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PAPER

Signaling of hypochlorous acid by selective deprotection of dithiolane†

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The selective signaling of hypochlorous acid by dithiolane-protected pyrene-aldehyde was investigated. Dithiolane derivative of pyrene-aldehyde was efficiently deprotected by hypochlorous acid to its corresponding aldehyde, which resulted in a prominent UV-vis and turn-on type fluorescence signaling. The signaling was not affected by the presence of other common alkali, alkaline earth metal ions, and anions. Interference from Hg^{2+} ions could be successfully circumvented by using Chelex-100 resin. Dithiolane also provided selectivity toward hypochlorous acid over other commonly used oxidant of hydrogen peroxide.

Introduction

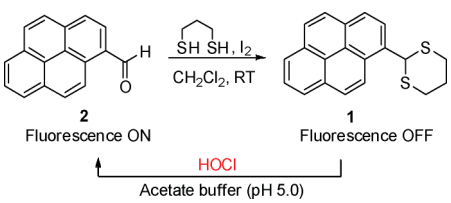
There is increasing research effort to develop optical signaling and visualizing techniques for a variety of important metal ions, anions, and biologically active species.[1] For this purpose, chemical probes have attracted much attention because of their specificity and cumulative signaling effects.[2] They are based on target analyte-induced specific reactions or the transformation of probes that are accompanied by changes in absorption or fluorescence behavior. Among these, the sensing or visualizing of chemically and biologically important oxidants, such as hydrogen peroxide,[3] peroxynitrite,[4] hypochlorous acid,[5] and peracids[6] have recently attracted particular interest.

Hypochlorous acid (HOCl) is widely used as a bleaching agent and disinfectant in occupational health, hygiene, and sanitation.[7] It is also suited for applications in laboratory syntheses and scaled-up processes,[8] and has been shown to be a useful oxidant for the conversion of various functional groups including epoxidation of alkenes,[9] oxidation of alcohols to aldehydes,[10] and dihydroxylation of various terminal and internal olefins.[11] Although HOCl is widely used, a convenient optical signaling system for the identification of HOCl is rare;[12] iodometric,[13] amperometric,[14] and potentiometric[15] methods are most commonly used. Recently, a BODIPY-based fluorescent probe that is based on a specific reaction of *p*-methoxyphenol with HOCl was designed.[5b] Ferrocene-anthracene based fluorescent probe[16] and rhodamine-hydroxamic acid were also successfully used for biological imaging of HOCl.[5c] In addition, rhodamine B-based hydrazide[17] and thiospirolactone[18] exhibited efficient HOCl signaling behavior.

The dithiolane group is one of the most useful protecting tools in synthetic organic chemistry.[19] As a carbonyl protection group, its dithioacetal function has found wide use in organic synthesis due to its easy access and high stability in both acidic and basic conditions.[20] Among many reagents used for the deprotection of dithiolane, various oxidants, such as *m*CPBA, SeO_2, ceric ammonium nitrate, *t*-BuOOH, $NaBO_3$, $PhI(O_2CCCl_3)_2$, $Tl(NO_3)_3$, and Oxone are known to be effective.[19] In this paper, we report a simple and selective signaling system for HOCl based on the facile deprotection of dithiolane by HOCl, which is an unprecedented. HOCl-selective signaling was observed in the presence of representative metal ions as well as common anions. The designed probe seems to be suitable for the signaling of HOCl in various samples of practical importance, such as bleaching agent, disinfectant, as well as oxidant in synthetic and industrial applications.

Results and discussion

Compound **1**[21] was prepared by the reaction of 1-pyrenecarboxaldehyde with 1,3-propanedithiol using iodine (yield, 98%) (Scheme 1).[22] The UV-vis spectrum of dithiolane **1** showed strong absorption bands at 315, 329, and 345 nm in 50% aqueous acetonitrile. Upon treatment of **1** with 10 equiv HOCl, absorption bands of **1** disappeared and new bands at 361 and 395 nm appeared (Fig. 1) due to deprotection of the dithiolane group of **1** by HOCl to yield aldehyde **2** (Scheme 1). Commonly encountered metal ions and anions revealed almost no signaling except for Hg^{2+}, which is known as a good deprotecting species for dithiolane (Fig. S1, ESI†).[19] This observation is in line with the



Scheme 1 Preparation of dithiolane probe **1** and its HOCl-selective signaling.

