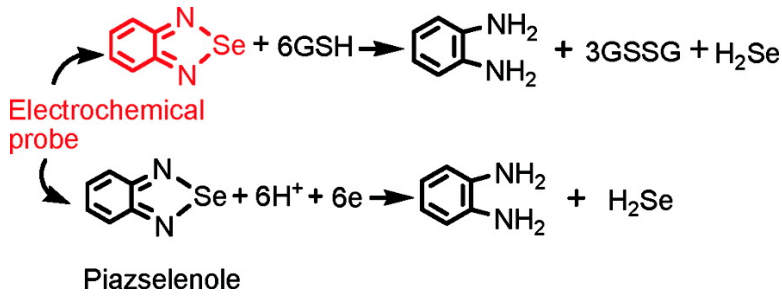
Communication

Determination of Physiological Thiols by Electrochemical Detection with Piazselenole and Its Application in Rat Breast Cancer Cells 4T-1

Wei Wang, Lin Li, Shufeng Liu, Cuiping Ma, and Shusheng Zhang

J. Am. Chem. Soc., **2008**, 130 (33), 10846-10847 • DOI: 10.1021/ja802273p • Publication Date (Web): 25 July 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 07/25/2008

Determination of Physiological Thiols by Electrochemical Detection with Piazselenole and Its Application in Rat Breast Cancer Cells 4T-1

Wei Wang,[†] Lin Li,[†] Shufeng Liu,[†] Cuiping Ma,[‡] and Shusheng Zhang*[,†]

Key Laboratory of Eco-chemical Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R. China, and Department of Molecular Biology, School of Medicine and Pharmacy, Ocean University of China, Qingdao, 266003, P. R. China

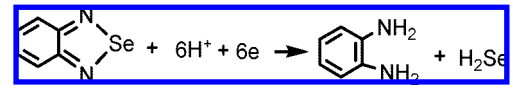
Received March 27, 2008; E-mail: shushzhang@126.com

The low molecular weight thiols, widely distributed in the tissues and cells, have been proven to play an important role in metabolism and cellar homeostasis.[1,2] Herein, glutathione is found to be the most abundant cellar thiol and exists in redox equilibrium between sulfhydryl (reduced form, GSH) and disulfide (oxidized form, GSSG) forms.[3] It has been extensively revealed to play a central role in combating oxidative stress and maintaining redox homeostasis that is pivotal for cell growth and function,[4] and its level has been directly linked to some diseases and cancers.[5−8]

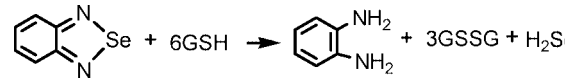
Various conventional techniques for the determination of GSH and related thiols such as high performance liquid chromatography coupled with different detection methods usually suffer from several substantial difficulties or drawbacks in terms of equipment cost, complexity, sample processing, and applicable feasibility in in vivo analysis.[9,10] In contrast, electrochemical detection poses an attractive solution because of the advantages of simplicity, sensitivity, and low instrumental cost, etc.[11] Direct electro-oxidation of thiol compounds on common solid electrodes limits its selectivity because of the similar oxidation potential of most biological reducing substances with that of thiol.[12] Alternatively, the mercury or mercury amalgam electrodes are employed,[13] but the toxicity with the involvement of mercury hinders further use for in vivo analysis. Recently, considerable interest has turned to the use of some organic or inorganic electroactive indicators as electron mediators for thiol compounds detection, yet the requirement of delicate and finely tuned experimental conditions for the success of the electrocatalytic reaction between the thiol compounds and the indicator limits their wide applications. Thereby, additional efforts are needed to create a more broadly applicable electrochemical approach that would allow accurate, sensitive, rapid, and low-cost determination of GSH and related thiols, especially suitable for in vivo analysis.

Recently, Tang et al. devised a novel fluorescence probe containing a Se−N bond and successfully utilized it for thiol determination.[14] Inspired by this strategy, we envisioned that an electrochemical probe containing a Se−N bond should be reasonably conceivable for thiol compound determination. As a proof of the notion, in present study, an electroactive piazselenole containing the Se−N bond was synthesized. The decreased current signal of piazselenole was observed with the increase of GSH concentration due to the consumption of electroactive piazselenole by the nucleophilic reaction of GSH. Thus, a novel electrochemical approach for thiol determination was developed. It was also further used in the application in cell extracts. As far as we know, this is the first case of electrochemical detection of GSH and application in a cell using electroactive species such as piazselenole containing the Se−N bond for the nucleophilic reaction of sulfhydryl.

Scheme 1. Electrochemical Reaction of Piazselenole



Scheme 2. Reaction of Piazselenole with GSH



The electrochemical behavior of piazselenole was studied by measuring the cyclic voltammogram on a Au electrode in pH 2.0 Britton−Robinson buffer (BR buffer) solution. As shown in the cyclic voltammogram of piazselenole (see Supporting Information, SI), only a cathodic signal (at −0.106 V) was observed. It was suggested that piazselenole followed a six-electron electrochemical reduction pathway on the Au electrode with the equation shown as follows (Scheme 1). The subsequent addition of GSH to the solution of piazselenole caused a decrease in the peak current, which could be ascribed to the consumption reaction of electrochemically active piazselenole with GSH, yielding the nonelectroactive species in the studied potential region according to Scheme 2.

