Optical Sensor Assistant with Voltage Enrichment for Ultrasensitive Detection of Mercury Ions

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ABSTRACT: Voltage enrichment-assisted surface-enhanced resonance Raman scattering (SERRS) was employed for monitoring mercury(II) ions based on a nanoporous gold (NPG)/aptamer hybrid sensor. The hybrid sensor based on the coordination chemistry of thymine−Hg^{2+}−thymine interaction and an applied voltage that pushed mercury ions toward the NPG film improved both the speed and sensitivity of Hg^{2+} ion detection. The detection limit can reach 0.1 pM, even in the aqueous solution containing 13 metal ions, and the sensor also possesses a fine reproducibility and stability, promising great potential in real-time sensing applications.

INTRODUCTION

Mercury ions (Hg^{2+}), which are extremely widespread in nature, pose a serious threat for human health and the ecosystem. The methylmercury ingested by aquatic organisms can accumulate in the body and continue to accumulate through the food chain, which would cause serious irreversible brain damage. Due to the adverse effects of Hg^{2+} ions on human health and safety, an ultrasensitive and real-time detection method of mercury ions is of great significance in environmental protection and health monitoring. Various methods have been developed to detect mercury ions, such as electrochemical sensing, colorimetric assays, atomic absorption spectroscopy, fluorescence detection, and surface-enhanced Raman scattering (SERS). Since Hg^{2+} is inactive to most optical spectroscopy, Hg^{2+}-sensitive molecular probes and tag labels have been applied for detection, among which a metal ion-specific aptamer was often used due to its excellent selectivity. In a previous study, a nanoporous gold/aptamer-based surface-enhanced resonance Raman scattering (SERRS) sensor showed sub-parts per trillion level heavy metal ion sensitivity. In this study, we further improved the detection efficiency of an aptamer-based SERRS method via voltage enrichment Raman spectroscopy. A Cy5-labeled aptamer-modified nanoporous gold was selected as the substrate, and a constant voltage was applied to guide the mercury ions toward the hybrid electrode to fasten the coordination of mercury ions with aptamers, leading to higher detection efficiency and detection limit. In addition, the voltage enrichment SERS does not influence the selectivity of the hybrid sensor, and a 0.1 pM detection limit for Hg^{2+} ions was achieved with interference of other heavy metal ions in drinking water and groundwater.

RESULTS AND DISCUSSION

Poly(thymine)-based aptamer-functionalized NPG has been proven to be an excellent heavy metal ion optical sensor due to the large surface-to-volume ratio and uniform intense local electromagnetic field. A Hg^{2+}-mediated base pair formed a thymine−Hg^{2+}−thymine (T−Hg^{2+}−T) duplex structure, and the variation of the structure was utilized for optical sensing. Consequently, the stabilizing effect of Hg^{2+} ions on T−T combination surpassed the effects of other metals and appeared to be highly specific. In this study, a constant voltage was applied to increase the density of the Hg^{2+} ions surrounding the sensor substrate, which also works as the negative electrode of the system. The application of voltage enrichment efficiently improved the detection rate, and Figure 1 demonstrates the working mechanism. Figure 1a shows the scanning electron microscopy (SEM) image of the NPG substrate used in this experiment, and the characteristic length of nanopores and ligament is \( \sim 27 \pm 2 \) nm, which is estimated by a fast Fourier transform method. Figure 1b illustrates the

**Received:** December 13, 2018  
**Accepted:** March 22, 2019  
**Published:** April 2, 2019
experimental setup for Hg\textsuperscript{2+} detection. DC stands for a portable electrochemical workstation, and PC is a computer connected to the workstation.

A labeled aptamer with 15 thymines (Apt15) was chosen to functionalize the NPG film, and a Cy5 probe, which is resonant with 633 nm laser excitation, is at the 3′ terminal of the oligonucleotide, and an alkanethiol moiety, which can bond to the gold surface via a thiol anchor, is at the 5′ terminal. The Apt15-functionalized NPG film served as the working electrode and was stabilized in an optical electrochemical cell for further measurement. As shown in Figure 1c, mercury ions are guided toward the NPG film by electrostatic forces, which increase the combination rate of mercury ions and aptamers.\textsuperscript{25}

