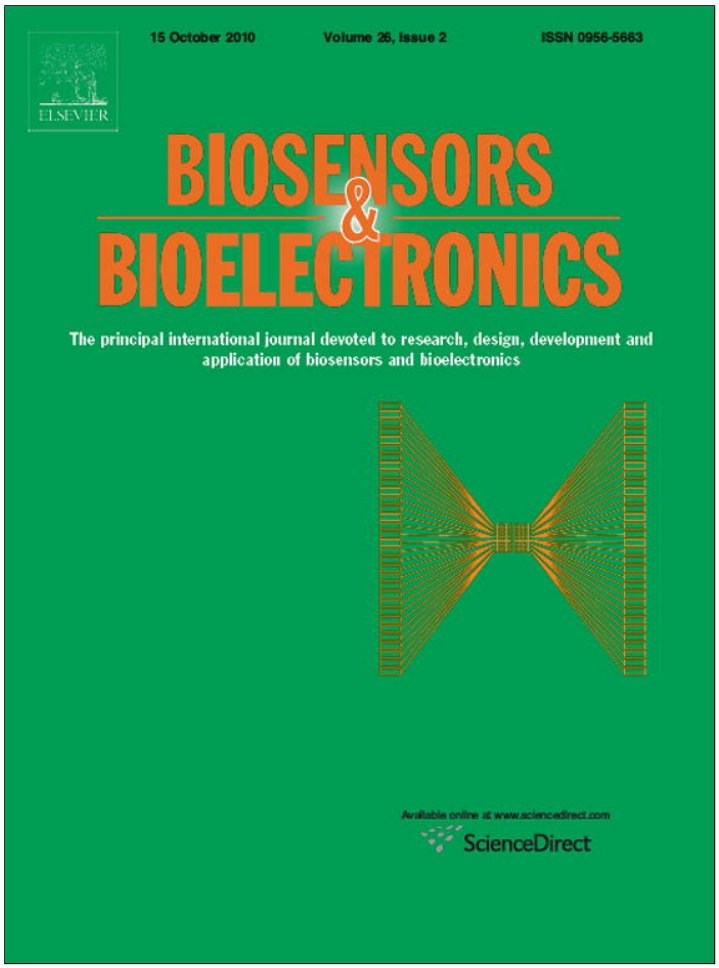

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Short communication

Rapid and highly sensitive detection of mercury ion (Hg^{2+}) by magnetic beads-based electrochemiluminescence assay

Qiang Li, Xiaoming Zhou, Da Xing*

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou 510631, China

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ABSTRACT

A novel and highly sensitive electrochemiluminescence (ECL) assay based on magnetic beads separation/collection process and thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) coordination chemistry has been designed to detect Hg^{2+} ions in aqueous solution. In this protocol, two amine-terminated complementary DNA probes with five thymine–thymine (T–T) mismatches were introduced. One was coupled with carboxyl-modified magnetic beads and the other was labeled with tris (2, 2-bipyridine) ruthenium (II) (TBR). The couple of DNA probes, in the presence of Hg^{2+}, can form double-stranded structure via the Hg^{2+}-mediated coordination of T–Hg^{2+}–T base pair. Therefore, they can be collected on the surface of electrode using a magnetic field. On the electrode, TBR labels and tripropylamine (TPA) could react to emit photons, which were detected by a custom-built ECL system. The detection limit for Hg^{2+} in this assay is 5 nM, which is below the upper limit of Hg^{2+} for drinkable water mandated by United States Environmental Protection Agency (EPA), 10 nM. To the best of our knowledge, this is the first example of ECL assay applying to detect Hg^{2+} with highly sensitivity and selectivity.

