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Magnetic beads-based DNA hybridization chain reaction amplification and DNAzyme recognition for colorimetric detection of uranyl ion in seafood

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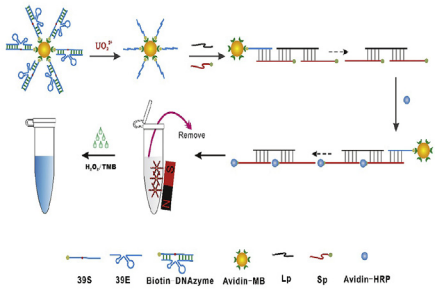
HIGHLIGHTS

- A visual biosensor for sensitive and specific detection of trace UO_2^{2+} in seafood was developed.
- All detecting procedures of the biosensor were carried out at room temperature.
- The biosensor can be used to detect as low as 2.5 ppb UO_2^{2+} in seafood by naked-eye observation.
- 1000-folds of other environmentally relevant metal ions do not interfere with the detection of UO_2^{2+}.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel colorimetric biosensor, which employs DNAzyme-functionalized magnetic beads (MBs) as recognition probe, enzyme-assisted catalytic oxidation of TMB (3,3′,5,5′-tetramethylbenzidine sulfate) as signal and DNA hybridization chain reaction as amplification strategy, has been developed for detecting trace uranyl ion (UO_2^{2+}) in seafood and aqueous environment with high sensitivity and specificity. We demonstrated that UO_2^{2+} can specifically cleave DNAzyme immobilized on MBs surface to release a short single-strand DNA (primer), and the released primer trigger DNA hybridization chain reaction to form a long one dimensional DNA concatamer on the MBs surface. The resulting long DNA concatamer could capture a large amount of HRP to generate the one UO_2^{2+}-to-multiple HRP amplification effect. Upon the addition of TMB-H_2O_2 solution, the HRP-tagged DNA concatamer-MBs conjugates could catalyze the H_2O_2-mediated oxidation of TMB, and thus results in a color change from colorless to blue in solution. This provided a sensitive and selective sensing platform for the visual or colorimetric detection of UO_2^{2+}. The proposed biosensor has high sensitivity and strong anti-interference capability, it can be used to detect as low as 2.5 ppb (9.25 nM) of UO_2^{2+} by naked-eye observation and 0.09 ppb (0.33 nM) of UO_2^{2+} by UV-visible spectrometry with no interference of other ions and a RSD $\leq$ 6% (n = 5). With the help of this method, we have successfully determined trace UO_2^{2+} in fish muscle and river water with a recovery of 93 −106%. High sensitivity and specificity, as well operation convenience, low cost and strong resistibility to the matrix, which makes our method a potential approach for the on-site detection of UO_2^{2+} in seafood and aqueous environment.

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1. Introduction

At present, human is facing the unprecedented energy crisis since the traditional and non-renewable fossil fuels such as oil and coal are gradually consumed. The nuclear energy is increasingly developed to resolve this crisis. Therefore, uranium, as one of the main fuels of nuclear energy, has been increasingly used in nuclear industry, such as nuclear power station and nuclear weapons etc [1]. However, the utilization of uranium minerals also brought uranium pollution in environment, and the pollution of uranium in environment will harm the mammalian reproduction and development in the long term since uranium not only has radioactive toxicity and chemical genotoxicity but also has a long half-life [2,3]. Although uranium may exist in the form of +3, +4, +5 or +6, the most stable species of uranium existing in the seafood, mammalian body and aqueous environment is uranyl ion (UO_2^{2+}) [4,5]. For above reasons, it is thus significant to establish a simple and convenient approach for the rapid and on-site determination of UO_2^{2+} in the seafood and aqueous environment, in order to prevent human being hurt by UO_2^{2+}.

