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Benzothiazoline based chemodosimeters for fluorogenic detection of hypochlorous acid

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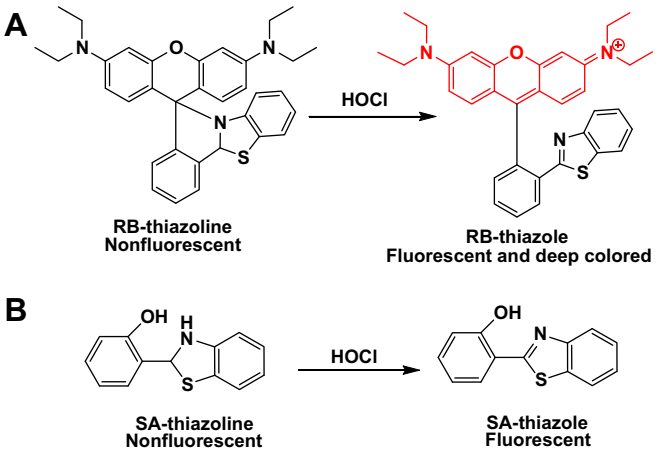
ABSTRACT

Two nonfluorescent and colorless chemodosimeters featuring benzothiazoline moiety were developed for chromo-fluorogenic detection of HOCl.

Hypochlorous acid (HOCl), with pK_a of 7.5, is poised to dissociate into hypochlorite (OCl^-) in aqueous media. Artificial HOCl or hyperchlorite is widely used as disinfection agents in civilized society. Biogenic HOCl, synthesized from hydrogen peroxide and chloride via the catalysis of myeloperoxidase in leukocytes, could also be directed against infectious microorganisms by the host immune systems. Despite its protective roles, abnormal levels of HOCl in vivo have been associated with multiple diseases including arthritis, neuron degeneration and cancers.[1] Imaging agents that allow facile detection or fluorescent tracking of HOCl will be valuable for investigations on HOCl related biological processes or monitoring the levels of HOCl in abiotic samples, for example, drinking water. The multiple roles of HOCl in the aforementioned phatho-physiological events had inspired the development of a number of chemodosimeters for HOCl detection via analyte triggered oxidation of integrated HOCl-responsive fuctionalities including hydroxamate, hydrazide, and thioester, etc.[2] Here we report the fluorogenic detection of HOCl with two benzothiazoline-based chemodosimeters. Specifically, SA-thiazoline, conjugate of salicylaldehyde with 2-aminothiophenol, undergoes HOCl mediated oxidation of the thiazoline to give SA-thiazole which is a conventional fluorophore employed in a number of studies.[3] In contrast, the rhodamine B-benzothiazoline diad (referred to as RB-thiazoline) gives highly fluorescent and deep colored species via oxidative opening of the intramolecular C–N bond (Scheme 1).

RB-thiazoline and SA-thiazoline, colorless and nonfluorescent, were respectively prepared via condensation of 2-aminothiophenol with 2-hydroxybenzaldehyde or rhodamine-aldehyde (Supplementary data). We first examined the influence of solvents on the fluorogenic reactions and identified that RB-thiazoline preferred polar organic solvents whereas SA-thiazoline was applicable in



Scheme 1. Detection of HOCl with RB-thiazoline (A) and SA-thiazoline (B).

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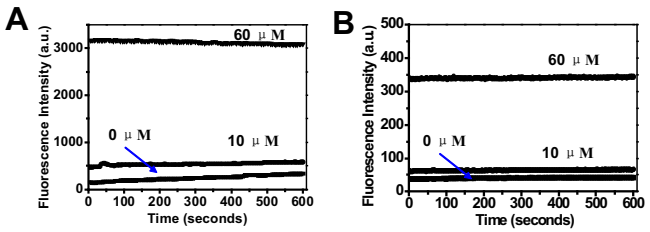

Figure 1. Kinetic profiles of the reactions between NaOCl and RB-thiazoline or SA-thiazoline. (A) The fluorescence emission of RB-thiazole (10 μM) in ethanol containing NaOCl (0, 10, or 60 μM) was recorded as a function of time (λ_{em}@590 nm; λ_{ex}@560 nm); (B) time correlated fluorescence emission of SA-thiazole (20 μM) at 462 nm in phosphate buffered saline (PBS)/ethanol (50%, v/v) containing NaOCl (0, 10 or 60 μM) was recorded using λ_{ex}@402 nm.

aqueous media. Time course monitoring of formation of fluorescent species in the optimized assay solutions by fluorometry showed that NaOCl triggered oxidation of the chemodosimeters was completed immediately upon addition of various levels of NaOCl (Fig. 1).

To ascertain the identities of the fluorescent species generated under the optimized assay conditions, the assay solutions of RB-thiazoline and SA-thiazoline were respectively, analyzed by mass spectrometry (MS). A major signal at 532.2412 was identified in the assay solution of RB-thiazoline (Fig. S1, Supplementary data), confirming the genesis of RB-thiazole ($C_{34}H_{34}N_3OS^+$; MW: 532.2417). In a parallel experiment, generation of SA-thiazole was also confirmed by MS (Fig. S2, Supplementary data). SA-thiazole was isolated by silica gel chromatography and then analyzed by ^{1}H NMR and ^{13}C NMR (Supplementary data). Collectively, these results supported the proposed HOCl-mediated oxidation mechanisms as described in Scheme 1.