The peak current decrease of piazselenole recorded by differential pulse voltammetry could be exploited as an assay for GSH concentration. With the GSH concentration changed in the range of 5.0×10^{-10}∼2.2×10^{-8} M (Figure 1), a good linearity was plotted with the regression equation as Δi_P ($\times 10^{-6}$ A) = 0.0952 +0.4287 × C_{GSH} ($\times 10^{-8}$ M) (γ = 0.9940), and a detection limit (3σ) of 83 pM was achieved with the proposed approach.

The effect of interference of a variety of biorelevant analytes on monitoring GSH was also studied for control experiments. The various bioanalytes employed mainly included metal ions, bioamines, and biological antioxidants listed below (tolerance ratio with 5.0 nM GSH and final concentration): Fe^{3+}, Al^{3+}, Mg^{2+}, Na+, Zn^{2+}, Cu^{2+}, K+, dopamine, histamine, *L*-adrenaline (1000, 5.0

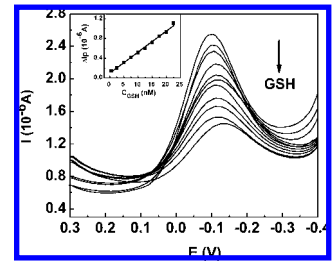


Figure 1. Differential pulse voltammograms of piazselenole (0.25 μM) toward different concentrations of GSH (final concentration: 0, 0.5, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.5 nM) after incubation at 25 °C for 20 min in 0.2 M BR buffer (pH 2.0).

[†] Qingdao University of Science and Technology.
[‡] Ocean University of China.

10.1021/ja802273p CCC: $40.75

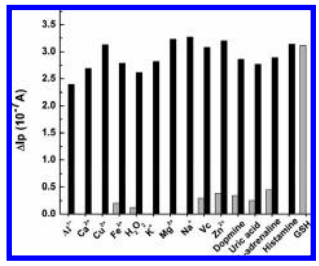


Figure 2. Electrochemical responses of piazselenole (0.25 μM) to diverse bioanalytes in 0.2 M BR buffer (pH 2.0). Light gray bars represent the addition of analytes: Fe^{3+}, Al^{3+}, Mg^{2+}, Na+, Zn^{2+}, Cu^{2+}, K+, dopamine, histamine, *L*-adrenaline (1000, 5.0 μM); Ca^{2+}, H_2O_2 (500, 2.5 μM); Vc (300, 1.5 μM); uric acid (200, 1.0 μM); GSH (5.0 nM). Black bars represent the subsequent addition of 5.0 nM GSH to the mixture. All data were obtained after incubation at 25 °C for 20 min.

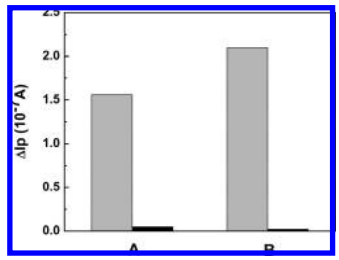


Figure 3. Electrochemical responses of piazselenole (0.25 μM) toward the supernatant of 4T-1 cells (A) and responses toward the protein extracts of 4T-1 cells (B). The gray bars represent no addition of NEM and the black bars represent the addition of 1.0 mM NEM.

μM); Ca^{2+}, H_2O_2 (500, 2.5 μM); ascorbic acid (Vc) (300, 1.5 μM); uric acid (200, 1.0 μM). The results indicated that various bioanalytes yielded no or low enough for ignorance interference with GSH detection. That is also to say, piazselenole could selectively respond to GSH compared with other analytes (Figure 2).

The electrochemical responses of piazselenole toward nonprotein thiols and protein thiols were further investigated. It was found that piazselenole showed a best response to GSH than other nonprotein thiols such as 2-mercaptoethanol (β-ME), cysteine (Cys), thioglycolic acid (TA), and dithiothreitol (DTT). Both protein thiols including glutathione reductase (GR) and metallothionein (MT) were observed to exhibit much higher peak current decreases of piazselenole than that of GSH.

To examine the capability and sensitivity of the method presented here in biological samples, the detection of intracellular nonprotein thiols and protein thiols in the extracts of rat breast cancer cells 4T-1 was performed, respectively, aiming at offering an effective method for clinical diagnosis. The experimental results demonstrated that piazselenole showed much higher reactive responses toward protein thiols in the protein extracts of rat breast cancer cells 4T-1 than those of nonprotein thiols in the supernatant when no thiol-blocking reagent *N*-ethylmaleimide (NEM) was added (Figure 3). After preincubation with 1.0 mM NEM, both the protein thiols and nonprotein thiols could be consumed by NEM. Then, the protein extracts of 4T-1 cells showed nearly no decrease in peak current of piazselenole, and just a slight decrease of peak current in the supernatant. Although the concentration of intracellular nonprotein thiols are much higher than that of protein thiols, the decrease of peak current of piazselenole induced by the latter is more distinct than that by the former. Taken together, piazselenole is more sensitive to protein thiols than nonprotein thiols. This conclusion is in good agreement with the result reported by Tang et al.[14] The different pK_a, and implicitly then the nucleophilicity of the sulfhydryl group surrounding the proteins, might account for the difference in sensitivity for protein thiols.

