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Ultrasensitive and selective gold film-based detection of mercury (II) in tap water using a laser scanning confocal imaging-surface plasmon resonance system in real time

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ABSTRACT

An ultrasensitive and selective detection of mercury (II) was investigated using a laser scanning confocal imaging-surface plasmon resonance system (LSCI-SPR). The detection limit was as low as 0.01 ng/ml for Hg^{2+} ions in ultrapure and tap water based on a T-rich, single-stranded DNA (ssDNA)-modified gold film, which can be individually manipulated using specific T–Hg^{2+}–T complex formation. The quenching intensity of the fluorescence images for rhodamine-labeled ssDNA fitted well with the changes in SPR. The changes varied with the Hg^{2+} ion concentration, which is unaffected by the presence of other metal ions. The coefficients obtained for ultrapure and tap water were 0.99902 and 0.99512, respectively, for the linear part over a range of 0.01–100 ng/ml. The results show that the double-effect sensor has potential for practical applications with ultra sensitivity and selectivity, especially in online or real-time monitoring of Hg^{2+} ions pollution in tap water with the further improvement of portable LSCI-SPR instrument.

1. Introduction

Hg^{2+} ions are one of the most toxic heavy metal ion pollutants, and are caustic and carcinogenic materials with high cellular toxicity. The Hg^{2+} ions can accumulate in the human body through the food chain and cause serious and permanent damage to the brain with both acute and chronic toxicity (Aragay et al., 2011; Knecht and Sethi, 2009). According to the US Environment Protection Agency (EPA) guidelines, Hg^{2+} ions must be at concentrations below 1 ng/ml (10 nM) in drinking water. The traditional testing for Hg^{2+} ions includes atomic absorption, atomic fluorescence, and inductively coupled plasma mass spectrometry (Leermakers et al., 2005; Pourreza and Ghanemi 2009; Acon et al., 2000; Bings et al., 2006). These methods are usually coupled with gas chromatography, high-performance liquid chromatography, and capillary electrophoresis. However, these instrument-based techniques are rather complicated, time-consuming, costly, and unsuitable for online and portable use. Recently, several methods for detecting Hg^{2+} ions based on chemiluminescence, nanotechnology, proteins, and isothermal nucleic acid amplification have been developed (Liu et al., 2009a; Li et al., 2008; I et al., 2012; Wang et al., 2010a; Wang et al., 2010b). Although ultra sensitivity can be achieved by organic small-molecule fluorescence sensors and surface-enhanced raman scatting (SERS) sensors with a 5–10 ng/ml detection limits (Wang et al., 2012; Han et al., 2010; Li et al., 2013), such technologies are still relatively underdeveloped for use as portable equipment for online real-time monitoring. Methods with a high degree of sensitivity and simplicity are still needed to meet practical requirements, especially for the development of ultraportable equipment with real-time applications.

Surface plasmon resonance (SPR) is the most widely used technique for in situ and real-time measurements of probe-target interactions at surfaces (Mazumdar et al., 2010; Bombera et al., 2012; Jose et al., 2010; Bolduc et al., 2010; Linman et al., 2008). The highly sensitive optical reflectivity of gold to dielectric changes in the environment enables the SPR sensor to detect traces of contaminants in water, food, air, and label-free biochemical assays (Matsunaga et al., 2007; Choi et al., 2009 Teramura and Iwata 2007; Jecklin et al., 2009). SPR sensing is particularly valuable in verifying interactions between biomolecules (such as antigen–antibody) and quantifying their equilibrium and kinetic constants, as well as the underlying energy, to understand the mechanics of the interaction (Ayela et al., 2007; Williams et al., 2010). During SPR sensing, the interaction process is deduced from optical response changes based on the resonance angle or wavelength that were dependent on the refractive index or thickness of the medium adjacent to the metal film. However, SPR results only show the average effect of the testing region. Non-specific adsorption influences its accuracy. SPR has been previously integrated with other methods to avoid non-specific adsorption and improve

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accuracy, such as SPR-mass spectrometry (SPR-MS), electrochemical SPR (EC-SPR), LSCI-SPR, etc. (Dobrin 2007; Wang et al., 2010b; Zhang et al., 2012). LSCI-SPR has two lasers, one is the light source of SPR and the other is used to excite fluorescence probe on SPR sensor's surface. This LSCI-SPR probe is different from SPFS (surface plasmon-enhanced fluorescence) and can inhibit the serious interfere between SPR and fluorescence spectrum (Attridge et al., 1991; Schmidt et al., 1998; Neumann et al., 2002).

In this paper, an ultrasensitive and selective quantitative detection method for Hg^{2+} ions based on T–Hg^{2+}–T complex formation and LSCI-SPR was developed by integrating a wavelength modulation SPR with an LSCI in real-time and in situ. Hg^{2+} ions can selectively bind between the thymine (T) bases of the two strands and promote these T–T mismatches to form stable T–Hg^{2+}–T base pairs. For the device used in this study, the specific strands were assembled on the gold-covered slide. The presence of Hg^{2+} ions in the sample solution triggered the movement of SPR signals in line with the change in the index and thickness of the gold film surface. The ssDNA folded into the T–Hg^{2+}–T mediated hairpin structure and this conformational change drew the rhodamine moiety close to the gold film surface. SPR on the gold film surface can quench fluorescence within a certain distance (Lu et al., 2006; Liaw et al., 2012). The quenching intensity of fluorescence for rhodamine labeled in a T-rich ssDNA fitted well with the changes in both the Hg^{2+} ions concentration and resonance in the complex formation process. This method showed high selectivity and ultra sensitivity for Hg^{2+} ions with the detection limit of 0.01 ng/ml in ultrapure and tap water, which has greatly surpassed the upper limit of Hg^{2+} levels defined by EPA of the USA. In addition, the detection process is much simpler than these works by the integration of SPR on the surface of the gold film and the local SPR of the gold nanoparticle (Chang et al., 2011; Wang et al., 2010).

