Introduction

As one of the most hazardous heavy metal contaminant, lead ion can cause serious impact on human health and environment [1]. Exposure to lead ion mainly through contaminated water in the environment, leaded gasoline, coal combustion, ceramic production and so on [2]. It can accumulate in the body through skin absorption, digestive tract or food chain, leading to nerve disorder, memory decay, or anemia even at very low level exposure, particularly in children [3,4]. The World Health Organization (WHO) regulate that the maximum acceptable concentration of lead ion in drinking water are 10μg/L [5]. Thus, the construction of simple, sensitive and rapid methods for the detection lead ion is of great value. There are many methods to analyze lead ion, such as atomic absorption spectrometry (AAS), inductive coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) have been reported for detection of lead ion [6-8]. Although these conventional analytical methods are highly sensitive and accurate, they are labor-intensive, time-consuming, expensive equipment, which restrict their wide applications [9]. Conversely, electrochemical sensor has been widely utilized in detection of lead ion owing to simple instrument, high selectivity, fast detection and low cost [10].

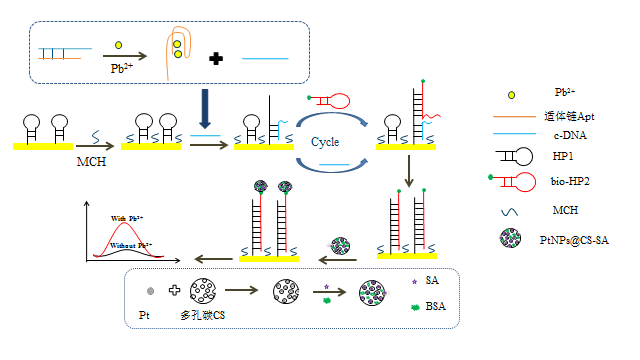
Aptamer, an artificial synthetic single-stranded DNA or RNA oligonucleotides, is selected in vitro through a SELEX (systematic evolution of ligands by exponential enrichment) [11]. Due to binding stability to targets, it has been employed in biosensors to analyze small molecules, heavy metal, proteins or cells [12-15]. There are different specific aptamers can be used in lead ion aptasensor design [16]. Because all of lead ion aptamer contain lots of guanines, they can product stable G-quadruplex structure when the presence of lead ion [17]. Based on this unique property, many electrochemical sensors were fabricated for ultrasensitive detection of lead ion [18, 19].

Recently, catalytic hairpin assembly (CHA) as an enzyme-free signal and isothermal nucleic acid amplification strategy has been broadly applied to detection of RNA or DNA in electrochemical sensing platforms [20, 21]. In the detection of lead ions, the lead ion DNAzyme was used for CHA, but the literature on CHA amplification using lead ion aptamers was not reported [22,23]. CHA not only overcomes the disadvantage of enzymatic amplification, such as complex operations, specific reaction conditions, and reaction-time dependent enzymatic activity，but exhibits the high sensitivity, simplifies the reaction conditions and reduces the experimental costs [24,25]. Hundred-fold catalytic amplification can be achieved by CHA reactions. CHA is powerful for amplifying and transducing signals at the terminus of nucleic acid amplification reactions [26-28].

Recently, platinum nanomaterials have been widely used in electrochemical biosensors due to their peroxidase-like properties. （表明可以催化过氧化氢）Platinum nanomaterials have unique properties such as large surface, good redox activity and better ability to catalyze H2O2 [29]. But it will aggregate, resulting in a gradual decrease in catalytic activity. To improve these problems, using a support material to enhance electron transfer capability, biocompatibility of Pt nanomaterials and catalytic activity is a useful approach.

group reported

In this work, a novel electrochemical sensor based on catalytic hairpin assembly (CHA) has been developed to detect lead ion. We select T30695 aptamer as lead ion specific aptamer. As shown in Scheme 1, the signal amplification strategy was conducted as follows: firstly, in the presence of lead ion, lead ion aptamer was transformed stable G-quadruplex structure with lead ion, leading to the release of cDNA. Then, the released cDNA opened and hybridized with thiol-modified hairpin probes (HP1) that were immobilized on the Au electrode. After another hairpin probes (bio-HP2) was added electrode, cDNA liberated from HP1 for the next cycle process and HP1/bio-HP2 duplex structure are formed. When the cyclic process completed, there are a large number of capture probe (HP1/bio-HP2) left on the electrode. Finally, amounts of PtNPs@CS-SA by the specific recognition between HP2 modified biotin and streptavidin (SA) were immobilized on the electrode. Thus, in the presence of H2O 2 , Pt-CS acted the same as horseradish peroxidase, which would catalyze the oxidation of H2O2 , further facilitating the conversion of HQ into BQ and improving electrochemical signal.



**Scheme 1.** Illustration of the electrochemical biosensor for detection of lead ion.

2. Experimental

*2.1. Reagents and materials*

Streptavidin,

The buffer: DNA hybridization buffer: 20 mM Tris-HCl, 140 mM NaCl, 5 mM MgCl2 (pH 7.4). Working buffer:

All DNA sequences were listed in Table.1 and were synthesized by Sangon biotechnology (Shanghai, China).

适体链与互补链以及发夹在95度加热5min后缓慢冷却至室温备用。

Table1.

|  |  |
| --- | --- |
| Name | Sequence (5’-3’) |
| Apt  c-DNA  HP1  bio-HP2 | GGGTGGGTGGGTGGGTAT  TCATACCCACCCACC TTTTGGGTGGGTATGACCACCGCCCACCCA  bio-TATGACCACCTGGGTGGGCGGTGGTCATACCCAC |

*2.2. Apparatus*

Electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were carried out with a CHI 660D electrochemical workstation (Shanghai Chenhua Instrument, China). A conventional three-electrode system: a platinum wire as counter electrode, a saturated calomel as reference electrode and the modified Au electrode (AuE, Φ=3 mm)) as working electrode.

*2.3. 纳米材料的准备 2.4. Preparation of ALP-PSC@AuNPs-SA bioconjugates*

*2.5.* *Fabrication of the electrochemical biosensor*

All the hairpin DNA were annealed at 95℃ for 5min and then slowly cooled to room temperature before using. HP1 在使用前要用TCEP处理block disulfide bonds。Firstly, Apt and its complementary chain (c-DNA) were hybridized with same proportions (3µL, 4µM) at (条件) . Secondly, 5 µL of HP1 hairpin probe DNA (HP)（浓度）was added onto the surface of AuE at room temperature for 12 h. After washing with Tris-HCl buffer (pH 7.4), the electrode was blocked with MCH for 30 min at room temperature to 封闭未结合位点. Then, containing Apt, c-DNA and different concentrations of Pb2+ （量）were added to electrode for 1.5h at 37℃. Next, 5 µL of HP2 was cast onto the electrode and incubated for 1.5h at 37℃. Finally, 5 µL of PtNPs@CS-SA was added to the electrode and incubated at 37 ° C for 2h. The electrode was rinsed with phosphate buffer for each step of the experiment.

*2.6. Electrochemical measurement*

The prepared electrochemical biosensor was performed by DPV in PBS (pH 7.4) containing 1.0 mM HQ and （） H2O2, with the scan range from . CV measurement were carried out in 1 mL PBS (0.1 M, pH 7.4) containing 5.0 mM [Fe (CN) 6 ] 3-/4- as redox probe with a potential range of and a scan rate of 100 mV s -1 .

3. Results and discussion

4. Conclusion

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