Microchemical Journal 131 (2017) 145–153

Contents lists available at ScienceDirect

Microchemical Journal

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

Review article

DNAzyme-based biosensor for detection of lead ion: A review

Gang Liang [a,b,c,d], Yan Man [a,b,c], An Li [a,b,c], Xinxin Jin [a,b,c], Xinhui Liu [d,*], Ligang Pan [a,b,c,**]

[a] Beijing Research Center for Agricultural Standards and Testing, Beijing Academy of Agricuture and Forestry Science, Beijing 100097 PR China
[b] Risk Assessment Lab for Agro-products (Beijing), Ministry of Agriculture PR China
[c] Beijing Municipal Key Laboratory of Agriculture Environment Monitoring, PR China
[d] State Key Laboratory of Water Environment Simulation, School of Environment, Beijing Normal University, Beijing 100875, PR China

ARTICLE INFO

Article history:
Received 27 August 2016
Received in revised form 23 November 2016
Accepted 4 December 2016
Available online 19 December 2016

Keywords:
Lead
Pb^{2+}-specific DNAzyme
G-quadruplex DNAzyme
Sensor

ABSTRACT

Lead ion (Pb^{2+}) is a kind of toxic metal pollutant that widely spread in the environment, which is of serious concern to human health. So it is of great significance to develop simple, fast method for the detection of Pb^{2+}. Up to now, a number of DNA sensors have been designed to recognize Pb^{2+}, especially DNAzyme-based sensors have attracted much attention due to its advantages, such as high selectivity and sensitivity. In this review, we summarize the recent progress of two types of DNAzyme-based sensors for the detection of toxic Pb^{2+}, including Pb^{2+}-specific DNAzyme and G-quadruplex DNAzyme sensors. On the basis of the sensor transducers and their signal types, development and applications of fluorescent DNAzyme sensors, colorimetric DNAzyme sensors and electrochemical DNAzyme sensors are to be discussed in this paper. These DNAzyme-based sensors are highly sensitive and selective toward Pb^{2+}, and has proved to be a useful tool in the potential application for on-site and real-time real sample (such as environmental and biological samples) monitoring in the future.

Contents

1. Introduction

Heavy metals ions, especially lead ion (Pb^{2+}), are ubiquitous environmental contaminants with high toxicity to humans, which make their presence undesirable [1]. The source of Pb^{2+} in the environment is mainly from the mining activities and metal smelting, during which the Pb^{2+} contained waste water and dust are arbitrarily discharged into the environment [2,3]. Studies show that Pb^{2+} can cause a number of adverse health effects even at low-level exposure [4], and eventually lead to disease or even death [5]. The U.S. Environmental Protection Agency (EPA) has set the safety limits of Pb^{2+} in drinking water as 72 nM [6]. Therefore, developing sensitive techniques that can accurate determine the Pb^{2+} at the trace level in environmental and biological samples has become increasingly important.

Commonly, the traditional methods applied for the quantification of the Pb^{2+} in the laboratory are mainly based on cold vapour atomic, atomic fluorescence spectroscopy (AFS) [7], atomic absorption spectroscopy (AAS) [8], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [9,10] and inductively coupled plasma-mass spectrometry

* Corresponding author.
** Corresponding author at: Beijing Research Center for Agricultural Standards and Testing, Beijing Academy of Agriculture and Forestry Science, Beijing, PR China.
E-mail addresses: xhliu@bnu.edu.cn (X. Liu), panlg@nercita.org.cn (L. Pan).

http://dx.doi.org/10.1016/j.microc.2016.12.010
0026-265X/

(ICP-MS) [11]. Although these techniques are precise but suffer from some disadvantages of time-consuming pretreatment of the sample, high cost, the requiring of sophisticated equipments and highly qualified technicians etc. [12]. To overcome these limitations, an achievement of sensitive, simple and economical approach for the detection of Pb^{2+} is highly imperative. Therefore, Pb^{2+} sensors have become a hot topic in analytical chemistry. In contrast, DNA biosensors are of particular interest and have been widely employed for the task of detection of contaminations or bioterrorism agents, analysis of food, point-of-care diagnostics due to its high selectivity and sensitivity [13]. Especially, DNAzyme-based biosensors that developed through the systematic selection is generalizable for a wide range of metal ions [14]. So the DNAzyme-based biosensors has the potential to fill this technology gap and is considered as a promising tool for Pb^{2+} detection. As ICP-MS technique is routinely used for the analysis of Pb^{2+} in environmental samples, the dis-/advantages of ICP-MS technique for determination of Pb^{2+} was shown in Table S1 for comparing the analytical performance features for Pb^{2+} determination from the current state of the art (ICP-MS) to the DNA sensors discussed in this review.

In this review paper, we intend to focus on the recent progress in the design of two types of Pb^{2+}-dependent DNAzyme and G-quadruplex DNAzyme sensors for toxic Pb^{2+} detection with fluorescent, colorimetric and electrochemical signal readout as the sensing signal. Furthermore, examples of the sensors' application for rapid, sensitive and selective Pb^{2+} detection in real samples, such as environmental sample (real environmental water, extracts of soil) and biological sample (blood), are also discussed in this review.

2. Pb^{2+}-dependent DNAzyme for Pb^{2+} detection

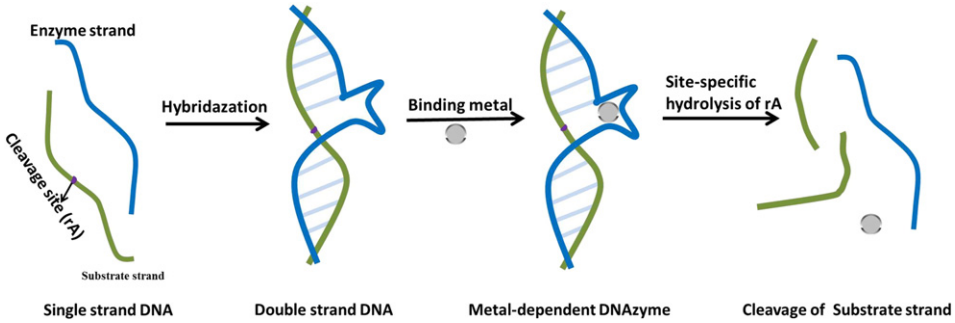
Metal-dependent DNAzyme, newest members of the enzyme family, was first reported in the 1990s [15]. The DNAzyme, such as Pb^{2+}-dependent 8–17 Enzyme [16], Mg^{2+}-dependent DNAzymes [17], Ca^{2+}-dependent Enzyme [18], is comprised of an enzyme strand and a substrate strand, which contains a single ribonucleobase (RNA) linkage (such as rA) that serves as the cleavage site (RNA phosphoester) followed by a G-T wobble pair [19]. Generally, the enzyme strands and the substrate strands were first hybridized to duplex in buffer solution. And then the enzyme strand can bind its target metal ions (such as Pb^{2+}, Ca^{2+}, Mg^{2+}) [20], which acts as cofactors, and exhibits high catalytic activities to recognize and cleave toward its rA-containing substrate strand (Scheme 1). By choosing proper signal reporter, the Pb^{2+} can be detected. Compared with the protein enzymes, these metal-dependent DNAzymes possess many merits of low production cost, simple preparation steps, and easy storage. All these features make the metal-dependent DNAzymes particularly attractive as a biosensor platform for metal ion detection. 8–17 DNAzyme-based sensor is the first DNAzyme sensor reported for Pb^{2+} detection [21]. Until now, a variety of DNAzyme-based sensors have been developed for the detection of Pb^{2+} ion with brilliant specificity toward Pb^{2+} due to its "lock-and-key" mode of catalysis for Pb^{2+}-dependent activity [20,22].

