

Cite this: *Chem. Commun.*, 2012, **48**, 3091–3093

www.rsc.org/chemcomm

COMMUNICATION

A reversible near-infrared fluorescence probe for reactive oxygen species based on Te–rhodamine†

Yuichiro Koide,^{‡a} Mitsuyasu Kawaguchi,^{‡a} Yasuteru Urano,^b Kenjiro Hanaoka,^a Toru Komatsu,^a Masahiro Abo,^a Takuya Teraï^a and Tetsuo Nagano^{*a}

Received 22nd December 2011, Accepted 6th February 2012

DOI: 10.1039/c2cc18011a

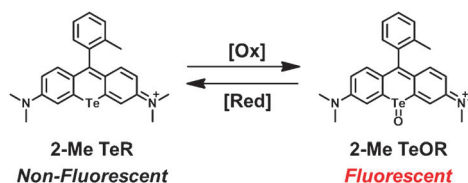
We have designed and synthesized a reversible near-infrared (NIR) fluorescence probe, 2-Me TeR, for reactive oxygen species (ROS), utilizing the redox properties of the tellurium (Te) atom. 2-Me TeR is oxidized to fluorescent 2-Me TeOR by various ROS, while the generated 2-Me TeOR is quickly reduced in the presence of glutathione to regenerate 2-Me TeR. This redox-induced reversible NIR-fluorescence response of 2-Me TeR allowed us to detect the endogenous production of ROS and subsequent homeostatic recovery of the intracellular reductive environment in hydrogen peroxide-stimulated HL-60 cells. This probe is expected to be useful for monitoring the dynamics of ROS production continuously *in vivo*.

Living cells are exposed to various stresses, such as reactive oxygen species (ROS), UV light, and metal ions.¹ Among them, ROS especially play important roles in a variety of biological and pathological systems.² Overproduction of ROS is associated with many diseases, including acute and chronic inflammation, cancer, cardiovascular disease, and neuro-degenerative disorders.³ On the other hand, ROS have key roles as signal transducing molecules.⁴ Therefore, visualizing ROS or oxidative environments would be a powerful approach for elucidating the biological functions of ROS and for diagnosis of related diseases. Generally, living cells are protected from oxidative stresses by intracellular redox systems, such as glutathione (GSH) and thioredoxin.⁵ An understanding of the mechanisms that maintain intracellular redox homeostasis would be helpful in developing novel therapeutics for various ROS-related diseases. Although reported fluorescence ROS probes are very useful for sensing ROS, the generated fluorescent products are generally irreversibly fluorescent,⁶ *i.e.*, the probes are unsuitable for continuous monitoring of real-time ROS dynamics. For this purpose, we require reversible ROS probes, which detect ROS (oxidative environment) and also GSH

(reductive environment). Development of such reversible fluorescence probes for ROS is currently one of the most challenging topics in the field.

On the other hand, Cy-PSe and BzSe-Cy, both of which contain selenium atoms as a redox-sensitive, fluorescence off/on switch moiety, have been reported as cyanine-based reversible fluorescence probes for peroxynitrite.⁷ We considered that compounds with various oxidation states, such as those containing chalcogen atoms, sulfur (S) and selenium (Se), might also be applicable to the design of novel reversible ROS probes.⁸ In particular, we focused on organic tellurium (Te)-containing compounds; we expected that these would be more sensitive to redox reactions than the Se-containing ones, because they exhibit both a high HOMO energy level and a low LUMO energy level.⁹ For example, Oba *et al.* reported that diaryl telluride was converted to diaryl telluroxide by singlet oxygen (¹O₂).^{9,10} Therefore, we considered that the Te atom would be suitable as the functional core of reversible ROS probes.

