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An electrochemical aptamer biosensor based on "gate-controlled" effect using β-cyclodextrin for ultra-sensitive detection of trace mercury

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ABSTRACT

A new strategy for construction an electrochemical biosensor for Hg^{2+} analysis with extremely high sensitivity was proposed based on "T–Hg^{2+}–T" coordination by aptamers and "gate-controlled" amplification by switch of the channels of the probe. SH-β-cyclodextrin was self-assembled on the gold electrode to form an orderly arranged molecularly layer with interspaces among β-CDs; thionine labeled aptamer was then linked on the assembled β-CD. When Hg^{2+} was added, the aptamer combined with Hg^{2+} and formed "T–Hg^{2+}–T" structure, which caused the aptamer folded and the labeled thionine covered the interspaces to block off the channel for probe entrance. The oxidative current of probe decreased, which provide the basis for the determination of Hg^{2+}. With the gate-controlled amplification, the changes of trace amounts of Hg^{2+} will produce great changes of the probe current. The sensor exhibited significantly higher sensitivity with a detection limit of 5.0×10^{-15} mol/L, which is lower than other reported methods.

1. Introduction

Heavy metal pollution produces serious harm to human health because accumulated metals can enter an organism and lead to chronic poisoning. Mercury contamination is a continuing concern because mercuric ions are environmental pollutants with severe toxicity (Cao et al., 2009) and resistance to degradation (Yuan et al., 2011). Mercuric ions often exist at trace levels and coexist with a million-fold excess of other ionic species. Thus, developing sensitive and selective methods for detecting heavy metal ions is of crucial significance. Although a number of analytical methods for Hg^{2+} analysis have been developed and several sensors employing colorimetry (Li et al., 2009b; Xue et al.,2008; He et al., 2008; Liu et al., 2010; Kanayama et al., 2011; Lee et al., 2007), fluorescence (Ando and Koide, 2011; Yang et al., 2009; Freeman et al., 2009), QD-fluorescence (Paramanik et al., 2013), SPR (Yu et al., 2004), SERS (Wang and Chen, 2009) and electrochemistry (Cao et al., 2009; Liu et al., 2009; Zhu et al., 2009) have been proposed, the detection limits of these methods and electrodes are not sufficiently low. As such, developing a method that is highly sensitive and analytical is an urgent necessity.

Oligonucleotides have attracted extensive research attention for sensing applications (Xiao and Plaxco, 2009). Aptamers are antibody-like nucleic acids (Tang et al., 2011) and synthetic nucleic acid ligands; they are of particular importance in analytical chemistry because of their excellent efficiency and selectivity (Huang et al., 2013; Bini et al., 2007; McCauley et al., 2003). Aptamer-based sensors can be used not only to detect biological macromolecules but also detect metal ions. The negatively charged DNA can bind with positively charged metal ions, such as Pb^{2+}, K^{+}, Hg^{2+}, and Ag^{+}, for selective determination of metal ions from stable metal-mediated DNA duplexes (Xiao and Plaxco, 2009; Pelossof, et al., 2012; Radi and O'Sullivan, 2006).

The strong coordination of Hg^{2+} to the thymine–thymine (T) mismatched pair has been extensively studied, and a large number of aptasensors have been fabricated based on T–Hg^{2+}–T coordination (Cao et al., 2009; Yuan et al., 2011; Liu et al., 2009; Ono and Togashi, 2004; Chiang et al., 2008; Tang et al., 2010). Improvements in the sensitivities of these aptasensors, however, are restricted because of the limited availability of sites on the aptamer used for probe labeling, which results in low detection signals. New strategies to prepare highly sensitive aptasensors must be developed.

The gate-controlled (Li et al., 2009a) effect has been proven to be an effective method of amplifying detection signals by controlling the probes that enter a gate-like entrance using analyte species; this effect results in an obvious amplification similar to the effects of a low voltage change applied between the grid and cathode on the amplified current between the anode and cathode

