Biosensors and Bioelectronics 78 (2016) 73–79

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

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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ARTICLE INFO

Article history:
Received 1 September 2015
Received in revised form
7 November 2015
Accepted 9 November 2015
Available online 12 November 2015

Keywords:
DNAzyme
Uranyl ion
Magnetic beads
Colorimetric biosensor
AuNPs

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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http://dx.doi.org/10.1016/j.bios.2015.11.024
0956-5663/

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

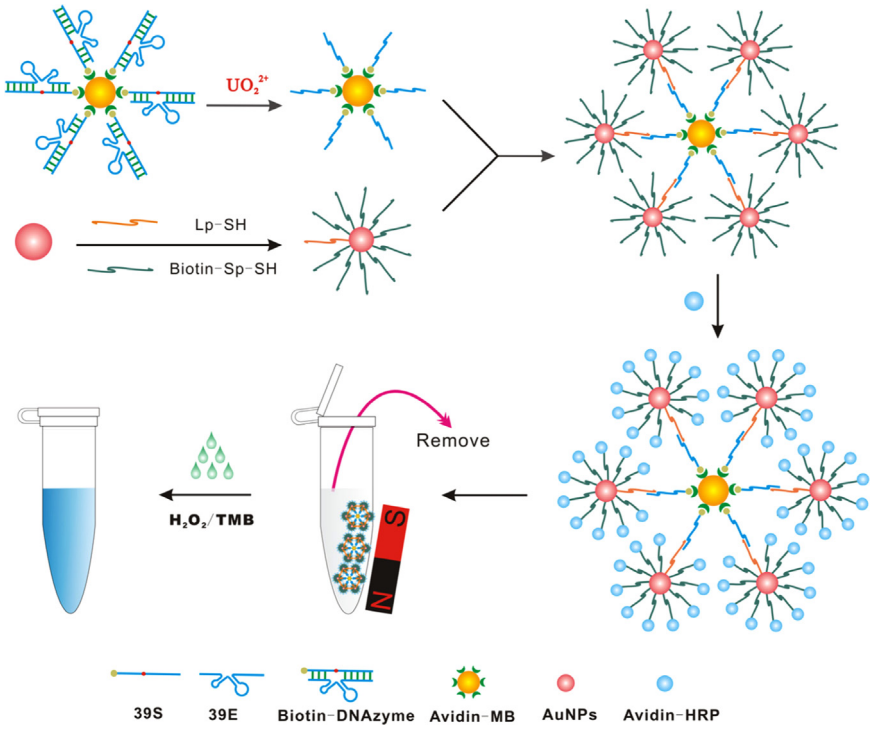
2.1. Apparatus and reagents

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.



Scheme 1. Schematic illustration of the experimental principle for the visual detection of UO_2^{2+} by using DNAzyme-functionalized MBs for UO_2^{2+} recognition and AuNPs-based enzymatic catalysis of TMB for signal generation.

2.3. Preparation of L_P/S_P-modified AuNPs

Firstly, the citrate-stabilized AuNPs (~13 nm) used in this experiment were prepared with the procedure described in previous literature (Hill and Mirkin, 2006; Liu and Lu, 2006). In brief, 3.5 mL of 10 mg/mL trisodium citrate solution was added to 100 mL boiling solution of $HAuCl_4$ (0.01% w/v) under plenty stirring. The solution was then kept in boiling and stirring state for 30 min. After being naturally cooled to room temperature, the resulting AuNPs was stored in 5 °C for the next use. Then, 20 μL of 4 μM L_P, 80 μL of 10 μM S_P and 100 μL of above prepared citrate-stabilized AuNPs were added into 800 μL of 10 mM PBS buffer solution (10 mM PBS-0.1 M NaCl-0.05% Tween-20, pH 7.4). After vortex for 2 min, the mixture was incubated at room temperature for 1 h to immobilize L_P and S_P on the surface of AuNPs via the S-Au interaction. The resulting L_P/S_P-modified AuNPs were further filtered through 10 K MWCO centrifugal filter device by centrifuging at 4000*g* for 30 min, and then were re-dispersed in 1 mL of 10 mM PBS buffer solution (pH 7.4). The final L_P/S_P-modified AuNPs solution was stored in 5 °C for the late use.

