ORGANIC LETTERS

2013
Vol. 15, No. 4
878–881

Hypochlorous Acid Turn-on Fluorescent Probe Based on Oxidation of Diphenyl Selenide

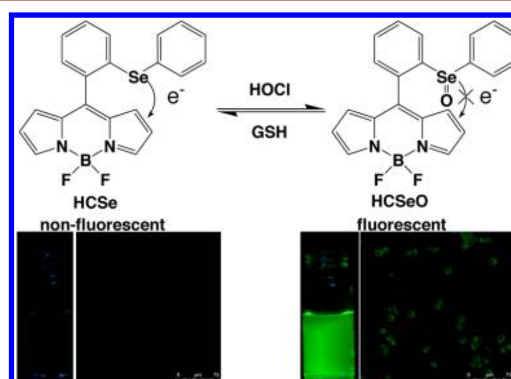
Shi-Rong Liu and Shu-Pao Wu*

Department of Applied Chemistry, National Chiao Tung University, Hsinchu, Taiwan 300, ROC

spwu@mail.nctu.edu.tw

Received January 2, 2013

ABSTRACT



A BODIPY-based fluorescent probe, HCSe, has been successfully developed for the rapid detection of hypochlorous acid based on the specific HOCl-promoted oxidation of diphenyl selenide in response to the amount of HOCl. Confocal fluorescence microscopy imaging using RAW264.7 cells showed that the new probe HCSe could be used as an effective fluorescent probe for detecting HOCl in living cells.

Hypochlorous acid (HOCl), a reactive oxygen species (ROS), is a powerful antimicrobial agent that plays an important role in the immune system.[1] Endogenous hypochlorous acid is mainly produced from the reaction of a chloride ion and hydrogen peroxide catalyzed by the enzyme myeloperoxidase (MPO)[2] in leukocytes including macrophages, monocytes, and neutrophils.[3] When microbial invasion happens, HOCl is produced as a defense mechanism in the immune system. However, an excess amount of HOCl causes several human diseases such as arthritis,[4] cancer,[5] and neurodegeneration.[6] Because of the biological importance of HOCl, the development of highly sensitive and selective probes for HOCl has become an important issue. Fluorescent probes that can respond reversibly to the concentration change of HOCl would be helpful in studying the dynamic distribution of HOCl in living cells.

Several fluorescent probes have been developed for HOCl detection recently, with most of them utilizing the strong oxidation property of HOCl in the design.[7] Herein, we report a highly sensitive and selective fluorescent probe for hypochlorous acid based on the HOCl-promoted oxidation of selenium. Selenium (Se) is an essential component found in the active sites of many enzymes and is also used as a dietary "antioxidant", as it can react with reactive oxygen species. Although several HOCl sensors have been available, fluorescent probes based on the redox cycle of selenium to detect HOCl in living cells have not been studied much.

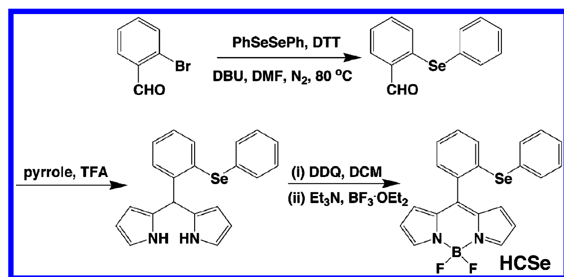
In this work, a novel BODIPY-based fluorescent probe **HCSe**, bearing an organoselenium group, was designed for HOCl detection. BODIPY, which stands for boron-dipyrromethene, is a fluorescent dye with a high molar absorption coefficient and fluorescence quantum yield.[8]

(1) Winterbourn, C. C.; Hampton, M. B.; Livesey, J. H.; Kettle, A. J. *J. Biol. Chem.* **2006**, *281*, 39860–39869.
(2) Fiedler, T. J.; Davey, C. A.; Fenna, R. E. *J. Biol. Chem.* **2000**, *275*, 11964–119671.
(3) Yap, Y. W.; Whiteman, M.; Cheung, N. S. *Cell Signal* **2007**, *19*, 219–228.
(4) Steinbeck, M. J.; Nesti, L. J.; Sharkey, P. F.; Parvizi, J. *J. Orthop. Res.* **2007**, *25*, 1128–1135.
(5) Pattison, D. I.; Davies, M. J. *Biochemistry* **2006**, *45*, 8152–8162.
(6) Pattison, D. I.; Davies, M. *J. Chem. Res. Toxicol.* **2001**, *14*, 1453–1464.

