Chinese Chemical Letters xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Communication

A fluorometric and mitochondrion-targetable probe for rapid, naked-eye test of hypochlorite in real samples

Ying Xia[a,1], Xiaoyu Liu[a,1], Dan Wang[a], Zechen Wang[a], Qiang Liu[a], Haibo Yu[a,*], Mingyan Zhang[b,*], Youtao Song[a,*]

[a] College of Environmental Sciences, Liaoning University, Shenyang 110036, China
[b] Liaoning Center of Disease Prevention and Control, Shenyang 110001, China

ARTICLE INFO

Article history:
Received 16 November 2017
Received in revised form 23 January 2018
Accepted 29 January 2018
Available online xxx

Keywords:
Colorimetric probe
Fluorescent probe
Rhodamine
Hypochlorite
Mitochondrion-tragetable

ABSTRACT

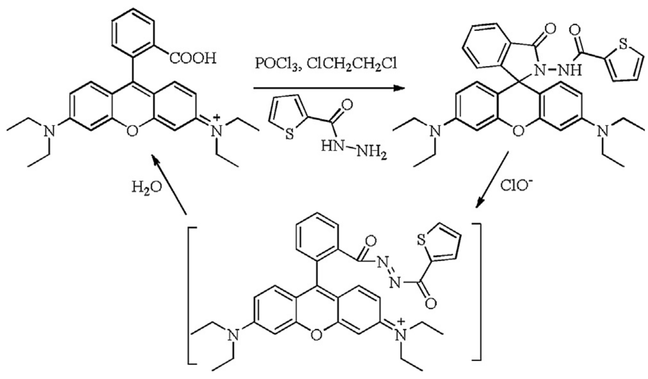
Hypochlorite anion is a ubiquitous reactive molecule in the terminal disinfection systems, inflammatory stress and immune systems. Thus, rapid and visual monitoring ClO^- in water and biological samples is very meaningful for water quality safety and toxicity assessment of contaminants. Herein, a colorimetric and fluorometric probe (Rh-ClO) based on rhodamine B fluorophore and thiophene-2-carbohydrazide has been unveiled and successfully utilized for ClO^- detection in water samples and HeLa cells. Upon addition of ClO^-, color changes of solution from colorless to pink were immediately visible to the naked-eyes, meanwhile, brilliant red fluorescence was observed under excited at UV light (365 nm). Rh-ClO displayed high selectivity and sensitivity for ClO^-, and the detection limit was 7 μmol/L calculated from the fluorescence titration. With the aid of its merits including rapid response to ClO^- within 10 s, Rh-ClO and its test paper could successfully detect ClO^- in water. Additionally, HeLa cells image co-stained with Rh-ClO and Rh123 demonstrated that Rh-ClO possessed excellent and fast cell-membrane permeability and mitochondrion-targetability. It was clearly confirmed that Rh-ClO would be a promising probe for rapid tracking of ClO^- in water samples and in mitochondria of living cells.

Sodium hypochlorite (NaClO), as well known as famous bactericide and oxidizing agent, play vital roles in many fields such as drinking water disinfection [1,2], dye decoloration [3,4], odor elimination [5], oxidation contaminates in soils [6,7], and so on. Especially, in water treatment, *e.g.*, reclaimed water and drinking water, sodium hypochlorite is now among the most widely used disinfectant, owing to its residual protection, low cost and ease of use [8–10]. The free chlorine residual can guarantee to kill bacteria and inactivate viruses, but it also can react with the constituents present to form disinfection by-products (DBPs) during water disinfection [11–13]. Some DBPs (*e.g.*, *N*-nitrosamines, trihalomethanes, haloacetic acids, haloacetonitriles, *etc.*) are of growing concern because they have been recently identified as probable human carcinogens and deformity [14,15]. Therefore, a reasonable control on free chlorine residual in water disinfection will be of great significance to guarantee for the health of populations. Besides that, hypochlorous acid produced by the enzyme myeloperoxidase in inflammatory stress [16] plays a critical role as one of the most important reactive oxygen species (ROS) in defending from the invasion of contaminants [17]. On the contrary, uncontrolled endogenous hypochlorite will further induces oxidation damage of the extracellular components, which may involve in some diseases, such as arthritis [18], kidney disease [19], lung injury [20], atherosclerosis [21] and cancer [22]. As well known, both hypochlorous acid and hypochlorite anion exist *in vivo* at almost equal concentrations. Measurement of hypochlorous acid and hypochlorite constitutes a fundamental aspect to evaluate the stress state exposed to different hazardous contaminants. Given the health risk of ClO^-, it is necessary to test and monitor hypochlorite residues both in real water samples and in biological specimens.