In summary, we provide for the first time with an extremely sensitive and specific electrochemical probe of piazselenole a method to detect GSH and related cellular thiols. The relatively low detection limit of 83 pM and a broad dynamic range of 5.0×10^{-10}~2.2×10^{-8} M were achieved. The novel developed electrochemical assay based on the strong nucleophilicity of sulfhydry to cleave Se−N bond for electrochemical response is considerably attractive for rapid, sensitive, and selective detection of cellular thiols.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (No.20775038), the Scientific and Technical Development Project of Qingdao (06-3-1-4-yx), and the National High-tech R&D Program (863 Program, No. 2007AA09Z113). We thank Fred R Miller for providing the murine breast tumor cell lines.

Note Added after ASAP Publication. After this paper was published ASAP July 25, 2008, the y-axis labels were corrected in Figure 1 and Figures S1-S3, S5, and S6 in the Supporting Information. The corrected versions were published July 29, 2008.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kleinman, W. A.; Richie, J. P., Jr. *J. Chromatogr., B* **1995**, *672*, 73–80.
(2) Pelletier, S.; Lucy, C. A. *J. Chromatogr., A.* **2002**, *972*, 221–229.
(3) Kizek, R.; Vacek, J.; Trnková, L.; Jelen, F. *Bioelectrochemistry* **2004**, *63*, 19–24.
(4) Rahman, I.; MacNee, W. *Free Radic. Biol. Med.* **2000**, *28*, 1405–1420.
(5) Curello, S.; Ceconi, C.; Cargnoni, A.; Cornacchiari, A.; Ferrari, R.; Albertini, A. *Clin. Chem.* **1987**, *33*, 1448–1449.
(6) Kleinman, W. A.; Richie, J. P. *Biochem. Pharmacol.* **2000**, *60*, 19–29.
(7) MacDonald, C. M.; Dow, J.; Moore, M. R. *Biochem. Pharmacol.* **1977**, *26*, 1529–1531.
(8) Buhl, R.; Jaffe, H. A.; Holroyd, K. J.; Wells, F. B.; Mastrangeli, A.; Saltini, C.; Cantin, A. M.; Crystal, R. G. *Lancet* **1989**, *2*, 1294–1298.
(9) (a) Chen, S. J.; Chang, H. T. *Anal. Chem.* **2004**, *76*, 3727–3734. (b) Kawakami, S. K.; Gledhill, M.; Achterberg, E. P. *TrAC-Trends Anal. Chem.* **2006**, *25*, 133–142. (c) Liu, J. F.; Roussel, C.; Lagger, G.; Tacchini, P.; Girault, H. H. *Anal. Chem.* **2005**, *77*, 7687–7694. (d) Wang, S. J.; Ma, H. M.; Li, J.; Chen, X. Q.; Bao, Z. J.; Sun, S. N. *Talanta* **2006**, *70*, 518–521. (e) Jiang, H.; Ju, H. X. *Anal. Chem.* **2007**, *79*, 6690–6696.
(10) (a) Ivanov, A. R.; Nazimov, I. V.; Baratova, L. *J. Chromatogr., A* **2000**, *895*, 157–166. (b) Haj-Yehia, A. I.; Assaf, P.; Nassar, T.; Katzhendler, J. *J. Chromatogr., A* **2000**, *870*, 381–388. (c) Amarnath, K.; Amarnath, V.; Amarnath, K.; Valentine, H. L.; Valentine, W. M. *Talanta* **2003**, *60*, 1229–1238. (d) Maccoss, M. J.; Fukagawa, N. K.; Mathews, D. E. *Anal. Chem.* **1999**, *71*, 4527–4533. (e) Guan, X.; Hoffman, B.; Dwivedi, C.; Matthees, D. P. *J. Pharm. Biomed. Anal.* **2003**, *31*, 251–261.
(11) (a) Mauzeroll, J.; Bard, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7862–7867. (b) Mauzeroll, J.; Bard, A. J.; Owhadian, O.; Monks, T. J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17582–17587.
(12) Kuninori, T.; Nishiyama, J. *Anal. Biochem.* **1991**, *197*, 19–24.
(13) Allison, L. A.; Shoup, R. E. *Anal. Chem.* **1983**, *55*, 8–12.
(14) Tang, B.; Xing, Y. L.; Li, P.; Zhang, N.; Yu, F. B.; Yang, G. W. *J. Am. Chem. Soc.* **2007**, *129*, 11666–11667.

JA802273P