2.4. The procedures for detecting UO_2^{2+}

For detecting UO_2^{2+}, firstly, above 20 μL different concentrations of UO_2^{2+} standard solution or sample solution and 10 μL of the as prepared DNAzyme-functionalized MBs solution were added into 20 μL MES buffer solution (pH 5.5). After mild vortex, the whole was incubated at room temperature for 60 min to finish the specific cleavage to release a short single-strand DNA (part of the substrate strand, we called it primer) on the surface of MBs. Subsequently, the MBs were separated and completely washed for 3 times with 100 μL MES buffer solution (pH 5.5) under an external magnet to remove the released substrate fragments and 39E, and 50 μL of the as prepared L_P/S_P-modified AuNPs solution was added. The whole was fully mixed for 3 min and then was incubated at room temperature for 60 min to obtain MBs-L_P-AuNPs-S_P conjugates via the hybridization of L_P and the released primer on the surface of MBs. After washing 3 times with 100 μL W&B buffer solution (pH 7.5), the resulting MBs-L_P-AuNPs-S_P conjugates was collected and re-dispersed in 50 μL of 10 mM PBS buffer solution (pH 7.4), and then 2.5 μL of 0.02 mg/mL streptavidin-functionalized HRP solution was added. The whole was incubated at room temperature for 30 min to tag HRP to MBs-L_P-AuNPs-S_P conjugates via the interaction of streptavidin and biotin. After tagging HRP to MBs-L_P-AuNPs-S_P conjugates, the resulting HRP-tagged MBs-L_P-AuNPs-S_P conjugates was separated by the external magnet and washed carefully with 200 μL 10 mM PBS buffer solution (pH 7.4) for 5 times to completely remove the excess HRP and non-specifically adsorbed HRP. Finally, 300 μL of 10 mM TMB-50 mM H_2O_2 solution was added into the HRP-tagged MBs-L_P-AuNPs-S_P conjugates. After 20 min, the color change of the TMB solution was recorded with a digital camera and the absorption spectrum of the TMB solution was determined in the range of 300–700 nm with a 1 cm path length cell using the UV–visible spectrophotometer. The concentration of UO_2^{2+} was quantified based on the absorption at 650 nm (A_{650}) or naked eye observation.

3. Results and discussions

3.1. The detailed principle of the proposed UO_2^{2+} biosensor

As we mentioned previously, a UO_2^{2+}-specific DNAzymes has been isolated (Brown et al., 2009), and it was reported that HRP can effectively catalyze the H_2O_2-mediated oxidation of TMB to cause the color change from colorless to blue (Zhang et al., 2011). Thus, a novel colorimetric biosensor for the visual and specific detection of trace UO_2^{2+} by using DNAzymes for UO_2^{2+} recognition and AuNPs-based enzymatic catalysis oxidation of TMB for signal generation was designed. As shown in Scheme 1, MBs were utilized to achieve the convenient separation and collection process

for the purpose of reducing the matrix interference in the complex environmental sample. In the MES buffer solution (pH 5.5), the biotin-modified substrate strand (39S) can completely hybridize with enzyme strand (39E) to form UO_2^{2+}-specific substrate–enzyme complex DNAzyme, and the UO_2^{2+}-specific substrate–enzyme complex DNAzyme can be easily immobilized on the surface of MBs via the streptavidin-biotin interaction. In the presence of UO_2^{2+}, the UO_2^{2+} can specifically cleave substrate–enzyme complex DNAzyme to release a short single-strand DNA (primer, the sequence is ATATAT TGT CCG TGC TAG AAG GAA CTC ACT AT) on the surface of MBs. The released primer on the surface of MBs can hybridize with L_P/S_P-modified AuNPs to form MBs-L_P-AuNPs-S_P conjugate since L_P was designed to be complementary to the released primer. The MBs-L_P-AuNPs-S_P conjugate can catch streptavidin-HRP via the biotin modified on the 3′ end of S_P to form HRP-tagged MBs-L_P-AuNPs-S_P conjugate, and the resulting HRP-tagged MBs-L_P-AuNPs-S_P conjugate can effectively catalyze the H_2O_2-mediated oxidation of TMB to give rise to a change in solution color from colorless to blue. This provided a sensitive and specific sensing platform for the simple and field-portable visual detection of trace UO_2^{2+} in aqueous environment.