We synthesized rhodamine-based 2-Me TeR as a candidate reversible ROS probe. It is known that the O atom at the 10 position of the xanthene moiety of typical rhodamines can be replaced by a Te atom to afford tellurium rhodamines (TeRs).¹¹ 2-Me TeOR was also synthesized by the oxidation of 2-Me TeR with H₂O₂ (Scheme S1 in the ESI†). 2-Me TeOR was relatively stable, so we could characterize its structure by means of NMR and MS. Our strategy to reversibly detect ROS with 2-Me TeR is illustrated in Scheme 1. As shown in Fig. 1, 2-Me TeR exhibited a relatively long λ_{abs} (600 nm) and showed no fluorescence ($\Phi_{\text{f}} < 0.001$), due to the heavy-atom effect.¹² In contrast, 2-Me TeOR, the oxidized form of 2-Me TeR, exhibited a large red shift ($\lambda_{\text{abs}} = 669$ nm) compared to 2-Me TeR and showed strong fluorescence ($\Phi_{\text{f}} = 0.18$). This result indicates that the heavy-atom effect in 2-Me TeOR owing to the Te atom was weakened by the binding of an oxygen atom. This in turn means that we can detect the oxidative environment



Scheme 1 Principle of our reversible fluorescence probe for ROS.

^a Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku Tokyo 113-0033, Japan.
E-mail: tlong@mol.f.u-tokyo.ac.jp; Fax: +81 3 5841 4855;
Tel: +81 3 5841 4850

^b Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku Tokyo 113-0033, Japan

† Electronic supplementary information (ESI) available: Detailed descriptions of materials and general methods, and synthetic procedures. See DOI: 10.1039/c2cc18011a

‡ These authors contributed equally to this work.

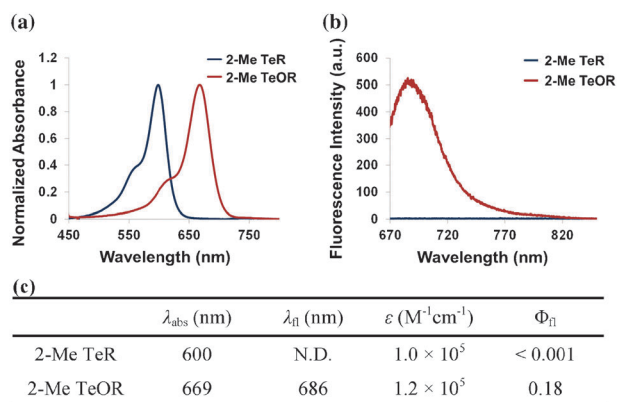


Fig. 1 Photophysical properties of 2-Me TeR and 2-Me TeOR. (a) Absorption and (b) emission spectra of 1 μM 2-Me TeR containing 3 μM GSH (reduced form) and 1 μM 2-Me TeOR in PBS at pH 7.4. Excitation wavelength for fluorescence spectra was 660 nm. (c) Photophysical properties of 2-Me TeR and 2-Me TeOR, measured in PBS at pH 7.4. For the determination of fluorescence quantum yields, Cy5.5 in PBS ($\Phi_{\text{fl}} = 0.23$) was used as a fluorescence standard. N.D. = not determinable.

as a large fluorescence increment (~ 200 -fold), together with a large red shift of the absorption spectrum of the probe.¹³ We also evaluated the effect of pH on the fluorescence. As shown in Fig. S1 and S2 in the ESI[†], the pH hardly influenced the fluorescence properties of 2-Me TeR and 2-Me TeOR under physiological conditions (pH 5.0 to 8.0).

Next, we examined the reactivity of 2-Me TeR with various ROS, as shown in Fig. 2. Upon addition of hydroxyl radical ($\cdot\text{OH}$), peroxynitrite (ONOO^-), or hypochlorite (OCl^-) in phosphate-buffered saline (PBS, pH 7.4), 2-Me TeR showed a large fluorescence increment and we confirmed that the resulting product was 2-Me TeOR (see Fig. S7 in the ESI[†]). On the other hand, in the presence of superoxide ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), or nitric oxide ($\cdot\text{NO}$), 2-Me TeR showed a weak fluorescence increment. We also examined whether or not 2-Me TeOR could be reduced to 2-Me TeR by an excess amount of GSH and found that 2-Me TeOR was quickly converted to

