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An ultra-sensitive colorimetric Hg^{2+}-sensing assay based on DNAzyme-modified Au NP aggregation, MNPs and an endonuclease

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ABSTRACT

This paper reports the development of an ultra-sensitive colorimetric method for the detection of trace mercury ions involving DNAzymes, Au nanoparticle aggregation, magnetic nanoparticles and an endonuclease. DNAzyme-sensing elements are conjugated to the surface of Au nanoparticle-2, which can crosslink with the T-rich strands coated on Au nanoparticle-1 to form Au nanoparticle aggregation. Other T-rich stands are immobilized on the surface of MNPs. The specific hybridization of these two T-rich strands depends on the presence of Hg^{2+}, resulting in the formation of a T-Hg^{2+}-T structure. Added endonuclease then digests the hybridized strands, and DNAzyme-modified Au NP aggregation is released, catalysing the conversion of the colourless ABTS into a blue-green product by H_2O_2-mediated oxidation. The increase in the adsorption spectrum of $ABTS^+$ at 421 nm is related to the concentration of Hg^{2+}. This assay was validated by detecting mercury ion concentrations in river water. The colorimetric responses were not significantly altered in the presence of 100-fold excesses of other metal ions such as Zn^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+}, Ca^{2+} and Ni^{2+}. The inclusion of both Au NP aggregation and an endonuclease enables the assay to eliminate interference from the magnetic nanoparticles with colorimetric detection, decrease the background and improve the detection sensitivity. The calibration curve of the assay was linear over the range of Hg^{2+} concentrations from 1 to 30 nM, and the detection limit was 0.8 nM, which is far lower than the 10 nM US EPA limit for drinking water.

1. Introduction

Mercury is a highly toxic global pollutant with an environmental residence time from 0.5 to 2 years [1,2]. This kind of persistent toxic substance occurs naturally and releases into the environment through both natural and anthropogenic processes. Mercury has a strong affinity to organic substances, which plays an important role in mobilization and transportation of mercury from forest ecosystems to water ecosystems [3,4]. Consumption of polluted water or seafood can lead to the accumulation of mercury in the human body, leading to some serious illnesses, including neuropsychological dysfunction and chronic mercury poisoning syndrome. Among various mercury pollutants, Hg^{2+} is the most stable inorganic form in water environments [5]. Moreover, methyl mercury, the most common organic source and bioaccumulative form of mercury, is generated from Hg^{2+} by microbial biomethylation [6]. Hence, the sensitive and specific detection of Hg^{2+} is important for water pollution control and drinking water safety.

The determination of trace amounts of mercury has commonly been carried out using cold vapor atomic absorption spectrometry (CVAAS) or inductively coupled plasma mass spectrometer (ICP-MS). These instrumental analysis of Hg require that all chemical forms of mercury are needed to reduce to elemental mercury (Hg^0) [4]. However, these extra pretreatment steps can lead to increased risk of contamination and/or mercury volatilization from sample. Therefore, it is very urgent to develop the novel and sensitive methods for the detection of Hg avoiding the environmentally hazardous pretreatment steps. Hg sensors based on T-Hg^{2+}-T conjugation have been developed to meet the requirement [7–10]. These electrochemical [11–13], fluorescence [14] and optical sensors [15–17] have been used to monitor aqueous Hg^{2+}. Due to its high sensitivity and low production costs, electrochemical sensing is one of the most popular Hg^{2+} detection system designs. However, its unstable performance, poor reproducibility, and high non-specific absorption lead to imperfect detection. While fluorescence is another attractive detection method, it requires sophisticated instrumentation, an expensive and complicated labelling procedure, and it tends to result in a high background. Compared with the methods described above, colorimetric systems are stable and easy to control, but less sensitive. Hence, to meet the requirements of Hg ion detection in drinking water (within the 10 nM US EPA limit), the sensitivity of the colorimetric method should be improved. In this work, we use Au nanoparticle aggregation modified with DNAzymes as signal amplification elements and demonstrate that this approach reduces the detection limit.

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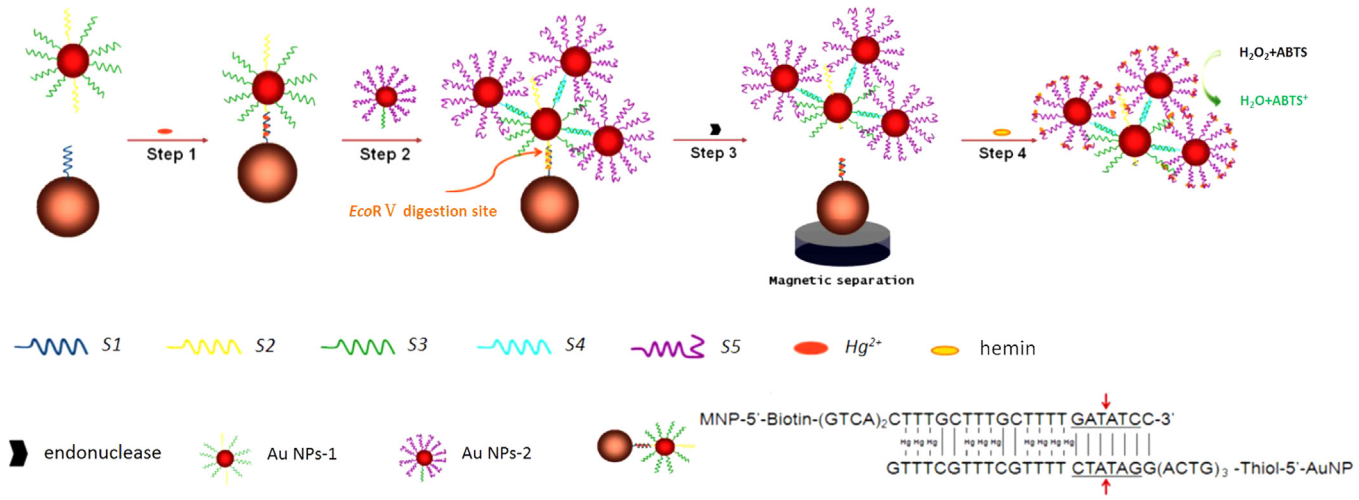

Fig. 1. A schematic illustration of the amplified Hg^{2+}-sensing assay.

