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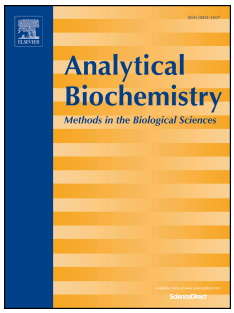

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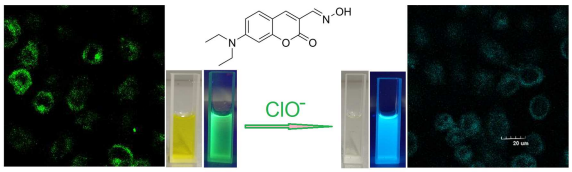
Graphical abstract



A fluorescent probe for hypochlorite with colorimetric and fluorometric characteristics and imaging in living cells

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Abstract

An excellent fluorescent probe with "turn on" phenomenon for sensitive monitoring hypochlorite (ClO^-) was prepared using the mild condensation reaction between coumarin and hydroxylamine (NH_2OH). The probe possessed potent selectivity to hypochlorite (ClO^-) with obvious color changes from yellow to light yellow and green to blue fluorescence emission, which could be noticed by the naked eye. Moreover, the probe has been succeeded in imaging ClO^- in living A549 cells and thus has the potential prospect in the visual detection of intracellular ClO^-.

Keywords: coumarin derivative; fluorescence probe, hypochlorite, colorimetric change, fluorescence imaging

1. Introduction

Hypochlorite (ClO^-) is one of the very important reactive oxygen species (ROS) mainly including hydroxyl ion (OH^-), oxygen ($^1O_2$), hydrogen dioxide (H_2O_2), nitrite (NO_2^-), hypochlorite (ClO^-) and son on in the human body, which is biologically generated from chloride ions (Cl^-) and H_2O_2 with the catalysis of enzyme myeloperoxidase.[1-3] ClO^- has an essential influence on cellular defense system and organism immunity such as the prevention of infection and the destruction of bacteria.[4-6] ┐However, when the concentration level of ClO^- is higher than normal, it will bring about oxidative stress and tissue damage along with a lot of related risks including immunodeficiency, myocardial damage, nerve injury, nephropathy, angiocardiopathy and tumor.[7-12] Therefore, the design and development of the convenient and fast analysis methods for the recognition of ClO^- is of vital significance.

At present, all kinds of detection methods have successfully been used to test ClO^-, such as potentiomeric analysis, electrochemical method, chemiluminescence method and fluorescence analysis.[13-17] In these methods, fluorescence probe has incomparable superiorities such as user-friendly control, time saving, simple processing, good specificity, high sensitivity and cell imaging.[18-20] Also some fluorescence probes of ClO^- have been successfully developed.[21-24] Nevertheless, some of them took too long time in the detection process, which limited their applications in real time detection,[25, 26] some of them possessed poor detection limits,[27-29] some of them did not achieve ClO^- detection in live cells,[26, 30] and others showed typical “turn off” characteristics, which is inconvenient to visual detection of ClO^-.[31] For instance, Yin et. al developed two fluorescence probe for ClO^- based on coumarin.[32] These both probes could be used in detecting intracellular ClO^-, however, they showed serious “turn off” appearance, which hindered application in the visual detection of ClO^-. Therefore, there are still a lot of works to be done in quantity and performance of fluorescence probe of ClO^-.

Herein we synthesized a simple compound (probe 1) based on coumarin and hydroxylamine (NH_2OH) without tedious chemical reactions,[33] which can be used as a dependable testing implement of ClO^- with both ratiometric and turn on characteristics. Coumarin was selected as a fluorescent chromophore to build up various kinds of fluorescence probes on the strength of its prominent characteristics including good luminous performance, low cost, low biotoxicity, easy to synthesis and excitation wavelength and emission wavelength are both in a visible light area.[34] Hydroxylamine of probe 1 could transform into a positive derivative formation with arresting fluorescence after interacting with ClO^-.[35] This probe has many advantages like as the low limit of detection (0.21 μM), higher selectivity for ClO^- over other species, quick response, and noteworthy transformations of color and fluorescence. More importantly, probe 1 has been implemented in the detection of ClO^- in living A549 cells.