2. Experiment

2.1. Materials and reagents

The sequence of the ssDNA purchased from Beijing Lu He Tong Trade Co. was 5′–HS-(CH_2)6-CTT GTT TCT CCCCCC TGT TTC TTG-rhodamine-3′, doubly labeled with thiol and rhodamine at the 5′- and the 3′-ends, respectively Mercury perchlorate and other metal perchlorate salts were obtained from Beijing Chemical Regents Co. and used without further purification. All solutions were prepared with ultrapure water (18.4 MΩ cm, Millipore Corp.) and tap water.

2.2. LSCI-SPR instrument

The schematic diagram of the LSCI-SPR experimental setup was similar to that described in our work (Zhang et al., 2012) (Fig. S1). The wavelength-dependent SPR sensor was built with modifications of two important devices, the polarizer and flow cell. White light from a LED (HLV-24SW-3W, Japan) was collimated and polarized by transverse-magnetic polarization using a Glan Taylor prism with the 5×10^{-5} extinction ratio. The flow cell was made with a 0.13 mm thick bottom to suit the confocal image requirement. The reflected light was collected into a fiber spectrometer (AH-ISW301, China) equipped with a photomultiplier tube. The angle of incidence was varied liberally by adjusting the rotation arms of the instrument by two rotary stages (KS432-75, Japan), and the resonance minimum at a given wavelength was positioned accordingly. The biosensor substrates were prepared by sputtering 2 nm Cr and 50 nm Au onto BK7 slides with much (111) plane and less (222) plane (Fig. S2). The side of slide with Au film was immersed in the flow cell with a 0.13 mm thick bottom, and the blank side was attached to the prism using refractive index matching liquid. The prism, substrate, and flow cell were fixed on the stage of the inverted microscope of the LSCI (Nikon C1 Si, Japan) system. A 40 × objective (Nikon CFI Plan Flour ELWD DM N. A. 0.6W.D. 3.7 mm to 2.7 mm) captured with a multi-line Ar laser the fluorescence image of the fluorescein-labeled sample at 488 nm. The focal plane was positioned on the gold film to detect the fluorescence image when Hg^{2+} ions were inserted in the flow cell, thereby inducing the ssDNA to form stable T–Hg^{2+}–T base pairs. The samples were then injected into the flow cell by a spring pump (Harvard 33 Twin Syringe Pump, USA). LSCI-SPR instrument intergraded a wavelength dependent SPR with a laser scanning confocal microscopy can successfully monitor the process of species interaction in real-time and in situ by combining the virtues (identify the interaction process of bimolecular and imagining) of the two techniques.

2.3. LSCI-SPR sensor-chip fabrication and characterization

Gold-covered slides were soaked in 0.3 mg/ml ssDNA water resolution for 24 h at 4 °C and attached to the prism on the side without the ssDNA self-assembly (SAM) film containing the index matching fluid. The coverage of the ssDNA on the gold film was estimated to be about 293 ng/cm^2 (3.68×10^{-11} mol/cm^2) by the method minded in the work of Warmack (Thundat et al., 1995; Chen et al., 1995). The FT-IR spectra of the ssDNA covered gold film exhibited the absorption peaks at 1716, 1647, 1556, 1420 and 1260 cm^{-1} (Fig. S3), which corresponded to the functional groups of ssDNA according the previous studies (Rafique et al., 2013; Ramulu et al., 2013). The ultrapure water or tap water flowed from the surface of the self-assembly film for about 10 s until the SPR signal (Fig. S4) and fluorescence intensity reach the steady-state, indicating the excess ssDNA which non-specifically bind on the gold-covered surface was removed. The different Hg $(ClO_4)_2$ concentrations were added to the flow cell at 37 °C.

2.4. Detection measurements

The fluorescence images and SPR curve were recorded during the formation of the T–Hg^{2+}–T base by focusing the LSCI-SPR system on the local sites of interest. With the same ssDNA-covered gold film, different Hg^{2+} concentrations were injected into the flow cell. The ssDNA used in our work are flexible as the referee's description (Han et al., 2010). The SPR signal data were collected with a Labview program and recorded the reflection intensity (I) fixed on a point of the resonance peak with the maximum slope. The changes of SPR reflection intensity (ΔI) were achieved by setting SPR reflection I of the ultrapure water or that of the tap water without metal ions as a reference. All the experiments are done at the same conditions. The change in the SPR reflectance intensity based on Hg^{2+} concentration was corrected to evaluate the detection limit and the linear relationship. Various competing divalent metal ions, such as Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+}, Cr^{3+} and Zn^{2+}, were inputted into the cell at 1000 ng/ml concentration respectively to prove the selective detection for Hg^{2+} at 100 ng/ml. The fluorescence images and SPR curve were recorded in real time. The original SPR peaks of different Hg^{2+} ion concentrations in ultrapure water and tap water are shown in Fig. S5 and S6, respectively.

3. Results and discussion

3.1. Principle of the LSCI-SPR sensor

The sensing principle is shown in Fig. 1. Generally, Hg^{2+} ions can involve a T-rich ssDNA through specific T–Hg^{2+}–T complex formation between the T bases of the two strands and promote