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A new, highly water-soluble, fluorescent turn-on chemodosimeter for direct measurement of hydrogen sulfide in biological fluids

Matthew C. T. Hartman^{a,b} and M. Michael Dcona^{a,b}

Matthew C. T. Hartman: mchartman@vcu.edu

^aDepartment of Chemistry, Virginia Commonwealth University (VCU), 1001 W. Main St. P.O. Box 842006, Richmond, VA 23284

^bMassey Cancer Center, Virginia Commonwealth University, 401 College St, Richmond, VA 23298-0037

Abstract

A new reaction-based fluorescent reporter for H₂S has been developed based on 8-aminopyrene-1,3,6-trisulfonate. This reporter shows high selectivity for H₂S over other ions and thiols, and can detect H₂S directly in serum without additives.

Although previously considered as a toxic gas, hydrogen sulfide has recently been discovered to be an important gaseous signaling compound. H₂S has been shown to be involved in a diverse and ever expanding array of biochemical processes such as inflammation, control of blood pressure, neurotransmission, and ischemia reperfusion.^{1–9} Several methods to determine the H₂S concentration in biological samples have been developed including those based on colorimetric^{10, 11}, electrochemical^{12, 13}, and other approaches^{14, 15}. However these methods often require extensive sample processing before measurement, complicating their use.

Because of the potential ability to monitor H₂S in situ, we and other groups have very recently become interested in investigating the use of fluorescence-turn-on reporters for H₂S^{16–29}. One successful strategy pioneered by the Chang group¹⁹ has been to use the ability of H₂S to rapidly and selectively reduce azides to amines as a means to turn on fluorescence^{22, 23, 26, 29}. Conveniently, many fluorescent molecules are quenched when their aryl amino groups are converted to the corresponding azides^{29–31}. In this paper our effort has focused on an azido analog of 8-aminopyrene-1,3,6-trisulfonic acid (APTS) and its use as an H₂S chemodosimeter. As compared to other profluorescent molecules used for H₂S sensing, 8-azidopyrene-1,3,6-trisulfonic acid (N₃-PTS) has the advantages of high selectivity vs. other ions and thiols, and extremely high water solubility. These features enable analysis of H₂S concentrations in biological samples without prior sample processing.

N₃-PTS was prepared in a single step from known fluorescent dye APTS using Sandmeyer conditions (Scheme 1). The resulting product could be readily purified using size exclusion

Correspondence to: Matthew C. T. Hartman, mchartman@vcu.edu.

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chromatography with Bio-Gel P-2. The ammonium salt of N₃-PTS is highly soluble in water at concentrations >100 mM.

The absorption spectrum of N₃-PTS shows a blue-shift relative to APTS (Fig. 1a) with peaks at 382 and 403 nm, vs. 427 nm for APTS (for further spectral parameters, See ESI Fig. S1 and Table S1). As seen with other fluorophores^{19, 22, 30, 31}, the presence of the electron-rich azide dramatically quenches the fluorescence (Fig. 1b) of N₃-PTS. At 425 nm excitation, the quantum yield of N₃-PTS is 25-fold lower than APTS (See ESI Fig. S2 and Table S2). By mixing N₃-PTS and APTS in known ratios, we determined that the excitation wavelength to maximize fluorescence enhancement was 435 nm (See ESI Table S1).

We expected that H₂S-mediated azide reduction would convert N₃-PTS to APTS, with a corresponding enhancement in fluorescence. A solution of 50 μM NaHS was prepared and added to 100 μM N₃-PTS in phosphate buffer. The reaction was monitored by fluorescence (Fig. 2) and was compared with the same reaction lacking NaHS. A time-dependent large increase in fluorescence was observed in the solution containing NaHS. After 90 minutes a 7-fold fluorescence enhancement was observed. For a time course of H₂S reactions at different concentrations see ESI Figure S3.

We then proceeded to test the selectivity of our potential H₂S chemodosimeter versus a collection of several common anions, reactive oxygen and nitrogen species, and thiols (Fig. 3). Significant fluorescence enhancement was observed only in the presence of 50 μM HS⁻. The fluorescence signal of other ions (OAc⁻, N₃⁻, HCO₃⁻, Cl⁻, OH⁻, NO₂⁻, SO₄²⁻, SO₃²⁻, S₂O₃²⁻), thiols (glutathione and cysteine), or reactive oxygen and nitrogen species (See ESI, Figure S4) gave little to no fluorescence enhancement when tested at 1 mM, a 20-fold higher concentration. Thus, N₃-PTS shows excellent selectivity for aqueous hydrogen sulfide in the presence of other ions and thiols encountered in biological environments.

