Talanta 182 (2018) 125–130

Contents lists available at ScienceDirect

Talanta

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

Highly sensitive and selective detection of Pb^{2+} using a turn-on fluorescent aptamer DNA silver nanoclusters sensor

Baozhu Zhang[a,b], Chunying Wei[a,*]

[a] Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education, Institute of Molecular Science, Shanxi University, Taiyuan 030006, PR China
[b] College of Chemistry and Chemical Engineering, Jinzhong University, Yuci 030619, PR China

ARTICLE INFO

Keywords:
DNA-Ag NCs
Aptamer
G-quadruplex
Pb^{2+}

ABSTRACT

A novel turn-on fluorescent biosensor has been constructed using C-PS2.M-DNA-templated silver nanoclusters (Ag NCs) with an average diameter of about 1 nm. The proposed approach presents a low-toxic, simple, sensitive, and selective detection for Pb^{2+}. The fluorescence intensity of C-PS2.M-DNA-Ag NCs enhances significantly in the presence of Pb^{2+}, which is attributed to the special interaction between Pb^{2+} and its aptamer DNA PS2.M. Pb^{2+} induces the aptamer to form G-quadruplex and makes two darkish DNA/Ag NCs located at the 3′ and 5′ terminus close, resulting in the fluorescence light-up. Moreover, Pb^{2+} can be detected as low as 3.0 nM within a good linear range from 5 to 50 nM (R = 0.9862). Furthermore, the application for detection of Pb^{2+} in real water samples further demonstrates the reliability of the sensor. Thus, this sensor system shows a potential application for monitoring Pb^{2+} in environmental samples.

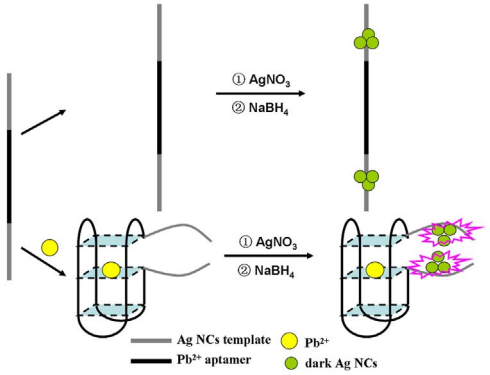
1. Introduction

Pb^{2+}, as a highly toxic heavy metal ion, causes adverse health effects to humans, including kidney, liver, and nervous system diseases, high blood pressure in adults, and delayed physical and mental development in infants and children [1,2]. Thus, it is increasingly important and necessary for the health of human beings to develop a highly sensitive and selective method for the detection of Pb^{2+} in drinking water and biosystem. Although instrumental techniques for Pb^{2+} detection are widely used, such as atomic absorption spectrometry (AAS) [3,4], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [5,6], and inductively coupled plasma mass spectrometry (ICP-MS) [7], they suffer from expensive and sophisticated instruments and/or complicated sample preparation processes. Therefore, to overcome these limitations and drawbacks, a variety of sensors such as colorimetry [8,9], fluorescence [10,11], and electrochemistry [12,13] have been developed to rapidly detect Pb^{2+} by utilizing organic fluorescent dyes, oligonucleotides, or Pb^{2+}-dependent RNA cleaving DNAzymes. Especially, conjugated nanomaterials are growing attention for detection of diverse metal ions based on their specific functionality by colorimetric method. For example, Awual's group has performed a lot of excellent researches in the detection and removal of diverse heavy metal ions [14–18] including Pb^{2+} [1,2,19–22] using ligands doped conjugate adsorbents. In addition, Pb^{2+} is able to induce the structural transition of a guanine (G)-rich single-stranded oligonucleotide into a G-quadruplex [23] that consists of planar stacks of four guanines stabilized by Hoogsteen hydrogen bonding [24], and some of biosensors of Pb^{2+} ions have been designed by exploiting this feature [25,26]. However, some disadvantages still existed, such as high toxicity, poor aqueous solubility, and complicated synthesis procedures [27]. Recently, fluorescent metal nanocluster (NCs) for Pb^{2+} sensing have drawn increasing attention because of their good biocompatibility, low cost, simple operation, and high sensitivity and selectivity [28–30]. Nevertheless, most of nanocluster sensors belong to “on-off’ and are not ideal sensors for being possibly interfered by various factors. Thus, the development of a turn-on fluorescent metal nanocluster probe for detecting Pb^{2+} is necessary.

