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Invited feature article

A self-assembled fluorescent nanoprobe for detection of GSH and dual-channel imaging

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ABSTRACT

We report a fluorescent nanoprobe based on the co-assembly of amphiphilic BODIPY and fluorescein for selective detection of GSH. The amphiphilic BODIPY derivative, consisting of hydrophobic BODIPY unit and hydrophilic PEG chains, co-assembled with fluorescein to give non-fluorescent nanoparticles due to the aggregation caused quenching. Upon addition of GSH, the thiol group of GSH reacted with BODIPY through nucleophilic substitution to afford highly polar GSH-substituted BODIPY with high solubility in water, resulting in the disassembly of the nanoparticles. The GSH-induced disassembly of the nanoparticles released the highly fluorescent BODIPY derivative and fluorescein, enabling the dual-emissive detection of GSH. This nanoprobe was applied for the dual-channel fluorescence imaging of GSH in living cells.

1. Introduction

Glutathione (GSH) is the most abundant intracellular nonprotein thiol and plays important role in maintaining the intracellular redox activity. Alterations in GSH concentrations are associated with many diseases, Parkinson's disease, immune dysfunctions, liver disease, and many types of cancer [1–3]. Consequently, the selective detection and quantification of GSH in biological system may provide valuable information for early diagnosis of some diseases. Fluorescent probes are considered as effective molecular tools that monitor and visualize intracellular analytes [4–6]. Great efforts have been devoted to develop small-molecules-based fluorescent sensors for the detection of thiols in living systems [7–10].

Alternatively, recent progress in nanotechnology enables the development of various nanoprobes for detection and imaging in biological microenvironments [11]. A variety of supramolecular nanostructures, such as micelles, vesicles and nanoparticles, have been conveniently constructed using building blocks including small organic fluorescent dyes [12,13], π-conjugated oligomers [14], amphiphilic polymers [15–17], peptides [18,19], DNA [20,21], etc. Researchers fabricate their nanoprobes on rational design by the assembly of these building blocks, which are sensitive to external stimuli because of the weak noncovalent interactions [16,22,23]. Self-assembled nanoprobes display superior characteristics compared to small organic molecules [24]. The supramolecular assembly approach offers an opportunity to easily contain various useful molecules, and their functions can be well tuned by simple modifications and changes of functional molecules. Many of the self-assembled structures possess unique properties and show specific response towards analytes [25].

Herein, we report a self-assembled nanoprobe for the selective detection of GSH in living cells. The assembly of amphiphilic **BODIPY-S-PEG (BSP)** consisting of hydrophobic BODIPY dye and hydrophilic tetraethylene glycol monomethyl ether chains generated **BODIPY-S-PEG nanoparticles (BSP NPs).** Such nanoprobe was non-fluorescent on account of aggregation caused quenching (ACQ). Upon addition of GSH, the nucleophilic substitution of the thiol moiety to the BODIPY dye resulted in GSH substituted BODIPY with high solubility in water, which induced the disassembly of the nanoparticles. The recovery of the fluorescence induced by disassembly of the nanoprobe enabled the turn on fluorescent detection of GSH. By contrast, the addition of Cys or Hcy yielded less hydrophilic products that nanoprobe remained the aggregate states, thus the selective detection of GSH was realized. Moreover, we encapsulated fluorescein into the aggregates to form **BSP-F NPs**. In the presence of GSH, both BODIPY and fluorescein were released, enabling the dual-emission detection of GSH. We

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employed it for dual-channel fluorescence imaging of GSH in living cells, confirming its potential biological applications.

2. Experiment section

2.1. Materials and instruments

All reagents and solvents were of commercial quality without further purification. ^{1}H and ^{13}C NMR spectra were recorded on an Advance Bruker 400 M or 600 M spectrometer and referenced to solvent signals. Mass spectra were obtained on a Bruker Apex IV Fourier Transform Mass Spectrometer. Size and size distribution were determined by DynaPro NanoStar for dynamic light scattering (DLS). The measurement was carried out at 25 °C and the scattering angle was fixed at 90°. The morphology was measured by scanning electron microscopy (SEM) performed on a Hitachi S-4800 with electron microscope operating at acceleration voltages of 100 kV. Fluorescence spectra were determined on a Hitachi 4500 spectrophotometer. Absorption spectra were determined on a Shimadzu UV-1601PC UV–vis spectrophotometer.

2.2. Cell culture and fluorescence imaging

HeLa cells were cultured in culture media (DMEM/F12 supplemented with 10% FBS, 50 unit/mL penicillin, and 50 μg/mL of streptomycin) at 37 °C under a humidified atmosphere containing 5% CO_2 for 24 h. The cells were seeded in a 6-well plate at a density of 104 cells per well in culture media. Confocal fluorescence imaging was performed with Nikon A1R MP multiphoton microscopy with a 60 × oil-immersion objective lens. Green fluorescence was excited at 488 nm with a Si laser and emission was collected by a 500–550 nm band pass filter. Red fluorescence was excited at 561 nm and emission was collected by a 570–620 nm band pass filter.