The traditional UO_2^{2+} detection techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), laser-atomic absorption spectrometry, energy dispersive X-ray fluorescence spectrometry and laser-induced kinetic phosphorimetry etc [6–9], have relatively high sensitivity and good accuracy. However, most of these techniques required costly device and/or tedious and laborious pre-treatment, which makes the rapid and on-site detection impossible [10]. Therefore, the development of novel methods for the cost-effective, rapid, convenient and on-site detection of trace UO_2^{2+} in the seafood and aqueous environment will be beneficial for minimizing uranium damage. For this purpose, various chemical sensors and biosensors, especially DNAzyme-based biosensors, have been fabricated for the rapid and portable detection of UO_2^{2+} since UO_2^{2+}-specific DNAzymes was reported [11–22]. Among these biosensors, the fluorescent DNAzyme-based UO_2^{2+} biosensors have relatively poor anti-interference ability and required a relatively costly fluorescent spectrometer, which make it did not meet the requirement of the rapid and on-site detection of trace UO_2^{2+} in seafood. The previous colorimetric biosensors, which based on UO_2^{2+}-specific DNAzyme and distance-dependence optical characteristic of gold nano-particles (AuNPs), are rapid, portable, low-cost and equipment-free, but they suffer from lower visual detection limit and widespread matrix interference in complex and/or colored samples [20,21,23]. Not long ago, a sensitive and selective visual method for the detection of trace UO_2^{2+} in water sample was reported by using magnetic beads (MBs)-based UO_2^{2+}-specific DNAzyme as recognition molecular and AuNPs-based enzymatic catalysis/oxidation of TMB as signal [24]. The method has high visual sensitivity and better specificity, but it needs a relatively complex operation process, and several single or double labeled DNA, MBs and AuNPs. Therefore, it is still a significant challenge to design a simpler, more portable and cost-effective visual method with strong disturbance resistibility for the sensitive, selective and on-site detection of trace UO_2^{2+} in complex samples such as seafood.

DNA hybridization chain reaction (HCR) is a simple and effective amplification biotechnology. In comparison with other amplification biotechnologies such as polymerase chain reaction (PCR), rolling circle amplification (RCA) and so on [25–30], it has obvious advantages such as no requirement of nuclease and no false-positive results. More importantly, the HCR of two auxiliary probes can be carried out in the long-range to form a long one dimensional DNA concatamer under the mild conditions via the rigorous and delicate design [31]. For above reasons, in recent years, it has been well used for signal amplification to further improve the sensitivity of biosensors by adjusting the length of DNA in situ [31,32]. In this study, we developed a sensitive and selective colorimetric biosensor with strong disturbance resistibility for trace UO_2^{2+} detection by taking the advantage of MBs, UO_2^{2+}-specific DNAzyme and DNA hybridization chain reaction amplification, hoping to provide a reliable and stable visual method for the on-site detection of trace UO_2^{2+} in seafood and water by only naked-eye observation.

2. Experimental

2.1. Materials and instruments

All oligonucleotides used in the experiment were obtained from Takara Bio Inc. (Dalian, China), and their base sequences were as follow: the substrate strand of UO_2^{2+}-specific DNAzyme (39S): 5'-biotin-ATATAT TGT CCG TGC *TAG AAG GAA CTC ACT AT* rA GGA AGA GAT GGA CGT G-3'; the enzyme strand of UO_2^{2+}-specific DNAzyme (39E): 5′-CAC GTC CAT CTC TGC AGT CGG GTA GTT AAA CCG ACC TTC AGA CAT AGT GAG TTC CTT CTA-3'; link probe (L_p): 5′-**TAC CGT CAG CCT A**AC AT CAT CAG TAG AAG GAA CTC AC-3' (the sequences with underline and black font match the underlined and black font sequences of S_p, respectively); signal probe (S_p): 5'-biotin- C**T AGG CTG ACG GTA** GAA CAT CAT C*AT AGT GAG TTC CTT CTA*-3' (italicized part is complementary to the italic parts of 39S). The buffer solutions used in the experiment are as follow: the washing and binding buffer (W&B buffer) is the mixture of 10 mM Tris–HCl, 1 mM EDTA and 2 M NaCl (pH 7.5); the hybridization buffer is 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) containing 900 mM NaCl (pH 5.5); the immobilizing buffer is 10 mM PBS (pH 7.4).

Other reagents and apparatus used in the experiments were showed in the supplementary information.

