

Imaging Different Interactions of Mercury and Silver with Live Cells by a Designed Fluorescence Probe Rhodamine B Selenolactone

Wen Shi, Shuna Sun, Xiaohua Li, and Huimin Ma*

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

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Rhodamine B selenolactone has been designed, synthesized, and characterized as a new fluorescent probe for imaging both Hg^{2+} and Ag^+ in live cells to better understand their distinct toxicities to organisms. The probe is designed based on the fact that selenium has a strong affinity for mercury and silver, and is constructed by incorporating a Se atom into the spirocyclic structure of rhodamine. It exhibits a rapid and specific spectroscopic off-on response to Hg^{2+} and Ag^+ instead of other species, with detection limits of 23 nM Hg^{2+} and 52 nM Ag^+ . Moreover, the probe is membrane-permeable, and can react with Ag^+ even in the presence of Cl^- because of the higher affinity of Se than Cl^- for Ag^+ , which makes it of potential use for imaging not only Hg^{2+} but also Ag^+ in live cells. This applicability has been demonstrated by imaging Hg^{2+} and Ag^+ in Hela cells. It is observed that the reaction of Ag^+ with the probe inside the cells occurs much slower than that of Hg^{2+} , which is ascribed to the high concentration of cellular chloride ions inhibiting the formation of sufficient free Ag^+ . The present finding is helpful to get an insight into the different interaction mechanism of Hg^{2+} and Ag^+ with cells, and more applications of the probe may be expected for studying the behaviors of Hg^{2+} and Ag^+ in various biosystems.

Introduction

Mercury has long been known to be a highly toxic metal, which may cause prenatal brain damage, cognitive and motion disorders, vision and hearing loss, and even death;¹ whereas silver (a similar *ds*-block element) hardly exhibits toxicity to humans, and has been widely used as a broad-spectrum antimicrobial agent.² To better understand their distinct toxicities to organisms, it may be useful to develop a

fluorescent probe capable of imaging both Hg^{2+} and Ag^+ in live cells, so as to eliminate the effect of different probes. So far, many excellent fluorescent probes have been proposed for the detection of Hg^{2+} or Ag^+ ,³ but very few are available for cell imaging.⁴ In particular, to the best of our knowledge, none of them is suited for imaging Ag^+ in live cells. The main difficulty of this is that high concentration (more than mM levels) of cellular chloride ions⁵ may lead to the precipitation of Ag^+ as AgCl , thereby hindering the reaction of Ag^+ with a fluorescent probe.

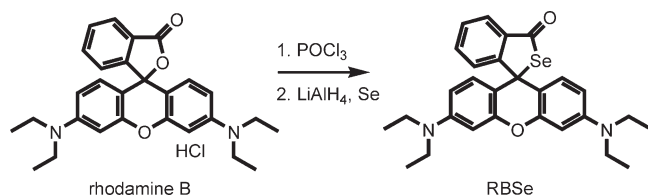
The combination of fluorochromes with cleavable active bonds has opened a novel alternate route to developing specific spectroscopic probes.⁶ Recently, rhodamine as an excellent fluorochrome has attracted considerable interest to prepare various spectroscopic off-on-type probes by virtue of its easy formation of a colorless and non-fluorescent

*To whom correspondence should be addressed. E-mail: mahm@iccas.ac.cn. Fax: +86-10-62559373.

