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A Ratiometric Fluorescent Probe for Hypochlorite Based on a Deoximation Reaction

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Abstract: In this work, we have successfully provided a novel strategy for the rational design and synthesis of a ratiometric fluorescent probe for hypochlorite. The strategy is based on the deoximation reaction, which has not yet been used in the fluorescent hypochlorite probe design. Interestingly, the probe showed a ratiometric fluorescent response to hypochlorite with the emission intensities ratio (I_{509}/I_{439}) increasing from 0.28 to 2.74. Furthermore, the probe displayed high selectivity for hypochlorite over other species due to the distinct deoximation conditions. The probe developed herein represents the first ratiometric fluorescent probe for hypochlorite.

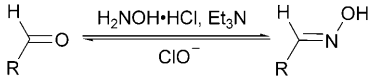
Keywords: charge transfer · fluorescence spectroscopy · fluorescent probes · hypochlorite · ratiometric

Introduction

Hypochlorite (ClO^-) is widely employed in our daily life, such as household bleach, disinfection of drinking water, cooling-water treatment, and cyanide treatment.[1] Typically, it is used in the concentration range of 10^{-5}–10^{-2} M.[1] However, concentrated hypochlorite solutions are a potential health hazard to human and animals.[2] On the other hand, hypochlorite is one of the important reactive oxygen species (ROS) in living organisms,[3] and it plays a critical role in the immune system. The endogenous hypochlorite is produced from peroxidation of chloride ions catalyzed by the enzyme myeloperoxidase (MPO).[4] However, the abnormal production of hypochlorite owing to variations in MPO levels can lead to a variety of diseases including cardiovascular diseases,[5] neuron degeneration,[6] arthritis,[7] and cancer.[8] Thus, it is of great interest to detect hypochlorite by sensitive and selective fluorescent probes. A limited number of selective hypochlorite/hypochlorous acid fluorescent probes has been constructed only very recently,[9] all of which respond to hypochlorite/hypochlorous acid with changes only in fluorescent intensity. A major limitation of intensity-based probes is that variations in the sample environment and probe distribution are problematic for quantitative measurements.[10] By contrast, ratiometric fluorescent probes allow the measurement of emission intensities at two different wavelengths, which should provide a built-in correction for environmental effects and can also increase the dynamic range of fluorescence measurement.[11]

To our best knowledge, no ratiometric fluorescent probes for hypochlorite have been reported thus far. We therefore wanted to develop a fluorescent probe which displayed a ratiometric response to hypochlorite using intramolecular charge transfer (ICT) as a signaling mechanism.[12] An ICT system contains an electron donor and an electron acceptor. As the ICT efficiency can be regulated by the variation in electron donor/acceptor strength, we decided to employ this key feature of ICT in our ratiometric fluorescent probe design.

Recently, we have demonstrated that a specific reaction promoted by an analyte could be employed to devise probes with high selectivity for the analyte.[13] Therefore, for a design of a highly selective probe for hypochlorite, we turned our attention to select a specific reaction promoted by hypochlorite. In organic synthesis, aldehyde groups can be protected as oximes,[14] which are rapidly deprotected by hypochlorite under mild conditions (i.e., at ambient temperature) (Scheme 1).[15]



Scheme 1. Protection of aldehydes as oximes and deprotection by ClO^-.