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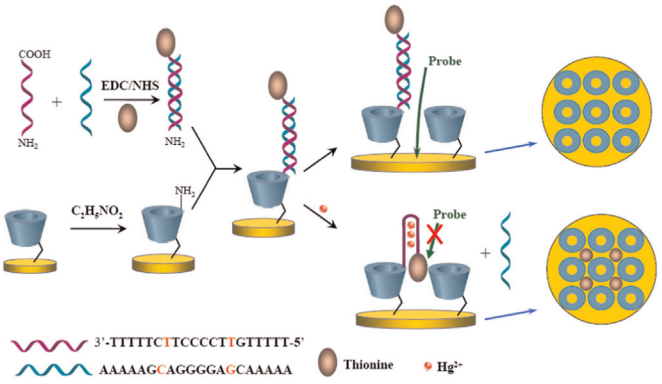

Scheme 1. Schematic of the electrochemical sensor for detecting Hg^{2+}.

in a triode vacuum tube. Thus, the gate-controlled effect provides an attractive alternative for sensitive detection. SH-β-cyclodextrin (SH-β-CD) can be self-assembled on a gold electrode to form a layer with honeycomb lattice structure, and left interspaces among the CD molecules (Henke et al., 1996; Cui et al., 2015), which could be used as the channels of probe passage. However, sensors based on the channels formed by assembled β-CD layer for analytes assay have not been reported.

In this study, a new strategy to detect Hg^{2+} is demonstrated through an electrochemical method based on T–Hg^{2+}–T (Pelossof et al., 2013) coordination and the gate-controlled effect of β-CD. This strategy was firstly introduced to design a novel aptasensor based on the gate-controlled effect with the channels among β-CDs for $[Fe(CN)_6]^{3-/4-}$ probe. The scheme of the strategy for the assay was shown in Scheme 1.

2. Experimental

2.1. Reagents and apparatus

HPLC-purified DNA oligonucleotides were provided by Shanghai Bioengineering Limited Company; these oligonucleotides had the following sequences:

(1) 3′NH_2-*TTTTTCTTCCCCTTGTTTTT*-COOH5′ (A_1);
(2) 3′AAAAAGCAGGGGAGCAAAAA5′ (A_2).

Italicizes letters in the sequence can form intermolecular double strands, as shown in Scheme 1. The oligonucleotides solutions were diluted to 10 μmol/L in pH 7.4 TE buffer containing 10 mmol/L Tris–HCl, 1 mmol/L EDTA (Yuan et al., 2011). A stock solution of 0.001 mol/L Hg^{2+} was prepared by dissolution of 0.3925 g of $Hg(NO_3)_2$ in 20 mL of 1 mol/L HNO_3 and dilution to 1000 mL. The stock solution was then diluted stepwise with pH=7.4 TE buffer solution to obtain the working solutions. All of the reagents were of analytical grade and used as received.

Double-distilled water purified by Milli-Q system was used throughout the experiments; all tests were conducted in a dustless laboratory. Water samples were collected locally and filtered through filter paper before use.

The CHI660 electrochemical workstation (Chenhua Instrument Limited Company, Shanghai, China), a traditional three-electrode system, was used with saturated Ag/AgCl/KCl as the reference electrode, a 3 mm diameter Au electrode as the working electrode, and Pt wire as the counter electrode.

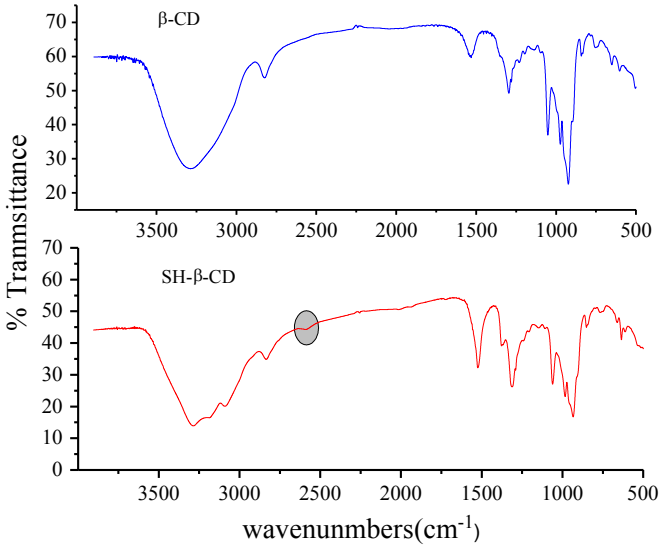

Fig. 1. Infrared spectra of β-CD and SH-β-CD.