2.1. Dual-channel fluorescence imaging of GSH

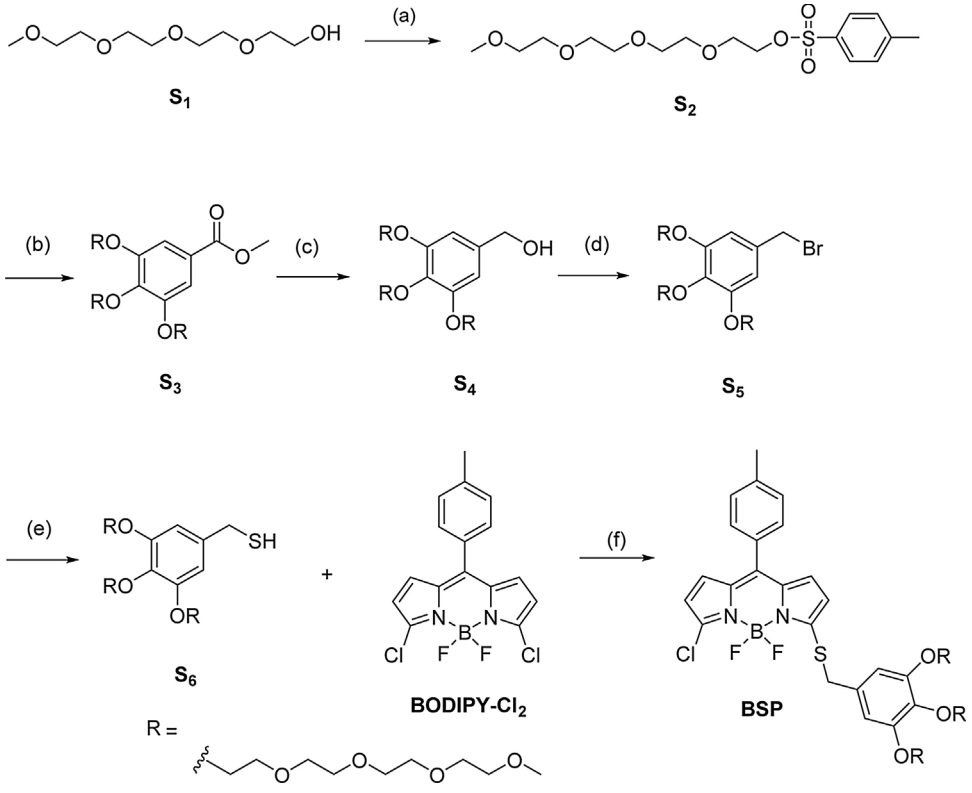
HeLa cells were treated with 1 mM GSH in culture media for 20 min at 37 °C in a humidified incubator. After washing with phosphate buffered saline (PBS), the cells were further incubated with **BSP-F NPs** in culture media for 20 min, After washing with PBS, 1 mL of cell culture fluid was added. For the control experiment, the cells were treated with 1 mM NEM for 20 min before treated with **BSP NPs** in culture media for 20 min at 37 °C in a humidified incubator, and after washing with PBS, 1 mL of cell culture fluid was added.

2.2. Synthesis of BSP and preparation of BSP NPs, BSP-F NPs

BODIPY-Cl_2 [26,27] and **S2-S5** [28] was synthesized according to the reported literature methods.

Synthesis of compound S2 [28]: *p*-Toluenesulfonyl chloride (7.60 g, 39.86 mmol) in THF (25 mL) was added dropwise into a two-phase system of water (25 mL) and THF (200 mL) of NaOH (2.33 g, 58.25 mmol) and tetraethylene glycol monomethyl ether (7.79 g, 37.43 mmol) at 0 °C. After addition, the mixture was stirred for another 8 h, then poured into ice-water and extracted with dichloromethane (3 × 50 mL). The organic layers were washed with water (pH = 1) and brine twice. After drying over Na_2SO_4, the solvent was evaporated in vacuo to yield the pure compound **S2** (11.92 g, yield: 88%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.16 (t, J = 4.8 Hz, 2H), 3.73-3.52 (m, 14H), 3.37 (s, 3H), 2.44 (s, 3H).

Synthesis of Compound S3 [28]: A mixture of **S2** (5.20 g, 14.36 mmol), methyl 3,4,5-trihydroxybenzoate (0.79 g,



Scheme 1. Synthesis of **BSP**. (a) *p*-Toluenesulfonyl chloride, NaOH, water, THF; (b) methyl 3,4,5-trihydroxybenzoate, K_2CO_3, DMF; (c) $LiAlH_4$, N_2, THF; (d) PBr_3, dry CH_2Cl_2; (e) (1) thiourea, DMF, N_2; (2) NaOH; (3) HCl; (f) triethylamine, acetonitrile.

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