(1) (a) Nolan, E. M.; Lippard, S. J. *Chem. Rev.* **2008**, *108*, 3443–3480. (b) Boening, D. W. *Chemosphere* **2000**, *40*, 1335–1351. (2) (a) Klasen, H. J. *Burns* **2000**, *26*, 117–130. (b) Berger, T. J.; Spadaro, J. A.; Chapin, S. E.; Becker, R. O. *Antimicrob. Agents Chemother.* **1976**, *9*, 357–358. (3) (a) Huang, W.; Song, C. X.; He, C.; Lv, G. J.; Hu, X. Y.; Zhu, X.; Duan, C. Y. *Inorg. Chem.* **2009**, *48*, 5061–5072. (b) Lyoshi, S.; Taki, M.; Yamamoto, Y. *Inorg. Chem.* **2008**, *47*, 3946–3948. (c) Lee, M. H.; Lee, S. W.; Kim, S. H.; Kang, C.; Kim, J. S. *Org. Lett.* **2009**, *11*, 2101–2104. (d) Choi, M. G.; Kim, Y. H.; Namgoong, J. E.; Chang, S. K. *Chem. Commun.* **2009**, 3560–3562. (e) Suresh, M.; Mishra, S.; Mishra, S. K.; Suresh, E.; Mandal, A. K.; Shrivastav, A.; Das, A. *Org. Lett.* **2009**, *11*, 2101–2104. (f) Li, K. H.; Xu, Z. T.; Fetting, J. C. *Inorg. Chem.* **2004**, *43*, 8018–8022. (g) Chatterjee, A.; Santra, M.; Won, N.; Kim, S.; Kim, J. K.; Kim, S. B.; Ahn, K. H. *J. Am. Chem. Soc.* **2009**, *131*, 2040–2041. (h) Coskun, A.; Akkaya, E. U. *J. Am. Chem. Soc.* **2005**, *127*, 10464–10466. (i) Swamy, K. M. K.; Kim, H. N.; Soh, J. H.; Kim, Y.; Kima, S. J.; Yoon, J. *Chem. Commun.* **2009**, 1234–1236. (j) Ho, M. L.; Chen, K. Y.; Lee, G. H.; Chen, Y. C.; Wang, C. C.; Lee, J. F.; Chung, W. C.; Chou, P. T. *Inorg. Chem.* **2009**, *48*, 10304–10311.

(4) (a) Ko, S. K.; Yang, Y. K.; Tae, J.; Shin, I. *J. Am. Chem. Soc.* **2006**, *128*, 14150–14155. (b) Suresh, M.; Shrivastav, A.; Mishra, S.; Suresh, E.; Das, A. *Org. Lett.* **2008**, *10*, 3013–3016. (c) Zhang, X.; Xiao, Y.; Qian, X. *Angew. Chem., Int. Ed.* **2008**, *47*, 8025–8029. (d) Yoon, S.; Miller, E. W.; He, Q.; Do, P. H.; Chang, C. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 6658–6661. (5) Incharoensakdi, A.; Takabe, T. *Plant Cell Physiol.* **1988**, *29*, 1073–1075. (6) (a) Richard, J. A.; Meyer, Y.; Jolivel, V.; Massonneau, M.; Dumeunier, R.; Vaudry, D.; Vaudry, H.; Renard, P. Y.; Romieu, A. *Bioconjugate Chem.* **2008**, *19*, 1707–1718. (b) Li, X. H.; Zhang, G. X.; Ma, H. M.; Zhang, D. Q.; Li, J.; Zhu, D. B. *J. Am. Chem. Soc.* **2004**, *126*, 11543–11548. (c) Chen, X. Q.; Sun, M.; Ma, H. M. *Curr. Org. Chem.* **2006**, *10*, 477–489.

Scheme 1. Synthesis of RBSe



spirocyclic structure.^{7,8} However, most of the rhodamine derivatives are spirolactams (constructed through a pH-sensitive N atom), which readily suffer from influence of acidity.^{7c} To overcome the problem, we previously synthesized rhodamine B thiolactone by replacing the N atom with a S atom (a weak H^+ -receptor), which can serve as a Hg^{2+} -selective fluorescent probe with higher tolerance against pH changes in aqueous media.^{8a} Nevertheless, the probe is unsuitable to be used for comparing the interacting behaviors of Hg^{2+} and Ag^+ with live cells by fluorescence imaging because of its insensitivity to Ag^+ .

