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A label-free SERS approach to quantitative and selective detection of mercury (II) based on DNA aptamer-modified SiO_2@Au core/shell nanoparticles

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ABSTRACT

A prototype of label-free SERS sensor, based on the formation of T-Hg^{2+}-T pairs and the corresponding orientation variation of DNA on Au shell surface, was developed for detection of Hg^{2+} in aqueous solution quantitatively and selectively. The DNA aptamer immobilized on the surface of SiO_2@Au core/shell nanoparticles (NPs) consisted of two segments, namely, the segment which has the consecutive thymines (T) severing as the Hg^{2+} recognition elements, and the segment which contains the guanine (G) and adenine (A) bases working as the signal reporter. With interaction of Hg^{2+} ions and the thymines between the adjacent single-stranded DNA (ssDNA), the DNA molecule adopted vertical orientation, resulting in increase of Raman intensity ratio I(660 cm^{-1})/I(736 cm^{-1}) with increase of Hg^{2+} concentration, which thus allowed to measure trace amounts of Hg^{2+} in aqueous solution selectively and quantitatively. Our results revealed that this label-free SERS sensor could sensitively respond to Hg^{2+} ions within a wide concentration range (from 1×10^{-8} to 1×10^{-3} M). This work therefore demonstrates that proper design of aptamer-modified SiO_2@Au core/shell NPs can be utilized for label-free SERS detection of heavy metal ions in the environment.

1. Introduction

Mercury (II) (Hg^{2+}) is a widespread pollutant in the aqueous environment and may cause serious harm to human health [1,2]. Mercury ions, particularly methyl mercury can be enriched in the human body through food chain, and induce permanent damages to kidney, central nervous system, digestive system, leading to a variety of chronic diseases [3,4]. Methyl mercury can be biomethylated from soluble mercury ions (Hg^{2+}) by aquatic sediments [3]. Therefore, quantitative and specific detection of Hg^{2+} has been of a long-standing concern of people. A variety of methods for Hg^{2+} detection have been developed including organic dye probes [5,6], conjugated polymers [7,8], electrochemical anodic stripping voltammetry [9,10]. These methods are normally dependent on coordination chemistry and oxidation-reduction properties of mercury ions, and so the detection systems are restricted by the conditions of chemical reactions. Recently, some bio-recognition materials are utilized to improve the detection specificity, which include aptamers DNA nucleases [11,12] and proteins [13]. For the DNA aptamers, the specific interaction of thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) [14] has gained special attention. Taking advantage of this interaction, researchers have developed some new Hg^{2+} detection probes and sensors via electrochemical, fluorescence spectroscopy and colorimetric detection approaches [15]. For example, Yu et al. used ferrocene-modified T-rich DNA sequence coupled with electrochemical method for specific detection of mercury ions by redox current changes [16]. Yang et al. have made use of fluorescein-modified single-stranded DNA and gold nanoparticles for Hg^{2+} ions detection by both fluorescent and colorimetric methods [17]. Chen et al. made use of paper analytical device based on colorimetric gold nanoparticles for detection of mercury ions [18].

On the other hand, surface-enhanced Raman spectroscopy (SERS) is an emerging technique for rapid, non-destructive and trace chemical measurements, and it can also be utilized for mercury ions detection in different ways. Compared with the con-

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ventional Raman spectroscopy, SERS not only possesses the feature of fingerprint identification of analytes [19–22], but also can provide very high detection sensitivity which normally reaches more than 6 orders of magnitudes, and in some cases even up to the level of single molecule detection [23]. To improve SERS detection sensitivity, a lot of efforts have been made to fabricate effective SERS substrates which can offer large enhancement effect, and many different kinds of SERS substrates such as single nanowire-on-film [24], gold nanoparticles/graphene heterojunctions [25], nanoporous gold film [26], Au/Ag core-shell nanoparticles [27], SERS-active ZnO/Ag nanoarrays [28], gold nanoparticles decorated silicon nanowire array [29], Au triangular nanoarrays/n-layer graphene/Au nanoparticles sandwich structure [30] have been achieved. For SERS application in Hg^{2+} detection, there have also been studies reporting the use of T-Hg^{2+}-T for mercury ion capture and detection. For example, people made use of SERS-active dye labeled T-rich DNA modified SERS substrates, and the SERS signal change of dyes was used to probe Hg^{2+} ions [30,31]. Han et al. devised a sensor to detect mercury ions with TAMRA-labeled-DNA modified single gold microshell and achieved the detection limit as low as 50 nM [32]. Ma et al. developed a 4-ATP labeled DNA functionalized self-assembled nanostar dimer as a SERS sensor for Hg^{2+} detection with even higher sensitivity [33]. But in these cases, extrinsic Raman labels were required which would inevitably raise the cost and complexity of the detection system.

