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A near-infrared fluorescence dye for sensitive detection of hydrogen sulfide in serum

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ABSTRACT

Cy-Cl, a cationic near-infrared cyanine dye, readily reacts with hydrogen sulfide (H_2S) via nucleophilic thiolation to give dose-dependent 'turn-off' fluorescence and colorimetric read-out, allowing selective detection of low levels of H_2S in serum and imaging of mitochondrial H_2S in living cells.
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Hydrogen sulfide (H_2S) is a gasotransmitter involved in a broad variety of biological processes including cell signaling, vasodilation and inflammation.[1] H_2S could be endogenously produced in multiple tissues such as brain and colon,[2] and the levels of H_2S is elevated in a number of pathological conditions.[3] As such, methods enabling sensitive detection of H_2S in biological fluids or mitochondria are highly desirable.

Optical probes are being actively pursued for detection of H_2S owing to their noninvasiveness and ease of manipulation.[4] Existing reaction-based probes often rely on the superior reductivity or the unique double nucleophilicity of H_2S to afford fluorescence readout.[5] Heptamethine cyanine (Cy) dye-based probes are attractive for bioimaging owing to their spectra located at the near-infrared (NIR) region where the biological milieu exhibits the least absorption and autofluorescence. As such, a number of Cy-derived chemodosimeters, responsive to the reductive potential of H_2S, have been constructed for imaging and detection of H_2S.[5h,z] Here we report the sensitive detection of H_2S in serum with Cy-Cl by analyte-dependent nucleophilic thiolation (Scheme 1), leading to dose-dependent 'turn-off' fluorescence and UV–vis absorbance.

To explore the sensing property of Cy-Cl for H_2S, the reaction rates of Cy-Cl with Na_2S in fetal bovine serum (FBS) were investigated. UV–vis absorption of Cy-Cl in FBS spiked with Na_2S, which is used as the donor of H_2S, was monitored over time. As shown in Figure 1, the reactions are completed in 10 min, and the absorbance at 790 nm decreased as a function of Na_2S concentration. In parallel assays, it was shown that the recognition Cy-Cl for Na_2S was also effective in distilled water, methanol and dimethylformamide (DMF) (Supplementary material, Fig. S2). To probe the sensing mechanism, the product generated from Cy-Cl and Na_2S in methanol was isolated and characterized by mass spectrometry, ^{1}H NMR and ^{13}C NMR (Supplementary material). In a control experiment, Cy-Cl was found to be inert to NaOH and amino acids under identical conditions (Supplementary material, Fig. S1). Collectively, these data support the formation of Cy-SH via Na_2S-triggered thiolation as proposed in Scheme 1. UV–vis spectrometry and fluorometry analysis showed that Cy-SH exhibits minimal absorption around 770–800 nm and is almost nonfluorescent (Supplementary material, Fig. S2) whereas CY-Cl displays strong absorption peak centered at 790 nm, suggesting the applicability of detection of H_2S in biological fluids by turn-off fluorescence and UV–vis absorption read-out.

To effectively detect H_2S in biological specimens. It is essential that Cy-Cl is immune to endogenous thiols such as glutathione, cysteine, and homocysteine. Hence the reactivity of Cy-Cl towards these biological thiols was investigated. Figure 2 showed that Cy-Cl could efficiently respond to Na_2S and no color change was

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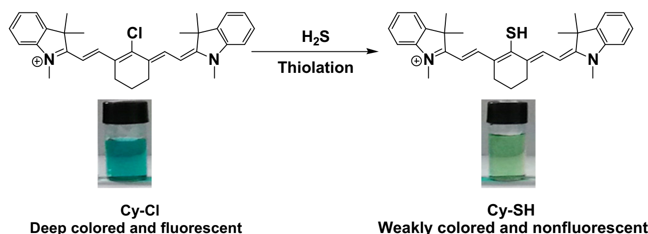

Scheme 1. Detection of H_2S with Cy-Cl. The insert shows the visual images of Cy-Cl (100 μM) in distilled water spiked with or without Na_2S (1 mM).

