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Aptamer-Based Electrochemical Biosensor for Mercury Ions Detection Using AuNPs-Modified Glass Carbon Electrode

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A mercury ion aptamer electrochemical biosensor based on a Thymine-Hg^{2+}-Thymine (T-Hg^{2+}-T) structure has been constructed and successfully used to detect mercury ions in tap water samples. The aptamer electrochemical biosensor was assembled using a mercury ion aptamer-functionalized AuNPs-modified glass carbon electrode (aptamer/$(AuNPs/CS)_2$/GCE) capable of specifically detecting mercury ions through a T-Hg^{2+}-T structure. The experimental results indicated the optimum electrochemical performance of the prepared aptamer biosensor when the $(AuNPs/CS)_2$/GCE surface was modified with 1.0 μM aptamer and incubated with Hg^{2+} for 60 min. Moreover, the aptamer biosensor exhibits a good linear relationship between the logarithm of the Hg^{2+} concentration and the DPV peak current in the range from 0.01 to 500 nM following the linearization equation I_p (μA) $= 2.59902 + 0.2097 \log C$ ($R^2 = 0.9994$) with a limit of detection as low as 0.005 nM. Therefore, the constructed aptamer biosensor provides a simple and sensitive approach for Hg^{2+} detection in aqueous solution with promising application for trace Hg^{2+} detection in real samples.

KEYWORDS: *Aptamer Electrochemical Biosensor, Mercury Ions, AuNPs, Detection.*

INTRODUCTION

Mercury compounds have a deleterious impact on both ecosystems and human health.[1,2] Mercury compounds can directly damage the root structure of plants and inhibit their development, which can seriously affect the production of crops and even cause death. While human mercury poisoning occurs mainly through skin contact or respiratory and digestive tract intake, the small ingestion of mercury ions can result in highly toxic mercuro-organic compounds under the action of the microbes in the body with serious effects to human organs. In addition, mercury poisoning can be deadly to the brain, kidneys, and digestive system, thus causing kidney damage, language barrier, oral inflammation, and neurological disorders. Therefore, the development of sensitive methods for trace Hg^{2+} detection is urgent. Traditional techniques such as fluorescence spectroscopy or colorimetric methods can detect mercury ions, but their expensive equipment and unsuitable detection limits restrict the on-site rapid detection of mercury ions.[3–5]

According to the recent literature, electrochemical biosensors have proven to be reliable techniques for heavy metal ion detection owing to their excellent characteristics, such as low cost, high sensitivity, simultaneous detection, and easy operation.[6, 7] In addition, it is well known that gold nanoparticles (AuNPs) display excellent catalytic properties, conductivity, and biocompatibility with enzymes, being widely used in electrochemical biosensors[8] to enhance their response, stability, and sensitivity.[9, 10] In addition, AuNPs can enhance electron transfer reactions and improve the analytical performance of the biosensors.[11]

Aptamers,[12, 13] a class of single strand nucleic acids, present high specificity[14] and affinity towards their respective target ions or molecules,[15–17] as well as many advantages over traditional recognition molecules, such as

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nontoxicity, lack of immunogenicity, and ease of synthesis and modification. At the same time, aptamer biosensors have been employed to overcome the limitations of the abovementioned methods owing to their excellent specificity and sensitivity and have been recently employed to detect heavy metal ions.[18, 19] Several studies have proposed aptamer biosensors for mercury ion detection. Tan et al.[20] constructed an aptamer-functionalized AuNPs-based fluorescence sensor for Hg^{2+} detection. Although the aptamer could improve the selectivity toward Hg^{2+} ions, the detection range of this fluorescence sensor was still rather narrow with a detection limit of 16 nM. Later, Tortolini et al.[21] developed an electrochemical biosensor based on polythymine bearing a methylene blue-modified electrode for the selective detection of Hg^{2+}, where a gold electrode was used as the electrochemical transducer, affording a limit of detection (LOD) of 0.1 nM with a detection range of 0.2–100 nM. Moreover, Mei et al.[22] fabricated a highly sensitive and selective electrochemical biosensor for Hg^{2+} detection by coupling T-Hg^{2+}-T base pairs to improve the selectivity by surface-initiated enzymatic polymerization and achieve high sensitivity. The proposed method was able to detect aqueous Hg^{2+} with a low detection limit and wide linear detection range.

