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Highly sensitive electrochemical detection of mercury (II) via single ion-induced three-way junction of DNA

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ABSTRACT

A "signal-on" electrochemical sensing strategy was designed for highly sensitive and selective detection of mercury (II) via its induction to three-way junction of DNA (DNA-TWJ). The TWJ consisted of the capture probe that was self-assembled on a gold electrode surface through S—Au bond, the signal probe that was labeled with ferrocene (Fc) and contained single T–T mismatch to capture probe, and an assistant probe for the formation of DNA-TWJ upon the presence of mercury (II). This process caused the Fc tag approaching the electrode for fast electron transfer and thus increased the oxidation current. The "signal-on" sensing method could detect Hg^{2+} ranging from 0.005 to 100 nM. The assay was simple and fast. It showed potential application in on-site and real-time Hg^{2+} detection.

1. Introduction

As one of the most well-known highly toxic heavy metal ions, water-soluble mercuric ion (Hg^{2+}) causes adverse effects on the environment and human health [1]. For example, mercury (II) can accumulate in the human body through the food chain, leading to the damage of immune, central nervous and endocrine systems [2]. Hence, effective methods featured with simplicity, high sensitivity and selectivity are urgently needed for Hg^{2+} detection.

Since Hg^{2+} can interact specifically with thymine bases (T) in two DNA strands to form stable T–Hg^{2+}–T structure, which is even more stable than the Watson–Crick adenine–thymine pair [3], various DNA-based Hg^{2+} sensors with fluorescent [4–7], colorimetric [8–12] or electrochemical [13–19] detection have been proposed. Among these sensors, the "one-step" detection strategies have attracted considerable attentions [3,4,11,19–21]. They can produce the "on" or "off" switch signal through the formation of T–Hg^{2+}–T base pairs. However, these simple methods need multiple T–Hg^{2+}–T coordination for forming one DNA switch structure, which limits their sensitivity. Thus different amplification strategies [22], such as exonuclease mediated metal ion cyclical reshuffling [18], have recently been employed to improve the detection sensitivity. However, the amplification steps increase the complexity of analytical procedures with strict detection conditions, prolong the assay time, and sometimes produce high background.

Recently a three-way DNA structure with conformational versatility [23–25] has been presented as a well-defined structural target for highly specific detection of drugs or small-molecules [25]. In this structure three double helical arms are equilibrated at a junction point [26–29]. Here a novel three-way junction of DNA (DNA-TWJ) was designed using a single T–Hg^{2+}–T base pair to induce the DNA hybridization and assembly, which means that the hybridization between complementary bases of these three different DNA strands depends on the presence of Hg^{2+}. As the formation of each DNA-TWJ structure was corresponded to a single T–Hg^{2+}–T coordination, the strategy possessed much higher sensitivity than multiple T–Hg^{2+}–T coordination. Moreover, the specific assembly led to high selectivity for the detection of Hg^{2+}.

No junction occurred among three probes due to the weak affinity of low number base pairs. However, the presence of Hg^{2+} could induce T–Hg^{2+}–T coordination and trigger the DNA hybridization and assembly to form the DNA-TWJ on electrode (Fig. 1), which led to the approach of Fc tag to the electrode surface, and thus produced a sensitive "signal-on" sensing method for one-step detection of Hg^{2+}. The "signal-on" method showed a wide detectable range of 0.005 to 100 nM without any additional amplification step. The simple and fast assay possessed high selectivity and good accuracy, providing potential application in on-site and real-time Hg^{2+} detection.

2. Experimental

2.1. Chemicals

Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) and 6-mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich (USA). Hg^{2+} stock solution (0.1 M) was prepared by dissolving

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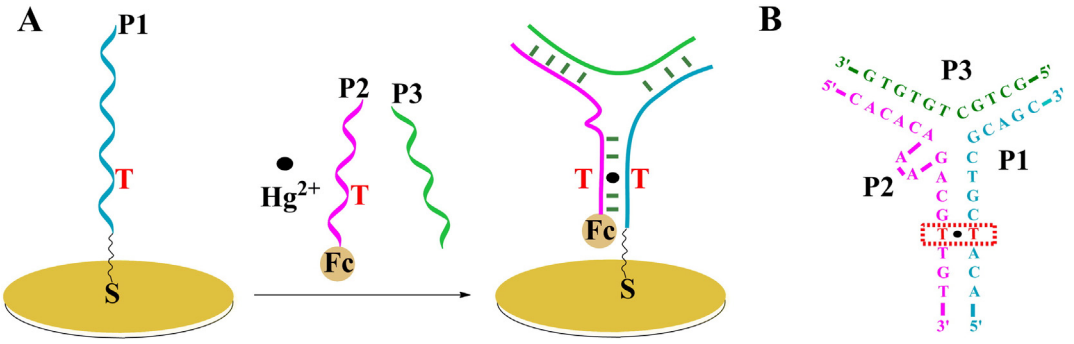

Fig. 1. Schematic diagram of (A) the "signal-on" electrochemical sensing platform of Hg^{2+} and (B) the single T–Hg^{2+}–T coordination induced synergy-dependent DNA three-way junction.

