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Electrochemical detection of arsenic contamination based on hybridization chain reaction and RecJ$_f$ exonuclease-mediated amplification

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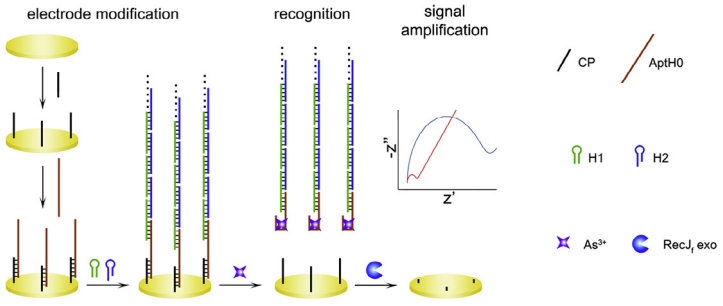
HIGHLIGHTS

- An ultrasensitive biosensor for the detection of arsenic contamination is developed.
- Hybridization chain reaction and RecJ$_f$ exonuclease-mediated amplification are involved.
- The limit of detection is as low as 0.02 ppb.
- The biosensor successfully distinguishes arsenic ions from other ions.
- The biosensor performs satisfactorily in a range of real samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Arsenic (As) is a type of highly toxic substance, which is widely distributed in environment due to both natural and anthropogenic sources. For health concerns, the allowable arsenic level must be strictly regulated. In this study, an ultrasensitive electrochemical biosensor for arsenic contamination is developed with signal amplification mediated by hybridization chain reaction (HCR) and RecJ$_f$ exonuclease catalyzed reaction. DNA assembly is firstly achieved on the surface of gold electrode prior to the analysis, which generates tremendous charge-transfer resistance (R_{ct}). In the presence of As^{3+}, aptamer sequence specifically binds As^{3+} and DNA dissociation occurs. The release of HCR product significantly decreases R_{ct}, which could be further enhanced by RecJ$_f$ exonuclease catalyzed digestion. Superior analytical performance for As^{3+} detection is obtained including the limit of detection (LOD) as low as 0.02 ppb and a wide linear range from 0.1 to 200 ppb, which complies with WHO regulation. It can be envisioned that in combination with a portable electrochemical instrumentation, the sensing strategy is suitable for field application of arsenic contamination monitoring.

1. Introduction

Arsenic (As) is one of the most abundant elements and its occurrences as composites with other minerals or metals has generated a large number of industrial applications. However, oxidation state of As are highly poisonous, especially in the form of As^{3+}. The intake of As^{3+} could lead to multiple organ failures such as lung, kidney and bladder [1,2]. Currently, arsenic contamination has affected a large number of

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Table 1
DNA sequences used in this work.

Name	Sequence (from 5′ to 3′)
CP	AAACAAACATCATTTTTT-$(CH_2)_3$-SH
AptH0	TGATGTTTGTTTACGCATGTGTGAGGAGAGGCTGGGGTGATGAATCCCAATCCC
H1	GGGATTGGGATTGGGATTGTGATGAATCCCAATCCC
H2	AATCCCAATCCCAATCCCGGGATTGGGATTCATCAC

people across the world [3,4]. As a result, it is of great importance to develop effective biosensors for the detection of arsenic contamination. Both of the World Health Organization (WHO) and the Environmental Protection Agency (EPA) published strict drinking water guidelines and set the standards of arsenic level. Up to now, some highly sensitive methods are established for the detection of As^{3+}, including inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and X-ray fluorescence (XRF) [5,6]. Nevertheless, they cannot meet the requirements of fast response and on-site measurement. Novel strategies for rapid, convenient and sensitive analysis of As^{3+} without sophisticated instrumentation are still highly desired.

Electrochemical analysis well fits with these requirements [7–12]. Electrochemical instruments are relatively simple, which can be facilely miniaturized for field measurements. The sensitivity is also high enough for practical use. For example, anodic stripping voltammetry (ASV) or square wave anodic stripping voltammetry (SWASV) are powerful electrochemical tools which can be used to determine traces of As^{3+} in different samples [13,14]. The process of preconcentration at certain reduction potential is involved to enrich target ions from the sample, thus high sensitivity is promised. On the other hand, different nanomaterials have been integrated for signal amplification. For instance, Podešva et al. presented a nanostructured gold microelectrode array with increased surface area for ultrasensitive detection of As^{3+} by ASV [15]. Wang et al. fabricated a DNA wrapped carbon nanotubes based electrochemical assay by comparing the peak currents of differential pulse voltammetry (DPV) curves [16]. Li et al. developed a field-effect transistor sensor by the investigation of the Schottky barrier influence on the MoS_2 device [17].

