Sensors and Actuators B 254 (2018) 214–221

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Selection of DNA aptamers for the development of light-up biosensor to detect Pb(II)

Yang Chen[a,b,1], Haohuan Li[a,c,1], Tian Gao[a], Tingting Zhang[b], Lijun Xu[a], Bing Wang[b], Jine Wang[a,*], Renjun Pei[a,*]

[a] CAS Key Laboratory of Nano-Bio Interface, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Suzhou, 215123, China
[b] School of Pharmacy, Xi'an Jiaotong University, Xi'an, 710061, China
[c] Nano Science and Technology Institute, University of Science and Technology of China, Suzhou, 215123, China

ARTICLE INFO

Article history:
Received 3 March 2017
Received in revised form 6 July 2017
Accepted 12 July 2017
Available online 14 July 2017

Keywords:
Aptamer
SELEX
Biosensor
Pb(II)

ABSTRACT

In order to develop a simple, facile, and cost-effective light-up biosensor with high specificity for Pb(II), a modified affinity chromatography SELEX (Systematic Evolution of Ligands by Exponential Enrichment) method based on target-induced release of strands was used to isolate aptamers of Pb(II). After 14 rounds positive selection and 4 rounds negative selection, two aptamers with high binding affinity and good specificity were obtained, which have the dissociation constant K_d values of 1.60 ± 0.16 μM and 0.76 ± 0.18 μM, respectively. Then we designed a biosensor for Pb(II), which is based on the Pb(II)-induced release of fluorescence-labeled aptamer from complex with a quencher-labeled short complementary sequence. The biosensor showed a Pb(II) concentration-dependent increase of fluorescence intensity in the low micromolar range and a detecting linear range from 100 to 1000 nM, and great specificity even in the existence of various interfering metal ions. Moreover, this simple and reliable biosensor obtained satisfying results for the detection of Pb(II) in lake water and serum samples.

1. Introduction

With the rapid development of industry and agriculture, heavy metal pollution has become a serious threat to human health due to the toxicity to humans and the environment. As one of the most toxic heavy metal ions, Pb(II) has neurotoxic effect and can impair brain development, such as memory loss, irritability, anemia and mental retardation, especially to children [1,2]. According to the recommended standard by the US Environmental Protection Agency (EPA) and the World Health Organization (WHO) regulations, the Pb(II) concentration limits in drinking water are 0.015 mg/L and 10 μg/L, respectively [3]. Currently, traditional analytical techniques are the most common methods for detection of Pb(II), such as atomic absorption/emission spectroscopy [4], inductively coupled plasma optical emission spectroscopy [5], inductively coupled plasma mass spectrometry [6], anodic stripping voltammetry [7], reversed-phase high-performance liquid chromatography [8]. Although they are reliable and sensitive, these methods generally measure only total lead content and require specialized equipments, complicated sample pre-treatment steps and a relatively long analysis time. They are neither readily available in developing countries nor capable of on-site field detections [9]. Therefore, it is still necessary to develop simpler and more cost-effective methods to detect Pb(II).

Functional nucleic acids are powerful and widely-used tools for designing sensors owing to their properties of high recognition and designability [10]. In recent years, functional DNA has been extensively used to design Pb(II) sensors, and can be divided into two main classes according to their mechanisms of action. One is Pb(II)-specific DNAzymes, which compose of an enzyme strand and a substrate strand, for example 8-17E and GR5 [11–13]. But the easy degradation and high cost of RNA substrate limits their wide applications. The other one is based on Pb(II)-induced formation of G-quadruplexes (G4) from G-rich sequences [14–18]. For example, Dong and co-workers designed different sensors to detect Pb(II) based on Pb(II)-induced conformational conversion of G-rich sequence [15,16], DasGupta and co-workers reported a simple and inexpensive method for Pb(II) detection based on Pb(II)-induced formation of Spinach's G-quadruplex [17]. However, the G-quadruplex structures are easy to be influenced by some metal ions, small molecules and proteins, *etc*, which limits their

* Corresponding authors.
E-mail addresses: jewang2012@sinano.ac.cn (J. Wang), rjpei2011@sinano.ac.cn (R. Pei).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.snb.2017.07.068
0925-4005/

potential in applications [19]. In addition, most of the reported sensors required relatively specialized design. It would be highly useful to develop a versatile sensor which could detect Pb(II) in the presence of interfering ions.