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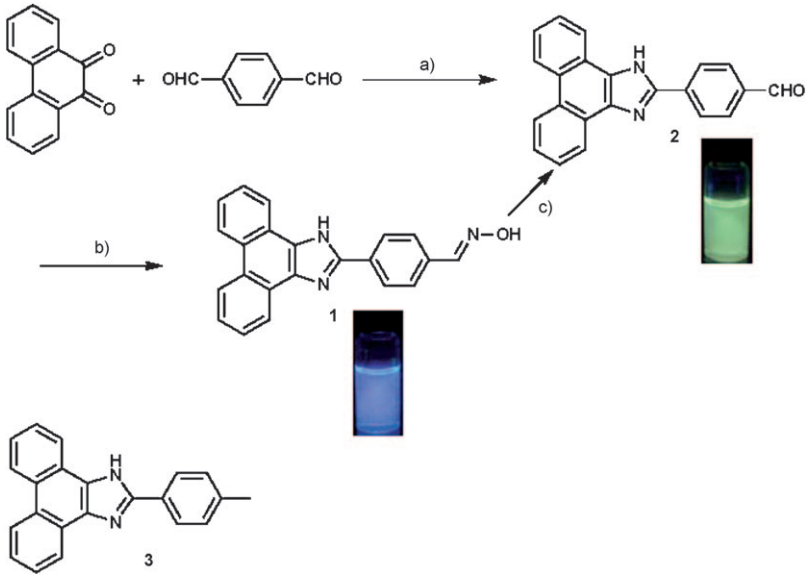
Although the mechanism of oxidative deoximation with hypochlorite has not been described in the literature thus far, we envisioned that this deprotection reaction could be exploited as an interesting platform for ratiometric fluorescent ClO^- probes. Notably, the deoximation reaction has not been previously employed in fluorescent ClO^- probe design. Based on this platform, compound **1** was designed as the first ratiometric fluorescent probe for hypochlorite (Scheme 2). Compound **1** is composed of a phenanthroimidazole dye[16] and an oxime protective group. We hypothesized that the oxime protective group could be removed by ClO^- to give the aldehyde group. Thus, oxime **1** is transformed into aldehyde **2**. As phenanthroimidazole is an electron-rich fluorophore[16] and the electron-withdrawing ability of the aldehyde group is stronger than that of the oxime group, it was expected that the emission of compound **2** should have a redshift relative to that of compound **1** due to stronger ICT. Therefore, "protected" **1** and "deprotected" **2** may be suitable for the development of ratiometric fluorescent ClO^- probes based on regulation of the electron-withdrawing ability of the electron acceptor in the ICT system.



Scheme 2. Synthetic route to compound **1** and the structure of control compound **3**. a) CH_3COONH_4, CH_3COOH, 100 °C, 30 min; b) hydroxylamine hydrochloride, Et_3N, ethanol, 60 °C, 3 h; c) NaOCl, potassium phosphate buffer/DMF, ambient temperature.

Results and Discussion

Synthesis: Probe **1** was prepared by a standard procedure (Scheme 2). Condensation of 9,10-phenanthrenequinone with terephthalaldehyde and NH_4OAc in AcOH by the Steck and Day procedure[17] gave compound **2** in 72.6% yield. The structure of the reference compound **3** is shown in Scheme 2. Probe **1** was obtained in excellent yields by reacting compound **2** with hydroxylamine hydrochloride in ethanol.

Absorption and emission spectra properties: The optical properties of probe **1** and compounds **2**, **3** were studied in 0.1 M potassium phosphate buffer, pH 9.0/DMF (v/v, 1:4) at room temperature. The absorption spectra of compounds **1** and **2** exhibited a redshift relative to that of the reference **3** (Figure 1). This bathochromic shift is apparently attributed to ICT between the phenanthroimidazole fluorophore and

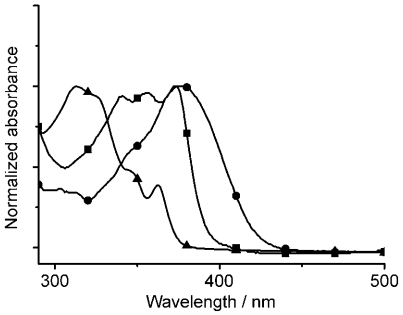


Figure 1. Normalized absorption spectra of compounds **1** (■), **2** (●), and **3** (▲) in 0.1 M potassium phosphate buffer, pH 9.0/DMF (v/v, 1:4).

the oxime or aldehyde group. Additionally, the absorption of compound **2** showed a longer redshift than that of compound **1**. This is consistent with the fact that the aldehyde group has stronger electron-withdrawing ability than the oxime group. In good agreement with the bathochromic shift in the absorption spectra, the fluorescence emission spectra of compounds **1** ($\Phi_f = 0.782$, in DMF, quinine sulfate as standard,[18] see Supporting Information) and **2** ($\Phi_f = 0.567$, in DMF) displayed a different extent of redshift when compared with that of compound **3** ($\Phi_f = 0.493$, in DMF) (Figure 2). Compounds **1** and **2** have maximal emission at 439 and 509 nm, respectively. The observation that the emission spectrum of compound **2** has a 70 nm redshift in comparison with that of compound **1** indicates that "protected" oxime **1** is promising ratiometric fluorescent probe for ClO^- provided that **1** could be deprotected by ClO^- to afford the "deprotected" aldehyde **2**.