10.1021/ol400011u © 2013 American Chemical Society
Published on Web 02/01/2013

It was used as the signal transduction unit in this study while the diphenyl selenide unit performed as a modulator to respond to the amount of HOCl. **HCSe** displays weak fluorescence with a quantum yield of $\Phi = 0.005$, because photoinduced electron transfer (PET) from the diphenyl selenide group to the BODIPY moiety takes place. However, the strong fluorescence of BODIPY is restored after the oxidation of selenium by HOCl. This new probe exhibits high selectivity and sensitivity toward HOCl over other ROS and reactive nitrogen species (RNS) in aqueous solution. Most importantly, **HCSe** shows good cell-membrane permeability and can be successfully applied to image endogenous HOCl in living cells.

Scheme 1. Synthesis of the Probe **HCSe**



The synthesis procedure for the probe **HCSe** is outlined in Scheme 1. 2-(Phenylselenyl)benzaldehyde was obtained from the reaction of diphenyl diselenide with *o*-bromobenzaldehyde in the presence of dithiothreitol (DTT). Treatment of 2-(phenylselenyl)benzaldehyde with excess pyrrole in the presence of trifluoroacetic acid (TFA) under N_2 yielded the corresponding dipyrromethane. In the next step, the compound 2-(phenylselenyl)phenyldipyrromethane was oxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to yield the corresponding dipyrromethene, which was transformed into the BODIPY skeleton in the presence of BF_3 under N_2. The structure of **HCSe** was confirmed using ^{1}H NMR, ^{13}C NMR, ^{77}Se NMR, and MS spectra.

The sensing ability of the probe **HCSe** was tested toward various ROS and RNS, including HOCl, $^{\bullet}$OH, H_2O_2, $^1O_2$, NO_2^-, NO_3^-, NO, $ONOO^-$, O_2^-, and *t*-BuOOH, in a phosphate-buffered saline (PBS) solution. It was found that the strong green fluorescence emission only occurred for the addition of HOCl to the **HCSe** solution; other ROS and RNS produced no change in fluorescence (Figure 1). The quantitative fluorescence spectra of **HCSe** were recorded in the presence of several ROS and RNS, but HOCl was the only reactive species to cause an obvious fluorescence enhancement (Figure 1).

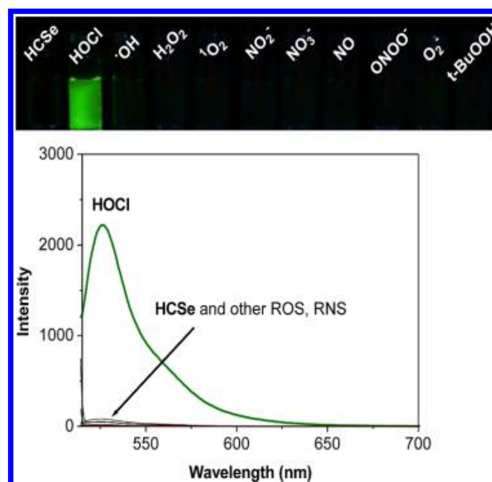


Figure 1. Fluorescence changes of **HCSe** (10 μM) in response to NaOCl (10 μM) and various ROS/RNS (100 μM) in a H_2O-CH_3CN (v/v = 99/1, 0.1 M PBS, pH 7.4) solution. The excitation wavelength was 510 nm.