2.1. Fluorescent Pb^{2+}-dependent DNAzyme sensor

With fluorescence as the signal transduction, DNAzyme-based fluorescent sensors has been extensively applied for Pb^{2+} detection by virtue of its advantages of high sensitivity, fast kinetics. However, DNA itself has no fluorescent property, so a fluorescence signal reporter is required, which is usually labeled in the ends of DNA strand (labels with fluorophores and/or quenchers) during the synthesis processes [23]. The general fluorescent sensor design based on Pb^{2+}-dependent DNAzyme for Pb^{2+} detection was shown in Fig. 1. Li et al. developed the first sensor for highly sensitive and selective detection of Pb^{2+} by labeling the 8–17 DNAzyme and its substrate with a quencher and fluorophore, respectively [21]. The fluorescent sensor was designed by labeling the 5′-end of the substrate with the fluorophore TAMRA (6-carboxytetramethylrhodamine) and the 3′-end of the enzyme strand with a fluorescence quencher Dabcyl (4-(4′-dimethylaminophenylazo) benzoic acid). Due to the specific recognition of Pb^{2+}, the assay exhibited a lower quantifiable detection limit of 10 nM and an excellent selectivity of 80-fold for Pb^{2+} over other metal ions. Furthermore, on the basis that single-stranded DNA containing different number of bases in length showed different affinity to graphene, Zhao et al. developed a GO-DNAzyme based biosensor for amplified fluorescence detection of Pb^{2+} for the first time [24]. The hybridized duplex of FAM-labeled DNAzyme and substrate acted as both a molecular recognition module and signal reporter. Due to the super fluorescence quenching efficiency of GO, the assay showed high sensitivity with a detection limit of 300 pM for Pb^{2+}. And the assay performed excellent selectivity than 8–17 DNAzyme sensor as a result of the use of Pb^{2+}-dependent GR-5 DNAzyme instead of 8–17 DNAzyme. Furthermore, the proposed sensor was applicable for practical Pb^{2+} detection in Xiang River water spiked with Pb^{2+}, with other potentially interfering species coexisting.

Some free organic compounds do not exhibit fluorescence, but can produce measurable fluorescence signal after interacting with the DNA strands [25]. For example, the dye Picogreen (PG) exhibits very weak fluorescence upon binding to single-stranded DNA while it displays much intense fluorescence when binding to double-stranded DNA. On the basis of the findings, Zhang et al. demonstrated a label-free, signal-off fluorescent Pb^{2+} assay using 8–17 DNAzyme and the double-strand-chelating PG [26]. In the absence of Pb^{2+}, PG is intercalated into the double strand DNA, displaying very strong fluorescence signal; while in the presence of Pb^{2+}, the substrate strand is cleaved to fragments, resulting in weak interactions of ss-DNA with PG, showing very weak fluorescence. The assay enables fluorescence detection of Pb^{2+} in the aqueous solution with sensitivity of 10 nM detection limit and selectivity over other metal ions of Hg^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+} and Cu^{2+}.

The solution based fluorescent assays allow the effective Pb^{2+} detection, but the background fluorescent signal is relatively high because of the partially unhybridized substrates strand and the enzyme strand at ambient conditions [20]. To overcome this inherent drawback, the surface-based assays have attracted people's attention due to that it can avoid the worry that the fluorescent signal may be quenched by other



Scheme 1. Preparation and working mechanism of metal-dependent enzyme.

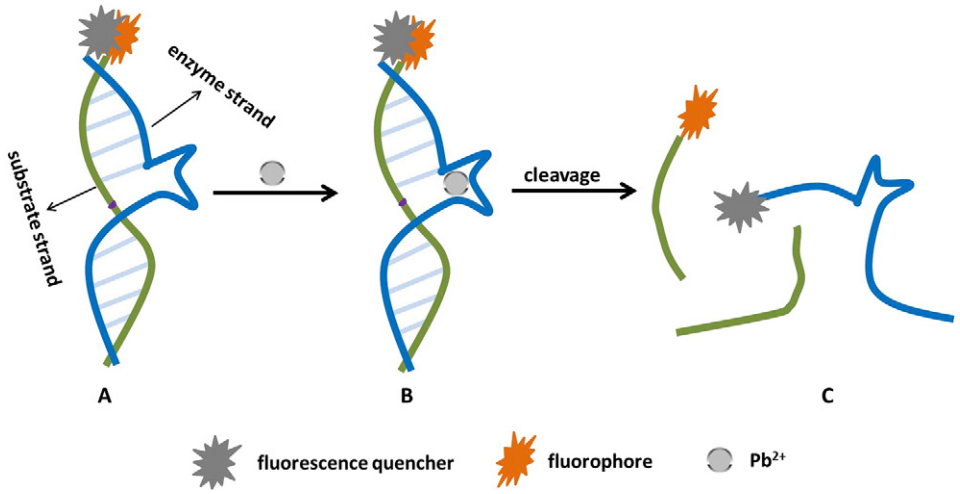


Fig. 1. Working principle of fluorescent Pb^{2+}-dependent DNAzyme sensor for Pb^{2+} detection. A: Quencher-labeled and fluorophore-labeled DNAzyme-substrate duplex (without fluorescence); B: Binding of Pb^{2+} in the specific binding site; C: Cleavage of the rA-containing substrate, releasing free fluorophore-labeled substrate and producing strong fluorescent signal.

potential interfering compounds [27]. This surface-immobilized fluorescent assays enable the long-term storage of the sensors, and the background fluorescence of the sensor system was decreased by one order of magnitude, which make it more practical applications in environmental samples. Swearingen et al. reported the first surface-based assay [28]. In their work, the fluorophore labeled substrate DNA was immobilized on the gold substrates, upon incubating with Pb^{2+} samples, the substrate strands were cleaved into fragments and the fluorophores were released, resulting in Pb^{2+}-dependent fluorescence change. The assay showed a wide linear response for Pb^{2+} from 1 nM to 10 μM and an improved sensitivity (lowers the detection limit from 10 nM to 1 nM) over the solution based fluorescent sensor. Recently, however, Fu et al. reported an amplified "turn-on" fluorescent sensor for Pb^{2+} detection with catalytic DNAzyme as label-free catalytic beacons [29]. Through enzymatic turnover with cleavage-induced G-quadruplex formation, then the zinc(II)-protoporphyrin IX/G-quadruplex complexes were formed by intercalating interaction with zinc(II)-protoporphyrin IX, which acted as the signal reporter unit. Based on the fluorescence signal change, the proposed DNAzyme sensor could achieve high selectivity and sensitivity, and showed a linear response for Pb^{2+} from 5 to 100 nM, with a low detection limit of 3 nM.