Sensing response to ClO^-: To examine whether probe **1** could detect ClO^- based on the deoximation reaction, probe **1** (10 μM) was treated with ClO^- at ambient temperature, and the progress of the reaction was monitored by fluorescence spectroscopy. In the absence of ClO^-, compound **1** ex-

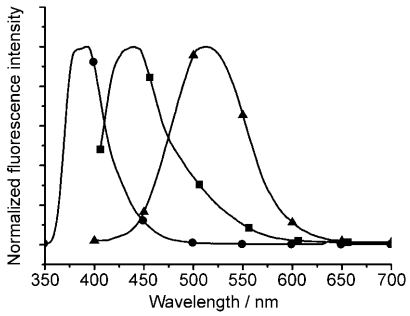


Figure 2. Normalized fluorescent emission spectra of compounds **1** (■), **2** (▲), and **3** (●) in 0.1 M potassium phosphate buffer, pH 9.0/DMF (v/v, 1:4), excited at 394, 394, and 326 nm, respectively.

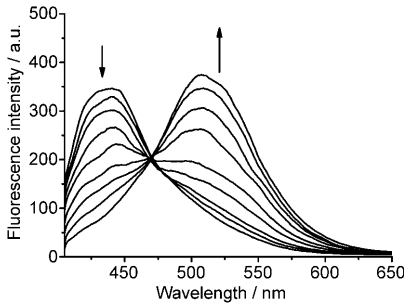


Figure 4. Fluorescence emission spectra of probe **1** (10 μM) in the presence of different concentrations of hypochlorite anion (0–30 equiv) in 0.1 M potassium phosphate buffer, pH 9.0/DMF (v/v, 1:4). Excitation wavelength was 394 nm.

hibited no visible variations in the ratios of emission intensities at 509 and 439 nm ($I_{509}/I_{439}=0.28$), suggesting that compound **1** was stable in the assay conditions and not converted to deprotected **2** (Figure 3). However, upon addition of ClO^- at room temperature, a marked increase in the ratio was observed within seconds, indicative of rapid deprotection of protected **1** to give deprotected **2**, as anticipated. Furthermore, the rapid conversion of **1** into **2** by ClO^- suggests that probe **1** can be used to monitor ClO^- in real time.

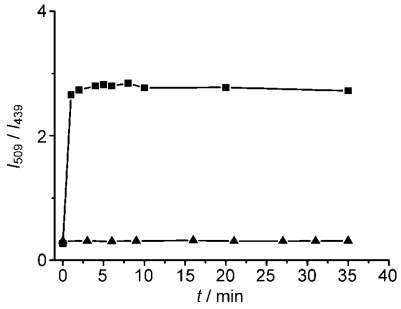


Figure 3. Reaction–time profile of probe **1** (10 μM) in the absence (▲) and presence (■) of 30 equiv of ClO^- in 0.1 M potassium phosphate buffer, pH 9.0/DMF (v/v, 1:4). Kinetic studies were performed at room temperature. The emission ratio changes (I_{509}/I_{439}) were continuously monitored at time intervals.

When increasing concentrations of ClO^- ions were introduced, the fluorescent spectra of probe **1** exhibited significant changes. The intensity of the emission maximum at 439 nm was gradually decreased with the simultaneous appearance of a new redshifted emission band centered at 509 nm (Figure 4). Importantly, the ratios of emission intensities at 509 and 439 nm (I_{509}/I_{439}) displayed a large increase from 0.28 to 2.74. Probe **1** is able to detect low micromolar concentrations of hypochlorite. As the pathophysiologically relevant concentrations of HOCl are in the low to medium micromolar concentrations,[5b] our probe is potentially suitable for medical and biological use. Furthermore, a definite isoemission point was observed at 470 nm which may suggest that only one new species was formed during the titration process. The reaction product was isolated and subject to the standard characterization. The ^1H NMR (Figure 5), Mass, absorption, excitation, and emission spectra of the isolated product are identical with those of the standard compound **2**, demonstrating that, indeed, probe **1** was deprotected by ClO^- ions to give aldehyde **2**.

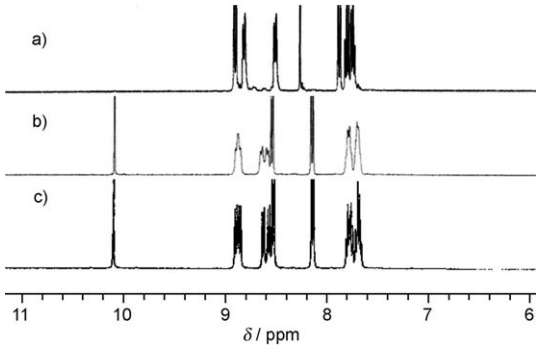


Figure 5. Partial ^1H NMR (400 MHz) spectra of a) probe **1**, b) the separated product of probe **1**+ClO^-, and c) the standard compound **2**.