Fluorescent noble metal nanoclusters, consisting of a few to roughly a hundred atoms, have attracted a great deal of interest for their unique chemical and physical features, such as owning sizes comparable to the Fermi wavelength of electrons and showing molecule-like properties, including discrete electronic transitions and strong fluorescence [31]. However, without stabilizer noble metal nanoclusters would strongly interact with each other and aggregate irreversibly to reduce their surface energy [32]. A number of methods have been developed to generate noble metal nanoclusters in the past decade [33,34], and DNA oligonucleotides, polymers, dendrimers and thiols can be exploited for stabilizing fluorescent metal nanoclusters [35]. Among these small molecules, DNA oligonucleotides provide a versatile scaffold for preparing fluorescent nanoclusters due to possessing optimizable strand

* Corresponding author.
E-mail address: weichuny@sxu.edu.cn (C. Wei).

https://doi.org/10.1016/j.talanta.2018.01.061
Received 7 September 2017; Received in revised form 21 December 2017; Accepted 27 January 2018
Available online 31 January 2018
0039-9140/



Scheme 1. Schematic illustration of the sensing method for the detection of Pb^{2+} by using ssDNA-scaffolded silver nanoclusters combined with PS2.M aptamer.

length and base sequence [36,37]. Particularly, DNA-scaffolded silver nanoclusters (DNA-Ag NCs) [38], as a new class of promising fluorescent nanomaterials and an ideal alternative to organic dyes and quantum dots, have been successfully applied in biosensing [39,40] and bioimaging [41,42] owing to their strong fluorescence emission, lower toxicity, excellent photophysical properties, and good biocompatibility.

It is found that the fluorescence intensity can enhance largely when two darkish DNA–Ag NCs are closed each other by their complementary linkers [43], herein, we construct a Pb^{2+} biosensor by a similar mode, and the working principle of the sensor is depicted as Scheme 1. DNA template consists of two segments: one is the Ag NCs-nucleation segment at the two termini, and the other is the aptamer segment of Pb^{2+} in the middle of DNA template. Upon addition of Pb^{2+}, the high specificity and affinity of the Pb^{2+} aptamer to Pb^{2+} results in the conformational change of the aptamer and makes the two darkish DNA–Ag NCs close, which enhances largely the fluorescence of Ag NCs and enables the sensitive, specific and turn-on detection of Pb^{2+}.

2. Experimental

2.1. Reagents and apparatus

The oligonucleotides employed in this work are provided by Sangon Biotechnology Inc. (Shanghai, China). The sequences and names of DNA are listed in Table S1. Sodium borohydride ($NaBH_4$, 98%), silver nitrate ($AgNO_3$, 99.8%), acetic acid, $CaCl_2$, $Cd(NO_3)_2$, $Co(NO_3)_2$, $Cr(NO_3)_3$, $Cu(NO_3)_2$, $Fe(NO_3)_3$, $Hg(NO_3)_2$, KNO_3, $Mg(NO_3)_2$, $Mn(NO_3)_2$, $NaNO_3$, $Pb(NO_3)_2$, $TbCl_3$, and $Zn(NO_3)_2$, are purchased from Aladdin Bio-chem technology Co. Ltd. (Shanghai, China). Tris (Tris-(hydroxymethyl) aminomethane) is obtained from Sigma-Aldrich (Shanghai, China). The standard reference materials for river sediment (GBW07310) and soil (GBW07403) are obtained from the National Institute of Metrology (Beijing, China). All chemical reagents are the analytical grade and used as received without further purification. All solutions are prepared using Milli-Q water.