2.2. Synthesis of 6-SH-β-CD

SH-β-CD was synthesized as described in reference (Jing et al., 2008). And the method was introduced in supplementary material. The IR spectrum of 6-SH-β-CD in Fig. 1 shows a weak shoulder peak between 2550 and 2590 nm, which is attributed to stretching vibrations of the mercapto group. The figure evidently indicates that 6-SH-β-CD was synthesized successfully.

2.3. Construction of the aptasensor

To design the sensor, the process was mentioned in supplementary material. Briefly, two single-stranded DNAs (ssDNAs; 10 μmol/L), A_1 and A_2 were used; these strands had the following sequences:

A_1: 3′NH_2-*TTTTTCTTCCCCTTGTTTTT*-COOH 5′,
A_2: 3′AAAAAGCAGGGGAGCAAAAA 5′.

As shown in Scheme 1, A_1 and A_2 were hybridized to constitute double-stranded DNA (dsDNA) with a rigid construction (Liu et al., 2009). Thionine was then cross-linked to the dsDNA using NHS/EDC. SH-β-CD was self-assembled on a gold electrode through Au–S bonds.

Finally, 2.5% glutaraldehyde solution was used to covalently cross-link the thionine-modified dsDNAs and amino-functionalized SH-β-CDs.

After reaction, the modified electrode was thoroughly cleaned and incubated in Hg^{2+} solution for 30 min. After the incubation, the electrode was took away from the solution, cleaned by water and then put into $[Fe(CN)_6]^{3-/4-}$ solution for Hg^{2+} determination.

2.4. Electrochemical measurements

Electrochemical measurements including differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were performed in 0.1 mol/L KCl containing 0.05 mol/L $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$. The scanning rate was 100 mV/s, and the potential ranged between −0.2 and 0.6 V *versus* Ag/AgCl. Hg^{2+} concentrations were determined by DPV measurement. All of the electrochemical measurements were performed at room temperature (25 °C).

3. Results and discussion

3.1. The self-assembly conditions

In the self-assembly process, the gold electrode surface was covered by modifier and the current of $[Fe(CN)_6]^{3-/4-}$ gradually decreased (Fig. S2a of supplementary materials), which indicates the formation of self-assembled SH-β-CD layer on electrode. The current decreased slowly with increasing self-assembly time and then remained constant after 80 min. The comprising of self-assembly temperatures at 17 and 30 °C are shown in Fig. S2. The curves obtained under 17 and 30 °C overlapped each other, which revealed that self-assembly could be performed in a wide temperature range.

3.2. Effect of pH value and time of reaction between Hg^{2+} and aptamer

The combination reactions of Hg^{2+} and aptamer, thionine and β-CD, and the oxidation of probe on gold electrode might be influenced by the pH value of solution. Considering the fact that β-CD and aptamer are more stable in alkaline solution in the presence of EDTA (Yuan et al., 2011), Tris–HCl buffer containing 1 mmol/L EDTA (TE) was chosen. The effect of TE buffer with pH values ranged from 7.2 to 8.0 on the responsive current was evaluated, the result was shown in Fig. S3. As a result, pH=7.4 was chosen.

The combination reaction time between aptamer and Hg^{2+} was examined. Fig. S7 showed that the current decreased rapidly with the reaction time because more and more interspaces were blocked by labeled thionine. After 30 min, the current got to a minimum value and remained unchanged. Therefore, 30 min was chosen for the reaction between Hg^{2+} and aptamer.

3.3. The response of the aptasensor

In the absence of Hg^{2+}, the rigid duplex make thionine molecule far away from β-CD, the probe can across through the interspaces among β-CDs and reach the gold electrode to produce an oxidative current. In the presence of Hg^{2+}, the dsDNA unwinds and Hg^{2+} combines with T oligonucleotides in A_1 to form a "T–Hg^{2+}–T" construction because the "T–Hg^{2+}–T" conjunction was more stable than the covalent bond between base pairs in oligonucleotides (Tang et al., 2010), then the ssDNA bends toward β-CD. The labeled thionine at the end of A_1 covered the interspaces and block off the channels. Herein thionine was selected as the molecule for the "gate-control" effect since the dimension of thionine has just the right size to the gaps and the "$-NH_2$" on the molecule could be used to bind with aptamer. With increasing Hg^{2+} concentration, a corresponding decrease in current is observed, similar to a gate closing or opening.

