Talanta 165 (2017) 274–281

Contents lists available at ScienceDirect

Talanta

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

A label–free GR–5DNAzyme sensor for lead ions detection based on nanoporous gold and anionic intercalator

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ARTICLE INFO

Keywords:
Label–free
Lead
Anionic intercalator
GR–5DNAzyme
Nanoporous gold

ABSTRACT

A label–free electrochemical sensor, based on a classic lead ions (Pb^{2+})–dependent GR–5DNAzyme as the catalytic unit, disodium–anthraquinone–2,6–disulfonate (AQDS) as DNA intercalator, and nanoporous gold (NPG) for signal amplification, was designed for sensitive and selective detection of Pb^{2+}. Firstly, NPG modified electrode surface were employed as a platform for the immobilization of thiolated probe DNA, and then, hybridized with DNAzyme catalytic beacons. The Pb^{2+}–induced catalytic reaction makes the substrate strand break at the cleavage sitGe irreversibly, which disturbs the formation of DNA strands. AQDS served as an indicator that intercalated into the base–pairing regions of DNAzyme, resulting in a strong electrochemical signal. In the presence of Pb^{2+}, the complementary regions were reduced, due to the fracture of the DNA strand, resulting in the release of AQDS. And a decreased current was obtained, corresponding to Pb^{2+} concentration. Taking advantage of the amplification effect of NPG electrode for increasing the reaction sites of thiol modified capture probe, the proposed electrochemical biosensor could detect Pb^{2+} quantitatively, in the range of 1–120 nM, with a limit of detection as low as 0.02 nM, which is much lower than the maximum contamination level for Pb^{2+} in drinking water defined by the U.S. Environmental Protection Agency. The electrochemical sensor was also used to detect Pb^{2+} from real water samples, and the results showed excellent agreement with the values determined by inductively coupled plasma mass spectroscopy. This biosensor showed a promising potential for on–site detecting Pb^{2+} in aqueous environment.

1. Introduction

Lead ions (Pb^{2+}) is an important pollutant mainly arising from lead–based paints and contaminated water, soils and foodstuffs [1,2]. Lead causes adverse health effects in human bodies, including neurological, cardiovascular, reproductive, and developmental disorders [3–5]. The maximum contamination level (MCL) for Pb^{2+} in drinking water is 72 nM according to the U.S. Environmental Protection Agency (EPA). In fact, even lower than 72 nM of Pb^{2+} is associated with children's neuro–developmental deficits [6–9]. Hence, it is of great significance to monitor its level in the environment.

Traditional quantitative methods, such as atomic absorption spectrometry (AAS) [10], and inductively coupled plasma mass spectroscopy (ICP–MS) [11], are the standard techniques utilized for Pb^{2+} determination. However, these methods need expensive and complex equipment, materials and include time consuming extraction steps to eliminate the excipients, contaminants and interfering ions [12]. Besides, traditional methods cannot be used as portable devices for on–site/in–site quantification, and meanwhile large–scale determination of heavy metals can be time consuming, labor intensive and costly. In contrast, electrochemical techniques have great potential for on–site/in–site detection of multiple heavy metals. Anodic stripping voltammetry (ASV) has been considered to be the most effective tool for the direct electrochemical detection of trace Pb^{2+} ions due to an effective pre-concentration step followed by electrochemical stripping measurements of the accumulated analytes [13–15]. However, the task for sensor to avoid interferences from complex environmental matrices is very hard.

