Biosensors and Bioelectronics 99 (2018) 646–652

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Novel ratiometric surface-enhanced raman spectroscopy aptasensor for sensitive and reproducible sensing of Hg^{2+}

Yan Wu[a,b], Tingting Jiang[a], Zhaoyang Wu[a,*], Ruqin Yu[a]

[a] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China

[b] Department of Chemistry and Chemical Engineering, Yangtze Normal University, Chongqing, Fuling 408100, China

ARTICLE INFO

Keywords:
Ratiometric
Surface-enhanced Raman scattering
Aptasensor
Mercury (II) ions
Au@Ag core-shell nanoparticles

ABSTRACT

It is important to precisely monitor mercury (II) ions (Hg^{2+}) for environment protection and human health monitoring. Although many strategies have been developed in the past decades, there still remains a challenge for developing an ultrasensitive, simple and reliable approach to detect Hg^{2+}. Herein, we report a ratiometric surface-enhanced Raman scattering (SERS) aptasensor by employing aptamer-modified Au@Ag core-shell nanoparticles (Au@Ag NPs) as highly functional sensing probes, allowing for ultrasensitive detection of Hg^{2+}. In principle, the thiolated 5′-Cy3 labeled aptamer probe (Cy3-aptamer) is firstly immobilized on the SERS substrate surface and then hybridizes with the 5′-Rox labeled complementary DNA (cDNA) to form a rigid double-stranded DNA (dsDNA), in which the Cy3 and Rox Raman labels are used to produce the ratiometric Raman signals. In the presence of Hg^{2+}, the aptamer DNA turns into the thymine (T)-Hg^{2+}-T mediated hairpin structure, leading to the dissociation of dsDNA. As a result, the Rox labels are away from the Au@Ag NP SERS substrate while Cy3 labels are close to it. Therefore, the intensity of SERS signal from Cy3 labels increases while that from Rox labels decreases. The ratio between the Raman intensities of Cy3 labels and Rox labels is linear with Hg^{2+} concentrations in the range from 0.001 to 1.0 nM, and the limit of detection is estimated to be 0.4 pM. The proposed strategy provides a new rapid, simple and reliable approach for sensitive detection of Hg^{2+} and may create a universal methodology for developing analogous aptasensors for a wide range of other analytes determination.

1. Introduction

Mercury and its compounds, one kind of widespread pollutant, have drawn significant attention in recent decades due to its accumulative and toxic properties in the environment. In particular, the water-soluble mercuric (II) ion (Hg^{2+}) is one of the most stable toxicity and stable forms of mercury pollutant because of its deleterious effects on environment and human health (Nolan and Lippard, 2008; Reardon and Bhat, 2007; Schneider et al., 2012). The maximum residue limit of Hg^{2+} is 2 ppb (10 nM) in drinkable water as stipulated by the U. S. Environmental Protection Agency (USEPA) (Kang et al., 2011). Therefore, a sensitive and selective method for monitoring of aqueous Hg^{2+} in environment is highly demanded. Although a multitude of modern analytical techniques for the detection of Hg^{2+} have been developed, e.g. atomic fluorescence spectrometry (Díez and Bayona, 2002; Leopold et al., 2008), atomic absorption spectrometry (Cizdziel and Gerstenberger, 2004), and inductively coupled plasma mass spectrometry (Jitaru and Adams, 2004), the majority of them still suffer from time-consuming, complicated sample handling process, and requiring sophisticated equipments. Therefore, it is still a challenge to develop rapid and simple methods for Hg^{2+} detection.

Considerable contributions have been made to explore mercury sensing assays based on different signal transduction mechanisms, including colorimetric (Lee et al., 2007a; Li et al., 2009; Xu et al., 2009; Xue et al., 2008), fluorescent (Che et al., 2008; Xu et al., 2016; Yang et al., 2005), electrochemical (Kong et al., 2009; Xie et al., 2016; Xuan et al., 2013), liquid crystal (Yang et al., 2013), and surface-enhanced Raman spectroscopy (SERS) (Ding et al., 2013; Han et al., 2010; Li et al., 2013; Ma et al., 2013; Sun et al., 2015). In particular, much attention was poured into the colorimetric and fluorescent approaches owing to their high sensitivity, fast response and easy operation. For example, Mirkin et al. developed a colorimetric sensor for Hg^{2+} detection, which is based on the aggregation of gold nanoparticles (AuNPs) and exhibited high sensitivity and selectivity for Hg^{2+} (Lee et al., 2007a). Liu et al. proposed a novel and practical system for colorimetric monitoring mercury at room temperature (Xue et al.,

