in vitro enzyme assays. Despite much progresses have been made in the development of H_2S-sensitive fluorescent probes, there are still of significance to rationally design sensors for rapid, facile detection in situ.

In previous work, the novel probe for H_2S, HSip-1, based on a fluorescein scaffold conjugated with an azamacrocyclic Cu^{2+}complex, has superior properties for biological imaging, but fluorescence "turn-on" response mechanism limited its application in accurate quantification [38]. Herein, we proposed a simple strategy to construct a ratiometric fluorescent probe for H_2S, in which the H_2S-response fluorescent molecular HSip-1 was immobilized onto the surface of BSA-AuNCs via a coupling reaction between amine groups from BSA and carboxyl groups from HSip-1. In our design of single-excitation (488 nm), dual-emission nanohybrid, intrinsically fluorescent AuNCs emitting at 644 nm was chosen as the reference signal which was independent on H_2S, while H_2S-sensitive fluorescent dye HSip-1 served as indicator which gave selective response to H_2S at 519 nm. Such a design not only took advantages of the excellent performance of HSip-1, but also avoided much of the variability in complicated biological media when used AuNCs as the built-in correction. The ratiometric measurements performed through monitoring the ratio of fluorescent emission intensities at two appropriately chosen wavelengths (I_{519}/I_{632}) in the presence or absence of H_2S. As we envisioned, the resulting ratiometric H_2S-nanosensor showed satisfactory sensitivity and presented a good linear relationship between intensity ratio and H_2S concentration. The feasibility of HSip-1@AuNC as a ratiometric probe for H_2S has been fully demonstrated by detection of H_2S in real serum samples with perfect recoveries.

2. Experimental section

2.1. Materials and reagents

Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$,99%), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide(NHS), $_L$-cysteine ($_L$-Cys), 2-mercaptoethanole (2-ME), dithiothreitol (DTT), 2,2′-(hydroxynitrosohydra-zono)bis-ethanimine (DETA/NO), sodium ascorbate (AA) were purchased from Sigm-Aldrich. $_{DL}$-Homocysteine ($_{DL}$-Hcy) was obtained from TCI AMERICA. 5-Aminofluorescein (5-AF), chloroacetyl chloride, *N*, *N*-diisopropylethylamine(DIEA), sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$), sodiumsulfite (Na_2SO_3), sodium thiosulfate ($Na_2S_2O_3$), sodium thiocyanate (NaSCN), sodium nitrite($NaNO_2$) were obtained from Aladdin Chemistry Co. Ltd. Bovine serum albumin (BSA), $_L$-glutathione(GSH), dialysismembrane (MWCO:3500) were obtained from Beijing BioDee BioTech Corporation Ltd. 1,4,7,10-Tetraazacyclododecane (cyclen) was obtained from Tianjin Heowns Biochemical Technology Co. Ltd. Metal salts, sodium hypochlorite (NaClO) were obtained from J&K Scientific Ltd. Sinopharm. All the reagents were used as received without further purification. Ultrapure water obtained from a Millipore Milli-Q system (18.2 MΩ cm) was used in all runs.

2.2. Instruments and methods

UV–vis spectra were obtained on a UV-2100 Spectrophotometer (Shimadzu, Japan). TEM images were recorded with a JEM-2100 microscope (HR-TEM, JEOL, Japan). The XPS images were performed by an X-ray photoelectron Spectrometer (XPS, PHI Quantera, Ulvac, Japan) equipped with an Al Kα source. Fluorescence spectra were measured on a Hitachi F-7000 Spectrophotometer. FT-IR spectra were recorded on a Bruker VERTEX70 FT-IR spectrometer. The kinetic curves were performed by Microplate reader (SpectraMax-M^3, Molecular Devices).

2.3. Preparation of HSip-1@AuNC

The synthesis of AuNCs and HSip-1 is shown in the Supporting Information. HSip-1@AuNC probe was prepared with following optimized procedures: 5 mM of EDC and 5 mM of NHS were introduced into 3.6 mL of MES (0.1 M, pH=6). After stirring for a while, 50 μM of HSip-1 was added to the solution and the solution was magnetically stirred for 2 h. Next, 400 μL of AuNCs (30 mg/mL) was added to the above solution with magnetic stirring for an additional 10 h to obtain the organic-inorganic hybrid probe. Finally, the nanohybrid probe was separated from unreacted HSip-1 and the byproduct of the reaction by dialysis in PBS (0.01 M, pH=7.4) for 2 days using dialysis membrane (MWCO: 3500). The modified clusters were hereafter denoted as HSip-1@AuNC and the standard solution (3 mg mL^{-1}) was stored at 4 °C. All the processes were kept in the room temperature.

2.4. Fluorescence spectroscopy

In the fluorescence assay, a cuvette with the length of 1 cm was used. The sample was excited at 488 nm, and the emission was collected from 500 to 800 nm. Na_2S was used as a hydrogen sulfide source in all experiments. After incubation with the probe for 1 h, the fluorescence spectrum were obtained on an F-7000 FL instrument.

For the selectivity experiment, hydroxyl radical ($^{\bullet}OH$) was generated by the Fenton reaction (Fe^{2+}/H_2O_2=100 μM/600 μM). Hypochlorite anion (ClO^-) was provided by NaClO (150 μM). Peroxynitrite ($ONOO^-$) was chemically generated by the reaction between H_2O_2 (50 μM) and $NaNO_2$ (50 μM). Nitric oxide (NO) derived from the solution of DETA/NO. All experiments were obtained after incubation with the appropriate ROS/RNS for 1 h at room temperature.

2.5. The detection of H_2S in fetal bovine serum

In a test tube, 50 μL of fetal bovine serum, 450 μL of HSip-1@AuNC standard solution were mixed. Different concentrations of Na_2S were spiked into these mixture and incubated for 1 h before fluorescence test.

3. Results and discussion

3.1. Characterization of AuNCs and HSip-1@AuNC probe

At the beginning of our study, the AuNCs was synthesized