Right now, colorimetric and fluorometric methods are available for detecting and monitoring hypochlorite residues in water [23,24]. Colorimetric methods that utilize color changes to quantitatively assess residual hypochlorite against a visual comparator standards or by usage of a colorimeter make themselves very adept in facilitating hypochlorite detection in real water samples but not *in vivo*. Fluorometric methods can offset

* Corresponding authors.
E-mail addresses: yuhaibo@lnu.edu.cn (H. Yu), lncdczmy@163.com (M. Zhang), youtaos@gmail.com (Y. Song).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cclet.2018.01.054
1001-8417/



Scheme 1. Synthetic route of Rh-ClO.

this imperfection in monitoring hypochlorite produced from intracellular micro-environment of flora and fauna. Developing colorimetric and fluorometric probes for hypochlorite is still highly demanded yet challenging, though a few probes for specific detection of hypochlorite have been reported [25–29]. Recently, rhodamine dyes have been the preferred chromophore and fluorophore in the design of colorimetric and fluorometric probes, owing to its brilliant red color, high quantum yields and excellent photophysical properties [30–33]. However, most of the reported rhodamine-based probes for ClO^- displayed either modest selectivity or delayed response time. Thus, it is still urgently needed to develop rapidly responsive, highly selective and sensitive fluorescent probes for ClO^-, which can be used in both colorimetric and fluorometric tests.

Herein, a rapidly responsive, selective and sensitive fluorescent probe for hypochlorite based on rhodamine B fluorophore has been developed and utilized to detect hypochlorite in real sample and living cells. A two-step-in-one-pot synthesis of Rh-ClO was very concise and highly efficient. Reaction of rhodamine B with $POCl_3$ followed by thiophene-2-carbohydrazide afforded Rh-ClO with 62% yield (Scheme 1). It is reported that hydrazide can be oxidized by hypochlorite and hydrolyze into carboxylic acid in aqueous solution [34]. Recently, rhodamine dyes have been widely employed to design 'turn-on' fluorescent probes for various analytes [35–40], owing to their outstanding ring-opening transformations from colorless and non-fluorescent spirolactam forms to rhodamine fluorophores with intensive absorptions and emissions in the visible spectrum range [30,31]. Thereupon, thiophene-2-carbohydrazide has been introduced into rhodamine B fluorophore through the spirolactam connection pattern to develop color and fluorometric probe Rh-ClO for hypochlorite.

Anion selectivity studies were first performed in Tris-HCl buffer solution (C_2H_5OH/H_2O = 4/6 (v/v), pH 7.4). The presence of 100 equiv. anions and reactive species such as $ONOO^-$, Cl^-, HPO_4^{2-}, $H_2PO_4^-$, SO_4^{2-}, NO_3^-, CO_3^{2-}, S^{2-}, $S_2O_3^{2-}$, H_2O_2, NO and •OH, did not cause any color changes of solution, which meant that the ring-opening reaction of spirolactam was not brought about in presence of these species, as show in Figs. 1a and b. Upon addition of 100 equiv. ClO^-, the solution color changed from colorless to pink and was immediately visible to the naked-eyes, and a red fluorescence was observed under excited at UV 365 nm light. Meanwhile, a new absorption peak at 556 nm and fluorescent peak at 578 nm appeared, which indicated that hypochlorite could induce a ring-opening reaction of rhodamine moiety, and the product rhodamine B had already generated, which was verified by HRMS (Fig. S1 in Supporting information). Furthermore, in Tris-HCl buffer solution (C_2H_5OH/buffer = 4/6 (v/v), pH 7.4), the presence of 100 equiv. various metal ions, such as K^+, Ca^{2+}, Na^+, Mg^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Cd^{2+}, did not cause any observable changes of absorption and emission spectra (Fig. S2 in Supporting information). The effect of competitive anions was also studied by adding hypochlorite to Rh-ClO solution in the presence of anions and reactive species, such as $ONOO^-$, Cl^-, HPO_4^{2-}, $H_2PO_4^-$, SO_4^{2-}, NO_3^-, CO_3^{2-}, S^{2-}, $S_2O_3^{2-}$, H_2O_2, NO, •OH, which were prepared and calibrated according to the methods reported in supplementary. The presence of anions and reactive species did not affect absorption and emission spectral responses between Rh-ClO and ClO^- (Fig. S3 in Supporting information). The good selectivity and anti-interference for ClO^- indicated that Rh-ClO would be a potential tool as both colorimetric and fluorometric probe for ClO^- test in practice.

