Short communication

Highly sensitive and selective photoelectrochemical DNA sensor for the detection of Hg^{2+} in aqueous solutions

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ABSTRACT

A “turn-on” photoelectrochemical sensor for Hg^{2+} detection based on thymine- Hg^{2+} -thymine interaction is presented by using a thymine-rich oligonucleotide film and a double-strand DNA intercalator, $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ (bpy =2,2'-bipyridine, dppz =dipyrido[3,2-a:2',3'-c]phenazine) as the photocurrent signal reporter. The presence of Hg^{2+} induces the formation of a double helical DNA structure which provides binding sites for $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$. The double helical structure was confirmed by circular dichroism and fluorescence measurements. Under the optimized conditions, a linear relationship between photocurrent and Hg^{2+} concentration was obtained over the range of 0.1 nM to 10 nM Hg^{2+} , with a detection limit of 20 pM. Interference by 10 other metal ions was negligible. Analytical results of Hg^{2+} spiked into tap water and lake water by the sensor were in good agreement with mass spectrometry data. With the advantages of high sensitivity and selectivity, simple sensor construction, low instrument cost and low sample volume, this method is potentially suitable for the on-site monitoring of Hg^{2+} contamination.

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1. Introduction

Mercury contamination is now widespread with both natural and anthropogenic sources including volcanic and oceanic emission (Renzone et al., 1998), coal and gold mining (Olaf, 1998), fossil fuel combustion (EPA, 2001), and so forth (Nolan and Lippard, 2008). Mercury is one of the most toxic elements that impacts human and ecosystem health. Once introduced into the water environment, inorganic mercury can also be converted to methylmercury by bacteria, which accumulates through food chain and is more toxic than inorganic mercury (Harris et al., 2003). Because of its persistence in the environment, bioaccumulation and high toxicity, mercury species have been listed as a priority pollutant by many countries and international agencies (Leopold et al., 2010). Therefore, routine monitoring of mercury in the environment is essential for risk assessment.

Traditional methods for Hg^{2+} analysis include cold vapor atomic absorption spectroscopy (Bloom et al., 2004), atomic fluorescence spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), and so forth. However, most of them require complicated sample preparation and expensive instruments, and are thus not suitable for routine monitoring. As alternatives, Hg^{2+} sensors by the use of chromophores, fluorophores and conjugated polymers have

been developed (Buica et al., 2009; Coronado et al., 2005; Nolan and Lippard, 2008; Rurack et al., 2000; Zhao and Zhong, 2006). Nevertheless, these chemical sensors are often hampered by interference from other metal ions and poor water solubility of the synthetic molecules, delayed response to Hg^{2+} , and/or insufficient sensitivity. As a result, it is still of great challenge to developing methods for Hg^{2+} analysis with high sensitivity, selectivity and simplicity.

Thymine (T) bases have been found to form T- Hg^{2+} -T base pair (Katz, 1952). By using ^{15}N NMR, ^{15}N - ^{15}N J-coupling across Hg^{2+} was observed and definite chemical structure of T- Hg^{2+} -T was determined by Ono (Tanaka et al., 2007). According to the report, thymine residues bind to Hg^{2+} through the formation of N- Hg^{2+} -N bond (N3 of thymine), accompanied with the release of two imino protons (Tanaka et al., 2007). The Hg^{2+} mediated T- Hg^{2+} -T pair is even more stable than the Watson-Crick (WC) A-T pair (Miyake et al., 2006). Based on this mechanism, DNA sensors have been developed for Hg^{2+} detection. Many of them took advantage of the competitive interaction of T-rich single-strand DNA (ss-DNA) with Hg^{2+} and nanomaterials to modulate colorimetric, fluorescent, circular dichroism (CD), resonance scattering or electrochemical signal (Gao et al., 2008; Jiang et al., 2009; Li et al., 2008; Miao et al., 2009; Wang et al., 2008; Xue et al., 2008; Zhang et al., 2010a). Fluorescent and electrochemiluminescent sensors were also reported by using organic dyes or DNA-intercalating ruthenium complexes (Ono and Togashi, 2004; Tang et al., 2010; Zhang et al., 2010c). Most of the DNA sensors demonstrated high selectivity toward Hg^{2+} due to the specific interaction between the metal ion

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and T bases. However, with only a few exceptions (Jiang et al., 2009; Tang et al., 2010; Zhang et al., 2010c), the detection limit of these sensors is at the nanomolar level.