$Hg(Ac)_2$ with 0.5% HNO_3 for preventing the formation of HgO particles. Prior to use, the pH of Hg^{2+} solution was adjusted to 6.0. All of the oligonucleotides were synthesized and purified with HPLC by Takara Biotech. Co. Ltd. (Dalian, China) and their sequences were listed as follows:

P1: 5′-SH-$(CH_2)_6$-ACATCGTC*GCAGC*-3′

P2: 5′-CACACAAAGACGTTGT-$(CH_2)_6$-Fc-3′

P3: 5′-*GCTGC*TGTGTG-3′

The binding regions between probes are shown in bold, italic and underlined, respectively. Their solutions were prepared in 10 mM Tris–HCl buffer containing 10 mM NaCl (pH 8.0).

2.2. Apparatus

All electrochemical measurements were performed on a CHI 660D electrochemical workstation (CH Instruments Inc., USA) at room temperature with a conventional three-electrode system composed of a platinum wire, a saturated calomel and the Hg^{2+} sensor as counter, reference and working electrodes, respectively.

2.3. Preparation of electrochemical sensor

The electrochemical sensor was constructed on a gold electrode. Prior to modification, the Au electrode was polished with 1.0, 0.3 and 0.05 μm α-Al_2O_3 slurry, respectively, followed by successive sonication with pure water and ethanol for 3 min, and electrochemically cleaned in 0.1 M H_2SO_4 by potential scanning between −0.2 and +1.6 V until a reproducible cyclic voltammogram was obtained. After washing with pure water and drying in a nitrogen stream, 6 μL of 1.0 μM P1 including 10 μM TCEP, which was included to reduce disulfide bonded oligos, was dropped on the electrode to incubate at room temperature for 2 h. After rinsing with 10 mM pH 7.4 Tris–HCl buffer and drying with nitrogen, 6 μL of 1 mM MCH was dropped on the electrode for 30 min to block the unmodified sites.

2.4. Electrochemical detection of Hg^{2+}

6 μL of mixture solution containing 1 μM P2, 1 μM P3 and Hg^{2+} at known or unknown concentrations was firstly dropped on the sensor surface. After reaction for 25 min, the sensor was rinsed with 10 mM Tris–HCl buffer (containing 0.1 M NaCl) and detected in 10 mM Tris–HCl buffer (containing 1.0 M $NaClO_4$, pH 7.4) with alternating current voltammetry (ACV), which was scanned from 0 to +700 mV with an amplitude of 25 mV, a frequency of 100 Hz and a step of 4 mV.

3. Results and discussion

3.1. Characterization of electrochemical sensing strategy

To confirm the DNA-TWJ mechanism based on the single Hg^{2+}-induced synergy-dependent hybridization (SDH), ACV response of the sensing system was tested (Fig. 2a). Similar to the control response of the sensor (curve 1), negligible response was observed in the absence of Hg^{2+} (curves 2–4) or assistant DNA probe (curve 5), while obvious oxidation peak of Fc was observed when the P2 and P3 coexisted with Hg^{2+} (curve 6), indicating that the formation of DNA-TWJ highly depended on both P3 and the presence of Hg^{2+} due to the mismatch of T base in P1 and P2.

