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An ultra-sensitive Au nanoparticles functionalized DNA biosensor for electrochemical sensing of mercury ions

Yanyan Zhang, Cong Zhang, Rui Ma, Xin Du, Wenhao Dong, Yuan Chen, Qiang Chen *

The Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Science, Nankai University, Weijin Road No. 94, Tianjin 300071, China

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ABSTRACT

The present work describes an effective strategy to fabricate a highly sensitive and selective DNA-biosensor for the determination of mercury ions (Hg^{2+}). The DNA 1 was modified onto the surface of Au electrode by the interaction between sulfydryl group and Au electrode. DNA probe is complementary with DNA 1. In the presence of Hg^{2+}, the electrochemical signal increases owing to that Hg^{2+}-mediated thymine bases induce the conformation of DNA probe to change from line to hairpin and less DNA probes adsorb into DNA 1. Taking advantage of its reduction property, methylene blue is considered as the signal indicating molecule. For improving the sensitivity of the biosensor, Au nanoparticles (Au NPs) modified reporter DNA 3 is used to adsorb DNA 1. Electrochemical behaviors of the biosensor were evaluated by electrochemical impedance spectroscopy and cyclic voltammetry. Several important parameters which could affect the property of the biosensor were studied and optimized. Under the optimal conditions, the biosensor exhibits wide linear range, high sensitivity and low detection limit. Besides, it displays superior selectivity and excellent stability. The biosensor was also applied for water sample detection with satisfactory result. The novel strategy of fabricating biosensor provides a potential platform for fabricating a variety of metal ions biosensors.

1. Introduction

Mercury, a potent toxin, can be accumulated in the vital tissues and organs binding with proteins and enzymes, which results in cell functions abnormality and all kinds of diseases [1,2]. The detection of mercury contaminants has played an important role in environmental bioinorganic chemistry, clinical toxicology, waste management and bioremediation of metal ions [3].

Recently, many detection techniques have been developed for detecting a variety of environmental factors, including fluorescence, surface plasmon resonance, electrochemistry, colorimetric sensors and other analytical techniques [4–6]. Among these methods, electrochemical method has been widely applied for accurate determination of metal ions for its high sensitivity, low detection limit and low cost [7–15]. Despite many advances in this field, there is still a quest for new schemes and strategies for improvement of the sensitivity, simplicity and selectivity of metal ion sensors.

It's clear that thymine bases (T) could specifically capture Hg^{2+} ions as the intrinsic interaction between Hg^{2+} ions and thymine bases [16–18]. So, a series of mercury biosensors based on T-Hg^{2+}-T have been appeared because this strategy possesses high selectivity towards Hg^{2+} ions against other related environmental heavy metal ions. Many reported mercury biosensors have obtained lower detection limit, such as the Mirkin's group used Au NPs to combine with DNA for detecting Hg^{2+} ions with the detection limit of 10 nM [19], the Liu's group synthesized hydrogel microparticles with covalently attaching DNA probes and detected Hg^{2+} ions by the fluorescence change with the detection limit of 10 nM [20,21] and the Zhang's group developed a graphene-DNA biosensor for detecting Hg^{2+} ions with the detection limit of 5 nM [22], however, it's difficult to meet the need of water sample detection. Besides, some other T-Hg^{2+}-T biosensors for Hg^{2+} ions detection are also reported [23,24]. As the limit amount of mercury ions in drinking water recommended by the World Health Organization (WHO) is 5 nM [25], it is urgent to develop an effective strategy for improving the sensitivity of analyzing mercury ions.

Electrochemical sensor has been widely applied to detect Hg^{2+} ions because of its superior sensitivity, facile fabrication and low cost. Herein, we develop an ultra-sensitive, facile and reusable electrochemical biosensor for detecting Hg^{2+} ions. Initially, DNA modified Au electrode captured complementary probe forming double helix structure, which blocks the electrochemical signal transfer. Once DNA probe was specifically combined with Hg^{2+} ions, the electrochemical signal would increase. Au NPs functionalized reporter DNA was combined with DNA 1 to achieve signal amplification. Although Au NPs as signal amplifier has been reported, in this paper, the strategy of fabricating biosensor is facile and displays high sensitivity. Methylene blue (MB) was used as a signal indicating molecule and specifically combined with guanine

* Corresponding author.
E-mail address: qiangchen@nankai.edu.cn (Q. Chen).

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(G) bases of reporter DNA [26,27]. The Au NPs functionalized DNA 3-DNA 2-DNA 1-modified electrode exhibits excellent performance.