2. Experimental

2.1 Reagents and chemicals

4-(Diethylamino)salicylaldehyde (99%), Hydroxylamine hydrochloride (98.5%), Diethyl malonate (99.5%), Hydrochloric acid (36-38%), Phosphorus oxychloride (99.5%), ethyl alcohol (99.5%), Triethylamine(99.5%), N,N-Dimethylformamide (99.9%), Acetic acid, glacial (99%) were purchased from commercial suppliers and used directly without further purification. Double-distilled water was used to prepare solution throughout the experiment. ClO^-, SiO_3^{2-}, $S_2O_3^{2-}$, $H_2PO_4^-$, MnO_4^-, F, ClO_4^- and NO_2^- were severally prepared using their metal salts (NaClO, Na_2SiO_3, $Na_2S_2O_3$, NaH_2PO_4, $KMnO_4$, KF, $NaClO_4$ and $NaNO_2$). O_2^-, $^1O_2$, and ·OH were produced on the basis of relevant literatures.[36-38] The pH value of PBS solution (10 mM) was set as 7.4 by adjusting the amount of NaOH solution. A549 cells and MCF-7 cells were purchased from ATCC.

2.2 Apparatus

Fluorescence spectra were gained by Cary Eclipse fluorescence spectrophotometer (Varian,

USA). ^{1}H and ^{13}C NMR spectra were measured using an AVANCE III HD NMR spectrometer (600 MHz, Bruker, Germany) and TMS as the internal standard. Melting point was examined by a SGW X-4 melting point apparatus (Shanghai precision instruments Co., China). UV-visible spectra were acquired using a Lambda 950 spectrometer (PerkinElmer, USA). The fluorescence images were acquired by a laser scanning confocal microscopy (Olympus IX 71, Japan). The testing conditions of all experiment were performed at room temperature.

2.3 General Procedure

2.3.1. Solution preparation

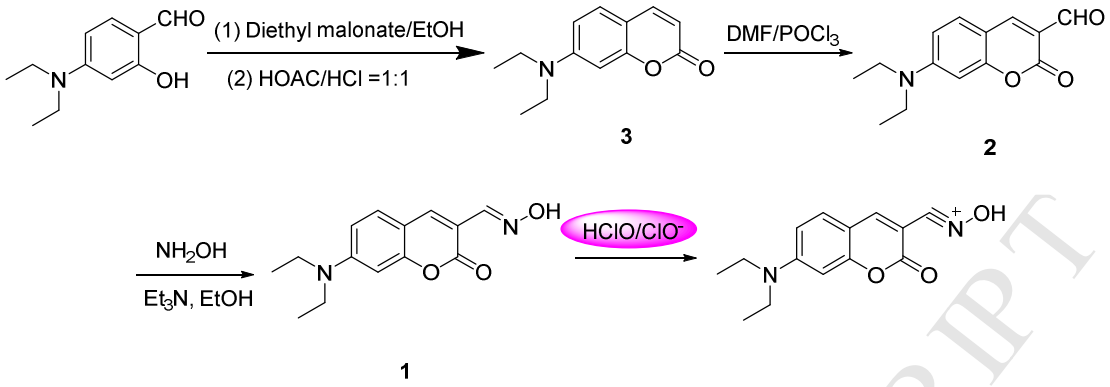
Probe 1 was easily dissolved in EtOH (10 mL) and was diluted with PBS solution (90 mL) to obtain a stock solution of probe 1 (1 mM). Then the stock solution of probe 1 was diluted with PBS-EtOH solution (9:1, v:v, 10 mM, pH = 7.4) to the final concentration according to the testing requirement. The testing samples of analytes were prepared in PBS-EtOH solution and were diluted to the target concentrations for test. The absorption and fluorescence spectra of probe 1 with various analytes were measured after equilibrated for 3 minutes. The excitation wavelength (λ_{ex}) of the fluorescence spectra was set as 365 nm.

2.3.2. Cell Imaging

A549 cells were cultivated for 30 minutes with probe 1 (10 μM) in DMEM culture at 37 °C, and washed three times with PBS, and then incubated with ClO^- (20 μM) for 5 minutes. After then, the cell images were photographed using green channel (500-600 nm) and blue channel (430-470 nm), respectively.

2.3.4. Synthesis

7-diethylamino coumarin (3) and 7-diethylamino coumarin-3-aldehyde (2) were synthesized according to the known methods (as shown in scheme 1).[39]



Scheme 1. Synthesis of compound 1.