In order to further assess pH effect, the absorption and emission spectral changes of Rh-ClO for pH were studied (Fig. S4 in Supporting information). When the pH value was above 6.0, there was no absorption peak in C_2H_5OH-H_2O (4/6, v/v) solution of Rh-ClO, indicating that Rh-ClO was suitable for usage at neutral and basic conditions. With the decrease of pH value, a new absorption peak at 556 nm was gradually enhanced, indicating that spirolactam of Rh-ClO underwent a ring-opening reaction in the protonation of Rh-ClO. Meanwhile, Rh-ClO showed 300-folds

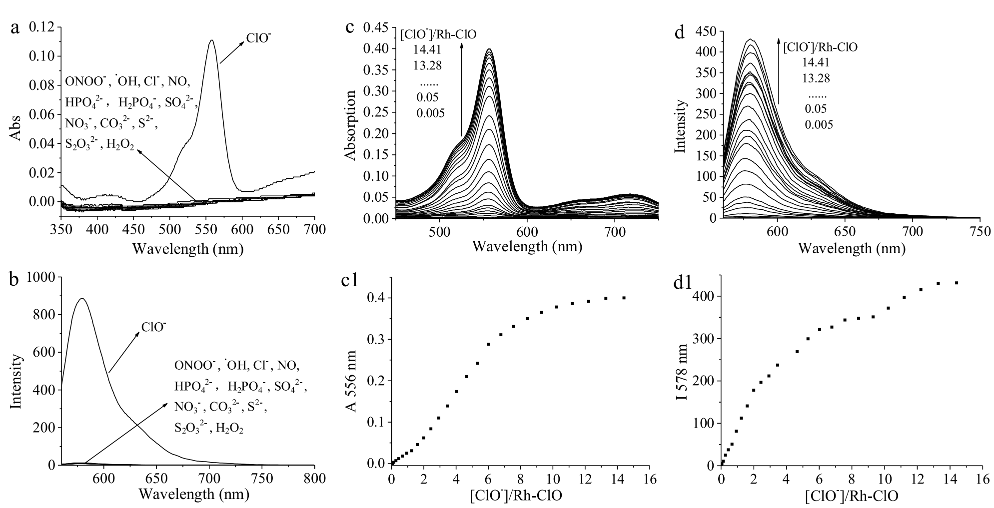


Fig. 1. Absorption (a) and emission (b) spectra of Rh-ClO in the presence of different anions. Changes in the absorption (c) and emission spectra (d) of Rh-ClO (20 μmol/L) in C_2H_5OH/H_2O (4/6, v/v) at pH 7.4 (0.1 mmol/L Tris-HCl buffer) with increasing of NaOCl solution from 0 to 300 μmol/L. (c1, d1) The response of absorption intensity (556 nm) and emission intensity (578 nm) in the concentration of NaOCl from 0 to 300 μmol/L.

Please cite this article in press as: Y. Xia, et al., A fluorometric and mitochondrion-targetable probe for rapid, naked-eye test of hypochlorite in real samples, Chin. Chem. Lett. (2018), https://doi.org/10.1016/j.cclet.2018.01.054