On the basis of the fact that selenium is a weaker H^+ -receptor and has a strong affinity for both mercury and silver,⁹ herein we design rhodamine B selenolactone (RBSe; Scheme 1) as a Hg^{2+} - and Ag^+ -specific fluorescent probe by incorporating a Se atom into the spirocyclic structure of rhodamine. The probe itself is nearly non-fluorescent because of its selenospirocyclic character. Upon addition of various species, however, the probe produces a rapid and specific fluorescence-on reaction with Hg^{2+} or Ag^+ only. Most notably, the probe is capable of reacting with Ag^+ in the presence of a high concentration of Cl^- because Se has a stronger binding ability toward Ag^+ than does Cl^- .¹⁰ Furthermore, the probe has been successfully used to compare the imaging behaviors of Hg^{2+} and Ag^+ in HeLa cells to gain a better understanding of their different toxicities.

Experimental Section

Apparatus and Reagents. A Hitachi F-2500 spectrofluorimeter was used for fluorescence measurements. The absorption spectra were recorded with a TU-1900 spectrophotometer (Beijing Purkinje General Instrument Co. LTD). NMR spectra were measured on a Bruker DMX-300 spectrometer at 300 MHz in $CDCl_3$ with tetramethylsilane as the internal standard. Electrospray ionization (ESI) mass spectra were measured with an LC-MS 2010A (Shimadzu) instrument. Elemental analyses were carried out with a Flash EA 1112 instrument. A single crystal was characterized on an R-Axis Rapid IP (Rigaku). A Delta 320 pH-meter [Mettler-Toledo Instruments (Shanghai) Co.,

China] was used for pH measurements. Fluorescence imaging experiments were performed on a FV 1000-IX81 confocal laser scanning microscope (Olympus, Japan) with FV5-LAMAR for excitation at 515 nm and a variable bandpass emission filter set to 530–630 nm through a 40×0.9 NA objective. Optical sections were acquired at $0.8 \mu m$.

Rhodamine B, selenium powder and $AgNO_3$ were purchased from Beijing Chemical Company. Mercuric chloride was obtained from Tianjin Jingjin Chemical Reagent Plant. All other chemicals used were local products of analytical grade. Distilled–deionized water was used throughout. The stock solution (1.0 mM) of RBSe was prepared by dissolving the requisite amount of it in 1,4-dioxane. Stock solutions (1–100 mM) of other species were prepared by dissolving their compounds in water or acidic solutions.

Synthesis of RBSe. To a stirred solution of rhodamine B (239 mg, 0.5 mmol) in 1,2-dichloroethane (5 mL) was added phosphorus oxychloride (0.3 mL) dropwise. After refluxing for 4 h, the reaction solution was cooled and evaporated in vacuo to give rhodamine B acid chloride as violet-red oil, which was used directly in the next step. Separately, a suspension of selenium powder (79 mg, 1.0 mmol) in dry tetrahydrofuran (THF, 10 mL) was prepared, and then $LiAlH_4$ (38 mg, 1.0 mmol) was added at $0^\circ C$ under an argon atmosphere. The mixture was stirred at $0^\circ C$ under an argon atmosphere for 30 min, yielding a heterogeneous grayish suspension. A solution containing crude rhodamine B acid chloride in 6 mL of THF was added dropwise to the selenium powder suspension. After stirring overnight at room temperature, the solvent was removed under reduced pressure to give a violet-red oil. Then, 5 mL of water was added to the oil, and the formed precipitate was filtered. The precipitate was washed several times with water and dried in air to give a violet-red powder. The crude product was purified by silica-gel column chromatography with petroleum ether (60– $90^\circ C$)/ethyl acetate (25:1, v/v) as eluent, affording 101 mg of RBSe (yield 40%). Mp 173 – $174^\circ C$. 1H NMR (300 MHz, $CDCl_3$, 298 K) δ 7.85 (dd, $J = 7.5$ Hz, 0.7 Hz, 1H), 7.52–7.41 (m, 2H), 7.26 (d, $J = 7.5$ Hz, 1H), 6.70 (d, $J = 8.7$ Hz, 2H), 6.32–6.27 (m, 4H), 3.32 (q, $J = 6.9$ Hz, 8H), 1.16 (t, $J = 6.6$ Hz, 12H); ^{13}C NMR (300 MHz, $CDCl_3$, 297 K) δ 201.2, 158.6, 152.3, 148.3, 140.9, 133.9, 130.0, 128.4, 128.0, 122.5, 111.2, 108.3, 97.7, 62.6, 44.3, 12.6; ESI-MS m/z 507.2 $[M + H]^+$. Elemental analysis, calcd. for RBSe ($C_{28}H_{30}N_2O_2Se$), C 66.53, H 5.98, N 5.54%; found, C 66.14, H 5.98, N 5.71%. Crystal data for RBSe: orthorhombic, $a = 16.1745(4)$, $b = 12.1930(2)$, $c = 12.1745(3)$ Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, $V = 2401.00(9)$ Å³, $T = 173(2)$ K, space group $Pna2(1)$, $Z = 4$, $D_c = 1.398$ g cm⁻³, 5287 reflections measured, $R1 [I > \sigma(I)] = 0.0373$, $wR2$ (all data) = 0.0900, GOF = 0.513. CCDC no. 737187. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