With the existence of Hg\textsuperscript{2+} ions, a pair of poly-T oligonucleotides hybridized through the T–Hg\textsuperscript{2+}–T coordination reaction, and the duplex-like complex is as relatively rigid as a normal DNA duplex and then draws the Cy5 tags far away from the NPG surface, leading to the decrease in Raman signals from Cy5 probes.\textsuperscript{26} Based on the mercury ion-induced hybridization, when voltage is applied, the amount of mercury ions around the NPG film can effectively increase in a short time and enables mercury ions to bind to the aptamer faster, thereupon improving the detection sensitivity and detection speed. The sensitive and quantitative detection of Hg\textsuperscript{2+} ions is

Figure 1. Nanoporous gold-based hybrid sensor and its working mechanism. (a) Typical SEM micrograph of nanoporous gold. (b) Experimental setup for Hg\textsuperscript{2+} detection. (c) Schematic description of the NPG/aptamer sensor for Hg\textsuperscript{2+} detection based on voltage enrichment.

Figure 2. Performances of the hybrid sensor. (a) SERRS spectrum of Apt15-modified NPG film and characteristic Raman peaks from Cy5 tags can be seen clearly. (b, c) Characteristic peaks of Cy5 at 555 and 1362 cm\textsuperscript{-1} with different concentrations of Hg\textsuperscript{2+}. The SERRS intensity of Cy5 reduces with the increase in Hg\textsuperscript{2+} concentration. (d, e) Normalized SERRS intensity variation (I/I\textsubscript{0}) of bands at 555 and 1362 cm\textsuperscript{-1} as a function of Hg\textsuperscript{2+} concentration in PBS buffer. I\textsubscript{0} is the SERRS intensity of Cy5 from the sensor in PBS buffer only. Square, sensor with an applied voltage in solution without Hg\textsuperscript{2+}; triangle, sensor without an applied voltage in solution with various Hg\textsuperscript{2+} concentrations; circle, sensor with an applied voltage in solution with various Hg\textsuperscript{2+} concentrations.
performed by monitoring the intensity changes of Raman signals with Hg\(^{2+}\) ion concentrations.

According to the correlation between the characteristic Raman peak intensity and the mercury ion concentration, the strongest Raman peak of Cy5 at 1362 cm\(^{-1}\) was selected for quantitative analysis. Figure 2a shows the baseline-corrected SERRS spectra of Cy5 tags obtained on the Apt15-modified NPG film. The relative intensity of the SERRS signals from Cy5 tags decreases with increasing concentration of Hg\(^{2+}\) ions, and the application of voltage further enlarges the intensity variation. Two relative stronger bands at 555 and 1362 cm\(^{-1}\) were chosen for quantitative analysis based on the intensity dependence of the characteristic Raman peaks on Hg\(^{2+}\) concentrations. As shown in Figure 2b,c, the variation ratios of the selected characteristic peaks are the same. When 0.1 pM Hg\(^{2+}\) is added, the detectable Raman peak intensity drop of \(\sim 5\%\) (Figure 2d,e) can be measured for the one without voltage; further, when voltage is applied, the intensity drops to \(\sim 13\%\) (Figure 2d,e). In general, the decrease in the Raman intensity with an applied voltage is more than twice as large as that without voltage. Apparently, the optical sensor assistant with voltage enrichment can improve the sensitivity by several orders of magnitude. In a previous study, the detection limit of 15-thymine aptamer-functionalized NPG was around 1 nM\(^{5}\); attractively, combining with voltage assistant ion beneficiation, the detection limit approached 0.1 pM with a dynamic detection range up to 10 µM, which is four orders of magnitude wider than that without an applied voltage. \(^{4}\)

To verify the tolerance of the hybrid sensor, not only the isolated Hg\(^{2+}\) ions but also mixtures that are analogous to the natural environments were detected. The T–Hg\(^{2+}\)–T interaction has high specificity and provides eminent selectivity for the detection of Hg\(^{2+}\) in water. As shown in Figure 3, the variation is less than 10%.

The function of applying a voltage pushes the Hg\(^{2+}\) ions toward the aptamer quickly, leading to the efficient and sensitive detection of Hg\(^{2+}\) ions. Compared to previous works about mercury detection based on NPG/aptamer SERRS sensors without voltage enrichment, we have proved that the hybrid method is more efficient and sensitive. Hg\(^{2+}\) ions can be detected at concentrations as low as 0.1 pM with a dynamic detection range of eight orders of magnitudes. Given these advantages, it is an excellent way to detect Hg\(^{2+}\) pollution in drinking water and groundwater and even in body fluids. This hybrid sensor is expected to have considerable potential in the application of real-time detection of Hg\(^{2+}\) ions.