Compound 2 (0.49 g, 0.2 mmol), hydroxylamine hydrochloride (0.21 g, 0.3 mmol) and triethylamine 0.5 mL were dissolved in anhydrous EtOH (20.0 mL). The precipitate taken shape after refluxed for 16 h, and then filtered off by suction filtration, washed with ice EtOH once again and dried in a vacuum drying chamber. The target product 1 as yellow solid could be given. Yield 0.23 g (44.2%). m.p. 205.1-206.8℃. ^{1}H NMR (DMSO-d_6, 600 MHz, δ ppm): 11.31 (s, 1H), 8.16 (s, 1H), 7.99 (s, 1H), 7.56, 7.54 (d, *J* = 12 Hz, 1H), 6.75, 6.73 (d, *J* = 12 Hz, 1H), 6.56 (s, 1H), 3.47-3.43 (q, *J* = 6 Hz, 4H), 1.14-1.12 (t, *J* = 6 Hz, 6H). ^{13}C NMR (DMSO-d_6, 150 MHz, δ ppm): 160.66, 156.72, 151.46, 143.32, 138.85, 130.81, 111.95, 109.96, 108.27, 96.84, 44.63, 12.81. EI-MS: 260.12 $[M+H]^+$. Elemental Analysis (%): C, 64.41; H, 6.32; N, 10.26, Calculated (%) for $C_{14}H_{16}N_2O_3$: C, 64.59; H, 6.19; N, 10.78.

3. Results and Discussion

3.1. Ultraviolet-visible spectrum of probe 1 with ClO^-

The ultraviolet-visible spectra of probe 1 with ClO^- were tested firstly through the titration experiment in PBS-EtOH solution (9:1, v/v, 10 mM, pH 7.4). As shown in Fig. 1, probe 1 (10 μM) showed an obvious absorption ban around 397 nm. After titration with different dopamine concentrations of ClO^- (0, 1 μM, 2 μM, 3 μM, 4 μM, 5 μM, 6 μM, 7 μM, 8 μM, 9 μM, 10 μM, 11 μM, 12 μM, 13 μM, 14 μM, 15 μM, 16 μM, 17 μM, 18 μM, 19 μM, 20 μM), the

ultraviolet-visible absorption bands at 397 nm gradually decreased. These changes suggested that probe 1 possesses prominent ultraviolet-visible spectral response towards ClO^-.

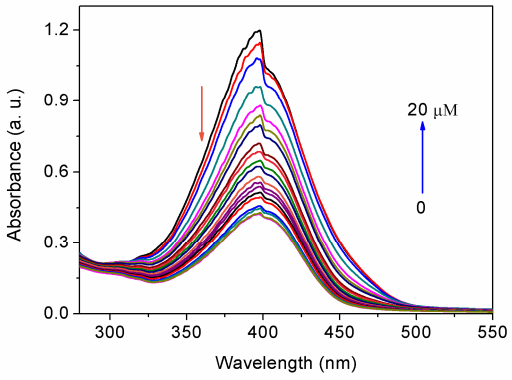


Fig. 1. The ultraviolet-visible spectra of probe 1 (10 μM) in PBS-EtOH solution with different levels of ClO^-.

3.2. The fluorescence selectivity of probe 1 for ClO^-

The fluorescence selectivity of probe 1 for ClO^- was carried out and the situation was shown in Fig. 2. Probe 1 displayed green fluorescence in the absence of other analytes. When 2 equiv. of ClO^- was added to the solution of probe 1, an outstanding enhancement of fluorescence intensity with blue shift of the emission peaks from 506 nm to 465 nm. Whereas ClO^-, SiO_3^{2-}, $S_2O_3^{2-}$, $H_2PO_4^-$, MnO_4^-, F, ClO_4^-, NO_2^-, O_2^-, $^1O_2$, ·OH and H_2O_2 did not has an evident influence to the emssion spectra of probe 1 at the same concentrations. The results of these tests demonstrated probe 1 has high selectivity for ClO^- over other ananlytes.

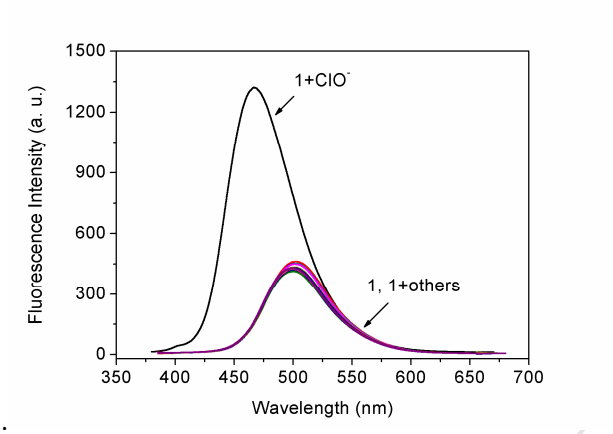


Fig. 2. Fluorescence spectra of probe 1 (10 μM) in PBS-EtOH solution with various analytes (20 μM).

