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Short communication

Gold-nanoparticle-based graphite furnace atomic absorption spectrometry amplification and magnetic separation method for sensitive detection of mercuric ions

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ABSTRACT

We have developed a sensitive gold-nanoparticle-based (AuNP-based) graphite furnace atomic absorption spectrometry (GFAAS) amplification and magnetic separation method for the detection of mercuric ions (Hg^{2+}). The assay relies on (i) a sandwich-type structure containing two thymine–thymine (T–T) mismatches for selectively recognizing Hg^{2+} ions; (ii) magnetic beads for homogeneous separation; and (iii) AuNP-based GFAAS amplification detection. The limit of detection (LOD) of this assay is 0.45 nM (0.09 $\mu g\,L^{-1}$) – one order of magnitude lower than the United States Environmental Protection Agency (US EPA) limit for Hg^{2+} in drinking water. Furthermore, because a shorter hybridization step and a simpler AuNP-based GFAAS amplification detection were employed, a faster analytical run time allowing us to analyze a batch of 24 samples within 0.5 h. We demonstrated the feasibility of the developed approach for the determination of Hg^{2+} in urine and aqueous environmental samples.

1. Introduction

Mercuric ions are toxic to humans because they accumulate in living tissues, even at very small doses. (Quig, 1998) Hg^{2+} ions are usually ingested by human beings through the food chain or from polluted water; Hg^{2+} is excreted from the human body mainly through urine, when exposure is high level. (Pavlogeorgatos and Kikilias, 2003) The mercury concentrations in human urine and serum have a positive correlation with exposure, so urinary mercury concentration is widely used as an exposure biomarker for mercury poisoning.(Chen et al., 2005) However, the concentrations of mercury in drinking water and in urine samples are in the range of micrograms per liter or lower [urine $\leq 10\,\mu g\,L^{-1}$; water $\leq 2\,\mu g\,L^{-1}$]; (World Health Organization, 2003; Santa Rosa et al., 2000) thus, it is important to develop sensitive and reliable analytical techniques capable of measuring nanogram levels of mercury.

To date, various novel sensing techniques have been developed for the determination of Hg^{2+}, using new probes such as small molecules (Huang and Chang, 2007), polymers (Liu et al., 2007), nanocrystals, (Zhu et al., 2005) and proteins (Wegner et al., 2007). However, some of these detection methods have limitations such as low sensitivity (LOD typically > 100 nM), poor water solubility, and poor selectivity with respect to the matrix ions. Recently, it was demonstrated that oligonucleotide sequences can specifically trap Hg^{2+} ions between two DNA thymine (T) bases and promote these T–T mismatches to form stable T–Hg^{2+}–T base pairs (Ono and Togashi, 2004). This phenomenon has been extensively employed in the determination of Hg^{2+} ions using polymerase-based amplification (Zhu et al., 2010), DNAzymes (Xiang et al., 2010), quantum dots (Li et al., 2007), AuNPs (Liu et al., 2008) and membrane blotting (Li et al., 2009). Lee et al. (2007) recently designed a colorimetric sensor to detect Hg^{2+} ions by quantifying the changes in the melting temperature of duplex containing T–Hg^{2+}–T complexes as a function of Hg^{2+} concentration. To simplify the method, Xue et al. (2008) and He et al. (2008) changed the structures or lengths of the DNA strands used, and developed an analytical procedure in which the duplexes without Hg^{2+} can be unwound and separated into single strands at ambient temperature. Although both the approaches described above have the advantage of being easily read with the naked eye, they are limited with respect to detection sensitivity. To enhance the detection capability of T–Hg^{2+}–T-mediated sensing systems, Mirkin's group has also taken advantage of the cooperative-binding, catalytic properties of DNA-functionalized AuNPs, and the selective binding of T–T mismatches for Hg^{2+}, to develop a more sensitive method using scanometric chip-based detection system by measuring the scattered light emitted from the resulting silver spots (LOD was 10 nM) (Lee and Mirkin, 2008). However, as described by Dai et al. (2008) the silver amplification method involves compli-

