Journal of Pharmaceutical and Biomedical Analysis 107 (2015) 258–264

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

A novel strategy for dual-channel detection of metallothioneins and mercury based on the conformational switching of functional chimera aptamer

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ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form
20 December 2014
Accepted 6 January 2015
Available online 14 January 2015

Keywords:
Functional chimera aptamer
Conformational switching
Dual-channel
Metallothioneins
Mercury

ABSTRACT

A novel strategy for dual-channel detection of metallothioneins (MTs) and Hg^{2+} has been proposed. In the absence of Hg^{2+}, the functional chimera aptamer (FCA) designed can form an intact G-quadruplex with flexibility, which was demonstrated to have peroxidase-like activities upon hemin binding. In the presence of Hg^{2+}, the formation of T–Hg^{2+}–T complex results in the conformational switching of FCA, which lost the peroxidase-like activities and cannot catalyze the oxidation of ABTS by H_2O_2. Upon addition of MTs in this solution, MTs could interact with Hg^{2+} to form a MTs–Hg^{2+} complex, leading to the recovery of the G-quadruplex DNAzyme. The color and absorbance of the sensing system were also changed accordingly. In the optimizing condition, ΔA was directly proportional to the concentration ranging from 8.84 nM to 1.0 μM for Hg^{2+}, and 7.82 nM to 0.462 μM for MTs with the detection limits of 2.65 nM and 2.34 nM, respectively. The proposed dual-channel method avoids the label steps in common methods, and allows direct analysis of the samples without costly instruments, and is reliable, inexpensive and sensitive.

1. Introduction

Metallothioneins (MTs) are low molecular weight, metal-binding proteins which have high cysteine content. The 20 cysteinyl sulfurs of mammalian MTs function as bridging and terminal ligands for the coordination of seven divalent metal ions, including both essential metals such as copper and zinc, and toxic metals (mercury and cadmium) in two metal-thiolate clusters [1]. Thus, MTs have been implicated in the detoxification of toxic metals due to their property of coordinating both mercury and cadmium in the cell [1,2]. Several factors can induce the synthesis of MTs in vivo including the presence of free mercury, cadmium and zinc [2,3]. Moreover, MTs level in human urine and blood is related to the hepatic and renal heavy metal burdens [4], and is also correlated with the levels of environmental pollution from metals [5,6]. Thereby, MTs have been widely used as a specific biomarker for disease such as renal tubular dysfunction, and metal pollution in the environment [3,7]. So, the detections of MTs and mercury are certainly challenging and intriguing in biomedicine, toxicology and environmental science fields.

Aptamers are functional oligonucleotides that could be in vitro generated by SELEX (systematic evolution of ligands by exponential enrichment) technology [8]. Compared with antibodies, aptamers possess several advantages including high affinity and specificity toward a variety of targets, easy labeling and long-term stability [8]. These unique characteristics of aptamers make them the ideal recognition elements in designing various types of biosensors. Recently, the aptamer-based methods for detecting mercury and MTs were also been reported [9,10]. Among these sensors, many are constructed by using a thymine (T)-rich oligonucleotide as a sensing element because of Hg^{2+}-mediated T–T base pair being able to fold single-stranded DNA (ssDNA) into duplexes [11]. Thus, this feature leads to the development of various biosensors for Hg^{2+} or MTs detection, such as colorimetry [9,12,13], fluorescence [14], electrochemistry [15], and resonance light scattering [16]. In most cases, an appropriate probe, such as gold nanoparticles or fluorophore, is tethered to oligonucleotides to indicate color or light intensity change. Such a labeling process would make experiments relatively more complex and expensive. Therefore, it is interesting and

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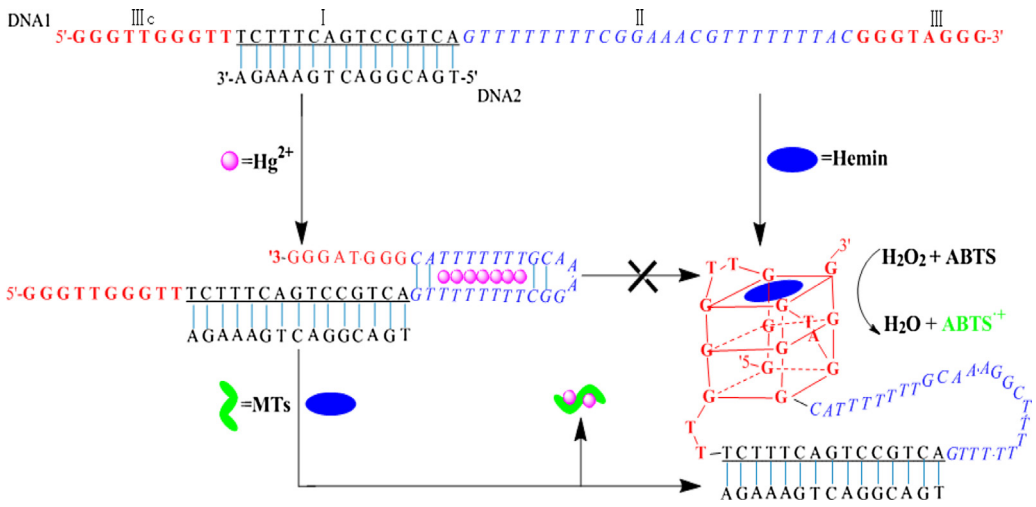
http://dx.doi.org/10.1016/j.jpba.2015.01.011
0731-7085/



Scheme 1. Schematic illustration of Hg^{2+} and MTs sensors based on the disruption and recovery of the G-quadruplex-hemin DNAzyme.

significant to develop a label-free approach for facile effective detection of mercury and MTs.

