A water-soluble fluorescence resonance energy transfer probe for hypochlorous acid and its application to cell imaging

JIA Jia & MA HuiMin*

Beijing National Laboratory for Molecular Sciences, Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China

Received February 28, 2011; accepted April 20, 2011

A water-soluble fluorescence resonance energy transfer (FRET) probe for hypochlorous acid (HOCl), dansyl rhodamine B piperazinoacetohydrazide, was designed, synthesized and characterized. The dansyl moiety in the probe acted as a FRET donor and the rhodamine moiety acted as a FRET acceptor. The two moieties were connected by a HOCl-cleavable active bond, and cleavage of this linker decreased the FRET efficiency and increased the fluorescence intensity of the donor at 501 nm. The water solubility of the probe was improved compared with other probes by introduction of the cationic rhodamine fluorophore. As a result, the probe could be used to detect HOCl in aqueous biosystems with a linear range of 2–10 $\mu\text{mol/L}$ and a detection limit of 80 nmol/L (signal- to-noise = 3). The probe was successfully applied to fluorescence imaging of HOCl in HeLa cells.

dansyl rhodamine B piperazinoacetohydrazide, water-soluble probe, hypochlorous acid, cell imaging, fluorescence resonance energy transfer

Citation: Jia J, Ma H M. A water-soluble fluorescence resonance energy transfer probe for hypochlorous acid and its application to cell imaging. *Chinese Sci Bull*, 2011, 56: 3266–3272, doi: 10.1007/s11434-011-4645-2

Hypochlorous acid/hypochlorite (HOCl/OCl^-) is a reactive oxygen species (ROS) and a powerful oxidant. Unlike most of other ROS, HOCl is widely used as a household bleach and disinfectant in our daily lives [1–3]. HOCl is also known to be essential to several biological processes [4–6]. Endogenous HOCl generated by the myeloperoxidase (MPO)- H_2O_2 -chloride system in activated leukocytes plays a critical role in immune systems, and can kill a wide range of pathogens [7]. For example, HOCl can be generated at 20–400 $\mu\text{mol/L}$ per hour by activated neutrophils under inflammatory conditions [8]. However, excess HOCl may cause tissue damage, and abnormal levels of HOCl are related to many pathological processes including cardiovascular disease, arthritis, neurodegeneration, and cancer [8–11]. To increase understanding of the biological effects of HOCl, many efforts have been made to develop selective and sensitive analytical methods for HOCl [1–3,12–20]. Among these

methods, fluorescent probes have received considerable attention because of their good time and spatial resolution [21]. To date, several fluorescent probes for HOCl sensing have been reported, which are designed based on HOCl-induced oxidation reactions [12–18]. These probes exhibit reasonable sensitivity and selectivity. However, most of them have poor water-solubility, which means that organic co-solvents have to be used. This makes detection rather inconvenient and sometimes even incompatible with aqueous biosystems.

Recently, we developed a spectroscopic reagent, rhodamine B piperazinoacetohydrazine (RBPH, Scheme 1), for carbonyl labeling [22,23]. Because of its electrically charged character, RBPH has good water-solubility and can be used in pure aqueous media. However, according to the previous observations, dibenzoylhydrazine can be selectively oxidized by HOCl to dibenzoyl diimide, which will undergo further decomposition in some nucleophilic solvents [13,24]. Based on these facts and the fluorescence resonance energy

*Corresponding author (email: mahm@iccas.ac.cn)

Scheme 1 Synthesis of DNS-RBPH.

transfer (FRET) principle, RBPH could be used to construct a water-soluble HOCl probe that possesses the structural feature of dibenzoylhydrazine. This would be expected to follow the HOCl-mediated oxidation pathway.

