An ultrasensitive electrochemical "turn-on" label-free biosensor for Hg^{2+} with AuNP-functionalized reporter DNA as a signal amplifier†

Rong-Mei Kong, Xiao-Bing Zhang,* Liang-Liang Zhang, Xiao-Yong Jin, Shuang-Yan Huan, Guo-Li Shen and Ru-Qin Yu*

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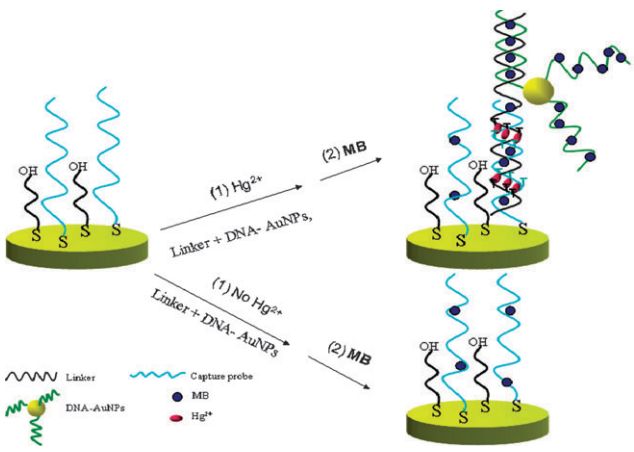
A highly selective electrochemical biosensor for the ultrasensitive detection of Hg^{2+} in aqueous solution has been developed based on the strong and specific binding of Hg^{2+} by two DNA thymine bases (T–Hg^{2+}–T) and the use of AuNP-functionalized reporter DNA to achieve signal amplification.

Mercury is a major environmental pollutant, as it is estimated by the United Nations Environment Programme (UNEP) that *ca.* 7500 tons of mercury is released into the environment annually.[1] Mercury also accounts for the majority of toxicity events in microorganisms and other species in the environment, even at low concentrations. The WHO and Ministry of Health of the P. R. China (MOH) define the toxic level of Hg^{2+} ions in drinking water as 0.001 mg L^{-1} or 5 nM. The development of methods for the highly sensitive and selective determination of mercury is therefore of significant importance for the environment and human health, and is the subject of current analytical chemical research. Towards this goal, many Hg^{2+}-sensitive sensors based on small molecule fluorophores,[2,3] proteins[4,5] and metal nanoparticles[6,7] have been reported. Although these methods have made great contributions toward the determination of Hg^{2+}, each of these approaches exhibits some features that limit its practical use, such as the poor aqueous solubility of some fluorophores, cross-sensitivity toward other metal ions and the sophisticated synthesis of probe materials. Therefore, it is desirable to develop new methods to overcome these limitations.

Since Ono *et al.* first reported that Hg^{2+} possesses a unique property to bind specifically to two DNA thymine bases (T) and promote these T–T mismatches to form stable base pairs,[8–10] the past five years have seen increasing interest in the development of fluorescent biosensors,[8,11,12] as well as gold nanoparticle-based colorimetric biosensors[13–17] using the unique strong T–Hg^{2+}–T interaction for the determination of Hg^{2+} in aqueous solution. All of these methods show excellent selectivity against other heavy and transition metal ions owing to the high specificity of the T–Hg^{2+}–T interaction. For environmental monitoring applications, such as the detection of Hg^{2+} ions in drinking water, a detection limit of lower than 5 nM is required. However, few reported mercury sensors could reach such a sensitivity. Lu *et al.* reported a uranium-specific DNAzyme-based catalytic beacon method for Hg^{2+} with signal amplification through allosteric interactions, giving a detection limit of 2.4 nM.[18] However, this method needs toxic uranium ions as co-factors. More recently, to overcome this drawback, they developed a structure-switching DNA-based fluorescent sensor for Hg^{2+} to achieve a detection limit of 3.2 nM.[19] Improving the sensitivity of oligonucleotide-based biosensors for Hg^{2+} is still an active field, as well as a challenge, in analytical chemistry[20–23]

Electrochemical biosensors have received increasing attention due to their remarkable features, such as high sensitivity, simple instrumentation, low production cost and promising response speed. Electrochemical biosensors for DNA, and aptamer-based biosensors for proteins and small molecules, have been extensively developed by Yu *et al.*[24–27] and many other groups.[28–33] AuNP-functionalized reporter DNA has been employed as an efficient amplifying tag for novel biosensors of various targets due to their unique properties.[34–37] However, to the best of our knowledge, no electrochemical biosensors based on the interaction of T–Hg^{2+}–T have been reported for Hg^{2+} assays. Herein, we describe a novel, sensitive and selective electrochemical biosensor for Hg^{2+}. The design of the electrochemical mercury biosensor is shown in Scheme 1. It is composed of three elements: a 5′-thiol-modified oligonucleotide containing six T-bases for Hg^{2+} binding was selected as a capture probe (DNA **1**); the second element was an appropriate oligonucleotide



Scheme 1 A description of the electrochemical Hg^{2+} sensing strategy based on the signal amplification of AuNP-functionalized reporter DNA.