Recently, many biochemical and biophysical studies have been carried out on DNAzymes. Important insights have been gained regarding metal binding sites, metal–dependent activity, and catalytic mechanisms for these DNAzymes. One of the most significant practical

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http://dx.doi.org/10.1016/j.talanta.2016.12.069
Received 10 October 2016; Received in revised form 19 December 2016; Accepted 24 December 2016
Available online 25 December 2016
0039-9140/

applications for DNAzymes is metal ion detection due to their high metal ion selectivity (avoid interferences from complex environmental matrices), such as the reported DNAzyme for Pb^{2+} is about 40,000–fold selective [16] over other competing metal ions, and the DNAzyme for UO_2^{2+} is more than 1,000,000–fold selective over other competing metal ions [17]. This kind of metal selectivity can be found in many other DNAzymes. Therefore, DNAzymes have been converted into sensors for a wide range of metal ions such as mercury(Hg^{2+}) [18], lead(Pb^{2+}) [19], uranyl(UO_2^{2+}) [20], magnesium(Mg^{2+}) [21], zinc(Zn^{2+}) [22], copper(Cu^{2+}) [23], calcium(Ca^{2+}) [24], silver(Ag^{+}) [25], and thallium(Tl^{3+}) [26]. Moreover, it is noteworthy that Liu's group found a series of metal ions–based DNAzymes, and elucidated the relationship between metal ion selectivity and DNAzyme sequence and structure in these systems [24–27]. These discoveries help to find and select the target metal ions–based DNAzyme, and contributed to the mental ions biosensor based DNAzyme. In addition, it has been found that 8–17DNAzyme and GR–5DNAzyme have the highest activity with Pb^{2+} and are by far the fastest RNA–cleaving DNAzymes. GR–5 reacts only with Pb^{2+} while 17E is also active with a number of other divalent metal ions [28]. Hence, GR–5DNAzyme was selected in this work.

Besides, in developing highly sensitive electrochemical sensors, amplified detection strategy is the central research topic. In recent years, various nanomaterials (e.g., graphene oxide, mesoporous carbon, carbon nanotubes, metal nanoparticles, quantum dots, magnetic nanoparticles, etc.) were employed as DNA immobilization substrates and recognition elements in biosensors [29–35]. These nanomaterials with large surface area, abundant binding sites, excellent biocompatibility and a synergic effect among conductivity, are beneficial to increase the amount of the immobilized DNA probe significantly, and thus, the amplified electrochemical detection signals are obtained. In our earlier studies, a novel label–free impedimetric sensing system was proposed for the detection of Pb^{2+} based on ordered mesoporous carbon–gold nanoparticle, and the detection limit of was 0.2 nm [32]. In addition, we also developed a DNA biosensor based on carboxylic acid group functionalized multiwalled carbon nanotubes and gold nanoparticles (GNPs) for the quantification of lead ions [36], and the detection limit of 0.0043 pM is much lower than the EPA limit of Pb^{2+} in drinking water [37]. These detecting systems are highly sensitive and selective at the cost of multiple assembly steps and multiple signal amplification, which may affect the operability of the biosensors. Moreover, in our previous work, we have also demonstrated new and simple strategies to increase the electrochemical signals and lower the detection limits using nanoporous gold (NPG) with the controllable three–dimensional nanoporous metal film and simple preparation as the platform for nucleotide immobilization [38,39]. Hence, based on our previous efforts, NPG was applied to fix DNA, and as a transducer to convert the recognition information into a detectable signal.

Besides, the choice of signal agent is also important for the DNA–based biosensors. It is well known that the indicators are classified into cationic and anionic intercalators, both single strand probe DNA (ssDNA) and double strands probe DNA (dsDNA) carry negative charges. Hence, there was large background signal of DNA–based biosensors using anionic intercalators because of the electrostatic attraction. In previous work, Zhang et al. introduced that employing the cationic intercalators of hexaammineruthenium(III) chloride (RuHex) as indicators may increase background signal, and oligonucleotide-functionalized gold nanoparticles as amplifying tags may cause blocking phenomenon in NPG based electrode [39]. Alternatively, anionic intercalators can reduce background signal. A comparison of anionic and cationic intercalators for the electrochemical transduction of DNA hybridization was studied as reported by Wong and coworkers [40]. They found that disodium–anthraquinone–2, 6–disulfonate (AQDS), a kind of anionic intercalators, can intercalate into the dsDNA but not into ssDNA, when only the ssDNA was present on the interface, there was no background electrochemical signal [40]. Thus, AQDS was used as the electroactive indicator in this work in order to improve the sensitivity, lower background signal.