In this work, a highly sensitive and selective aptamer electrochemical biosensor was developed based on a glass carbon electrode (GCE) modified with chitosan (CS), AuNPs, and an aptamer for the detection of Hg^{2+} (hereafter denoted aptamer/$(AuNPs/CS)_2$/GCE). Here, CS was used to immobilize AuNPs on the electrode, while the AuNPs were used to immobilize the aptamer as well as enhance the electron transfer to improve the analytical response. The aptamer was used to target the mercury ions for further improvement of the analytical performance. The mechanism of this aptamer biosensor is shown in Figure 1. As one can see, the presence of Hg^{2+} induces the folding of the aptamer forming a T-Hg^{2+}-T structure, bringing the electrochemical signal indicator ferrocene (Fc) close to the sensor surface to produce an electrochemical response. As a result, the proposed aptamer biosensor exhibits high sensitivity, acceptable stability, and low detection limit for Hg^{2+} detection, demonstrating its promising application for real sample analysis.

MATERIALS AND METHODS

Reagents and Apparatus

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$, Au $\geq$47.8%), potassium ferricyanide, trisodium citrate, sodium perchlorate, dipotassium hydrogen phosphate, and sodium dihydrogen phosphate were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Tris-(2-carboxyethyl) phosphinehydrochloride (TCEP), chitosan (CS, 85% deacetylation), Hg^{2+} standard solution (100 μg mL^{-1}), and 6-mercapto-1-hexanol (MCH) were purchased from Aladdin Biotech CO., Ltd. (Beijing, China). The thiolated Hg aptamer with the sequence 5′-HS-$(CH_2)_6$-TCATGTTTGTTTGTTGGCCCCCCTTCTTTCTTA-Fc-3′ was purchased from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. All reagents were of analytical grade and used without further purification. Solutions were prepared with double distilled (DI) water (18 MΩ cm).

Differential pulse voltammetry (DPV) experiments were performed on a PGSTAT302N electrochemical workstation (Metrohm, Shanghai, China). The conventional three-electrode system included a modified GCE as the working electrode, a platinum wire electrode as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode.

Preparation of the AuNPs/CS Modified Electrode

An aqueous suspension of citrate-stabilized AuNPs was prepared following the method reported by Fren.[23] CS (2 mg mL^{-1}) in 1% acetic acid solution was prepared and kept overnight to ensure its complete dissolution. The CS solution was then filtered through a 0.22 μM Millipore syringe filter and stored in a refrigerator at 4 °C for further use. The GCE was polished with 0.3 and 0.05 μM alumina slurry and washed with DI water, after which it was characterized in a 10 mM $[Fe(CN)_6]^{3-/4-}$ aqueous solution containing 0.1 M KCl to obtain a clean electrode in the potential range between −0.2 and 0.6 V at a scan rate of 0.1 V s^{-1}. The modified GCE was prepared following our previous work.[24] Briefly, 3 μL of a CS solution (2 mg mL^{-1}) was dropped on the GCE surface and

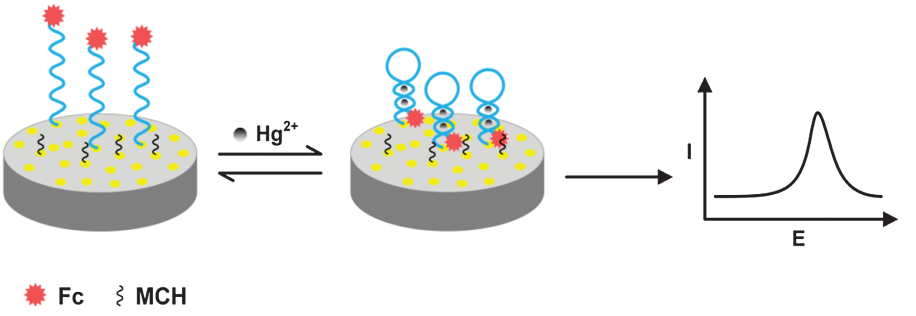

Figure 1. Mechanism of the aptamer electrochemical sensor for Hg^{2+} detection.

air-dried at room temperature; then, 3 μL of the AuNPs solution was dropped on the surface of CS/GCE and air-dried at room temperature. Further layer assembly on the working electrode afforded $(AuNPs/CS)_2$/GCE, which was washed with DI water and dried after each of the synthesis steps.

Preparation of the Electrochemical Hg^{2+} Aptasensor

The prepared $(AuNPs/CS)_2$/GCE was immersed in 85 μL of a 1.0 μM thiolated aptamer solution, which had been previously activated with 10 μM TCEP for 1 h, and kept overnight with 100% humidity to construct the aptamer/$(AuNPs/CS)_2$/GCE structure, which was subsequently washed with 10 mM PBS buffer (pH 7.4) three times. Then, the prepared aptamer biosensor was immersed in a 1 mM MCH solution for 2 h to block the electrode. All the solutions used above contained 1.0 M $NaClO_4$ to prevent the oxidation of ferrocene. The obtained biosensors were stored in a refrigerator at 4 °C when not in use.