2. Experimental

2.1. Reagents

The oligo-nucleotides were purchased from Dingguo Co. Ltd. (China). The sequence of DNA 1 is 5′-SH-AAAAAAAAAAAAAAAACGC GCG-3′, the sequence of probe DNA 2 is 5′-TTTTTTTTTTTTTTTT-3′ and the sequence of reporter DNA 3 is 5′-SH-CGCGCGCGCGCG-3′. Sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) were purchased from Tianjin Yingda Rare Chemical Reagent Co. (China). Au electrodes (3 mm diameter) were purchased from Tianjin Aidahengsheng technology Co. Ltd. (China). $HAuCl_4$, $HgCl_2$, $PdCl_2$, $NiCl_2$, $CuCl_2$, $CoCl_2$, $MnCl_2$ and $CdCl_2$ were purchased from Aldrich Co. (USA). All aqueous solutions were prepared by double distilled water. All other reagents used in this work were analytical grade without further purification.

2.2. Measurements

A 283 Potentiostat–Galvanostat electrochemical workstation (EG & GPARC with M 270 software) and Lanlike 2010 electrochemical workstation (Lanlike chemical & electronic high-tech Co. Ltd.) were applied to electrochemical experiments. Au NPs functionalized reporter DNA 3-DNA 2-DNA 1-modified Au electrode (3 mm diameter) was used as working electrode, Ag/AgCl (saturated with KCl) was used as reference electrode and a Pt wire was applied as auxiliary electrode which develops a conventional three-electrode system.

2.3. Preparation of Au NPs functionalized reporter DNA 3

Au NPs were synthesized by a traditional method [28]. Briefly, 39.97 mg of $HAuCl_4$ was dissolved in 25 mL of distilled water, then, 2.5 mL of sodium citrate (19.4 mM) was added to above boiling solution obtained dark amaranth solution. Au NPs functionalized reporter DNA by S—Au bond was prepared by incubating 50 μL of sulfydryl-modified reporter DNA (10 μM) with 5 mL of Au NPs solution at 37 °C. After that, 100 μL of 6-mercapto-hexanol was added to the solution, which blocked the sulfydryl group of reporter DNA to directly adsorb onto the surface of Au electrode.

2.4. Fabrication of DNA modified mercury biosensor

Au electrodes employed in this report were polished by powder of aluminum oxide and cleaned in the solution of H_2SO_4 (0.5 M) and H_2O_2 (30%) (3/1, v/v) for 10 min, then, ultrasonically rinsed with distilled water. The polished electrodes were dried with nitrogen gas. 50 μL of sulfydryl-modified DNA 1 was mixed with 5 mL of tris (2-carboxyethyl) phosphine (TCEP) (1 mM) to prevent the disulfide bond producing and assure the DNA 1 stayed on the electrode as single layer.

DNA 1-modified Au electrode was obtained by immersing the cleaned Au electrode into the above DNA 1 solution for 2 h at 37 °C. Then, the modified electrode was incubated with Au NPs functionalized reporter DNA 3 for 2 h at 37 °C. DNA 2 probes were incubated with various concentrations of Hg^{2+} for 2 h. Thereafter, Au NPs functionalized DNA 3-DNA 1 modified electrode was immersed into above DNA 2 probe for 2 h. At last, the modified electrode was immersed into 5 mg/mL MB for 1 h followed by being cleaned with distilled water. The approach of the fabricated biosensor is illustrated in Scheme 1. As shown in Scheme 1, when Hg^{2+} was added to DNA 2 probe, Hg^{2+}-mediated thymine bases formed mismatched T-Hg^{2+}-T stable bases pairs and the conformation of DNA probe changed from line to hairpin, which resulted in less DNA probes adsorbing onto DNA 1. Therefore, the electrochemical signal increased and the electrochemical responses of the fabricated biosensor were in accordance with various Hg^{2+} concentrations.

3. Results and discussion

3.1. Electrochemical characterization of the DNA modified electrode

In order to study the signal indicating role of MB, the Au NPs functionalized DNA 3-DNA 2-DNA 1-modified Au electrode without being immersed into 5 mg/mL MB (a) and with being immersed into 5 mg/mL MB (b) for detecting 100 nM Hg^{2+} ions were investigated. As shown in Fig. 1, the biosensor immersed into MB (b) displays a sharp reduction peak whereas the reduction peak of (a) is weak. This phenomenon can be explained that the MB possesses excellent reduction property under the electrochemical environment and the reduction current is increased obviously.