Changes in current during gate opening/closing produced by changes of Hg^{2+} concentrations were measured by cyclic voltammetry (CV) in the presence of 0.05 mol/L $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ in 0.1 mol/L KCl.

As shown in Fig. 2, curve a illustrates the CV curve of a bare gold electrode, which shows a pair of redox peaks. When the gold electrode was modified with β-CD/aptamers, the peak current decreased because the efficient electrode area (curve b) decreased. When 1×10^{-13} mol/L Hg^{2+} was added to the test sample, the peak current decreased (curve c) because of T–Hg^{2+}–T coordination and entrance blockage, which prevent the probes from reaching the electrode surface. When Hg^{2+} was increased to 2×10^{-13} mol/L, the current decreased continuously (curve d).

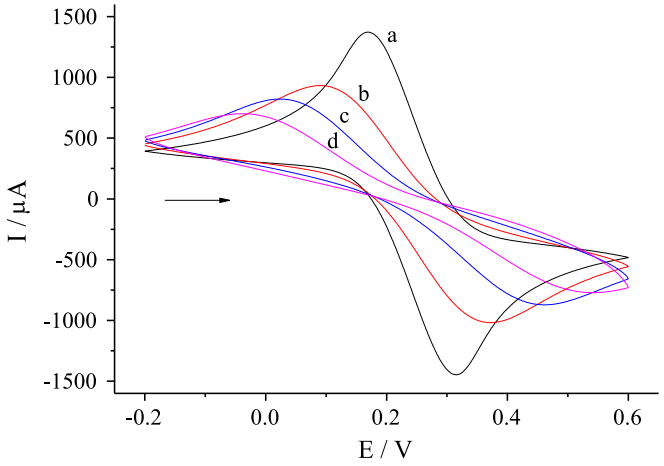

Fig. 2. CV curves of (a) the gold electrode, (b) the β-CD/aptamer modified gold electrode, (c) the β-CD/aptamer modified gold electrode with 1×10^{-13} mol/L Hg^{2+}, and (d) the β-CD/aptamer modified gold electrode with 2×10^{-13} mol/L Hg^{2+} in 0.1 mol/L KCl containing 0.05 mol/L $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$.

3.4. Calibration curve

The analytical performance of the assay based on the sensor was investigated. A corresponding change in current was recorded when the sensor was incubated in Hg^{2+} solutions with concentrations ranging from 1.0×10^{-14} mol/L to 2.0×10^{-12} mol/L. A linear equation of $i=2.1\times10^{-4}$ lgc_{Hg2+}(mol/L)+0.0023 with a linear correlation of $r=0.9960$ was also derived (Fig. 3). The detection limit was 5.0×10^{-15} mol/L at a signal-to-noise ratio of 3.

3.5. Selectivity of the aptasensor

The selectivity of the assay for Hg^{2+} was also evaluated. Several metallic ions that are environmentally relevant were investigated, including Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Cr^{3+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Ba^{2+}, Ag^{+}, K^{+} and SO_4^{2-}; each of the ions solution diluted with pH=7.4 TE buffer solution. Then, added different kinds of interference mental ions to 1×10^{-13} mol/L Hg^{2+} solution and determined the response of sensor to Hg^{2+}. The result shows that more than 1000 times of Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, Mn^{2+} and K^{+} did not affect the result, and 300 times Cu^{2+}, Zn^{2+}, Cr^{3+}, Co^{2+}, 200 times Ni^{2+}, Ba^{2+}, Ag^{+}, 100 times Cd^{2+}, Pb^{2+} did not affect the result. Anions such as SO_4^{2-} and PO_4^{3-} did not interference the assay.

