Biosensors and Bioelectronics 72 (2015) 95–99

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Fluorescent detection of Hg^{2+} and Pb^{2+} using GeneFinder™ and an integrated functional nucleic acid

Shenshan Zhan [a], Hanchu Xu [a], Dongwei Zhang [b], Bing Xia [b], Xuejia Zhan [a], Lumei Wang [a], Jing Lv [a], Pei Zhou [a,*]

[a] School of Environmental Science and Engineering; Key Laboratory of Urban Agriculture (South), Ministry of Agriculture; School of Agriculture and Biology, and Bor S. Luh Food Safety Research Center, Shanghai Jiao Tong University, Shanghai 200240, PR China
[b] School of Chemical and Environmental Engineering, Shanghai Institute of Technology, Shanghai 201418, PR China

ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form
3 April 2015
Accepted 10 April 2015
Available online 13 April 2015

Keywords:
Fluorescent assay
Functional nucleic acid
Hg^{2+} detection
Pb^{2+} detection
T–Hg^{2+}–T
G-quadruplex

ABSTRACT

This paper reports a simple fluorescent assay for the determination of Hg^{2+} and Pb^{2+} by using a DNA intercalator GeneFinder™ (GF) and an integrated functional nucleic acid (FNA). In the absence of Hg^{2+} and Pb^{2+}, GF intercalated with the FNA and released moderate strong fluorescence. While in the presence of Hg^{2+} or Pb^{2+}, the FNA would be induced to form T–Hg^{2+}–T or G-quadruplex structure, interacted with which the GF would exhibit extremely strong or very weak fluorescence. By monitoring the fluorescence changes upon addition of these two ions, the Hg^{2+} and Pb^{2+} could be selectively detected as low as 3.23 ppb and 2.62 ppb. As the main advantage of this assay is simplicity and the feasibility was demonstrated by detecting Hg^{2+} and Pb^{2+} in spiked water samples, this assay holds great potential for the development of a cost effective and useful tool for environmental monitoring.

1. Introduction

With the rapid development of industries and competitive use of fresh water in many parts of worlds, water contaminated by heavy metal ions has became a serious global issue nowadays (Que et al., 2008; Tofighy and Mohammadi, 2011). Mercury ions (Hg^{2+}) and lead ions (Pb^{2+}) are two of the most metal pollutants and may cause adverse effects on human health even at low concentration (Zhu and Zhang, 2014). For example, Hg^{2+} can damage the heart, brain, kidney, stomach, nervous system, endocrine system and many other organs (Huang and Chang, 2006; Wu et al., 2012). And Pb^{2+} exposure to the human body, especially in children may cause a variety of symptoms such as memory loss, irritability, anemia and muscle paralysis (Godwin, 2001). Consequently, the development of accurate and sensitive methods for these two heavy metal ions is getting considerable attention worldwide (Zhu and Zhang, 2014).

Conventional methods for Hg^{2+} and Pb^{2+} determination include atomic absorption spectrometry, atomic fluorescence spectrometry, inductively coupled plasma mass spectrometry, and selective cold vapor atomic fluorescence spectrometry (Deibler and Basu, 2013; Leopold et al., 2010). These methods ensure the high sensitivity and accuracy, but often require thorough multistage sample preparation procedure and sophisticated expensive equipment, falling to meet the requirements of real-time and on-site detection (Zhu et al., 2014). Therefore, it is necessary to develop sensitive and selective methods which are as well as simple and low-cost.

Recently, the functional nucleic acids (FNAs)-based sensors have shown great potential in the detection of heavy metal ions (Famulok et al., 2007). Generally, the FNAs include aptamers, DNAzymes and specific oligonucleotide ligands, among which the aptamers and DNAzymes were obtained from combinatorial oligonucleotide libraries by in vitro selection, and the specific oligonucleotide ligands were designed by researchers based on the specific interactions between ions and the nucleic acid bases (Liu et al., 2009; Zhao et al., 2013). As for the Hg^{2+} detection, the most widely used FNAs is thymine (T)-rich oligonucleotides which would form T–Hg^{2+}–T mismatched base pairs on interacting with Hg^{2+} (Miyake et al., 2006). And for the Pb^{2+} detection, there are mainly two types of FNAs that could be applied as the Pb^{2+} recognition elements. The first type is the RNA-cleaving Pb^{2+}-specific 8–17 DNAzyme that selected by Li and Lu (2000), and the other one is guanine (G)-rich oligonucleotides which would convert into G-quadruplex structure when induced by Pb^{2+} (Guo et al., 2012). Since the specific recognition of these FNAs to their targets was demonstrated, omnifarious FNAs-based sensors

* Corresponding author. Fax: +86 2134205762.
E-mail address: zhoupei@sjtu.edu.cn (P. Zhou).