To study the electrochemical response of DNA modified electrode towards Hg^{2+} ions and the signal amplified role of Au NPs, cyclic voltammetry (CV) responses of the Au NPs functionalized DNA 3-DNA 2-DNA 1-modified electrode towards 100 nM Hg^{2+} ions (a) and without Hg^{2+} ions (b) and DNA 1-modified biosensor towards 100 nM Hg^{2+} ions (c) were recorded in 0.1 M phosphate buffer solution (PBS) (pH 7.0) (Fig. 2). For Au NPs functionalized DNA 3-DNA 2-DNA 1-modified electrode, an obvious reduction peak is appeared at appropriately −0.9 V owing to the reduction of MB. Effective electro-active area is a vital factor for evaluating electrochemical behavior. According to the following Randles-Sevcik equation [29], microscopic effective electro-active area could be calculated.

$$I_p = 2.69 \times 10^5\, AD^{1/2}n^{3/2}\gamma^{1/2}C \quad (1)$$

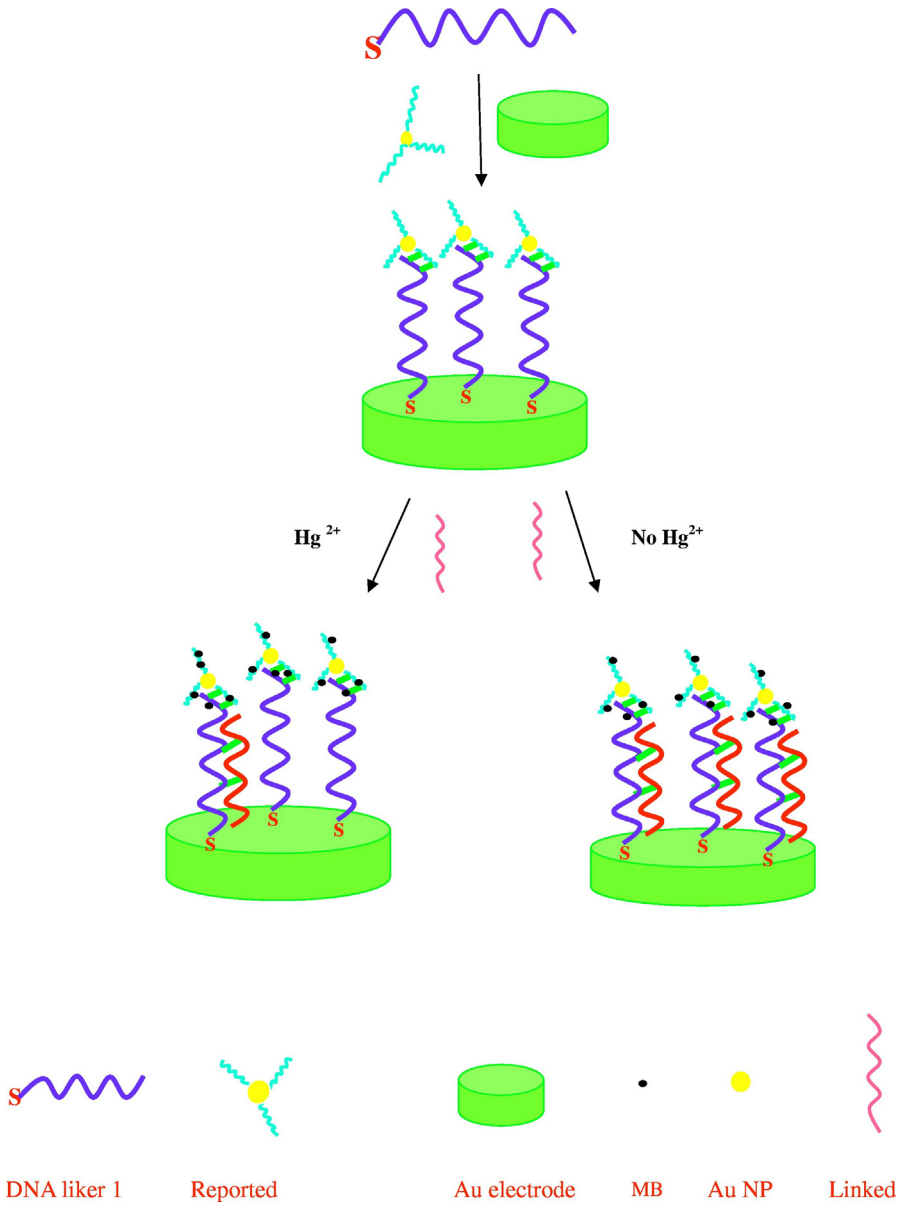
In this equation, A corresponds to effective electro-active surface area of working electrode (cm^2); D represents the diffusion coefficient in solution and it approximately is 6.70×10^{-6} cm^{-2} s^{-1}; n is the amount of electron which transfer in the reaction, the value is equal to 1; γ is the scan rate ($V\,s^{-1}$); C is the concentration of detected molecule, in this system the concentration of detected molecule is 100 nM and I_p represents the current of redox peak. By calculating, the effective electro-active area of the Au NPs functionalized DNA 3-DNA 2-DNA 1-modified electrode towards 100 nM Hg^{2+} ions is 2 and 1.5 times higher than without Au NPs functionalized electrode and without addition of Hg^{2+} ions, respectively. In Fig. 2, the reduction peak current of (a) is much higher than that of (c), indicating that the Au NPs can be regarded as signal amplifier which is in accordance with the reported literature [30]. Besides, the electrochemical response of the biosensor with adding of Hg^{2+} ions is much higher than that of without Hg^{2+} ions. This phenomenon could be caused by Hg^{2+} ions insert into the T-T base pairs forming stable T-Hg^{2+}-T interaction, which results in the DNA 2 conformation changing into double helix structure. Thereafter, while DNA 1 modified Au electrode was incubated with DNA 2, the electrochemical response was decreased, which resulted of that single linker was easier to electron transfer than double helix structure. Thus, more Hg^{2+} ions are added to DNA 2 solution, higher reduction current is appeared. Excellent electrochemical response of the modified electrode verifies that this strategy is worthy for detecting mercury ions.

