Biosensors and Bioelectronics 53 (2014) 245–249

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Detection of lead(II) ions with a DNAzyme and isothermal strand displacement signal amplification

Wenying Li [a,b], Yue Yang [a,b,c], Jian Chen [a], Qingfeng Zhang [a,b], Yan Wang [a,b], Fangyuan Wang [a,b], Cong Yu [a,b,*]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China
[b] University of the Chinese Academy of Sciences, Beijing 100049, PR China
[c] College of Biological and Agricultural Engineering, Jilin University, Changchun 130022, PR China

ARTICLE INFO

Article history:
Received 24 August 2013
Received in revised form
23 September 2013
Accepted 24 September 2013
Available online 8 October 2013

Keywords:
DNAzyme
Lead
Strand displacement amplification
Fluorescence
Exonuclease III

ABSTRACT

A DNAzyme based method for the sensitive and selective quantification of lead(II) ions has been developed. A DNAzyme that requires Pb^{2+} for activation was selected. An RNA containing DNA substrate was cleaved by the DNAzyme in the presence of Pb^{2+}. The 2′,3′-cyclic phosphate of the cleaved 5′-part of the substrate was efficiently removed by Exonuclease III. The remaining part of the single stranded DNA (9 or 13 base long) was subsequently used as the primer for the strand displacement amplification reaction (SDAR). The method is highly sensitive, 200 pM lead(II) could be easily detected. A number of interference ions were tested, and the sensor showed good selectivity. Underground water samples were also tested, which demonstrated the feasibility of the current approach for real sample applications. It is feasible that our method could be used for DNAzyme or aptazyme based new sensing method developments for the quantification of other target analytes with high sensitivity and selectivity.

1. Introduction

Lead (Pb^{2+}) is a wide-spread and highly toxic contaminant in the biological environment. Accumulation of Pb^{2+} in the human body has diverse detrimental effects on human health (Needleman, 1991, 2004). The detection and quantification of Pb^{2+} with high sensitivity and selectivity has attracted growing attentions over the years. Various analytical techniques for Pb^{2+} detection have been developed, such as atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS) (Wu and Boyle, 1997), and X-ray fluorescence spectrometry, etc. However, these traditional analytical methods usually require sophisticated instruments, complicated operation and sample preparation/pretreatment procedures, and are also quite expensive (Elfering et al., 1998). The development of new sensitive and selective techniques for Pb^{2+} quantification is therefore of great importance.

Nucleic acid enzymes include ribozymes (catalytic RNA) and deoxyribozymes (catalytic DNA, or DNAzymes) that can catalyze a variety of reactions, such as RNA or DNA cleavage, RNA or DNA ligation, phosphorylation, capping, aldol reaction, porphyrin metalation, etc (Liu et al., 2009; Breaker, 1997; Robertson and Ellington, 1999). A nucleic acid enzyme can have very high catalytic efficiency. For sensing applications, DNAzymes are more frequently used because of their easy synthesis, high stability, and cost-effectiveness. DNAzymes in many cases require a cofactor (for example, a metal ion) for activation, which forms the basis for the design of a variety of sensing methods (Elbaz et al., 2008; Liu and Lu, 2007; Xiao et al., 2007; Wang et al., 2010). Many DNAzyme based Pb^{2+} detection techniques have been developed in recent years, such as the colorimetric (Mazumdar et al., 2010; Wang et al., 2008; Liu and Lu, 2004, 2005), electrochemical (Xiao et al., 2007; Yang et al., 2010; Tang et al., 2013; Shen et al., 2008), fluorometric (Lan et al., 2010; Xu et al., 2013; Zhang et al., 2011; Zhang et al., 2010; Xiang et al., 2009), surface-enhanced Raman scattering (Wang and Irudayaraj, 2011), microarray (Zuo et al., 2009), femtoliter-well reactor (Wang et al., 2009), and dynamic light scattering techniques (Miao et al., 2011). And many of which utilized advanced materials such as gold nanoparticles (Mazumdar et al. 2010; Wang et al. 2008; Liu and Lu, 2005, 2004; Miao et al., 2011), graphene (Wen et al., 2011; Zhao et al., 2011), and quantum dot (Wu et al., 2010), etc. And some recently reported dynamic light scattering, rolling circle amplification, and exonuclease aided recycling amplification based methods provide quite good detection sensitivities (Miao et al., 2011; Tang et al., 2013; Xu et al., 2013). However, certain limitations still exist. For example, many of the reported literature methods are less sensitive, such as the gold nanoparticle aggregation based colorimetric

