A specific fluorescence probe for hypochlorous acid based on rhodamine 101 thiosemicarbazides and its application for living cells imaging

Bo-Song Zhu¹, Zhi-Xiang Han^{1, a}, Tong-Liang Wu¹, and Xiang-Yang Wu¹

¹ School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, 212013, P. R. China

^azhixianghan69@126.com

Keywords: Rhodamine 101; Hypochlorous Acid; Bioimaging; Living cells

Abstract. Utilizing the mechanism of HOCl-mediated cyclization of rhodamine 101 thiosemicarbazide (non-fluorescent) to rhodamine 101 oxadiazoles (fluorescent), we successfully developed a novel off-on far-red fluorescent probe (**1**) toward HOCl with high selectivity, great functionality at physiological pH and good cell-membrane permeability. Probe **1** was linearly proportional to the concentration of HOCl in the range of 1-10 μM and the detection limit is 0.3 μM . Moreover, the proposed probe (**1**) was successfully used for the detection of hypochlorous acid in HepG2 cells by fluorescent imaging.

Introduction

Owing to the variety functions in biological processes, reactive oxygen species (ROS) have received more and more considerable attention of chemist and biologist in recent years ^[1]. Hypochlorous acid (HOCl) is a biologically significant ROS that exists in equilibrium with hypochlorite (OCl⁻) at physiological pH. Normal level HOCl is a critical microbicidal agent in natural defense ^[2]. Abnormal levels of HOCl would lead to many inflammation-related diseases ^[3], including cardiovascular diseases, damage of human red blood cells, lung injury, rheumatoid arthritis, and cancer. Thus, develop the detection of HOCl is of great importance.

In comparison with traditional methods, fluorescence method is well known for its high sensitivity, high selectivity, simple manipulation and lack of a requirement for sophisticated instrumentation ^[4]. In addition, in conjunction with microscopy, bio-imaging applications can be used in living cells. Based on the reported ClO⁻ probes ^[5-9], they have the drawbacks like unsuitable pH range for cells ^[7, 9], lack of selectivity between ClO⁻ and Cu²⁺ or H₂O₂ ^[8] or UV excitation ^[9]. Therefore, it has profound meanings to develop one bioimaging probe for HOCl with an excellent selectivity and sensitivity that is applicable to various biological systems.

Due to the spiro lactam structural property, rhodamine derivative framework is an ideal model to construct "turn-on" fluorescent chemosensors ^[10]. However, rhodamine B or 6G is the main fluorophore to construct off-on probe for various metal ions, anions or ROS and so on. Obviously, the development of rhodamine analogues with longer absorption and emission in far-red or near infrared region still presents ample opportunities and will be more suitable for biological application. Rhodamine 101 has two amino groups rigidly linked to the xanthene skeleton at 3 and 6-position by multiple *n*-propylene bridges which elongate its emission to far-red region (>600 nm).

Inspired by Lin's work ^[11], in this paper, we described a novel HOCl-amplified fluorescent probe based on HOCl-promoted cyclization of rhodamine 101 thiosemicarbazide to rhodamine 101 oxadiazoles reaction mechanism with high selectivity and its application in cell imaging.

Experimental

Reagents and apparatus. Before used, N, N-dimethylformamide (DMF) was freshly distilled after dried by anhydrous MgSO_4 for 24h. All other reagents used were of analytical reagent grade. Twice distilled water was used throughout all experiments.

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 spectrometer and LC-MS analysis was performed using an LXQ Spectrometer (Thermo Scientific, USA). UV-vis absorption spectra were recorded on a Shimadzu UV-2600 spectrophotometer. All fluorescence measurements were conducted on a Thermo Scientific Lumina fluorescence spectrometer with excitation slit set at 5.0 nm and emission at 5.0 nm. Fluorescence images of HepG2 cells were carried out with an inverted fluorescence microscope (Carl Zeiss, Axio ObserverA1).

Scheme 1. Chemical structure and synthetic route of compound **1**.