The reaction of **HCSe** with HOCl was fast; addition of NaOCl(aq) to the solution of the probe **HCSe** caused an immediate, strong change in fluorescence intensity (see Figure S11 in Supporting Information). During HOCl titration with **HCSe**, a new band centered at 526 nm formed (Figure 2). The emission intensity reached its maximum after the addition of 1 equiv of HOCl. The quantum yield of the oxidized form **HCSeO** was $\Phi = 0.690$, which is 138-fold higher than that of **HCSe**, at $\Phi = 0.005$. The structure of the oxidized form **HCSeO** was also confirmed by ^{77}Se NMR and MS spectra. Notably, there was a good linear correlation between the fluorescence intensity and the concentration of NaOCl (0−9 μM). Furthermore, it was found that **HCSe** has a detection limit of 7.98 nM (see Figure S12 in Supporting Information), which makes it sufficiently sensitive for application in living systems.

Density functional theory (DFT) calculation was applied to determine the detecting mechanism of **HCSe** for HOCl. As shown in Scheme 2, the highest occupied molecular orbital (HOMO) of the diphenyl selenide moiety (electron donor) matches that of the fluorophore BODIPY (electron acceptor); the HOMO energy level (−5.75 eV) of the diphenyl selenide moiety is higher than that of the fluorophore BODIPY (−5.98 eV). Consequently, when the BODIPY moiety is photoexcited, the intramolecular electron transfer from the diphenyl selenide moiety to the BODIPY moiety is energetically favorable. Hence, the

(7) (a) Kenmoku, S.; Urano, Y.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2007**, *129*, 7313–7318. (b) Koide, Y.; Urano, Y.; Kenmoku, S.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2007**, *129*, 10324–10325. (c) Chen, X.; Wang, X.; Wang, S.; Shi, W.; Wang, K.; Ma, H. *Chem.—Eur. J.* **2008**, *14*, 4719–4724. (d) Sun, Z.-N.; Z.-N. Liu, Z.-N.; Chen, Y.; Tam, P. K. H.; Yang, D. *Org. Lett.* **2008**, *10*, 2171–2174. (e) Yang, Y.-K.; Cho, H. J.; Lee, J.; Shin, I.; Tae, J. *Org. Lett.* **2009**, *11*, 859–861. (f) Lin, W.; Long, L.; Chen, B.; Tan, W. *Chem.—Eur. J.* **2009**, *15*, 2305–2309. (g) Panizzi, P.; Nahrendorf, M.; Wildgruber, M.; Waterman, P.; Figueiredo, J. L.; Aikawa, E.; McCarthy, J.; Weissleder, R.; Hilderbrand, S. A. *J. Am. Chem. Soc.* **2009**, *131*, 15739–15744. (h) Cui, K.; Zhang, D. Q.; Zhang, G. X.; Zhu, D. B. *Tetrahedron Lett.* **2010**, *51*, 6052–6055. (i) Lou, X. D.; Zhang, Y.; Li, Q. Q.; Qin, J. G.; Li, Z. *Chem. Commun.* **2011**, *47*, 3189–3191. (j) Cheng, X.; Jia, H.; Long, T.; Feng, J.; Qin, J.; Li, Z. *Chem. Commun.* **2011**, *47*, 11978–11980. (k) Koide, Y.; Urano, Y.; Hanaoka, K.; Terai, T.; Nagano, T. *J. Am. Chem. Soc.* **2011**, *133*, 5680–5682. (l) Zhou, Y.; Li, J.; Chu, K.; Liu, K.; Yao, C.; Li, J. *Chem. Commun.* **2012**, *48*, 4677–4679. (m) Chen, G.; Song, F.; Wang, J.; Yang, Z.; Sun, S.; Fan, J.; Qiang, X.; Wang, X.; Dou, B.; Peng, X. *Chem. Commun.* **2012**, *48*, 2949–2951.
(8) Loudet, A.; Burgess, K. *Chem. Rev.* **2007**, *107*, 4891–4932.

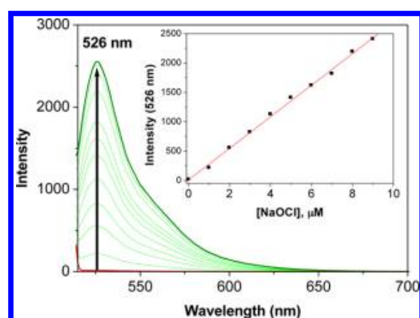
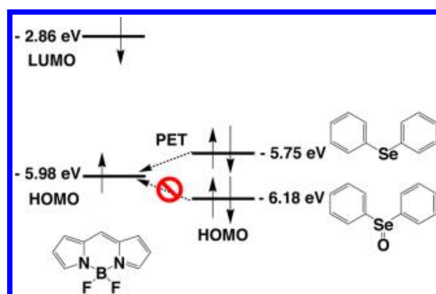


Figure 2. Fluorescence changes of **HCSe** (10 μM) in the presence of various equivalents of NaOCl in a H_2O-CH_3CN (v/v = 99/1, 0.1 M PBS, pH 7.4) solution. The excitation wavelength was 510 nm.