2.2. Fabrication of the colorimetric biosensor and the detection of UO_2^{2+}

For fabricating DNAzyme-based colorimetric biosensor of UO_2^{2+}, in first, 1 μL of 2 μM biotin-modified substrate strand (39S), 1 μL of 8 μM enzyme strand (39E) and 16 μL of hybridization buffer solution (pH 5.5) were mixed together in a vial. Then, the 39S and 39E were annealed by warming the mixture at 90 °C for 5 min and subsequently cooling to room temperature with a speed of 1 °C/min to form UO_2^{2+}-specific DNAzyme. Secondly, 2 μL of 1 mg/mL magnetic beads (MBs) dispersed suspension, which was pre-washed with W&B buffer (pH 7.5) for twice, was added into the above UO_2^{2+}-specific DNAzyme solution, and the mixture was kept at gentle shaking for 30 min under room temperature to immobilize UO_2^{2+}-specific DNAzyme on the MBs surface through the interaction between streptavidin and biotin. The resulting DNAzyme-modified MBs were collected with an external magnet, and were washed twice with hybridization buffer to remove all excess DNAzymes and 39E. Subsequently, the DNAzyme-modified MBs were incubated in 3% bovine serum albumin (BSA) solution for 30 min at room temperature to block the space site of MBs surface. After thorough washing, the DNAzyme-modified MBs were re-suspended in 20 μL of hybridization buffer and stored at 0–4 °C for later use.

For detecting UO_2^{2+}, 10 μL of UO_2^{2+} standard or sample solutions were added into 20 μL of above prepared DNAzyme-modified MBs suspension in a vial. After gentle vortex, the mixture was incubated at room temperature for 15 min to finish the specific cleavage. After magnetic separation and complete washing for 3 times with 100 μL of hybridization buffer, a short single-strand DNA (italicized part of 39S, we called it primer) was released on the surface of MBs. Then, 16 μL of hybridization buffer, 2 μL of 100 μM Sp and 2 μL of 100 μM

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Lp probe were added into the vial, and the whole was incubated for 2.5 h at room temperature to perform DNA hybridization chain reaction. After the separation and washing with hybridization buffer, 2 μL of 0.02 mg/mL streptavidin-HRP solution and 48 μL of immobilizing buffer were added, and the whole was continuously incubated for 20 min at room temperature to tag HRP on the DNA concatamer via the interaction of streptavidin-HRP and biotin on the S_p. Finally, the HRP-tagged DNA concatamer-MBs conjugates were separated with an external magnet and washed carefully with 200 μL of immobilizing buffer for five times, then, 300 μL of 10 mM TMB-50 mM H_2O_2 solution was added into the HRP-tagged DNA concatamer-MBs conjugates. After reacting for 15 min, the color of the TMB solution was captured with digital camera and the absorption spectrum of the solution was measured with spectrophotometer from 470 to 770 nm. The amount of UO_2^{2+} was calculated on the basis of absorption at 650 nm (A_{650}) or the solution color observed by naked-eye observation.

2.3. Determination of fish muscle and natural water

The UO_2^{2+} concentration in the natural water was directly detected with above method after the water sample was filtered through a 0.22 μm membrane filter to remove any sediment. For the fish muscle sample, the UO_2^{2+} in about 4.0 mg of dried fish muscle was firstly extracted with 500 μL of Milli-Q H_2O for two times, then the extracting solution was filtered through 0.22 μm membrane filter to remove any sediment. The filtrate was collected and used for the determination of UO_2^{2+} with above method.