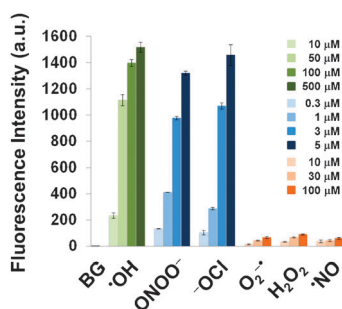


Fig. 2 Fluorescence intensity of 5 μM 2-Me TeR after reaction with various ROS in PBS (pH 7.4), measured at 690 nm with excitation at 660 nm. $\cdot\text{OH}$: ferrous perchlorate (10, 50, 100, and 500 μM) and H_2O_2 (500 μM) were added at rt. ONOO^- : ONOO^- (final 0.3, 1, 3, and 5 μM) was added and the mixture was stirred at 37 $^\circ\text{C}$. OCl^- : NaOCl (final 0.3, 1, 3, and 5 μM) was added and the mixture was stirred at 37 $^\circ\text{C}$. $\text{O}_2^{\cdot-}$: KO_2 (10, 30, and 100 μM) was added and the mixture was stirred at 37 $^\circ\text{C}$ for 30 min. H_2O_2 : H_2O_2 (10, 30, and 100 μM) was added and the mixture was stirred at 37 $^\circ\text{C}$ for 30 min. $\cdot\text{NO}$: NOC7 (10, 30, and 100 μM) was added and the mixture was stirred at 37 $^\circ\text{C}$ for 30 min. The results are mean \pm SD ($n = 3$). BG = background. For details, see Fig. S3–S8 in the ESI[†].

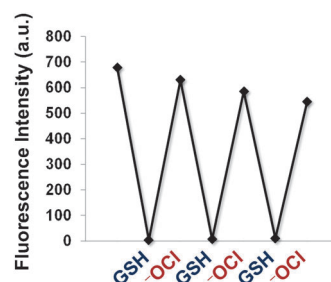


Fig. 3 Reversibility of the reactions of the probe with OCl^- and GSH. Reactions were performed with 5 μM 2-Me TeOR in PBS (pH 7.4) at 37 $^\circ\text{C}$. GSH (final 3 μM each) and OCl^- (final 10 μM each) were successively added to the reaction mixture three times each. The shown fluorescence intensities are averages after each addition. The fluorescence intensity was measured at 690 nm with excitation at 660 nm.

2-Me TeR by GSH (see Fig. S9 and S10 in the ESI[†]). These results suggest that 2-Me TeR can work as a reversible ROS probe with high sensitivity ($\text{FI}_{\text{HROS}}/\text{FI}_{\text{background}} = \sim 200$ -fold).

We further examined the reversibility of the reaction of 2-Me TeOR with GSH. As shown in Fig. 3, 2-Me TeOR was reduced upon addition of GSH and exhibited almost no fluorescence. The generated 2-Me TeR showed reactivity with subsequently added OCl^- and exhibited a large fluorescence increment, with recovery of the fluorescence almost to the original level. Furthermore, the generated 2-Me TeOR in turn showed the same reactivity with GSH as before. This cycle could be repeated at least three times with only a modest fluorescence decrement (20% of 2-Me TeOR was bleached during three cycles). These results suggest that 2-Me TeR could be used to detect ROS continuously *in vitro*.

Finally, we applied 2-Me TeR to live HL-60 cells, which are known to produce large amounts of ROS, such as OCl^- , upon H_2O_2 stimulation (Fig. 4).^{6c,14} Although 2-Me TeR existed as the reduced form in HL-60 cell suspension, a fluorescence increment was observed following stimulation of the cells with 100 μM H_2O_2 . This fluorescence increment was short-lived, because the added H_2O_2 was consumed by myeloperoxidase (MPO) and/or quenched by intracellular reductants. This result indicates that 2-Me TeR was oxidized in the oxidative environment after addition of H_2O_2 , then reduced as the

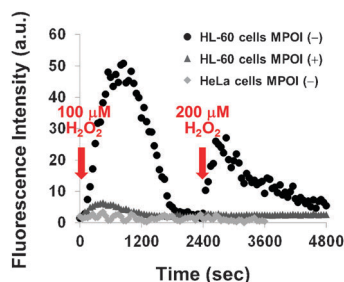


Fig. 4 Time courses of the change in fluorescence intensity observed with 5 μM 2-Me TeR for H_2O_2 -stimulated or unstimulated HL-60 cells (2.5×10^5 cells mL^{-1}), or H_2O_2 -stimulated HeLa cells (2.5×10^5 cells mL^{-1}) in HBSS at 37 $^\circ\text{C}$. H_2O_2 (100 μM and 200 μM final concentration) was added at 100 s and 2400 s, respectively. MPOI is aminobenzoic acid hydrazide (ABAH; 2 mM), added as a myeloperoxidase inhibitor. The fluorescence intensity was measured at 690 nm with excitation at 660 nm.

