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RESEARCH PAPER

A Förster Resonance Energy Transfer Ratiometric Probe Based on Quantum Dot-Cresyl Violet for Imaging Hydrogen Sulfide in Living Cells

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Abstract: Hydrogen sulfide (H_2S) has been confirmed as a significant endogenous gaseous signaling molecule involved in various physiological processes. In order to monitor H_2S in living cells, a Forster resonance energy transfer (FRET) ratiometric probe based on quantum dot-cresyl violet was developed. In this work, the quantum dot nanospheres via a facile ultrasonication emulsion strategy, and the mixture chloroform solution containing hydrophobic quantum dots and COOH-functionalized amphiphilic polymer were successfully transferred into the oil-in-water micelle. The negatively charged quantum dot nanospheres with quantum dots embedded in the polymer matrixes were successfully fabricated after the evaporation of chloroform. And then, these quantum dot nanospheres were condensed with positively charged cresyl violet-azide (CV-N_3) via electrostatic interaction to obtain the complexes (QDS-N_3). The as-prepared QDS-N_3 complexes were monodispersed nanospheres with an average diameter of about 120 nm. These complexes were taken up by the cell through endocytosis, and they were still stable even in wide pH range. In addition, the QDS-N_3 complexes exhibited no cellular toxicity which was verified by MTT assay. In this ratiometric probe, CV-N_3 as a FRET acceptor was conjugated to quantum dot nanospheres. The quantum dots emitted at 591 nm and served as the FRET donor; once the aryl azide on the CV-N_3 was reduced by H_2S to aniline, the probe emitted at 620 nm. The ratiometric probe allowed the elimination of interference of excitation intensity, intracellular environment and other factors. Furthermore, this method also offered a general protocol for preparing nanosensors for monitoring various small molecular in living cells.

Key Words: Quantum dot; Cresyl violet; Ratiometric fluorescence; Cell imaging; Hydrogen sulfide

1 Introduction

Hydrogen sulfide (H_2S) is the third endogenous signaling gasotransmitter following nitric oxide and carbon monoxide[1–4]. Abnormal level of endogenous H_2S has been found to be closely associated with a number of pathophysiological processes[5–12]. Consequently, monitoring of H_2S is of significance for the profound understanding of vital process and pathogenesis. Several methods have previously been explored for detection of H_2S, such as gas chromatography[13], electrochemical analysis[14], colorimetry[15], and UV absorbance[16], etc. However, these methods usually require destruction of the sample. In contrast, fluorescence detection has attracted much attention because of its great temporal and spatial sampling capability as well as high sensitivity for *in situ* and noninvasive analysis[17,18]. Although several fluorescent off-on H_2S probes have been developed[19–22], they are difficult to give precisely quantitative information on the H_2S concentration, because molecular emission intensity can be distinctly affected by excitation intensity, photobleaching, microenvironments, and local probe concentration[23]. By comparison, the ratiometric probe allows

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the elimination of these interferences based on Förster resonance energy transfer (FRET).

In recent years, semiconductor quantum dots (QDs) have attracted much attention in the fields of biological imaging, targeted delivery and cancer diagnosis based on their unique optical properties of broad excitation and narrow, symmetric emission spectra with high photostability and long lifetime[24–30]. In this work, a FRET ratiometric probe based on quantum dot-cresyl violet was developed. Quantum dot nanospheres via a facile ultrasonication emulsion strategy was first synthesized, and the mixture chloroform solution containing hydrophobic quantum dots and COOH-functionalized amphiphilic polymer were successfully transferred into the oil-in-water micelle. The negatively charged quantum dot nanospheres with quantum dots embedded in the polymer matrixes were successfully fabricated after evaporation of chloroform. And then, these quantum dot nanospheres were condensed with positively charged cresyl violet-azide (CV-N_3) via electrostatic interaction to obtain QDS-N_3 complexes. Meanwhile, the H_2S imaging photo was obtained via the ratio of red fluorescence to green fluorescence. Most importantly, this functional nanoprobe showed almost no cellular toxicity.

