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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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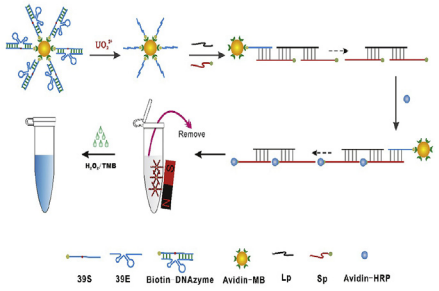
HIGHLIGHTS

- 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