Herein, the aim of this work was to develop a simple biosensor with sensitivity and selectivity for the quantification of Pb^{2+} based on GR–5DNAzyme, NPG and AQDS. This strategy for Pb^{2+} quantification is highly accurate, relatively simple to operate, and to exploit strong resistance of the sensor to environmental impact disturbance. NPG was carefully coated onto a pretreated electrode via physical adsorption, and then modified with Pb^{2+}–DNAzyme. AQDS was selected as an electroactive signal indicator. Furthermore, Pb^{2+} detections in landfill leachate and tap water samples are performed to demonstrate the practical use of this sensor. The developed sensor with high sensitivity and selectivity may be an alternative method for Pb^{2+} ion detection in environmental, biomedical, and other applications.

2. Experimental

2.1. Chemicals and reagents

Disodium–anthraquinone–2, 6–disulfonate was purchased from TokyoChemical Industry Co., Ltd. (Tokyo, Japan). Tris (2–carboxyethyl) phosphinehydrochloride (TCEP), 6–mercaptohexanol (MCH) and tris–(hydroxymethyl) aminomethane were purchased from Sigma–Aldrich (USA). $Pb(NO_3)_2$, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, and all aqueous solutions were prepared using ultrapure water (18.2 MΩ·cm, Milli–Q, Millipore). 50 mM tris–acetate buffer (pH 8.0) containing 300 mM NaCl and phosphate buffer saline (PBS, 0.15 M KH_2PO_4 and 0.15 M Na_2HPO_4) were used in this work. All oligonucleotides used in our experiment were synthesized and high performance liquid chromatography (HPLC)–purified by Sangon Biotech. Co., Ltd. (Shanghai, China). Their base sequences are as follows: 5′–SH–C_6–ACAGACATCATCTCTGAAGTAGCGCCGCCGTATAGTGAG–3′ (S1, capture probe) and 5′–CTCACTATArGGAAGAGATGATGTCTGT–3′ (S2, a complementary substrate oligonucleotide of the DNAzyme which contains a single, scissile ribo–adenine) [28]. Probes were dissolved in 50 mM tris–acetate buffer (pH 8.0) containing 300 mM NaCl and kept at −20 °C for further use. Phosphate buffer saline (pH 7.0) containing 0.2 M NaCl was used to store the 1 mM AQDS, in the dark. NPG was prepared according to our previous method. Briefly, Au/Ag alloy leaf was sandwiched between two supporting papers, and cut into small pieces of any size, subsequently, floated onto concentrated nitric acid for dealloying about 1 h and then water for rinsing 3 times [38,41].

2.2. Apparatus

All electrochemical measurements, such as cyclic voltammograms (CVs), electrochemical impedance spectroscopy (EIS) and different pulse voltammetric (DPV), were performed in aconventional three–electrode cell at room temperature with a CHI760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., China). A model pHSJ–3 digital acidimeter (Shanghai Leici Factory, China) was used to measure the solution pH. A Sigma 4K15 laboratory centrifuge, a vacuum freezing dryer and a mechanical vibrator were used in the assay.

2.3. Sensor fabrication and detection process

Probe was activated by 2 mM TCEP (which isincluded to reduce disulfide bonded oligomers), and diluted by 50 mM tris–acetate buffer (pH 8.0). The bare glass carbon electrode (GCE) was polished in alumina slurry firstly, and then rinsed with deionized water. Finally, the electrode surface was treated by H_2SO_4 (0.5 M) with cyclic voltammetry scan (between 0 and 1.2 V at the scan rate of 50 mV s^{-1}) until a reproducible scan was obtained. After being dried, the nanoporous gold (prepared by selective dissolution of Ag from Ag/Au) was carefully coated onto a pretreated GCE via physical adsorption, and then, washed with ultrapure water to neutral pH [38].