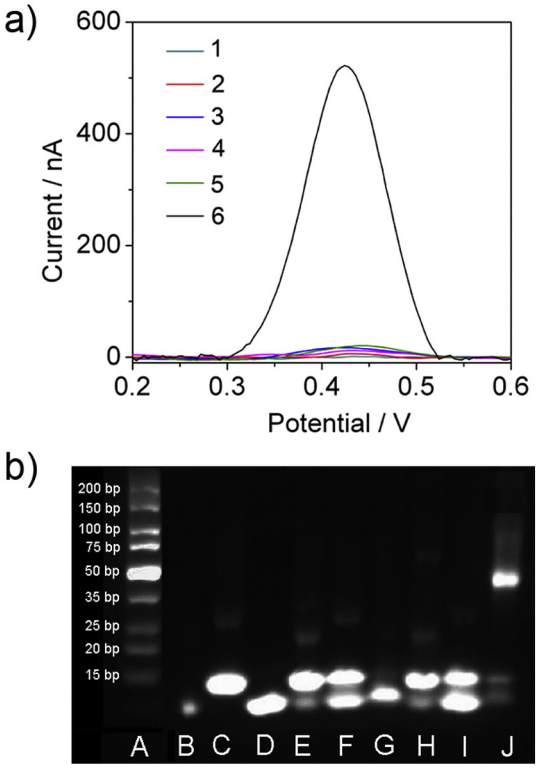

Fig. 2. (a) ACV responses of the sensor in 10 mM Tris–HCl buffer (pH 7.4) containing 1.0 M $NaClO_4$ without (1) and with (2) 1 μM P2, (3) 1 μM P3, (4) 1 μM P2 and P3, (5) (2) + 100 nM Hg^{2+}, and (6) (4) + 100 nM Hg^{2+}, and (b) PAGE analysis of (A) DNA ladder marker, (B) P1, (C) P2, (D) P3, the mixture of (E) P1 and P2, (F) P2 and P3, (G) P1 and P3, (H) P1+ P2 + 100 nM Hg^{2+}, (I) P1, P2 and P3, and (J) P1 + P2 + P3 + 100 nM Hg^{2+} at P1, P2 and P3 concentration of 10 μM.

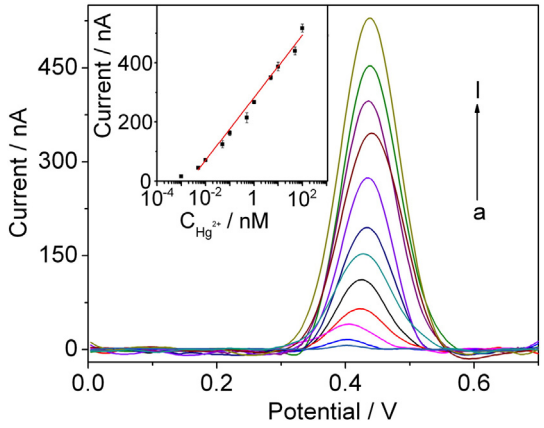

Fig. 3. ACV responses of the proposed sensor to Hg^{2+} at 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 nM (a-l) in 10 mM Tris–HCl buffer (pH 7.4) containing 1.0 M $NaClO_4$. Inset: Plot of peak current vs logarithm (log) value of Hg^{2+} concentration. Results were expressed as the average of three independent experiments and error bars represent standard deviations (S.D.).

The formation of the DNA-TWJ was further confirmed with PAGE analysis (Fig. 2b). P1, P2 and P3 exhibited individual clear band, respectively, while all the mixture of two probes including P1–P2, P2–P3 and P1–P3 pairs with and without Hg^{2+} displayed two bands located in the same position as the pure probes, indicating no hybridization was observed between P1–P2, P1–P3 and P2–P3 pairs even in the presence of Hg^{2+} (band H). Similar band distribution was also observed for the mixture of P1, P2 and P3 (band I), indicating that no hybridization complex was formed in the absence of Hg^{2+}, which led to a low background for the electrochemical detection of Hg^{2+}. Upon the addition of Hg^{2+} to the mixture of P1, P2 and P3, a new bright band with a slow migration speed appeared at ~40 bp, while the bands for the remaining P1, P2, and P3 were also observed at the similar band position as their mixture (band H). This result should be contributed to the formation of DNA-TWJ product, verifying the DNA-TWJ mechanism triggered by the single Hg^{2+}-induced SDH.

3.2. Detection of Hg^{2+} with DNA-TWJ strategy

The effective area of DNA-TWJ modified Au electrode could be measured to be 0.0105 cm^2 with chronocoulometry using 0.1 mM $K_3[Fe(CN)_6]$ ($D = 7.6 \times 10^{-6}$ cm^2 s^{-1}). At the reaction time of 25 min, the ACV response increased with the increasing concentration of Hg^{2+} (Fig. 3), which implied that more three-way DNA structures along with the electroactive Fc group were formed on the sensor surface upon the increase of Hg^{2+} concentration. The half-peak width for the oxidation of attached Fc group was about 100 mV, close to 90 mV for a reversible electrode process, demonstrating the fast electron transfer. The calibration plot showed a good linear relationship between the peak current and the logarithm (log) value of Hg^{2+} concentration ranging from 0.005 to 100 nM with a correlation coefficient of 0.9930 (inset in Fig. 3). The lowest detectable concentration of 5 pM, as the detection limit, was more than 1000 times lower than the standard level permitted by the United States Environmental Protection Agency and WHO for drinking water (10 nM and 30 nM). This detection limit was also at least 1.0 times lower than other electrochemical sensing methods [15–19,30,31], 49 times lower than T–Hg^{2+}–T-based electrochemiluminescent detection [32,33], 9 times lower than T–Hg^{2+}–T-based chemiluminescence assay [34], 9900 times lower than T–Hg^{2+}–T-based colorimetric methods [8–11], and even 35 times lower than the T–Hg^{2+}–T-based fluorescent analysis [4–7]. The ultra-low detection limit, high sensitivity and wide linear range over 4 orders of magnitude indicated promising application of the proposed Hg^{2+} sensor based on mercury (II)-induced DNA-TWJ.