Effect of pH: The ratiometric responses of the probe toward ClO^- at different pH conditions were investigated. In the absence of ClO^-, the probe was stable over a wide range of pH values from 2.5 to 10.5 (Figure 6). However, the ratiometric responses of the probe toward ClO^- were pH-dependent. Notably, the fluorescent ratio was increased dramatically above pH 7.5. As the pK_a of HOCl is 7.6, we reasoned that probe **1** senses ClO^- instead of HClO.[9a–b] We decided to study the photophysical and sensing properties of probe **1** at pH 9.0, as the probe was much more sensitive. However, the probe could still show a marked ratiometric response to ClO^- at pH as low as 7.8 (Figure S1), indicating that probe **1** may also be used in the near physiological pH range. Unlike the HClO probes which function well under acidic conditions,[9b–c] the ClO^- probes work better at basic conditions due to the fact that the pK_a of HOCl is 7.6. For example, an intensity-based ClO^- probe was reported[9a] to be used at pH 12.

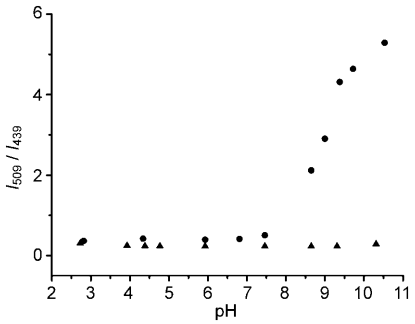


Figure 6. Ratios of the fluorescent emission intensities at 509 and 439 nm (I_{509}/I_{439}) of probe **1** (10 μM) in the absence (▲) or presence (●) of ClO^- (30 equiv) at various pH values. Excitation wavelength was 394 nm.

Selectivity studies: Probe **1** was treated with a wide variety of cations, anions, and oxidants to examine the selectivity. As shown in Figure 7, the addition of ClO^- induced a significant redshift of the fluorescence emission spectra. However, representative species such as Li^+, Na^+, K^+, Mg^{2+}, Zn^{2+}, Cu^{2+}, Al^{3+}, Cl^-, CH_3COO^-, SO_4^{2-}, CO_3^{2-}, NO_2^-, ClO_3^-, ClO_4^-, and H_2O_2 elicited almost no changes in the fluorescence spectra. Although Cu^{2+}, H_2O_2, or NO_2^- are known to be able to deprotect oximes into aldehydes, the deprotection by these species have to be performed under microwave irradiation,[19] in the presence of other catalysts,[20] or at elevated temperature.[21] Thus, distinct reaction conditions required for deoximation could account for the high selectivity of the probe for ClO^- over other species. Furthermore, the visual fluorescence response of probe **1** to various species (Figure 8) demonstrates that the probe can be used conveniently for hypochlorite detection by simple visual detection. We further examined the ratiometric response of the probe toward ClO^- in the presence of other potentially competing species. Most of other species only displayed minimum interference (Figure 9). This clearly indicates that probe **1** is useful to sense ClO^- in the presence of other related species.

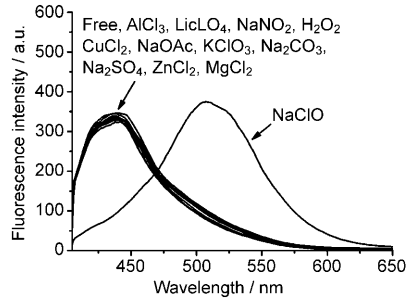


Figure 7. Fluorescence spectra of probe **1** (10 μM) in the absence and presence of 30 equiv of various species in 0.1 M potassium phosphate buffer, pH 9.0/DMF (v/v, 1:4). Excitation wavelength was 394 nm.

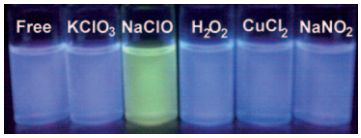


Figure 8. Visual fluorescence changes of **1** (10 μM) in the absence and presence of different species (30 equiv) in 0.1 M potassium phosphate buffer, pH 9.0/DMF (v/v, 1:4). The photos were taken under a handheld UV (365 nm) lamp.