The antijamming capability of probe 1 (10 μM) to the special recognition of ClO^- against other analytes was also identified by means of competition experiment. The peak values of fluorescence spectra of probe 1 with ClO^- (20 μM) and other competition analytes (20 μM) (including a: ClO^-, b: SiO_3^{2-}, c: $S_2O_3^{2-}$, d: $H_2PO_4^-$, e: MnO_4^-, f: F^-, g: ClO_4^-, h: NO_2^-, i: O_2^-, j: $^1O_2$, k: ·OH, and l: H_2O_2) were revealed in Fig. 3. Obviously, it can be easily observed that the fluorescence intensities of probe 1 with ClO^- showed no evident changes between with and without competition species. In other words, the tested competitive analytes didn't adversely disturb the detection capability of probe 1 to ClO^-. It can thus be concluded that this probe has high selectivity in the determination of ClO^- even when other species coexisted in abundance.

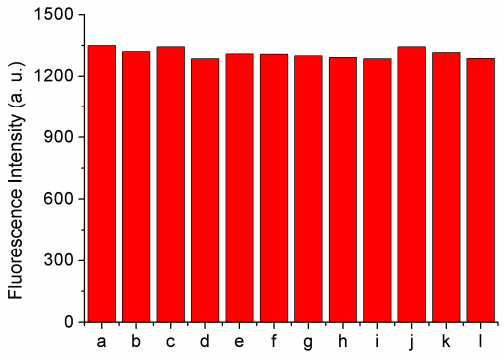


Fig. 3. The peak values of fluorescence spectra of probe 1 (10 μM) with ClO^- coexisted with other competing analytes. a: ClO^-, b: SiO_3^{2-}, c: $S_2O_3^{2-}$, d: $H_2PO_4^-$, e: MnO_4^-, f: F^-, g: ClO_4^-, h: NO_2^-, i: O_2^-, j: $^1O_2$, k: ·OH, and l: H_2O_2.

3.3. The fluorescence sensitivity of probe 1 for ClO^-

After verified the high selectivity of probe 1 towards ClO^-, we are more than happy to take our interests to the sensitivity. The fluorescence spectra of probe 1 towards different gradient concentrations of ClO^- (0, 2 μM, 4 μM, 6 μM, 8 μM, 10 μM, 12 μM, 14 μM, 16 μM, 18 μM, 20 μM) were collected and displayed in Fig. 4. The fluorescence intensities of probe 1 appeared a trend of enhancement accompanied by the concentrations of ClO^-. In addition, the fluorescence intensity reached the steady value when 2 equiv. of ClO^- was transferred to the solution of probe 1. More importantly, the changes of the maximum fluorescence intensities of the probe 1 linearly heightened accompanied by the accumulation of ClO^- concentrations. A straight-line formula could be established between the fluorescence ratiometric signals F_{max}/F_0 and the concentrations of ClO^- on a scale of 0 to 18 μM: $y=0.17+0.18x$ ($R^2=0.9832$, Fig. 4 Inset). The detection limit (DL) of probe 1 for ClO^- was defined as 0.21 μM when the signal-to-noise ratio (S/N) was 3.[40] Interestingly enough, with the concentration increasing of ClO^-, the maximum emission wavelength of probe 1 solution also have a considerable blue-shift trend drastically (Fig. 5). These results indicated that probe 1 is very sensitive to ClO^- and can be used for the quantitative determination in biological systems.

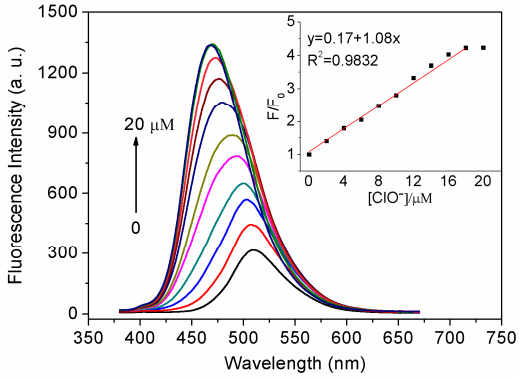


Fig. 4. Fluorescence spectra of probe 1 (10 μM) with different concentrations of ClO^-. Inset shows the linearity between the peak values of fuorescence and the concentrations of ClO^-.

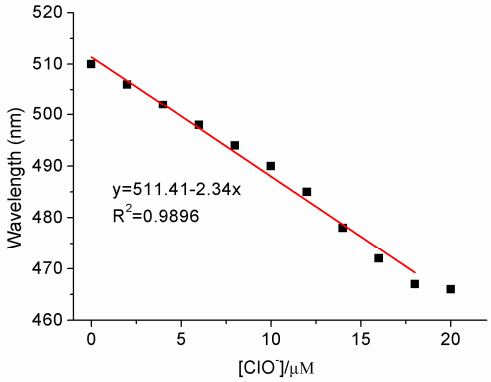


Fig. 5. Linear relationship between the maximum emission wavelength of probe 1 (10 μM) in PBS-EtOH solution and the concentration of ClO^-.