Here we report the design, synthesis, and characterization of dansyl rhodamine B piperazinoacetohydrazide (DNS-RBPH, Scheme 1) as a water-soluble FRET probe for HOCl. This probe includes a dansyl (DNS) fluorophore as a donor and RBPH as an acceptor. The two fluorophores are connected by an *N'*-acysulfonohydrazide linker, which can be cleaved by HOCl. Selective cleavage of the linker by HOCl should change the FRET efficiency and fluorescence intensity of the donor at 501 nm. The influence of the cationic RBPH unit on the probe's water solubility, and the selectivity of the probe for HOCl over other ROS were investigated. This probe could be used to detect HOCl in aqueous bio-systems, and it was applied to fluorescence imaging of HOCl in HeLa cells.

1 Experimental

1.1 Materials

Dansyl chloride (DNS-Cl, $\geq 99.0\%$) was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Acetonitrile and methanol (HPLC grade) were purchased from Thermo Fisher Scientific (Waltham, MA). RBPH was synthesized as reported previously [22]. Hypochlorous acid was prepared by distillation from the 5% commercial sodium hypochlorite solution. The hypochlorous acid was stored for up to one week at 4°C as a 100 mmol/L solution, and the pH was adjusted to 11 by addition of sodium hydroxide. Before use, sodium hypochlorite was assayed using a molar absorptivity of 391 L mol⁻¹ cm⁻¹ at 292 nm [25]. The fluorescence quantum yield was determined using rhodamine B in ethanol ($\Phi = 0.69$) as the standard [26]. A stock solution (1 mmol/L) of DNS-RBPH was prepared in water, and was stable for at least one week when stored at 4°C in the dark. Other reagents were of analytical grade. Deionized and distilled water was used in all the experiments.

1.2 Apparatus

¹H NMR was measured on a Bruker DMX-300 spectrometer

in D₂O. Electrospray ionization (ESI) mass spectra were recorded in either positive or negative mode with a Shimadzu LCMS-2010 (Kyoto, Japan). High-resolution ESI analysis was performed using a Bruker Apex IV FTMS (Billerica, MA). Fluorescence spectra were recorded on a Hitachi F-2500 spectrophotometer in 10 mm × 10 mm quartz cells (Tokyo, Japan), with excitation and emission slit widths of 10 nm. Absorption spectra were recorded in 1 cm cells with a TU-1900 spectrophotometer (Beijing, China). HPLC analyses were performed on an Inertsil ODS-SP (250 mm × 4.6 mm I.D., GL Sciences, Torrance, CA) column using a Shimadzu HPLC system consisting of two LC-20AT pumps and a SPD-M20A diode array detector. A model HI-98128 pH-meter (Hanna Instruments Inc., Woonsocket, RI) was used for pH measurements. Fluorescence images were obtained with a FV1000-IX81 fluorescence microscope (Olympus Corp., Tokyo, Japan).

1.3 Synthesis and characterization of DNS-RBPH

DNS-RBPH can be readily synthesized by direct coupling of dansyl chloride and RBPH (Scheme 1). Briefly, RBPH (200 mg, 0.32 mmol) and anhydrous K₂CO₃ (50 mg, 0.36 mmol) were added to a stirred solution of dansyl chloride (100 mg, 0.39 mmol) in CH₂Cl₂ (50 mL). The mixture was stirred vigorously at room temperature under an Ar atmosphere overnight. The resulting heterogeneous solution was filtered. The filtrate was concentrated under reduced pressure to give a purple residue, which was rinsed several times with diethyl ether to remove excess dansyl chloride. The retained solid was dissolved in diluted HCl (pH 5), saturated with NaCl, and extracted three times with CH₂Cl₂. The combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to yield a crude product. The product was then purified by silica gel column chromatography with CH₃OH/CH₂Cl₂ (1:10, v/v), affording DNS-RBPH (170 mg, 60%) as a dark purple solid. ¹H NMR (300 MHz, D₂O): δ = 8.41–8.36 (m, 2H), 8.24 (d, *J* = 6.9 Hz, 1H), 7.84–7.77 (m, 2H), 7.58–7.47 (m, 4H), 7.28 (d, *J* = 7.5 Hz, 1H), 7.18–7.10 (m, 2H), 6.93–6.73 (m, 4H), 3.56–3.53 (m, 8H), 3.10 (brs, 4H), 2.83 (s, 5H), 2.71 (s, 1H), 1.64 (brs, 5H), 1.27–1.25 (m, 1H), 1.21–1.17 (t, *J* = 6.9, 12H). HR-ESI-MS: calcd for [C₄₆H₅₄N₇O₅S]⁺: *m/z* 816.3907; found: *m/z* 816.3885.

