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Graphene oxide-assisted Au nanoparticle strip biosensor based on GR-5 DNAzyme for rapid lead ion detection

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ABSTRACT

This study has reported that a GR-5 DNAzyme based lead ion strip biosensor could exhibit an enhanced specificity with the assistance of graphene oxide (GO). This enhancement results from the specific π-stacking interaction between the ribose rings of the nucleobases and the carbon hexagons in GO which can reduce the false positive interference by removing unhybridized ssDNA during the annealing of GR-5 DNAzyme. Meanwhile, conjugate pad was sprayed with two kinds of AuNP-DNA probes, and nitrocellulose membrane test zone and control zone were immobilized with two kinds of biotin-DNA probes, respectively. The limit of detection of this strip biosensor was estimated to be about 0.05 nM (S/N = 3) and 1 nM (with naked eyes) with a linear range from 0.01 to 100 μM. Furthermore, the strip biosensor exhibited excellent selectivity toward Pb^{2+} in the presence of other divalent metal ions. For real soil samples, the obtained recoveries were in the range from 91.5% to 113.1%.

1. Introduction

Lead pollution that is caused by industrial activities and urbanizations has attracted increasing concern, because it may cause severe adverse effects on human marrow hematopoietic system, cardiovascular system, immune system, nervous system and digestive system [1–3]. As a central nervous system poison, it had worse on children [3]. Most of lead that released into the environment will enter the soil, and then enter the human body through foods. Many countries have made provision for lead level in the environment and food. Therefore, it is critical to develop a simple, fast, low cost and portable lead detection device with high sensitivity and selectivity. The commonly used lead detection methods include graphite furnace atomic absorption spectrometry [4,5], fluorescence spectroscopy [6–9], ultraviolet spectrophotometry [10–12], electrochemical analysis [13–16], inductively coupled plasma mass spectrometry [17,18] and dithizone colorimetric method [19], etc. Though these methods can provide good enough performance in lead detection, they always require expensive equipment and very experienced professionals. Furthermore, most of these equipments and operation processes have to be conducted only at laboratory and are not adaptive for in field scenarios.

On the other hand, the lateral flow test strip is a convenient, low cost and prompt approach that have been successfully applied to a wide range of analytes such as biomolecules, chemical contaminants, heavy metals [20–23]. Various molecules including antibody, aptamer…, have been employed as receptors in test strip because of their high affinity and specificity to their corresponding ligands. Recently, DNAzymes were reported to been utilized to construct test strip for the detections of metal ions including Pb^{2+}, Mg^{2+}, Zn^{2+}, Ca^{2+}, Mn^{2+} and Cu^{2+} [24–26]. DNAzymes are a class of DNA fragments that can own various catalytic functions including cleavage of nucleic acid, cleavage of phosphoramidate bond, igation of nuclei acid, formation of RNA branch or lariat, formation of nucleopeptide bond, DNA phosphorylation, DNA adenylation, DNA depurination, and porphyrin metallation. Among these DNAzymes, 8–17 DNAzyme can make specific response to Pb^{2+} in which the substrate strand of 8–17 DNAzyme can be cleaved at rA [12,27–34]. Zeng et al. [24] sprayed conjugate pad with one AuNP-DNA probe, binding cleaved fragment H1-H2-biotin through a series of toehold-mediated strand displacement reaction, achieved the detection of lead ion. However, further studies found besides lead ion, 8–17 DNAzyme was also active for Zn^{2+}, Mn^{2+}, Cr^{2+} and Co^{2+}, which meaned the interfere problem. Recently, Lu et al. [35] found that GR-5 DNAzyme had higher selectivity for lead ion. The results showed this DNAzyme had ~40,000 times selectivity for Pb^{2+} over Zn^{2+}, which

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Table 1
Sequences of oligonucleotides used in the study.

Name	Sequence (5'-3')
Substrate strand (GR-5S)	GGTCTCACTATrAGGAAGAGATGATGTCTGTCAGATGTAG
DNAzyme strand (GR-5E)	GACATCATCTCTGAAGTAGCGCCGCCGTATAGTGAGACC
AuNP-DNA probe a'	GACATCATCTCT-$(CH_2)_6$-SH
AuNP-DNA probe c'	SH-$(CH_2)_6$-GGTCTCACTATA
Biotin-DNA probe a	AGAGATGATGTCAAAAAA-biotin
Biotin-DNA probe c	biotin-AAAAAATATAGTGAGACC
Biotin-DNA probe b'	biotin-AAAAAACTACATCTGACA
Biotin-DNA probe d'	CGGCGCTACTTCAAAAAA-biotin

has been greatly improved than ~160 times of 8–17 DNAzyme. Therefore, it can be expected GR-5 DNAzyme may exhibit a better performance in the application of test strip based Pb^{2+} detection.