The above mentioned fluorescent method is not suitable for high throughput applications. Combining the lead-specific DNAzymes with the microarray technique should enable the high-throughput and efficient determination of lead ions at minute concentrations. Later, by immobilizing 8–17 DNAzymes and substrates on microarrays, Liu et al. designed a facile microarray-based fluorescent sensor for the detection of Pb^{2+} based on the catalytic cleavages of the substrates by the Pb^{2+}-dependent 8–17 DNAzyme [30]. The sensor showed a quantifiable detection range from 1 nM to 1 mM with 1 nM detection limit, and a selectivity of 20-fold for Pb^{2+} over other metal ions of Hg^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+} and Cu^{2+}. Zuo et al. constructed a sensor arrays for the high-throughput detection of Pb^{2+}, the sensor arrays combines the high sensitivity and selectivity of DNAzymes with the high throughput and parallel analysis of microarray technology, revealing a sensitivity of 10 nM for Pb^{2+} (linear range from 10 nM to 100 μM.) [31]. Furthermore, the proposed method was successfully performed for analyzing spiked river water samples.

2.2. Colormetric Pb^{2+}-dependent DNAzyme sensor

In addition to fluorescence color, with absorption color as the signal output, the Pb^{2+}-specific DNAzyme have also been applied for colorimetric detection of Pb^{2+} with the advantages of eliminating or minimizing most costs associated with fluorescence detection, as the visible sensor can be directly read out by eye observation without any instrumentation, and this merit make its on-site, real-time detection easier. So far, various colorimetric Pb^{2+}-specific DNAzyme sensors have been constructed for Pb^{2+} detection.

Previous researches have shown that different aggregation states of metal nanoparticles can result in distinctive color changes, such as gold nanoparticles [32,33], semiconductor nanoparticles [34]. The gold nanoparticles exhibit very high extinction coefficients and are regarded as ideal candidates for incorporation into colorimetric sensors [35]. Until now, the DNA-functionalized Au particles have been widely applied for economically viable sensors in bioanalytical areas. The first colorimetric Pb^{2+} sensor based on gold nanoparticles and DNAzymes was developed in 2003 [36]. In this work, Liu et al. reported a colorimetric Pb^{2+} sensor with the gold nanoparticles as the colorimetric reporter group. The sensing principle of the colorimetric sensor was shown in Fig. 2. In the absence of Pb^{2+}, the gold nanoparticles were crosslinked as aggregates by DNAzyme substrates through DNA hybridization, and the system showed a blue color with an absorption band about 700 nm; in the presence of Pb^{2+}, the substrate strands were readily cleaved, and the dispersed gold nanoparticles displayed a red color with an absorption band around 522 nm. Therefore, the ratio of E522/E700 was used as a measure for the detection of Pb^{2+}. The sensor showed a sensitivity to 100 nM for Pb^{2+}. However, the sensor was time-consuming, and 2 h was needed to observe the system color change. Later, their group optimized the lengths of DNAzymes, gold nanoparticles size and temperatures, and finally the "tail-to-tail" nanoparticle alignment and gold nanoparticles size (42 nm) were proved to be the major determining factors, demonstrating a new improved sensor that allowed rapid detection of Pb^{2+} at room temperature (<10 min). The sensor showed good selectivity over other divalent metals, such as Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} or Cd^{2+} [37].

Instead of cross-linking gold nanoparticles by DNA hybridization, Zhao et al. aiming to design a facile colorimetric sensor by DNAzymes cleavage of the substrate strands on well-dispersed gold nanoparticles, which could cause aggregation of the nanoparticles. In the assay, the DNAzyme modified AuNPs are well dispersed in salt solution in the absence of Pb^{2+} due to the electrostatic and steric stabilization. However, the addition of Pb^{2+} destabilizes the AuNPs because of the enzymatic cleavage of DNA chains on the AuNP surface, resulting in a rapid aggregation, and accompanied with a color change from red color to purple color. Thus, the Pb^{2+} could be detected with response time of merely 2 min [38]. The sensor also showed good selectivity over other metals of Mg^{2+}, Mn^{2+}, Ca^{2+}, and Cd^{2+}. Compared with previous studies in which the gold nanoparticles aggregates (DNAzyme-cross-linked gold

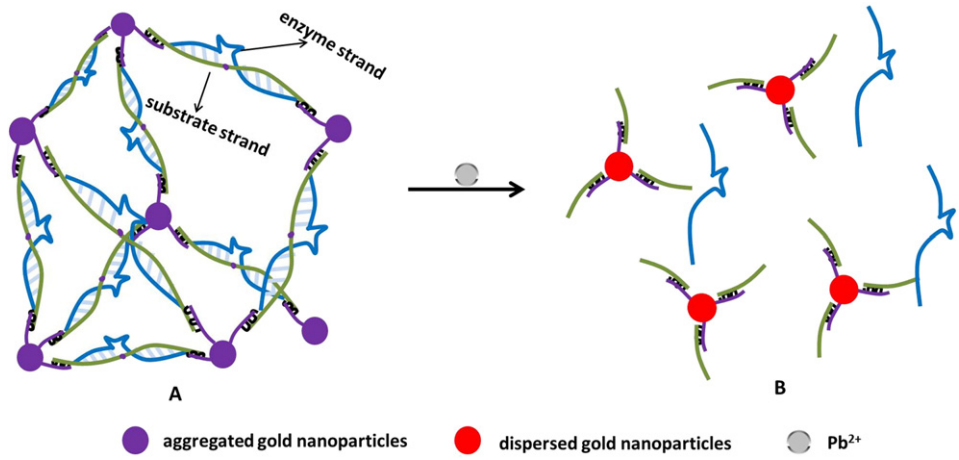


Fig. 2. Working principle of colorimetric Pb^{2+}-dependent DNAzyme sensor for Pb^{2+} detection. A: Gold nanoparticles, which are crosslinked as aggregates by DNAzyme-substrates through DNA hybridization, show a blue color with an absorption band about 700 nm; B: Site-specific hydrolysis of rA in the presence of Pb^{2+}, producing dispersed gold nanoparticles, which display a red color with an absorption band around 522 nm.

nanoparticles) were converted into dispersed gold nanoparticles by DNAzyme cleavage of substrate DNA crosslinkers, the improved colorimetric sensor was technically simpler. Furthermore, the accessibility of DNA to enzymes on well-dispersed gold nanoparticles in this work appeared to be better than that embedded inside aggregates [38].

The above mentioned sensors were designed on the basis of that the thiolated DNA can self-assembled onto the gold electrode via strong S—Au covalent bonding. In addition to thiol-gold interactions, the DNA strand can also bind to gold nanoparticles noncovalently via its nucleotide bases [39], with much higher binding affinities to gold for single-strand DNA than fully complementary double-strand DNA due to the exposed nucleotide bases [40]. Accordingly, the label-free colorimetric sensors for Pb^{2+} have been developed utilizing Pb^{2+}-specific DNAzymes and unmodified gold nanoparticles. For example, Wei et al. developed a rapid, sensitive and label-free DNAzyme-based sensor for Pb^{2+} detection [41]. In the presence of Pb^{2+}, the substrate strand was cleaved into fragments (single-stranded DNA) by the DNAzyme, which could be adsorbed on the citrate protected gold nanoparticles; while in the absence of Pb^{2+}, the hybridized double-stranded DNA of substrate and DNAzyme could not, the color change of gold nanoparticles could be measured, thereby Pb^{2+} detection was realized with a detection limit of 500 nM. The modification-free colorimetric sensor was successfully applied for detection of Pb^{2+} within 20 min and showed good selectivity over Mg^{2+}, Mn^{2+}, Ca^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+} and Cu^{2+}.