1. Introduction

Mercury ion is a highly toxic environmental pollutant and has lethal effects on human health such as damaging the brain, heart, kidney and many other organs (Tchounwou et al., 2003; Hoyle and Handy, 2005). Moreover, mercury contamination is widespread coming from a variety of anthropogenic and natural sources including coal and gold mining, solid waste incineration, wood pulping, fossil fuel combustion, and chemical manufacturing (Nolan and Lippard, 2008). Divalent mercuric ion (Hg^{2+}) is one of the most stable form for mercury pollution. Therefore, it is critical to design a sensitive, rapid and simple assay for the monitoring of Hg^{2+}. Recently, a variety of techniques for monitoring Hg^{2+} have been developed, including colorimetric method (Lee et al., 2007; Xue et al., 2008; Li et al., 2008; Liu et al., 2008; Xu et al., 2009), fluorometric method (Liu et al., 2009a,b; Ye and Yin, 2008; Wang et al., 2008; Liu, 2008; Chiang et al., 2009; Wang and Liu, 2008) and electrochemical method (Zhu et al., 2009; Kong et al., 2009; Miao et al., 2009; Cao et al., 2009; Liu et al., 2009a,b), since the property of thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) coordination chemistry (Ono and Togashi, 2004; Sugiyama et al., 2005; Tanaka et al., 2007) was proposed. Compared with the classical methods for mercury detection such as inductively coupled plasma mass spectrometry (ICPMS) (Li et al., 2006) and atomic absorption/emission spectroscopy (Han et al., 2006) which require sophisticated instrumentation and time-consuming sample pretreatment, these methods taking advantage of Hg^{2+} mediated thymine–thymine (T–T) bases pair have high selectivity. However, most of these approaches exhibit some features that limit its practical use. The gold nanoparticle based colorimetric assay can operated at room temperature, demonstrating the property of simplicity and rapidity. But their sensitivity is poor (about 1 μM). The fluorometric methods for mercury still need a sophisticated instrumentation though it has a high sensitivity. Electrochemical biosensor needs the immobilization of the probe on the electrode surface resulting in a complicated pretreatment process.

Herein, we described a novel and highly sensitive electrochemiluminescence (ECL) assay for the detection of Hg^{2+} ions in aqueous solution. This assay takes advantage of the selective thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) coordination chemistry and magnetic beads separation/collection process. The specifically interaction that Hg^{2+} ions can interact with thymine bases to form T–Hg^{2+}–T complexes has been used to design oligonucleotide-based probes for Hg^{2+} detection. Magnetic bead labeled with capture probes have been used to separate the products for Hg^{2+} detection and then collect them on the electrode, making the method simple and high sensitive. The experiment was operated on a custom-built ECL system which was designed by our group (Yan et al., 2004; Zhou et al., 2009). Due to the introduction of magnetic beads separation/collection process, electrode can be reused after rinsed out the reactants with deionized water, making the method time- and cost-effective. Result shown that the limit of

* Corresponding author. Tel.: +86 20 8521 0089; fax: +86 20 8521 6052.
E-mail address: xingda@scnu.edu.cn (D. Xing).

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Hg^{2+} detection in this assay is 5 nM, which is below the upper limit of Hg^{2+} mandated by United States Environmental Protection Agency (EPA), 10 nM.

2. Materials and methods

2.1. Materials

All chemicals were of analytical reagent grade and were used as received. 5′-NH_2-$(A)_{20}$TTCGTGTTGTGTTCG-3′ was used as the magnetic beads-capture probe, and 5′-NH_2-GTCGTTCTCAACTCGTA-3′ was used for tris (2, 2-bipyridine) ruthenium (II) (TBR) labeling, which were synthesized and HPLC-purified by Shanghai Sangon Biological Engineering & Technology Services Co. Ltd. (SSBE). The carboxyl-terminated magnetic beads (2–3 μm) were acquired from Tianjin BaseLine ChromTech Research Centre. 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES, 99%) was obtained from Sigma (St. Louis, MO, USA). $Hg(ClO_4)_2$ was purchased from Alfa Aesar (Royston, England). tripropylamine (TPA) and the chemicals to synthesize the $Ru(bpy)_3^{2+}$ N-hydroxysuccinimide ester (TBR-NHS ester) were got from Sigma (Louis, MO, USA). The TBR-NHS ester, synthesized in our laboratory according to Terpetschnig's et al. (1995)paper, was introduced into TBR-probe as described by Kenten et al. (1991).