Photoelectrochemistry based detection has a number of inherent advantages. Due to the separation of excitation source (light) and detection signal (photocurrent), photoelectrochemical detection has low background and high sensitivity. Because it can use low-powered light source and simple electronic circuit, the instrumentation cost is very low. Not surprisingly, photoelectrochemical biosensors for DNA damage, DNA hybridization and immunoassays have advanced rapidly in recent years (Haddour et al., 2006; Liang and Guo, 2007; Liang et al., 2006; Okamoto et al., 2005; Tokudome et al., 2005; Zhang et al., 2011; Zhao et al., 2012; Zhao et al., 2011). In comparison, photoelectrochemical detection of small molecules has been rare (Chamier et al., 2010; Zhang et al., 2010b). In this work, we developed a Hg^{2+} sensor by combining the sensitivity and simplicity of photoelectrochemical detection with the specificity of T– Hg^{2+} –T interaction. The sensor has the potential to be employed for on-site environmental monitoring.

2. Material and methods

2.1. Materials and reagents

Poly-(diallyldimethyl ammonium chloride) was from Sigma-Aldrich (St. Louis, MO, USA). TiO_2 nanoparticles (15 nm) were obtained from Alfa Aesar (Ward Hill, MA, USA). $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ (bpy = 2,2'-bipyridine, dppz = dipyrrodo[3,2-a:2',3'-c]phenazine) was synthesized previously (Liang and Guo, 2007). All other chemicals and solvents were of analytical grade. Buffer used to dissolve oligonucleotides was 10 mM Tris–HCl (pH 8.0). The stock solution of Hg^{2+} was prepared from HgCl_2 , with a concentration of 1 mM. Water used for the preparation of solutions and buffers was from a Millipore Milli-Q (Biocel) water purification system. Indium tin oxide (ITO) conductive glass was from Weiguang Corp. (Shenzhen, Guangdong Province, China) and cut into 2.5×0.5 cm sized electrodes. The synthetic oligonucleotides were obtained from Sangon Corp. (Shanghai, China) and purified by PAGE. Their sequences are listed as follow:

- Probe T: 5'-TTTTTTTTTTTTTTTTTTTTTT-3'
- Probe A: 5'-AAAAAAAAAAAAAAAAAAAAA-3'
- Probe R: 5'-ATCGTTAGACTCCAACATCA-3'
- Probe S1: 5'-TTCGTGTTGTGTTTCTGTTGCG-3'
- Probe S2: 5'-CGCATTACAGATTCTACTCGTA-3'

2.2. Sensor preparation and Hg^{2+} detection

SnO_2 nanoparticle modified ITO electrodes were prepared as previously described (Liang et al., 2006). DNA film was assembled on the SnO_2 nanoparticle modified ITO electrode surface by layer-by-layer electrostatic adsorption, with poly-cationic PDDA as the first layer and poly-anionic nucleic acid as the second layer (Liang and Guo, 2007). The morphology of SnO_2 nanoparticle modified ITO electrode was characterized by scanning electron microscopy and atomic force microscopy (AFM) (Figs. S1 and S2). The morphology of DNA film on PDDA modified surface was characterized by AFM in our previous work (Wang et al., 2009). Oligonucleotide concentration for film deposition was 50 μM . After the film was assembled, a 10 μL solution of Hg^{2+} was added on the electrode and reacted at 25 °C for 30 min. After rinse, a 10 μL solution of 50 μM $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ was added on the electrode and reacted for 30 min. Finally, photocurrent was measured.

2.3. Apparatus

Photocurrent was measured on a CHI 830B electrochemical analyzer (CH Instruments, Austin, TX, USA) in 30 mM oxalate, pH 5.8, using a Pt flag counter electrode, a Ag/AgCl (3 M KCl) reference electrode, and a bias voltage of +0.1 V. The excitation source was a 473 nm light emitting diode (Lamp Inc., Shenzhen, China) with an illumination area of 0.2 cm^2 . Fluorescence measurements were performed on a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon, Edison, NJ, USA), using 450 nm excitation, 610 nm emission, and a slit width of 5 nm. UV–vis absorption spectra were measured on an Agilent 8453 UV–visible spectrophotometer (Palo Alto, CA, USA). CD spectra were recorded on a JASCO J-815 spectrometer (JASCO Corporation, Tokyo, Japan). The chemical analysis of Hg^{2+} was performed using an Agilent 7500a ICP-MS (Palo Alto, CA, USA).

