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Rhodamine–propargylic esters for detection of mitochondrial hydrogen sulfide in living cells

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ABSTRACT

Flow cytometric detection of mitochondrial H_2S was achieved with propargylic esters of rhodamine B which selectively react with H_2S via cationic rhodamine-moiety directed thiolysis of the propargylic esters to give nonfluorescent rhodamine thio-spirolactone.

Hydrogen sulfide (H_2S) is an endogenous gaseous messenger in biological systems and regulates a broad variety of biological events, for example vasodilation and inflammation.[1] Generally considered to be synthesized in cytosol, H_2S was recently shown to be produced in mitochondria in vascular smooth-muscle cells due to the translocation of cystathionine-β-synthase, a H_2S-producing enzyme, from the cytosol into mitochondria under hypoxic conditions.[2] Despite the cytotoxicity of high levels of H_2S,[3] physiological levels of H_2S exhibited distinct effects on mitochondria ranging from sustaining energy production, depolarization, to inhibition of cellular respiration.[3,4] As such, agents that could selectively report the levels of intra-mitochondrial H_2S is highly desired to probe the aforementioned roles of H_2S.

Current reaction based probes rely on the double nucleophilicity or the reducing potential of H_2S to distinguish the analyte from interfering biological thiols such as cysteine (Cys) and glutathione (GSH).[5] Despite the advances in fluorescent imaging of H_2S, probes suitable for mitochondrial H_2S have been largely unexplored. Recently, a coumarin–cyanine diad was reported for imaging of H_2S in mitochondria for the first time.[6] In the investigation of Au(III) catalyzed rearrangement of propargylic esters,[7] we serendipitously found that the propargylic esters of rhodamine B (RB-PEs) quickly react with H_2S to give a colorless product (Scheme 1). The documented accumulation of cationic rhodamine derivatives in mitochondria[8] prompted us to explore the feasibility to detect mitochondrial H_2S with RB-PEs by dose-dependent 'turn-off' fluorescence.

The red color of RB-PEs quickly disappeared upon treatment with sodium sulfide (Na_2S), which is used as the donor of H_2S, in dimethylformamide (DMF). Analysis of the reaction media by TLC revealed the formation of a colorless product. Mass spectrometry analysis revealed a major peak located at 459.2 (Fig. S1, Supplementary data), which is consistent with the theoretical molecular weight of the proposed product ($C_{28}H_{30}N_2O_2S$, MH^+: 459.2) (Scheme 1). The product was further isolated by silica gel column chromatography and characterized by ^1H NMR and ^{13}C NMR (Supplementary data). The analytical data were found to be identical to that of reported rhodamine thiospirolactone,[9] confirming the molecular identity of the product as described in Scheme 1.

To probe the influences of propargylic moieties, three rhodamine B derivatives containing different propargylic moieties were prepared (Scheme 1) and assayed for their relative reaction rates with Na_2S by monitoring the fluorescence emission as a function of time (Fig. 1). It was shown that the fluorescence of RB-PE-1, RB-PE-2 and RB-PE-3 quickly faded in DMF upon addition of Na_2S whereas the methyl ester of rhodamine B (RB-ME) was largely unaffected (Fig. 1), suggesting the critical role of the propargylic moieties in sensing of H_2S. In a separate experiment, the propargylic ester of benzoic acid was found to inactive to Na_2S in DMF under the assay conditions, revealing the requirement of

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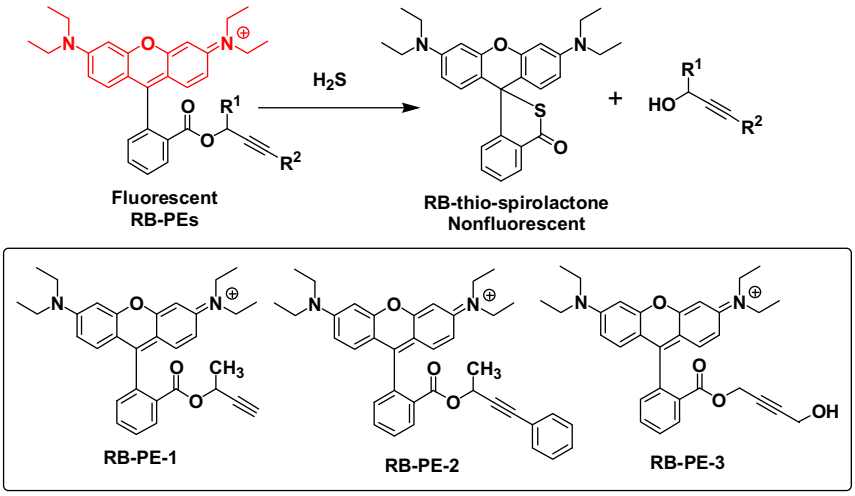


Scheme 1. Reaction of H_2S with RB-PEs.