The transfer coefficient (α) value was calculated via Eq. (2) [31]. α was found to be 0.41.

$$E_p - E_{p/2} = 48/(\alpha n_a) \quad (2)$$

3.2. Optimization of the fabricated biosensor

For achieving the optimal performance of the biosensor, scan rate, pH value of PBS solution, incubation time of DNA 1-modified electrode with DNA 2 solution and incubation time of DNA 1 with Au electrode



Scheme 1. A schematic illustration for the fabrication of Au NPs functionalized DNA 3-DNA 2-DNA 1-modified Au electrode.

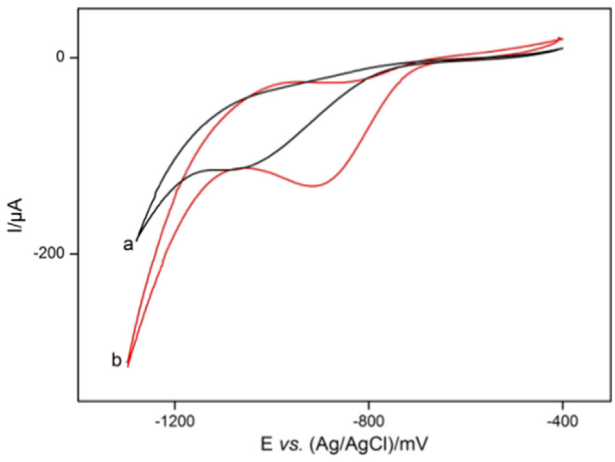


Fig. 1. CV responses of biosensor without being immersed into 5 mg/mL MB (a) and immersed into 5 mg/mL MB (b) for detecting of 100 nM Hg^{2+} ions.

were studied by changing single parameter while others were fixed. The effect of scan rate on electrochemical response of Au NPs functionalized DNA 3-DNA 2-DNA 1-modified biosensor towards 100 nM Hg^{2+} ions was studied by cyclic voltammetry (Fig. 3A).

In Fig. 3A, with the increasing of scan rate, the cathodic peak current of Hg^{2+} ions increases, confirming that the electrochemical reaction process of the biosensor towards Hg^{2+} ions is diffusion controlled (In order to assure the stability of the experiment process, we still chose 50 mV s^{-1} as the subsequent scan rate). In addition, the reduction peak current (I_p) is proportional to square root of scan rate ($v^{1/2}$) in the range of 10 mV s^{-1} to 100 mV s^{-1} (Fig. 3B). This observation is a clear evidence of that the process of catalysis is diffusion controlled rather than surface controlled under the condition of sufficient potential. According to the Andrieux and Save'ant theoretical model [32], I_p is proportional to the $v^{1/2}$ as following:

$$I_p = 0.496\, nFAC_s D_s^{1/2} v^{1/2} (nF/RT)^{1/2} \quad (3)$$

Besides, pH value of the PBS solution plays an important role in the detection of mercury ions. In 0.1 M PBS with pH 4, 5, 6, 7 and 8, the effect

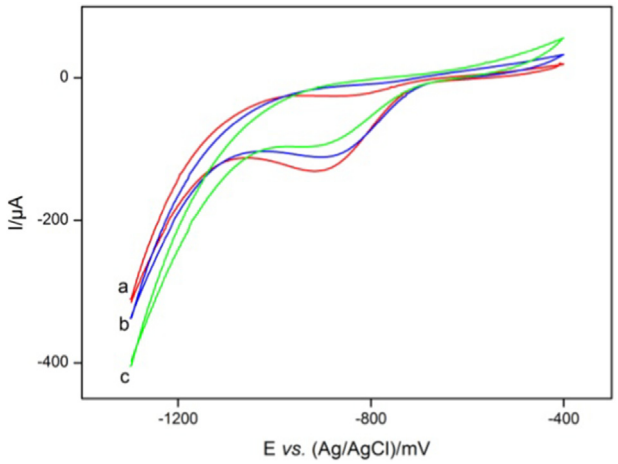


Fig. 2. CV responses of the Au NPs functionalized DNA 3-DNA 2-DNA 1-modified biosensor towards 100 nM Hg^{2+} ions (a) and without addition of Hg^{2+} ions (b) and DNA 1-modified biosensor towards 100 nM Hg^{2+} ions (c) in 0.1 M phosphate buffer solution (pH 7.0). Scan rate: 50 mV s^{-1}.

