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Sensitive SERS detection of lead ions via DNAzyme based quadratic signal amplification

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ABSTRACT

Highly sensitive detection of Pb^{2+} is very necessary for water quality control, clinical toxicology, and industrial monitoring. In this work, a simple and novel DNAzyme-based SERS quadratic amplification method is developed for the detection of Pb^{2+}. This strategy possesses some remarkable features compared to the conventional DNAzyme-based SERS methods, which are as follows: (i) Coupled DNAzyme-activated hybridization chain reaction (HCR) with bio barcodes; a quadratic amplification method is designed using the unique catalytic selectivity of DNAzyme. The SERS signal is significantly amplified. This method is rapid with a detection time of 2 h. (ii) The problem of high background induced by excess bio barcodes is circumvented by using magnetic beads (MBs) as the carrier of signal-output products, and this sensing system is simple in design and can easily be carried out by simple mixing and incubation. Given the unique and attractive characteristics, a simple and universal strategy is designed to accomplish sensitive detection of Pb^{2+}. The detection limit of Pb^{2+} via SERS detection is 70 fM, with the linear range from 1.0×10^{-13} M to 1.0×10^{-7} M. The method can be further extended to the quantitative detection of a variety of targets by replacing the lead-responsive DNAzyme with other functional DNA.

1. Introduction

The present food and drinking water are often contaminated with heavy metal ion pollution. It becomes a severe threat in human health. As one of the most toxic heavy metals, lead ions (Pb^{2+}), cause various neurotoxic effects, such as memory loss, irritability, anemia and mental retardation, even at very low levels (100 mg/L in blood) [1–3]. Children are more vulnerable to the effects of Pb^{2+} than adults because of higher rate of intestinal absorption and retention [4]. Although the level of Pb^{2+} is regulated by the Environmental Protection Agency with a maximum content in drinking water of 0.015 mg/L (72.4 nM) and 0.1 mg/L (483 nM) in the blood, recent studies have shown that no threshold exists for the adverse effect of lead, which is widely recognized as toxic at concentrations as low as 1 pM [5]. Therefore, the development of ultrasensitive method for rapid and accurate detection of lead is very necessary for water quality control, clinical toxicology, and industrial monitoring.

Traditional methods for Pb^{2+} quantification, include atomic absorption spectrometry (AAS) [6–8], inductively coupled plasma mass spectrometry (ICPMS) [9–12], and inductive coupled plasma atomic emission spectrometry (ICP-AES) [13,14]. These methods are capable of reaching the defined detection limit [15], but require sophisticated instruments and complicated sample preparation.

In recent decades, various sensing methods for Pb^{2+} have been reported, including Pb^{2+}dependent DNAzyme-based sensors [16]. DNAzymes are nucleic acids with RNA nuclease activity to cleave the ribonucleotide in the substrate. Most DNAzymes depend on certain metal ions or neutral molecules as specific cofactors [17,18]. The cofactor-dependent activity of catalytic nucleic acids has enabled them to act as reporter molecules for biosensing applications [19–22]. The Pb^{2+}-dependent DNAzyme has high catalytic activity for Pb^{2+} as its cofactor. The DNAzyme has been utilized in the detection of lead ion via different detection methods, including electrochemical signal [23–26], fluorescence [23,27,28], and chemiluminescence [29,30]. However, these methods are still limited by the need for complicated experiments, skilled operators. Notwithstanding, the detection limits of these sensors are normally limited in ~nM levels.

Surface-enhanced Raman spectroscopy (SERS) is a highly promising analytical tool due to its high sensitivity resulting from 10^{6-9} enhancement of Raman signal [31,32], narrow and sharp Raman bands leading to minimal background noise, and robust stability in diverse environments and complex biological systems [33–37]. SERS also have been utilized for detection of Pb^{2+} with DNAzyme in sensitive, nondestructive and low-cost manners [38–40]. Although most of such

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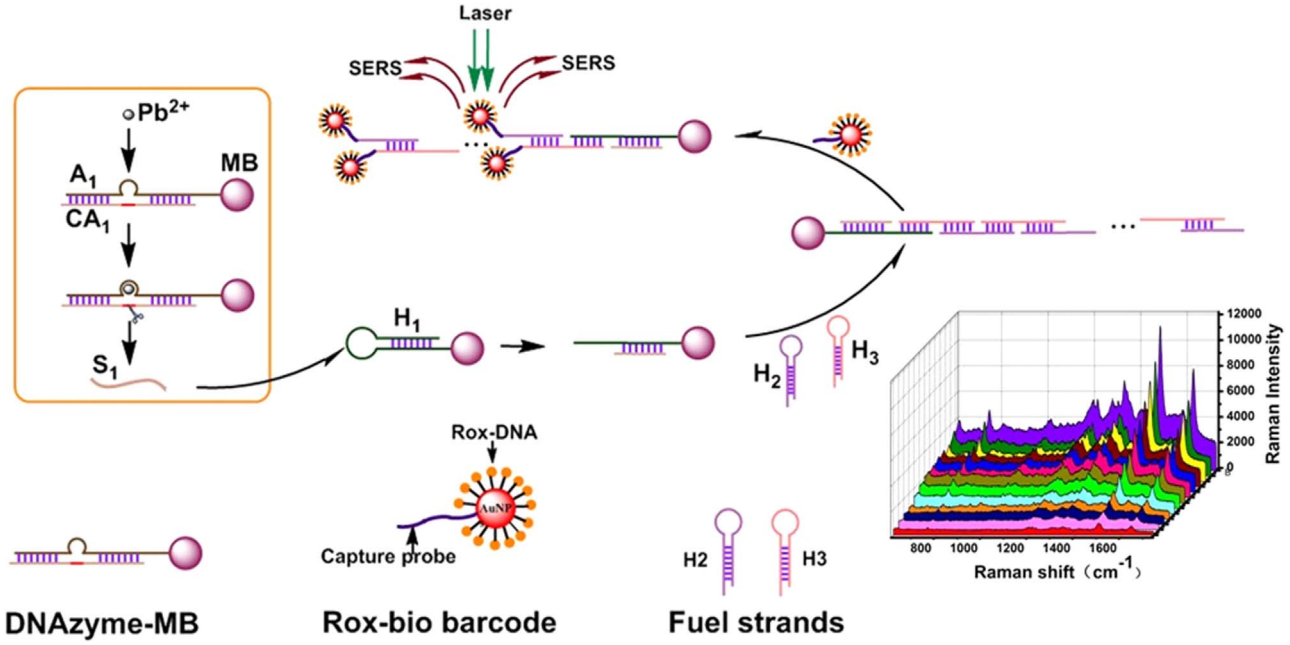