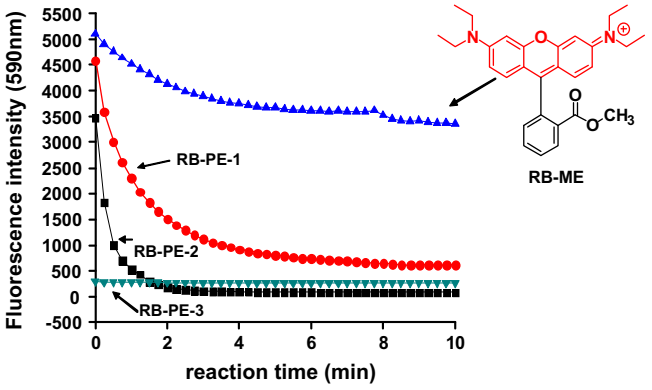
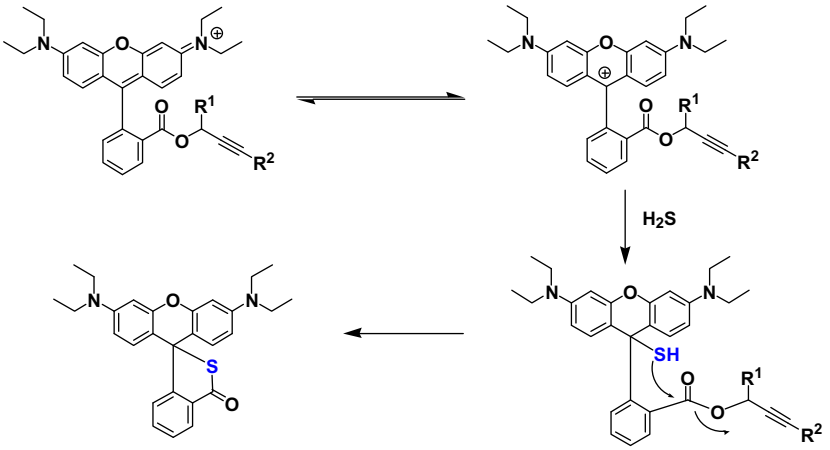


Figure 1. Kinetic profiles of the reactions between RB-PE-1, RB-PE-2, RB-PE-3 or RB-ME (5 μM) and Na_2S (20 μM) in DMF. The reaction rates were monitored by recording the fluorescence emission@590 nm as a function of time (λ_{ex}@560 nm).

the rhodamine B moiety in detection of H_2S. Taken together, these results revealed the synergistic effects of the rhodamine moiety and the propargylic group in effective sensing of the analyte. Albeit the exact reaction mechanism is not clear, it is likely that the cationic rhodamine moiety could direct the anionic suifide to nucelophilic attack the carbonyl moiety of the propargylic ester to give the colorless product (Scheme 2).

With a few exceptions,[5f,6,10] tens of minutes or hours are required for the assays of many reported probes. As H_2S is volatile and poised to air oxidation, the quick response of RB-PE-3 to Na_2S suggests its utility for real-time studies of H_2S-generating biological processes (Fig. 1). To access the detection range, various amounts of Na_2S was spiked into DMF containing RB-PE-1, RB-PE-2 or RB-PE-3. The solutions were mixed and then analyzed by fluorometry. Figure 3A showed that the fluorescence emission@590 nm decreased as a function of Na_2S concentrations. As expected, RB-ME failed to react with Na_2S under identical conditions (Fig. S4, Supplementary data). In mitochondria, cytochrome C is half maximally inhibited by 20 μM H_2S.[11] The titration revealed that 0–20 μM H_2S can be effectively detected by RB-PEs where subtle alterations of H_2S concentrations can be discerned (Fig. 2A), suggesting that the applicability of these probes for detection of H_2S in mitochondria.

Biological thiols are ubiquitous within mammalian cells. For instance, cytosol contains high levels of glutathione (GSH) while mitochondria contain abundant cysteine (Cys).[12] It is essential that RB-PEs are immune to these thiols. Hence, the reactivity of these probes was investigated towards representative biological thiols. Figure 2B showed that all the RB-PEs could efficiently detected Na_2S whereas no obvious changes on fluorescence emission were observed for RB-PEs cultured with GSH, Cys, or homocysteine (HCY), suggesting their stringent selectivity for H_2S over these biological thiols.



Scheme 2. Possible sensing mechanism of RB-PEs for H_2S.