1.4 General procedure for fluorescence analysis

Unless otherwise stated, a 50 mmol/L $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$ solution at pH 7.4 (referred to as phosphate buffer) was used in the fluorescence analysis. Briefly, 20 μL of the stock solution (1 mmol/L) of DNS-RBPH was added to a 10 mL tube, followed by addition of 5 mL of phosphate buffer (0.1 mol/L) and 4 mL of water. Then an appropriate volume of HOCl sample solution was added, and the final volume was adjusted to 10 mL with water. After standing for 20 min, the fluorescence intensities/spectra of the mixture were measured at $\lambda_{\text{ex/em}} = 370/501$ nm. As a control, the same procedure was performed in the absence of the analyte.

1.5 Preparation of ROS

Various ROS were produced following known methods [6,27–29].

1.6 Fluorescence imaging of HeLa cells

HeLa cells were grown on glass-bottom culture dishes (MatTek Co., Ashland, MA) using Dulbecco's modified eagle media supplemented with 10% (v/v) fetal bovine serum, penicillin (100 $\mu\text{g mL}^{-1}$), and streptomycin (100 $\mu\text{g mL}^{-1}$) in a humidified 37°C, 5% CO_2 incubator. Before use, the adherent cells were washed with phenol red-free Dulbecco's modified eagle media three times. For HOCl imaging, the cells were first loaded with 10 $\mu\text{mol/L}$ DNS-RBPH at 37°C for 20 min, washed with phosphate buffered saline (pH 7.4) three times to remove the free DNS-RBPH, and then incubated with 5 $\mu\text{mol/L}$ of sodium hypochlorite for 15 min or 30 min. Fluorescence images were captured with a cooled digital CCD camera attached to an inverted fluorescence microscope (objective lens 60 $\times$, $\lambda_{\text{ex}} = 330\text{--}385$ nm).

2 Results and discussion

2.1 Design of DNS-RBPH

The DNS-RBPH probe was designed by incorporating DNS and RBPH into a dyad using *N'*-acylsulfonohydrazide as a linker. The dyad is expected to exhibit FRET, because the absorption spectrum of RBPH (acceptor) overlaps with the emission spectrum of DNS (donor), which is favorable for FRET (Figure 1) [30,31]. When connected by the linker, the two fluorophores are positioned close to each other, and the fluorescence of the donor can be quenched effectively through the FRET process. The *N'*-acylsulfonohydrazide moiety in the probe may be selectively oxidized and cleaved by HOCl, and this cleavage will separate the two fluorophores and increase the fluorescence of the donor. Furthermore, the cationic unit of RBPH increases the water solubility of the probe, so that it could be applied in a pure aqueous medium.

Figure 1 Normalized absorption and emission spectra of DNS and RBPH. Absorption (a) and emission (a') spectra of DNS; absorption (b) and emission (b') spectra of RBPH. The shaded area indicates the spectral overlap between the emission of DNS and the absorption of RBPH.

2.2 Optimization of experimental conditions

Various factors affecting the reaction of DNS-RBPH with HOCl in an aqueous medium were investigated. The pH did not influence the fluorescence response in the range pH 6–10. Consequently, the physiological pH of 7.4 was used in this work, and the solution was maintained at this pH using 50 mmol/L phosphate buffer. Under acidic (pH<5) conditions, the fluorescence intensity decreased, which may be caused by the destruction of the fluorophores by the stronger oxidative ability of HOCl. Time course studies revealed that at either room temperature or 37°C, HOCl addition resulted in a rapid increase in the fluorescence intensity at 501 nm from the DNS unit up until 20 min, and then remained stable for at least 1 h. As a result, a reaction time of 20 min and room temperature were chosen for this study.