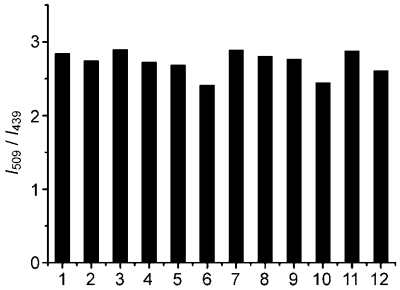


Figure 9. Fluorescence ratiometric response of probe **1** (10 μM) to 30 equiv of NaClO in the presence of 30 equiv of different species in 0.1 M potassium phosphate buffer, pH 9.0/DMF (v/v, 1:4): 1) the organic aqueous buffer; 2) $ZnCl_2$; 3) $AlCl_3$; 4) $NaNO_2$; 5) $CuCl_2$; 6) $LiClO_4$; 7) NaOAc; 8) $KClO_3$; 9) Na_2SO_4; 10) $NaCO_3$; 11) $MgCl_2$; 12) H_2O_2. Excitation at 394 nm.

Conclusion

In summary, we have rationally designed compound **1** as a ratiometric fluorescent probe for hypochlorite via the deprotection of the oxime into the aldehyde. Notably, this deoximation reaction has not been previously used in fluorescent hypochlorite probe development. Probe **1** was readily synthesized in two steps. Importantly, the probe exhibited a ratiometric fluorescent response to hypochlorite with the emission intensities ratio (I_{509}/I_{439}) increasing from 0.28 to 2.74. Additionally, the probe showed high selectivity for hypochlorite over other species due to distinct deoximation conditions. To our best knowledge, the probe developed herein represents the first ratiometric fluorescent probe for hypochlorite. As the probe features a ratiometric fluorescent signal and a low micromolar detection limit, it may be favorable for interesting applications in many environmental and biological settings.

Experimental Section

General information: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified and dried by standard methods prior to use. Twice-distilled water was used throughout all experiments. Electronic absorption spectra were obtained on a Shimadzu UV-2450 spectrometer. Photoluminescent spectra were recorded with a Hitachi F4500 fluorescence spectrophotometer with the excitation and emission slit widths at 2.5 nm. Melting point of compounds were measured on a Beijing Taike XT-4 microscopy melting point apparatus, all melting points were uncorrected. Mass spectrometry analyses were performed using an Agilent 1100 HPLC/MSD spectrometer. ^{1}H NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. Elementa-

ry analyses were obtained on a Vario El III Elemental Analyzer. TLC analyses were performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals. Stock solution of probe **1** was prepared at 5×10^{-4} M in DMF. The solutions of various testing species were prepared from $ZnCl_2$, $CuCl_2{\cdot}2H_2O$, $MgCl_2{\cdot}6H_2O$, $NaNO_2$, $LiClO_4{\cdot}3H_2O$, $KMnO_4$, Na_2CO_3, CH_3COOK, $AlCl_3{\cdot}6H_2O$, Na_2SO_4, $KClO_3$, 10% NaClO, 30% H_2O_2, respectively. K_2HPO_4 and KH_2PO_4 were utilized to prepare potassium phosphate buffer. Test solutions in 0.1 M potassium phosphate buffer, pH 9.0/DMF (v/v, 1:4) were prepared by placing 0.1 mL of probe **1** stock solution, 1 mL potassium phosphate buffer solution, and an appropriate aliquot of each species stock into a 5 mL volumetric flask, and then diluting the solution to 5 mL with DMF. The resulting solution was shaken well before recording the absorption and emission spectra.

4-(1*H*-Phenanthro[9,10-*d*]imidazol-2-yl)benzaldehyde oxime (1): Compound **2** (50 mg, 0.155 mmol), hydroxylamine hydrochloride (16 mg, 0.233 mmol), and Et_3N (23.6 mg, 0.233 mmol) in ethanol were heated to 60 °C for 3 h. After the reaction, the solvent was removed under reduced vacuum. The resulting residue was purified by silica gel column chromatography (acetone/methanol, 1:1) to afford the light yellow product (52.9 mg, 91.2%). M.p. 240 °C (decomp); ^{1}H NMR (400 MHz, [D$_6$]DMSO, TMS): $\delta=8.94$ (d, $J=8.4$ Hz, 2H), 8.85 (d, $J=8$ Hz, 2H), 8.54 (d, $J=7.6$ Hz, 2H), 8.30 (s, 1H), 7.91 (d, $J=8.8$ Hz, 2H), 7.76–7.86 ppm (m, 4H); UV/Vis (DMF): λ_{max} (ε) = 370 nm (10500 mol^{-1} dm^3 cm^{-1}); MS (ESI): *m*/*z*: 338.1 $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{15}N_3O$: C 78.32, H 4.48, N 12.46; found: C 78.14, H 4.70, N 12.35.

Acknowledgements

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