To probe the assay sensitivity, RB-thiazoline and SA-thiazoline were respectively, added into a serial of solutions containing varied levels of NaOCl. The fluorescence emission intensities of the solutions were recorded by fluorometry. Figure 2A showed that fluorescence emission of RB-thiazole centred at 590 nm intensified as a function of analyte concentrations. The highly fluorescent RB-thiazole is of deep red color, which indicated its utility in visual estimation of HOCl in selected samples, for example, tap water. The fluorescence emission of SA-thiazole, maximal at 462 nm, proportionally increased as a function of HOCl concentration (Fig. 2C and D). In both assays, 10 μM of HOCl can be detected.

Reactive oxygen species (ROS) could be generated by mammalian cells in responses to environmental stress. Demonstrated to be responsive to HOCl, RB-thiazoline and SA-thiazoline were further evaluated for their selectivity for other representative ROS. Figure 3 showed that SA-thiazoline and RB-thiazoline were both inert to H_2O_2, nitric oxide (NO), **.**OH, ROO**.**, and $.O_2^-$. 2-(2-Pyridyl)benzothiazoline was used for fluorogenic detection of superoxide anion.[4] It was shown that SA-thiazoline was highly responsive to HOCl as compared to superoxide anion radical. (Fig. 3 and Fig. S4, Supplementary data). Historically, rhodmaine-deoxylactams, derivatives of rhodamine featuring intramolecular deoxylactams have been used to stain lysosomes with acidic intracompartmental pH in live cells by proton mediated opening of the intramolecular deoxylactams.[5] We therefore probed the fluorogenic responses of SA-thiazoline and RB-thiazoline to pH and various ions that are often present in biological specimens. It was shown that SA-thiazoline exhibited negligible fluorescence towards all ions tested which included H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cl^-, HPO_4^{2-}, and HCO_3^- and SO_4^{2-} (Fig. 3B), indicating the stringent selectivity of SA-thiazoline for HOCl. In contrast, RB-thiazoline displayed significant fluorogenic response to Fe^{3+} (Fig. 3A) and proton (pH 4), presumably due to cation promoted opening of the intramolecular deoxylactam. Taken together, the findings showed that RB-thiazoline with moderate preference for HOCl whereas SA-thiazoline is highly selective for HOCl, indicating its utility for HOCl imaging in biological specimens.

To probe the impact of the hydroxyl moiety of SA-thiazoline in sensing of HOCl, benzyl-thiazoline, which is an structural analog of

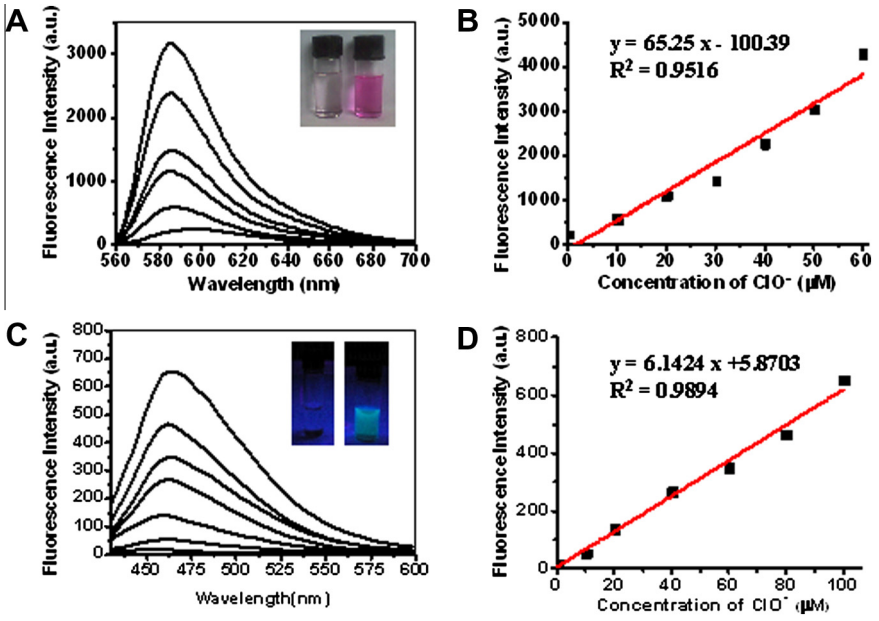

Figure 2. Titration of HOCl with RB-thiazoline and SA-thiazoline. (A) The fluorescence emission spectra of RB-thiazoline (10 μM) in ethanol containing various levels of NaOCl (60, 50, 40, 30, 20, 10, and 0 μM, from top to bottom) (λ_{ex}@560 nm); the insert showed the visual images of the assay solution of RB-thiazoline before and after addition of NaOCl (60 μM); (B) titration curve of RB-thiazoline based assay was plotted by fluorescence emission intensity@590 nm versus NaOCl concentrations; (C) fluorescence emission of SA-thiazoline (20 μM) in PBS buffered ethanol (1:1, v/v) spiked with NaOCl (100, 80, 60, 40, 20, 10, and 0 μM; from top to bottom) (λ_{ex}@402 nm); the insert showed images of SA-thiazole solution with or without addition of NaOCl (60 μM) under UV-illumination; (D) the titration curve of SA-thiazoline was plotted using fluorescence emission intensity@462 nm as a function of NaOCl concentrations.