2.2. Immobilization of capture DNA on to magnetic beads

Magnetic bead-capture probes were prepared according to the literatures (Miao et al., 2008; Fan et al., 2008) with certain modifications. Briefly, 100 μL of carboxyl-modified magnetic beads were washed 3 times with 500 μL of 100 mM imidazole buffer (pH 7.0), and then activated in 1000 μL of 100 mM imidazole buffer containing 30 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) with gently shaking for 30 min. 5 μL (10 μM) of capture probe DNA was added into the mixture and incubated for 2 h at 37 °C with gentle mixing. Magnetic bead-capture probes were washed 3 times with 500 μL wash buffer (7 mM Tris–HCl, pH 8.0, 170 mM NaCl, 0.05% Tween20) and resuspended in 250 μL buffer (20 mM Tris–HCl, pH 8.0, 500 mM NaCl) before use.

2.3. ECL detection

The ECL assay procedure was as follows: 1 μL Hg^{2+} with different concentration was added to 99 μL mixture of TBR-probes and magnetic bead-capture probes (both 10 nM) in HEPES buffer (50 mM pH 7.0), containing 100 mM $NaNO_3$, and incubated at room temperature for 15 min. The reactant was separated and collected using magnetic field, and washed three times with HEPES buffer (50 mM pH 7.0), containing 100 mM $NaNO_3$. 100 μL TPA buffer was added to the sample and shaked gently, then transferred it to the ECL detection cell. The magnetic beads-capture probes and TBR-probe form strong and stable duplexes as Hg^{2+} can specifically interact with T–T bases, and then captured and temporarily immobilized on the working electrode by a magnet under it. The applied potential of the ECL reaction was fixed at 1.25 V and photon signal was measured.

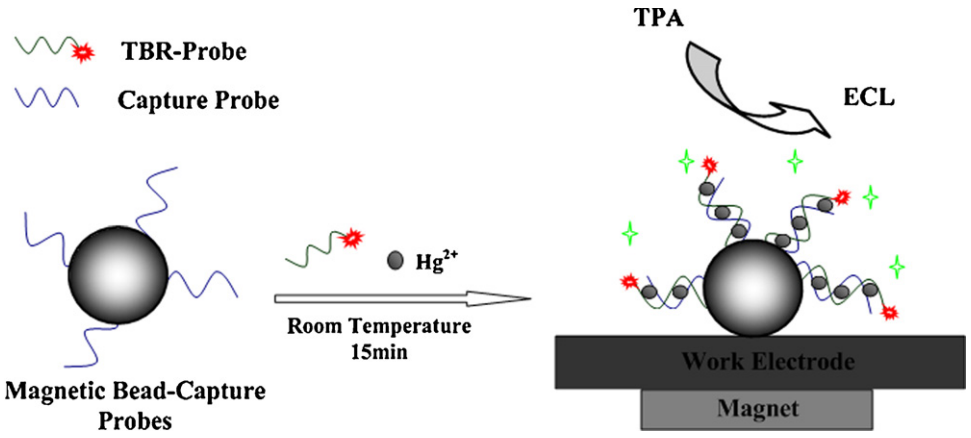
The cut-off value for Hg^{2+} detection was calculated based on the average ($V_{control}$) and standard deviation ($V_{stdev(con)}$) of the ECL values of the negative control, shown as formula (1). A sample with an ECL value higher than the cut-off value was considered to be positive for Hg^{2+} detection.

$$V_{cut\text{-}off} = V_{control} + 3V_{stdev(con)} \quad (1)$$

3. Results and discussion

Scheme 1 depicts the design of the ECL assay for Hg^{2+} ions detection. Two amine-terminated complementary DNA probes with five T–T mismatches were introduced in this report. One was coupled with carboxyl-modified magnetic beads and the other was labeled with TBR. The two DNA probes, in the presence of Hg^{2+}, form the double-stranded structure via the Hg^{2+}-mediated coordination of T–Hg^{2+}–T base pairs. Therefore, they can be collected on the surface of electrode using a magnetic field, and the TBR labels were reacted with the most efficient coreactant, TPA. Photons were produced and detected by a custom-built ECL system. In the absence of Hg^{2+}, the TBR-labeled probe does not hybridize with the magnetic bead-capture probe because the operation temperature is higher than the melting temperature (Tm) due to the T–T mismatches. Accordingly, few TBR-labeled probe are bound to magnetic beads after separating with a magnetic field, resulting in a weak ECL signal when it was reacted with the TPA in detection cell. In this assay, multiple samples can be detected continuously after rinsed out the reactants with deionized water from the work electrode.