The excellent recognition ability of probe 1 to ClO^- was visible with the naked eye. As shown in Fig. 6, only when ClO^- (20 μM) was added, the color of the probe 1 solution became colorless from obvious yellow, however, the other species including SiO_3^{2-}, $S_2O_3^{2-}$, H_2PO^{4-}, MnO_4^-, F, ClO_4^-, NO_2^-, O_2^-, $^1O_2$, ·OH and H_2O_2 did not brought the color change. Moreover, the specific fluorescence color change of probe 1 towards ClO^- could be clearly distinguished under a hand-held UV Lamp. Upon the addition of ClO^-, there was a significant fluorescence change of

probe 1 from green to blue. However, all of the competing species have no noticeable influence on the fluorescence color of probe **1**. Based on the above results, the probe 1 could be used as a simple and convenient visible probe for ClO^-.

Fig.6. Photographs of the color and fluorescent emission of probe 1 in H_2O-EtOH solution after addition different common anions under sunlight and 365 nm UV-lamp, respectively. 1: probe 1, 2: ClO^-, 3: SiO_3^{2-}, 4: $S_2O_3^{2-}$, 5: H_2PO^{4-}, 6: MnO_4^-, 7: F^-, 8: ClO_4^-, 9: NO_2^-, 10: O_2^-, 11: $^1O_2$, 12: ·OH, 13: H_2O_2.

3.4. The detection time of probe 1 for ClO^-

The detection time of probe is an important factor in practical application. Therefore, the response time of probe 1 to ClO^- was investigated. The kinetic characteristic of fluorescence intensity over action time between probe 1 and ClO^- was displayed in Fig. 7. When 20 μM of ClO^- was added to 10 μM of probe 1 solution, the emission maximum increased dramatically and was nearly maximized in about 2 min. This demonstrated that probe 1 could be served as a rapid identification tool for monitoring ClO^-.

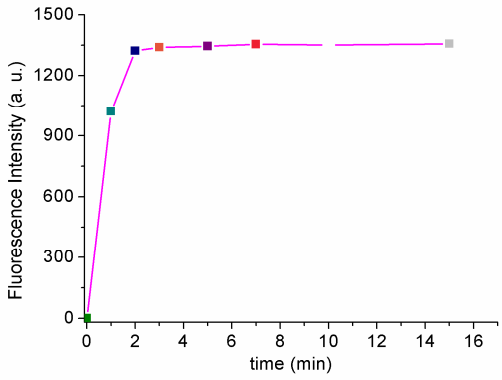


Fig. 7. The dynamic characteristic of probe 1 (10 μM) in the present of ClO^- (2 equiv.).

3.4. Cell imaging and cytotoxicity assay

In order to examine the application performance in biological sample, the fluorescence imaging experiments of probe 1 with and without ClO^- were further performed in living cells. When A549 cells were incubated with probe 1 for 30 minutes, the strong green fluorescence could be observed (Fig. 8b and 8c). And then incubated with 2 equiv. ClO^-, the green fluorescence (Fig. 8e) of cells faded away completely and arresting blue fluorescence appeared after 5 minutes (Fig. 8f). In addition, A549 cells have almost no fluorescence when they were incubated with ClO^- for 5 min (Fig. 8h and 8i). The cytotoxicity of the probe 1 was also evaluated via MTT assay of MCF-7 cells.[41] The results of MTT assays indicated that cell viability was over 85% when probe 1 was used below 80 μM (Fig. 9). These results demonstrated that the probe had significant fluorescence change and low cytotoxicity, which is suitable for the detection of ClO^- in living cells.

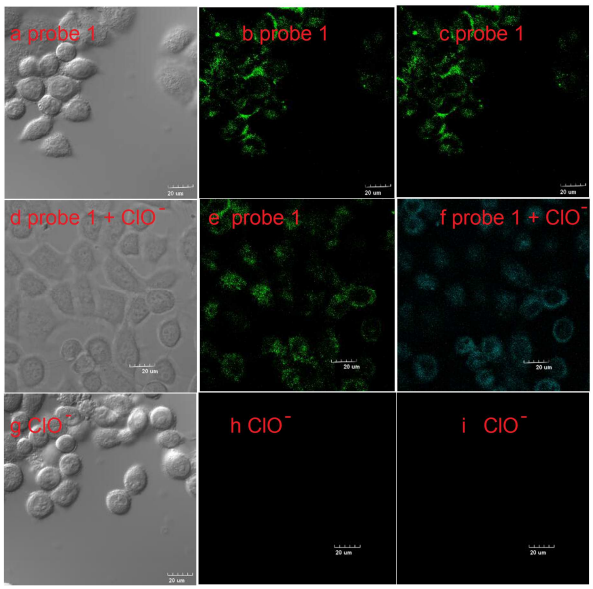


Fig. 8. Confocal microscopy images of A549 cells. Cells incubated with 10 μM probe 1: bright-field image (a) and fluorescence image (b, c). Cells incubated with 10 μM probe