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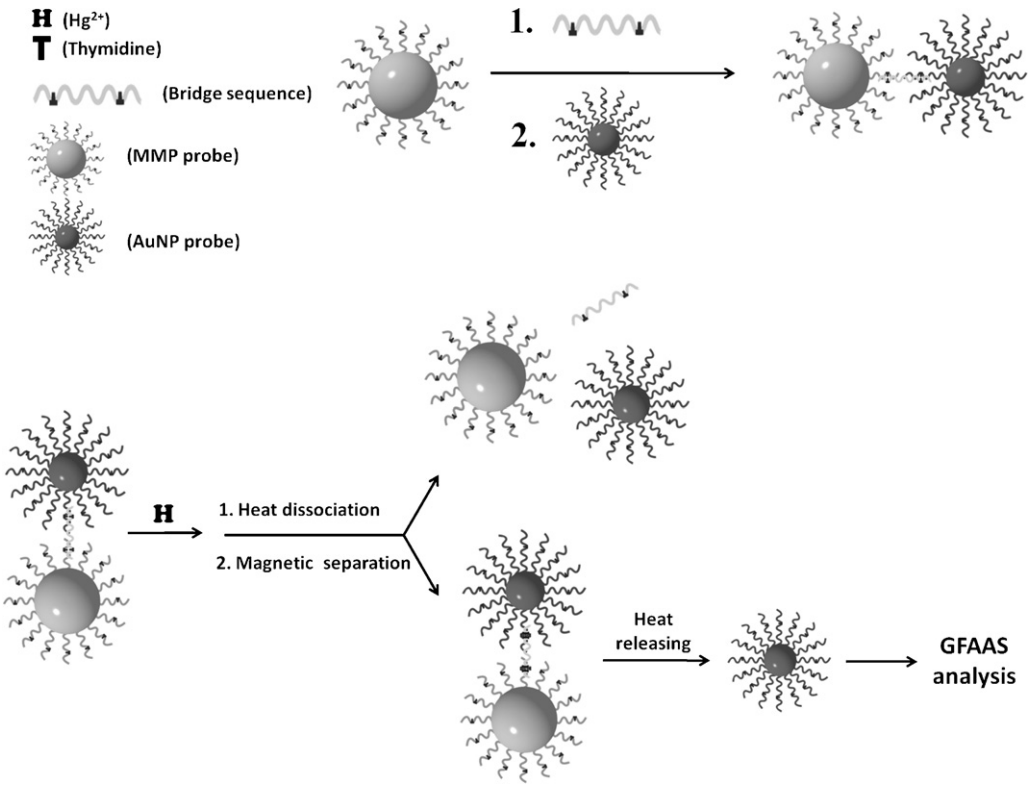

Scheme 1. AuNP-based GFAAS amplification method for the sensitive detection of Hg^{2+} Ions.

cated procedures that are not only time consuming but also often cause problems in reproducibility. In dealing with the health and environmental issues of mercury, the challenge of developing simple, environmentally friendly, and reliable analytical techniques to measure nanogram levels of Hg^{2+} in biological and environmental water samples remains. Because a 20 nm spherical nanoparticle contains approximately 10^5 atoms, to improve the simplicity and analytical sensitivity of T–Hg^{2+}–T-mediated sensing systems, we developed a highly sensitive AuNPs-based GFAAS amplification method for the detection of Hg^{2+} in urine and aqueous samples. The experimental results showed that a detection limit at the subnanogram-per-milliliter level was achieved, which is lower than the concentration (10 nM) in drinking water accepted by the US EPA.

2. Experimental

2.1. Instrumentation

Au determination was performed using a GFAAS (Analyst 600, Perkin-Elmer, Norwalk, CT, USA). The instrument operating conditions selected for the Au determination are listed in Table S1. Absorbance measurements for oligonucleotides and AuNPs were collected using a Helios Delta UV–vis spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Quadrupole ICP-MS (Agilent 7500a, Agilent Technologies, Inc., Santa Clara, CA, USA) was used to determine the original concentrations of Hg^{2+} in water samples.

2.2. Chemicals and materials

AuNPs (20 nm), solvents, and reagents were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). A solution of amino-functionalized magnetic microparticles (MMPs, 2.8 μm; Dynabeads® M-270 Amine) and a magnetic particle concentrator (Dynal MPC™-9600) were obtained from Invitrogen (Carlsbad, CA, USA). Oligonucleotides were purchased from Integrated DNA Technology (Coralville, IA, USA).

2.3. Detection procedure

The assay was conducted using a typical sandwich format (Scheme 1). In a typical experiment, the MMP probes (50 μL, 0.5 mg mL^{-1}) were placed in a 1.5 mL microcentrifuge tube and mixed with the bridge sequence (20 μM, 1 μL) for 2 h. After removing the residual bridge sequence, the AuNP probes (1 nM, 30 μL) were mixed with the complex. After 2 h, the unhybridized AuNP probes were removed by washing the sandwich structure with a washing buffer solution. The hybridization efficiency of the AuNPs was calculated by measuring the concentration of AuNPs released from the MMP surfaces. The results showed that there were approximately 4×10^{-15} mol of AuNP probes (a 20 nm AuNP is approximately 8×10^{-17} g) hybridized to the MMP probes before we used the T–T-mismatch-containing sandwich structure to recognize Hg^{2+}. Subsequently, different concentrations of Hg^{2+} (1 mL) were examined in parallel. The incubation time at room temperature for the formation of stable T–Hg^{2+}–T complexes was set at 5 min. The sample solution was then replaced by washing buffer and the mixtures were heated at 58 °C for 5 min under gentle agitation to release the AuNP probes from the uncomplexed sandwich structures. After washing with washing buffer (1 mL, 5 times), the sandwich structures were re-suspended in distilled water (100 μL) and heated for 10 min at 70 °C to release the AuNP probes from the Hg^{2+}-complexed sandwich structures. Finally, the resultant solutions were removed and mixed with aqua regia [1% (v/v), 100 μL]. The dissolved Au ions were then introduced into the GFAAS