3.2. Characterization of the proposed UO_2^{2+}biosensor

In order to verify whether the biosensor worked as expected, the UV–visible spectra of TMB solutions and the TEM and SEM images of MBs-L_P-AuNPs-S_P conjugates were investigated in the absence and presence of UO_2^{2+}, respectively. As the results shown in Fig. 1, in the absence of UO_2^{2+} (curve a), the TMB solution was colorless and only has a negligible absorption peak at 650 nm in the UV–visible spectra. On the contrary, in the presence of UO_2^{2+} (curve b), the solution became blue and the absorbance at 650 nm increased dramatically, which indicated that the DNAzyme had been specifically cleaved by UO_2^{2+} to release primer on the surface of MBs, and the released primer on the MBs surface has successfully captured L_P/S_P-modified AuNPs and streptavidin-HRP to form HRP-tagged MBs-L_P-AuNPs-S_P conjugate. The formation of the MBs-L_P-AuNPs-S_P conjugate was also confirmed by the SEM and TEM images (see Fig. 2). As shown in Fig. 2-A and -B, in the absence of UO_2^{2+}, no AuNPs were observed on the surface of DNAzyme-modified MBs after it reacted with L_P/S_P-modified AuNPs; whereas, in the presence of UO_2^{2+}, numerous AuNPs were observed on the surface of DNAzyme-modified MBs after it reacted with L_P/S_P-modified AuNPs (see Fig. 2-C and -D). All above facts suggested that the proposed UO_2^{2+} biosensor indeed worked as our expectation.

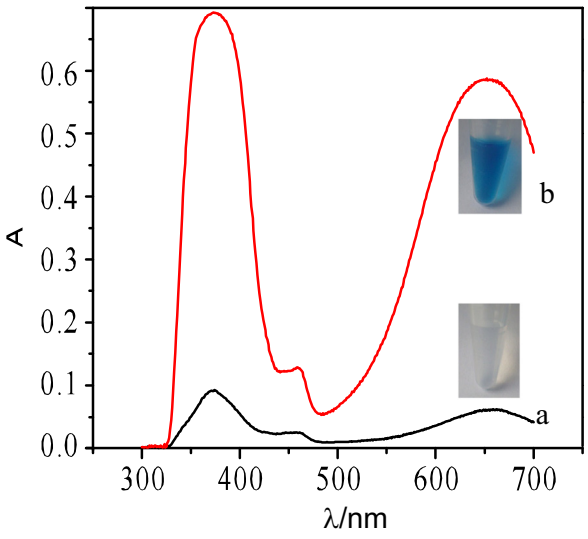


Fig. 1. The UV–visible absorption spectra and photographs of TMB-H_2O_2 solution by detecting blank solution (a) and UO_2^{2+} standard solution (b), respectively. The concentration of UO_2^{2+} is 15 ppb.

3.3. Optimization of the experimental conditions

As we mentioned previously, the specific interaction between DNAzyme and UO_2^{2+} play a vital role in the biosensor. Therefore, several key parameters including the density of DNAzyme immobilized on the surface of MBs, the pH and time of interaction between DNAzyme and UO_2^{2+} was investigated in detail to achieve the best performance of the biosensor. A high density of DNAzyme will lead to a big spatial hindrance and hinder the formation of MBs-L_P-AuNPs-S_P conjugate, and finally result in a low sensitivity. Whereas, a low density of DNAzyme can not provide adequate DNAzyme for the specific cleavage of UO_2^{2+} to form adequate MBs-L_P-AuNPs-S_P conjugate, and finally lead to a low sensitivity too. In this experiment, the density of DNAzyme on the surface of the MBs was controlled by adjusting the concentration of DNAzyme solution, and the results shown in Fig. S1 (see supporting information) clearly indicate that the proposed biosensor has a highest sensitivity when MBs were incubated in 0.2 μM DNAzyme solution.

The effect of pH on the interaction between DNAzyme and UO_2^{2+} was also investigated in the range of 5.0-6.5 in consideration of that the activity of DNAzyme to UO_2^{2+} greatly depends on the pH. As the results shown in Fig. S2 (see supporting information), when the pH = 5.5, DNAzyme has a highest activity to UO_2^{2+}. This result is consistent with that reported previously (Brown et al., 2009). The most possible explanation of this result is that the pH has an impact on the UO_2^{2+} speciation in solution, and pH 5.5 produced a UO_2^{2+} species that is the most effective cofactor for carrying out catalysis of DNAzyme (Brown et al., 2009). If the pH value is higher than 5.5, hydrolyzed species such as $UO_2(OH)^+$ might begin to dominate, which might not be the optimal cofactor for catalysis (Moulin and Decambox, 1995). Therefore, pH 5.5 was chosen as the optimal pH for the detection of UO_2^{2+} in this work.

