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Notes & tips

A novel electrochemical biosensor for selective determination of mercury ions based on DNA hybridization

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ABSTRACT

An electrochemical biosensor was developed for Hg^{2+} determination based on DNA hybridization. In the presence of Hg^{2+}, the target and probe DNAs with thymine–thymine (T–T) mismatches could hybridize by forming T–Hg^{2+}–T complex. This induced DNA hybridization led to the decrease in reduction peak currents of ethyl green (EG) as electroactive label, which could be used for determination of Hg^{2+}. The difference in the value of the peak currents of EG before and after DNA hybridization (ΔI) was linear with the concentration of Hg^{2+} in the range of 9.0×10^{-11}–1.0×10^{-9} M. The detection limit was 3.08×10^{-11} M.

Heavy metal ions are considered as the serious sources of environmental pollutants and cause many diseases [1]. The upper limits of Hg^{2+} as a toxic and harmful heavy metal in drinking water mandated by United States Environmental Protection Agency (EPA) and the World Health Organization (WHO) are 10 and 5 nM, respectively [2–4]. Therefore, sensitive and selective determination of this ion is very important. Various electrochemical [2,3], spectroscopic [5] and optical [6] analytical techniques have been developed for the determination of Hg^{2+}. Among these methods, electrochemical methods have attracted great interests because of excellent properties, such as low cost, short analytical time, high sensitivity and selectivity [1,2].

Some heavy metal ions can form complexes with certain nucleic acid bases [1]. Hg^{2+} can interact specially with thymine (T) to form stable T–Hg^{2+}–T structure [1–4]. This approach could develop a special biosensor for detection of Hg^{2+} [2–4,7]. In this work, we applied two single-strand poly-T DNAs as probe and target to consider the ability of Hg^{2+} to hybridize two oligonucleotides with full T–T mismatches. Carbon paste electrode (CPE) was modified with silver nanoparticle in order to improve the efficiency of biosensor.

Several kinds of labels are commonly applied to detection of DNA hybridization [8]. The changes in the electrochemical signal of labels after DNA hybridization are dependent on the mode of DNA-label interaction. If the interaction mode be electrostatic; the signal of label at probe-modified electrode largely decreases after hybridization, whereas, in the situation of intercalating or groove bonding modes, the signal of label largely increases after hybridization [9]. Based on results of our recent work, dominant DNA-dye ethyl green (EG) interaction mode is electrostatic [8]. The Hg^{2+}-induced DNA hybridization of these oligonucleotides led to the decrease in the reduction peak currents of EG, which could be used for electrochemical determination of Hg^{2+}.

Materials and reagents, apparatus, preparation of silver nanoparticles-modified carbon paste electrode (SN-CPE) and characterization of silver nanoparticles were described in the online supplementary material and Fig. S1 of the supplementary material. Electrochemical activation of working electrodes was performed by imposing optimized potential (1.8 V vs. SCE) during optimized time (5 min) [9] in 0.5 M acetate buffer solution (pH 4.8) comprising 20 mM NaCl without stirring. Following activation of the SN-CPE, the probe was immobilized on the activated electrode by applying 0.50 V potential vs. SCE for 5 min in 50.0 mM Tris–HCl buffer solution, pH 7.40 containing 10^{-6} M probe DNA and 20 mM NaCl with stirring. Then immobilized probe/NS-CPE was immersed in a solution containing 10^{-6} M of DNA target, 20 mM of NaCl and a series of different concentrations of Hg^{2+} for 5 min with stirring and applying potential of 0.5 V vs. SCE to the working electrode. After immobilization of probe on the electrode, the label was accumulated on the probe-modified electrode by immersing the electrode in 10 mM

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Tris–HCl buffer solution (pH 7.0) containing 1.0 mM EG for 5 min with stirring and without applying any potential to SN-CPE. In the presence of Hg^{2+}, the hybridization of probe with target by forming T–Hg^{2+}–T complex led to the decrease in the peak currents of EG. The difference in the value of the EG currents before and after hybridization, could be used for sensing of Hg^{2+} (Fig. 1A). The significant increase in the peak current of EG after immobilization of probe was attributed to the strong interaction between EG and the single strand DNA. When no Hg^{2+} was added into the hybridization solution, the probe and target did not hybridize because of the mismatched bases. To make sure about this behavior, the change in the signal of EG was recorded during the hybridization of probe with real target DNA, too (Fig. 1A (curve c)). The electroanalytical performance of the sensor for different concentrations of Hg^{2+} was investigated and the limit of detection was calculated (Fig. 1B). The EG reduction peak was obviously decreased with increasing the amount of the Hg^{2+}. To compare the efficiency of nanoparticles modified CPE with CPE as biosensors, the voltammetric determination of Hg^{2+} was performed on both electrodes. The results for CPE were shown in Fig. S2. ΔI was linearly dependent on the concentrations of Hg^{2+} in a range from 1.0×10^{-10} to 1.0×10^{-9} M and 9.0×10^{-11} to 1.0×10^{-9} M, respectively for biosensor and nanoparticles modified-biosensor. The detection limits 1.58×10^{-10} and 3.08×10^{-11} M, for biosensor and nanoparticles modified-biosensor, respectively. These values proved that the efficiency of biosensor was improved by the presence of nanoparticles in the carbon paste electrode. Table S1 shows the performance of some similar electrochemical DNA-based sensors for Hg^{2+} determination.