Sample Detection

DPV experiments were recorded in 10 mL PBS buffer (10 mM pH 7.4) containing 1.0 M $NaClO_4$ in the potential window from −0.2 to 0.8 V. Hg^{2+} detection was performed according to the following procedure: the obtained aptamer/$(AuNPs/CS)_2$/GCE biosensor was incubated in PBS buffer (10 mM, pH 7.4) containing different concentrations of the standard Hg^{2+} solution for 60 min, after which it was washed with 10 mM PBS buffer (pH 7.4) three times and then transferred to the electrochemical cell for DPV measurement. Before each experiment, the buffer solution was thoroughly purged with extra-pure nitrogen.

RESULTS AND DISCUSSION

Effect of the Incubation Time

In order to obtain excellent sensitivity for mercury ion detection, the effect of the contact time between mercury ions and the aptamer was optimized in the presence of 10 nM Hg^{2+}. As expected, the mixing time greatly influenced the DPV peak current of the electrochemical signal indicator Fc. Therefore, a wide range of time lengths from 20 to 80 min was studied. From Figure 2, it can be seen that the DPV peak current increases gradually with the contact time, reaching a plateau after 60 min, indicating the saturation of the binding between Hg^{2+} and the aptamer. Thus, 60 min was selected as the optimum mixing time for the detection of Hg^{2+}.

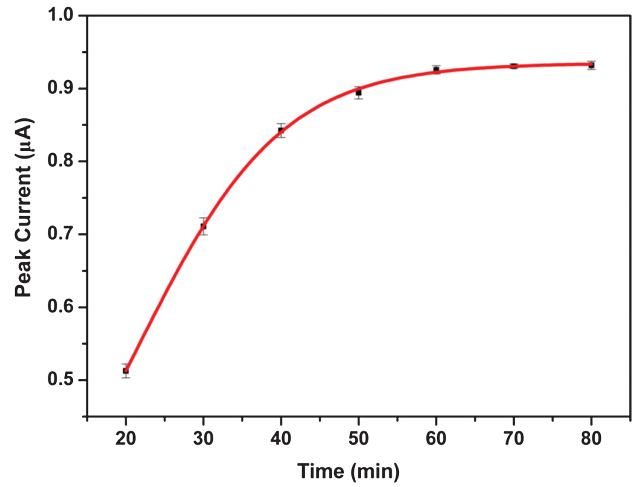

Figure 2. Effect of the reaction time on the DPV peak current for the proposed aptamer biosensor.

Effect of the Aptamer Concentration

The aptamer concentration determines its final density on the sensor surface, thus influencing the combination reaction with Hg^{2+}, which in turn has a direct effect on the electrochemical performance of the aptamer biosensor. Therefore, the electrochemical properties of the constructed sensor modified at different aptamer concentrations were investigated in the presence of 10 nM Hg^{2+}. As shown in Figure 3, it is clear that the DPV peak current increases with the aptamer concentration up to 2.0 μM at 10 nM Hg^{2+}, but such increasing trend slows down as the aptamer concentration increases from 1.0 to 2.0 μM. Finally, the DPV peak current decreases upon further increasing the Hg-aptamer concentration. An explanation to this phenomenon would be that fewer T-Hg^{2+}-T structures are formed at lower Hg-aptamer concentrations, affording fewer electrochemical signal indicators on the sensor surface and a subsequent weaker electrochemical response. In contrast, very high aptamer concentrations would result in steric hindrance of the T-Hg^{2+}-T structure, thus resulting in a reduced response. Therefore,

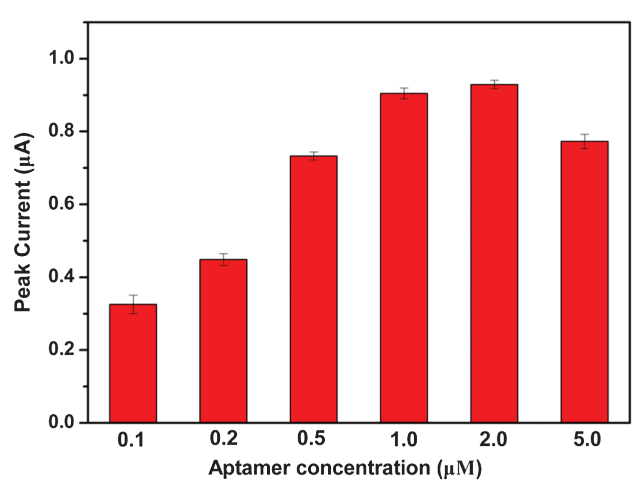

Figure 3. Effect of different concentrations of Hg-aptamer on the DPV peak current for the prepared aptamer biosensor in the presence of 10 nM Hg^{2+}.