With the absorption response of gold nanoparticles as the detection signal, the described colorimetric sensors can exhibit only moderate sensitivity with nanomolar detection limits [42]. To meet these challenges and greatly improve the detection sensitivity, enzymatic and hairpin based amplification processes have been utilized for Pb^{2+} detection. For example, Yun et al. designed a label-free ultra-sensitive colorimetric Pb^{2+} sensor using molecular beacon and DNAzyme for signal amplification [42]. By taking advantage of recycle using Pb^{2+}-dependent enzyme, the sensor exhibited high sensitivity for Pb^{2+} in a linear range from 0.05 nM to 5 nM with a detection limit of 20 pM by UV–vis spectrometer. The selectivity of this sensor was also evaluated by testing the other divalent metal ions, such as Mg^{2+}, Zn^{2+}, Ca^{2+}, Hg^{2+}, Fe^{2+}, Co^{2+}, Sn^{2+} and Cu^{2+}. Furthermore, this sensing strategy was also applied to determine Pb^{2+} in Fujing River (Jiangyou, China) water samples with satisfying results.

It is noteworthy that environmental water samples with color is not suitable for colorimetric and fluorescent detection of Pb^{2+}, that is because the coexisting colored compounds can not only produce strong background signal, but also effect the DNAzyme catalyzed H_2O_2-mediated oxidation of the substrates. Thus, the colorimetric/fluorescent signal intensity measured in the colored water samples cannot be used for Pb^{2+} quantification.

2.3. Electrochemical Pb^{2+}-dependent DNAzyme sensor

Electrochemical assays have been widely applied for the on-site detection of contaminations [12]. Due to their outstanding characteristics, such as high sensitivity, low cost, simple instrumentation, and rapid response time, electrochemical methods have been employed to design DNAzyme-based sensors for Pb^{2+} [43–45]. As the DNA cannot produce strong electrochemical signal on the electrode, thereby a electrochemical signal reporter is usually required [46–48]. The general electrochemical sensor design based on Pb^{2+}-dependent DNAzyme for Pb^{2+} detection was shown in Fig. 3.

For example, Plaxco et al. reported a DNAzyme-based electrochemical biosensor for Pb^{2+} detection with methylene blue (MB) as redox-active compound (signal reporter) [49]. First, the Pb^{2+}-specific DNAzyme strand was functionalized with MB and immobilized onto the gold electrode surface via a Au—S bonding. Then, the DNAzyme modified electrode was immersed in the complementary substrate DNA solution and hybridized to duplex DNA that could prohibit any contact between MB and the electrode, producing weak current signal. In the presence of Pb^{2+}, the substrate was cleaved and released from the electrode surface, thus the DNAenzyme strand was more flexible and facilitated the electrochemical communication between MB and the electrode, producing an enhanced electrochemical signal, which was proportional to the concentration of Pb^{2+}. The detection limit of the proposed sensor for Pb^{2+} was 300 nM after 60 min incubation, with a linear range from 0.5 μM to 10 μM. The specificity of the DNAzyme sensor was challenged with metal ions of Mn^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+}, Cu^{2+} and Hg^{2+}, and little signal change was observed. Thus the sensor appeared to be specific to Pb^{2+}. Furthermore, the sensor was successfully applied for Pb^{2+} detection in extracts of soil spiked with lead, with a detection limit of 300 nM, illustrating the sensor's applicability.

To improve the sensitivity, nanoparticles were usually modified in the substrate strand to enhance the electrochemical signal [50]. For example, Shen et al. developed a electrochemical sensing assay by taking advantage of catalytic reactions of the DNAzymes that binding to Pb^{2+} and the DNA-Au bio-bar codes for signal enhancement. After incubation with Pb^{2+} for 60 min, the electrochemical DNAzyme sensor enabled the detection limit of Pb^{2+} to be 1 nM with a linear calibration ranging from 5 nM to 100 nM by measuring differential pulse voltammetry (DPV) signals of $Ru(NH_3)_6^{3+}$ [51]. Later, Yang et al. developed a novel strategy for constructing an amplified DNAzyme sensor for the electrochemical

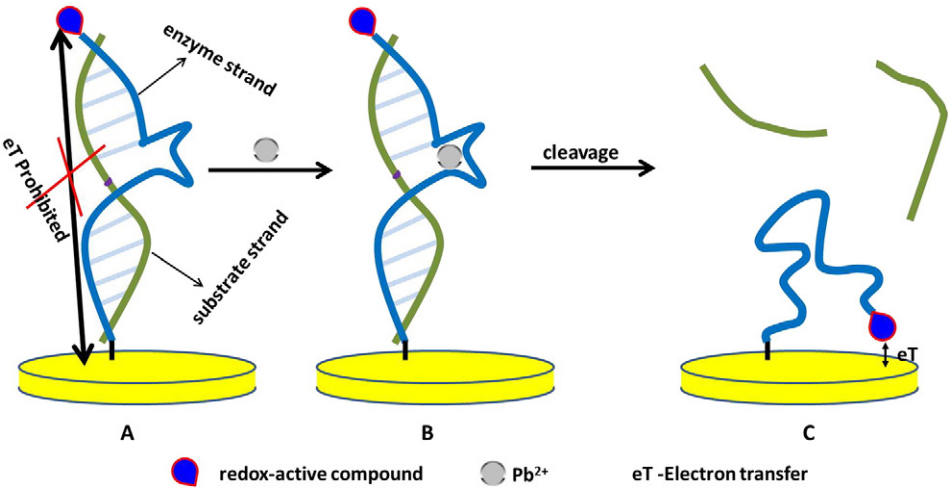


Fig. 3. Working principle of electrochemical Pb^{2+}-dependent DNAzyme sensor for Pb^{2+} detection. A: Immobilization of methylene blue (MB)-functionalized Pb^{2+}-specific DNAzyme and substrate duplex onto the gold electrode, prohibiting the contact between MB and the electrode; B: Binding of Pb^{2+} in the specific binding site, and exhibits high catalytic activities; C: Cleaving toward its rA-containing substrate strand, releasing flexible DNAenzyme strand, thus facilitated the electrochemical communication between MB and the electrode.

detection of Pb^{2+} [52]. The DNAzyme strand was immobilized onto the gold nanoparticles, and then hybridized it to substrate strand. In the presence of Pb^{2+}, the substrate was cleaved and released from the gold nanoparticles, then the DNAzyme strand modified on the gold nanoparticles could hybridize with the capture DNA modified electrode film, which resulting in an enhanced electrochemical signal. Based on the chronocoulometric (CC) signals of $Ru(NH_3)_6{}^{3+}$, a linear relationship between Q and Pb^{2+} concentration was obtained from 0.1 nM to 35 nM with a detection limit of 0.028 nM. The specificity of the sensor was determined by challenging it with other metal ions of Mn^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+} and Cu^{2+}, and the results indicated the sensor was highly specific to Pb^{2+}.