* Corresponding author at: State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China. Tel./fax: +86 431 85262710.
E-mail addresses: congyu@ciac.ac.cn, congyu@ciac.jl.cn, congyu1@gmail.com (C. Yu).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.09.055

assays (Liu and Lu, 2004, 2005, detection limit 400 nM, 100 nM, respectively), fluorometric assays (Lan et al., 2010, detection limit 3.7 nM; Zhang et al., 2011, detection limit 10 nM; Xiang et al., 2009, detection limit 4 nM), electrochemical methods (Xiao et al., 2007, detection limit 300 nM), surface-enhanced Raman scattering based assay (Wang and Irudayaraj, 2011, detection limit 20 nM). Some methods require covalent labeling of the substrate strand and the DNAzyme, and immobilization of the DNA strand on gold electrode is time consuming.

Herein we report the development of an amplified DNAzyme based method for the sensing of Pb^{2+}. A lead ion dependent DNAzyme was selected (GR-5 DNAzyme) (Lan et al., 2010). Upon binding to Pb^{2+}, the DNAzyme can cleave an RNA containing substrate (GR-DS, Table 1) with high efficiency. The 2′,3′-cyclic phosphate of the 5′-part of the cleaved substrate was removed by exonuclease III. The remaining part of the substrate was subsequently used as a primer for the strand displacement amplification reaction (SDAR). SYBR Green I was used for the detection of the SDAR products. Real-time emission intensity changes were detected, which could be directly related to the concentration of Pb^{2+} in the assay solution. The method is very sensitive, 200 pM Pb^{2+} could be easily detected. And it is also very selective, and could be used for the assay of complex assay mixtures (underground water samples). It is envisioned that our method could be used for DNAzyme and aptazyme based new biosensing technology developments.

2. Materials and methods

2.1. Materials

The RNA containing oligonucleotide GR-DS was obtained from TaKaRa Biotechnology Co., Ltd. (Dalian, China). All other oligonucleotides used in the current investigation were synthesized and ULTRAPAGE purified by Sangon Biotechnology Co., Ltd. (Shanghai, PR China) (Table 1). The enzymes were obtained from the New England Biolabs (Ipswich, MA, USA). $Pb(OAc)_2$ were from Sinopharm Chemical Reagent Co., Ltd (Beijing, PR China). Ultrapure Acetic acid, NaCl and Tris were from Merck KGaA (Darmstadt, Germany). 10000 × SYBR Green I was obtained from Beijing Dingguo Biotechnology Co., Ltd. (Beijing, China). All other chemicals were of analytical grade and used as received. The oligonucleotide stock solutions were stored at 4 °C before use. All stock and buffer solutions were prepared using water purified with a Milli-Q A10 filtration system (Millipore, Billerica, MA, USA) and disinfected in a autoclave (120 °C, 15 min).

Table 1
Oligonucleotides used in the current investigation.