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† Electronic supplementary information (ESI) available: General experimental details with additional chemosignaling behaviors of **1**–HOCl system and ^1H and ^{13}C NMR spectra. See DOI: 10.1039/c1ob06012k

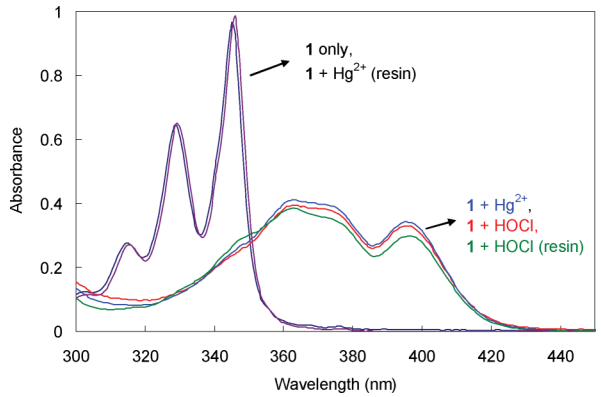

Fig. 1 UV-vis spectra of **1** and **1** in the presence of HOCl and Hg^{2+}. [**1**] = 2.0×10^{-5} M, [HOCl] = [Hg^{2+}] = 2.0×10^{-4} M in a mixture of CH_3CN and acetate buffer solution (pH 5.0, 10 mM), (1 : 1, v/v). Resin means treatment with Chelex-100 chelating resin.

work of Kim *et al.* that reported a Hg^{2+}-selective signaling behavior of coumarin-based dithiolane using Hg^{2+}-induced deprotection.[23] Response of **1** toward Hg^{2+} ions might be a serious drawback and limited its general use for the analysis of HOCl. However, the interference from Hg^{2+} ions could be successfully circumvented by using Chelex-100 resin, which is noteworthy for its ability to bind transition metal ions.[24] Interfering Hg^{2+} ions could be effectively sequestered by using this high affinity resin and the selective signaling of HOCl is not interfered any further (see **1** + Hg^{2+} and **1** + Hg^{2+} (resin) in Fig. 1).

The fluorescent signaling behavior of **1** toward HOCl and representative metal ions and anions was surveyed (Fig. 2 and Fig. S2, ESI†). Compound **1** exhibited weak fluorescence emissions around 378, 389, and 398 nm, which is characteristic of a pyrene moiety. Upon treatment of **1** with 10 equiv HOCl, a new strong emission at 457 nm evolved. Fluorescence enhancement observed at 457 nm was 145-fold. Upon interaction with metal ions, dithiolane **1** showed a pronounced fluorescence enhancement (155-fold) at 457 nm exclusively with Hg^{2+} ions as observed in absorption measurements. Other metal ions and common anions that were surveyed revealed almost no response. The fluorescence intensity ratio I/I_o of **1** observed at 457 nm varied within a narrow range, between 0.7 (Fe^{3+}) and 1.1 (Ag^{+}) for metal ions, and 0.7 (OAc^{-}) and 1.0 (Br^{-}) for anions, that resulted in pronounced HOCl selectivity (Fig. S3 and S4, ESI†). Interference from Hg^{2+} ions could also be effectively removed by using Chelex-100, and the fluorescence intensity ratio I/I_o of **1** + Hg^{2+} (resin) observed at 457 nm was 1.1 (see **1** + Hg^{2+} and **1** + Hg^{2+} (resin) in Fig. 2 and Fig. S3, ESI†). A time course plot showed that the signaling of HOCl by **1** was relatively fast and was completed within 1 min (Fig. S5, ESI†). Furthermore, signaling of HOCl by **1** was independent of pH in the range of 3.1 to 9.1 (Fig. 3).