Synthesis of compound 1. Synthetic routes of probe **1** are depicted in Scheme 1, and it was fully characterized by ^1H NMR, ^{13}C NMR, and mass spectrum. Briefly, Phenyl isothiocyanate (20 mg, 0.15 mmol) was added to the solution of rhodamine 101 hydrazide^[12] (50.4 mg, 0.1 mmol) in DMF (2 mL) in potions and then heated to 50 °C with stirring. After 12h, the mixture was cooled and the solvent was removed under reduced pressure, then the residue was purified on silica gel chromatography eluted with $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (3:1, v/v) to afford the desired product as a light purple solid (49 mg, yield 76.7%).

^1H NMR (400 MHz, CDCl_3): δ 1.77-1.88 (m, 8H), 2.41-2.47 (m, 4H), 2.84-3.01 (m, 4H), 3.08-3.13 (m, 4H), 3.17(t, J = 5.6Hz, 4H), 6.03 (s, 2H), 6.99 (s, 1H), 7.10 (d, J = 7.6Hz, 2H), 7.16 (d, J = 7.2Hz, 1H), 7.24(s, 1H), 7.28 (d, J = 3.2Hz, 2H), 7.57-7.61 (m, 2H), 7.63-7.67 (m, 1H), 8.02 (d, J = 7.2Hz, 1H). ^{13}C NMR (100 MHz, CDCl_3): δ 21.137, 21.238, 21.724, 27.286, 49.402, 49.799, 68.001, 104.068, 108.339, 117.892, 123.308, 123.709, 124.898, 124.973, 126.005, 128.322, 128.875, 134.257, 137.765, 144.330, 149.157, 150.753, 167.358, 182.661. MS: $[\text{M}+\text{H}]^+ = 640.23$, calculated: $[\text{M}+\text{H}]^+ = 640.27$.

Results and discussion

Fig.1 Changes in fluorescence emission (a, $\lambda_{\text{ex}}=550$ nm) and absorption (b) spectra of **1** (10 μM) upon addition of increasing concentrations (0~10 μM , from bottom to top) of NaOCl in test system. Inset (a): The plot of F/F_0 as a function of the concentration of NaOCl. Inset (b): Job's plots for **1** in test syetem (the total concentration of **1** and NaOCl was 20 μM).

Spectral Characteristics. The fluorescence titration of NaOCl was performed with a solution of 10 μM of probe **1** in test system (pH = 7.40 PB / DMF, 1:1, v/v). As shown in Fig. 1a, the probe **1** displayed almost no considerable emission bands above 600 nm, indicating that it exists as the ring-closed form. With increasing concentration treatment of NaOCl with probe **1**, a strong visible emission band centered at 620 nm with 25-fold fluorescence enhancement (treated by 10 μM NaOCl) was observed, suggesting that NaClO promotes the transformation of probe **1** from the ring-closed form to the ring-opened form. Meanwhile, a slight red shift from 617 nm to 620 nm also can be observed at the maximum emission. The fluorescent response of probe **1** towards NaOCl was calculated to cover a linear range from 1 to 10 μM (Fig. 1a, inset), with a detection limit of 0.3 μM (based on $S/N=3$). Figure 1b showed the variation of probe **1** on the gradual addition of NaClO in test system. The colorless probe **1** shows almost no absorption in visible region. Upon the gradual addition of NaClO to the solution of probe **1**, a new absorption peak at 585 nm emerged with increasing intensity. The solution displayed a clear change from colorless to pink simultaneously, implying that the conversion from spirolactam to the ring-opened amide occurred. Moreover, the absorbance enhanced accompanied with the increase of NaOCl concentration. Job's method for the absorbance was applied to determine the stoichiometry of the **1**-NaOCl complex by keeping the total concentration of NaOCl and **1** at 20 μM and continuously changing the molar ratio of NaOCl from 0 to 1. As shown in Fig. 1b inset, a maximum absorption was observed when the molar fraction of $[\text{NaOCl}]$ vs. $([\text{1}] + [\text{NaOCl}])$ was 0.5, which strongly indicated that a 1:1 adduct was formed with **1**.