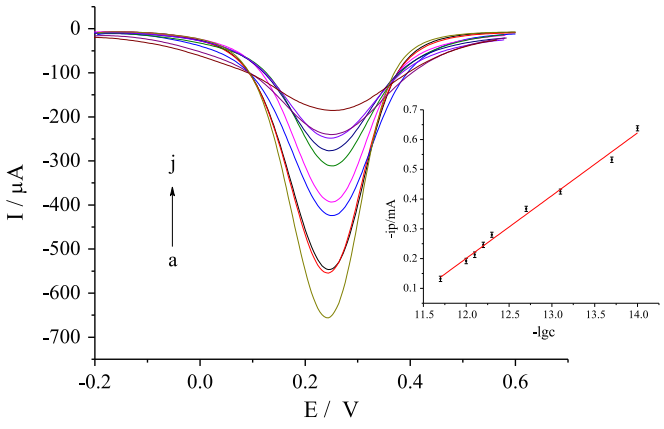

Fig. 3. Differential pulse voltammetry (DPV) curves of the different Hg^{2+} concentrations reacted with the modified gold electrode. From a to j, the concentrations of Hg^{2+} were 0, 1×10^{-14}, 2×10^{-14}, 8×10^{-14}, 1.5×10^{-13}, 2.0×10^{-13}, 5.0×10^{-13}, 6.0×10^{-13}, 1.0×10^{-12}, 2.0×10^{-12} mol/L. All of the Hg^{2+} solutions were mixed with 3 mL of PBS (pH 7.4) during preparation.

Table 1
Comparison of the analytical performance of T-oligonucleotide-based Hg^{2+} biosensors.

Sequences used	Detection methods	Linear ranges (mol/L)	Detection limits	References
T	EIS	1×10^{-9}–1×10^{-3}	1×10^{-10}	Cao et al. (2009)
T/$Ru(phen)_3^{2+}$	ECL	5×10^{-10}–2.5×10^{-6}	2.5×10^{-10}	Yuan et al. (2011)
T/ferrocene	DPV	1×10^{-9}–2.0×10^{-6}	5×10^{-10}	Liu et al. (2009)
T/Quantum Dots	Fluorescence	2×10^{-10}–1×10^{-6}	1.8×10^{-10}	Huang et al. (2013)
T/β-CD	DPV	1×10^{-14}–2×10^{-12}	5.0×10^{-15}	This work

EIS: electrochemical impedance spectroscopy; ECL: electrochemical chemiluminescence; DPV: differential pulse voltammetry.

Table 2
Analytical results of Hg^{2+} ions in various.

Samples	Found (mol/L)	Added (mol/L)	Total found (mol/L)	RSD ($n=5$)	Recovery
Tap water	3.56×10^{-14}	4.0×10^{-14}	7.72×10^{-14}	5.2%	104.0%
Reference material of water*	5.8×10^{-14}	–	–	4.1%	–
Rainwater	Not found	5.0×10^{-14}	5.12×10^{-14}	3.6%	102.4%

* Reference material of water (No. GSB04-1729-2004) was diluted stepwise to 6.2×10^{-14} mol/L before determination.

3.6. Comparison of the analytical performances with other methods

A comparison of the analytical performances of several aptamer-based methods is shown in Table 1. Significant improvements in sensitivity were achieved by the sensor proposed in this work, as evidenced by the considerably lower detection limit provided by this sensor compared with those reported in other references. The enhancement of the sensitivity is obviously obtained by the β-CD/aptamer/thionine system which acts like a gate for the probes similar to a grid of a triode vacuum tube, the changes of trace amounts of Hg^{2+} will produce great current changes of the probe current with higher signal to noise ratio.

3.7. Determination of Hg(II) in real samples

To evaluate the practical applicability of the aptasensor developed in this work, samples of rainwater, tap water, and the standard reference material for water (No. GSB04-1729-2004) were used in the analysis. The analytical results are shown in Table 2. Recoveries of 102.4% and 116.0% were obtained for rainwater and tap water, respectively. The analytical result of Hg^{2+} in the reference material corresponded to the standard value with a relative deviation of −6.45%, which indicates the feasibility of the assay for Hg^{2+} analysis in practical water samples.

3.8. Reproducibility and stability

Five sensors were prepared under similar conditions and used to detect 1×10^{-13} mol/L Hg^{2+}; the reproducibility obtained was satisfactory, with a relative standard deviation (RSD, $n=5$) of 9.51%. Storing the aptasensor at a constant temperature of 4 °C in a refrigerator did not change the electric current of DPV, which obviously demonstrates the stability of the developed biosensor.

4. Conclusion

In conclusion, the β-CD-derived electrochemical aptamer sensor for Hg^{2+} based on T–Hg^{2+}–T coordination and the gate-controlled effect exhibited significantly higher sensitivity and a lower detection limit than other reported methods. The proposed strategy shows potential use in the sensitive determination of other analytes.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2015.06.061.

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