■ CONCLUSIONS

We have developed a method for constructing an optical sensor assistant with voltage enrichment to detect Hg\(^{2+}\) ions. The function of applying a voltage pushes the Hg\(^{2+}\) ions toward the aptamer quickly, leading to the efficient and sensitive detection of Hg\(^{2+}\) ions. Compared to previous works about mercury detection based on NPG/aptamer SERRS sensors without voltage enrichment, we have proved that the hybrid method is more efficient and sensitive. Hg\(^{2+}\) ions can be detected at concentrations as low as 0.1 pM with a dynamic detection range of eight orders of magnitudes. Given these advantages, it is an excellent way to detect Hg\(^{2+}\) pollution in drinking water and groundwater and even in body fluids. This hybrid sensor is expected to have considerable potential in the application of real-time detection of Hg\(^{2+}\) ions.

■ EXPERIMENTAL METHODS

Materials and Equipment. The synthesized aptamer was supplied by TaKaRa Biotech. Mercury pernitrate and 69% nitric acid were purchased from Sinopharm Chemical Reagent Co., Ltd. The electrochemical workstation (Chi1230C) was produced by Chenhua. Ultrapure water (18.25 MΩ*cm) was regenerated by immersing in 100 mM ascorbic acid solution for 1 h and then washed with 33 mM PBS buffer (pH= 6.9) solution for 15 min. Figure 4 displays seven representative cycles of the hybrid sensor for detecting 0.1 nM Hg\(^{2+}\) in aqueous solution. Compared to the original Raman peak, it is observed that the Raman peak with 0.1 nM Hg\(^{2+}\) is reduced to 70–80% with the variation ratio about 5–8% within each cycle. Although the relative intensity of the SERRS signal from the immobilized aptamers cannot be fully recovered, the variation is less than 10%.

Figure 4. Normalized Raman peak intensities of the 1362 cm\(^{-1}\) band with 0.1 nM in the aqueous solution for seven cycles.
produced by a machine from Chengdu Youpu Biotechnology Co., Ltd. A scanning electron microscope (SEM, Thermo-Fisher Scientific, USA) and a homemade Raman spectroscope were used for microstructure characterization and spectral measurement.

**Preparation of Nanoporous Gold Films.** The 100 nm-thick NPG films were prepared by selective corrosion of Ag from Ag65Au35 (atom %) alloy leaves, making use of 69% nitric acid for 10 min at room temperature.27,28 The prepared films were washed several times with ultrapure water to wash away the residual nitric acid. Then, the NPG films were fixed on the indium tin oxide (ITO) glass and annealed at 100 °C for 2 h to ensure that the films could be stably combined with it.

**Aptamer–NPG Hybrid Optical Sensor.** The sequence of synthetic aptamer (5′-SH-(CH2)6-(TTT...)_15-Cy5-3′) was synthesized by Shanghai Haojia Technology Development Co., Ltd. The NPG substrates fixed on the ITO glass were immersed in the 0.5 mL aptamer solution containing 100 nM aptamer and 1.0 M NaCl in 10 mM PBS buffer (pH = 7.4) for 12 h at room temperature to allow aptamers to self-assemble on the substrates. After washing with PBS buffer (pH = 6.9) containing 0.5 M NaCl and 0.1 M NaClO4 several times, the sensor was preserved in PBS buffer at 5 °C and can maintain the activity for beyond 30 days.

**Raman Spectroscopy.** A Raman spectrometer with 632.8 nm laser excitation was used for Raman measurements. The experimental device is shown in Figure 1, the NPG films covered with the ITO glass was connected to the working electrode, and the platinum wire was inserted into one side of the round sample stage working as the counter electrode, and then a PBS buffer (pH = 6.9) solution with different concentrations of metal ions was filled in the stage as the electrolyte. The power of laser for Raman measurements was set to 0.03 mW to avoid excessive damage of the molecule. The SERRS spectrum was the average of at least five spectra obtained on the substrate after applying a constant voltage (−0.5 V) for 5 min.

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**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**
This work was partly supported by the National Natural Science Foundation of China (61675133, 11561121003, 11727812, and 51502092), the Shanghai Municipal Science and Technology Commission (17ZR1447500), and the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning (TP2015028).

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