Fig.2. Fluorescence response of **1** (10 μM) to 10 μM of selected analytes in test system. Bars represent the emission intensity ratios after (F) and before (F_0) addition of each analyte. (1) NaOCl; (2) blank; (3) Br^- ; (4) SO_4^{2-} ; (5) ClO_4^- ; (6) Cl^- ; (7) F^- ; (8) NO_3^- ; (9) PO_4^{3-} ; (10) I^- ; (11) CO_3^{2-} ; (12) OAc^- ; (13) H_2O_2 . Excitation wavelength was 550 nm.

Selectivity. Selectivity is an important aspect to evaluate the performance of probe **1**. As shown in Fig. 2, probe **1** displayed a remarkable selectivity only for HOCl over others biologically relevant species represented by F^- , Cl^- , Br^- , I^- , CH_3COOOH , NO_2^- , ClO_4^- , NO_3^- , CO_3^{2-} , SO_4^{2-} , HCO_3^- , H_2O_2 .

Fig. 3. Bright-field transmission image (a) and fluorescence image (b) of HepG2 cells incubated with **1** (10 μM) for 30 min. (c) Fluorescence image of the cells above further incubated with 10 μM NaOCl for another 30 min.

Detection of NaOCl in living cells. To explore the potential practical applications, probe **1** was employed for *in vitro* HOCl detection in living cells on an inverted fluorescence microscope. In the absence of NaOCl, no significant fluorescence could be observed (Fig. 3b). After incubation with HOCl, however, a bright fluorescence was observed in living cells (Fig. 3c). These results reveal that probe **1** was well permeable to efficiently image intracellular hypochlorous acid under fluorescence microscope.

Conclusions

Rhodamine 101 - thiomicarbazides has been developed as a novel fluorescent probe for HOCl. Its excellent characteristics, including high selectivity, working at physiological pH, emission in far-red region, enable us to measure HOCl in living cells by fluorescence technique with successful results.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (21105038), the Opening Project of State Key Laboratory of Chemo/Biosensing and Chemometrics of Hunan University (2010007, 2014015), China Postdoctoral Science Foundation (2013M541604), Jiangsu Postdoctoral Science Foundation (1301047B), the Scientific Research Foundation for the Talents in Jiangsu University (10JDG035) and collaborative innovation center of water treatment technology & material of Jiangsu province.

References

- [1] J.D. Lambeth. Free Radic Biol Med. Vol. 43 (2007), p. 332.
- [2] J.M. Albrich, C.A. McCarthy, and J.K. Hurst. Proc. Natl. Acad. Sci. U. S. A. Vol. 78 (1981), p. 210.
- [3] B.C. Dickinson, and C.J. Chang. Nat. Chem. Bio., Vol. 7 (2011), p. 504, and references therein.
- [4] A.P. Demchenko, Introduction to Fluorescence Sensing, Springer, 2008;
- [5] X.Q. Chen, X.Z. Tian, I. Shin et al. Chem. Soc. Rev., Vol. 40 (2011), p. 4783.
- [6] L. Yuan, L. Wang, B.K. Agrawalla, et al. J. Am. Chem. Soc. Vol. 137 (2015), p. 5930.
- [7] X.Q. Chen, X.C. Wang, S.J. Wang, et al. Chem. Eur. J. Vol. 14 (2008), p. 4719.
- [8] Y.L. Liu, Y. Sun, J. Du, et al. Org. Biomol. Chem. Vol. 9(2011), p. 432.
- [9] W.Y. Lin, L.L. Long, B.B. Chen, et al. Chem. Eur. J. Vol. 15 (2009), p. 2305.
- [10] X.Q. Chen, T. Pradhan, F. Wang et al. Chem. Rev. Vol. 112(2012), p. 1910, and references therein.
- [11] L. Yuan, W.Y. Lin, Y.N. Xie, et al. Chem. Eur. J. Vol. 18(2012), p. 2700.
- [12] P.H. Xie, F.Q. Guo, D. Li, et al. J. Lumin. Vol. 131 (2011), p. 104.