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An off-the-shelf sensor for colourimetric detection of sulfide

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ABSTRACT

The cheap, accessible spiropyran nitroBIPS is a sensitive, selective colourimetric sensor for quantitative determination of sulfide in aqueous media at neutral pH.

Introduction

There is now considerable evidence that supports hydrogen sulfide as being a third physiological gasotransmitter, alongside nitric oxide and carbon monoxide.[1–3] Contemporary research has implicated endogenous hydrogen sulfide release in many important pathologies[4–7] and various hydrogen sulfide donor molecules are undergoing scrutiny as therapeutic agents.[8,9] To support this expanding research field, there has been a tandem development in methods for the detection and quantification of hydrogen sulfide in solution.[10–12] Perhaps the key approach to this goal has been the synthesis of small molecule probes which enable colourimetric and/or fluorimetric measurement of hydrogen sulfide concentration in solution.[11,12] Although the challenges that this presents are manifold, the principal difficulty that an effective probe molecule must overcome is that physiologically relevant hydrogen sulfide concentrations are low (<1 μM)[13] and occur against a high background concentration of molecules that present similar chemical reactivity (e.g. thiols such as cysteine, homocysteine and glutathione; intracellular glutathione concentration is in the mM range).[14] Several classes of small molecule hydrogen sulfide probe have recently emerged which meet these stringent requirements for selectivity and sensitivity and have been employed with reasonable success in biological media;[11,12] nevertheless, none of these molecules have been adopted widely. We speculate that this is a matter of cost and convenience; there is a need for cheap, "off-the-shelf" probes for the majority of workers, who do not have access to specialist synthetic chemistry facilities. Recent development of a benzofurazan-based sulfide reporter has, in part, addressed this requirement;[15] herein we present a complementary approach.

Spiropyrans (e.g. **SP-1**; Scheme 1) are a class of spiro-fused indolochromene photochromes, commonly deployed in molecular/optical switching and sensing applications.[16–18] The photochromic behaviour displayed by a spiropyran is based upon the light-dependent equilibrium with its zwitterionic merocyanine isomer (e.g. **MC-1**): whereas spiropyrans are usually colourless in solution, merocyanines are fully conjugated, highly coloured and fluorescent (Scheme 1). Spiropyran/merocyanine-based H_2S probes, such as **2** (Fig. 1),[19] rely upon nucleophilic attack of HS^- upon the iminium ion present within the merocyanine structure, with the consequent disruption of the extended merocyanine conjugation resulting in reduced visible absorption/fluorescence emission (Scheme 1). As such, spiropyran/merocyanine-based H_2S sensors are often "switch-off" in nature. Such probes discriminate between H_2S and biologically competitive thiols on the basis of acidity: at physiological pH the lower pK_a value of H_2S (~7.0) compared with other biologically competitive thiols (e.g. cysteine, glutathione, $pK_a > 8.5$) ensures a greater degree of dissociation, hence a high concentration of strongly nucleophilic HS^-.

1′,3′-Dihydro-1′,3′,3′-trimethyl-6-nitrospiro[2*H*-1-benzopyran-2,2′-(2*H*)-indole] 1, commonly known as nitroBIPS, is a cheap and readily accessible spiropyran, available both commercially or through facile synthesis.[20] Although the response of nitroBIPS to H_2S has not been investigated, the same molecule has been used as a sensitive, selective probe for anionic cyanide.[21] In this capacity, nitroBIPS could accurately detect [CN^-] to a minimum of

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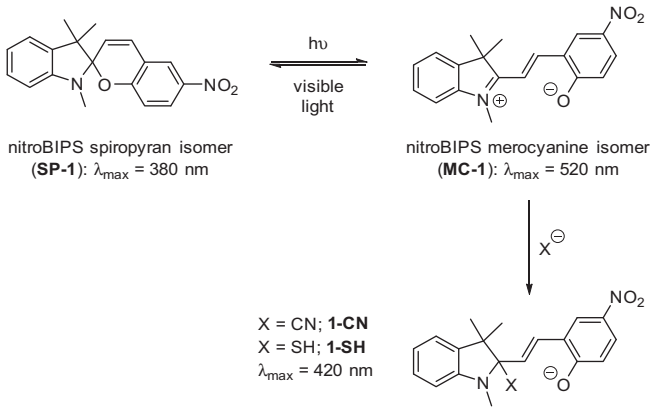


Scheme 1. Spiropyran-merocyanine equilibrium and reaction with cyanide/sulfide.

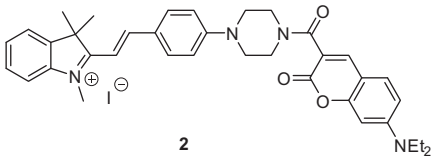


Fig. 1. Sulfide probe based upon a merocyanine-coumarin conjugate.

