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Label-free colorimetric detection of Hg^{2+} based on Hg^{2+}-triggered exonuclease III-assisted target recycling and DNAzyme amplification

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ABSTRACT

This work reported a label-free colorimetric assay for sensitive detection of Hg^{2+} based on Hg^{2+}-triggered hairpin DNA probe (H-DNA) termini-binding and exonuclease III (Exo III)-assisted target recycling, as well as hemin/G-quadruplex (DNAzyme) signal amplification. The specific binding of free Hg^{2+} with the thymine–thymine (T–T) mismatches termini of H-DNA could immediately trigger the Exo III digestion, and then set free G-quadruplex segments and Hg^{2+}. The Exo III impellent recycling of ultratrace Hg^{2+} produced numerous G-quadruplexes. The corresponding DNAzymes catalyzed efficiently the H_2O_2-mediated oxidation of the $ABTS^{2-}$ to the colored product in the presence of hemin. Using the color change as the output signal, and the Exo III-aided Hg^{2+} recycling and DNAzyme as the signal amplifier, the ultrasensitive assay system successfully achieved visual detection of Hg^{2+} as low as 1.0 nM by the naked eye, and was suitable for field monitoring. The calibration curve was linear in the range of 50.0 pM to 20.0 nM for Hg^{2+} (R=0.9962) with a detection limit of 10.0 pM. Moreover, this proposed strategy showed excellent selectivity, portability and low-cost, and was successfully applied to colorimetric detection of Hg^{2+} in laboratory tap water and Jialing river water samples.

1. Introduction

Mercury pollution has always been an important worldwide issue for years due to its high toxicity at low concentrations and non-biodegradability throughout the food chain as well as bioaccumulative effect on human health (Zahir et al., 2005; Tchounwou et al., 2003). It has been recognized that water-soluble divalent mercuric ion (Hg^{2+}) is one of the most usual and stable form of mercury pollution (Li et al., 2009a). Due to the ever-increasing environmental and health concerns about Hg^{2+}, it is necessary to develop a monitoring routine for trace Hg^{2+} detection with high sensitivity, selectivity, cost efficiency and easy operation.

It is well known that Hg^{2+} interacts selectively with two thymine (T) residues of DNA to form stable T–Hg^{2+}–T complexes (Ono and Togashi, 2004). Because the structure of T–Hg^{2+}–T is stabilized by covalent N–Hg bonds and even more stable than the Watson–Crick hydrogen-bonding (Miyake et al., 2006), many relative schemes have been provided to specifically detect Hg^{2+} in recent years, for example, various selective fluorophotometries (Deng et al., 2011; Qi et al., 2012; Huang et al., 2013a), electrochemical sensors (Lin et al., 2011; Yuan et al., 2011; Wang et al., 2012), and colorimetric methods (Zhang et al., 2012; Li et al., 2009a). However, most of these methods are of unsatisfactory sensitivity. In order to improve the sensitivity, the enzyme-aided target recycling strategy has been used to develop different amplified platforms for Hg^{2+} detection. Previous studies have revealed that the activity of nicking endonuclease (Li et al., 2011), polymerase (Urata et al., 2010; Park et al., 2010) and ligase (Bi et al., 2013) toward DNA duplexes containing T–T mismatches could be triggered by Hg^{2+}, meanwhile Hg^{2+} was released from the T–Hg^{2+}–T complexes and initiated a new cycle. However, polymerase chain reaction is a primer-dependent and complex multi-step process, and nicking endonuclease cleavage is sequence-specific, which limit their wide applications. In contrast, exonuclease III (Exo III) does not require a specific enzymatic recognition sequence and is able to digest duplex DNA from the blunt end, recessed 3′ end and nicked sites when the base number of the protruding 3′ end is less than 4 (Zuo et al., 2010; Zhao et al., 2011; Xuan et al., 2012). Recently, it has been demonstrated that catalysis activity of Exo III can be activated by T–Hg^{2+}–T base pairing (Xuan et al., 2013; Wang et al., 2014; Chen et al., 2014).