Oligonucleotide	Sequence (5′→3′)
GR-DS	AGA AGA AGA AAG ACT CAC TAT rA GGA AGA GAT GAT GTC TG*T
GR-E	ACA GAC ATC ATC TCT GAA GTA GCG CCG CCG TAT AGT GAG
Oligo(X′-Y′)	TCG CTA TCA GTT TCT TGG AAT T ‖ AA CTG ACT CTT ATA GTG AGT CTT TCT TCT TCT
Oligo(Y′-Y′)	TCG CTA TCA GTT TCT TGG AAT T ‖ AA CAG ACT CTT CGC TAT CAG TTT CTT GGA ATT
Oligo-4	AGA AGA AGA AAG ACT CAC
Oligo-8	AGA AGA AGA AAG AC
Oligo-12	AGA AGA AGA A
Oligo-14	AGA AGA AG

"rA" and "*" denote ribonucleotide and phosphorothioate labels, respectively. -GACTC- is the nicking endonuclease Nt.BstNBI recognition sequence, and ‖ is the nicking site of the complementary DNA strand. Oligo-4, oligo-8, oligo-12, and oligo-14 were used as the markers.

2.2. Instruments

UV–vis absorption spectra were obtained with a Cary 50 Bio spectrophotometer (Varian, USA). Oligonucleotides were quantified by UV–vis absorption at 260 nm. Emission spectra were obtained using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA) with an excitation wavelength of 497 nm. Excitation and emission bandwidths were both of 3 nm for all the measurements. ICP-MS (X Series 2, Thermo Fisher Scientific Inc., Germany) was used for real sample analysis.

2.3. Assay procedures

The DNAzyme (GR-E, 40 nM), substrate (GR-DS, 10 nM), and Pb^{2+} ions of a specific concentration were mixed in an aqueous buffer solution (50 mM Tris–HAc 100 mM NaCl, pH 7.2) and incubated at 30 °C for 50 min (total sample volume: 11 μL). 10 U exonuclease III (1 μL) and 25 mM $MgCl_2$ (1 μL) were added, and the sample was incubated at 37 °C for 2 h. Exonuclease III was then inactivated at 70 °C for 20 min (total sample volume, 13 μL).

Two separated sample mixtures were prepared (sample A and sample B). Sample A contained Nt.BstNBI reaction buffer, ThermoPol buffer, amplification templates [oligo(X′-Y′) and oligo(Y′-Y′)], dNTPs, SYBR Green I. Sample B contained the nicking endonuclease Nt.BstNBI, and Bst DNA polymerase. Sample A and sample B were freshly prepared for each set of assays.

82 μL of sample A was mixed with the above-mentioned assay solution (13 μL). The sample solution was transferred into an emission cell and equilibrated at 55 °C. 5 μL of Sample B (cooled on ice) was added, and the emission intensity changes at 520 nm were monitored in real time with data points taken every 5 s for 400 s. The final assay solution (total sample volume, 100 μL) contained 80 nM oligo(X′-Y′), 20 nM oligo(Y′-Y′), dNTPs (dATP, dTTP, dCTP, dGTP) each at 250 μM, Nt.BstNBI (0.2 U μL^{-1}), Bst DNA polymerase (0.04 U μL^{-1}), and 0.5 × SYBR Green I.

2.4. Underground water sample analysis

Underground water samples were collected from Changchun, Jilin province, PR China. The concentrations of Pb^{2+} in the water samples were determined by the above-mentioned assay procedures. Conditions: 40 nM DNAzyme (GR-E,), 10 nM substrate (GR-DS), incubation at 30 °C for 50 min. 10 U exonuclease III and 1.92 mM $MgCl_2$ were added to remove the cyclic phosphate. Exonuclease III was then inactivated. The DNAzyme assay solution was used to trigger the strand displacement amplification reaction.

Pb^{2+} concentrations in the underground water samples were also determined by ICP-MS to validate our assay results.

3. Results and discussion

3.1. Selection of the DNAzyme

Metal ion dependent DNAzymes have been discovered as a promising class of biomolecules for the selective detection of metal ions since the early 1990s. A variety of metal ion dependent DNAzymes have been obtained through an in vitro selection process (Liu et al., 2009; Breaker, 1997; Robertson and Ellington, 1999). Among them, a lead selective DNAzyme termed as 8-17 has often been used for the selective detection of lead ions