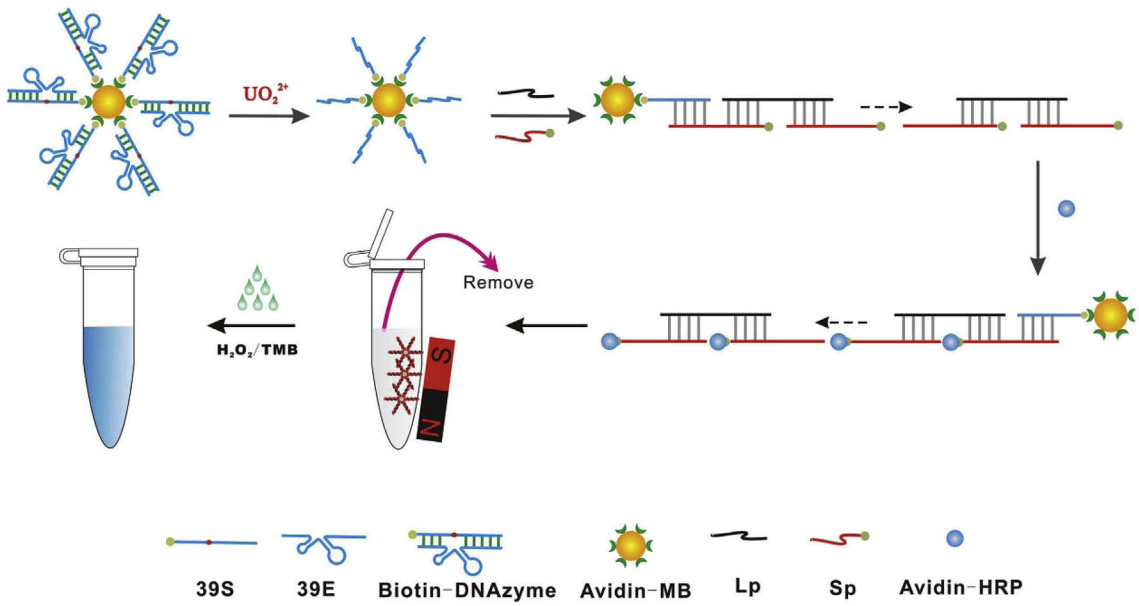
3. Results and discussions

3.1. The designing principle of the colorimetric biosensor

The detailed principle of the proposed colorimetric biosensor was illustrated in Scheme 1. In the design, MBs were utilized to achieve the convenient separation and collection for the purpose of reducing the matrix interference in the complex sample, and the UO_2^{2+}-specific DNAzymes were immobilized on the MBs surface for recognizing the target and triggering the DNA hybridization chain reaction amplification. As Scheme 1 showed, the link probe (L_p) and signal probe (S_p) was designed to be able to alternately hybridize (the underlined segment at the 3′ end of L_p can hybridize with the underlined sequences of S_p, the bold segment at the 5′ end of L_p can hybridize with the bold part of S_p), and S_p was also designed to be able to hybridize with part of 39S (the italicized segment at the 3′ end of S_p can hybridize with the italicized part of 39S). Thus, in the presence of UO_2^{2+}, the substrate-enzyme complex DNAzyme, which was immobilized on the MBs surface, could be specifically cleaved into two DNA fragments at the ribonucleotide site by UO_2^{2+}. After magnetic separation and washing, the short cleaved substrate strand fragments from 39 s at 5′ end (italicized part, we called it as primer) was left on the MBs surface. The released primer on the MBs surface will trigger DNA hybridization chain reaction to form a long one dimensional DNA concatamer, which analogous to alternating copolymers, in the presence of L_p and S_p. The resulting long DNA concatamer, which linked onto the MBs surface, could capture a large amount of HRP by the interaction between streptavidin-HRP and biotin on the S_p, and such generate the one UO_2^{2+}-to-multiple HRP amplification effect. Upon the addition of TMB-H_2O_2 solution, the HRP-tagged DNA concatamer-MBs conjugates could catalyze the H_2O_2-mediated oxidation of TMB, and thus results in a color change from colorless to blue in solution. This provided a sensitive and selective sensing platform for the visual or colorimetric detection of UO_2^{2+}.

3.2. Feasibility characterization of the proposed UO_2^{2+} colorimetric biosensor

To demonstrate the feasibility of our design, the UV–visible absorption spectrum of the sensing system under different conditions were investigated in detail. As depicted in Fig. 1, in the absence of UO_2^{2+} (curve a), an inappreciable absorption at 650 nm was observed on the absorption spectrum and the solution is almost colorless correspondingly. This result might be attributed to that the DNAzyme immobilizing on the MBs surface was not cleaved in the absence of UO_2^{2+}, and the DNA hybridization chain reaction was not triggered. Whereas, in the presence of UO_2^{2+} (curve c), the absorption at 650 nm increase remarkably and the solution



Scheme 1. Schematic illustration of the experimental principle of the colorimetric biosensor.

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color changed to blue obviously. This result demonstrated that the DNAzyme immobilizing on the MBs surface has been successfully cleaved by UO_2^{2+} to release primer on the MBs surface, and the DNA hybridization chain reaction of L_p and S_p has been triggered to form a long one dimensional DNA concatamer on the MBs surface, which leads to a large amount of HRP was captured on the MBs surface. Furthermore, as a control, the assay was performed without the introduction of L_p into the system but in the presence of UO_2^{2+} (curve b). In this case, the absorption at 650 nm only rise slightly in comparison with curve a. It is because that DNA hybridization chain reaction can not be formed without L_p and correspondingly only a few HRP was carried onto MBs, although the recognition and cleavage of DNAzyme was conducted.