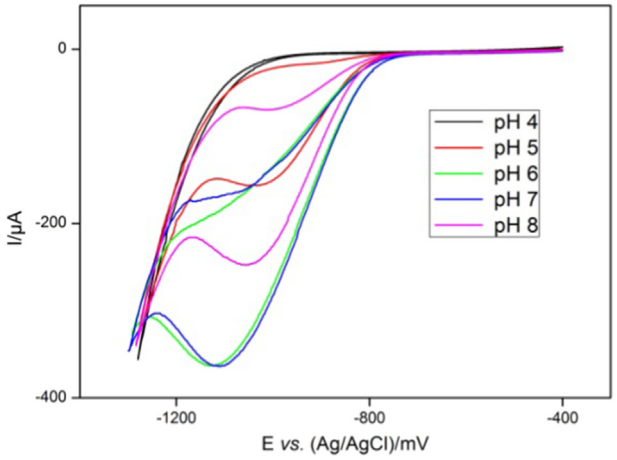


Fig. 4. The influence of pH on the biosensor towards 100 nM Hg^{2+} ions in PBS (scan rate: 50 mV s^{-1}).

of pH value on the as-prepared biosensor towards 100 nM Hg^{2+} ions was surveyed. Fig. 4 presents that the electrochemical response current increases with pH value of PBS solution from 4 to 7.0 and in pH 7.0 reaches maximum, thereafter decreases. It because that excessive acid results in the alkali MB losing of reduced property and excessive alkali is easier to bring out the precipitation of MB. The environment with excessive acid or excessive alkali restrains the reduction process of MB. Hence, PBS solution of pH 7.0 was used in the subsequent experiments to achieve optimized performance.

According to the principle of DNA 1 interacting with DNA 2, the amount of A-T base pairs can directly affect the electro-activity of the biosensor. The optimal incubation time of DNA 1-modified electrode

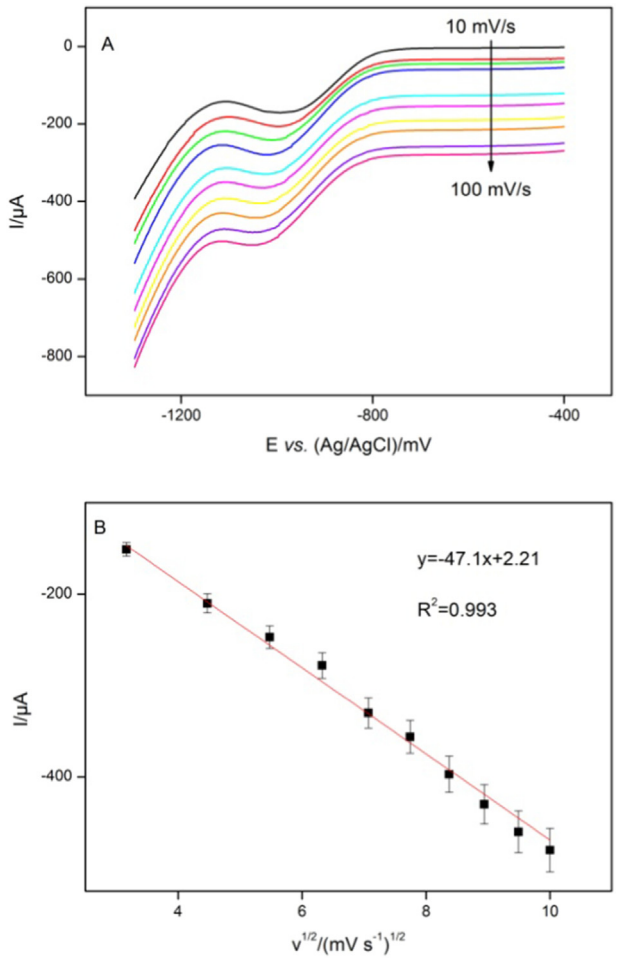


Fig. 3. CV responses of the biosensor towards 100 nM Hg^{2+} ions in PBS at various scan rates of 10 to 100 mV s^{-1} respectively (A), calibration curve of cathodic peak current vs. $v^{1/2}$ (B).