As a field-portable and on-site detecting method, the rate of interaction between DNAzyme and UO_2^{2+} is another key consideration. The effect of the reaction time on the absorbance at 650 nm was investigated in detail. The results shown in Fig. S3 (see supporting information) revealed that the absorbance increased with the increasing of the reaction time in the range of 10–60 min, and then the absorbance tended to level off when the time is longer than 60 min, indicating that the reaction between DNAzyme and UO_2^{2+} has completed within 60 min. To save analytical time, 60 min was chosen as the optimal reaction time.

In this study, L_P/S_P-modified AuNPs was used to tag HRP for generating and amplifying signal. In order to obtain the best sensitivity, the ratio of L_P/S_P modified on the surface of AuNPs was optimized in the range of 10:1 to 1:20. As exhibited in Fig. S4 (see supporting information), the optimal ratio of L_P to S_P was L_P: S_P = 1:10. Inadequate L_P will affect the formation of MBs-L_P-AuNPs-S_P conjugates, and finally reduce the sensitivity. Whereas, the excess L_P will decrease the amount of S_P, and thus tag less HRP, which resulting in a lower sensitivity too.

The hybridization between primer-modified MBs and L_P/S_P-modified AuNPs directly affects the formation of MBs-L_P-AuNPs-S_P conjugate, and finally affect the sensitivity of the biosensor. The effect of temperature, which used for the hybridization of primer-modified MBs and L_P/S_P-modified AuNPs, on the sensitivity of biosensor was investigated in the range from room temperature to 37 °C in detail. The experimental results showed that the biosensor has a stable and similar sensitivity

