Biosensors and Bioelectronics 87 (2017) 59–65

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Ultrasensitive sliver nanorods array SERS sensor for mercury ions

Chunyuan Song [a], Boyue Yang [a], Yu Zhu [b,*], Yanjun Yang [a], Lianhui Wang [a,*]

[a] Key Lab for Organic Electronics & Information Displays (KLOEID), Institute of Advanced Materials (IAM), Synergetic Innovation Center for Organic Electronics and Information Displays, Nanjing University of Posts & Telecommunications, Nanjing 210023, China
[b] Suzhou Institute of Nano-Tech & Nano-Bionics (SINANO), Chinese Academy of Sciences, Suzhou 215123, China

ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form
12 July 2016
Accepted 27 July 2016
Available online 28 July 2016

Keywords:
Surface-enhanced Raman spectroscopy (SERS)
Optical sensor
Mercury ions
Oligonucleotide
Sliver nanorods array

ABSTRACT

With years of outrageous mercury emissions, there is an urgent need to develop convenient and sensitive methods for detecting mercury ions in response to increasingly serious mercury pollution in water. In the present work, a portable, ultrasensitive SERS sensor is proposed and utilized for detecting trace mercury ions in water. The SERS sensor is prepared on an excellent sliver nanorods array SERS substrate by immobilizing T-component oligonucleotide probes labeled with dye on the 3′-end and –SH on the 5′-end. The SERS sensor responses to the specific chemical bonding between thymine and mercury ions, which causes the previous flexible single strand of oligonucleotide probe changing into rigid and upright double chain structure. Such change in the structure drives the dyes far away from the excellent SERS substrate and results in a SERS signal attenuation of the dye. Therefore, by monitoring the decay of SERS signal of the dye, mercury ions in water can be detected qualitatively and quantitatively. The experimental results indicate that the proposed optimal SERS sensor owns a linear response with wide detecting range from 1 pM to 1 μM, and a detection limit of 0.16 pM is obtained. In addition, the SERS sensor demonstrates good specificity for Hg^{2+}, which can accurately identify trace mercury ions from a mixture of ten kinds of other ions. The SERS sensor has been further executed to analyze the trace mercury ions in tap water and lake water respectively, and good recovery rates are obtained for sensing both kinds of water. With its high selectivity and good portability, the ultrasensitive SERS sensor is expected to be a promising candidate for discriminating mercury ions in the fields of environmental monitoring and food safety.

1. Introduction

As one of the most toxic metallic pollutants, mercury, released from natural emissions and anthropogenic contaminants, gradually accumulates in human body through food chains and drinking water (Castilhos et al., 2006; Nolan and Lippard, 2008). Excessive internal mercury accumulation will lead to various chronic diseases such as headache, deafness, visual impairment, and even irreversible damage of brain and central nervous system (Harada, 1978; Onyido et al., 2004; Palmer et al., 2006). Thus, sensitive monitoring of mercury ions in aquatic ecosystems has become essential because these contaminants can cause fearful human health problem and environmental pollution.

Up to now, several testing strategies of Hg^{2+} have already been implemented and a brief overview is listed in Table S1. Generally, traditional analytic methods for mercury ions are rapid and sensitive with a detection limit of sub-ppt level, including inductively coupled plasma mass spectrometry (ICP-MS) (Legrand et al., 2004), cold vapor atomic absorption spectrometry (CVAAS) (Pourreza and Ghanemi, 2009) and cold vapor atomic fluorescence spectrometry (CA-AFS) (Yu and Yan, 2004). However, the shortcoming of these technologies is also obvious, e.g., tedious pretreatment, expensive costs, and not suitable for on-site testing. Other detection methods have been reported to be convenient, fast and cheap, such as colorimetric, fluorescence, electrochemistry and resonance scattering (Fan et al., 2009; Henglein and Brancewicz, 1997; Wu et al., 2010, 2011; Ye and Yin, 2008). Nonetheless, some of them still face the problem of particles' non-specific aggregation, signal quenching and photo bleaching, cross-sensitivity toward other metal ions, or insufficient sensitivity. Therefore, it is still highly desirable to develop ultrasensitive and selective methods for Hg^{2+} detection with simple procedures, high stability and good portability.