To further confirm the formation of the DNA hybridization chain reaction, the gel electrophoresis assay was used to characterize the obtained DNA concatamer. As the results shown in Fig. 2, compared with the length of un-reacted S_p (lane 5, 30 bp), the DNA concatamer obtained with 2.5 μM of S_p and L_p has a longer length (up to 500 pb) together with less short strands by-products (lane 2). The length of the DNA concatamer obtained with 10 μM of S_p and L_p (lane 3) increased more markedly (up to 1000 pb), indicating that the DNA hybridization chain reaction was performed as our expectation and a remarkable signal amplification effect was achieved.

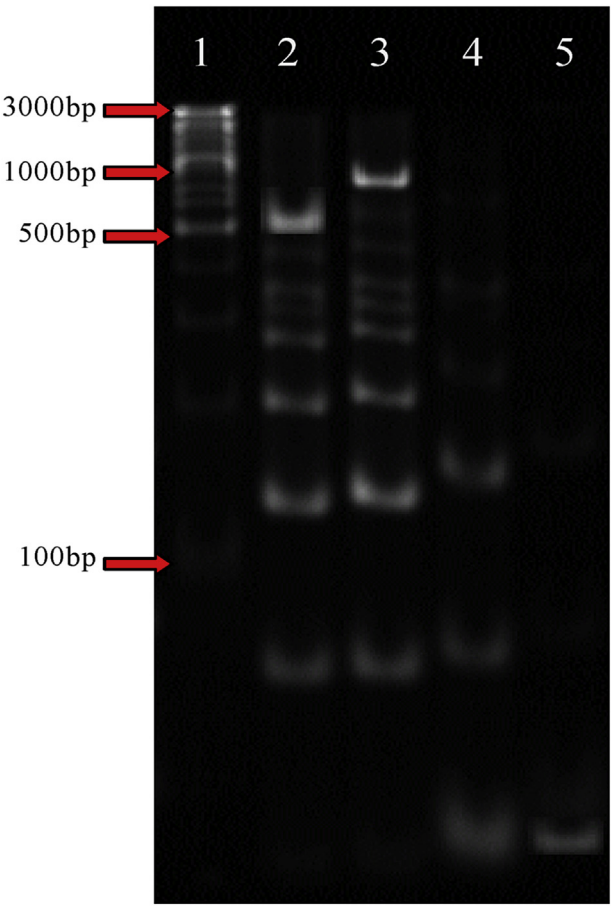

Fig. 2. The image of gel electrophoresis experiment. Lane 1: Markers; Lane 2: the DNA hybridization chain reaction products obtained with 2.5 μM of S_p and L_p; Lane 3: the DNA hybridization chain reaction products obtained with 10 μM of S_p and L_p. Lane 4: the DNA hybridization chain reaction products obtained with only 10 μM of S_p; Lane 5: link probe (L_p). Image was obtained by using 0.8% agarose gels and TBE buffer (0.04 M Tris, 0.04 M H_3BO_3, 1 mM EDTA, pH 8.0).

3.3. Optimization of experimental conditions

To obtain the best analytical performance, the absorbance at 650 nm (A_{650}) of the proposed biosensor was monitored under different conditions. As we mentioned above, the specific interaction between DNAzyme and UO_2^{2+} play a crucial role in the biosensor. In the previous work [17,24], we have repeatedly demonstrated that the DNAzyme has a greatest activity to UO_2^{2+} under pH 5.5 since the pH of solution has an impact on the uranyl ion speciation. Therefore, two other vital parameters including the density of DNAzyme immobilized on the MBs surface and the interacting time between DNAzyme and UO_2^{2+} was investigated in detail under pH 5.5. High-density of DNAzyme on the MBs surface will hinder the DNA hybridization chain reaction due to the spatial hindrance, and finally leads to a low sensitivity. Whereas, low-density of DNAzyme on the MBs surface can not provide adequate DNAzyme for the specific cleavage of UO_2^{2+} to release adequate primer on MBs surface to obtain massive DNA concatamers after hybridization chain reaction, and finally result in a low sensitivity too. In the experiment, the density of DNAzyme on the MBs surface was adjusted by controlling the concentration of DNAzyme solution. As seen in Fig. S1 (see supplementary information), the proposed biosensor has a highest sensitivity when MBs were incubated in 0.1 μM DNAzyme solution.