General Procedure for Detection of Metal Ions. Unless otherwise noted, all the measurements were made according to the following procedure. In a 10 mL glass tube, 5 mL of 20 mM HEPES buffer (pH 7.2) and 50 μL of the stock solution of RBSe were mixed, followed by addition of an appropriate volume of Hg^{2+} or Ag^+ sample solution. The final volume was adjusted to 10 mL with the HEPES buffer, and the reaction solution was mixed well. After 5 min at room temperature, a 3 mL portion of the reaction solution was transferred to a quartz cell of 1 cm optical length to measure absorbance or fluorescence intensity/spectrum with $\lambda_{ex/em} = 520/580$ nm and both excitation and emission slit widths of 10 nm. In the meantime, a blank solution containing no Hg^{2+} or Ag^+ was prepared and measured under the same conditions for comparison.

General Procedure for Cell Imaging. The HeLa cells were grown on glass-bottom culture dishes (MatTek Co.) in Dulbecco's modified eagle media (DMEM) supplemented with 10% (v/v) fetal bovine serum, penicillin (100 $\mu g/mL$), and streptomycin

(7) (a) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386–7387. (b) Kim, H. N.; Lee, M. H.; Kim, H. J.; Kim, J. S.; Yoon, J. *Chem. Soc. Rev.* **2008**, *37*, 1465–1472, and references therein. (c) Beija, M.; Afonso, C. A. M.; Martinho, J. M. G. *Chem. Soc. Rev.* **2009**, *38*, 2410–2433, and references therein. (d) Zhao, Y.; Zhang, X. B.; Han, Z. X.; Qiao, L.; Li, C. Y.; Jian, L. X.; Shen, G. L.; Yu, R. Q. *Anal. Chem.* **2009**, *81*, 7022–7030.

(8) (a) Shi, W.; Ma, H. M. *Chem. Commun.* **2008**, 1856–1858. (b) Chen, X. Q.; Nam, S. W.; Jou, M. J.; Kim, Y.; Kim, S. J.; Park, S.; Yoon, J. *Org. Lett.* **2008**, *10*, 5235–5238. (c) Zhan, X. Q.; Qian, Z. H.; Zheng, H.; Su, B. Y.; Lan, Z.; Xu, J. G. *Chem. Commun.* **2008**, 1859–1861.

(9) (a) Yoneda, S. J.; Kazuo, T. S. *Toxicol. Appl. Pharmacol.* **1997**, *143*, 274–280. (b) Tran, D.; Moody, A. J.; Fisher, A. S.; Foulkes, M. E.; Jha, A. N. *Aquat. Toxicol.* **2007**, *84*, 11–18.

(10) (a) Mehra, M. C.; Gubeli, A. O. *Can. J. Chem.* **1970**, *48*, 3491–3497. (b) F. Séby, F.; Potin-Gautier, M.; Giffaut, E.; Borge, G.; Donard, O. F. X. *Chem. Geol.* **2001**, *171*, 173–194. (c) Cypionka, H. *Arch. Microbiol.* **1989**, *152*, 237–243. (d) Tsiouras, N.; Rix, C. J.; Brady, P. H. *Clin. Chem.* **1995**, *41*, 87–91.