Results of selectivity experiment indicated that the response signal to Hg^{2+} was not significantly affected by the addition of other mixed metal ions, demonstrating a satisfactory selectivity for Hg^{2+} detection (Fig. S3). To demonstrate the applicability of the biosensor, the sensor was used in the analysis of Hg^{2+} in tap water that was directly spiked with certain amounts of Hg^{2+}. The recovery values demonstrated the accuracy of the proposed method for the detection of low concentration of Hg^{2+} in real samples (Table S2). The utility of the proposed biosensor was also tested by the determination of Hg^{2+} concentration in dental amalgam. The result was described in the online supplementary material.

In summary, we designed an electrochemical DNA biosensor for determination of Hg^{2+}. The hybridization of the two oligonucleotides through T–Hg^{2+}–T coordination led to the decrease in the peak currents of EG. We have determined Hg^{2+} successfully, selectively and sensitively by the difference in the value of peak currents of label before and after DNA hybridization.

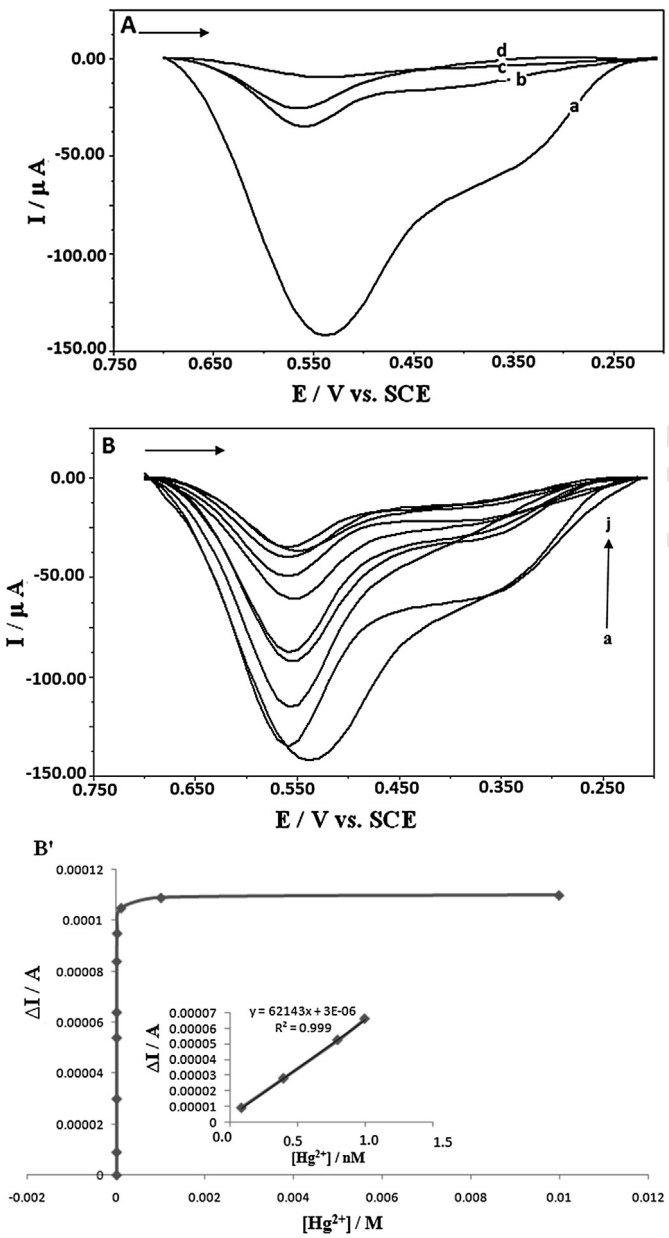

Fig.1. (A) Differential pulse voltammograms of 10^{-3} M EG accumulated on the (a) probe-immobilized working electrode, (b) probe-immobilized working electrode after hybridization with target in the presence of 0.01 M Hg^{2+}, (c) after hybridization with real target and (d) bare activated working electrode at SN-CPE. (B) Differential pulse voltammograms of 10^{-3} M accumulated EG on the probe-immobilized working electrode after hybridization with target in the presence of different concentrations of mercuric ions from 0 to 0.01 M (a–j) in 10 mM Tris–HCl buffer solution (pH 7.0). (B′) Variation of DPV responses vs. mercuric ions concentration at SN-CPE, inset: related calibration plot of Hg^{2+} concentration in the range of 0.09–1.00 nM. Modulation amplitude: 0.04995 and step potential: 0.01005 V vs. SCE.

Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.ab.2015.07.011.

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