Scheme 2. Energy Diagram for the Reaction of **HCSe** with HOCl



fluorescence of the BODIPY moiety is quenched through a PET process ($\Phi < 0.01$). In contrast, upon the oxidation of **HCSe** by HOCl, the HOMO energy level of the diphenyl selenoxide moiety (−6.18 eV) is lower than that of the BODIPY unit; therefore, the PET process is restricted and the fluorescence of BODIPY is restored.

A pH-dependence experiment of **HCSe** was conducted to investigate a suitable pH range for HOCl sensing. Figure 3a shows that the emission intensities of **HCSe** are very low at a pH range of 4−10. After addition of 1 equiv of HOCl, the emission intensity at 526 nm becomes significantly higher at a pH range of 5.5−8.0, which means that the probe could be used under physiological conditions. When the pH exceeded 8.5, the emission intensity dropped sharply. This is because the pK_a of HOCl is 7.6;[7a,d] hypochlorite (ClO^-) is dominant at pH > 8 and shows poor reactivity with **HCSe**. To investigate the redox cycling capacity of selenium in **HCSe**, the reducing reagent glutathione (GSH) was used to test whether the oxidized product **HCSeO** could be reduced to its original state by GSH. Figure 3b shows the reaction of the oxidized product **HCSeO** with GSH. A remarkable fluorescence decrease was observed after addition of GSH. This observation indicates the reversibility of **HCSe**, which can be used to monitor the dynamic changes of HOCl in living cells.

The potential of the probe **HCSe** for imaging HOCl in living cells was next investigated. Murine RAW264.7

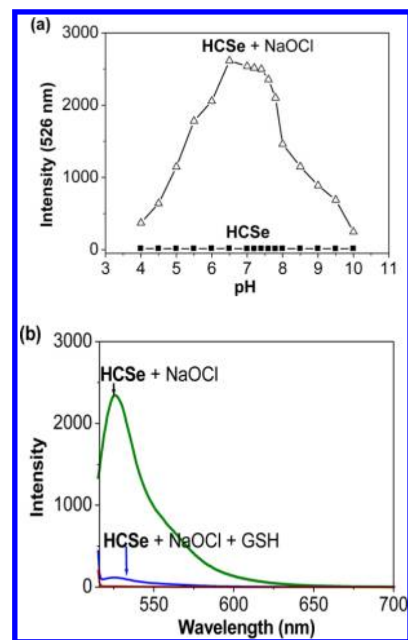


Figure 3. (a) Fluorescence response of free probe **HCSe** (10 μM) and after addition of NaOCl (10 μM) in a H_2O-CH_3CN (v/v = 99/1) solution as a function of different pH values. (b) Fluorescence response of **HCSe** (10 μM) to NaOCl (10 μM) in a H_2O-CH_3CN (v/v = 99/1, 0.1 M PBS, pH 7.4) solution. The fluorescence emission vanished after addition of the reducing agent GSH (200 μM) to the solution.