For strip biosensor, there are two important issues that need to be addressed, one is how to improve the sensitivity, the other is how to exclude false-positive results. For Pb^{2+} test strips based on DNAzyme, the previous studies usually immobilized one corresponding complementary sequence on strip that directed at one cleaved small fragment of DNAzyme, the remaining fragments did not play any role in the chromatographic process. In this work, it is the first time to detect lead ions using two different AuNP-DNA probes on conjugate pad and two different biotin-DNA probes on test zone (TZ) and other two biotin-DNA probes on control zone (CZ), which were all complementary with different part of the cleaved small DNA fragments respectively. This method improved the utilization efficiency of the cleaved fragments, meanwhile acquired higher detection sensitivity.

For interference of false-positive results, in the studies of lead ion detection strip based on DNAzyme, the two single strands of DNAzyme (DNAzyme strand and substrate strand) were usually synthesized separately and then hybridized to form a certain structure. During the hybridization process, complete reaction of those two single strands could not be achieved, there was always remaining single strand in solution, it would combine with complementary probes on the strip and cause false positive result, so how to overcome this interference is very important. In recent years, carbon nanotube, graphene oxide (GO) and other nano-carbon materials have increasingly been applied in biological field. GO is used to combine with gold nanoparticles to increase the electrochemical signal to enhance the detection sensitivity [36,37]. In our study, we use the characteristic that the surface unsaturated carbon atom of GO can form a large π bond with the bases of single-stranded DNA by non-covalent π stacking interaction, which can make GO bind single-stranded DNA closely; however, the interaction of GO and double-stranded DNA is very weak [38–41]. Based on this mechanism, herein, we used GO to process the sample after the hybridization of the two single strands, it was found free single-stranded DNA could be cleared absolutely, and the procedure had no effect on enzyme activity. The results also confirmed that this approach reduced interference of false positive result effectively. Compared with other strips for the detection of Pb^{2+} [24,25], the design in our study could make full use of the substrate strand and enzyme strand by employing dual AuNP-DNA probes and could reduce the false positive by introducing GO. After applying the above two novel points, high sensitivity and no false positive results of strip for lead ion detection was realized, and the detection limit was 0.05 nM (S/N = 3) and 1 nM (with naked eyes), which could meet the testing requirement of real samples. In addition, the single testing cost of strip was about 1.8 RMB, indicating it had a very good application potential.

2. Experimental section

2.1. Apparatus

Conjugate pad, TZ and CZ were obtained with HM3030 dispenser (Shanghai Kinbio Tech., China), strips were cut by a programmable strip cutter (Shanghai Kinbio Tech.,China), the normalized intensities of the red bands on the TZ and CZ were recorded by strip image analyzer (Shanghai Kinbio Tech. Co., Ltd. Shanghai, China). UV–vis absorption spectra were performed with UV 2450 spectrophotometer (Shimadzu, Japan), ultrapure water (≥18.2 MΩ cm) was produced by the Milli-Q system (Millipore, USA).

2.2. Materials and solutions

Chloroauric acid ($HAuCl_4{\cdot}4H_2O$), sodium citrate, sodium chloride, trisodium phosphate, Tween 20, sucrose, polyethylene glycol octylphenol ether (Triton X-100), sodium dodecyl sulfate, polyethylene glycol (PEG 4000), trisodium phosphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, Tris(hydroxymethyl)aminomethane, glacial acetic acid, boric acid, edetate disodium ($EDTA_2Na$), silver nitrate, ethanol, nitric acid, formaldehyde, sodium carbonate, acrylamide, bisacrylamide, ammonium persulfate, *N,N,N′,N′*-tetramethylethylenediamine, sodium hydroxide, mercuric chloride, barium chloride, zinc chloride, copper nitrate, cobalt chloride, nickel sulfate, manganese chloride and calcium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); 4-hydroxyethyl piperazine acetic acid (HEPES), lead nitrate, cadmium nitrate were purchased from Aladdin (Shanghai, China); GO (2 mg/mL, 50–200 nm) was acquired from XF Nanotechnology Co., Ltd. (Nanjing, China); streptavidin-biotin (1 mg) was purchased from BBI Life Sciences Corporation (Shanghai, China). All chemicals were of analytical-reagent grade; absorbent paper (SX42), glass fiber (CB06), PVC plastic adhesive backing (SMNF31-25) were obtained from Shanghai Kinbio Tech. Co., Ltd (Shanghai, China); nitrocellulose membrane (CN140) was purchased from Sartorius (Goettingen, Germany); the sequences of the oligonucleotides used in this study were shown in Table 1. All sequences were synthesized and purified (HPLC) by Sangon Biotech Co., Ltd. (Shanghai, China).

2.3. Preparation of Au nanoparticle

Au nanoparticle (AuNP) was prepared by a previously reported trisodium citrate reduction method [42,43]. Briefly, 100 mL $HAuCl_4$ solution (0.01%) was heated to boiling and then added 2 mL trisodium citrate solution (1%) rapidly. The color of solution turned from yale yellow to wine-red, and after heating for another 10 min, the solution was slowly cooled to room temperature, filtered with a 0.22 μm