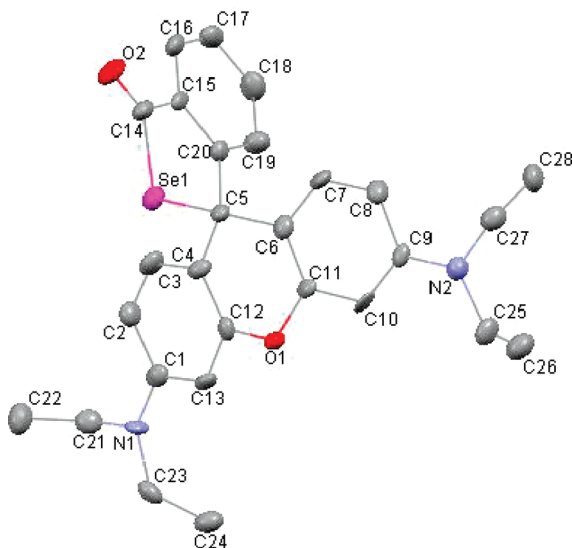


Figure 1. View of the structure of RBSe with displacement atomic ellipsoids drawn at the 20% probability level, excluding H atoms.

(100 $\mu\text{g/mL}$) at 37 $^{\circ}\text{C}$. Before use, the adherent cells were washed three times with phenol red-free DMEM. For mercury imaging, cells were first loaded with 10 μM RBSe in DMEM at 37 $^{\circ}\text{C}$ for 20 min, washed three times with phosphate buffered saline solution (pH 7.4) to remove the free RBSe, and then incubated in phosphate buffered saline media with 10 μM HgCl_2 for 10 or 20 min. For silver imaging, unless otherwise stated, a Cl^- -free buffer of 20 mM HEPES (pH 7.4) containing 0.14 M NaNO_3 was used to avoid the formation of insoluble AgCl precipitates. Hela cells were prepared in a similar manner as for mercury imaging, except that the Cl^- -free buffer was employed for the last cell washing and AgNO_3 loading.

Results and Discussion

Synthesis and Characterization of RBSe. The probe RBSe was prepared by treating rhodamine B with phosphorus oxychloride, and then letting the formed rhodamine B acid chloride react with selenium powder¹¹ in the presence of LiAlH_4 . The structure of RBSe was confirmed by ^1H NMR, ^{13}C NMR, MS, and X-ray analyses (Figures S1 and S2 in the Supporting Information). The single crystal of the probe was well grown as a yellow needle from the ethyl acetate/petroleum ether (1:25, v/v) solution, which has a unique selenospirocyclic structure (Figure 1).

Spectroscopic Properties of RBSe. A detailed investigation was conducted on spectroscopic properties of RBSe and their variations upon addition of different substances. Slightly different from rhodamine B thiolactone, RBSe has a very weak fluorescence ($\Phi \approx 0.01$)¹² with a molar absorptivity of about $1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ at 560 nm, which might result from the different property between Se and S. Various metal ions, oxidizing and reducing agents were examined under the same conditions to assess the specificity of the fluorescence-on reaction. As shown in Figure 2, RBSe reacts with only Hg^{2+} and Ag^+ instead of the other common species tested, accompanying a great

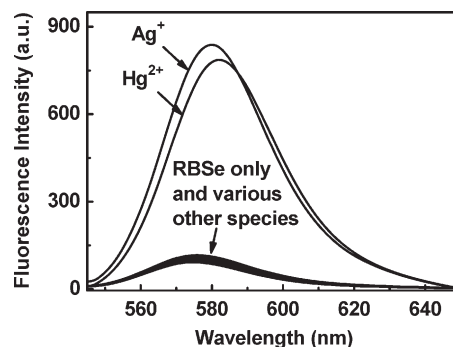


Figure 2. Fluorescence spectra ($\lambda_{\text{ex}} = 520 \text{ nm}$) of RBSe (5 μM) in 20 mM HEPES buffer (pH 7.2) containing 0.5% (v/v) 1,4-dioxane in the presence of various species (50 μM of Ag^+ , Al^{3+} , Cd^{2+} , Cu^{2+} , Fe^{3+} , Hg^{2+} , Pb^{2+} , Zn^{2+} , H_2O_2 , or ClO^- ; 0.2 M of NaCl ; 0.15 M of K^+ ; 2 mM of Ca^{2+} ; 1 mM of NO_3^- ; 50 mM of PO_4^{3-} ; 30 mM of CO_3^{2-} ; 10 mM of SO_4^{2-} ; 1.5 mM of Mg^{2+} or reduced glutathione).