To evaluate the sensitivity of the ECL assay for Hg^{2+} detection, various concentrations (1 nM, 5 nM, 10 nM, 50 nM, 100 nM, 150 nM, 200 nM, and 250 nM) of Hg^{2+} from one stock solution were tested. The same reaction system without Hg^{2+} was also detected as a negative control, which ECL signal intensity was 19.61 ± 11.31 counts per second (cps). The ECL signal intensity increased in the presence of increasing Hg^{2+} concentration within the range from 1 nM to 250 nM (shown in Fig. 1A). Fig. 1B depicts the relationship between the ECL signal intensity and the concentration of Hg^{2+} over the concentration range from 5 nM to 250 nM. According to formula (1), the cut-off value for Hg^{2+} detection was 53.54 cps. Thus, the present



Scheme 1. Schematic illustration of the magnetic beads-based ECL assay for detection of Hg^{2+}.

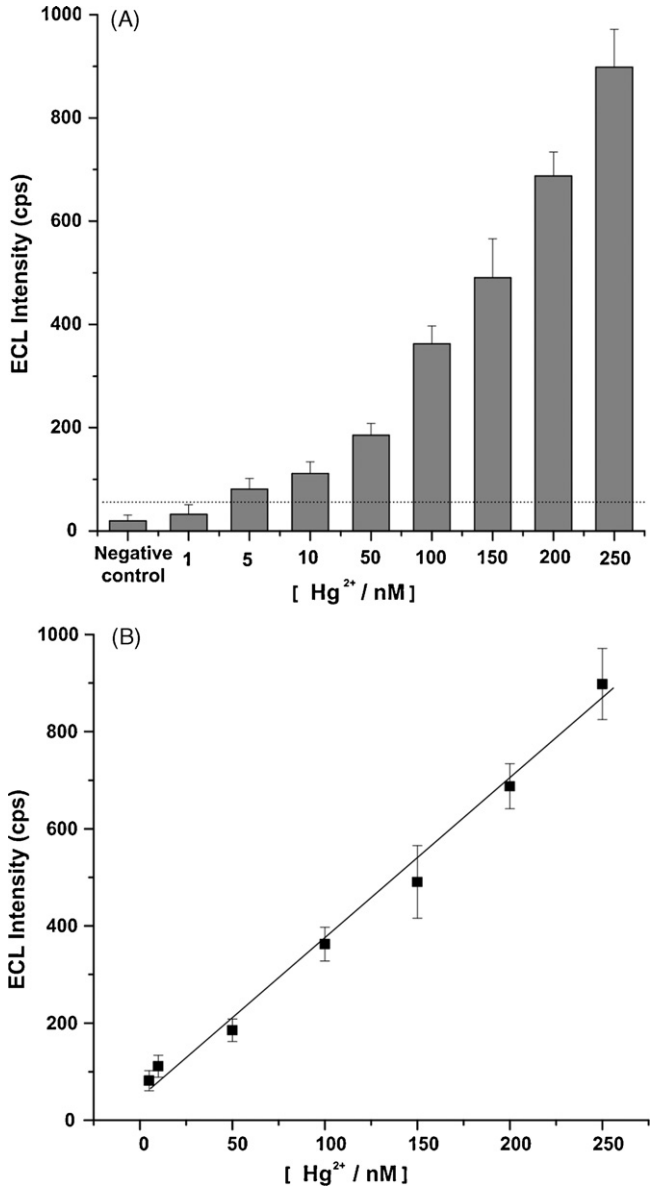


Fig. 1. (A) ECL detection of different concentration of Hg^{2+} (1 nM, 5 nM, 10 nM, 50 nM, 100 nM, 150 nM, 200 nM, 250 nM). The same reaction system without Hg^{2+} was also detected as a negative control. All ECL signal value were subtracted the blank control signal (ECL assay buffer). (B) The corresponding linear response of ECL detection of Hg^{2+} over the concentration range from 5 nM to 250 nM.

limit of detection for this assay is approximately 5 nM Hg^{2+} ions (the ECL signal intensity (81.18 ± 20.78 cps) is higher than the cut-off value), making it suitable for drinking water monitoring, according to the United States Environmental Protection Agency (EPA) limit for Hg^{2+} ions (10 nM).