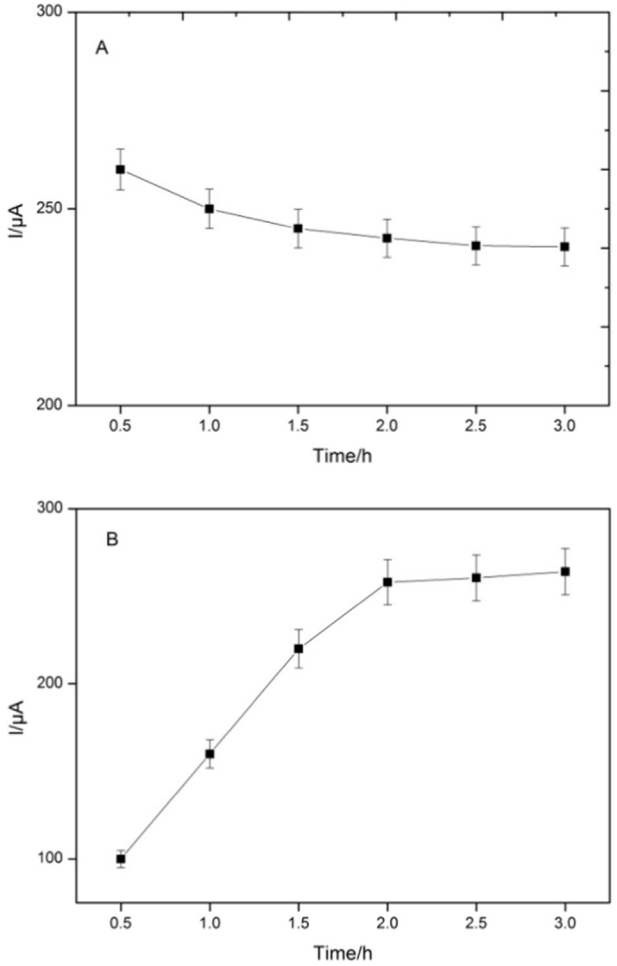


Fig. 5. The influence of incubation time between DNA 1 and DNA 2 (A) and incubation time between DNA 1 and Au electrode (B) on the biosensor towards 100 nM Hg^{2+} ions in PBS (scan rate: 50 mV s^{-1}).

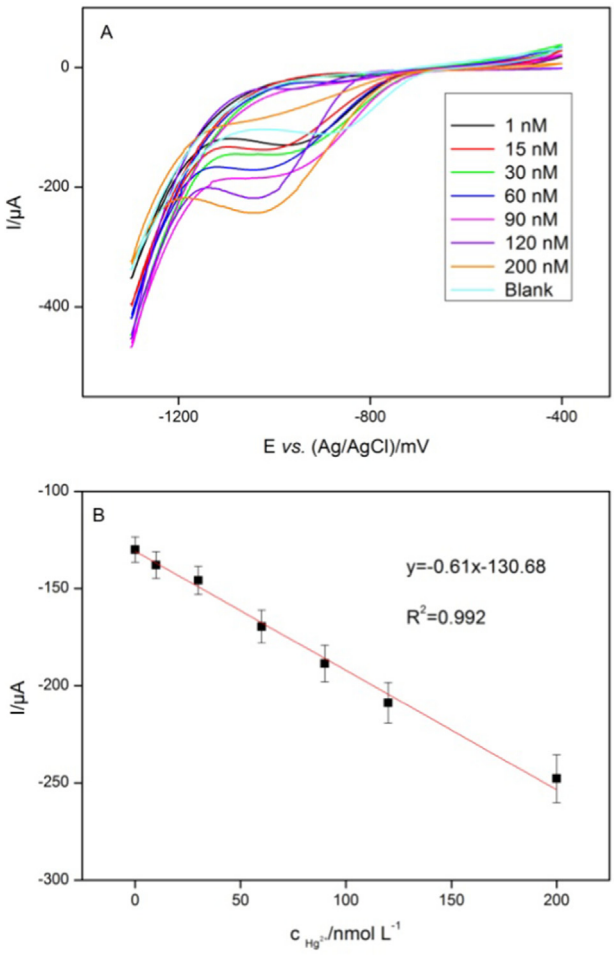


Fig. 6. CV responses of the biosensor towards different concentrations of Hg^{2+} ions (0, 0.1, 10, 30, 60, 90, 120 and 200 nM) (A); calibration curve of the concentration of Hg^{2+} ions vs. current response in the range of 0.1 nM–200 nM (B).