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China. E-mail: xbzhang@hnu.cn, rqyu@hnu.cn; Fax: +86 731-8822782; Tel: +86 731-8822577

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linker (DNA **2**), with the 3′-terminal partially complementary with the capture probe sequence close to the 5′-terminal, except six T–T mismatches; the third element was 5′-thiol-modified DNA **3** functionalized AuNPs (AuNP-functionalized reporter DNA) that could specifically hybridize with the partial linker sequence close to the 5′-terminal. The capture probe was first immobilized on the gold electrode surface *via* the thiol–Au affinity, and then treated with 6-mercaptohexanol to block the non-specific sites on the surface and obtain an optimal oligonucleotide orientation for specific hybridization with complementary oligonucleotides.[38] When the sensing interface was incubated with solutions containing Hg^{2+} and the linker DNA, the capture probe could hybridize with the linker *via* the specific T–Hg^{2+}–T interaction, and the AuNP-functionalized reporter DNA (AuNPs modified with a large number of "*G*-rich" oligonucleotide strands) subsequently hybridized with the linker. The modified electrode was then immersed into a PBS solution containing methylene blue (MB). MB can specifically bind with guanine bases to form MB–guanine complexes,[39,40] resulting in a significant increase in the electrochemical signal. In the absence of Hg^{2+}, the linker does not hybridize with the capture probe because the melting temperature (T_m) is lower than the incubating temperature due to the T–T mismatches. Accordingly, few MB molecules are bound to the capture probe, resulting in a weak electrochemical signal. Here, it is important to point out that the thiophilic nature of Hg^{2+} does not replace the thiolated DNA on the Au surface and thus does not affect the stability of the biosensor, as proven by Mirkin *et al*. using a fluorophore-labeled oligonucleotide strategy.[13]

In order to acquire a larger signal-to-background ratio for Hg^{2+} sensing, AuNP-functionalized reporter DNA was adopted to provide considerable signal amplification.[34–36] Fig. 1 depicts the DPV signal of the AuNP-functionalized reporter DNA-based sensor, as compared with that of the direct T–Hg–T interaction. With the participation of the AuNP-functionalized reporter DNA, a large reduction peak current was observed (curve c). Meanwhile, a much smaller peak current was obtained in the direct assay (curve b). Therefore, we conclude that the AuNP-functionalized reporter DNA really plays an amplification role in the proposed sensing route.

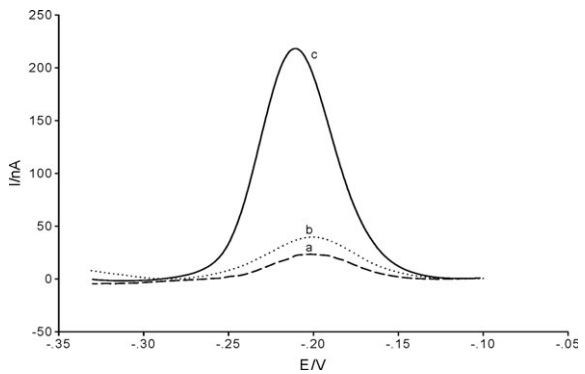

Fig. 1 DPV responses of the sensing interface: (a) in the absence of Hg^{2+}, (b) in the presence of Hg^{2+} without AuNP-functionalized reporter DNA and (c) in the presence of Hg^{2+} and AuNP-functionalized reporter DNA. The concentration of Hg^{2+} was 100 nM.

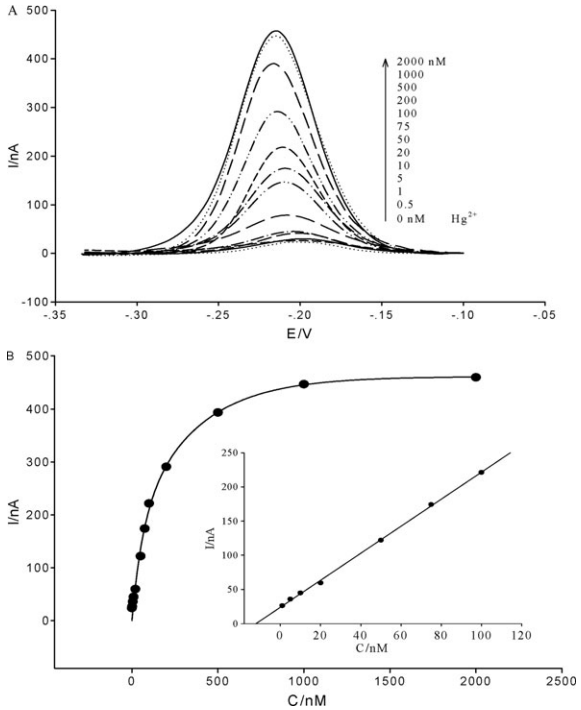

Fig. 2 A: DPV responses of the sensor after the addition of different concentrations of Hg^{2+} ions (0, 0.5, 1, 5, 10, 20, 50, 75, 100, 200, 500, 1000 and 2000 nM). B: Calibration curves of peak current as a function of Hg^{2+} concentration. Inset: the linearity of the relative peak current with respect to the Hg^{2+} concentration over the concentration range 1–100 nM.