The high sensitivity comes from the response patterns of TBR to TPA, and the enrichment process of magnetic beads. On the one hand, a single TBR-probe hybridized on the magnetic bead-capture probe via Hg^{2+}-mediated coordination of T–Hg^{2+}–T base pairs can be recycled and emitted many photons in the reaction. The mechanism for the ECL reaction of TBR and TPA is described before (Zhu et al., 2004; Zhou et al., 2008;). On the other hand, due to the introduction of magnetic bead, the Hg^{2+}-mediated magnetic bead-capture probe and TBR-probe complexes can be enriched on the electrode surface by magnetic field, resulting in a construction of a highly condensed TBR domain and then improve the sensitivity.

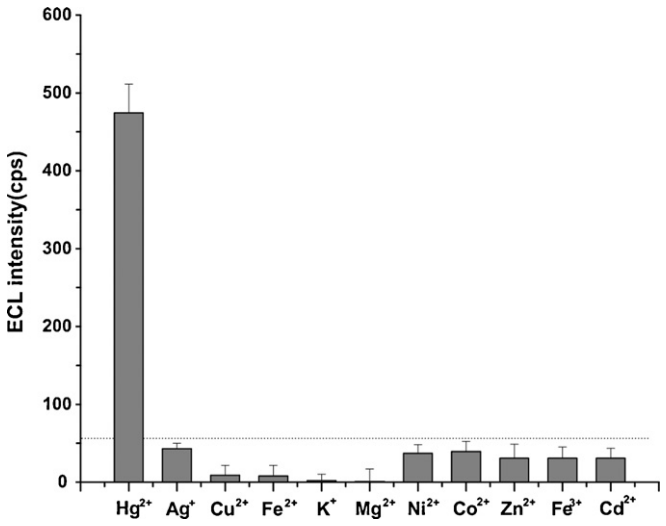


Fig. 2. ECL detection of 100 nM Hg^{2+} and 10 μM other metal ions. All ECL signal value were subtracted the blank control signal (ECL assay buffer).

In addition to sensitivity, the selectivity of the ECL assay for Hg^{2+} detection has also been investigated. The other metal ions including Ag^{+}, Cu^{2+}, Fe^{2+}, K^{+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Fe^{3+}, Cd^{2+} at a concentration of 10 μM was tested under the same conditions as in the case of Hg^{2+}. The experiment results have been compared with Hg^{2+} detection at a concentration of 100 nM. As is shown in Fig. 2, weak ECL signal were observed with other metal ions whereas Hg^{2+} has a apparent ECL signal though it has the concentration 100 time lower than the other metal ions. The ability of Hg^{2+} to chelate T–T mismatches, resulting in the formation of stable T–Hg^{2+}–T complexes, contributes to the high selectivity.

4. Conclusions

In summary, we for the first time have developed an ECL assay for detection of mercury ions in aqueous solution, based on magnetic beads separation/collection process and thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) coordination chemistry. This sensor has several advantages. Firstly, this method is high selectivity and sensitivity though the assay is enzyme-free and does not required any amplification process. Secondly, this approach is cost- and time-effective because it does not require sophisticated equipment other than a simple custom-built ECL system and took only about 20 min to measure Hg^{2+} for the entire process. Thirdly, the work electrode can reproducible as introduction of magnetic bead-capture probe instead of immobilization of the capture probe on the electrode surface, as the ECL values (17.68 ± 5.49 cps) drop to the negative control (19.61 ± 11.31 cps) after washing. Thus, it was expected to hold considerable potential to develop a Hg^{2+} detection kit for monitoring Hg^{2+} in drinking water.

Acknowledgments

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