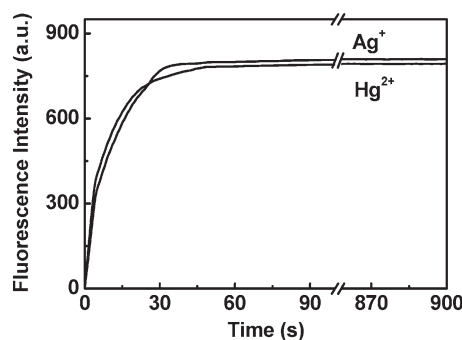


Figure 3. Change of fluorescence intensity at 580 nm of RBSe (5 μM) with time in the presence of 10 μM of Hg^{2+} or Ag^+ in 20 mM HEPES buffer (pH 7.2) at room temperature.

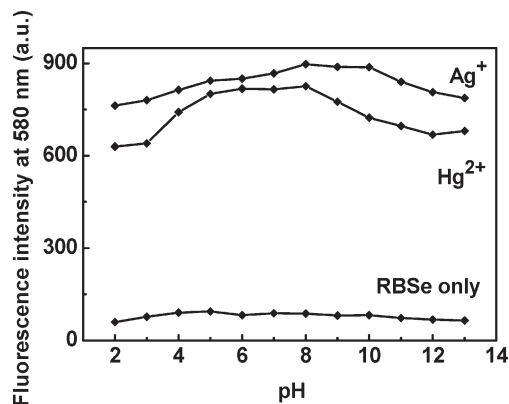


Figure 4. Effect of pH on fluorescence intensities ($\lambda_{\text{ex/em}} = 520/580 \text{ nm}$) of RBSe (5 μM) and its reaction solution with 50 μM of Hg^{2+} or Ag^+ . The lower fluorescence intensity in the case of Hg^{2+} may result from its stronger quenching effect.

fluorescence enhancement (~ 10 fold) at about 580 nm and the retrieval of magenta color characteristic of rhodamine B. Time course studies revealed that the reaction was fast and nearly complete in 30 s in both cases of 2 equiv. Hg^{2+} and Ag^+ (Figure 3); the resulting absorbance and fluorescence signals remained constant for at least 24 h. The pH of reaction media varying from 4 to 9 causes negligible changes in the fluorescence intensity, and the probe itself is rather stable over a wider range of pH (Figure 4). Then, we examined the sensitivity of

(11) Ishihara, H.; Koketsu, M.; Fukuta, Y.; Nada, F. *J. Am. Chem. Soc.* **2001**, *123*, 8408–8409.

(12) Fluorescence quantum yield Φ was determined according to the literature: Karstens, T.; Kobs, K. *J. Phys. Chem.* **1980**, *84*, 1871–1872.

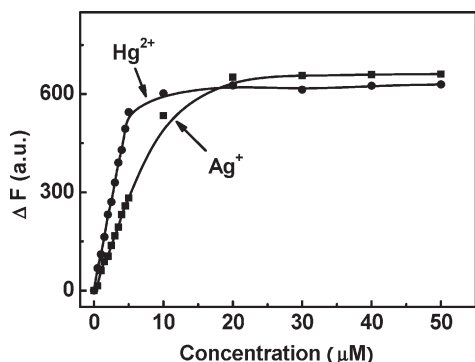
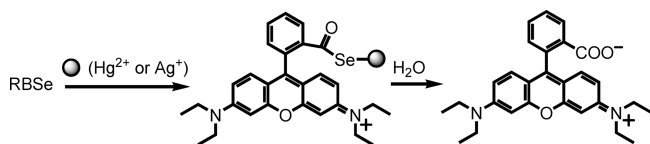


Figure 5. Change in fluorescence intensity (ΔF) of RBSe ($5 \mu\text{M}$) reacting with Ag^+ and Hg^{2+} for 5 min at varied concentrations. $\Delta F = F - F_0$, where F_0 and F are the fluorescence intensity at 580 nm before and after a species was added to the probe solution, respectively.