3. Results and discussion

In our sensor design (Fig. 1A), the photoelectrochemical signal reporter, $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$, is a DNA intercalator which binds to a double-stranded DNA (ds-DNA) film on SnO_2 electrode by inserting its phenazine ring into the space between adjacent base pairs with high affinity (binding constant $K = 10^6$ – 10^7 M^{-1}) (Jenkins et al., 1992). Upon illumination, $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ absorbs photon energy and becomes excited. Injection of the excited electrons into the conduction band of SnO_2 produces photocurrent. Oxalate in the electrolyte acts as an electron donor to regenerate $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ and amplify photocurrent signal (Dong et al., 2004). In the absence of Hg^{2+} , the oligonucleotide maintains a single-strand structure. As there is no base pairs for the binding by $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$, the DNA film on the electrode binds very little signal reporter molecule, resulting in low photocurrent response (Liang and Guo, 2007). However, in the presence of Hg^{2+} , a double helical DNA structure is formed through T– Hg^{2+} –T interactions, which facilitates $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ intercalation, leading to a Hg^{2+} concentration dependent enhancement of photocurrent response.

Photocurrent response of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ bound to four types of oligonucleotide films was investigated, including a T-rich oligonucleotide (Probe T), an A-rich oligonucleotide (Probe A), a random sequence oligonucleotide (Probe R), and a 1:1 mixture of the two complementary oligonucleotides with eight T–T mismatches (Probe S1/Probe S2) which were used in previous studies (Xue et al., 2008; Zhang et al., 2010c). After separate reaction with Hg^{2+} and $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$, photocurrent enhancement was observed only on Probe T and Probe S1/Probe S2 films under the experimental conditions. However, the latter produced a high background response in the absence of Hg^{2+} . Therefore, Probe T was selected for sensor development.

Conformational change of Probe T induced by Hg^{2+} was investigated by CD and fluorescence spectroscopy. As shown in Fig. 1B, in the absence of Hg^{2+} , Probe T in 10 mM Tris–HCl buffer (pH 8.0) exhibited a negative and a positive CD band at 250 and 278 nm, respectively. However, incubation of Probe T with Hg^{2+} at 37 °C for 30 min led to a red shift of the negative band with increased intensity and the disappearance of the positive band. This change is similar to that of $d(\text{T}_3)$ reported by Miyake et al. (2006), and can be attributed to the binding of Hg^{2+} to T bases and subsequent formation of double helical structure. $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ is known as a molecular “light switch”, displaying no fluorescence by itself but lighting up after intercalating into ds-DNA (Jenkins et al., 1992). It is thus that $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ is very popular for the research of DNA structure by photoluminescence. We hypothesize that in an aqueous solution containing Hg^{2+} , Probe T might form a helical structure,

Fig. 1. (A) Schematic diagram of T-Hg²⁺-T based photoelectrochemical sensor. (B) CD spectra of 5 μM Probe T solution (a), and 5 μM Probe T and 100 μM Hg²⁺ reacted for 30 min at 37 °C (b). (C) Fluorescence spectra of a mixture of 5 μM Probe T and 5 μM Ru(bpy)₂(dppz)²⁺ after incubating with 0, 0.1, 2, 5 and 10 nM Hg²⁺ (from (a) to (e)) for 30 min at 25 °C.

Fig. 2. (A) Photocurrent response of the sensor in the presence of Hg²⁺ (from a to f: 0, 0.1, 1, 2, 5, 10 nM). Inset: linear relationship between photocurrent and Hg²⁺ concentration. (B) Effect of other metal ions on the detection of 5 nM Hg²⁺. The bars represent 5 nM Hg²⁺, PB (20 mM phosphate, pH 7.4), 1 μM another metal ion, and a mixture of 5 nM Hg²⁺ and 1 μM each of the ten other metal ions, respectively. Error bars represent the standard deviation of 3 replicates.

which would provide more overlaps for Ru(bpy)₂(dppz)²⁺ to bind on, resulting in a luminescent enhancement. As expected, the fluorescence of Ru(bpy)₂(dppz)²⁺ in the presence of Probe T increased progressively with the addition of Hg²⁺, indicating the formation of DNA duplex (Fig. 1C).