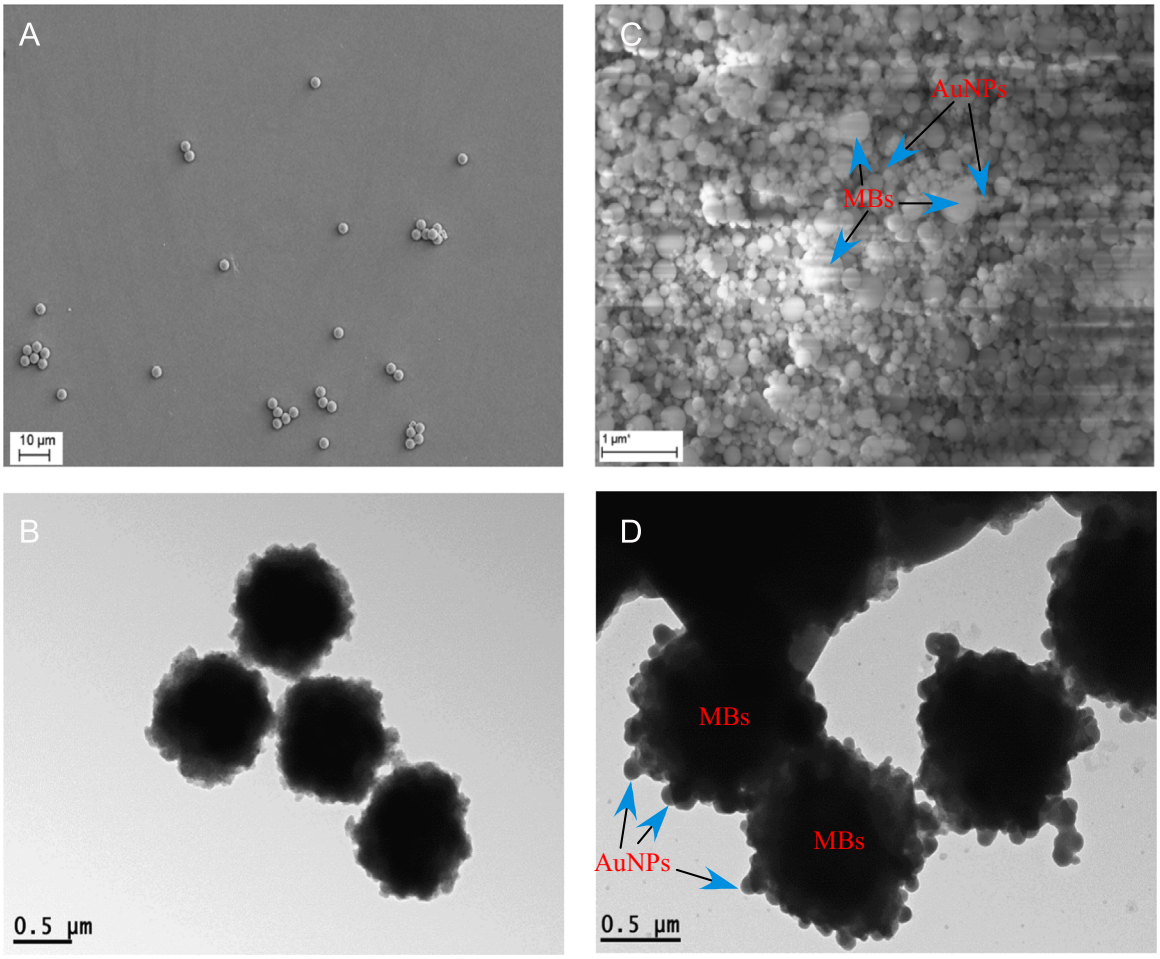


Fig. 2. The SEM image (A) and TEM image (B) of DNAzyme-functionalized MBs after it reacted with L_P/S_P-modified AuNPs in the absence of UO_2^{2+}. The SEM image (C) and TEM image (D) of DNAzyme-functionalized MBs after it reacted with L_P/S_P-modified AuNPs in the presence of UO_2^{2+}.

when the hybridization of primer-modified MBs and L_P/S_P-modified AuNPs was carried out in the range from room temperature to 37 °C, since L_P has been designed to have a melting temperature of about 40 °C with the primer (see Fig. S5 in supporting information). Therefore, the hybridization of primer-modified MBs and L_P/S_P-modified AuNPs was carried out at room temperature.

3.4. The selectivity of the proposed biosensor

It is possible that the existence of various metallic ions in aqueous environment will interfere with the detection of UO_2^{2+}. In order to evaluate the selectivity of our method, we challenged the other environmentally relevant metal ions including Ba^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, Cr^{3+}, Mg^{2+}, Cu^{2+}, Fe^{3+}, Ag^{+}, Pb^{2+}, Ni^{2+}, Hg^{2+}, La^{3+} and Lu^{3+} in 1000-fold concentration vs. UO_2^{2+}. In the detection of Ag^{+}, we replaced the original buffer solution (50 mM MES-300mM NaCl, pH 5.5) with the buffer solution of 50 mM MES-300mM $NaNO_3$ (pH 5.5) in order to avoid the generation of AgCl precipitation, since it is demonstrated that the specific cleavage of UO_2^{2+} to DNAzyme can also be completed successfully in 50 mM MES-300 mM $NaNO_3$ (pH 5.5) buffer solution (Brown et al., 2009). As the results shown in Fig. 3, only UO_2^{2+} can specifically cleave DNAzyme to release primer on the surface of MBs to form HRP-tagged MBs-L_P-AuNPs-S_P conjugate, which can catalyze the H_2O_2-mediated oxidation of TMB to give rise to apparent change in solution color from colorless to blue and a high absorbance at 650 nm. No color change and only a negligible absorbance at 650 nm was observed for other metallic ions, even if their concentrations were 1000-folds higher than that of UO_2^{2+}, indicating that other metallic ions can not cleave DNAzyme and thus do not interfere with the detection of UO_2^{2+}. The experimental results revealed that the proposed biosensor, which is based on the specific DNAzyme and AuNPs-based enzymatic catalysis