* Corresponding author.
E-mail address: zywu@hnu.edu.cn (Z. Wu).

http://dx.doi.org/10.1016/j.bios.2017.08.041
Received 22 June 2017; Received in revised form 4 August 2017; Accepted 17 August 2017
Available online 19 August 2017

2008). A fluorescent method was developed based on the fluorescence quenching of Au nanoclusters by Hg^{2+}-Au^{+} interactions (Xie et al., 2010), and exhibited a limit of detection (LOD) for 0.5 nM Hg^{2+}. Additionally, Jung et al. (Kim et al., 2008, 2010; Lee et al., 2007b; Park et al., 2010) fabricated a series of nanomaterials, including acyclic receptor immobilized mesoporous silica (Kim et al., 2008), amino-naphthalimide-functionalized Fe_3O_4@SiO_2 core/shell magnetic nanoparticles (Park et al., 2010), and so on, to detect and remove toxic ions, which recognized target ions with high sensitivity and selectivity among heavy metal ions in aqueous solution. Most of these methods can detect Hg^{2+} in the range from part-per-trillion (ppt) to part-per-billion (ppb). However, more sensitive and simple approaches for Hg^{2+} detection are urgently needed because Hg^{2+} still do harm to human health and environment after long-term accumulation.

SERS has received prominent attention due to its distinctive advantages including high sensitivity, specific Raman fingerprinting spectra and rapid detection capability without complicated sample preparation. Therefore, taking advantages of these unique features, SERS is considered as a promising alternative to sensitively monitor Hg^{2+}. Recently, several methods based on SERS for Hg^{2+} have been reported. For example, Zhang et al. developed a surface-enhanced resonance Raman scattering sensor by employing dealloyed nanoporous gold as plasmonic substrate and Cy5-labeled aptamer as optical tags for the ultrasensitive detection of Hg^{2+} with a LOD of 1.0 pM (0.2 ppt) (Zhang et al., 2013). Sun et al. reported a SERS sensing platform based on DNA technology, enabling ultrasensitive, rapid detection of trace Hg^{2+} (Sun et al., 2015). Among those methods, most of them are developed by employing the thymine containing oligonucleotides as sensing platforms and Raman-labeled DNA is used as the single signal probe. Although these methods can achieve the advantages of simple, rapid, ultrasensitive, and selective for Hg^{2+} detection simultaneously, they have to suffer from the unstable and poor reproducibility of SERS signals.

The ratiometric method is an effective way to surmount the instability of single analytical signal intensity. More recently, several ratiometric sensors such as electrochemical, fluorescent, and SERS have been proposed for Hg^{2+} detection (Jin et al., 2017; Peng et al., 2016; Wegner et al., 2007; Xiong et al., 2015), and they have been demonstrated to permit signal rationing and provided built-in correction for environment effects. Furthermore, our group has developed an aptasensor platform based on ratiometric SERS for the detection of ATP using aptamer-modified AuNPs as SERS substrate (Wu et al., 2017); however, it is still suffer from weak enhancement factor of AuNP SERS substrate. Herein, inspired by the aforementioned advantages of ratiometric strategy, we try to propose a novel ratiometric SERS aptasensor to detect Hg^{2+} using aptamer-modified Au@Ag core-shell nanoparticles (Au@Ag NPs) as SERS substrate. In the ratiometric sensing strategy, a thiolated 5′-Cy3 labeled recognition aptamer used as the signal probe is immobilized on the Au@Ag NP surface and a 5′-Rox labeled complementary DNA (cDNA) hybridizes with the signal probe to form a rigid double-stranded DNA (dsDNA) to construct a ratiometric SERS biosensing platform, in which the Rox Raman labels on the cDNA are close to SERS substrate and produce strong SERS signals while the Cy3 Raman labels on the signal probe are away from the substrate and hardly has SERS signal. In the presence of Hg^{2+}, the signal probe forms a more stable T-Hg^{2+}-T mediated hairpin structure, which remarkably reduced the distance between the Cy3 Raman labels and nanoparticle surface and then produced strong Cy3 SERS signal. At the same time, the cDNA dissociated from dsDNA and released from nanoparticle surface, making the Rox Raman labels away from substrate surface and decrease the Rox SERS signal. The ratio value between the Raman signals of two labels is used to quantify the Hg^{2+} concentration. As we all known, this is the first report of the aptasensor platform for the detection of Hg^{2+} based on the ratiometric SERS strategy, which will well improve the sensitivity for Hg^{2+} detection and the reproducibility of SERS signal and thus open a novel avenue to promote the practical application of aptasensors for other analytes detection.