2.3 Fluorescence properties and analytical characteristics of DNS-RBPH for HOCl

The fluorescence spectra of DNS-RBPH and its reaction solution with various concentrations of HOCl are shown in Figure 2(a). As expected, the fluorescence of the DNS unit in the probe was quenched by intramolecular FRET, and only the fluorescence ($\lambda_{\text{em}} = 585$ nm) of the RBPH unit was observed. When HOCl was added to the probe solution the DNS fluorescence ($\lambda_{\text{em}} = 501$ nm) appeared, which may be ascribed to the decrease in the FRET efficiency via the removal of the RBPH unit. Surprisingly, the fluorescence intensity of the RBPH unit also increased. A possible explanation for this phenomenon may be the fact that RBPH also has a relatively weak absorption band in the ultraviolet region (curve b in Figure 1), and can be excited at 370 nm. However, the acceptor RBPH has a higher fluorescence quantum yield ($\Phi = 0.19$ in water) [22] than DNS-RBPH ($\Phi = 0.07$ in water). Therefore, the reaction solution containing the released RBPH unit has the increased fluorescence

Figure 2 Analytical performance of DNS-RBPH. (a) Fluorescence spectra of DNS-RBPH (2 $\mu\text{mol/L}$, curve 1) and its reaction solutions with various concentrations of HOCl (2–40 $\mu\text{mol/L}$, curves 2–9) according to the general procedure. (b) Fluorescence intensity change (ΔF) of DNS-RBPH against the HOCl concentration from 0 to 10 $\mu\text{mol/L}$ at $\lambda_{\text{ex/em}} = 370/501$ nm.

intensity at 585 nm.

Because of the low background signal, the quantification of HOCl is based on the enhancement (ΔF) of the fluorescence intensity from the DNS unit at 501 nm. This is directly proportional to the HOCl concentration from 2 to 10 $\mu\text{mol/L}$ with a regression equation of $\Delta F = 0.704 \times C + 0.097$ and a linear correlation coefficient of 0.998 (Figure 2(b)). The detection limit was 80 nmol/L HOCl ($S/N = 3$).

2.4 Selectivity studies

To test the selectivity of the probe, the reactivity of DNS-RBPH was studied with various ROS in 50 mmol/L phosphate buffer (pH 7.4), including $\cdot\text{O}_2^-$, $\text{HO}\cdot$, $^1\text{O}_2$, H_2O_2 , and HOCl. The reactions were conducted in parallel at room temperature for 20 min, and the fluorescence intensity at 501 nm was monitored. The probe displayed high selectivity towards HOCl against other ROS (Figure 3(a)). In addition, the reaction of DNS-RBPH with other common ions (Al^{3+} , Ca^{2+} , Cu^{2+} , Fe^{3+} , Fe^{2+} , Hg^{2+} , K^+ , Mg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , Cl^- , NO_3^- , and CO_3^{2-}) was also examined under the same conditions (Figure 3(b)). None of the ions led to a noticeable fluorescence response, which suggests that the

Figure 3 Selectivity studies of DNS-RBPH. (a) Comparison of the fluorescence intensities ($\lambda_{\text{ex/em}} = 370/501$ nm) of 2 $\mu\text{mol/L}$ DNS-RBPH (probe) and its reaction solutions with HOCl (20 $\mu\text{mol/L}$) and other ROS (100 $\mu\text{mol} \cdot \text{O}_2^-$, 100 $\mu\text{mol/L}$ H_2O_2 , 20 $\mu\text{mol/L}$ $\text{HO}\cdot$, and 100 $\mu\text{mol/L}$ $^1\text{O}_2$). (b) Fluorescence spectra ($\lambda_{\text{ex}} = 370$ nm) of 2 $\mu\text{mol/L}$ DNS-RBPH in 50 mmol/L phosphate buffer of pH 7.4 with various species: 20 $\mu\text{mol/L}$ HOCl; 10 $\mu\text{mol/L}$ Fe^{2+} , Fe^{3+} , Hg^{2+} or Pb^{2+} ; 100 $\mu\text{mol/L}$ Cu^{2+} , Mn^{2+} , Ni^{2+} , Zn^{2+} , Al^{3+} or ClO_3^- ; 1 mmol/L Ca^{2+} or Mg^{2+} ; 0.1 mol/L K^+ , Cl^- , SO_4^{2-} or NO_3^- .

probe may be used to detect HOCl directly in the presence of other ROS and common ions.