In order to use N₃-PTS to quantify HS⁻ in biological samples it is desirable that the fluorescence be linearly related to the sulfide concentration. Indeed, treatment of N₃-PTS (100 μM) with several different concentrations of HS⁻ led to the expected linear relationship (Fig. 4). HS⁻ was easily quantifiable by fluorescence when its concentration was between 2–100 μM.

With the linearity and selectivity studies completed, we turned our attention H₂S detection in biological milieu. First, we investigated the extent to which APTS fluorescence was quenched in fetal bovine serum (FBS) vs. standard phosphate buffer. FBS showed little, if any, quenching (See ESI, Figure S5), suggesting that the measurement of H₂S could be accomplished directly in the media without prior removal of proteins. Moreover, the high water solubility of N₃-PTS could in principle allow measurement of fluorescence without addition of any organic co-solvents or detergents. We then titrated NaHS into FBS containing N₃-PTS. After 90 minutes, the fluorescence was measured showing good linear dependence (Fig. 5). The point at which the fluorescence was >2-fold above background was 9 μM.

Using N₃-PTS, we can measure the H₂S concentration in FBS in the range of 10–100 μM. This concentration range corresponds exactly with the range of H₂S concentrations found in human blood³². We also observed that the fluorescence enhancement in FBS at the same concentration of NaHS was diminished compared to phosphate buffer. This reduced sensitivity was presumably due to the quenching of the added HS⁻ with serum electrophiles³³. We surmise, therefore, that derivatizing N₃-PTS to maximize its reaction rate with H₂S will lead to enhanced sensitivity in serum.

In summary, we have developed a new chemodosimeter for hydrogen sulfide based on H₂S conversion of profluorescent N₃-PTS to APTS. N₃-PTS can be prepared in good yield in a single step from commercially available APTS. N₃-PTS shows very high selectivity for H₂S and is extremely water soluble. These two properties have allowed us to measure H₂S levels in serum directly without any processing of the sample or addition of detergents or cosolvents. The compatibility of N₃-PTS with aqueous solutions distinguishes it from other fluorescent methods for H₂S detection and makes it well suited for in situ analysis of H₂S levels in a variety of biological fluids.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Spectral parameters of APTS and N₃-PTS. (A) The absorbance spectral scans of N₃-PTS (red) and APTS (blue), both at 50 μ M, are shown. (B) The fluorescence spectral emission scans ($\lambda_{\text{ex}} = 435$ nm) of N₃-PTS (red) and APTS (blue), both at 5 μ M, are shown.

Fig. 2.

Enhancement in fluorescence of N₃-PTS solutions in the presence of H₂S. A solution of 100 μ M N₃-PTS and either 50 μ M NaHS or water were incubated in phosphate buffer (pH 7.8) at room temperature for various times. Fluorescence emission scans ($\lambda_{\text{ex}} = 435$ nm) were taken every 5 minutes.

Fig. 3. Comparison of the fluorescence of N_3 -PTS after incubation in 50 mM phosphate buffer (pH 7.8) along with with NaHS (50 μ M) or NaOAc, NaN_3 , $NaHCO_3$, NaCl, NaOH, $NaNO_2$, Na_2SO_4 , Na_2SO_3 , $Na_2S_2O_4$, reduced glutathione or L-cysteine hydrochloride (all 1 mM). Fluorescence emission ($\lambda_{ex} = 435$ nm) was measured after 90 min.

Fig. 4. Plot of H₂S concentration vs. N₃-PTS fluorescence. N₃-PTS (100 μM) and H₂S (0–100 μM) were incubated in 50 mM phosphate buffer pH 7.8 at room temperature for 90 minutes. Error bars denote one standard deviation from the mean of three experiments.

Fig. 5. Linear relationship between H₂S concentration and N₃-PTS fluorescence in 90% fetal bovine serum (FBS). N₃-PTS (100 μM) and H₂S (0–100 μM) were incubated in FBS at room temperature for 90 minutes. Error bars denote one standard deviation from the mean of three experiments.

Scheme 1.
Synthesis of 8-azidopyrene-1,3,6-trisulfonic acid