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Magnetic beads-based DNAzyme recognition and AuNPs-based enzymatic catalysis amplification for visual detection of trace uranyl ion in aqueous environment

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ABSTRACT

We herein developed a novel biosensor for the visual detection of trace uranyl ion (UO_2^{2+}) in aqueous environment with high sensitivity and specificity by using DNAzyme-functionalized magnetic beads (MBs) for UO_2^{2+} recognition and gold nano-particles (AuNPs)-based enzymatic catalysis oxidation of TMB (3,3′,5,5′-tetramethylbenzidine sulfate) for signal generation. The utilization of MBs facilitates the magnetic separation and collection of sensing system from complex sample solution, which leads to more convenient experimental operation and more strong resistibility of the biosensor to the matrix of sample, and the utilization of AuNPs-based enzymatic catalysis amplification greatly improved the sensitivity of the biosensor. Compared with the previous DNAzyme-based UO_2^{2+} sensors, the proposed biosensor has outstanding advantages such as relative high sensitivity and specificity, operation convenience, low cost and more strong resistibility to the matrix of sample. It can be used to detect as low as 0.02 ppb (74 pM) of UO_2^{2+} in aqueous environment by only naked-eye observation and 1.89 ppt (7.0 pM) of UO_2^{2+} by UV–visible spectrophotometer with a recovery of 93–99% and a RSD $\leq$ 5.0% (n=6) within 3 h. Especially, the visual detection limit of 0.02 ppb (74 pM) is much lower than the maximum allowable level of UO_2^{2+} (130 nM) in the drinking water defined by the U.S. Environmental Protection Agency (EPA), indicating that our method meets the requirement of rapid and on-site detection of UO_2^{2+} in the aqueous environment by only naked-eye observation.

1. Introduction

As the main fuel of nuclear energy, uranium has been widely used in many fields such as nuclear power station, nuclear weapons, industrial and medical fields (Li and Zhang, 2012; Yazzie et al., 2003). In the past few decades, the worldwide uranium consumption significantly increased due to the continuous development of nuclear energy. However, mining and processing of uranium mineral resources has also brought a large area of uranium pollution, and the pollution of uranium has caused increasing attention due to its high toxicity and radioactivity. It was reported that uranium not only has radioactive toxicity, which could lead to long-term harm to mammalian reproduction and development (Domingo, 2001), but also has chemical genotoxicity, which is similar to that of hexavalent chromium (Yazzie et al., 2003). Although uranium can exist in oxidation states +3, +4, +5 or +6, the most stable species of this element existing in the aqueous environment and mammalian body is uranyl ion (UO_2^{2+}). For the reasons mentioned above, it is thus crucial to develop a portable and simple method for the rapid and on-site detection of UO_2^{2+} in the aqueous environment, in order to protect people from uranium damage.

Traditional analysis techniques for the trace UO_2^{2+} included inductively coupled plasma mass spectrometry (ICP-MS) (Lorber et al., 1996), energy dispersive X-ray fluorescence spectrometry (Rao et al., 2012), laser-atomic absorption spectrometry (Quentmeier et al., 2001), and laser-induced kinetic phosphorimetry etc (Brina and Miller, 1992). These methods have relative high sensitivity and accuracy, however, most of them required tedious and laborious pre-treating procedures, and/or sophisticated and costly

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instruments, which make on-site and real-time detection difficult (Yin et al., 2013). The limitations of traditional techniques have pushed researchers to develop novel methods for the rapid, sensitive and specific detection of UO_2^{2+} in the field. Along this goal, several chemical sensors, especially colorimetric sensors, have been developed for the visual and on-site detection of UO_2^{2+} due to the outstanding analytical advantages of the visual method, such as portability, low cost, short detection time and lack of requirement for sophisticated equipment (Chen et al., 2014; Drogat et al., 2014; Safavi and Bagheri, 2005; Sessler et al., 2004; Zhang et al., 2015a, 2015b). However, the previous colorimetric chemical sensors have lower sensitivity and relatively poor selectivity, which do not meet the requirement of rapid and on-site detection of trace UO_2^{2+} in aqueous environment. So, improving the sensitivity of the visual methods is a key factor for the on-site detection of UO_2^{2+}.

DNAzymes isolated via in vitro selection have emerged as a promising sensing platform for designing metal ions biosensors due to its unique specificity for a series of metal ions (Silverman, 2008; Willner et al., 2008). Compared to protein enzymes, DNAzymes are easier to synthesize, more economic and thermally stable (Kosman and Juskowiak, 2011). To date, a number of highly specific DNAzymes have been isolated and used for detecting metal ions such as Pb^{2+} (Li et al., 2014), Zn^{2+} (Qian et al., 2014), Cu^{2+} (He et al., 2014), Ce^{3+} (Huang et al., 2014a, 2014b) and Mn^{2+} (Liu et al., 2003) etc. In recent years, some DNAzyme-based fluorescent and colorimetric biosensors have also been developed for the rapid and specific detection of UO_2^{2+} (Lee et al., 2008; Liu et al., 2007; Yin et al., 2013; Zhang et al., 2015a, 2015b) since the first report of UO_2^{2+}-specific DNAzymes (Brown et al., 2009). The fluorescent UO_2^{2+} biosensors previously reported have relatively high sensitivity. However, they required a fluorescent spectrometer and their sensitivity still is not high enough for the direct detection of trace UO_2^{2+} in aqueous environment, which make rapid and on-site detection difficult. The previous colorimetric biosensors, which based on DNAzyme and gold nano-particles (AuNPs), are rapid, portable and low-cost. However, most of them have lower sensitivity and poor resistibility to matrix of sample, which do not meet the requirement of visual and on-site detection of trace UO_2^{2+} in aqueous environment (Lee et al., 2008; Zhou et al., 2013). Moreover, the AuNPs colorimetric assay is also limited by the species and concentration of salt in the solution, which hindering its further development (Lin et al., 2013). Therefore, it is still a significant challenge to design simple, portable and cost-effective sensors with strong disturbance resistibility for the sensitive, selective and on-site detection of trace UO_2^{2+}.