2 Experimental

2.1 Instruments and reagents

HT7700 transmission electron microscope (Hitachi, Japan), FluoroMax-4 spectrophotometer (Horiba Jobin Yvon, French) equipped with a plotter unit and a quartz cell, F50 ELISA (Tecan, Switzerland) and Ti-E inverted microscope (Nikon, Japan) were used in this study. Dynamic light scattering (DLS) particle size analysis as well as zeta potentials were measured using a Zetasizer Nano-ZS90 (Malvern, UK) zeta and size analyzer.

Methacrylic acid (MAA, ≥ 98.0), styrene (St, ≥ 99.5%), 2,2′-azobis(isobutyronitrile) (AIBN), oleic acid (Analytical reagent), $ZnCl_2$ (≥ 98.0%), $MnCl_2·4H_2O$ (≥ 99.0%), NaOH (Analytical reagent), $Na_2S·9H_2O$ (Analytical reagent), $Na_2EDTA·2H_2O$ (Analytical reagent) and chloroform (≥ 99.0%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Cresyl violet was obtained from Sigma-Aldrich. All the chemicals were analytical grade and used as received without further purification. Ultrapure water (> 18.2 MΩ cm) was prepared with a Milli-Q filtration system (Millipore, USA).

2.2 Experimental methods

2.2.1 Synthesis of cresyl violet-azide

$NaNO_2$ solution (1.0 mmol) was dropwise added to cresyl violet (1.0 mmol) in 2 M HCl aqueous solution at 0–5 ºC. After stirring for 20 min, NaN_3 solution (2.0 mmol) was added slowly. Subsequently, the reaction mixture was stirred for 24 h at room temperature. The as-prepared brown precipitate was filtrated and recrystallized from acetonitrile. After that, the brown powder was dried in vacuum drying oven and kept for further use.

2.2.2 Synthesis of QDs nanosphere

The oil phase QDs were prepared based on the previous researches[31]. Then the amphiphilic copolymer was prepared as follows[32]. In brief, St (5.0 g), MAA (46.0 mg), and AIBN (96.0 mg) were added into chloroform (35.0 mL). Herein, St and MAA were used as monomers and AIBN as initiator. The mixture solution was then transferred into Teflon lined autoclave and heated at 100 ºC for 10 h. Then, methanol was added to precipitate the white products. The copolymer was purified by washing with chloroform, precipitating with methanol, and then centrifuging. This purification cycle was repeated at least twice. The amphiphilic copolymer was dried in 70 ºC for 3 h and kept for further use. Finally, the as-prepared QDs (15.0 mg) and amphiphilic copolymer (50.0 mg) were dispersed into chloroform to obtain transparent solution. Thereafter, the mixture solution was transferred into NaOH aqueous solution (pH 10) with ultrasonic treatment. The QDs nanospheres were obtained via removing chloroform by evaporating. The obtained QDs nanospheres product were centrifuged. The precipitate was redispersed into ultrapure water, and stored for later use. The final concentration of the colloidal solution is ca. 19.4 mg mL^{-1}.

2.2.3 Synthesis of QDS-N_3 ratiometric probe

By simply mixing and stirring, QDS-N_3 ratiometric probe was obtained via electrostatic interaction between the negatively charged QDs nanosphere and positively charged CV-N_3. And then, QDS-N_3 product was centrifuged for three times. The precipitate was redispersed into ultrapure water.

2.2.4 Cell viability test

Mammalian cell lines (HeLa and MCF-7) were cultured in Dulbecco's modified eagle medium supplemented with 10% of heat-inactivated fetal bovine serum and 1% antibiotics penicillin/streptomycin (100 U mL^{-1}) in a humidified incubator with 5% CO_2 at 37 ºC.

The 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to evaluate cell viability. MCF-7 cells were seeded in a 96-well microplate with 10,000 cells per well with five parallel wells. The microplates were incubated at 37 ºC for overnight. Subsequently, cells were treated with different concentrations (0–200 μg mL^{-1}) of QDS-N_3 ratiometric probe at 37 ºC for 24 h. After the medium