Furthermore, quantum dots have also been widely used as signal amplifiers for constructing ultrasensitive DNA sensors. For example, Zhang et al. developed an electrochemical DNAzyme-based strategy for monitoring of Pb^{2+} by anodic stripping voltammetry (ASV) with PbS quantum dots layer-by-layer assemblies as signal amplification labels [53]. The proposed Pb^{2+} sensing approach combined the catalytic activity of the Pb^{2+}-specific DNAzymes, and enabled to detect Pb^{2+} with a dynamic range from 1 nM to 1000 nM (detection limit of 600 pM) after 60 min incubation with Pb^{2+}. The sensor showed good selectivity for Pb^{2+} against other metal ions of Mg^{2+}, Mn^{2+}, Zn^{2+}, Hg^{2+} and Cu^{2+}, and was successfully employed to monitor Pb^{2+} in real waste water samples. Tang et al. constructed a simple, ultrasensitive sensor for electrochemical detection of Pb^{2+} by ASV [54]. Due to the DNA-assisted cascade of hybridization reaction with the CdS quantum dots for signal amplification, the detection limit for Pb^{2+} can be as low as 6.1 pM after 60 min cleavage time of the biosensor in the presence of Pb^{2+}. And the DNAzyme sensor has high selectivity toward Pb^{2+} over other metal ions commonly coexist with Pb^{2+}, such as Mg^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Hg^{2+} and Cu^{2+}. Furthermore, they also applied the proposed sensing system to detect Pb^{2+} in Minjiang River (Fuzhou, China) water samples. Zhuang et al. designed a novel magneto-controlled electrochemical DNA biosensor for ultrasensitive Pb^{2+} detection with ferrocene as signal reporter by coupling the Pb^{2+}-specific DNAzyme with DNA-based hybridization chain reaction. Based on the square wave voltammetry response (SWV), a linear relationship was observed between the peak currents and the logarithm of Pb^{2+} concentrations in the range of 0.1 nM to 75 nM with a low detection limit of 37 pM [47]. The selectivity of the designed sensor was evaluated by challenging it against other metal ions including Hg^{2+}, Co^{2+}, Cu^{2+}, Ag^{+}, Ni^{2+}, Zn^{2+}, Fe^{2+}, Cd^{2+} and Mg^{2+}, and the results revealed that the sensor exhibited high specificity to Pb^{2+}. Ge et al. developed a multi-amplified paper-based electrochemical DNAzyme sensor with HRP/GOx bioconjugations as the signal amplification unit for Pb^{2+} detection [55]. The developed sensor exhibited a wide linear response from 0.005 nM to 2000 nM, with a detection limit of 2 pM.

Electrochemical impedance spectroscopy (EIS) has been proven to be sensitive tool for probing the features of DNA-modified electrodes with the advantages of ease of signal quantification, less damage to the biological interactions, and label-free on the DNA strand [56]. And EIS biosensors have been widely applied to heavy metals detection. Zhou et al. developed a label-free impedimetric sensor for Pb^{2+} determination based on DNAzyme catalytic beacons and ordered mesoporous carbon–gold nanoparticle sensing platform. The sensor showed a linear relation with the Pb^{2+} concentration in a wide range of 0.5 nM to 50 μM, with a detection limit of 0.2 nM [57]. Then the sensor was employed for Pb^{2+} determination in Taozi lake water and Xiangjing River water samples, indicating the potential of the sensor as a reliable analytical method for environmental Pb^{2+} detection. Our group reported a label-free, simple and sensitive assay for detection of Pb^{2+} by EIS [58]. The pre-hybridized duplex DNA of DNAzyme and the complementary substrate DNA was immobilized onto the gold electrode via Au—S bonding. Upon addition of Pb^{2+}, changes in the DNA structure led to reduced charge transfer resistance, based on which allowed to selectively detect at 0.1 pM, which is much lower than other electrochemical DNAzyme sensors. The sensor was also successfully challenged in natural lake water sample (Baiyangdian Lake, China).

Commonly, traditional Pb^{2+}-specific DNAzyme based electrochemical biosensors need the immobilization of DNA on the electrode surface, and this procedure is time-consuming and tedious. Tan et al. proposed a simple but sensitive immobilization free DNAzyme based electrochemical sensor for Pb^{2+} detection [59]. The established sensor was ease of operation because it is immobilization free and has high selectivity due to the DNAzyme specificity to Pb^{2+}. The electrochemical signal has a linear relationship with the Pb^{2+} concentration over the range of 0.05 μM to 1 μM with a 18 nM detection limit. Furthermore, the sensor has been applied to detect Pb^{2+} in Minjiang River (Fuzhou China) water samples with satisfactory results.

3. G-quadruplex DNAzyme for Pb^{2+} ion detection

A single-stranded guanine-rich DNA can transform into G-quadruplex in the presence of cation ions (such as K^{+}, Pb^{2+}) that is found to complex tightly with hemin to form the ion-stabilized hemin/G-quadruplex complex [60]. The hemin/G-quadruplex complex

displays robust peroxidase activity and acts as horseradish peroxidase-mimicking DNAzyme (G-quadruplex DNAzyme), which can catalyze the oxidation reaction between the target molecules and hydrogen peroxide, and result in the appearance of an oxidized product [61, 62]. Compared with the Pb^{2+}-specific DNAzyme, which is high cost of DNAzyme preparation and the use of unstable molecules (RNA) [63], the G-quadruplex DNAzyme possesses the advantages of less expensive, stable against hydrolysis, high thermal stability, renatured several times without losing their activity [64]. Therefore, the G-quadruplex DNAzyme has been extensively applied for the design of numerous sensors. In the followings, we will focus on the strategies for designing of fluorescent, colorimetric and electrochemical sensors based on the signal amplification of hemin/G-quadruplex DNAzyme.

3.1. Fluorescent G-quadruplex DNAzyme sensor

Studies have confirmed that Pb^{2+} is capable of stabilizing the G-quadruplex structure [65–67], and the formed Pb^{2+}-stabilized G-quadruplex can also bind with hemin and then show peroxidase activity. However, to develop a fluorescent sensor, a fluorescent signal probe is required, which has no fluorescence property itself but the oxidation product displays strong fluorescence signal when oxidized by G-quadruplex DNAzyme. Following this principle, G-quadruplex DNAzyme-based fluorescent sensor for selective detection Pb^{2+} was developed. Nevertheless, a few fluorescent sensors have been developed so far.

Li et al. developed a G-quadruplex DNAzyme-based fluorescence assay for the detection of Pb^{2+} ions [68]. The detection mechanism based on G-quadruplex DNAzyme for Pb^{2+} detection was shown in Fig. 4. In their work, Amplex UltraRed (AUR) was selected as the signal reporter and AGRO100 was employed for preparation the G-quadruplex DNAzyme. In the absence of Pb^{2+}, the catalytic activity of hemin in the presence of AGRO100 was very weak, and the fluorescence intensity of the system showed very low. In the presence of Pb^{2+}, the fluorescence intensity of the system increased significantly, which was due to the formation of Pb^{2+}-stabilized hemin/G-quadruplex complexes (G-quadruplex DNAzyme) that possesses high catalytic activity and can act as a cofactor to catalyze H_2O_2-mediated oxidation of non-fluorescent Amplex Ultra Red (AUR) to form a highly fluorescent oxidized AUR product, resulting in increased fluorescent intensity. The assay exhibited excellent selectivity of 100-fold toward Pb^{2+} over other tested metal ions and high sensitivity for Pb^{2+} with a linear detection range from 0 nM to 1000 nM, detection limit of 0.4 nM. Besides, they also validated the practicality of the sensing probe for Pb^{2+} determination in extracts of soil samples, and the results indicated that the proposed method was comparable to the ICP/MS-based analysis for Pb^{2+}.