1.0 μM Hg-aptamer was chosen as the optimum concentration.

Analytical Performance of the Aptamer Biosensor

The DPV peak current response at different Hg^{2+} concentrations were measured under optimum conditions to validate the proposed biosensor system. As shown in Figure 4, the DPV peak current of Fc increased with the concentration of Hg^{2+} due to the larger number of T-Hg^{2+}-T structures formed on the sensor surface at higher Hg^{2+} concentrations. The aptamer biosensor was found to exhibit a good linear relationship between the DPV peak current and the logarithm of the Hg^{2+} concentration, following the linearization equation I_p (μA) = 2.59902 + 0.2097 log$C$ (R^2 = 0.9994) in the range from 0.01 to 500 nM (Fig. 5). The LOD was as low as 0.005 nM, as calculated by the equation LOD = $3S_B/b$ (where S_B is the standard deviation of 20 independent blank samples and b is the sensitivity of the calibration graph). Finally, the properties of the aptamer biosensor were compared to those of other methods reported in the literature, as summarized in Table I. Our strategy is more sensitive than other electrochemical methods for Hg^{2+} detection. Moreover, the linear range of our strategy is wider than that of most other methods.

Repeatability, Reproducibility, and Stability Studies

The repeatability and reproducibility of the aptamer biosensor were evaluated by detecting 10 nM Hg^{2+} under optimum conditions using three electrodes. The intraassay and interassay relative standard deviation (RSD) values were determined to be 3.2% (n = 25) and 4.6% (n = 6), respectively, indicating that the proposed biosensor displays good accuracy and acceptable reproducibility for the detection of Hg^{2+}.

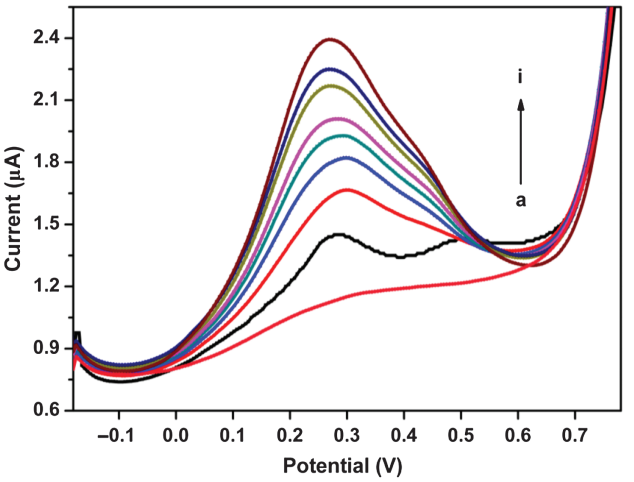

Figure 4. DPV response of the aptamer biosensor against Hg^{2+} at concentrations of 0, 0.01, 0.1, 1.0, 5.0, 10, 100, 200, and 500 nM (from a to i) in PBS buffer.

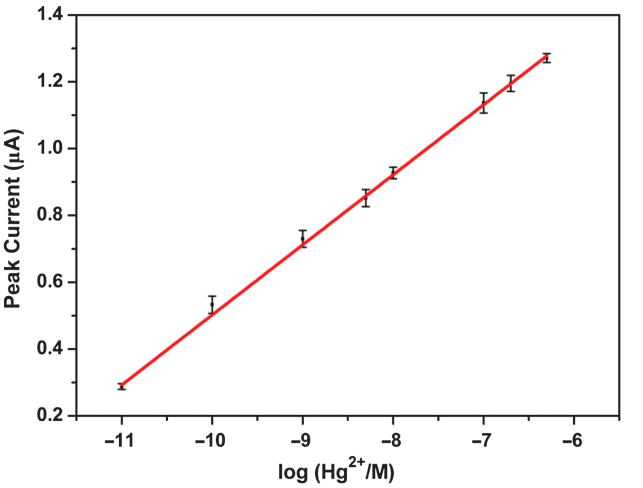

Figure 5. Relationship between the logarithm of the Hg^{2+} concentration and DPV peak current.

The proposed aptamer biosensor was stored in a refrigerator at 4 °C for further use. No obvious decrease in the DPV peak current was observed in the first seven days. After storage for 30 days, the aptamer biosensor retained 92% of its initial DPV peak current response, indicating the good stability of this biosensor and the possibility of long-term storage.