Aptamers are short single-stranded DNA (ssDNA) or RNA molecules, which were selected in vitro via Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [20–23]. Since 1990, aptamers for various targets from small molecules to whole cells have been selected [23,24]. Because of their high affinity and selectivity to targets, aptamers have been widely used for designing biosensors. Some DNA and RNA aptamers for metal ions have been selected and used to detect metal ions [25–28]. For example, Ellington and Rajendran developed a fluorescent aptamer beacon that can be used for the detection of Zn^{2+} [26]. Zhou and co-workers selected a DNA aptamer with high affinity for Cd(II) and offered a new substitute for the detection of Cd(II) [28]. But there are still some factors which could limit the probable applications in designing biosensors, such as high cost and easy degradation from RNA aptamer sequences and the long sequences of DNA aptamers. Recently our group has selected a short DNA aptamer for Cd(II) and designed a biosensor to detect Cd(II) [29]. However, there was still no DNA aptamer which has been selected by SELEX for Pb(II).

In this study, we selected DNA aptamers for Pb(II) by a modified affinity chromatography SELEX method which is based on the target-triggered release of aptamers with some modifications as previous reports [30,31]. The ssDNA library was coupled on streptavidin-coated agarose beads through the hybridization with a biotin labeled complementary oligonucleotide. After incubation with Pb(II), the sequences strongly binding with Pb(II) would be released from agarose beads and the sequences with weakly binding or no binding would still fix on agarose beads. The enriched ssDNA pool was amplified by PCR to generate new ssDNA pool for the next round of selection. An additional negative selection step was performed using other competitive metal ions to improve the selectivity of the aptamer candidates. Two aptamers with low micromolar dissociation constants (K_ds) and good specificity were achieved after 18 rounds of positive and negative selection. The FAM-aptamer based biosensor was further designed for Pb(II) analysis. The correlation of Pb(II) concentration and fluorescence intensity was found to be linear with the range of 100–1000 nM with a limit of detection of 60.7 nM, and great specificity even in the existence of various interfering metal ions. Such biosensor is simple, rapid, reliable, and then further used for Pb(II) detection in real samples and obtained satisfying results.

2. Experimental

2.1. Materials

Lead(II) nitrate was obtained from Fu Chen Chemical Reagents Factory (Tianjin, China). Streptavidin-agarose beads were purchased from Thermo Fisher Scientific (USA). SYBR® Green I was purchased from Invitrogen Technology (Shanghai, China). Agarose was purchased from Biowest (Spain). BL21 Escherichia coli was purchased from American Type Culture Collection (ATCC, USA). 20 bp DNA Marker, 6 × Loading buffer, 10 × PCR buffer, deoxynucleotide triphosphates (dNTPs), rTaq polymerase and pMD18-T Vector Cloning Kit were all purchased from Takara Biotechnology (Dalian, China).

In this experiment, all the oligonucleotide, including the initial ssDNA library (5′-GGAGGCTCTCGGGACGAC-N30-GTCGTCCCGATGCTGCAATCGTAAGAAT-3′) containing 30 random nucleotides, the forward primer P1 (5′-GGAGGCTCTCGGGACGAC-3′), the reverse primer P2 (5′-ATTCTTACGAATTGCAGCATCGGGAC-3′) and the biotin labeled reverse primer Biotin-P2 (5′-Bio-ATTCTTACGAATTGCAGCATCGGGAC-3′), the 3′-biotin labeled capture-oligonucleotide (5′-GTCGTCCCGAGAGCCATA-biotin-3′) and quenching oligonucleotide strands were synthesized and purified by Sangon Biotechnology (Shanghai, China). All other chemical reagents were purchased from Sinopharm Chemical Reagent (Shanghai, China), and were analytical grade. Milli-Q water (18.2 MΩ.cm) was used throughout.

