Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 151 (2015) 315–321

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Dual-channel detection of metallothioneins and mercury based on a mercury-mediated aptamer beacon using thymidine–mercury–thymidine complex as a quencher

Si-Han Chen, Yong-Sheng Wang *, Yun-Sheng Chen, Xian Tang, Jin-Xiu Cao, Ming-Hui Li, Xiao-Feng Wang, Yu-Feng Zhu, Yan-Qin Huang

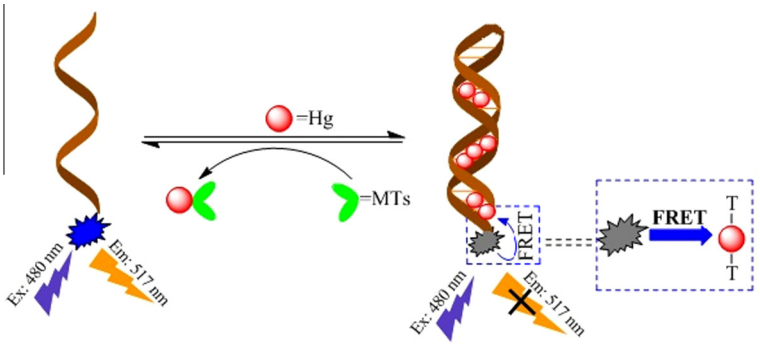
College of Public Health, University of South China, Hengyang 421001, PR China

HIGHLIGHTS

- A mercury-mediated aptamer beacon was proposed using T–Hg–T as a quencher.
- The proposed strategy can detect nM MTs and Hg^{2+}.
- This method avoids the label of a quencher, without tedious procedure.
- The mechanism of the conformational switching of MAB was also discussed.

GRAPHICAL ABSTRACT

The presence of Hg^{2+} can result in a fluorescence quenching of the mercury-mediated aptamer beacon by FRET. The addition of MTs into this solution leads to an increase of the fluorescent intensity of the system due to the disruption of the T–Hg^{2+}–T complex.



ARTICLE INFO

Article history:
Received 16 November 2014
Received in revised form 26 June 2015
Accepted 28 June 2015
Available online 29 June 2015

Keywords:
Dual-channel
Mercury-mediated aptamer beacon
Thymidine–mercury–thymidine quencher
Metallothioneins
Mercury

ABSTRACT

A novel dual-channel strategy for the detection of metallothioneins (MTs) and Hg^{2+} has been developed based on a mercury-mediated aptamer beacon (MAB) using thymidine–mercury–thymidine complex as a quencher for the first time. In the presence of Hg^{2+}, the T-rich oligonucleotide with a 6-carboxyfluorescein (TRO-FAM) can form an aptamer beacon via the formation of T–Hg^{2+}–T base pairs, which results in a fluorescence quenching of the sensing system owing to the fluorescence resonance energy transfer (FRET) from the fluorophore of FAM to the terminated T–Hg^{2+}–T base pair. The addition of MTs into this solution leads to the disruption of the T–Hg^{2+}–T complex, resulting in an increase of the fluorescent signal of the system. In the optimizing condition, ΔF was directly proportional to the concentrations ranging from 5.63 nM to 0.275 μM for MTs, and 14.2 nM to 0.30 μM for Hg^{2+} with the detection limits of 1.69 nM and 4.28 nM, respectively. The proposed dual-channel method avoids the label steps of a quencher in common molecular beacon strategies, without tedious procedure or the requirement of sophisticated equipment, and is rapid, inexpensive and sensitive.

* Corresponding author.
E-mail address: yongsheng.w@tom.com (Y.-S. Wang).

http://dx.doi.org/10.1016/j.saa.2015.06.106
1386-1425/

1. Introduction

Metallothioneins (MTs) are a family of metal-binding proteins with a high cysteine content [1], in which the 20 cysteinyl sulfurs of mammalian MTs function as bridging and terminal ligands for the coordination of seven divalent metal ions in two metal-thiolate clusters [2]. MTs play a key role in metal ion homeostasis and the detoxification of toxic metals including mercury and cadmium in the cell [2,3]. The synthesis of MTs in vivo is able to be induced by free mercury, cadmium and so on, and their levels in human urine and blood are related to the hepatic and renal heavy metal burdens [3–7]. Thereby, MTs have been widely used as a specific biomarker for disease such as renal tubular dysfunction, and metal pollution in the environment [4,8,9]. Therefore, the detections of MTs and mercury are intriguing and high desired in environmental science, biological science and toxicology fields.