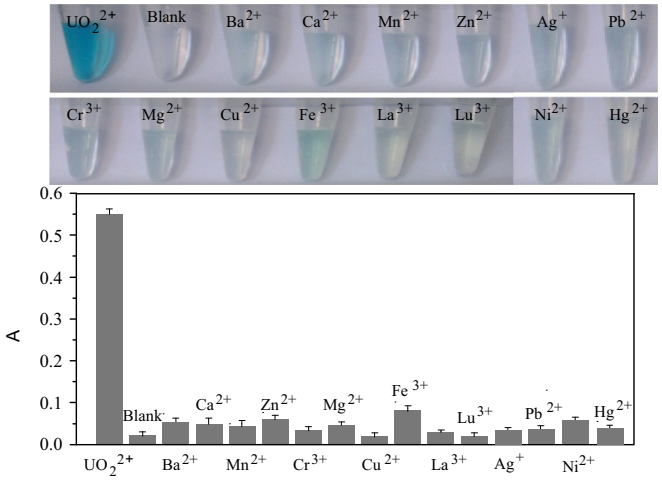


Fig. 3. The photographs and absorption at 650 nm of TMB-H_2O_2 solution by detecting various metal ions. The concentration of UO_2^{2+} is 15 ppb, and the concentrations of other ions are all 15 ppm.

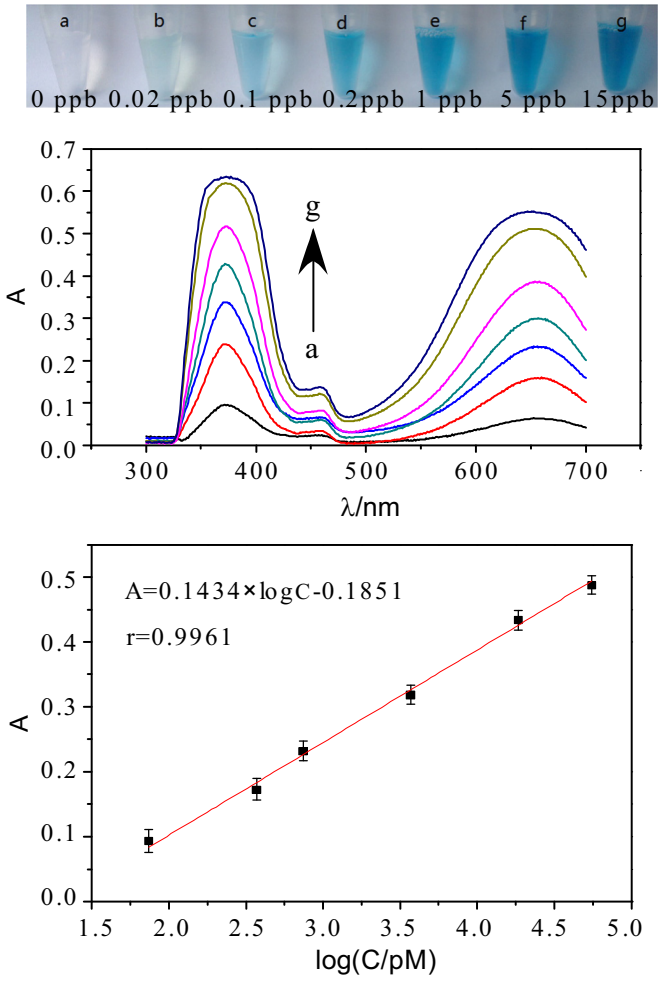


Fig. 4. The photographs, absorption spectra and calibration curve for detecting UO_2^{2+}. The concentrations of UO_2^{2+} are 0, 0.02, 0.10, 0.20, 1.00, 5.00 and 15.0 ppb.

amplification, has excellent specificity.

3.5. The linear range, sensitivity and reproducibility of the proposed biosensor

Under the previously discussed optimum conditions, the color change and absorption spectra of the H_2O_2-TMB solution induced by UO_2^{2+} was monitored by UV–visible spectroscopy to obtain calibration curve. As the results shown in Fig. 4, upon the addition of UO_2^{2+} from 0.02 to 15 ppb (74 pM–56 nM), the absorbance of the solution at 650 nm increased obviously. At the same time, the color of the solution changed from colorless to deep blue step by step. As the photographs in Fig. 4 show, the color change could be observed with the naked eye when the concentration of UO_2^{2+} was 0.02 ppb, i.e., the visual detection limit of UO_2^{2+} was about 0.02 ppb (74 pM). The absorption at 650 nm (A_{650}) showed a good linear relationship with the concentrations of UO_2^{2+} in the range of 0.02 to 15 ppb (74 pM–56 nM). The equation was $A_{650} = 0.1434 \times \log C - 0.1851$ ($r = 0.9961$), where C is the concentration of UO_2^{2+} (pM). The detection limit ($3\sigma/S$) was calculated to be 7.0 pM for UO_2^{2+} and the relative standard deviation (RSD, $n = 5$) was calculated to be less than 6% for the detection of 50 pM UO_2^{2+}.