2.5 Study of the reaction mechanism

The reaction mechanism of the present system was studied. Cleavage of the linker in the probe, and the subsequent separation of the DNS and RBPH fluorophores may be responsible for the fluorescence response. To prove this, the reaction products of DNS-RBPH with HOCl were analyzed by HPLC. As shown in Figure 4, the probe DNS-RBPH gave a chromatographic peak at 16.67 min (peak C in curve b). After reaction with HOCl (curve a), the DNS-RBPH peak decreased markedly, and two new chromatographic peaks emerged at 3.75 min (peak A) and 6.69 min (peak B). These peaks represented the formation of two major products, whose absorption spectra were recorded with the diode array detector. The product for peak A exhibited a characteristic absorption of the DNS fluorophore at 320 nm, and the absorption (561 nm) of the product for peak B was characteristic

Figure 4 HPLC chromatograms of different reaction products in 50 mmol/L phosphate buffer (pH 7.4). Curve a, 50 $\mu\text{mol/L}$ DNS-RBPH plus 100 $\mu\text{mol/L}$ HOCl. Curve b, 50 $\mu\text{mol/L}$ DNS-RBPH. Curve c, 100 $\mu\text{mol/L}$ HOCl. The peaks can be assigned as follows: A, 3.75 min, dansyl acid; B, 6.69 min, rhodamine B piperazinoacetic acid; C, 16.67 min, DNS-RBPH.

of the RBPH fluorophore (Figure 5). This indicates that the two products contain the DNS and RBPH skeletons, respectively. The two products were then obtained separately by collecting the corresponding chromatographic peaks, and subjected to ESI mass spectral analysis. The results indicated that dansyl acid (m/z 250.1 $[\text{M-H}]^-$) was present in the peak A fraction and rhodamine B piperazinoacetic acid (m/z 569.7 $[\text{M}]^+$) was present in the peak B fraction. Based on the above findings and the known HOCl-induced oxidation of dibenzoylhydrazine [24], the reaction of this system is proposed to proceed mainly through the route depicted in Scheme 2, which releases the separated DNS and rhodamine fluorophores.

2.6 Fluorescence imaging of HOCl in HeLa cells

Because DNS-RBPH has good water solubility it may be applied in aqueous biosystems. This was investigated by taking fluorescence images of HOCl with HeLa cells as the model biosystem. Because HeLa cells cannot produce a substantial amount of endogenous HOCl, they were exposed to HOCl before imaging. As shown in Figure 6, intact HeLa cells gave negligible autofluorescence under the imaging conditions, and DNS-RBPH loaded cells displayed only a relatively weak red-orange fluorescence. After incubation with NaOCl (5 $\mu\text{mol/L}$) for 15 or 30 min, the fluorescence intensity of the DNS-RBPH loaded cells increased and their color changed from red-orange to yellow-orange as the incubation time increased (Figure 6(c) and (d)). These results illustrate that DNS-RBPH can readily permeate the cell membrane and can be used to image the presence of HOCl in living cells.

Figure 5 Absorption spectra obtained by a diode array detector in a Shimadzu HPLC system. (a) Absorption spectrum of the product for peak A in curve a of Figure 4. (b) Absorption spectrum of the product for peak B in curve a of Figure 4.

Scheme 2 Possible reaction mechanism of DNS-RBPH with HOCl.

Figure 6 Fluorescence imaging of HOCl in HeLa cells. (a) Intact HeLa cells. (b) HeLa cells treated with 10 $\mu\text{mol/L}$ DNS-RBPH. The DNS-RBPH-loaded HeLa cells were then incubated with 5 $\mu\text{mol/L}$ NaOCl for 15 min (c) and 30 min (d).