Magnetic beads (MBs) can be easily collected with a magnet to extract the target analyte from the matrix of the samples. More importantly, the use of MBs can minimize matrix effect due to the improved washing and separation steps, which allows the analysis of complex samples without any pre-enrichment or purification procedures (Zacco et al., 2006). In view of the above properties of MBs, it has been extensively used for fabricating electrochemical (Barthelmebs et al., 2011) and colorimetric biosensor etc (Huang et al., 2014a, 2014b). Herein, we developed a sensitive and selective colorimetric biosensor with strong disturbance resistibility for UO_2^{2+} detection by taking the advantage of MBs, UO_2^{2+}-specific DNAzyme and AuNPs-based enzymatic catalysis amplification, in hope of providing a reliable and stable visual method for the on-site detection of trace UO_2^{2+} in aqueous environment by only naked-eye observation.

2. Experimental section

2.1. Apparatus and reagents

The UO_2^{2+}-specific DNAzyme used in the experiment is the same as that reported by Lu and his co-workers (Liu et al., 2007). High-performance liquid chromatography (HPLC) purified enzyme strand (39E), biotin-modified substrate strand (39S), ligation probe (L_P), and signal probe (S_P) were synthesized by TaKaRa BioInc (Dalian, China). Their concentrations were accurately quantified by OD260 based on their individual absorption coefficients. Their base sequences were as follows: 39E: 5′-CAC GTC CAT CTC TGC AGT CGG GTA GTT AAA CCG ACC TTC AGA CAT AGT GAG T-3′; 39 S: 5′-biotin-ATATAT TGT CCG TGC TAG AAG GAA CTC ACT AT rA GGA AGA GAT GGA CGT G-3′; L_P: 5′-HS-AAA AAT AGT GAG TTCC-3′; S_P: 5′-biotin-ATATAT TGT CCG TGC TAG AAG-SH-3′. L_P was designed to contain one region (underlined) that is complementary to part of 39 S (underlined). Streptavidin-functionalized MBs (1 μm diameter, 10 mg/mL) were obtained from Invitrogen Co., Ltd. (Oslo, Norway). The streptavidin-HRP (2 mg/mL) was purchased from Boisynthesis Biotech Co., Ltd. (Beijing, China). The solution of 10 mM 3,3′,5,5′-tetramethylbenzidine sulfate (TMB)-50 mM H_2O_2 was purchased from Neogen Corporation (USA). Bovine serum albumin (BSA) was purchased from Sangon Biotech (Shanghai, China). Gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$) and trisodium citrate were obtained from Aladdin Reagent Inc (Shanghai, China). The stock standard solution of 1 ppm UO_2^{2+} was provided by Fujian Environmental Radiation Supervision Station. More dilute standard solutions of UO_2^{2+} were prepared by serially diluting the stock standard solution to the desired concentration with buffer solution. The washing and binding buffer (W&B buffer) used in the experiment is the mixture of 10 mM Tris–HCl, 1 mM EDTA and 2 M NaCl (pH 7.5). The hybridization buffer and cleavage buffer solution used in the experiment is 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) containing 300 mM NaCl (pH 5.5). All reagents are of analytical grade and used without further purification. The sterilized Milli-Q water (18 MΩ/cm) was used in all experiment.

A TU-1950 UV–visible spectrophotometer (Persee, China) was used to obtain the UV–visible spectra, and a Zeiss Sigma scanning electron microscope (Zeiss, Germany) was used to obtain the scanning electron microscopy (SEM) images. The transmission electron microscopy (TEM) images were obtained with a Tecnai G2 F20 transmission electron microscope (FEI, USA).

2.2. Preparation of DNAzyme-functionalized MBs

For immobilizing the DNAzyme on the surface of MBs, firstly, 1 μL of 10 μM biotin-modified substrate strand (39S) and 2 μL of 10 μM enzyme strand (39E) was added into 47 μL of MES buffer solution (pH 5.5). The whole was then annealed by heating it at 85 °C for 2 min and then cooling it to room temperature with a rate of 1 °C/min to form substrate–enzyme complex DNAzyme. Subsequently, 10 μL of streptavidin-functionalized MBs solution (1 mg/mL), which was pre-washed twice with 100 μL W&B buffer (pH 7.5), was added into the DNAzyme solution. The whole was incubated at room temperature for 30 min under full vortex to immobilize DNAzyme on the surface of MBs via the streptavidin–biotin interaction. The resulting DNAzyme-functionalized MBs was separated and washed for three times with 100 μL MES buffer under a magnet, then, the as prepared DNAzyme-functionalized MBs were further treated with 2% BSA for 1 h to block the space site of MBs surface in order to reduce non-specific adsorption. After washing with 100 μL MES buffer under a magnet, the DNAzyme-functionalized MBs were re-suspended in 50 μL MES buffer (pH 5.5) and stored at 5 °C for later use.