G-quadruplex DNAzymes are peroxidase-like complexes formed by hemin and G-rich nucleic acid sequences [17], which are able to effectively catalyze the H_2O_2-mediated oxidation of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) [17]. This strategy based on G-quadruplex DNAzymes was also used for Hg^{2+} detection [18,19]. However, the main drawbacks of these methods based on G-quadruplex are the need to avoid the interference of some metal ions such as K^+, Na^+ and Pb^{2+} due to their higher efficiency for stabilizing G-quadruplex [20]. To the best of our knowledge, there is no report on a dual-channel sensing system for the determination of mercury and MTs based on the disruption and recovery of the G-quadruplex DNAzymes.

The goal of this work is to develop a dual-channel strategy for the continuous monitoring of mercury and metallothioneins in human urine, which could be utilized to assess health risks owing to heavy metal exposure such as mercury. For this purpose, we rationally designed a novel functional chimera aptamer (FCA) by incorporating a thymine (T)-rich sequence and two halves of split G-quadruplex into a chimera oligonucleotide (Scheme 1). Using this FCA, a novel method for MTs and mercury was proposed based on Hg^{2+} and MTs modulated conformational switching of FCA to lead to the disruption and recovery of the G-quadruplex DNAzyme. Accordingly, the peroxidase-like ability of the G-quadruplex DNAzyme for catalyzing the oxidation of ABTS by H_2O_2 was lost or recovered, resulting in the change of the color and absorbance of the sensing system. Thereby, a sensitive, selective and label-free method for MTs and Hg^{2+} was established. The method described here could detect nM MTs and Hg^{2+}, without tedious procedure or the requirement of sophisticated equipment. This strategy can be easily expanded by replacing corresponding aptamers to provide a highly sensitive and cost-effective detection platform for analyzing various analytes in the biomedical studies and clinical diagnosis.

2. Materials and methods

2.1. Reagents

The oligonucleotide strands (DNA1: 5′-GGG TTG GGT TTC TTT CAG TCC GTC AGT TTT TTT TTC GGA AAC GTT TTT TTT ACG GGT AGG G-3′ and DNA 2: 5′-TGA CGG ACT GAA AGA-3′) were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China). Their concentrations were represented as single-stranded concentrations, which were determined by measuring the absorbance at 260 nm. Molar extinction coefficients were determined using a nearest neighbor approximation (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer). Both DNA1 and DNA2 solutions were dissolved with appropriate water and then diluted to the final concentration of 10 μM. MTs (from rabbit liver) were obtained from Beijing Yong Kang Jiaxin Biotechnology Co. Ltd. (Beijing, China) and the final concentration was 15.4 μM. 2,2′-Azinobis (3-ethylbenzothiozoline)-6-sulfonic acid (ABTS), hemin and $Hg(Cl)_2$ were supplied by Sigma–Aldrich (St. Louis, MO, USA), the concentrations of their working solutions were 5 mM for ABTS, 20 μM for hemin, and 1 mM for $Hg(Cl)_2$, respectively. Both ABTS and hemin were protected from light and stored in freezer. Tris–HAc buffer was bought from Aladdin Chemistry Co. Ltd. (Shanghai, China), and the concentration of its working solution was 200 mM with 300 mM KNO_3, 5 mM $Mg(Ac)_2$ and 1 M NaAc (pH 5.5). All chemicals used were analytical grade, and sterilization doubly distilled water (18.25 MΩ) was used throughout this work.

2.2. Apparatus

Shimadzu UV-2550 spectrophotometer (Kyoto, Japan) was used to obtain the UV–vis absorption spectra of the system with 1.0 cm quartz cuvette. A Jasco J-815 circular dichroism spectrometer (Tokyo, Japan) was applied for measuring the circular dichroism spectra. A pH meter (Sartorius AG, Germany) was used to pH adjustment.

2.3. Circular dichroism measurements

Circular dichroism (CD) spectra were measured at room temperature. The mixture of DNA1 (4 μM) and DNA2 (12 μM) was added to 15 μL of Tris–HAc buffer (pH 5.5) and heated at 95 °C for 5 min, then cooled slowly to room temperature and incubated for 30 min. After that, $HgCl_2$ and MTs were added, and the final concentrations were 10 μM for $HgCl_2$, and 3.7 μM for MTs. The CD spectra were collected ranging from 200 to 300 nm at 0.05 nm interval in 1 mm pathlength cuvettes. Three scans with a scanning speed of 200 nm min^{-1} were accumulated and averaged.

2.4. Pretreatment of urine samples

Urine samples, which were collected from five inpatients of the First Affiliated Hospital of the University of South China and two