Interference Studies

The selectivity of this aptamer sensing system was evaluated by comparing the DPV peak current of solutions containing 10 nM Hg^{2+}or other metal ions at concentrations 10 times higher, such as Mn^{2+}, As^{3+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, and Ba^{2+}. A PBS solution without any of the above metal ions was used as the blank. As shown in Figure 6, a remarkable DPV peak

Table I. Comparison of the present biosensor with other reported biosensors for the detection of Hg^{2+}.

Analytical method	Linear range/nM	Detection limit/nM	Ref
Aptamer-based chemiluminescence sensor	–	0.016	[19]
Aptamer-based fluorescence biosensor	1–2000	0.5	[20]
DNA-based electrochemical biosensor	0.2–100	0.2	[21]
DNA-based electrochemical biosensor	6–1066	1	[25]
DNA-based electrochemical biosensor	1–300	1	[26]
DNA-based electrochemical biosensor	8–100	5	[27]
DNA-based electrochemical biosensor	0.5–5000	0.08	[28]
Aptamer-based electrochemical biosensor	0.01–500	0.005	This work

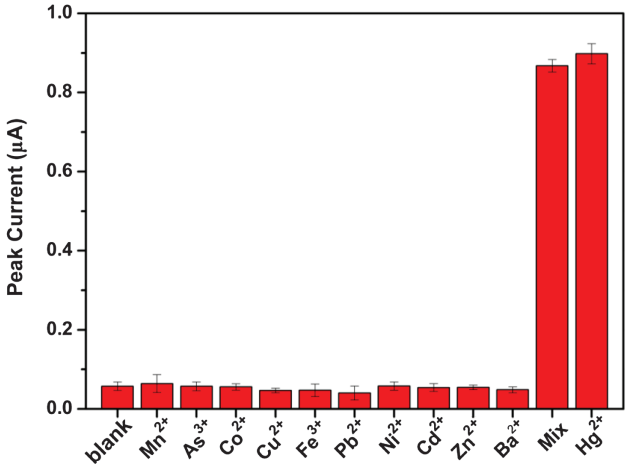

Figure 6. Effect of interference ions on Hg^{2+} detection. DPV peak currents of the aptamer biosensor at 100 nM Mn^{2+}, As^{3+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, Ba^{2+}, and 10 nM Hg^{2+}. Blank and Mix denote the solution without Hg^{2+} and the solution containing 100 nM of the above interfering ions and 10 nM Hg^{2+}, respectively.

current response was obtained for the solution containing Hg^{2+}, while no significant DPV peak current changes were observed for the solutions containing other metal ions at 100 nM. The negligible interference of those ions with Hg^{2+} indicates that the developed aptamer biosensor displays high selectivity toward Hg^{2+}.

Detection of Hg^{2+} in Water Samples

In order to validate and evaluate the accuracy as well as practical application of the constructed aptamer biosensor, tap water samples collected from our laboratory were tested. The procedure details are as follows: the tap water samples were filtered through a 0.24 μm membrane (Millipore) and then diluted with 20 mM PBS buffer (pH 7.4) containing different amounts of a mercury ion standard solution in a ratio of 1:1 to simulate Hg^{2+}-contaminated water. The DPV electrochemical measurement results are shown in Table II. It was confirmed that the developed Hg-aptamer biosensor displays excellent capability for the accurate detection of Hg^{2+} in water samples. The high recovery percentage with acceptable RSD values below 5% demonstrate that the Hg^{2+} detection is not influenced by the tap water composition, thus demonstrating that the proposed aptamer biosensor may be applied to the analysis of real samples.

Table II. Detection of Hg^{2+} in tap water samples.

Sample	Added Hg^{2+}/nM	Detected/nM	Recovery/%	RSD/% ($n = 3$)
Tap water	1.0	0.97	97	2.5
	5.0	4.93	99.2	1.2

CONCLUSION

In summary, a novel aptamer electrochemical biosensor based on an aptamer-functionalized AuNP-modified GCE was successfully developed for the quantitative determination of Hg^{2+}. By combining the advantages of AuNPs and an aptamer, our proposed aptamer/$(AuNPs/CS)_2$/GCE biosensor displays a wider linear range (0.01–500 nM) and lower detection limit (0.005 nM) for the detection of Hg^{2+} than other reported sensors. Moreover, the present biosensor displays excellent sensitivity and selectivity, and good stability and reproducibility, with no interference from other metals during the detection of Hg^{2+} in aqueous samples, demonstrating that the fabricated aptamer biosensor is a promising candidate for Hg^{2+} detection with great potential for practical applications.

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