1 and 20 μM ClO^-: bright-field image (d) and fluorescence image (e, f). Cells incubated with 20 μM ClO^- bright-field image (g) and fluorescence image (h, i). Scale bar represents 20 μm in every image.

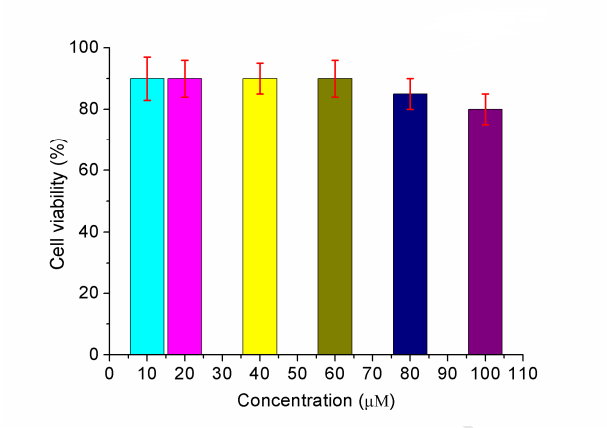


Fig. 9. The changes of MCF-7 cell viabilities after incubation with probe 1 in range of 10 μM to 100 μM. Error bars represent the standard deviations of 3 trials.

4. Conclusions

In conclusion, a coumarin-based spectroscopic probe with both colorimetric and fluorometric features for the detection of ClO^- has been successfully prepared. Along with addition of ClO^-, the color of the probe solution showed a marked turnaround from yellow to colorless, while other competing species hardly displayed any noteworthy color change. Additionally, the fluorescence of the probe can turn into blue color from green color with the enhancement of fluorescence intensity. The probe also has a fast response process for detection of ClO^- about 2 minutes. Based on the excellent virtues of rapid detection, remarkable color and fluorescence changes, low toxicity, low limit of detection and high specificity, the probe has a good application prospect in the fluorescence testing of ClO^- in living cells.

Acknowledgements

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References

1. Roos D, Winterbourn CC. Immunology - Lethal weapons. Science. 2002;296(5568):669-671. doi:DOI 10.1126/science.1071271.

2. Winterbourn CC, Hampton MB, Livesey JH, Kettle AJ. Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome - Implications for microbial killing. J Biol Chem. 2006;281(52):39860-39869. doi:10.1074/jbc.M605898200.

3. Bhattacharya S. Reactive Oxygen Species and Cellular Defense System. 2015:17-29. doi:10.1007/978-81-322-2035-0_2.

4. Pattison DI, Davies MJ. Evidence for rapid inter- and intramolecular chlorine transfer reactions of histamine and carnosine chloramines: Implications for the prevention of hypochlorous-acid-mediated damage. Biochemistry-Us. 2006;45(26):8152-8162. doi:10.1021/bi060348s.

5. Maden M, Ertugrul IF, Orhan EO, Erik CE, Yetis CC, Tuncer Y et al. Enhancing antibacterial effect of sodium hypochlorite by low electric current-assisted sonic agitation (vol 12, e0183895, 2017). Plos One. 2017;12(11). doi:ARTN e0188672 10.1371/journal.pone.0188672.

6. Harrison JE, Schultz J. Studies on the chlorinating activity of myeloperoxidase. J Biol Chem. 1976;251(5):1371-1374.

7. Khatib S, Musa R, Vaya J. An exogenous marker: A novel approach for the characterization of oxidative stress. Bioorgan Med Chem. 2007;15(11):3661-3666. doi:10.1016/j.bmc.2007.03.052.

8. Yap YW, Whiteman M, Cheung NS. Chlorinative stress: An under appreciated mediator of neurodegeneration? Cell Signal. 2007;19(2):219-228. doi:10.1016/j.cellsig.2006.06.013.

9. Sam CH, Lu HK. The role of hypochlorous acid as one of the reactive oxygen species in periodontal disease. J Dent Sci. 2009;4(2):45-54. doi:Doi 10.1016/S1991-7902(09)60008-8.

10. Maruyama Y, Lindholm B, Stenvinkel P. Inflammation and oxidative stress in ESRD - the role of myeloperoxidase\. J Nephrol. 2004;17:S72-S76.