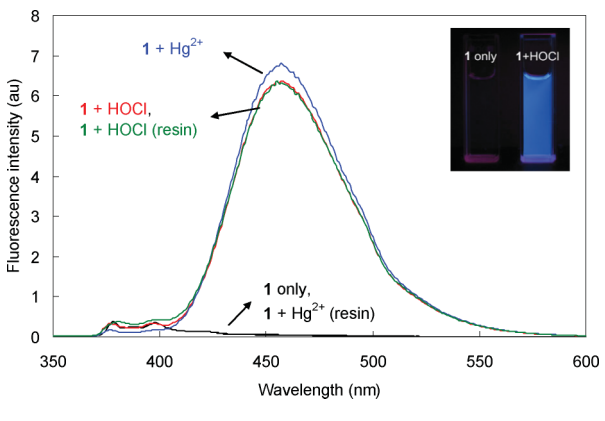

Fig. 2 Changes in fluorescence spectra of **1** and **1** in the presence of HOCl and Hg^{2+}. [**1**] = 5.0×10^{-6} M. [HOCl] = [Hg^{2+}] = 5.0×10^{-5} M in a mixture of CH_3CN and acetate buffer solution (pH 5.0, 10 mM), (1 : 1, v/v). λ_{ex} = 340 nm. Resin means treatment with Chelex-100 chelating resin.

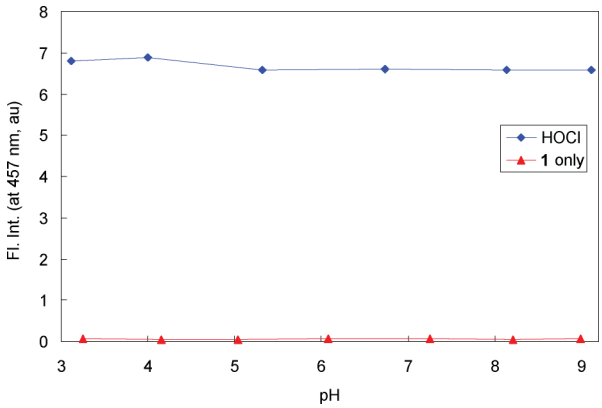

Fig. 3 Effect of pH on the fluorescence signaling of HOCl by **1**. Measured at 457 nm. [**1**] = 5.0×10^{-6} M, [HOCl] = 5.0×10^{-5} M in a mixture of CH_3CN and water, (1 : 1, v/v). λ_{ex} = 340 nm. Solution pH was adjusted by HCl or NaOH.

As described earlier, signaling was due to the HOCl-triggered deprotection of the dithiolane group to aldehyde (Scheme 1). The suggested transformation was evidenced by the ^{1}H NMR, UV-vis, and fluorescence measurements. The ^{1}H NMR spectrum of **1** obtained for the purified reaction product of **1** with 10 equiv HOCl was almost that of **2**; the methine proton at 6.23 ppm (highlighted in red star) and methylene protons around 2.0–3.4 ppm of the dithiolane moiety of **1** disappeared and a new peak at 10.8 ppm (highlighted in blue star) appeared, which is characteristic of the aldehyde proton of **2** (Fig. 4). In addition, the UV-vis and fluorescence spectra of **1** in the presence of 10 equiv HOCl were almost identical to those of **2** (Fig. S6 and S7, ESI†).

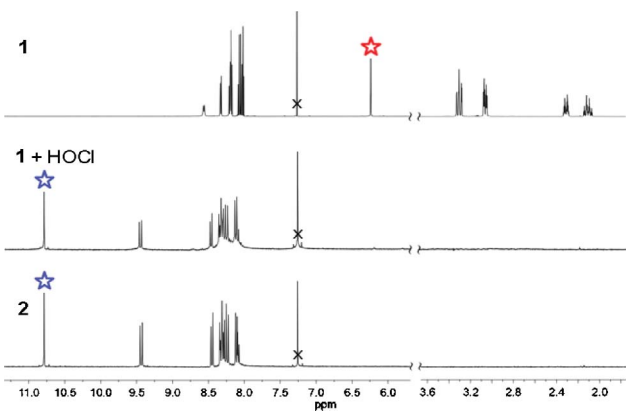

Fig. 4 Partial ^{1}H NMR spectra of **1**, **1** upon reaction with HOCl, and **2** in $CDCl_3$. [**1**] = [**2**] = 2.0×10^{-3} M. The middle spectrum (**1** + HOCl) was obtained for the purified reaction product of **1** with HOCl (10 equiv) in 50% aqueous acetonitrile.