Recently, surface-enhanced Raman spectroscopy (SERS) has received widespread attention for its advantages of ultra-sensitivity and good specificity. SERS is considered as a novel, ultrasensitive optical technique for its possibility of testing trace analytes down to single molecule (Kneipp et al., 1997; Nie and Emory, 1997). So far, several SERS strategies have been reported to sensor

* Corresponding authors.
E-mail addresses: yzhu2011@sinano.ac.cn (Y. Zhu), iamlhwang@njupt.edu.cn (L. Wang).

http://dx.doi.org/10.1016/j.bios.2016.07.097
0956-5663/

Hg^{2+}. Generally, SERS-based Hg^{2+} sensors consist of three parts: Hg^{2+} capture molecules, SERS substrate and Raman reporters. For instance, in Wang's work (Feng et al., 2013), Au NPs were labeled with Hg^{2+} capture molecule L-cysteine and SERS signal reporter first, and then mixed with Hg^{2+} liquid sample. Due to the combination between L-cysteine and Hg^{2+}, Au NPs aggregated, generating an enhanced SERS signal. In other cases, once the SERS sensors incubate with Hg^{2+} solution, capture molecules can fall off from the metal surface for the stronger competitive binding with Hg^{2+} than noble metal. Based on this principle, some SERS signal-off strategies for detecting Hg^{2+} were developed. Representatively, Du proposed a Hg^{2+} detection strategy on the Au@AgNPs modified silicon wafer SERS substrate (Du et al., 2013). In their work, 4,4′-dipyridyl molecules (Dpy) served as both capture molecules and Raman reporters were immobilized onto Au@AgNPs *via* Au–N bond. Once mercury ions exist in the aqueous sample, Dpy can preferentially combine with Hg^{2+} than Au and release from the metal nanoshells, quenching the SERS signal of Dpy. Imperfectly, when the above-mentioned strategies come to the detection of Hg^{2+} in complex samples, these methods still suffer from complex lab procedures, poor specific, or narrow adaptability of pH range. On the other hand, researchers found that thymine in oligonucleotides could ultra-selectively and strongly capture Hg^{2+} to form stable DNA duplexes by T–Hg^{2+}–T pairs (Fujimoto et al., 2006; Ding et al., 2013; Yoshiyuki et al., 2007). The T–Hg^{2+}–T coordination compound is formed by proton substitution reaction between Hg^{2+} and nitrogen in thymine (Kosturko et al., 1974), with the advantages of high stability and specificity. Recently, strategies utilizing oligonucleotides functionalized metallic nanoparticles have been widely reported, which make them becoming prevalent methods for detecting Hg^{2+} (Lee and Choo, 2011; Li et al., 2015). A summary of the recent progress of the SERS-based Hg^{2+} detections is presented in Table S2. Generally, these approaches using noble metal colloids usually have complicated sensor structures, uncontrollable signal homogeneity and suffer from unavoidable aggregation for many factors such as temperature, salts and organic compounds, which restrains them from further practical applications. Therefore, available strategies that avoid the undesirable aggregation were performed on solid bases (Kang et al., 2012; Kang et al., 2011; Zhang et al., 2013). However, the solid SERS sensors are still facing great challenges, especially limited to low enhancement performance and dissatisfactory uniformity of SERS signal, which restricts their wide and practical usage.

In our previous works, we deposited a sliver nanorods (Ag NRs) array by oblique angle deposition technique (Song et al., 2012). The Ag NRs array has good SERS reproducibility (~10% relative variation), high SERS enhancement factor (up to 10^9), and large uniformity (Abell et al., 2011; Driskell et al., 2008). The Ag NRs array shows excellent SERS performance, but its application as SERS sensor for Hg^{2+} has not yet been reported. In the present work, a novel and portable SERS sensor for Hg^{2+} was prepared on the Ag NRs array SERS substrate for the first time by modifying its surface with the T-component oligonucleotide probes. The preparation and application conditions of the sensor were optimized to achieve ultrasensitive detection of Hg^{2+} with the limit of detection low to 0.16 pM. The specificity of SERS sensor was also verified by testing other ten metal ions. Finally, the SERS sensors were utilized to test tap water and lake water respectively, and the results indicate that the proposed SERS sensor can be a powerful and reliable tool for monitoring trace Hg^{2+} in natural water.