intracellular reductive environment was homeostatically restored. After the fluorescence intensity had returned to the original level, a second cycle of fluorescence increment and decrement was observed upon further addition of 200 μM H_2O_2 . This result demonstrates that 2-Me TeR was not bleached by ROS during the first redox cycle. Both fluorescence increments were suppressed in the presence of a myeloperoxidase inhibitor, aminobenzoic acid hydrazide (ABAH).¹⁵ Also, no fluorescence increment was observed in the case of H_2O_2 -stimulated HeLa cells; this is as expected, because HeLa cells do not produce ROS upon H_2O_2 stimulation.^{6e} In contrast, APF, which is a previously reported fluorescence probe for highly reactive oxygen species (hROS), produced only a monotonic fluorescence increment in the H_2O_2 -stimulated HL-60 cell assay (see Fig. S11 in the ESI†).^{6d} Taken together, these results indicate that 2-Me TeR allows the reversible detection of endogenously produced ROS in HL-60 cells with high sensitivity and reversibility.

In conclusion, we have developed 2-Me TeR as a reversible NIR fluorescence probe for ROS, based on the redox properties of the Te atom. 2-Me TeR is oxidized by various ROS and quickly converted to fluorescent 2-Me TeOR. In turn, 2-Me TeOR is reduced by GSH, quickly regenerating 2-Me TeR. This redox cycle between 2-Me TeR and 2-Me TeOR can be run repeatedly. This system was confirmed to work in living cells. Interestingly, the reactivity of this Te-rhodamine-based reversible ROS probe toward hROS is different from that of Se-cyanines, which exhibit selective reactivity toward ONOO^- . Furthermore, the photochemical properties of 2-Me TeOR, whose absorption and emission maxima are in the NIR region of 650–900 nm, are favorable, because light in this region shows high tissue penetration.¹⁶ We believe that the redox character and reversible NIR-fluorescence response of 2-Me TeR mean that this probe will be useful for monitoring the dynamics of ROS production continuously *in vivo*.

This research was supported in part by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant No. Specially Promoted Research 22000006 to T.N.), and by a grant from the Industrial Technology Development Organization (NEDO) of Japan (to T.T.). K.H. was supported by Inoue Foundation for Science, Konica Minolta Science and Technology Foundation and The Asahi Glass Foundation. Y.K. and M.K. were supported by a Grant-in-Aid for JSPS Fellows.