11. Malle E, Buch T, Grone HJ. Myeloperoxidase in kidney disease. Kidney Int. 2003;64(6):1956-1967. doi:DOI 10.1046/j.1523-1755.2003.00336.x.

12. Steinbeck MJ, Nesti LJ, Sharkey PF, Parvizi J. Myeloperoxidase and chlorinated peptides in osteoarthritis: Potential biornarkers of the disease. J Orthop Res. 2007;25(9):1128-1135. doi:10.1002/jor.20400.

13. Adam LC, Gordon G. Direct and Sequential Potentiometric Determination of Hypochlorite, Chlorite, and Chlorate Ions When Hypochlorite Ion Is Present in Large Excess. Anal Chem. 1995;67(3):535-540. doi:Doi 10.1021/Ac00099a009.

14. Thiagarajan S, Wu ZY, Chen SM. Amperometric determination of sodium hypochlorite at poly MnTAPP-nano Au film modified electrode. J Electroanal Chem. 2011;661(2):322-328. doi:10.1016/j.jelechem.2011.08.009.

15. Shi LL, Li X, Zhou M, Muhammad F, Ding YB, Wei H. An arylboronate locked fluorescent probe for hypochlorite. Analyst. 2017;142(12):2104-2108. doi:10.1039/c7an00467b.

16. Ingle DFMaJD. Determination of Chlorine in Water by Luminol Chemiluminescence. Anal Chem. 1981;53(3):455-458. doi:10.1021/ac00226a015.

17. Yan LQ, Hu CJ, Li JP. A fluorescence turn-on probe for rapid monitoring of

hypochlorite based on coumarin Schiff base. Anal Bioanal Chem. 2018;410(28):7457-7464. doi:10.1007/s00216-018-1352-8.

18. Yan LQ, Cui MF, Zhou Y, Ma Y, Qi ZJ. A Simplified and Turn-on Fluorescence Chemosensor Based on Coumarin Derivative for Detection of Aluminium(III) Ion in Pure Aqueous Solution. Anal Sci. 2015;31(10):1055-1059. doi:DOI 10.2116/analsci.31.1055.

19. Chen XQ, Wang F, Hyun JY, Wei TW, Qiang J, Ren XT et al. Recent progress in the development of fluorescent, luminescent and colorimetric probes for detection of reactive oxygen and nitrogen species. Chem Soc Rev. 2016;45(10):2976-3016. doi:10.1039/c6cs00192k.

20. Liu W, Dong H, Zhang LM, Tian Y. Development of an Efficient Biosensor for the In Vivo Monitoring of Cu+ and pH in the Brain: Rational Design and Synthesis of Recognition Molecules. Angew Chem Int Edit. 2017;56(51):16328-16332. doi:10.1002/anie.201710863.

21. Gong YQ, Yu B, Yang W, Zhang XL. Phosphorus, and nitrogen co-doped carbon dots as a fluorescent probe for real-time measurement of reactive oxygen and nitrogen species inside macrophages (vol 79, pg 822, 2016). Biosens Bioelectron. 2016;83:379-379. doi:10.1016/j.bios.2016.03.058.

22. Fan JL, Mu HY, Zhu H, Wang JY, Peng XJ. Light up ClO- in live cells using an aza-coumarin based fluorescent probe with fast response and high sensitivity. Analyst. 2015;140(13):4594-4598. doi:10.1039/c5an00777a.

23. Gong HL, Jiang Y, Hou RC, Ding XQ. A Sensitive and Selective Fluorescent Coumarin-Based Probe for Detection of Hypochlorite ion and its Application to Cellular Imaging. J Fluoresc. 2016;26(2):403-406. doi:10.1007/s10895-015-1726-7.

24. Ding SS, Zhang Q, Xue SH, Feng GQ. Real-time detection of hypochlorite in tap

water and biological samples by a colorimetric, ratiometric and near-infrared fluorescent turn-on probe. Analyst. 2015;140(13):4687-4693. doi:10.1039/c5an00465a.

25. Huang X, Li ZP, Cao TT, Cai Q, Zeng CC, Fu H et al. A methylene blue-based near-infrared fluorescent probe for rapid detection of hypochlorite in tap water and living cells. Rsc Adv. 2018;8(26):14603-14608. doi:10.1039/c8ra01037d.

26. Ji RX, Qin K, Liu AK, Zhu Y, Ge YQ. A simple and fast-response fluorescent probe for hypochlorite in living cells. Tetrahedron Lett. 2018;59(24):2372-2375. doi:10.1016/j.tetlet.2018.05.027.