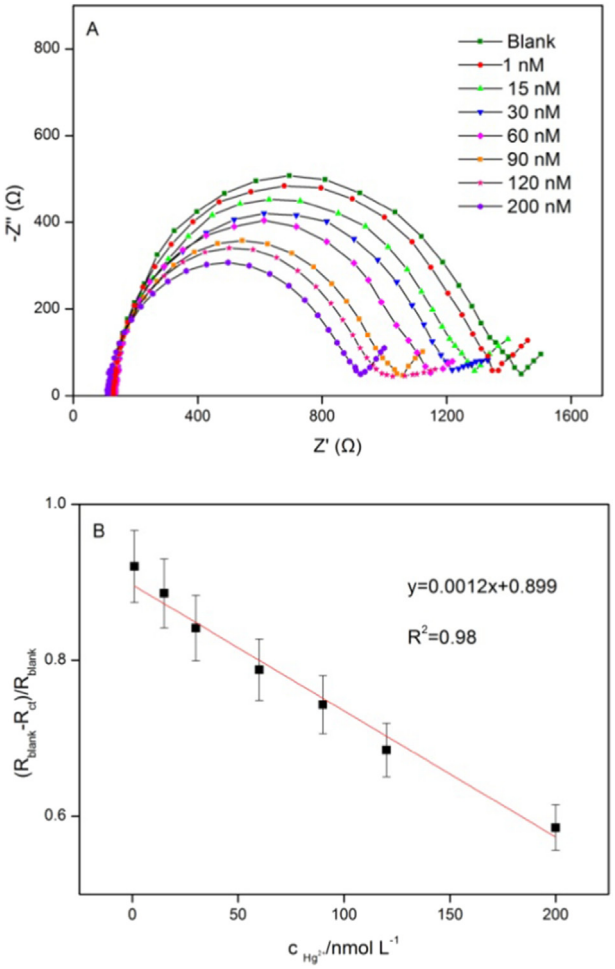


Fig. 7. Nyquist plots for EIS detection of the biosensor in PBS solution towards different concentrations of Hg^{2+} ions (0, 0.1, 10, 30, 60, 90, 120 and 200 nM) (A); Plot of a relationship between Hg^{2+} ions concentration and $(R_{blank} - R_{ct})/R_{blank}$ (B).

immersing into DNA 2 was investigated. As shown in Fig. 5A, the reduction peak current decreases as the incubation time increases from 1 to 2 h, thereafter, no obvious change has been observed in the current response after 2 h. Moreover, the interaction between DNA 1 and DNA 2 reaches a stable condition. Besides, the incubation time of DNA 1 with Au electrode was also optimized. As shown in Fig. 5B, the reduction peak current increases as the incubation time extends from 1 to 2 h, thereafter, the reduction peak current keeps stable. The phenomenon may be caused that more DNA 1 has stayed on Au electrode, more Au NPs functionalized DNA 3 has adsorbed on DNA 1. Therefore, the electrochemical response increases from 1 to 2 h. Thereafter, the fixed electrode area restricts the amount of DNA 1 on electrode which results in the stable reduction current.

Table 1
Comparison of some DNA-based electrochemical biosensor for Hg^{2+} ions determination.

Electrode	Liner range (nM)	Detection limit (nM)	Reference
DNA-functionalized silica nanoparticles	0–500	20	[33]
Molecular beacon	–	19	[34]
graphene-DNA	8–100	5	[22]
mercury specific oligonucleotide probe	0–100	0.5	[35]
Aptamer-functionalized hydrogel microparticles	1–200	10	[21]
Oligonucleotide modified electrode	100–2000	100	[36]
Quaternary ammonium group-capped gold nanoparticles	0–600	30	[24]
Au NPs functionalized DNA 3-DNA 2-DNA 1	0–200	0.05	This work

3.3. Electrochemical response of DNA modified electrode towards different concentration of mercury ions

In order to evaluate the performance of the biosensor, different concentrations of Hg^{2+} ions were added to DNA 2 solution. CV responses of the biosensors were investigated in PBS solution (pH 7.0) under optimal experiment condition.

Fig. 6A clearly indicates that the reduction peak current increases as the amount of Hg^{2+} ions increases, which is owing to the less amount of double helix structure on Au electrode. The above result is consent with the previous hypothesis. Fig. 6B depicts the concentrations of Hg^{2+} ions versus current response is proportional relation in the range from 0.1 nM to 200 nM. The detection limit is calculated by 3 × blank variance/slope (S/N = 3) to be 0.05 nM which is much lower than many

Table 2
Tests of Hg^{2+} in real samples based on Au NPs-RGO-DNA-modified electrode.

Sample	Added Hg^{2+} (nM)	R.S.D. (%, $n = 6$)	Recovery (%)
Tap water 1	2	2.35	102
Tap water 2	6	2.91	98.8
River water 1	2	3.02	97.3
River water 2	6	3.17	96.9

Table 3
Interference of other metal ions to the biosensor towards 100 nM Hg^{2+} ion.