Concentration-dependent signaling of HOCl was measured by the treatment of **1** with varying amounts of analytes.[25] Fluorescence intensity increased linearly as a function of HOCl concentration up to 1.0×10^{-5} M (Fig. 5). Based on this result, the detection limit of **1** for the determination of HOCl was estimated as 9.2×10^{-7} M (0.048 ppm) in 50% aqueous acetonitrile.[26]

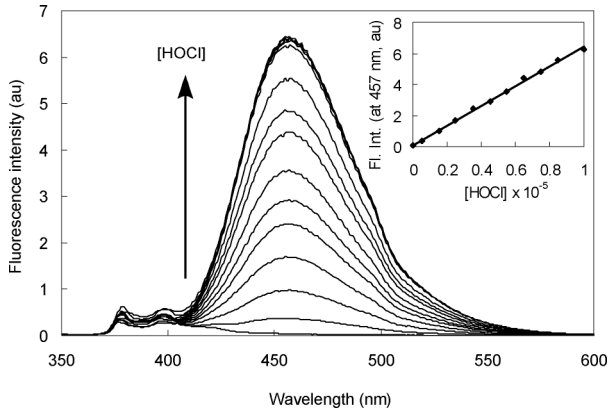

Fig. 5 Concentration-dependent fluorescence signaling of HOCl by **1**. [**1**] = 5.0×10^{-6} M in a mixture of CH_3CN and acetate buffer solution (pH 5.0, 10 mM), (1 : 1, v/v). λ_{ex} = 340 nm.

Practical applicability of **1** as a HOCl signaling device was verified by competitive signaling experiments with representative metal ions and anions (Fig. 6). Compound **1** exhibited unaltered signaling for HOCl in the presence of commonly encountered metal ions as background except for Ag^+ ion. That might be because Ag^+ ion forms white AgCl precipitate with Cl^- derived from HOCl. On the other hand, with the commonly encountered anions as background, fluorescence signaling was considerably reduced by bromide and iodide. This observation might be due to the direct reaction of HOCl with bromide and iodide to form free bromine and iodine.[27] In the presence of bromide or iodide ions, HOCl in the analyte will be consumed by these ions, and the analysis of HOCl itself would be meaningless. Other anions were found not interfere at all.

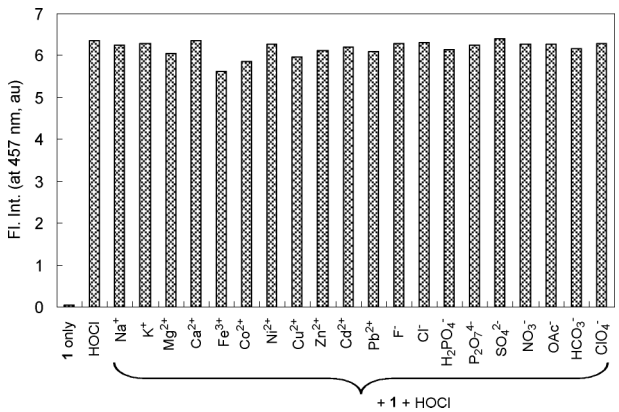

Fig. 6 Competitive fluorescent signaling of HOCl by **1** in the presence of metal ions and anions as background. [**1**] = 5.0×10^{-6} M, [HOCl] = [M^{n+}] = [A^{n-}] = 5.0×10^{-5} M in a mixture of CH_3CN and acetate buffer solution (pH 5.0, 10 mM), (1 : 1, v/v). λ_{ex} = 340 nm. Fluorescence intensities were measured at 457 nm.