3.3. Selectivity and stability of sensor

The selectivity of the proposed sensor for Hg^{2+} was evaluated by exposing the sensor to the aqueous solution containing other environmentally relevant metal ions, such as Mg^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+} and Ag^{+}. As shown in Fig. 4a, the sensor showed obvious response to the solution containing Hg^{2+}, while negligible response was observed in the solutions containing interference ions even at a 100-fold higher concentration than Hg^{2+} (10 μM *vs.* 0.1 μM). In addition, the current responses of both 5 and 100 pM Hg^{2+} in the presence of interfering ions were similar to those without interfering ion (insert in Fig. 4a), indicating the proposed mercury (II)-induced DNA-TWJ sensing strategy possessed high selectivity for Hg^{2+} detection and potential application for analysis of complex samples.

After the detection of Hg^{2+}, the used sensor could be conveniently regenerated by immersing it in 100 nM Na_2S for 10 min. After the immersion treatment, the current response decreased instantly to the blank level, suggesting that the DNA-TWJ was broken because Hg^{2+} was taken away from the T–Hg^{2+}–T coordination by S^{2-} to produce HgS precipitate [31], leading to the release of Fc-labeled P2, as well as P3, from the sensor surface to regenerate the sensor. The regenerated

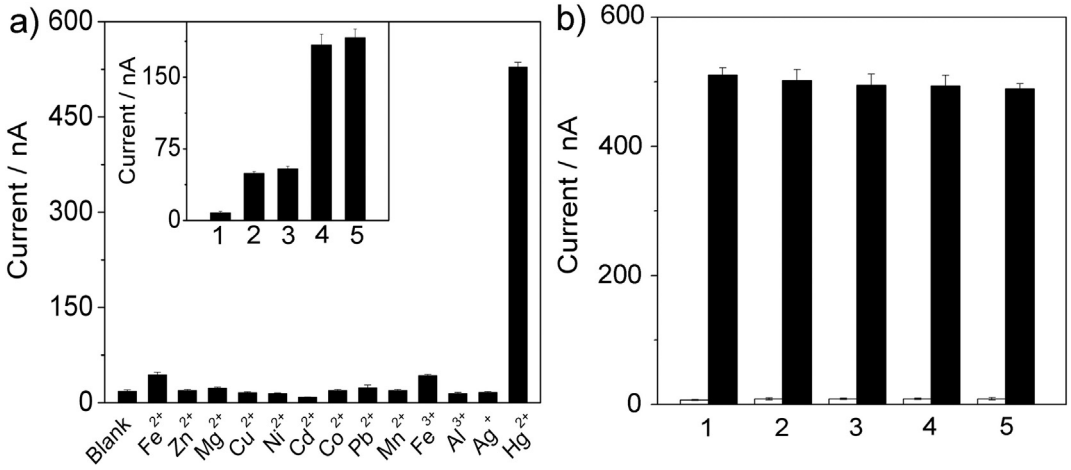

Fig. 4. (a) ACV responses at 0.1 μM Hg^{2+} and 10 μM other metal ions. Inset: ACV responses of (1) blank, (2) 5 pM Hg^{2+}, (3) (2) + the mixture of other metal ions at 500 pM, (4) 0.1 nM Hg^{2+}, (5) (4) + the mixture of other metal ions at 10 nM. (b) Typical ACV peak currents at 100 nM Hg^{2+} upon the sensor regeneration with 100 nM Na_2S for 10 min in 5 regeneration runs.

sensor retained its original performance after five regeneration cycles (Fig. 4b), indicating that the proposed sensor could be almost completely recovered to the original state by Na_2S and recycled for Hg^{2+} sensing.

4. Conclusions

A three-way structure of DNA with single mismatched thymine was designed for highly sensitive and selective electrochemical sensing of Hg^{2+}. By combining the T–Hg^{2+}–T coordination with the assembly of DNA-TWJ structure, the proposed electrochemical sensing method exhibited the following features: (1) it adopted a one-step assay format, and thus was simple and fast; (2) only the T–Hg^{2+}–T coordination could induce the SDH and the formation of DNA-TWJ, leading to extremely low nonspecific background signal and excellent detection selectivity for Hg^{2+}; and (3) the single T–Hg^{2+}–T induced SDH and DNA-TWJ led to a wide linear range (4 orders of magnitude) for Hg^{2+} with a detectable concentration down to 5 pM. The assay method possessed potential application in on-site and real-time Hg^{2+} detection.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgments

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