http://dx.doi.org/10.1016/j.bios.2015.04.021
0956-5663/

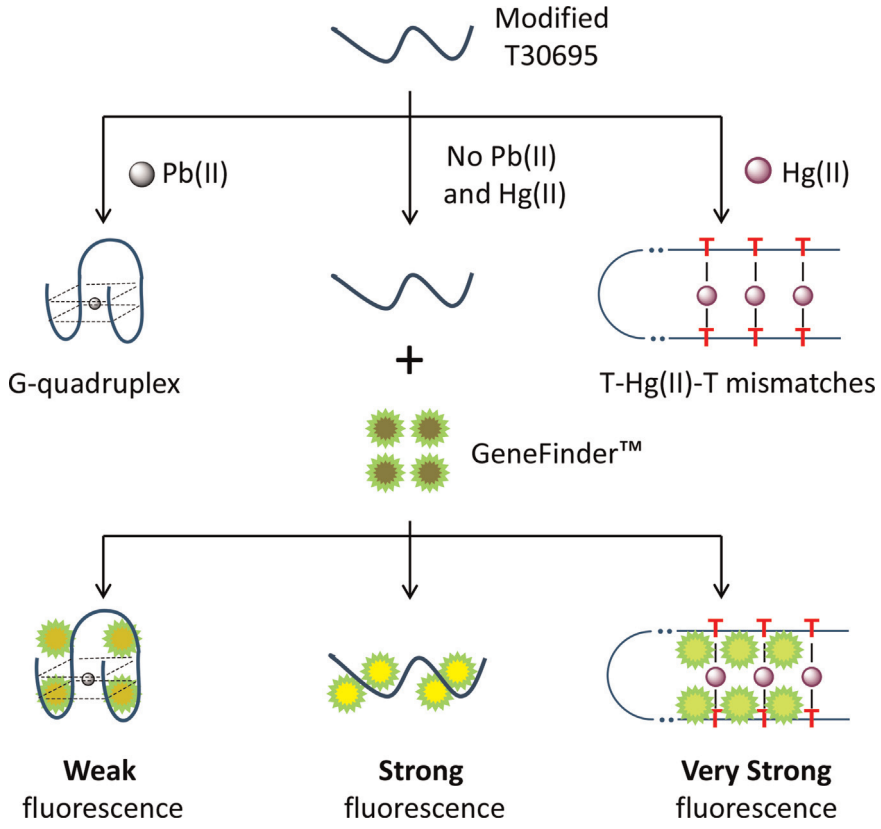
have been developed for the Hg^{2+} or Pb^{2+} detection by transforming the recognition behavior into scattering (Fu et al., 2012; Ling et al., 2011; Yue et al., 2013), colorimetric (Liu, 2004; Li et al., 2008, 2010), fluorescent (Xiang et al., 2009; Xu et al., 2011; Zhan et al., 2014), electrochemical (Guo et al., 2011; Li et al., 2011; Pelossof et al., 2012), or chemiluminescent signals (Cai et al., 2011; Li et al., 2010; Ma et al., 2011). Respectable innovation and high sensitivity is shown by these sensors, but the disadvantage should not be neglected, that is most of these sensors could only detect one target analyte with the fixed components, which considerably limit their application in the field of analysis where multiplexed analyses are highly needed (Xiang et al., 2011). Therefore numerous FNAs-based simultaneous detection methods for heavy metal ions have emerged one after another. For example, Lin et al. (2011b) constructed an impedimetric DNA-based sensor for simultaneous detection of Pb^{2+}, Ag^{+} and Hg^{2+} by using three different FNAs and two masking agents. Wang et al. developed a multi-walled carbon nanotube-based fluorescent biosensor for the detection of Hg^{2+}, Ag^{+} and Pb^{2+} in homogeneous solution by labeling three different FNAs with various dyes (Wang and Si, 2013). And based on the assembly of Tween 20-stabilized gold nanoparticles, Tseng's group established a rapid colorimetric method for the highly selective detection of Hg^{2+} and Ag^{+} (Lin et al., 2010). All these sensors successfully realized the goal of multiplexed analyses, while one impressive drawback is the complexity comes along with the modification of the FNAs and the involvement of diversified FNAs or masking agents. Yang et al. made great progress in simplifying the detection system by just utilizing a G-quadruplex DNA for simultaneous sensing of Pb^{2+} and Ba^{2+}, without using any masking agents, but the preparation process involved complicated and time-consuming protein expression and purification (Yang et al., 2013). Thus much effort should be put on developing multiplexed analyses sensing system which is composed of simpler components.

In this paper, a simple fluorescent assay which could detect Hg^{2+} and Pb^{2+} was established by applying an integrated functional nucleic acid (named modified T30695) as the sensing element and GeneFinder™ (GF) as the signal reporter. As depicted in Scheme 1, when there was no Hg^{2+} and Pb^{2+} in the sensing system, the single-stranded DNA (ssDNA) modified T30695 would keep in random-coil status. As GF was subsequently added into the system, it would intercalate with the ssDNA and release moderate strong fluorescence. When Pb^{2+} was introduced into the system, it would induce the ssDNA to form G-quadruplex structure and lead to the fluorescence of GF changing from strong to weak. While if the modified T30695 was treated with Hg^{2+}, Hg^{2+} would turn the random-coil ssDNA into a hairpin structure through the formation of T–Hg^{2+}–T base pair, interacted with which the GF would exhibit very strong fluorescence. By monitoring the variation of the fluorescence, a simple fluorescent method for Hg^{2+} and Pb^{2+} detection was thus developed.

2. Materials and methods

2.1. Reagents and instruments

The integrated functional nucleic acid which named modified T30695 was designed by adding six T to both ends of T30695, thus its sequence is 5'-TTTTTTGGGTGGGTGGGTGGGTTTTTTT-3'. Tris (Tris-(hydroxymethyl)aminomethane), acetic acid and cationic compounds such as nitrates of K^{+}, Na^{+}, Mg^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+}, Ag^{+}, Fe^{3+} and sulfates of Fe^{2+}, Mn^{2+}, Cu^{2+} were obtained from commercial sources and used without further purification. Standard solution (1 mg mL^{-1}, 1000 ppm) of Pb^{2+}, Hg^{2+} and Cd^{2+} were purchased from Merck Co., Inc. (Germany) and used after diluted to appropriate concentration with ultrapure water. GF (10000 × concentrate in DMSO) was purchased from Shanghai



Scheme 1. Schematic description of the fluorescent assay for detection of Hg^{2+} and Pb^{2+} based on GeneFinder™ and an integrated functional nucleic acid.