2.2. Selection procedure

Before each round of selection, the micro-spin column (Bio-Rad) filled with streptavidin-agarose beads (200 μL) was washed and pre-equilibrated by 3 vols of SELEX buffer (20 mM Tris-HAc, 10 mM NaAc, pH = 7.4) for 5 times. In order to immobilize the ssDNA library onto the surfaces of streptavidin-agarose beads, the ssDNA library was mixed with the 3′-biotinylated capture-oligonucleotide in a mole ratio of 1:2 in SELEX buffer, denatured under 95 °C for 5 min and then cooled down slowly to room temperature (RT). Then, the hybridized DNA was transferred to the column and incubated with streptavidin-agarose beads for 10 min at RT, 5 times.

In order to wash away the poor bound or unbound ssDNA, the column above was subjected to 3 vol of SELEX buffer. And subsequently, 200 μL 100 μM $Pb(NO_3)_2$ in SELEX buffer was added into the column for 3 times to obtain the eluted sequences, which are supposed to bind Pb(II). Then, the column was washed two more times by 200 μL of SELEX buffer. The above eight elutions were collected into 1.5 mL eppendorf tubes respectively.

In order to improve the selectivity of aptamers to Pb(II), after several rounds of positive selection, the negative selection step was introduced. The ssDNA pool immobilized on the surfaces of streptavidin-agarose beads was successively washed with 200 μL of SB-buffer (100 μM $Al(NO_3)_3$, 100 μM $Cu(NO_3)_2$, 100 μM $Co(NO_3)_2$, 100 μM $Zn(NO_3)_2$, 100 μM $Ca(NO_3)_2$ and 100 μM $Ba(NO_3)_2$ in SELEX buffer) two times, 200 μL of SELEX buffer, 200 μL of 100 μM $Pb(NO_3)_2$ in SELEX buffer three times, 200 μL of SELEX buffer two times. And the above eight elutions were collected into 1.5 mL eppendorf tubes respectively.

To monitor the efficiency of selection process, the above collected elutions were PCR-amplified and checked by electrophoresis. Each PCR mixture contained 400 μM dNTPs, 1 μM P1, 1 μM P2, 0.025 unit/μL rTaq polymerase and 1/50 vol template in 1 × PCR buffer (with Mg^{2+}). The PCR program was: 14 cycles of denaturation (92 °C, 15 s), annealing (58 °C, 30 s), and extension (72 °C, 20 s), followed by a final 5 min extension period at 72 °C. After amplification, the PCR products were verified and estimated using electrophoresis on a 4% agarose gel stained with SYBR Green I and imaged using a LAS4000 mini (FujiFilm).

Then, the 3 Pb(II) elutions were mixed as the enriched ssDNA pool and PCR-amplified by Bio-P2 with the PCR reaction condition mentioned above. The new ssDNA pool was generated using streptavidin-agarose beads and alkaline denaturation and used for the next round of selection. The procedure of preparation was as following: the PCR products were firstly incubated with streptavidin-agarose beads (150 μL) in a micro-spin column (Bio-Rad) for 10 min 3 times. The column was then washed with 800 μL of SELEX buffer 3 times to remove unbound DNA. The ssDNA strands were eluted by incubating with 0.15 M NaOH for 3 min 2 times, and then desalted and concentrated by ethanol precipitation.

2.3. DNA cloning and sequencing

After 18 rounds of selection, the enriched ssDNA pool was PCR-amplified under the same condition described above. The PCR product was ligated into pMD18-T vectors and transformed into BL21 E. coli cells. About 30 clones were randomly picked and cul-