Interference	Response change(%)[a]	R.S.D.(%)
Pd^{2+}	4.35	2.8
Ni^{2+}	3.27	2.3
Co^{2+}	2.86	3.1
Cu^{2+}	3.12	2.9
Mn^{2+}	1.98	3.5
Fe^{2+}	2.34	2.7
Cd^{2+}	3.16	3.2

[a] The values were obtained by averaging the values from five successive determination.

previously reported biosensors (Table 1). The method of fabricating biosensor provides a favorable platform for monitoring the drinking water.

For further proof, electrochemical impedance spectroscopy (EIS) was employed to detect Hg^{2+} ions under the optimal condition. As shown in Fig. 7A, as Hg^{2+} ions are added into DNA 2 solution, the semicircle referred to electron-transfer resistance decreases obviously, suggesting that Hg^{2+} ions prevents DNA 2 from adsorbing on DNA 1. So, the rate of electron transfer becomes accelerated.

Various mercury ions concentrations associates with the charge transfer resistance is shown in Fig. 7B. $(R_{blank}–R_{ct})/R_{blank}$ was plotted as a function of Hg^{2+} ions concentrations (R_{ct}: the charge transfer). The changes of charge transfer resistance are proportional to the concentrations of Hg^{2+} ions between 0.1 nM and 200 nM with a linear correlation coefficient (R^2) of 0.982. The result is consistent with the CV response, which further confirms the value of the as-prepared biosensor.

This biosensor was applied to detection of Hg^{2+} ions in tap water and river water for investigating its practical application. The recovery study towards Hg^{2+} ions was performed through addition different known concentrations of Hg^{2+} ions into the river water and tap water (Table 2). The recovery values are in the range of 96.9% to 102% and the relative standard deviations (RSD) are in the range of 2.35% to 3.17%, confirming that the proposed biosensor could be successfully applied to monitor the Hg^{2+} ions in water samples.

3.4. Selectivity, stability and reproducibility of the fabricated biosensor

The general problem of detecting Hg^{2+} ions is the interference by other heavy metal ions and transitional metal ions. To study the selectivity of the as-fabricated biosensor against these possible interfering metal ions, CV responses of biosensor towards 100 nM Hg^{2+} ions and various high concentrations of other metal ions between −1.3 V and −0.4 V were compared. As we can see in Table 3, the electrochemical responses to 1 μM Pd^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Mn^{2+} and Fe^{2+} could be negligible, indicating that the DNA-based biosensor possesses excellent selectivity for Hg^{2+} ions. This is mainly due to the unique specific interaction between Hg^{2+} ions and T-T mismatches.

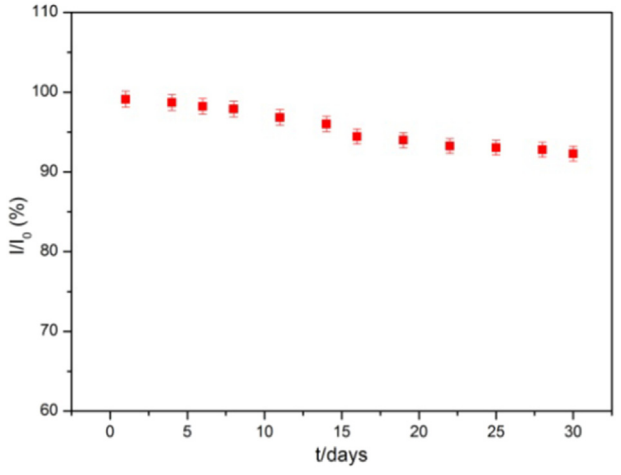


Fig. 8. The stability of DNA-based biosensor for 100 nM Hg^{2+} ions on successive one month (stored at 4 °C).

The reproducibility is also an important parameter for biosensor. In this work, the reproducibility was evaluated by detecting 100 nM Hg^{2+} ions of five electrodes respectively and the relative standard deviation found to be 2.7%. After one month, the electrochemical response of the biosensor for Hg^{2+} ions reached to 92.3% of its original response (Fig. 8). As we can see, the biosensor possesses a favorable long-term stability. In addition, the biosensing interface could be easily regenerated only be two step and the working interface could be regarded as "chip" and changes easily. To the best of our knowledge, no reported biosensors have possessed the property of easily fabrication and reusable application. Therefore, the as-prepared biosensor is hopeful of using for practical detection.

4. Conclusions

In this work, a facile and reusable approach has been developed for preparing DNA biosensor on the basis of the change of electrochemical signal. DNA modified electrode captured complementary probe forming double helix structure to block electron transfer. Hg^{2+} ions form strong T-Hg^{2+}-T interaction with T-T mismatches, which is applied to detect Hg^{2+} ions. In addition, the DNA-based biosensor displays ultrasensitivity, lower detection limit and reusable ability towards Hg^{2+} ions. Moreover, the biosensor exhibits excellent performance in water sample detection. We believe this approach of fabricating DNA-based biosensors developed in this work can provide a valuable platform for metal biosensor fabrication.

Acknowledgments

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