Later, using the similar strategy, they designed an improved fluorescence assay for quantification of Pb^{2+} [69]. In this work, gold nanoparticles were employed for DNAzyme oligonucleotide (T30695) immobilization. Due to the large surface of the gold nanoparticles, many Pb^{2+}-stabilized hemin/G-quadruplex complexes formed on the gold nanoparticle, which significantly enhanced the catalytic activity of the system to catalytic oxidization the H_2O_2-AUR probe. Thus, a lower detection limit of 0.05 nM for Pb^{2+} is achieved, and the linear response of its fluorescence intensity with respect to the Pb^{2+} concentration was over the range 0 nM to 100 nM. Furthermore, the assay allowed detection of Pb^{2+} in blood samples without conducting tedious sample pretreatment. The results showed that the T30695–Au NPs/AUR assay had the potential for on-site and real-time detection of Pb^{2+} ions in biological samples.

3.2. Colormetric G-quadruplex DNAzyme sensor

Unlike the colorimetric Pb^{2+}-dependent DNAzyme sensors that colorimetric reporters (nanoparticles) are required, many other agents (substrate of the G-quadruplex DNAzyme) are available for colorimetric G-quadruplex DNAzyme sensors, such as 3,3′,5,5′-Tetramethylbenzidine (TMB) [70], *o*-phenylenediamine (OPD) [71] and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [72]. The G-quadruplex DNAzyme can catalyze oxidation of the substrates to colored products with excessive H_2O_2. Therefore, a series of colorimetric sensors for the sensitive and selective detection of Pb^{2+} were prepared.

For example, Li et al. developed a colorimetric G-quadruplex DNAzyme (named PS2. M) sensor for Pb^{2+} with ABTS as the signal reporter [73]. The principle of sensing Pb^{2+} via G-quadruplex DNAzyme was shown in Fig. 5. In the presence of K^+, the K^+-stabilized G-quadruplex DNAzyme exhibited a superior peroxidase activity and effectively catalyzed the H_2O_2-mediated oxidation of ABTS to the colored $ABTS^{\bullet-}$ (radical form). Upon addition of Pb^{2+}, the K^+-stabilized G-quadruplex converted to the more stable Pb^{2+}-stabilized G-quadruplex but with low DNAzyme activity, which is reflected by an obvious decrease in readout signal. This ABTS-H_2O_2 colorimetric system allowed to quantitative detection Pb^{2+} in aqueous with a detection limit of 32 nM. To demonstrate the application potential of the G-quadruplex DNAzyme probe in environmental analysis, Pb^{2+} in the freshwater system was tested, and the results suggested that the introduced DNAzyme-based method could be applied to the analysis of Pb^{2+} in environmental samples.

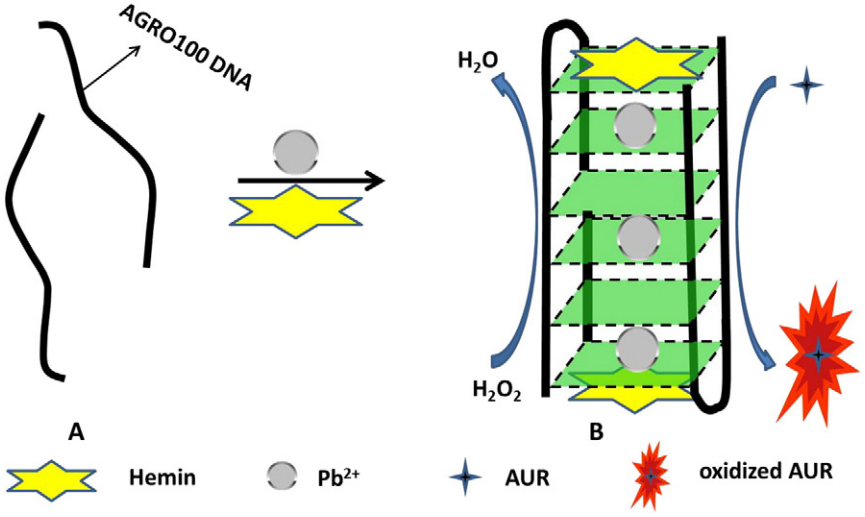


Fig. 4. Sensing principle of fluorescent G-quadruplex DNAzyme sensor for Pb^{2+} detection. A: Free single DNAzyme strands (AGRO 100) in buffer; B: Formation of Pb^{2+}-stabilized hemin/G-quadruplex complexes (G-quadruplex DNAzyme) in the presence of Pb^{2+} and hemin (acts as a cofactor), which show high catalytic activity and catalyze the H_2O_2-mediated oxidation of non-fluorescent Amplex Ultra Red (AUR) to form a highly fluorescent oxidized AUR product.

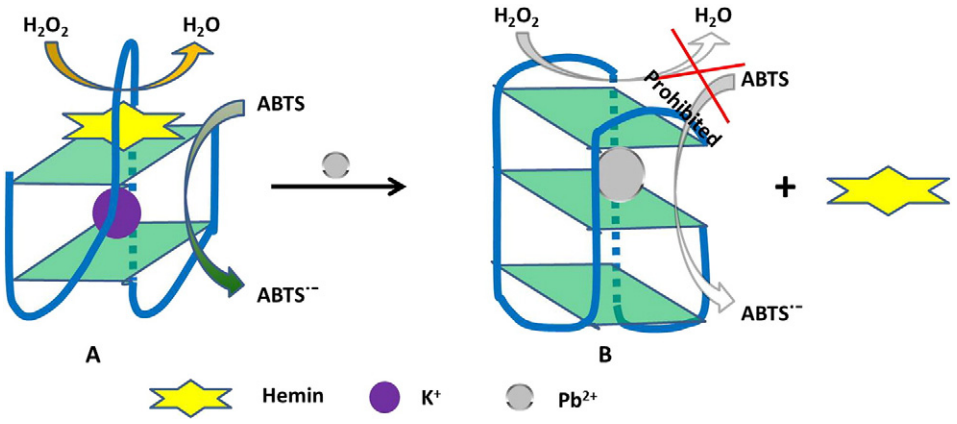


Fig. 5. Illustration of Pb^{2+}-induced allosteric G-quadruplex DNAzyme for colorimetric detection of Pb^{2+}. A: The K^+-stabilized G-quadruplex DNAzymes exhibit peroxidase activity and catalyze the H_2O_2-mediated oxidation of ABTS to the colored ABTS•$^-$; B: Transformation of the K^+-stabilized G-quadruplex into the more stable Pb^{2+}-stabilized G-quadruplex but with low DNAzyme activity, decreasing the color of the sensing system.

Similarly, based on the difference in binding abilities and G-quadruplex DNAzyme activities of the G-quadruplex toward K^+ and Pb^{2+} [74], Wang et al. reported a colorimetric G-quadruplex DNAzyme (named PS2.M) sensor for Pb^{2+} detection with ABTS as the signal reporter [75]. Upon addition of Pb^{2+} to the K^+-stabilized G-quadruplex/hemin complex system, K^+ was replaced by Pb^{2+} in the G-quadruplex, resulting in the loss of the binding ability toward hemin and the inhibition of the G-quadruplex DNAzyme activity. Based on the color change of the catalyzed H_2O_2-mediated oxidation of ABTS, this assay enabled Pb^{2+} to be determined in the range from 0.05 μM to 1.2 μM, with a detection limit of 27 nM. The assay was successfully applied for Pb^{2+} detection in real water samples.