2. Experimental section

2.1. Materials

$Hg(NO_3)_2$ was purchased from Xiya Reagent Co., Ltd (Chengdu, China), and the Hg^{2+} standard solution (0.01 M) was prepared by dissolving 3.25 g $Hg(NO_3)_2$ into 10 mL of 1 M HNO_3 (Sinopharm Chemical Reagent, Shanghai, China) and then diluted to 1000 mL with water. The addition of HNO_3 can extend the preservation time of the mercury ion in the aqueous solution by preventing adsorption of Hg^{2+} on the bottle wall. The Cyanine 5 (Cy5) labeled T-component oligonucleotide probe, 5′-thiol-TTT TTT TTT TTT TTT-Cy5-3′ (Cy5-ssDNA), was synthesized and HPLC-purified by Takara Biotechnology (Dalian, China). The following ten metal ions were chose as interfering ions to test the specificity of the Hg^{2+} sensor: $CaCl_2$ and $CoCl_2$ were obtained from Sinopharm Chemical Reagent Co., Ltd (Bejing, China), LiCl, $MnCl_2$, $CrCl_3$, $FeCl_3$ and $NiCl_2$ were acquired from Aladdin Chemistry Co., Ltd (Shanghai, China), $ZnCl_2$ and $Cu(NO_3)_2$ were obtained from Xinbao Chemical Co., Ltd (Shanghai, China), and $BaCl_2$ was purchased from Xilong Chemical Co., Ltd (Shantou, China). Unless otherwise specified, all chemicals were used without further purification, and the solutions were prepared using ultrapure Millipore water (18.2 MΩ cm). The lake water was collected from Yangshan Lake (Qixia, Nanjing, China).

2.2. Preparation of silver nanorods array

Silver nanorods (Ag NRs) array was prepared by the oblique angle deposition (OAD) technology according to Song et al. (2012). Briefly, 9 mm × 9 mm clean glass slides were loaded into the deposition chamber with the substrate normal antiparallel to the incident vapor direction. Firstly, a 20 nm layer of Ti was deposited at a rate of 0.2 nm/s, followed by a 200 nm Ag film deposited at 0.3 nm/s. Then, the substrate normal was rotated to 86° relative to the incident vapor direction, and a final 2000 nm layer of Ag NRs was deposited at a rate of 0.3 nm/s. The entire evaporation process was conducted under high vacuum conditions ($< 3 \times 10^{-6}$ Torr).

2.3. Preparation of mercury ions SERS sensor

Silver nanorods array (i.e. SERS substrate) was rinsed by ultrapure water and dried by N_2 flow before use. The SERS sensor was prepared by assembling oligonucleotide probes onto the substrate through the Ag–S covalent bond. Briefly, twenty microliters of Cy5-ssDNA were incubated with the SERS substrate at 25 °C in a chamber with ~80% humidity for 3 h. Then the Cy5-ssDNA modified substrate was rinsed by ultrapure water for 3 times to remove unreacted and physically deposited probes. After that, the prepared SERS sensor was stocked in 10 mL of 1 mM phosphate buffer (0.001 M NaCl, pH 7.4) at room temperature for following tests.

2.4. Procedure of sensing Hg^{2+}

The original SERS signal of the sensor before incubation with Hg^{2+} was recorded first. Briefly, the sensor was removed from the stock solution, followed by immediately SERS measurements. Then the SERS sensor was immersed into 10 mL as-prepared Hg^{2+} solution in a well of 6-well plate at room temperature. After 3 h, the sensor was taken out and the SERS spectra were collected. All spectra were subtracted the baselines by software Wire 4.0.

2.5. Instruments

The morphological characterization of Ag NRs array was characterized by field emission scanning electron microscopy (FESEM)