Table 1 Comparison of fluorescent probes for HOCl

Probes	Linear range ($\mu\text{mol/L}$)	Detection limit	Detection media	Application	Reference
HKOCI-1	3–8	Not available	Phosphate buffer-DMF (0.5%), pH 7.5	Cell imaging	[3]
(9-Anthryl)ethenyl-ferrocene	10–100	300 nmol/L	Phosphate buffer-THF (50%), pH 7.4	Cell imaging	[12]
N-Benzoyl rhodamine B-hydrazine	1–10	27 nmol/L	$\text{Na}_2\text{B}_4\text{O}_7/\text{NaOH}$ buffer-THF (30%), pH 12	–	[13]
Rhodamine 6G hydroxylamide	Not available	25 nmol/L	PBS buffer-DMF (0.1%), pH 7.4	Cell imaging; bio-imaging in zebrafish	[14]
4-(Diphenylamino)-benzaldehyde	100–180	Not available	Phosphate buffer-DMF (80%), pH 9.0	–	[16]
DNS-RBPH	2–10	80 nmol/L	Phosphate buffer, pH 7.4	Cell imaging	This work

2.7 Comparison with other methods

Table 1 summarizes the parameters for different fluorescent probes for HOCl. Compared with the other probes, DNS-RBPH displays excellent water solubility, which may make it more suitable for biological studies.

3 Conclusions

In summary, we have developed a water-soluble FRET probe, DNS-RBPH, which exhibits high selectivity for HOCl over other ROS and common ions. The probe can be used in pure aqueous media, and is suited for the selective detection of HOCl in biosystems. The probe was successfully applied to fluorescence imaging of HOCl in HeLa cells. This combination of a cleavable bond and intramolecular FRET process may provide a useful strategy for the design of fluorescent probes for other species.

This work was supported by the National Natural Science Foundation of China (20935005, 90813032, and 20875092), National Basic Research Program of China (2010CB933502, 2011CB935800), and the Chinese Academy of Sciences (KJCX2-EW-N06-01).

- Yan Y, Wang S H, Liu Z W, et al. CdSe-ZnS quantum dots for selective and sensitive detection and quantification of hypochlorite. *Anal Chem*, 2010, 82: 9775–9781
- March J G, Simonet B M. A green method for the determination of hypochlorite in bleaching products based on its native absorbance. *Talanta*, 2007, 73: 232–236
- Sun Z N, Liu F Q, Chen Y, et al. A highly specific BODIPY-based fluorescent probe for the detection of hypochlorous acid. *Org Lett*, 2008, 10: 2171–2174

- Gomes A, Fernandes E, Lima J. Fluorescence probes used for detection of reactive oxygen species. *J Biochem Biophys Methods*, 2005, 65: 45–80
- Fink M P. Role of reactive oxygen and nitrogen species in acute respiratory distress syndrome. *Curr Opin Crit Care*, 2002, 8: 6–11
- Li X H, Zhang G X, Ma H M, et al. 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: 11543–11548
- Pullar J M, Vissers M C M, Winterbourn C C. Living with a killer: The effects of hypochlorous acid on mammalian cells. *IUBMB Life*, 2000, 50: 259–266
- Yap Y W, Whiteman M, Cheung N S. Chlorinative stress: An under appreciated mediator of neurodegeneration? *Cell Signal*, 2007, 19: 219–228
- Sugiyama S, Okada Y, Sukhova G K, et al. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. *Am J Pathol*, 2001, 158: 879–891
- Steinbeck M J, Nesti L J, Sharkey P F, et al. Myeloperoxidase and chlorinated peptides in osteoarthritis: Potential biomarkers of the disease. *J Orthop Res*, 2007, 25: 1128–1135
- Pattison D I, Davies M J. Evidence for rapid inter- and intramolecular chlorine transfer reactions of histamine and carnosine chloramines: Implications for the prevention of hypochlorous-acid-mediated damage. *Biochemistry*, 2006, 45: 8152–8162
- Chen S M, Lu J X, Sun C D, et al. A highly specific ferrocene-based fluorescent probe for hypochlorous acid and its application to cell imaging. *Analyst*, 2010, 135: 577–582
- Chen X Q, Wang X C, Wang S J, et al. A highly selective and sensitive fluorescence probe for the hypochlorite anion. *Chem Eur J*, 2008, 14: 4719–4724
- Yang Y K, Cho H J, Le J, et al. A rhodamine-hydroxamic acid-based fluorescent probe for hypochlorous acid and its applications to biological imagings. *Org Lett*, 2009, 11: 859–861
- Lin W Y, Long L L, Chen B B, et al. Ratiometric fluorescent probe for hypochlorite based on a deoxygenation reaction. *Chem Eur J*, 2009, 15: 2305–2309
- Shi J, Li Q Q, Zhang X, et al. Simple triphenylamine-based luminophore as a hypochlorite chemosensor. *Sens Actuators B*, 2010, 145: 583–587
- Kenmoku S, Urano Y, Kojima H, et al. Development of a highly spe-