Fig. 2A shows the DPV response of the sensor to target Hg^{2+} at different concentrations under optimal experimental conditions (see the ESI†). It is clear that the introduction of Hg^{2+} at different concentrations to the sensing system induced different increases in peak current, associated with the quantity of adsorbed MB molecules. Fig. 2B depicts the relationship between the response current and the increasing Hg^{2+} concentration, ranging from 0.5 nM to 2 μM. The Hg^{2+} could be quantified perfectly over a concentration variation from 1 nM to 0.1 μM, and a detection limit of 0.5 nM Hg^{2+} could be estimated using the 3σ rule. The low detection limit is lower than those obtained in T–Hg–T-based colorimetric sensors with gold nanoparticles,[13] fluorescent probes with FRET[8] and fluorescent probes based on a DNAzyme catalytic beacon,[18] making it suitable for drinking water monitoring.

The selectivity of the sensor for Hg^{2+} was investigated by recording the change of electrochemical signal before and after adding other environmentally-relevant divalent metal ions. As indicated in Fig. 3 (black bar), the sensor showed an almost neglectable response to other metal ions at 1 μM compared with that of Hg^{2+} at 100 nM. In addition, 100 nM of Hg^{2+} and 1 μM of another metal ion were added together in 10 mM PBS (pH 7.4). The electrochemical response of the Hg^{2+}–M^{2+} pair (Fig. 3, gray bar) suggests excellent selectivity for Hg^{2+} over other metal ions as well. All of these selective results indicate that our proposed biosensor could meet the selective requirements for biomedical and environmental applications.

Reusability is also an important issue for sensors. In the present study, the sensing interface could be easily regenerated

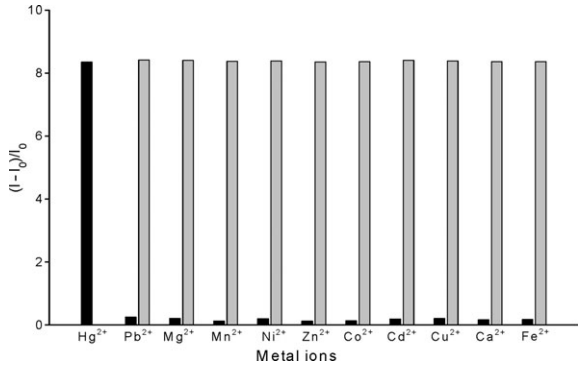

Fig. 3 DPV signal changes for the sensor after being treated with 100 nM of Hg^{2+} or 1 μM of other divalent metal ions (black bar), and mixtures of 1 μM of another metal ion with 100 nM of Hg^{2+} (gray bar). I_0 and I are the peak current of the sensor in the absence and presence of metal ions, respectively.

Table 1 The concentration of Hg^{2+} in water samples detected using the proposed biosensor

Sample	Added $[Hg^{2+}]$/nM	Proposed method mean[a] ± SD[b]/nM	Recovery (%)
Tap water 1	2.0	2.08 ± 0.14	104.0
Tap water 2	5.0	4.86 ± 0.34	97.2
River water 1	10.0	9.67 ± 0.28	96.7
River water 2	50.0	48.92 ± 0.46	97.8

[a] The mean of three determinations. [b] SD = standard deviation.

by immersing the electrode into 1 M NaOH at 50 °C for 15 min. It was found that the sensor could be reused more than three times without significant loss of its original sensing efficiency. The stability of the sensor for storage was also investigated, and it could still be used for the measurement of Hg^{2+} without any significant change of current response after being stored at 4 °C for two weeks. Therefore, the developed biosensor would have sufficient stability.

The practical application of the designed sensor was evaluated by determination of the recovery of spiked Hg^{2+} in both river and tap water samples. The analytical results are shown in Table 1. We observed that the results obtained in real water samples showed good recovery values, which confirmed that the proposed sensor was applicable for practical Hg^{2+} detection, even with concentrations lower than 5 nM.

In summary, a label-free and "turn-on" biosensor has been developed for the electrochemical detection of Hg^{2+} ions. With amplification by AuNP-functionalized reporter DNA, a detection limit of 0.5 nM could be achieved for Hg^{2+}, which makes the detector favorable for Hg^{2+} assays in practical samples of very low concentration. The proposed method does not require costly equipment and reagents, and has many salient advantages, including high specificity, a low detection limit, a simple-to-implement procedure and an easy regeneration process. The proposed strategy is expected to hold potential for the construction of ultrasensitive electrochemical sensor arrays for the detection of various toxic metallic ions.

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