With G-quadruplex DNAzyme as the biocatalyst for the amplified readout, dual DNAzyme-based sensors were developed on the basis of the Pb^{2+}-specific DNAzyme and G-quadruplex DNAzyme [76,77]. For instance, Zhu et al. designed a novel aptamer-DNAzyme hairpin structure, composed of Pb^{2+}-dependent DNAzyme and HRP-mimicking DNAzyme (G-quadruplex DNAzyme), which could be used as a sensor for colorimetric detection of Pb^{2+} [78]. In the absence of Pb^{2+}, the hairpin could not be open, and thus the formation of the G-quadruplex was inhibited, whereas the presence of Pb^{2+} induced the conformational change of the hairpin structure and activated the HRP-mimicking DNAzyme. The formed G-quadruplex DNAzyme then catalyzed the H_2O_2-mediated oxidation of ABTS to the colored ABTS•$^-$. Based on the color change, the concentration of Pb^{2+} could be determined with a detection limit of 10 nM. Selectivity results showed that the sensor had a highly specific response to Pb^{2+}. The proposed method was applied to evaluate Pb^{2+} concentration in Minjiang River (Fuzhou China) water samples. The results indicated that this proposed method could be used to detect Pb^{2+} in real water samples. Similarly, Zhang et al. designed an intramolecular stem-loop structure with the DNA/RNA-cleaving DNAzyme in the loop portion and the G-quadruplex DNAzyme sequence was caged in the double-stranded stem portion. In the presence of Pb^{2+}, the substrate strand is cleaved to fragments, resulting in the release of the DNAzyme sequence and subsequent formation of a G-quadruplex DNAzyme. Due to the dual signal amplifications of the Pb^{2+}-specific DNAzyme and the G-quadruplex DNAzyme, the sensing system exhibited high level of sensitivity with a detection limit of 14 nM [79].

The above mentioned Pb^{2+}-promoted G-quadruplex DNAzyme has been widely used for Pb^{2+} detection in laboratory, but it has never been applied to design a detection kit for Pb^{2+} detection. With this motivation, Liu et al. developed a detection kit using guaiacol (GA) as ideal colorimetric probe for facile detection of Pb^{2+} [80]. The detection limit had a working range of 10 nM to 100 nM with a low detection limit of 1 nM for Pb^{2+}. The shelf-life of the kit was 180 days at 4 °C, and the reliability of the kit was evaluated for Pb^{2+} determination in real food samples. The excellent performance characteristics of the detection kit indicated that it holds a promising application for low-cost and sensitive Pb^{2+} detection in foods.

3.3. Electrochemical G-quadruplex DNAzyme sensor

A very important feature of the G-quadruplex DNAzyme is the peroxidase-like activity, which is able to effectively catalyze the H_2O_2-mediated oxidation of the substrates with an obvious color change or lead to the generation of fluorescence in the presence of H_2O_2-AUR. Taking advantage of these properties, a number of colorimetric or fluorescent G-quadruplex DNAzyme-based biosensors have been developed for the amplified biosensing of Pb^{2+}. However, electrochemical assay is an interesting and convenient technique with high sensitivity and selectivity and electrochemical detection of small molecules by using the simple, low cost devices have recently received a good deal of attention, in comparison with the spectroscopic methods [81].

The peroxidase can catalyze oxidation polymerization of aniline to PANI [82], which is one of the most important conducting polymers due to its remarkable electrochemical and redox properties [83]. On the basis of this principle, Li et al. developed the first electrochemical G-quadruplex DNAzyme sensor for highly sensitive and selective detection of Pb^{2+} with aniline as the substrate [84]. The sensing principle for Pb^{2+} was shown in Fig. 6. First, the guanine-rich DNA was self-assembled on the electrode surface. Upon addition of Pb^{2+}, the guanine-rich DNA transformed into G-quadruplex structure, and the G-quadruplex DNAzyme with peroxidase catalytic activity was successfully achieved after binding with hemin. Subsequently, the G-quadruplex DNAzyme catalyzed oxidation of aniline to PANI in the G-quadruplex structure in the presence of H_2O_2, resulting in a readily measurable "turn-on" amperometric signal. The constructed assay exhibited good response toward Pb^{2+} with a low detection limit of 0.5 nM.

Afterwards, Zhang et al. also designed an in-situ amplified electrochemical sensing of Pb^{2+} based on G-quadruplex DNAzyme-catalyzed polymerization of aniline [85]. In the presence of Pb^{2+}, the Pb^{2+}-specific DNAzyme was activated and then cleaved the substrate strand at the RNA site (rA) into two parts, which triggering strand-induced DNA hybridization chain reaction events, and forming a nicked double-helix. Upon addition of hemin, the hemin-binding aptamer could form the DNAzyme. Hence, a large number of DNAzymes were cascaded through the double-helix DNA. Due to the generation of polyaniline through the large number of DNAzymes-catalyzed polymerization, the catalytic currents of the as-produced polyaniline were linearly dependent on target Pb^{2+} concentration from 0.05 nM to 50 nM with a low detection limit of 32 pM. The sensor was successfully applied for determination of Pb^{2+} in

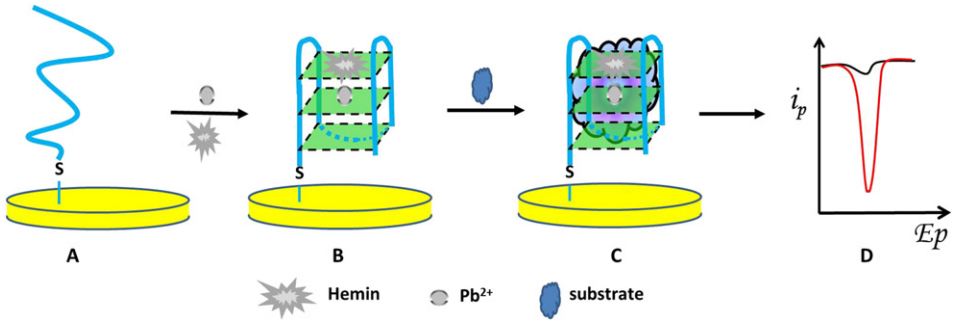


Fig. 6. Schematic illustration of Pb^{2+} sensing principle based on G-quadruplex DNAzyme-catalyzed deposition of PANI. A: Preparation of guanine-rich DNA modified gold electrode; B: Immersing the DNA film into Pb^{2+} and hemin solution, successively, to prepare G-quadruplex DNAzyme film; C: Catalyzed oxidation of aniline to PANI in the G-quadruplex structure in the presence of H_2O_2; D: Amperometric measurement.

Minjiang River (Fuzhou, China) water samples and showed good specificity and selectivity to Pb^{2+} over other metal ions, e.g., Zn^{2+}, Cu^{2+}, Cd^{2+}, Mn^{2+}, Mg^{2+}, Co^{2+} and Ni^{2+}.