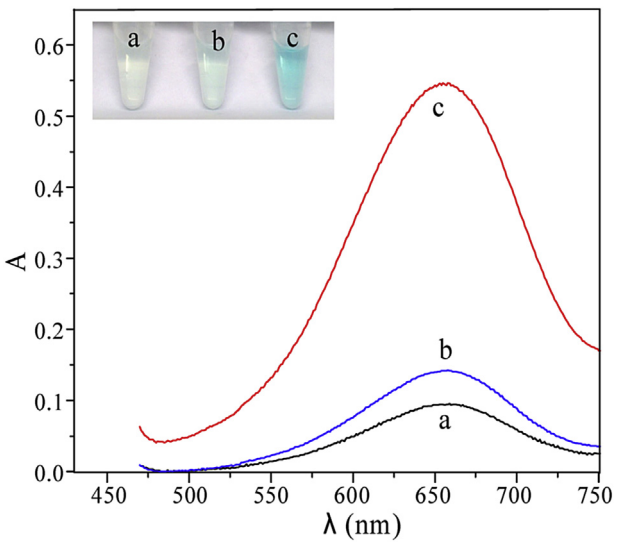

Fig. 1. The UV-visible absorption spectra and photographs of TMB-H_2O_2 solution by detecting blank solution (a), UO_2^{2+} standard solution without the introduction of L_p (b) and UO_2^{2+} standard solution in the presence of both L_p and S_p, respectively. The concentration of UO_2^{2+} is 15 ppb.

As a visual biosensor for the on-site detection of trace UO_2^{2+}, the interacting time between DNAzyme and UO_2^{2+} is another key consideration. The results shown in Fig. S2 (see supplementary information) revealed that the absorbance at 650 nm (A_{650}) continued to enhance with the increasing of the reaction time during 5–10 min, and then the absorbance kept on a plateau when the time is longer than 10 min, indicating that the interaction between DNAzyme and UO_2^{2+} has completed within 10 min. To save analytical time, 15 min was chosen as the optimal reaction time in this work.

The time of the DNA hybridization chain reaction directly affect the length of the obtained DNA concatamer, which will influence the amount of tagging HRP and thus influence the sensitivity of the biosensor. The hybridization time was optimized in detail in the range of 30–180 min, and the results shown in Fig. S3 (see

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supplementary information) indicated that the sensitivity of the biosensor increase with the increasing of hybridization time when the time is less than 150 min. However, the sensitivity did not increase obviously when the hybridization time is longer than 150 min. By considering the analytical time, the DNA hybridization chain reaction was carried out for 150 min in this study.

In this proposal, the amplified signal mainly derives from the HRP tagged on the DNA concatamer, which was obtained by the hybridization chain reaction with L_p and S_p probes. Therefore, the length of the obtained DNA concatamer directly affects the amount of tagging HRP, and thus affected the final sensitivity of the biosensor. According to the previous literature [31], the length of the DNA concatamer obtained with the hybridization chain reaction can be controlled by changing the concentration of L_p and S_p probes. Results in Fig. S4 (see supplementary information) showed the effect of the hybridization chain reaction on the sensitivity of the biosensor under various concentrations of L_p and S_p. It was observed that the sensitivity increased significantly when the L_p and S_p concentrations increased from 0 to 10.0 μM. However, the sensitivity of the biosensor did not further increase significantly when the concentrations of L_p and S_p further increased from 10.0 μM to 15.0 μM. Therefore, 10.0 μM was chosen as the optimum L_p and S_p concentration for the hybridization chain reaction.

3.4. The selectivity of the UO_2^{2+} colorimetric biosensor

In general, it is possible that the existence of various metallic ions in seafood and aqueous environment will interfere with the detection of UO_2^{2+}. To test the selectivity of the proposed method, we challenged with various environmentally relevant metal ions including Na^+, K^+, Ba^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, Cr^{3+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, La^{3+}, Lu^{3+} and Th^{3+} in 1000-fold concentration versus UO_2^{2+}. As results shown in Fig. 3, only UO_2^{2+} can recognize and cleave DNAzyme to release primer on the MBs surface, and thus trigger the hybridization chain reaction of L_p and S_p to form a long one dimensional DNA concatamer to capture massive HRP, which gives rise to an apparent color change in TMB/H_2O_2 solution and a high absorbance at 650 nm. For other metallic ions, a negligible absorbance at 650 nm and no color change was observed even if their concentrations were 1000-fold higher than that of UO_2^{2+}, indicating that the proposed method possesses an excellent specificity and anti-interference capability. Such an outstanding specificity and anti-interference capability should be attributed to the utilization of UO_2^{2+}-specific DNAzyme and MBs-based separation in this proposal.