Possibility of selective signaling of hypochlorous acid with **1** over other commonly used oxidants was tested. As can be seen from Fig. 7, compound **1** showed a pronouncedly selective HOCl signaling over important oxidant of hydrogen peroxide and *tert*-butyl hydroperoxide (TBHP). However, as expectedly, dithiolane **1** also revealed considerable responses toward other important oxidants of peracetic acid (PAA), and superoxide. In spite of these responses, after 1 min of sample preparation, there was significant selectivity toward HOCl ($I_{HOCl}/I_{Oxidant}$ at 457 nm = 164 for hydrogen peroxide, 124 for TBHP, 99 for PAA, 37 for superoxide). In practical purpose, discrimination of HOCl over other oxidant seems to be not so critical, because HOCl is frequently used as a single component oxidant. All these observations suggest that the dithiolane **1** could be used as a selective fluorescent signaling system for HOCl in aqueous environments. Another possible application of dithiolane **1** for the cell imaging, however, is not feasible due to the fact that short wavelength irradiation at 340 nm was required for the excitation.

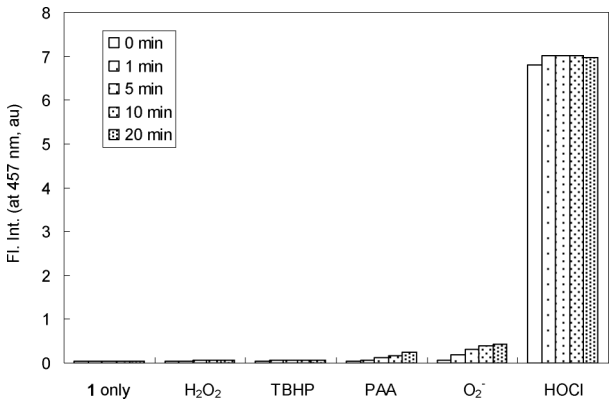

Fig. 7 Time dependent changes in fluorescent intensity at 457 nm of **1** in the presence of HOCl and other oxidants. [**1**] = 5.0×10^{-6} M. [Oxidant] = 5.0×10^{-5} M in a mixture of CH_3CN and acetate buffer solution (pH 5.0, 10 mM), (1 : 1, v/v). λ_{ex} = 340 nm. TBHP: *tert*-Butyl hydroperoxide, PAA: Peracetic acid.

Finally, applications of **1** for the analysis of real samples of daily household items were tried. Two commercial bleach products obtained from the local market were analyzed. The determined concentrations of hypochlorite were compared with those obtained by the recommended iodometric titration with thiosulphate (Fig. 8). They are in good agreement in the range up to 1.0×10^{-5} M of HOCl. In addition to this, analysis of hypochlorite in tap water was tried. As shown in Table 1, present method could be used for the analysis of 0.18 ppm of hypochlorite which is present in tap water. Recovery between the present method and the well-known DPD method was in good agreement within ±2.5%.[28]

Conclusions

In summary, a simple chemosignaling system for the widely-used HOCl oxidant was devised based on the deprotection of dithiolane to aldehyde. The pyrene-dithiolane derivative demonstrated efficient UV-vis and fluorogenic signaling of HOCl in a mixed aqueous solution. The devised compound also exhibited a pronounced and selective signaling behavior in the presence of

Table 1 Comparative results of HOCl detection in tap water[a]

Samples	Conc.[b] (ppm)	Conc.[c] (ppm)	Relative Error (%)
Tap water 1	0.18	0.179	0.48
Tap water 2	0.19	0.182	4.18
Tap water 3	0.18	0.175	2.68
Average	0.183	0.179	2.45

[a] Measured at 457 nm. [**1**] = 5.0×10^{-6} M in a mixture of CH_3CN and acetate buffer solution (pH = 5.0, 10 mM), (1 : 1, v/v). λ_{ex} = 340 nm. [b] Measured by DPD-method.[28] [c] Measured by **1**.

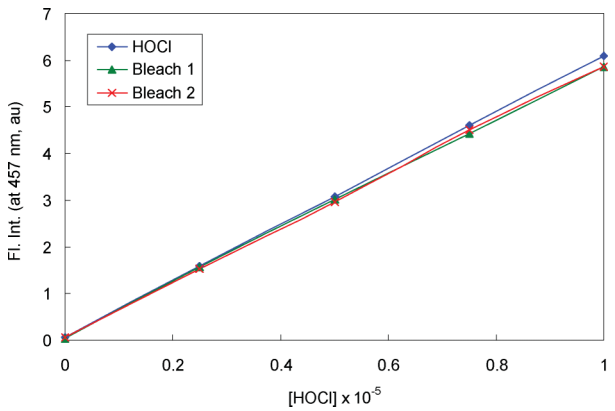

Fig. 8 Concentration-dependent fluorescence signaling of HOCl and commercial bleaches by **1**. Measured at 457 nm. [**1**] = 5.0×10^{-6} M in a mixture of CH_3CN and acetate buffer solution (pH = 5.0, 10 mM), (1 : 1, v/v). λ_{ex} = 340 nm.