Scheme 2. Possible Reaction Mechanism of RBSe with Hg^{2+} or Ag^+



RBSe to Hg^{2+} and Ag^+ . The change in fluorescence intensity of RBSe ($5 \mu\text{M}$) was found to be directly proportional to the concentration of $0.1\text{--}5 \mu\text{M}$ Hg^{2+} and $0.1\text{--}10 \mu\text{M}$ Ag^+ , with detection limits of 23 nM Hg^{2+} and 52 nM Ag^+ ($S/N = 3$), respectively. Although the corresponding stability constants can not be obtained because of the irreversibility of the reactions (vide infra), the observed apparent stoichiometric ratios of RBSe to Hg^{2+} and Ag^+ were 1:1 and 1:2 (Figure 5), respectively, which are in agreement with those in their typical complexes HgSe and Ag_2Se .

Reaction Mechanism. To study the reaction mechanism of the present system, the reaction products of RBSe with Hg^{2+} and Ag^+ were subjected to electrospray ionization mass spectral analysis, confirming the generation of rhodamine B ($m/z = 443 [\text{M}+\text{H}]^+$) as a major final product (Figure S3 in the Supporting Information); the introduction of KI^8 into the system can not produce a colorless solution. This indicates that the reactions of RBSe with Hg^{2+} and Ag^+ are irreversible, and may proceed through the following way: the selenium atom of RBSe recognizes and binds Hg^{2+} and Ag^+ , and the subsequent complexation of Hg^{2+} or Ag^+ promotes hydrolytic cleavage of the selenolactone bond, causing the release of the rhodamine B and thereby the retrieval of the fluorescence (Scheme 2).

Reaction of RBSe with Ag^+ in the Presence of Cl^- . We investigated the reaction behavior of RBSe with Ag^+ in the environment of high Cl^- concentration. As can be seen from Figure 6, the spectrum of sample (b) is nearly identical to that of sample (c), which indicates that, in the presence of 0.15 M Cl^- , RBSe can still react with trace free Ag^+ dissociated from the AgCl precipitate, and the reaction is not affected by their adding order. The results from samples (d) and (e) show that, with the increase of reaction time, RBSe can gradually strip Ag^+ from AgCl , though the reaction rate is slower than

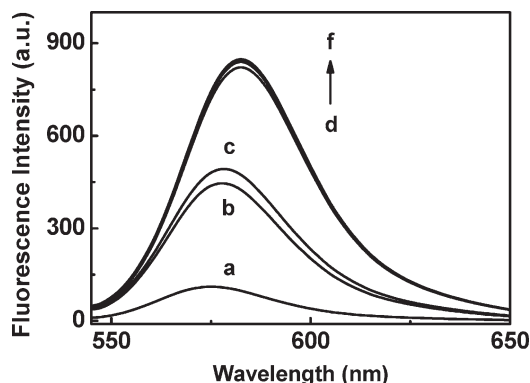


Figure 6. Fluorescence spectra of RBSe ($5 \mu\text{M}$) reacting with Ag^+ for 5 min in the environment of high Cl^- concentration. (a) RBSe in 0.15 M NaCl aqueous media without Ag^+ (control experiment). (b) RBSe was added to the mixture of 0.15 M NaCl and $10 \mu\text{M}$ AgNO_3 . (c) AgNO_3 (final concentration $10 \mu\text{M}$) was added to the mixture of 0.15 M NaCl and $5 \mu\text{M}$ RBSe. (d) The spectrum of the sample (b) was recorded after 4 h. (e) The spectrum of the sample (c) was recorded after 4 h. (f) RBSe in the presence of $10 \mu\text{M}$ AgNO_3 without NaCl .