As we mentioned above, to date, several biosensors that based on UO_2^{2+}-specific DNAzyme including fluorescent sensors (Liu et al., 2007; Xiao et al., 2015; Zhang et al., 2015a, 2015b), colorimetric sensors (Lee et al., 2008), magnetoelastic sensors (Yin et al., 2013) and resonance light scattering sensors (Zhou et al., 2013) have been developed for detecting UO_2^{2+}. As shown in Table S1 of supporting information, compared to the previous fluorescent sensors, the proposed biosensor is more simple and cost-effective because there is no need to label expensive fluorescent group and no need to use spectrometer. In comparison with previous distance-dependent colorimetric sensors, magnetoelastic sensors and RLS sensors, the proposed biosensor has outstanding resistance to the matrix and higher sensitivity. The sensitivity of the proposed method was approximately three orders of magnitude higher than that of AuNPs distance-based colorimetric methods although the proposed method needs a longer analysis time (Lee et al., 2008). The higher sensitivity of our method is due to the ternary amplification effect of the MBs-L_P-AuNPs-S_P. Moreover, the visual detection limit of our method is also 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 convenient and on-site detection of UO_2^{2+} in the aqueous environment by only naked-eye observation.

3.6. Analysis of UO_2^{2+} in river water samples

To further investigate the applicability and reliability of the developed biosensor, we challenged the river water samples collected from Minjiang river, Fujian of China. The river water was firstly filtered through a 0.22 μm membrane filter to remove any sediment, and then the amount of UO_2^{2+} in the filtered water was directly determined with our method by naked-eye observation and UV–visible spectrometry, respectively. The results obtained were compared with the results obtained by ICP-MS, a traditional method for UO_2^{2+} determination. As results shown in Table 1, the UO_2^{2+} in the river water can be easily detected with our method by only naked-eye observation, with a concentration of about 0.2 ppb. The UO_2^{2+} in the river water obtained with our method by UV–visible spectrometry is 0.22 ppb with a recovery of 93–99% and a RSD ≤ 5.0% ($n = 6$). The results obtained with our method were consistent with those detected by ICP-MS, indicating that the proposed biosensor is reliable and can be applied to practical

Table 1
The analytical results of UO_2^{2+} in water samples collected from Minjiang river, Fujian of China.

Sample	UO_2^{2+} added (ppb)	Color	UO_2^{2+} detected (ppb)	Recovery (%)	RSD ($n = 6$, %)	UO_2^{2+} detected with ICP-MS
1	0.0		0.22	–	4	0.21 ppb
2	1.0		1.15	93	5	1.13 ppb
3	5.0		5.17	99	4	5.11 ppb

detection of UO_2^{2+} ions in aqueous environment. The success of this study provides a promising approach for simple and on-site detection of UO_2^{2+} ions in aqueous environment by only naked-eye observation.

4. Conclusions

In summary, we herein developed a sensitive and selective biosensor for the visual detection of trace UO_2^{2+} in aqueous environment by using DNAzyme-functionalized MBs for UO_2^{2+} recognition and AuNPs-based enzymatic catalysis of TMB for signal generation. The utilization of MBs facilitated the magnetic separation and collection of sensing system from complex sample solution, and finally considerably reduced the matrix interference and simplified the experimental operation. The utilization of AuNPs-based enzymatic catalysis amplification greatly improved the sensitivity of the biosensor. The proposed biosensor 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 our method is also 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). Above features, as well as its fabrication easiness, operation convenience and low cost, make our sensor a promising approach for the simple and on-site detection of trace UO_2^{2+} in aqueous environments by only naked-eye observation.

Acknowledgments

The authors gratefully acknowledge the NSFC (21377025, 81202914), Ministry of Science and Technology for the People's Republic of China (2011DFA31770), Ministry of Education, Science and Technology Development Center (20123514110001), Fujian Provincial Department of Science and Technology (2014Y0042), and National Key Technologies R & D Program of China during the 12th Five-Year Plan Period (NO. 2012BAD29B06) for financial support.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.11.024.

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