common metal ions and anions. Interference from Hg^{2+} ions could be readily circumvented by using Chelex-100 resin. Furthermore, selectivity over another important oxidant of hydrogen peroxide was also observed.

Experimental section

1-Pyrenecarboxaldehyde **2**, 1,3-propanedithiol, and iodine were purchased from Aldrich Chemical Co. and used without further purification. All solvents were purchased from Aldrich Chemical Co. as 'anhydrous' or 'spectroscopic grade'. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were obtained on a Varian VNS NMR spectrometer and referenced to the residual solvent signal. UV-Vis spectra were recorded with a Jasco V-550 spectrophotometer equipped with a Peltier temperature controller. Fluorescence spectra were measured on an Aminco-Bowman Series 2 Spectrophotometer. Mass spectrum was obtained on a JMS-AX505WA (JEOL).

Preparation of 1

To a solution of 1-pyrenecarboxaldehyde **2** (0.23 g, 1.0 mmol) and 1,3-propanedithiol (0.12 g, 1.1 mmol) in CH_2Cl_2 (5 mL) was added iodine (0.025 g, 0.1 mmol) and the resulting mixture was stirred at room temperature for 1 h. The reaction was quenched by the successive addition of aqueous solutions of $Na_2S_2O_3$ (0.1 M, 5 mL) and NaOH (10%, 5 mL). The resulting reaction mixture was extracted with CH_2Cl_2 and the organic layer was separated and washed with water. The combined organic solution was dried over anhydrous $MgSO_4$ and filtered. Evaporation of the solvent and concentration in vacuum gave the crude product. Yellowish product **1** was purified by crystallization from CH_2Cl_2 and CH_3OH. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.56 (d, J = 12.0 Hz, 1H), 8.33 (d, J = 6.0 Hz, 1H), 8.21–8.16 (m, 4H), 8.08–8.00 (m, 3H), 6.23 (s, 1H), 3.32–3.27 (m, 2H), 3.07–3.03 (m, 2H), 2.33–2.28 (m, 1H), 2.14–2.06 (m, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 132.2, 131.4, 131.3, 130.7, 127.9, 127.9, 127.8, 127.3, 126.0, 125.9, 125.5, 125.3, 125.2, 124.9, 124.8, 122.7, 48.9, 32.9, 25.4. HRMS (DIP) m/z calcd for $C_{20}H_{16}S_2$ $[M]^+$ 320.0693; found, 320.0693.

Analysis of hypochlorite in bleach and tap water

Concentration of HOCl in commercial bleaches was determined by iodometry.[13] Stock solutions of each sample (1.0 mM) were prepared in distilled water. Stock solution of **1** (5.0×10^{-4} M) in CH_3CN and acetate buffer solution (1.0 M) in distilled water were also prepared. Test solutions were prepared by placing 30 μL of stock solution of **1**, 30 μL of acetate buffer solution, and various volume (0–30 μL) of each sample containing HOCl (1.0 mM) in a vial. The resulting solutions were diluted to 3.0 mL with distilled water and CH_3CN to make a final composition of 1 : 1, v/v. The final concentration of **1** and acetate buffer was 5.0×10^{-6} M and 1.0×10^{-2} M, respectively. Free chlorine in tap water was measured by published procedure using DPD-method.[28] To measure HOCl concentration in tap water with present method, test solutions were prepared by placing 30 μL of stock solution of **1**, acetate buffer solution, and 1.47 mL of tap water in a vial. The test solutions were diluted to a final volume of 3.0 mL with CH_3CN. Concentration of HOCl in tap water was calculated by using a linear calibration plot obatined by measuring fluorescence intensities at 457 nm. Measurements of each solution were carried out in triplicate.

Acknowledgements

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