Aptamers are functional oligonucleotides generated by an in vitro selection technology called systematic evolution of ligands by exponential enrichment (SELEX) [10]. They possess several advantages including high affinity and specificity toward a variety of targets and easy labeling. These characteristics make them the ideal recognition elements for disease diagnosis and biological analysis and so on [10,11]. Although aptamers have the advantages mentioned above, they lack the signal transduction mechanism of molecular beacons [12]. Therefore, aptamer beacons have drawn much interests owing to them having both the signal transduction mechanism of molecular beacons and the molecular recognition specificity of aptamers. They have been applied for the detection of biomacromolecule and small molecules [13–15]. Analogous to molecular beacons, aptamer beacons would exist in a quenched stem-loop structure in the absence of a target molecule, and a fluorophore–quencher pair is used to report the changes in the conformation induced by target binding. In these strategies, DABCYL [(4-(4-dimethylamino phenylazo)benzoic acid)] and gold nanoparticles are customarily used as quenchers, which are tethered to oligonucleotides to indicate the change of fluorescence intensity [14]. Such a labeling process would make experiments relatively more complex and expensive. So, it is interesting and significant to develop a label-free aptamer beacon for facile effective detection of mercury and MTs. Recently, many aptamer-based biosensors were constructed by using a thymine (T)-rich oligonucleotide as a sensing element, and used for detecting mercury, MTs and so on [16–23]. To the best of our knowledge, there is no report on a dual-channel detection of metallothioneins and mercury based on a mercury-mediated aptamer beacon using thymidine–mercury–thymidine complex as a quencher.

Herein, we rationally designed a novel mercury-mediated aptamer beacon (MAB), which is constructed by a thymine (T)-rich oligonucleotide with a 6-carboxyfluorescein (TRO-FAM) at 5′-end and a 3′-end thymidine as a unique quencher upon the formation of T–Hg^{2+}–T complex in the presence of mercury(II) ions. When MTs is introduced, Hg^{2+} prefers to form a MTs–Hg^{2+} complex rather than a T–Hg^{2+}–T complex, triggering the conformational switching of MAB. As a result, the fluorescence intensity of the system was enhanced accordingly. Thereby, a novel dual-channel sensing strategy for MTs and mercury was proposed based on a mercury-mediated conformational switching of aptamer beacon. The method described here could detect nM MTs and Hg^{2+}, without tedious procedure or the requirement of sophisticated equipment. This strategy can in principle be expanded to develop an universal approach for various targets in the biomedical studies and clinical diagnosis.

2. Materials and methods

2.1. Reagents

The oligonucleotide strand (5′FAM-CGC TTG TTT GTT CGC ACC CGT TCT TTC TT-3′) was purchased from Sangon Biotech. Co. Ltd. (Shanghai, China), and was dissolved with an appropriate amount of water and then diluted to the final concentration of 5 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. The concentration of $Hg(Cl)_2$ working solution was 1.0 mM. Tris–HCl buffer was bought from Aladdin Chemistry Co. Ltd. (Shanghai, China), and the concentration of its working solution was 100 mM (pH 8.5). All chemicals used were analytical grade, and sterilization doubly distilled water (18.25 MΩ cm) was used throughout this work.

2.2. Apparatus

All fluorescence spectra were obtained with a Shimadzu F-4500 spectrofluorometer (Tokyo, Japan). 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 pH meter (Sartorius AG, Germany) was used to pH adjustment.

2.3. Pretreatment of urine sample

Urine samples, which were collected from five inpatients of the Second Affiliated Hospital of the University of South China, were pretreated according to the literature with appropriate modifications [7,24]. Firstly, an appropriate volume of the urine samples were mixed with an equal volume of 10 mM Tris–HCl buffer solution of pH 8.5 in a centrifuge tube, with shaking and centrifuging (4000 rpm, 20 min, at 4 °C). Subsequently, the supernate fluid was incubated at 80 °C for 10 min and cooled to room temperature slowly. After centrifuging at 4000 rpm for 20 min at 4 °C, the supernatant was mixed with an anhydrous ethanol (1:3, *v/v*), and was kept in a refrigerator at −20 °C for 12 h. Then the mixture was centrifuged (4000 rpm, 30 min, at 4 °C) again. Thereafter, the residue was dissolved in a Tris–HCl buffer solution (pH 8.5), and the isopyknic chloroform–ethanol (0.08:1, *v/v*) solution was added in this solution, centrifuging at 4000 rpm for 20 min at 4 °C. After the aforementioned process being repeated, the residue obtained was redissolved with doubly distilled water and centrifuged (4000 rpm, 30 min, at 4 °C). The supernatant obtained was analyzed directly by using the proposed method.

2.4. Assay for Hg^{2+} (a) and MTs (b)

Into a 2-mL EP tube, a 30 μL of 1.0 μM TRO-FAM was mixed with 45 μL Tris–HCl buffer (pH 8.5). (a) 20 μL different concentrations of Hg^{2+} ions were added, followed by adding an appropriate amount of water to a volume of 400 μL. After 10 min, the fluorescence spectra were obtained by scanning from 350 to 650 nm at λ_{ex} = 480 nm. The spectral bandwidths of both the excitation and emission slits were set to 5.0 and 10.0 nm, respectively. The fluorescence intensity of the system was measured at λ_{em} = 517 nm, and represented as $\Delta F = F_0 - F$, here F_0 and F were the fluorescence intensities of the system without and with Hg^{2+}, respectively. (b) A 12 μL of 10 μM $HgCl_2$ was added in the TRO-FAM-Tris–HCl solution prepared above, reacting for 10 min. Thereafter, 20 μL different concentrations of MTs and 293 μL of doubly distilled water were added, and incubated for 10 min. The fluorescence intensity of