Nowadays a new trend of SERS application is to develop the so-called label-free SERS detection approach, in which no Raman label is required in the detection system. In this work, therefore, we attempted to develop a novel label-free SERS sensor which was based on aptamer-modified SiO_2@Au core/shell NPs for Hg^{2+} detection, in which we could use intrinsic Raman signals of adenine and guanine bases of the aptamer DNA for sensing Hg^{2+} when the mercury ions interact with the thymines in the aptamer. For this purpose, we designed the DNA aptamer immobilized on the surface of SiO_2@Au core/shell NPs which consisted of two segments, namely, the consecutive thymines severing as the Hg^{2+} recognition and capture elements, and the segment which contained the guanine (G) and adenine (A) bases working as the intrinsic signal reporters. The T-rich segment was arranged near the thiol binding site for capturing Hg^{2+} specifically, and when Hg^{2+} ions interacted with the T bases between the adjacent DNA strands to form T-Hg^{2+}-T pairs, the DNA molecule adopted vertical orientation, resulting in change of relative Raman intensities of A and G bases in the DNA strands, which thus allowed us to measure trace amounts of Hg^{2+} in a specific and quantitative way.

2. Experimental

2.1. Materials

Tetraethyl orthosilicate (TEOS, 99.9999%), tetrakishydroxymethylphosphonium chloride (THPC), (3-aminopropyl)-trimethoxysilane (APTES)were purchased from Sigma-Aldrich Co, Ltd. Potassium carbonate (K_2CO_3), tetrachloroauric acid ($HAuCl_4{\cdot}3H_2O$), ethanol, ammonium hydroxide and formaldehyde were obtained from Sangon Biotechnology Inc. (Shanghai, China). All metal salts listed as $Zn(NO_3)_2{\cdot}6H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, $Fe(NO_3)_3{\cdot}9H_2O$, $Ba(NO_3)_2$,$AgNO_3$, $Fe_2SO_4{\cdot}7H_2O$, $CdCl_2{\cdot}2.5H_2O$, LiCl, $NaNO_3$, KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$, $Cu(NO_3)_2{\cdot}6H_2O$, $Pb(NO_3)_2$ (analytical reagent grade) used in this work were purchased from Aladdin. The stock solutions were prepared by dissolving these metal salts in distilled, deinized water. All DNA with an alkanethiol moiety (-SH) at the 5′ terminal were HPLC grade and were synthesized by Takara Bio.

2.2. SiO_2@Au NPs synthesis and characterization

The SiO_2@Au NPs were fabricated according to the multi-step synthesis methods developed by Halas et al. [34], with the revised protocol described in detail previously elsewhere [35]. Briefly, Au seeds [36,37] were synthesized and conjugated to APTES modified silica NPs prepared using Stöber method [38], followed by the formation of thin Au shell from the Au seeds through chloroauric acid reduction. Then, the as-fabricated SiO_2@Au NPs were immobilized on APTES modified quartz glasses [39] to form the SERS substrates without aptamer modification [35]. The synthesized SiO_2@Au NPs were characterized by UV–vis absorption measurements with SHIMADZU, UV-2550 spectrophotometer. The surface morphology and core/shell structure of these NPs were examined by scanning electron microscope (SEM) with Hitachi S-4800 machine and transmission electron microscope (TEM) though JEOL 2010 machine. Typically, the diameter of silica was about 100 nm and the thickness of Au shell was about 10 nm. The maximum absorbance peak of this dimension SiO_2@Au NPs was located at 750 nm which is in particular suitable for 785 laser excitation. More details about nanoparticle synthesis, characterization and substrate fabrication can be found in the SI.

2.3. Fabrication of SERS substrates for Hg^{2+} detection

The DNA aptamer used in this work was designed as HS-$(CH2)_6$-TTTTTTTTTTGGGGGGGGAAAAAAAA. Prior to the conjugation of single stranded DNA (ssDNA) on the substrates, the thiolated DNA was treated with thiol-activation and desalting. All the DNA samples were heated by hot water at 90 °C for 10 min followed with rapid cooling in ice-water bath. After this treatment, DNA tended to "stand on" the surface of the gold shell and thus the uniform DNA SERS spectra could be achieved [40]. Next, 5 μL of DNA samples were dropped on each 0.4 cm × 0.6 cm freshly prepared substrates and then were incubated in 4 °C refrigerator with high humidity overnight. Before the Raman spectral recording, these aptamer-modified SERS substrates were washed carefully in ultrapure water several times and dried in fume hood without interruption.

2.4. SERS measurements and spectral analysis

For SERS detection of mercury (II) ions, $HgCl_2$ was dissolved in ultra-pure water with different concentrations 1×10^{-3} M – 10^{-8} M. Freshly prepared DNA-modified SERS substrates were in placed in Hg^{2+} solution during the SERS measurements. The SERS spectra were recorded by XploRA Raman microspectrometer (Horiba Jobin Yvon) with a 785 laser. The diameter of the laser spot used here was about 0.7 μm. The laser power focusing on the samples was approximately 0.2 mW so the possible molecular damage and fluorescence interference could be avoided. For the spectral analysis, multiple spectra recorded from randomly chosen locations of the substrate were averaged. The intensities of peaks were evaluated by Bruker OPUS 7.0 software.

2.5. DFT computation of structural and spectral features of T-Hg^{2+}-T pairs

To interpret the Raman spectral changes with presence and absence of Hg^{2+} ions, DFT computation was performed using M06-2X (thymine,b3lyp/aug-cc-pvtz; T-Hg-T, b3lyp/6–311 + G** + MWB60) for searching the stable optimization geometries and calculating the Raman vibration modes. This modeling has been successfully applied in studying main-group noncovalent interactions, spectroscopy and so on. All the calculations were performed by using *Gaussian 09* software.