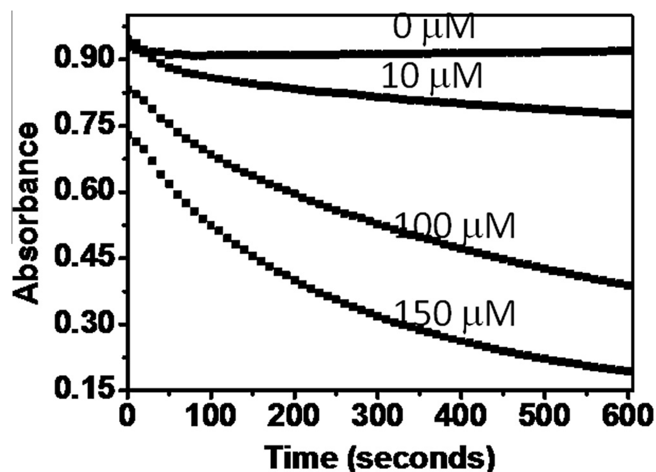

Figure 1. Kinetic profiles of the reaction between Cy-Cl and Na_2S in FBS. To FBS containing Cy-Cl (10 μM) was supplemented with various levels of Na_2S (0–150 μM as indicated) in FBS at rt. The absorbance at 790 nm of the assay solutions was recorded as a function of time.

observed in FBS supplemented with glutathione, cysteine or homocysteine, suggesting the stringent selectivity of Cy-Cl for Na_2S over these naturally occurring biological thiols.

To determine the limit of Cy-Cl based assay, various amounts of Na_2S were spiked into FBS containing Cy-Cl. The solutions were incubated for 10–20 min and then analyzed by fluorometry and UV–vis spectrometry. Figure 3 showed that UV–vis absorption and fluorescence emission of Cy-Cl decreased as a function of Na_2S concentration where 2–100 μM of Na_2S can be easily detected. As 10–100 μM H_2S were typically present in serum,[9] the titrations indicate the applicability of Cy-Cl for detection of H_2S in serum by fluorometry or UV–vis spectrometry.

Mitochondria play critical roles in H_2S metabolism. Generally considered to be synthesized in cytosol, H_2S was recently suggested to be produced in mitochondria owing to the translocation of cytosolic cystathionine-β-synthase into mitochondria under hypoxia conditions.[6] To date, H_2S has been documented to exhibit distinct effects on mitochondrial activity including cytoxicity, depolarization, and inhibition of respiration.[6,7] Currently, probes amenable for selective-imaging of mitochondrial H_2S have been largely unexplored.[8]

Demonstrated to be able to selectively detect Na_2S in serum, we further explore the utility of Cy-Cl for imaging of H_2S in mitochondria. Cationic and lipophilic dyes tend to accumulate in mitochondria owing to the negative transmembrane potentials of mitochondria.[10] Cy-Cl, a cationic NIR dye, was therefore probed for the feasibility to sense mitochondrial H_2S in living cells. Confocal fluorescence microscopic images of HeLa cells co-stained with Cy-Cl and rhodamine-123 (Rho 123) showed that the intracellular fluorescence of Cy-Cl colocalized with that of rhodamine-123, a mitochondria specific marker (Fig. 4). The colocalization confirmed regioselective accumulation of Cy-Cl in mitochondria in live cells. Next, HeLa cells pre-stained with Cy-Cl were incubated in phosphate buffered saline (PBS) supplemented with various levels of

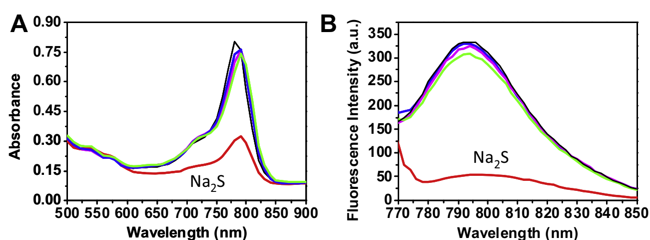

Figure 2. Selectivity of Cy-Cl for Na_2S over biological thiols. UV–vis absorption spectra (A) and fluorescence emission spectra (B) of Cy-Cl (10 μM) that have been incubated for 10 min (A) or 20 min (B) at rt in FBS with or without addition of glutathione (0.5 mM, in blue), cysteine (0.5 mM, in magenta), homocysteine (0.5 mM, in green), or Na_2S (100 μM, shown in red).