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

Under above optimized conditions, the UV-visible absorption spectra and the color change of TMB/H_2O_2 solution was monitored by detecting various concentrations of UO_2^{2+} (0.5, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 ppb) with the proposed method. The relationship between the color change/absorbance and UO_2^{2+} concentrations was shown in Fig. 4. It was apparent that there was a light-to-dark color change when the concentration of UO_2^{2+} increased from 0.5 to 15.0 ppb. In addition, the color change could be clearly observed with the naked eyes when the concentration of UO_2^{2+} is 2.5 ppb (9.25 nM), i.e., the visual detection limit of UO_2^{2+} was about 2.5 ppb. The absorbance at 650 nm (A_{650}) showed a good linear correlation with the concentration of UO_2^{2+} in the range from 0.5 to 15.0 ppb. The regression equation was $A = 0.034 \times C + 0.014$ ($R^2 = 0.9965$), where C is the concentration of UO_2^{2+} (ppb). The detection limit ($3\sigma/S$) for UO_2^{2+} was calculated to be 0.09 ppb, and the relative standard deviation (RSD, n = 5) was calculated to be less than 6% for the

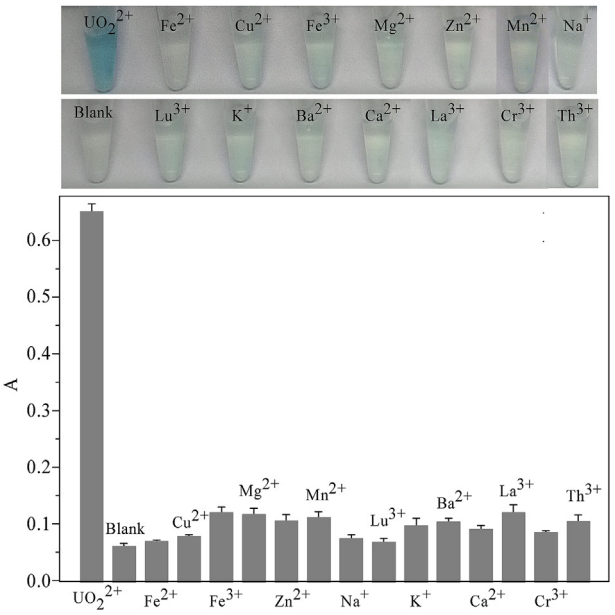

Fig. 3. The photographs and absorption at 650 nm of TMB-H_2O_2 solution by detecting various metallic ions. The concentration of UO_2^{2+} is 15 ppb, and the concentrations of other ions are all 15 ppm.

detection of 2.5 ppb UO_2^{2+}. The higher sensitivity of this method should mainly attribute to the amplification effect of the DNA hybridization chain reaction. Even a small number of DNAzyme was recognized and cleaved by UO_2^{2+}, the released primer on the MBs surface could catch a large quantity of HRP by the interleaving embedded S_p in the long DNA concatamer, and the high catalytic efficiency of HRP produced a dramatic color change of the solution and enhanced detection sensitivity. To date, several biosensors based on UO_2^{2+}-specific DNAzyme including fluorescent sensor [16–19], colorimetric sensor [20,24], magnetoelastic sensor [10], and resonance light scattering sensor have been developed for

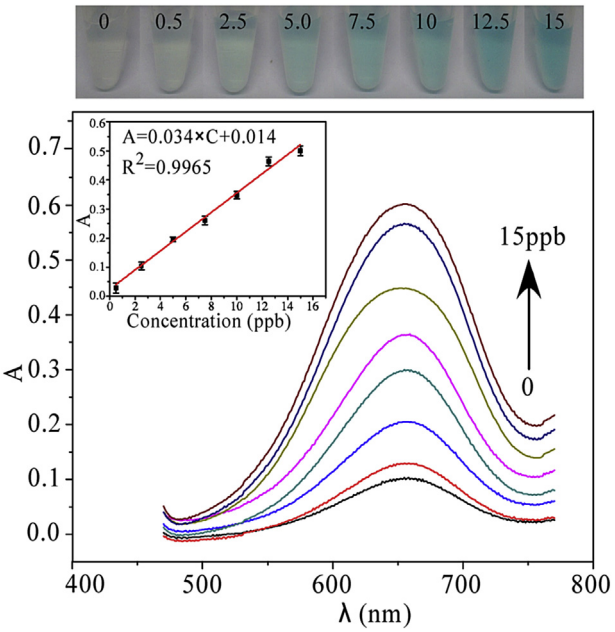

Fig. 4. The photographs, absorption spectra and calibration curve for detecting UO_2^{2+}. The concentrations of UO_2^{2+} are 0.5, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 ppb.