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Thus, Exo III-assisted target recycling tactic has been employed to develop signal-amplified sensor platform for Hg^{2+} detection with preferable selectivity and sensitivity.

In most cases, the requirements for sophisticated instruments, complicated probe modification or time-consuming sample pretreatment offer some advantages, but it may not be suitable for on-site detection and rapid quantification. There is still an urgent demand for robust Hg^{2+} detection with high sensitivity, selectivity, and portability. In recent years, label-free colorimetric methods based on G-quadruplexes have attracted great interest because of low-cost, convenience and rapidness. A series of classic strategies has been constructed for the formation of G-quadruplex using guanines in the stem of hairpin DNA. Target binding with stem opened the hairpin DNA, and then the G-quadruplex/hemin complex, a horseradish peroxidase (HRP) mimicking DNAzyme (denoted as DNAzyme), can be obtained in the presence of hemin. The DNAzyme exhibits higher peroxidase activity than hemin alone and is able to catalyze the H_2O_2-mediated oxidation of 2,2′-azino-bis(3-ethylbenzothiazo- line-6-sulfonate) disodium salt ($ABTS^{2-}$) to the colored free-radical product $ABTS^{\bullet-}$, resulting in increase of the absorbance signal with a maximal absorption at 419 nm (Travascio et al., 1998,1999). Up to now, G-quadruplex labels have been utilized extensively for detection of a number of analytes including small molecules (Li et al., 2009b, 2007a; Kong et al., 2010; Zhou et al., 2010; Elbaz et al., 2008), metal ions (Zhang et al., 2012; Li et al., 2009a, 2007b, 2009c; Zhou et al., 2010; Elbaz et al., 2008; Kong et al., 2009), protein (Li et al., 2007a, 2009b; Huang et al., 2013b) and DNA (Deng et al., 2008; Willner et al., 2008; Nakayama and Sintim, 2009).

In the present work, a simple, label-free and ultrasensitive colorimetric assay was developed for selective detection of Hg^{2+} based on DNAzyme-signal amplification and Exo III-assisted continuous recycling of ultratrace Hg^{2+}. In brief, free Hg^{2+} specifically binding with T–T mismatches of the hairpin DNA (H-DNA) could immediately trigger Exo III digestion of H-DNA duplex to release G-quadruplexes and Hg^{2+} which initiated a new recycling, and thus produced cascades of DNAzyme in the presence of hemin. The color change resulting from DNAzyme-catalyzed H_2O_2-mediated $ABTS^{2-}$ oxidation herein was served as a signal output and can be instantly recognized by the naked eye and chromometer. It was worth mentioning that the proposed ultrasensitive sensing platform accomplished the sensitive quantization and visual detection of Hg^{2+} as low as 1.0 nM just by the naked eye, which has important application value to the local detection of Hg^{2+} in potable water. Moreover, the proposed colorimetric assay is simple, easy operating and low-cost, and could be applied to detect ultratrace Hg^{2+} in real water samples.

2. Material and methods

2.1. Materials and apparatus

Hemin, $ABTS^{2-}$, and 2-amino-2-(hydroxymethyl)-1,3-propanediol (tris) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Exo-III (specific activity, 20,000 units/mL) was purchased from Sangon (Shanghai, China). Other reagents of analytical reagent grade were purchased from Chengdu Kelong Chemical Reagents Factory (China), and used as received. The hemin stock solution was prepared in dimethyl sulfoxide (DMSO) and stored at −20 °C. Tris–HAc buffer (20 mM Tris, 100 mM NaAc, 50 mM KAc, and 0.05% Triton X-100, pH 7.4). For all experiments, ultrapure water (18.2 MΩ cm) was used. The experiment temperature was kept with a SD-101-005DB super digital thermostat bath (Sida Experimental Equipment Ltd., Chongqing, China). Ultraviolet-visible (UV–vis) absorption spectra were recorded from 500 to 400 nm using a Shimadzu UV–vis 2450 spectrophotometer (Japan). The tungsten–halogen (electric power: 50 W; wavelength range: 300–2600 nm; absorbance resolution: 0.1 nm) was used as a continuous light source. The pH values of solutions were measured with a pH meter (PHS-3 C, Shanghai Leici Instrument Company, Ltd., China). DNA oligonucleotides were synthesized by Sangon (Shanghai, China) and used without further purification. The sequences of oligonucleotides are listed as follows:

H_1: TTT*T*GTGGGTAGGGCGGGTTGGACCCTACCCAC*T*AAA;
H_2: TT*TT*GTGGGTAGGG CGGGTTGGACCCTACCCAC*TT*AA;
H_3: T*TTT*GTGGGTAGGGCGGGTTGGACCCTACCCAC*TTT*A;
H_4: *TTTT*GTGGGTAGGGCGGGTTGGACCCTACCCAC*TTTT*;
H_3': *TTT*GTGGGTAGGGCGGGT TGGACCCTACCCAC*TTT*.

All oligonucleotides samples were prepared by dissolving in Tris–HAc buffer solution and heated to 90 °C for 5 min and then allowed to cool to room temperature for 1 h before use.

2.2. Analytical procedure for Hg^{2+}

The detailed procedure for Hg^{2+} detection was as follows. First, the incorporated reaction was performed by mixing H-DNA (50 μL, 2.0 μM) with 50 μL of Hg^{2+} (0.0 nM or 10.0 nM) and 10 μL of Exo III (2.0 U/μL). The mixture was incubated for 40 min at 35 °C and heated to 80 °C for 10 min to inactivate the Exo III. Subsequently, hemin (100 μL, 10% DMSO) was added to the cooled mixture and obtained the final concentration of 2.0 μM hemin and then the mixture was incubated for 30 min at 4 °C to form the hemin/G-quadruplex structures. Finally, colorimetric measurement was performed after mixing 200 μL of the above prepared G-quadruplex-hemin DNAzyme solution with 100 μL of H_2O_2 (10.0 mM) and 100 μL of $ABTS^{2-}$ (5.0 mM) solution for 10 min at 25 °C. For evaluation of the sensitivity, different concentrations of Hg^{2+} were added to the system. To investigate the selectivity of the assay, the colorimetric responses of the blank and 10.0 nM Hg^{2+} were compared with those environmentally relevant metal ions (Ag^{+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, and Fe^{3+}) at high concentrations (1.0 μM).

2.3. Gel electrophoresis

Gel electrophoresis was used to confirm the Hg^{2+}-triggered digestion of the hairpin DNA probe three (H_3) by Exo III. Samples for gel electrophoresis assays were prepared as follows: (1) the H_3 (10.0 μM) was used as sample one; (2) the mixture of H_3 (10.0 μM) and 10 units Exo-III was incubated and used as sample two; (3–5) the mixtures of H_3 (10.0 μM), 20 units Exo-III, and Hg^{2+} (10.0, 100.0 or 1000.0 nM) were incubated and used as samples three to five; (6) the mixture of H_3 (10 μM) and 100.0 nM Hg^{2+} was incubated and used as sample six. All samples were prepared similarly at 35 °C for 40 min and heated to 80 °C for 10 min to inactivate the Exo III. All reaction mixtures (without addition of hemin, $ABTS^{2-}$, and H_2O_2) were assessed using 12% native polyacrylamide gel electrophoresis (PAGE). All the sample solutions were allowed to slowly equilibrate to room temperature for about 30 min and then loaded into lanes for PAGE analysis. Before loading, DNA samples were mixed with isopyknic DNA loading buffer. A potential of 120 V was applied for gel electrophoresis separation in 1 × TBE buffer for about 70 min at 25 °C. After separation, PAGE gel visualized with ethidium bromide staining was imaged using a Gel Doc XR+ system.

2.4. Analysis of environmental water samples

Tap water was obtained from our laboratory. River water was taken from the Jialing River (Chongqing, China). The river water