After optimization of oligonucleotide and Ru(bpy)₂(dppz)²⁺ concentration for sensor fabrication, the linear range and detection limit of the Hg²⁺ sensor was evaluated. The photocurrent increased with increasing Hg²⁺ concentration, and the dependence was linear in the range of 0.1 to 10 nM Hg²⁺ with an R^2 of 0.9969 (Fig. 2A). Benefiting from the use of oxalate as the electron

donor to amplify the photocurrent, a calculated detection limit of 20 pM was obtained, which is among the most sensitive sensors reported to date (Jiang et al., 2009; Tang et al., 2010) and is comparable with the result of ICP-MS coupled with electrochemistry (Ugo et al., 2001). The United States Environmental Protection Agency (EPA) has mandated an upper limit of 10 nM for Hg²⁺ in drinking water (EPA, 2001). Our sensor greatly exceeds the sensitivity requirement.

Selectivity of the sensor was assessed by measuring the photocurrent of the sensor in the presence of other metal ions (Na⁺, K⁺, Mg²⁺, Ca²⁺, Fe²⁺, Pb²⁺, Cu²⁺, Al³⁺, Fe³⁺, Mn²⁺). As shown in

Table 1

Determination of Hg^{2+} spiked into environmental water samples using photoelectrochemical sensor and ICP-MS.

Sample	Hg^{2+} added (nM)	Sensor (nM) ^a	Recovery (%)	ICP-MS (nM) ^a	Recovery (%)
Tap water	0	n.d. ^b	–	n.d. ^b	–
	2.00	2.05 ± 0.15	102	2.02 ± 0.50	101
	5.00	5.16 ± 0.09	103	5.21 ± 0.17	104
Lake water	0	0.46 ± 0.02	–	0.37 ± 0.15	–
	2.00	2.27 ± 0.04	92	2.24 ± 0.02	94
	5.00	5.41 ± 0.03	99	5.48 ± 0.05	102

^a Mean of three measurements $\pm$ standard deviation.

^b N.d. not detectable.

Fig. 2B, even with 200 times excess of another metal ion ($1 \mu\text{M}$ versus 5 nM Hg^{2+}), the photocurrent was substantially lower than with Hg^{2+} . This excellent selectivity can be attributed to highly specific interaction of T- Hg^{2+} -T. The response of the sensor in 5 nM Hg^{2+} did not change appreciably between pH 5–10 and between 0 and 100 mM NaCl, illustrating remarkable robustness. Compared with the homogeneous assays such as fluorescent methods, one advantage of the photoelectrochemical sensor is that it suffers less environmental interference, for the electrodes can be rinsed after every operation step, which also avoids the worry that signal response may be quenched by other metal ions.

The applicability of the sensor in the detection of environmental samples was demonstrated by measuring Hg^{2+} spiked into tap water and lake water from Taihu Lake, Jiangsu Province, China. The samples were measured by both the sensor and ICP-MS method. As shown in Table 1, both the mean concentration and recovery determined by the sensor are in excellent agreement with ICP-MS results, showing the potential practicality of the sensor for environmental samples.

4. Conclusions

By using a T-rich oligonucleotide film for specific T- Hg^{2+} -T interaction and a ds-DNA intercalator as the signal reporter, a “turn-on” photoelectrochemical DNA sensor was developed for the detection of Hg^{2+} . The detection principle was verified by the conformational change of the probe monitored CD and fluorescence spectroscopy in the presence of Hg^{2+} . Under the optimized conditions, a detection limit of 0.02 nM was obtained. Interference from other metal was negligible, and practicality of the sensor was demonstrated by detection of Hg^{2+} in tap water and lake water. This sensor is highly sensitive, selective and robust. In addition, it uses a low-cost instrument with a cheap light-emitting diode and simple electronics, and requires low sample volume ($10 \mu\text{L}$). It can be configured readily into a portable device for the routine on-site monitoring of Hg^{2+} in the aquatic environment. With the reported metal-base pairs, the sensor can easily be extended to the analysis of other metal ions (Clever et al., 2007).

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