27. Gong YQ, Yu B, Yang W, Zhang XL. Phosphorus, and nitrogen co-doped carbon dots as a fluorescent probe for real-time measurement of reactive oxygen and nitrogen species inside macrophages. Biosens Bioelectron. 2016;79:822-828. doi:10.1016/j.bios.2016.01.022.

28. Tong HJ, Zhang YJ, Ma SN, Zhang MH, Wang N, Wang R et al. A pinacol boronate caged NIAD-4 derivative as a near-infrared fluorescent probe for fast and selective detection of hypochlorous acid. Chinese Chem Lett. 2018;29(1):139-142. doi:10.1016/j.cclet.2017.07.007.

29. Lou XD, Zhang Y, Qin JG, Li Z. Colorimetric hypochlorite detection using an azobenzene acid in pure aqueous solutions and real application in tap water. Sensor Actuat B-Chem. 2012;161(1):229-234. doi:10.1016/j.snb.2011.10.024.

30. Lin WY, Long LL, Chen BB, Tan W. A Ratiometric Fluorescent Probe for Hypochlorite Based on a Deoximation Reaction. Chem-Eur J. 2009;15(10):2305-2309. doi:10.1002/chem.200802054.

31. Wang KN, Sun PZ, Chao XJ, Cao DX, Mao ZW, Liu ZQ. A coumarin Schiff's base two-photon fluorescent probe for hypochlorite in living cells and zebrafish. Rsc Adv. 2018;8(13):6904-6909. doi:10.1039/c8ra00093j.

32. Xiong KM, Huo FJ, Zhang YB, Wen Y, Chao JB, Yin CX. A novel recognition mechanism based on aldehyde group oxidized into carboxyl group by hypochlorite for the materials of fluorescent probes. Sensor Actuat B-Chem. 2018;255:2378-2383. doi:10.1016/j.snb.2017.09.042.

33. Giannella M, Gualtieri F, Stein ML. On the reaction between coumarins and hydroxylamine. Journal of Heterocyclic Chemistry. 1971;8(3):397-403. doi:10.1002/jhet.5570080308.

34. Yan LQ, Ma Y, Cui MF, Qi ZJ. A novel coumarin-based fluorescence chemosensor containing L-histidine for aluminium(III) ions in aqueous solution. Anal Methods-Uk. 2015;7(15):6133-6138. doi:10.1039/c5ay01466b.

35. Nguyen KH, Hao YG, Zeng K, Fan SN, Li F, Yuan SK et al. A benzothiazole-based fluorescent probe for hypochlorous acid detection and imaging in living cells. Spectrochim Acta A. 2018;199:189-193. doi:10.1016/j.saa.2018.03.055.

36. Ma Z, Sun W, Chen LZ, Li J, Liu ZZ, Bai HX et al. A novel hydrazino-substituted naphthalimide-based fluorogenic probe for tert-butoxy radicals. Chem Commun. 2013;49(56):6295-6297. doi:10.1039/c3cc42052c.

37. Li XH, Zhang GX, Ma HM, Zhang DQ, Li J, Zhu DB. 4,5-Dimethylthio-4'-[2-(9-anthryloxy)ethylthio]tetrathiafulvalene, a highly selective and sensitive chemiluminescence probe for singlet oxygen. J Am Chem Soc. 2004;126(37):11543-11548. doi:10.1021/ja0481530.

38. Koide Y, Urano Y, Hanaoka K, Terai T, Nagano T. Development of an Si-Rhodamine-Based Far-Red to Near-Infrared Fluorescence Probe Selective for Hypochlorous Acid and Its Applications for Biological Imaging. J Am Chem Soc. 2011;133(15):5680-5682. doi:10.1021/ja111470n.

39. Yoon S, Albers AE, Wong AP, Chang CJ. Screening mercury levels in fish with a

selective fluorescent chemosensor. J Am Chem Soc. 2005;127(46):16030-16031. doi:10.1021/ja0557987.

40. Yan LQ, Kong ZN, Shen W, Du WQ, Zhou Y, Qi ZJ. A label-free turn-on fluorescence probe for rapidly distinguishing cysteine over glutathione in water solution. Anal Biochem. 2016;500:1-5. doi:10.1016/j.ab.2016.01.027.

41. Yan LQ, Nan D, Lin C, Wan Y, Pan Q, Qi ZJ. A near-infrared fluorescent probe for rapid detection of carbon monoxide in living cells. Spectrochim Acta A. 2018;202:284-289. doi:10.1016/j.saa.2018.05.059.

Highlights

1. Observable colorimetric and fluorometric changes.
2. Fast response time;
3. Highly selective and sensitive response to ClO^-;
4. Strong contrast imaging in living cells.