that of sample (f). This is due to the higher affinity of Se than Cl^- for Ag^+ , since the solubility product of Ag_2Se ($K_{sp} = 1.6 \times 10^{-56}$) is much lower than that of AgCl ($K_{sp} = 1.56 \times 10^{-10}$).¹⁰

Cell Imaging. The probe RBSe is a less polar lactone derivative, which is favorable for penetrating the cell membrane; while its reaction product, rhodamine B, is a more polar zwitterion, which would facilitate its stay in cells. Together with the above observations, it can be thus concluded that RBSe not only displays a highly selective and sensitive fluorescence response to both Hg^{2+} and Ag^+ in water but also may be suitable for imaging these two ions in cells. Figure 7 shows fluorescence images of RBSe-loaded Hela cells in the absence and presence of Ag^+ . Surprisingly, after treatment for 10 min, Ag^+ only switches on fluorescence of the cell surface. A parallel experiment for Hg^{2+} with the same incubation time of 10 min reveals that Hg^{2+} can turn on the fluorescence of the whole cell (Figure S4 in the Supporting Information). Moreover, in the case of Hg^{2+} , we cannot capture the similar image that fluoresces only on the surface but not inside, even if image time interval was decreased to 0.5 min. Nevertheless, a longer incubation time of 20 min leads to a brighter and broader fluorescence image in both cases of Hg^{2+} and Ag^+ (Figure S4 in the Supporting Information). The above results indicate that the reaction rate of Ag^+ with RBSe is noticeably slower than that of Hg^{2+} in intracellular environments. The possible reason for this phenomenon is that the high concentrations of cellular chloride ions inhibit the fast formation of sufficient free Ag^+ , which is consistent with our observation in the above *in vitro* experiments (Figure 6). Moreover, higher extracellular Cl^- concentrations (above 100 mM) in intact multicellular organisms even prevent free Ag^+ from accessing cells effectively (Figure S5 in the Supporting Information), thus scarcely showing fluorescence enhancement. However, it is understandable that, in common aqueous environments containing lower Cl^- concentration, Ag^+ may enter unicellular organisms in a higher concentration, exerting the well-known antimicrobial effect.

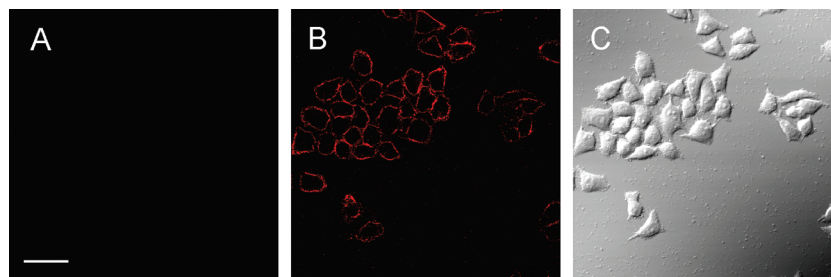


Figure 7. Confocal fluorescence images of Ag^+ in HeLa cells. (A) HeLa cells loaded with $10\ \mu\text{M}$ RBSe for 20 min. (B) RBSe-loaded cells incubated with $10\ \mu\text{M}$ Ag^+ for 10 min. (C) The corresponding differential interference contrast (DIC) image of sample B. Scale bars, $50\ \mu\text{m}$.

Conclusion

In summary, RBSe with a unique selenospirocyclic structure has been developed as a new fluorescent probe, which can produce a fluorescence-on reaction with both Hg^{2+} and Ag^+ through deselenation. Fluorescent confocal imaging reveals for the first time that the reaction of Ag^+ with RBSe in live cells is much slower than that of Hg^{2+} , which is ascribed to the high concentration of cellular chloride ions prohibiting the presence of sufficient free Ag^+ . These different interactions in cells may be responsible for the very different toxicities of Hg^{2+} and Ag^+ . Further applications of the probe to various biosystems may provide more direct

evidence to evaluate the claimed safety of silver as a disinfectant and medicine.

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Supporting Information Available: X-ray structural data of RBSe in CIF format, NMR spectra of RBSe, electrospray ionization mass spectral analysis, and other supplementary figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.