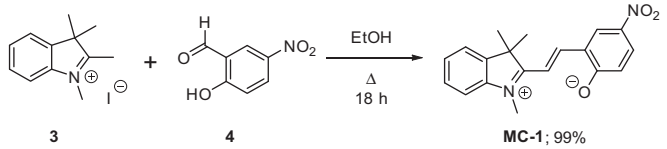
1.7 μM in 1:1 acetonitrile–water and showed a high degree of selectivity for cyanide against a range of potentially competitive anions. It is notable, however, that HS^- was not included and that these experiments were buffered to pH = 9.3 (the authors noted that sensitivity to NC^- was considerably diminished at neutral pH). The product of cyanide addition to nitroBIPS displayed a strong, blue-shifted absorbance distinct from that of the merocyanine; hence the cyanide response could be quantified either by a reduction in merocyanine absorbance and fluorescence (as a switch-off sensor) or by an increase in addition product absorbance (switch-on).

In light of the above studies, nitroBIPS presented an obvious candidate for investigation as an easily-accessible colourimetric/fluorimetric hydrogen sulfide probe. In this work, we assess the performance of nitroBIPS as a colourimetric probe for hydrogen sulfide in aqueous solution at physiological pH.

Results and discussion

NitroBIPS was synthesised *via* the straightforward condensation of tetramethylindolium iodide **3** and nitrosalicylaldehyde **4** in ethanol at reflux (Scheme 2).[20] The merocyanine isomer precipitated from the reaction mixture in excellent yield and high purity and following filtration, no further purification was necessary.

Given the photochromic nature of nitroBIPS and that previous work has identified the merocyanine isomer, and not the spiropyran, as the sole reactive electrophile in detection of cyanide,[21] our initial investigations identified appropriate conditions for use of nitroBIPS as a sulfide sensor by promoting merocyanine formation.



Scheme 2. Synthesis of nitroBIPS.

Preparation of a 0.1 mM solution of nitroBIPS in 1:1 acetonitrile–water (PBS 5 mM; pH = 7.4) resulted in a pink solution which displayed a strong absorbance in the visible region (518 nm), corresponding to the merocyanine isomer **MC-1**. This solution decolourised when exposed to standard ambient light for 10 min, with the corresponding loss of the 518 nm absorption band attributed to complete isomerisation to spiropyran **SP-1**. If the initially pink solution was kept in darkness, the initial merocyanine concentration remained approximately constant over 30 min. Consequently, we adopted dark conditions for the use of nitroBIPS as a sensor molecule in acetonitrile–water. This contrasts somewhat with previous work, where nitroBIPS in 1:1 acetonitrile–water (CHES 100 mM; pH = 9.3) required UV irradiation to ensure the presence of merocyanine; only spiropyran was present in darkness.[21]

NitroBIPS was then assessed as a sulfide sensor, as follows. Aqueous solutions of sulfide or other potentially competitive species (50 equiv.) were added to individual aliquots containing nitroBIPS (0.1 mM in 1:1 acetonitrile–water; PBS 5 mM; pH = 7.4) then left in darkness for 30 min before being analysed by UV–visible spectroscopy. From the results shown in Fig. 2, it is apparent that nitroBIPS is unreactive towards most nucleophiles under these conditions: solutions were produced that reflected modulation of the spiropyran-merocyanine equilibrium but did not suggest nucleophilic addition (addition of sodium sulfite resulted in a change in absorbance line shape but without apparent erosion of spiropyran or merocyanine absorbance and without appearance of further visible absorbance peaks). In contrast, in the presence of cyanide, a yellow solution was produced which absorbed strongly at 418 nm and displayed no merocyanine absorbance. A similar result was observed when nitroBIPS was treated with sulfide; however, the resulting solution displayed a further absorbance at 286 nm. On the basis of these results, nitroBIPS can be viewed as a selective sensor for sulfide; competition is only observed from cyanide (with weak interference from sulfite), and sulfide addition can be distinguished from that of cyanide by observation of the λ_{286} absorbance. It is important to note that quantitative sulfide detection *in the presence of cyanide* is not possible under these conditions, which would result in depletion of **MC-1** by cyanide and hence under-reporting of the λ_{286} absorbance. NitroBIPS is reactive towards sulfide but shows no reactivity towards apparently similar sulfur-based nucleophiles such as mercaptoethanol, mercaptopropionic acid, cysteine and glutathione. This distinction is absolutely crucial because cellular sulfide determination must occur against a high background concentration of cysteine and glutathione.

Quantitative determination of sulfide concentration was achieved by titration of sulfide against nitroBIPS and ratiometric analysis of the characteristic absorbance peaks assigned to **1-SH** (286 nm) and **MC-1** (518 nm). A linear relationship (R^2 = 0.9927) between Na_2S and **1-SH**/**MC-1** was observed, indicating that accurate measurement of sulfide is possible in this range (Fig. 3). The lower limit of detection was defined by the average of 5 repetitions of the blank experiment (i.e. in the absence of sulfide) + 3 standard deviations from the mean. In this instance, with [nitroBIPS] = 0.1 mM, this corresponded to a limit of detection of [HS^-] = 10.7 μM or, to provide generality in terms of sensor probe concentration, 1 equivalent of nitroBIPS provides a limit of detection of 0.11 equivalents sulfide. Ultimately, the absolute lower limit of detection will depend upon the lowest possible [nitroBIPS] detectable by the spectrophotometer. Using an entry level spectrophotometer (Jenway 7315), nitroBIPS could be detected to a minimum concentration of 5 μM, at which point sulfide detection remained effective (Fig. 2, inset). Consequently, applying our lower limit of detection to this experiment, we anticipate that statistically relevant detection of [SH^-] = 550 nM is possible.