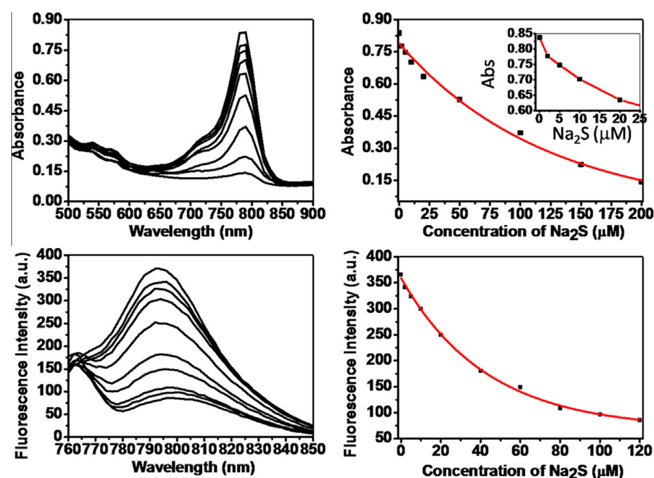

Figure 3. Quantitation of Na_2S with Cy-Cl. To FBS containing Cy-Cl (10 μM) was spiked with various amounts of Na_2S. The solutions were incubated for 10–20 min at rt and then analyzed by UV–vis spectrometry and fluorometry. (A) UV–vis absorption spectra of Cy-Cl (10 μM) that has been incubated for 10 min in FBS supplemented with Na_2S (0, 2, 5, 10, 25, 50, 100, 150 and 200 μM, from top to bottom); (B) the titration curve was plotted by absorbance at 790 nm vs Na_2S concentration. (C) Fluorescence emission spectra of Cy-Cl (10 μM) that has been incubated for 20 min in FBS containing Na_2S (0, 2, 5, 10, 20, 40, 60, 80, 100 and 120 μM, from top to bottom; (D) the titration curve was plotted by fluorescence emission intensity at 800 nm (λ_{ex} at 760 nm) vs analyte concentration.

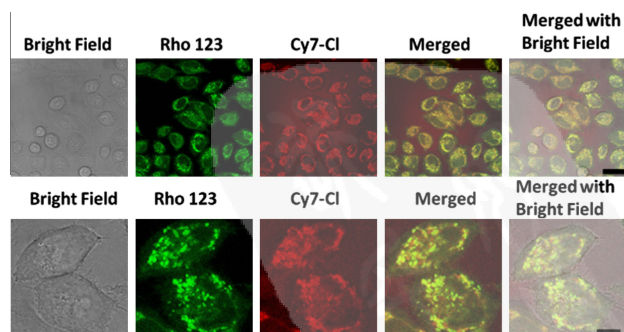

Figure 4. Intracellular distribution of Cy-Cl in HeLa cells (A: broad view; B: signal cell). Cells co-stained with rhodamine 123 (1 μM) and Cy-Cl (10 μM) were visualized by confocal fluorescence microscopy. The fluorescence of Rho 123 was shown in green and that of Cy-Cl was shown in red. Colocalization of both signals was shown in yellow. Bars: 20 μm in A; 5 μm in B.

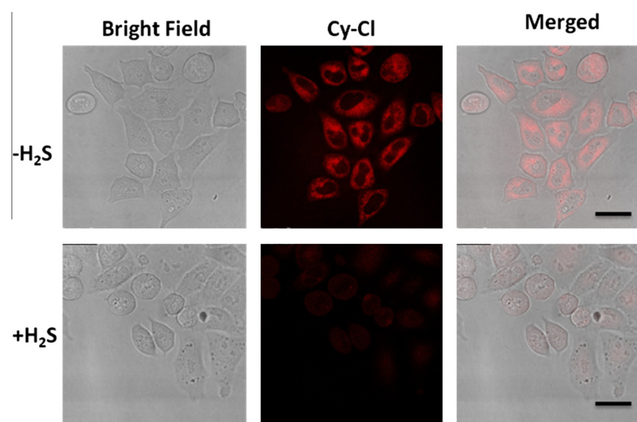

Figure 5. Detection of mitochnodrial H_2S in HeLa cells with Cy-Cl. HeLa cells were incubated with Cy-Cl (10 μM) in PBS for 10 min. The cells were washed and then treated with or without Na_2S (200 μM) in PBS for 10 min. The cells were washed and then analyzed by confocal fluorescence microscopy. The emission at 700–790 nm was recorded using λ_{ex} at 633 nm. Bar: 20 μm.

Na_2S. The cells were washed with PBS and then probed by confocal fluorescence microscopy. Figure 5 showed that cells treated Cy-Cl and Na_2S exhibited analyte-dependent 'turn-off' fluorescence emission, suggesting that Cy-Cl was suitable for fluorescent sensing of H_2S in mitochondria.

In summary, Cy-Cl selectively reacts with H_2S in serum by analyte-triggered thiolation, leading to dose-dependent 'turn-off' of NIR fluorescence. In addition, cationic Cy-Cl accumulates in mitochondria in live cells and selectively senses mitochondrial H_2S which was recently shown to be generated under hypoxia conditions. With the applicability of this assay in serum and the NIR fluorescence emission of Cy-Cl that could effectively avoid the interference of the autofluorescence of the biological samples, the stringent selectivity of Cy-Cl towards H_2S suggested the broad potentials of Cy-Cl in the studies of H_2S biology, for example, determination of H_2S levels in serum and studies of H_2S biogenesis in mitochondria.

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Supplementary data

Supplementary data (the synthesis and characterization of Cy-SH, and detailed assay procedures) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.11.016.

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