In this work, a novel electrochemical biosensor is proposed, which involves As^{3+}-specific aptamer for recognition and signal amplification mediated by hybridization chain reaction (HCR) and $RecJ_f$ exonuclease catalyzed reaction. Aptamers are single-stranded DNA or RNA molecules which can bind their targets with high affinity and specificity similar to antigen–antibody interactions [18,19]. The binding between aptamers and their targets rely on the complementary shape interactions and three dimensional folding. Aptamers are usually selected by the process of systematic evolution of ligands by exponential enrichment (SELEX) from large libraries of oligonucleotides. In this study, the applied aptamer towards As^{3+} can promise the high selectivity of the method. In addition, the proposed strategy does not need any preconcentration process or nanomaterials. Exonuclease-mediated reactions always significantly improve the sensitivities [20,21], which have been introduced in this work. Electrochemical signal originates from the variation of charge-transfer resistance (R_{ct}) across the modified electrode. Ultrasensitive detection of As^{3+} content is achieved by electrochemical impedance spectroscopy (EIS) instead of commonly used voltammetry techniques. The limit of detection (LOD) is determined as low as 0.02 parts per billion (ppb), which is 500 times below the content limit of 10 μg/L (10 ppb) recommended by WHO for drinking water. The developed biosensor can be used as an effective candidate for As^{3+} monitoring in environmental samples.

2. Experimental

2.1. Materials and chemicals

Potassium hexacyanoferrate (III), potassium hexacyanoferrate (II), tris(2-carboxyethyl)-phosphine hydrochloride (TCEP), and mercaptohexanol (MCH) were obtained from Sigma (USA). $RecJ_f$ exonuclease was obtained from New England Biolabs Ltd (Beijing, China). NaCl, KCl, $AgNO_3$, $MgCl_2$, $CaCl_2$, $CdCl_2$, $PbCl_2$, $NiSO_4$, $CoSO_4$, $CuSO_4$, $FeCl_2$, $FeCl_3$, and $AlCl_3$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Arsenic standard solution was purchased from National Sharing Platform for Reference Materials (Beijing, China, www.ncrm.org.cn). Other reagents were of analytical reagent grade and used as received. Ultrapure water (18.2 MΩ cm) was purified with a Millipore system (Millipore Co., USA). DNA probes used in this study were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). Detailed sequences and modifications were listed in Table 1.

2.2. Electrode modification

The gold electrode (2 mm) was firstly incubated with piranha solution (98% H_2SO_4: 30% H_2O_2 = 3: 1) for about 5 min (*Caution: Piranha solution reacts vigorously with organic solvents and should be handled with great care!*). After careful rinsing, it was polished to mirror smooth on P5000 sand paper and then 1, 0.3, 0.05 μm alumina slurries, respectively. Subsequently, the electrode was cleaned by ultrasonication in ethanol and then pure water, each for 5 min. It was then incubated in 50% HNO_3 for 30 min, followed by electrochemical treatment with 0.5 M H_2SO_4 to remove any remaining impurities. After careful rinsing, the electrode was dried by nitrogen. The cleaned electrode was incubated with CP (1 μM, 10 mM Tris–HCl, 1 mM EDTA, 10 mM TCEP, and 100 mM NaCl, pH 7.4) for 8 h [22]. Subsequently, it was treated with 0.1 M MCH for 30 min. After rinsed with double-distilled water, it was incubated with AptH0 (1 μM, 10 mM phosphate buffer, 250 mM NaCl, pH 7.4) for 1.5 h. Then, it was further incubated with the mixture of H1 and H2 (0.5 μM each, 10 mM phosphate buffer, 250 mM NaCl, pH 7.4) for another 1.5 h.

2.3. Arsenic recognition and signal amplification

As^{3+} standard solutions with a series of concentrations from 0.1 to 500 ppb were prepared using 10 mM phosphate buffer (pH 8.0). The modified electrode was incubated with the As^{3+} solutions for 2 h. Afterward, it was immersed in the solution of $RecJ_f$ exonuclease (1 unit/μL, 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9) at 37 °C. After 30 min, the reaction was terminated by heating to 65 °C for 20 min.

2.4. Real sample analysis

Tap water, lake water and sea water samples were collected as real samples in this study. The water samples were firstly centrifuged at 10,000 rpm for 5 min. After removing the solid impurities, they were filtered using a 0.22 μm membrane and the pH was adjusted to 8.0. Standard addition method is used. Different amounts of As^{3+} were spiked in the samples. Subsequently, they were analyzed by the