Due to their favourable biocompatibility, it is easy to modify magnetic nanoparticles (MNPs) with biologically active groups to achieve the enrichment and separation of components of interest [18]. MNPs have been employed in this way to powerful effect in a number of applications [19–21]. However, MNPs have been reported to exhibit an intrinsic peroxidase-like activity, which could cause a background signal in colorimetric assays [22]. Moreover, the use of MNPs in an optical detection system could lead to uneven scattering, leading to some bias in the results. To eliminate these disadvantages, this assay uses an endonuclease with a specific recognition and hydrolysis ability to release the sensing element into the liquid phase for UV-visible spectrophotometric detection. This paper reports the development of a novel, selective and sensitive colorimetric Hg^{2+}-sensing assay based on Au NP aggregation coated with DNAzyme, MNPs and an endonuclease (*Eco*RV). As illustrated in Fig. 1, this strategy uses several DNA strands and consists of four main steps. First, in the presence of Hg^{2+}, MNPs coated with the T-rich S1 strands (S1/MNPs) specifically hybridize with AuNP-1s modified with the T-rich strands (S2) and with AuNP-1s modified with cross-linking strands (S3). Second, the Au NP-2s covered with a different type of cross-linking strand (S4) and with DNAzyme strands (S5) are added to the Au NP aggregation formed in the first step. The excess AuNP-1s, AuNP-2s and interfering substances are then removed by magnetic separation. Then, *Eco*RV is used to digest the DNA double-strand to release Au NP aggregation, contributing to the amplification of the optical signal. After incubating the supernatant of the digested Au NP aggregation with hemin, the colorimetric measurement is carried out, with the optical signal of $ABTS^+$ at 421 nm directly related to the concentration of the Hg ion in solution.

2. Experimental procedures

2.1. Materials

An HZQ-C Swing bed machine (Donglian Electrical Technology Co. Ltd., China) was used to assemble the oligonucleotide strands onto the surfaces of the Au NPs and MNPs. Colorimetric measurements were performed on a Cary 50 UV–visible Spectrophotometer (Varian, Palo Alto, USA). A JEM-2100F field emission electron microscope (JEOL, Japan) was used to observe the structure of the nanoparticles.

Tris-(2-carboxyethyl) phosphine (TCEP), saline sodium citrate (SSC) buffer (15 mM SSC, 150 mM NaCl, pH 7.0), bovine serum albumin (BSA) and hemin were purchased from Sigma Chemical Co. (Shanghai, China). Chloroauric acid and 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Sangon Biotechnology Co. (Shanghai, China). *Eco*RV and 100 × BSA were purchased from New England Biological Technology Co. Ltd. (Beijing, China). Streptavidin-coated magnetic nanoparticles (MNPs, hydrodynamic diameter of 100 nm, 10 mg/mL) were obtained from Chemicell GmbH (Germany). All oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. and purified by HPLC. The oligomer sequences and the buffers used are listed in the supporting information.

2.2. Preparation of oligonucleotide-modified MNPs

A 100-μL solution of MNPs modified with streptavidin (10 mg/mL) was washed three times with SSC buffer to remove sodium azide. Then, 0.38 nmol S1 in 200 μL of SSC buffer was added to the collected MNPs. After 1 h of gentle shaking at room temperature, the mixture was washed to remove free DNA. A 1% BSA solution was used to block the MNP surface and eliminate further non-specific adsorption. After 1 h of blocking treatment, these S1/MNPs were stored in 200 μL of Hg^{2+} capture buffer at 4 °C.

2.3. Preparation of oligonucleotide-modified Au NPs

Au NPs with an average diameter of 13 nm (estimated by TEM) were prepared by the citrate reduction method [23]. S2 and S3 (0.36 nmol and 3.6 nmol, respectively) were treated with TCEP (2.5 mM, dissolved in 0.1 M Tris-HAc, pH 7.4) for 1 h. Then, 200 μL Au NPs (10.4 nM) were added to the reduced S2 and S3 solution. The mixture was gently stirred at room temperature for 42 h while the salt concentration was gradually adjusted to a final value of 0.3 M NaAc. The solution was centrifuged (13000 rpm, 15 min) and rinsed with water twice to remove the excess oligonucleotides. Then, 10 μL of 20% BSA was added for 1 h to block the surface of the Au NPs. The resulting solution was suspended in 200 μL capture buffer and named Au NPs-1. The Au NPs-2 (S4 to S5 ratio of 1:100) and Au NPs-3 (S2 to S5 ratio of 1:10) were prepared by the same procedure and dispersed in hybridization or capture buffer, respectively. All of the Au NPs were stored at 4 °C until further use.

2.4. Hg^{2+} recognition

The recognition of Hg^{2+} was carried out by following steps. Briefly, 10 μL S1/MNPs, 40 μL S2/S3/Au NPs and certain volumes of Hg^{2+} were added to a tube and gently shaken at room