2. Materials and methods

2.1. Chemicals and reagents

Gold chloride trihydrate ($HAuCl_4$), 6-mercapto-1-hexanol (MCH), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), sodium citrate, silver nitrate, L-ascorbic acid and sodium citrate tribasic dehydrate were purchased from Sigma-Aldrich. Metal salts ($Hg(NO_3)_2$, $CaCl_2$, $FeCl_2$, $CdCl_2$, $BaCl_2$, $MgCl_2$, KCl, $CuCl_2$, $CoCl_2$, $ZnCl_2$, $MnCl_2$, $Pb(NO_3)_2$, and $Ni(NO_3)_2$) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All of these reagents were used without further purification. The thiolated oligonucleotide DNA (5′-Cy3-TTC TTT GTT CCC CTT CTT TGT TCC CCC CCC CC-SH-3′ (Cy3: Cyanine 3), aptamer DNA) and its complementary DNA (cDNA) (5′-Rox-AAC AAA GAA-3′ (Rox: 5-carboxy-X-rhodamine)) were synthesized by TaKaRa Biotechnology Co. Ltd. (Dalian, China). The molecule structures of Cy3 and Rox are listed in Fig. S1 in Supporting information. Ultra-pure water (≥18 MΩ cm), which was obtained from ZOOMWO water purification system, was used in all procedures.

2.2. Apparatus

A transmission electron microscope (TEM) (JEM-3010, Japan) was used for characterization of the as-prepared AuNPs and Au@Ag NPs. All Raman and SERS spectra were collected using a Renishaw InVia micro-Raman spectrometer (Renishaw, Wotton under Edge, U.K.) equipped with a Peltier charge-coupled device (CCD) detector, a Leica microscope, and spectra was obtained using a 633 nm He-Ne laser at 25 °C and a 50×long objective lens. For Raman measurement, the laser output power was 20 mW and the acquisition time was 3 s for all SERS spectra. JEM-3010 TEM and Shimadzu UV-2450 spectrophotometer UV–vis absorption spectra were used to characterize the nanoparticles.

2.3. Preparation of dsDNA-embedded Au@Ag NPs

AuNPs with a size of 30 nm were synthesized according to the citrate reduction method reported by Frens (Frens, 1972). Typically, a 50 mL of 0.01% $HAuCl_4$ solution was heated to the boiling point under stirring, then a 0.5 mL of 1.0% tri-sodium citrate dehydrate solution was added under vigorous stirring. The color of the solution changed from faintly blue to brilliant red in a few seconds, indicating the formation of the AuNPs. The solution was refluxed for about 30 min until the color became wine red and cooled to room temperature under stirring. The solution was filtered through 0.22 μm Millipore membrane and stored at 4 °C for further use. The thiolated probe DNAs were immobilized on the surface of AuNP by Au-S bonds. Here, a 3.0 μL of 100 μM thiolated oligonucleotide DNA (aptamer DNA) was mixed with 3.0 μL of 5.0 mM TCEP in 100 mM Tris-HCl (pH 7.0). The mixture was incubated at room temperature for 1 h. The solution was then added to 3.0 mL AuNPs solution, and the mixture was incubated at 4 °C for 24 h. Unbound chemicals and DNA strands were removed by centrifugation at 12,000 rpm for 15 min, and then re-suspended in 3.0 mL of ddH_2O (the method and results for estimation of Cy3-aptamer on AuNP and quantification of surface coverage are described in Supporting information). Aptamer DNA-embedded AuNPs were encapsulated with silver to enhance Raman signals. A 60 μL of 10 mM ascorbic acid was mixed into the solution and silver nitrate was slowly added under stirring. To form the dsDNA-Au@Ag core-shell conjugate, 100 μM cDNA was added to the aptamer-immobilized Au@Ag core-shell NPs. This solution was placed in a water bath at 95 °C for 10 min and then gradually cooled to room temperature before use. The solution was centrifuged at 12,000 rpm for 15 min to remove un-reacted reagents.