Notes and references

- 1 V. Jamier, L. A. Ba and C. Jacob, *Chem.–Eur. J.*, 2000, **16**, 10920.
- 2 (a) S. J. Stohs and D. Bagchi, *Free Radical Biol. Med.*, 1995, **18**, 321; (b) M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur and J. Telser, *Int. J. Biochem. Cell Biol.*, 2007, **39**, 44.
- 3 (a) B. D'Autr aux and M. B. Toledano, *Nat. Rev. Mol. Cell Biol.*, 2007, **8**, 813; (b) H. Wiseman and B. Halliwell, *Biochem. J.*, 1996, **313**, 17; (c) J. M. McCord, *Science*, 1974, **185**, 529; (d) J. Berliner, *Vasc. Pharmacol.*, 2002, **38**, 187; (e) I. Dalle-Donne, R. Rossi, R. Colombo, D. Giustarini and A. Milzani, *Clin. Chem.*, 2006, **52**, 601.
- 4 (a) T. Finkel and N. Holbrook, *Nature*, 2000, **408**, 239; (b) T. Matoba, H. Shimokawa, H. Kubota, K. Morikawa, K. Fujiki, I. Kunihiro, Y. Mukai, Y. Hirakawa and A. Takeshita, *Biochem. Biophys. Res. Commun.*, 2002, **290**, 909.
- 5 (a) C. M. Grant, *Mol. Microbiol.*, 2001, **39**, 533; (b) J. F. Curtin, M. Donovan and T. G. Gotter, *J. Immunol. Methods*, 2002, **265**, 49.
- 6 (a) H. Kojima, N. Nakatsubo, K. Kikuchi, S. Kawahara, Y. Kirino, H. Nagoshi, Y. Hirata and T. Nagano, *Anal. Chem.*, 1998, **70**, 2446; (b) S. Kenmoku, Y. Urano, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2007, **129**, 7313; (c) T. Ueno, Y. Urano, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2006, **128**, 10640; (d) K. Setsukinai, Y. Urano, K. Kakinuma, H. J. Majima and T. Nagano, *J. Biol. Chem.*, 2003, **278**, 3170; (e) Y. Koide, Y. Urano, S. Kenmoku, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2007, **129**, 10324; (f) H. Maeda, Y. Fukuyasu, S. Yoshida, M. Fukuda, K. Saeki, H. Matsuno, Y. Yamauchi, K. Yoshida, K. Hirata and K. Miyamoto, *Angew. Chem., Int. Ed.*, 2004, **43**, 2389; (g) E. Sasaki, H. Kojima, H. Nishimatsu, Y. Urano, K. Kikuchi, Y. Hirata and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 3684; (h) D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2010, **132**, 2795; (i) M. Abo, Y. Urano, K. Hanaoka, T. Terai, T. Komatsu and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 10629.
- 7 (a) Y. Yamada, Y. Tomiyama, A. Morita, M. Ikekita and S. Aoki, *ChemBioChem*, 2008, **9**, 853; (b) K. Lee, V. Dzubeck, L. Latshaw and J. P. Schneider, *J. Am. Chem. Soc.*, 2004, **126**, 13616; (c) F. Yu, P. Li, G. Zhao, T. Chu and K. Han, *J. Am. Chem. Soc.*, 2011, **133**, 11030; (d) K. Xu, H. Chen, J. Tian, B. Ding, Y. Xie, M. Qiang and B. Tang, *Chem. Commun.*, 2011, **47**, 9468.
- 8 (a) J. L. Dutton and P. J. Ragogna, *Chem.–Eur. J.*, 2010, **16**, 12454; (b) L. A. Ba, M. D ring, V. Jamier and C. Jacob, *Org. Biomol. Chem.*, 2010, **8**, 4203.
- 9 M. Oba, Y. Okada, M. Endo, K. Tanaka, K. Nishiyama, S. Shimada and W. Ando, *Inorg. Chem.*, 2010, **49**, 10680.
- 10 P. Sergueievski and M. R. Detty, *Organometallics*, 1997, **16**, 4386.
- 11 (a) D. J. Del Valle, D. J. Donnelly, J. J. Holt and M. R. Detty, *Organometallics*, 2005, **24**, 3807; (b) B. Calitree, D. J. Donnelly, J. J. Holt, M. K. Gannon, C. L. Nygren, D. K. Sukumaran, J. Autschbach and M. R. Detty, *Organometallics*, 2007, **26**, 6248.
- 12 (a) M. J. Kasha, *Chem. Phys.*, 1952, **20**, 71; (b) S. P. McGlynn, R. Sunseri and N. J. Christodouleas, *Chem. Phys.*, 1962, **37**, 1818; (c) J. Nag-Chaudhuri, L. Stoessell and S. P. McGlynn, *J. Chem. Phys.*, 1962, **38**, 2027; (d) S. P. McGlynn, J. Daigre and F. J. Smith, *J. Chem. Phys.*, 1963, **39**, 675; (e) S. P. McGlynn, T. Azumi and M. Kasha, *J. Chem. Phys.*, 1964, **40**, 507.
- 13 T. Egawa, Y. Koide, K. Hanaoka, T. Komatsu, T. Terai and T. Nagano, *Chem. Commun.*, 2011, **47**, 4162.
- 14 S. Izumi, Y. Urano, K. Hanaoka, T. Terai and T. Nagano, *J. Am. Chem. Soc.*, 2009, **131**, 10189.
- 15 A. J. Kettle, C. A. Gedy and C. C. Winterbourn, *Biochem. J.*, 1997, **321**, 503.
- 16 (a) R. Weissleder and V. Ntziachristos, *Nat. Med.*, 2003, **9**, 123; (b) R. Weissleder, *Nat. Biotechnol.*, 2001, **19**, 316.