Fluorescence measurements are performed using Fluoromax-4 Spectrofluorometer (Horiba Jobin Yvon Inc., France) or a Cary Eclipse Fluorescence spectrophotometer (Varian Inc. CA) at room temperature, and the slit widths are 5.0 nm and 10 nm for excitation and emission, respectively. UV–vis absorption spectra are recorded at room temperature with a Cary 50 Bio spectrophotometer (Varian Inc., CA). CD spectra are performed at room temperature on a Chirascan Circular Dichroism Spectrometer (Applied Photophysics Ltd., Surrey, UK), and the spectrum is obtained within the range of 220–320 nm at 1 nm intervals employing 1 mm optical path-length quartz cell and an instrument scanning speed of 120 nm/min. Time-resolved fluorescence measurements were performed using FL920 fluorescence lifetime spectrometer (Edinburgh Instruments, Livingston, UK) operating in the time-correlated single photon counting (TCSPC) mode using a semiconductor laser (405 nm) as the excitation source. Commercial software by Edinburgh Instruments is used for data analyses. When $\sum_{i=1}^{n} A_i = 1$, the average excited state lifetime is expressed by the equation $\tau_{avg} = \sum_{i=1}^{n} A_i \tau_i$. The reported spectrum of each sample represents the average of three scans. The morphologies and average size of the DNA–Ag NCs are recorded by using a JEOL JEM-2100 transmission electron microscope with an acceleration voltage of 200 kV. The real samples are analyzed by the classic inductively coupled plasma-mass spectrometry (ICP-MS) on a NexION350X instrument (PerkinElmer Inc. USA).

2.2. Sample preparation

The certified reference material (GBW07310 and GBW07403) is digested according to the previous literature [44]. 0.05 g of sample powder is accurately weighed into a 50 mL container and 4.0 mL of concentrated nitric acid and 12 mL of concentrated hydrochloric acid (aqua regia) is added. A watch glass is put on the container and the mixture is evaporated on a hot plate at 95 °C almost to dryness. Then 8.0 mL of aqua regia is added to the residue and the mixture is again evaporated to dryness (but not baked). After cooling, the resulting mixture is filtered through a 0.45 μm polytetrafluo-roethylene (PTFE) millipore filter. The sample is diluted to 50 mL with double distilled water and analyzed by the proposed procedure.

2.3. Preparation of DNA-Ag NCs and fluorescence assay of Pb^{2+}

DNA-Ag NCs are synthesized according to our previous reported method [38]. Briefly, The DNA oligonucleotides (0.3 μM) with various concentrations (0–500 nM) of Pb^{2+} ions in tris-acetate buffer (5 mM, pH 7.0) were first heated at 85 °C for 15 min and then gradually cooled to 4 °C. Then $AgNO_3$ was added to the DNA solution, and the mixture was kept away from light at 4 °C for 20 min, followed by reduction with the freshly prepared $NaBH_4$ and by the vigorous shaking of the solution for 1 min. The final concentrations of DNA, $AgNO_3$, and $NaBH_4$ were 0.3, 1.8, and 1.8 μM, respectively. The final mixture was kept in the dark at 4 °C for 1 h. The incubation time was calculated from after the addition of $NaBH_4$. The fluorescent spectrum of each sample was recorded at room temperature.

2.4. Selective detection of Pb^{2+}

To investigate whether the other metal ions could interfere with the detection of Pb^{2+}, the selectivity of the fluorescence assay was measured. The metal ions such as Na^+, K^+, Ca^{2+}, Cr^{3+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, Mg^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, Tb^{3+} and Fe^{3+} were used, and the concentrations of Pb^{2+} and the other metal ions were 0.1 and 1 μM, respectively. The assay method was same as that of Pb^{2+}.

2.5. Circular dichroism measurements

C-PS2.M (5.0 μM) with 20 nM Pb^{2+} in tris-acetate buffer (5 mM, pH 7.0) were first heated at 85 °C for 15 min and gradually cooled to 4 °C. Then C-PS2.M-DNA-Ag NCs were prepared according to the method described above (see Section 2.3), subsequently, CD spectra were measured.

2.6. Analytical applications

To demonstrate the practical application of the C-PS2.M-DNA-Ag NCs detection system, metal ions in tap water and lake water (from Shanxi University) were detected by using this sensing system. The samples were spiked with standard Pb^{2+} solution at different concentration levels, and the actual samples were measured using the same approach as described above.