Scheme 1. The principle of the SERS detection for lead ions via DNAzyme based quadratic signal amplification.

biosensors show satisfying sensitivity, more sensitive and simple assays are desired for complex environmental or biological samples analysis.

In this work, a novel DNAzyme-based SERS amplification detection method is developed for Pb^{2+}. Compared to the DNAzyme-based SERS methods, this method is unique in some characteristics, which are as follows: (i) Coupled DNAzyme-activated hybridization chain reaction (HCR) with bio-barcodes, a quadratic amplification method is designed. The SERS signal is significantly amplified. This method is rapid with a detection time of 2 h. (ii) The problem of high background induced by excess bio-barcodes is circumvented by using magnetic beads (MBs) as the carrier of signal-output products, and this sensing system is simple in design and can easily be carried out by simple mixing and incubation.

2. Method

2.1. Principle of the SERS assay for lead ions via DNAzyme based quadratic signal amplification

In Scheme 1, the method for detection of Pb^{2+} is depicted. The system includes one kind of DNAzyme-MB complex, H1-MB complex (block DNA (H1) immobilized on MB), fuel DNAs (hairpin DNA, H2, H3), and a SERS active bio-barcode (SERS probe). The DNAzyme consists of an enzyme strand (black) immobilized on MB and a substrate strand (purple) complementary to the enzyme strand. The SERS bio-barcode probe is composed of the capture probes and Raman dye labeled DNA (Rox-DNA) immobilized on gold nanoparticles (AuNPs). The capture DNA is complementary to the stem of hairpin DNA. Rox-DNA as a signal probe is immobilized on AuNPs to generate an intense SERS signal.

In the presence of Pb^{2+}, a DNA-Pb^{2+} complex are formed. The complex can perform a catalytic reaction that is the cleavage of the substrate strand at the scissile ribonucleic acid adenosine (rA) [16,41,42]. The substrate is cleaved, the separated duplex regions lack thermal stability, and the trigger strand region is released into the solution. Trigger strand is complementary to the loop of the block DNA (H1). Through the strand binding with trigger strand, the hairpin structure of the block DNA is opened, and the initiate strand of the stem is unlocked. It can trigger enzyme-free autonomous cross-opening of hairpin probes H2 and H3 via HCR to form a dsDNA polymer. This is a key dsDNA polymer in this construct, because of its unique framework for further assembly of SERS bio barcode for signal amplification. The sticky ends of H2 and H3 are complementary to the capture DNAs (C1) of Rox-bio-barcode (the sequence of the stick end is shown in Table S1), so abundance of SERS bio-barcodes is attached to MB. By magnetic separation, the excess bio barcodes were remained in the solution. The resulting MBs were separated for SERS detection. Thus, the unwanted background was removed from excess bio-barcodes.

As a result, Pb^{2+} could be detected quantitatively by measuring the Raman signal of Rox attached on MB after quadratic signal amplification through the combination of the bio-barcode and HCR after magnetic separation. The detailed experiment is described in the Supporting information.

3. Results and discussion

3.1. The investigation of the H1 probe as the trigger strand

As a block probe, the H1 probe plays an important role in the HCR. It blocks the trigger strand stem in the HCR. Thus, to achieve optimal performance for the detection of Pb^{2+}, the sequences and the immobilization concentration of the H1 probe were investigated (Fig. 1A, the sequences of the optimal H1 probe in Table S1, ESI†). In Fig. 2A, the laser excitation of the samples provided characteristic peaks of Rox molecules, at 1344, 1451 and 1644 cm^{-1}. The strongest Raman band at 1451 cm^{-1} was used for the quantitative evaluation of Pb^{2+}. An important parameter in the assay is the number of complementary base pairs of stem sequences, which have an effect on the binding and release of initiate strand. Initially, the optimal design of the stem domain of H1 was also to prevent the nonspecific hybridization of the H2 and the H1 probe. The number of complementary base pairs (bps) was designed in the range from 5 to 11. In Fig. 1A, it has been shown that the hairpin folding can exist stably in the range from 8 to 11. The H1 probe with 8 bps is the optimal probe. When the number of complementary bases was smaller than 8 bps, the hairpin folding of the H1 probe was not exist stably. The hairpin structure of the block DNA was opened easily. The initiate strand of the stem was unlocked, and easy to hybrid with H2, then triggered the HCR reaction in the absence of Pb^{2+}. As a result, the experiment exhibited low specificity,