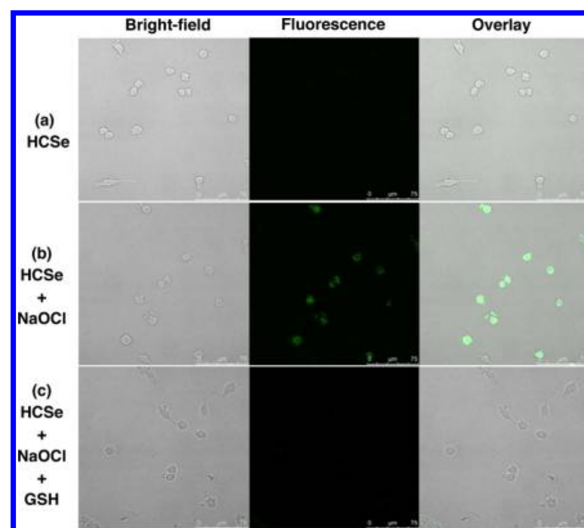


Figure 4. Fluorescence images of RAW264.7 cells. (Left) Bright field image; (Middle) fluorescence image; and (Right) merged image. (a) The cells incubated with **HCSe** (10 μM) for 30 min. (b) Subsequent treatment of the cells with NaOCl (10 μM) for 10 min. (c) Further incubation with GSH (200 μM) for 30 min.

macrophages were used as a model because macrophages are known to generate ROS and RNS in the immune system. The images of cells were obtained using a confocal

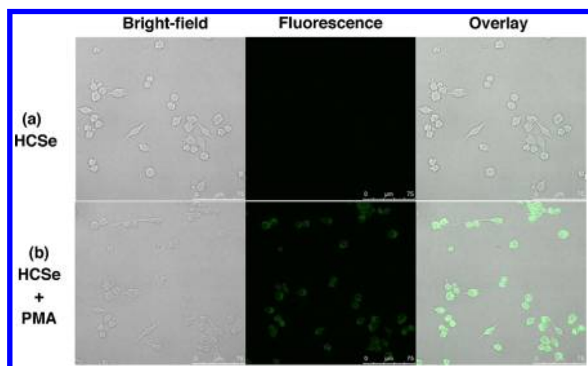


Figure 5. Detection of PMA-induced HOCl production in RAW264.7 cells. (a) The cells incubated with **HCSe** (10 μM) for 30 min. (b) The cells treated with stimulant PMA (25 ng/mL) for 2 h in the presence of **HCSe**.

fluorescence microscope. First, when RAW264.7 cells were incubated with **HCSe** (10 μM), no fluorescence was observed (Figure 4a). After the treatment of NaOCl, bright green fluorescence was observed in the RAW264.7 cells (Figure 4b). An overlay of fluorescence and bright-field images shows that the fluorescence signals are localized in the intracellular area, indicating a subcellular distribution of HOCl and the good cell-membrane permeability of **HCSe**. Further addition of GSH (200 μM) into the culture medium caused the intracellular fluorescence in RAW264.7 cells to disappear (Figure 4c). This indicates that the oxidized probe (**HCSeO**) was reduced to the nonfluorescent probe (**HCSe**) by GSH.

Finally, **HCSe** was used to detect PMA-induced endogenous HOCl production in RAW264.7 cells. Phorbol myristate acetate (PMA) is known to activate the generation of ROS and RNS in macrophage cells, including HOCl.[9] After stimulation with PMA (25 ng/mL) for 2 h in the presence of **HCSe**, strong green fluorescence was observed in RAW264.7 cells (Figure 5b). These results demonstrated that **HCSe** is able to visualize PMA-induced endogenous HOCl production in the macrophages.

In summary, we have developed a BODIPY-based green fluorescent probe **HCSe** that exhibits a rapid, highly selective, and sensitive response to HOCl over other reactive species. The system utilizes the HOCl-promoted oxidation of diphenyl selenide to respond to the amount of HOCl. **HCSe** is rapidly oxidized by HOCl with an emission enhancement, but the oxidized form **HCSeO** can then be reduced by GSH back to **HCSe**. Confocal fluorescence microscopy imaging using RAW264.7 cells showed that the probe **HCSe** could be used to evaluate the important roles of HOCl in biological systems.

Acknowledgment. We gratefully acknowledge the financial support of the National Science Council (ROC) and National Chiao Tung University.

Supporting Information Available. Synthesis, experimental details, and characterization of **HCSe**. This material is available free of charge via the Internet at http://pubs.acs.org.

(9) (a) Wolfson, M.; McPhail, L. C.; Nasrallah, V. N.; Snyderman, R. *J. Immunol.* **1985**, *135*, 2057–2062. (b) Yang, Y.; Lu, H.; Wang, W.; Liau, I. *Anal. Chem.* **2011**, *83*, 8267–8272.

The authors declare no competing financial interest.