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Table 1
The analytical results of UO_2^{2+} in the water collected from Minjiang river in Fujian of China and in the fish muscle samples collected from coastal area of Fujian, China.

Samples		Added UO_2^{2+} (ppb)	Detected concentration				
			Naked-eye observation (ppb)	UV-visible spectrometry			ICP-MS (ppb)
				Con. (ppb)	Recovery	RSD (n = 5)	
River water	1	0.0	<0.5	0.2	–	6%	0.21
	2	0.5	0.5	0.73	106%	5%	0.78
	3	5.0	5.0	5.01	96%	3%	5.18
Fish muscle	1	0	–	–	–	–	–
	2	10	10	9.30	93%	6%	9.77

detecting UO_2^{2+} [21]. Compared to the previous fluorescent sensor, the proposed biosensor is easy-operated and cost-effective because there is no need to label expensive fluorescent group. Although the detection time of about 3 h in this method is longer than that needed in a few methods, our method possesses the outstanding advantages such as excellent specificity, operation convenience, low cost and strong resistibility to the matrix. Moreover, the visual detection limit of our method is lower than the maximum allowable level of 130 nM UO_2^{2+} in the drinking water defined by the U.S. Environmental Protection Agency (EPA) [16], indicating that the proposed method can be used for the direct detection of UO_2^{2+} in the seafood and aqueous environment with the naked-eye observation.

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

To further demonstrate the feasibility of the practical application of this biosensor, we tried our method to determine the UO_2^{2+} in the river water and dried fish muscle sample. The recovery was obtained by detecting the same river water and dried fish muscle, which spiked with different concentrations of UO_2^{2+}, with the proposed method too. The analytical results were shown in Table 1 together with their pictures. As the data shown in Table 1, the UO_2^{2+} in river water can be directly detected with the proposed biosensor by naked-eye observation or UV-visible spectrometry, with a recovery of 96–106% and a relative standard deviation (RSD, $n = 5$) $\leq$ 6%. For fish muscle samples, the recovery was 93% and the RSD ($n = 5$) was smaller than 6%. Moreover, the results obtained with the proposed biosensor consisted well with that obtained with ICP-MS. These facts revealed that the proposed biosensor is reliable and have the potential application to practical detection of UO_2^{2+} ions in aqueous environment and seafood samples. The success of this study provides a promising alternative for on-site and naked-eye detection of UO_2^{2+} ions in aqueous environment and sea food samples.

4. Conclusions

In conclusion, we herein developed a highly sensitive and selective biosensor for the on-site and visual detection of UO_2^{2+} in aqueous environment and seafood by employing DNAzyme-modified MBs as recognition probe, enzymatic catalysis of TMB as signal and DNA hybridization chain reaction as amplification strategy. The utilization of MBs considerably reduced the matrix interference, and the employment of DNA hybridization chain reaction amplification greatly improved the sensitivity. Moreover, all of the detecting procedures could be carried out at room temperature, which makes the on-site detection easier. The proposed biosensor can be used to detect as low as 2.5 ppb (9.25 nM) of UO_2^{2+} by naked-eye observation and 0.09 ppb (0.33 nM) of UO_2^{2+} by UV-visible spectrometry within 3 h with a RSD $\leq$ 6% ($n = 5$). The proposed biosensor also has excellent specificity and strong anti-interference ability. 1000-folds of other environmentally relevant metal ions do not interfere with the detection of UO_2^{2+}. With the help of this method, we have successfully determined trace UO_2^{2+} in river water and fish muscle with a recovery of 93–106%. High sensitivity and excellent specificity, as well as stability, operation easiness, low-cost and no requirement of instrument, which makes our method a potential approach for the on-site detection of UO_2^{2+} in aqueous environment and seafood.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.12.021.

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