- cific rhodamine-based fluorescence probe for hypochlorous acid and its application to real-time imaging of phagocytosis. *J Am Chem Soc*, 2007, 129: 7313–7318
- 18 Shepherd J, Hilderbrand S A, Waterman P, et al. A fluorescent probe for the detection of myeloperoxidase activity in atherosclerosis-associated macrophages. *Chem Biol*, 2007, 14: 1221–1231
- 19 Panizzi P, Nahrendorf M, Wildgruber M, et al. Oxazine conjugated nanoparticle detects *in vivo* hypochlorous acid and peroxynitrite generation. *J Am Chem Soc*, 2009, 131: 15739–15744
- 20 Cui K, Zhang D Q, Zhang G X, et al. A highly selective naked-eye probe for hypochlorite with the *p*-methoxyphenol-substituted aniline compound. *Tetrahedron Lett*, 2010, 51: 6052–6055
- 21 Chen X Q, Sun M, Ma H M. Progress in spectroscopic probes with cleavable active bonds. *Curr Org Chem*, 2006, 10: 477–489
- 22 Jia J, Wang K, Shi W, et al. Rhodamine B piperazinoacetohydrazine: A water-soluble spectroscopic reagent for pyruvic acid labeling. *Chem Eur J*, 2010, 16: 6638–6643
- 23 Jia J, Chen W, Ma H M, et al. Use of a rhodamine-based bifunctional probe in N-terminal specific labeling of *Thermomyces lanuginosus* xylanase. *Mol Biosyst*, 2010, 6: 1829–1833
- 24 Leffler J E, Bond W B. The influence of polar substituents and polar solvents on radical decomposition reactions. *J Am Chem Soc*, 1956, 78: 335–341
- 25 Kanofsky J R. Singlet oxygen production from the reactions of alkylperoxy radicals—evidence from 1268-nm chemiluminescence. *J Org Chem*, 1986, 51: 3386–3388
- 26 Fery-Forgues S, Lavabre D. Are fluorescence quantum yields so tricky to measure? A demonstration using familiar stationary products. *J Chem Educ*, 1999, 76: 1260–1264
- 27 Zhao H T, Kalivendi S, Zhang H, et al. Superoxide reacts with hydroethidine but forms a fluorescent product that is distinctly different from ethidium: Potential implications in intracellular fluorescence detection of superoxide. *Free Radic Biol Med*, 2003, 34: 1359–1368
- 28 Setsukinai K, Urano Y, Kakinuma K, et al. Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. *J Biol Chem*, 2003, 278: 3170–3175
- 29 Aubry J M, Cazin B, Duprat F. Chemical sources of singlet of oxygen. Peroxidation of water-soluble singlet oxygen carriers with the hydrogen-peroxide molybdate system. *J Org Chem*, 1989, 54: 726–728
- 30 Kikuchi K, Takakusa H, Nagano T. Recent advances in the design of small molecule-based FRET sensors for cell biology. *Trend Anal Chem*, 2004, 23: 407–415
- 31 Lee M H, Kim H J, Yoon S, et al. Metal ion induced FRET OFF-ON in tren/dansyl-appended rhodamine. *Org Lett*, 2008, 10: 213–216

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.