Until now, unfortunately, there has been very few reports with respect to G-quadruplex DNAzyme-catalyzed deposition of substrate on the electrode surface in constructing Pb^{2+} sensors. So developing electrochemical G-quadruplex DNAzyme sensors for the detection of Pb^{2+} is still of a great challenge. Unlike PANI that possesses outstanding conductivity, we have previously confirmed that some phenolic compounds can also be catalytically oxidized by G-quadruplex DNAzyme in the presence of H_2O_2 and produce insoluble products precipitated on the electrode surface that reduced the conductivity of the electrode, based on which "signal-on" electrochemical impedance spectroscopy (EIS) sensors were developed with the merits of high sensitivity and very short response time [86,87]. Therefore, G-quadruplex DNAzymes have great potential in designing ultrasensitive Pb^{2+} sensors by using proper substrates (such as phenolic compounds) as signal amplifiers.

4. Conclusions

DNAzymes have attracted growing interest as catalytic labels for sensing events, which have become part of numerous applications in many areas. And they also provide a broad platform for constructing biosensors and have been customized for use in various signaling transduction strategies. This review presents fundamentals concerning the properties of the Pb^{2+}-specific DNAzyme and G-quadruplex DNAzyme, and their sensing principle of diverse type sensors. Examples of application of different DNAzymes sensors for the detection of Pb^{2+} with fluorescent, colorimetric and electrochemical readout are also described in this review. The rapid development of the DNAzyme-based sensing field is undoubtedly a result of their outstanding properties, and thereby the DNAzymes have the potential to be further applied to construct robust recognition and catalytic units for bioanalysis and nanobiotechnology in the future.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.microc.2016.12.010.

Acknowledgement

We are grateful for financial support from the Special Projects of Construction of Science and Technology Innovation Ability of Beijing Academy of Agriculture and Forestry Sciences (KJCX20170420, KJCX20150408), the Open Project of Beijing Research Center for Agricultural Standards and Testing (ZBZXKFKT201511) and National Basic Research Program of China (2013CB430400).

References

[1] D.O. Carpenter, K. Arcaro, D.C. Spink, Understanding the human health effects of chemical mixtures, Environ. Health Perspect. 110 (2002) 25.
[2] G. Liu, L. Tao, X. Liu, J. Hou, A. Wang, R. Li, Heavy metal speciation and pollution of agricultural soils along Jishui River in non-ferrous metal mine area in Jiangxi Province, China, J. Geochem. Explor. (2013).
[3] G. Liu, J. Wang, E. Zhang, J. Hou, X. Liu, Heavy metal speciation and risk assessment in dry land and paddy soils near mining areas at southern China, Environ. Sci. Pollut. Res. 23 (2016) 8709–8720.
[4] J.M. Llobet, G. Falcó, C. Casas, A. Teixidó, J.L. Domingo, Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain, J. Agric. Food Chem. 51 (2003) 838–842.
[5] P.C. Hsu, Y.L. Guo, Antioxidant nutrients and lead toxicity, Toxicology 180 (2002) 33–44.
[6] http://www.epa.gov/safewater/contaminants/index.html.
[7] Z. Wan, Z. Xu, J. Wang, Flow injection on-line solid phase extraction for ultra-trace lead screening with hydride generation atomic fluorescence spectrometry, Analyst 131 (2006) 141–147.
[8] R. Ma, W. Van Mol, F. Adams, Determination of cadmium, copper and lead in environmental samples. An evaluation of flow injection on-line sorbent extraction for flame atomic absorption spectrometry, Anal. Chim. Acta 285 (1994) 33–43.
[9] H. Elfering, J. Andersson, K. Poll, Determination of organic lead in soils and waters by hydride generation inductively coupled plasma atomic emission spectrometry, Analyst 123 (1998) 669–674.
[10] M. Ochsenkühn-Petropoulou, K.M. Ochsenkühn, Comparison of inductively coupled plasma–atomic emission spectrometry, anodic stripping voltammetry and instrumental neutron-activation analysis for the determination of heavy metals in airborne particulate matter, Fresenius J. Anal. Chem. 369 (2001) 629–632.
[11] A. Cocherie, M. Robert, Direct measurement of lead isotope ratios in low concentration environmental samples by MC-ICP-MS and multi-ion counting, Chem. Geol. 243 (2007) 90–104.
[12] G. Liang, T. Li, X.H. Li, X.H. Liu, Electrochemical detection of the amino-substituted naphthalene compounds based on intercalative interaction with hairpin DNA by electrochemical impedance spectroscopy, Biosens. Bioelectron. 48 (2013) 238–243.
[13] G. Bagni, S. Hernandez, M. Mascinl, E. Sturchio, P. Boccla, S. Marconi, DNA biosensor for rapid detection of genotoxic compounds in soil samples, Sensors 5 (2005) 394–410.
[14] X.B. Zhang, R.M. Kong, Y. Lu, Metal ion sensors based on DNAzymes and related DNA molecules, Annu. Rev. Anal. Chem. 4 (2011) 105–128.
[15] R.R. Breaker, G.F. Joyce, A. DNA, Enzyme that cleaves RNA, Chem. Biol. 1 (1994) 223–229.
[16] A.K. Brown, J. Li, C.M.B. Pavot, Y. Lu, A lead-dependent DNAzyme with a two-step mechanism, Biochemistry 42 (2003) 7152–7161.
[17] R.R. Breaker, G.F. Joyce, A. DNA, Enzyme with Mg^{2+}-dependent RNA phosphodiesterase activity, Chem. Biol. 2 (1995) 655–660.
[18] W. Zhou, Y. Zhang, J. Ding, J. Liu, In vitro selection in serum: RNA-cleaving DNAzymes for measuring Ca^{2+} and Mg^{2+}, ACS Sensors (2016).
[19] Y. Lu, New transition-metal-dependent DNAzymes as efficient endonucleases and as selective metal biosensors, Chem. Eur. J. 8 (2002) 4588–4596.
[20] Y. Xiang, Y. Lu, DNA as sensors and imaging agents for metal ions, Inorg. Chem. 53 (2013) 1925–1942.
[21] J. Li, Y. Lu, A highly sensitive and selective catalytic DNA biosensor for lead ions, J. Am. Chem. Soc. 122 (2000) 10466–10467.
[22] X. Miao, L. Ling, X. Shuai, Ultrasensitive detection of lead (II) with DNAzyme and gold nanoparticles probes by using a dynamic light scattering technique, Chem. Commun. 47 (2011) 4192–4194.
[23] C.W. Liu, C.C. Huang, H.T. Chang, Highly selective DNA-based sensor for lead (II) and mercury (II) ions, Anal. Chem. 81 (2009) 2383–2387.
[24] X. Zhao, R. Kong, X. Zhang, H. Meng, W. Liu, W. Tan, G. Shen, R. Yu, Graphene–DNAzyme based biosensor for amplified fluorescence "turn-on" detection of Pb^{2+} with a high selectivity, Anal. Chem. 83 (2011) 5062–5066.
[25] M. Long, H. Deng, G. Tian, C. Song, H. Liu, Y. Shen, C. Lv, A novel detection of radon based on its decay product inducing conformational changes of an aptamer probe, Anal. Chim. Acta (2016) http://dx.doi.org/10.1016/j.aca.2016.06.025.
[26] L. Zhang, B. Han, T. Li, E. Wang, Label-free DNAzyme-based fluorescing molecular switch for sensitive and selective detection of lead ions, Chem. Commun. 47 (2011) 3099–3101.

[27] G. Liang, Y. Man, X. Jin, L. Pan, X. Liu, Aptamer-based biosensor for label-free detection of ethanolamine by electrochemical impedance spectroscopy, Anal. Chim. Acta 936 (2016) 222–228.
[28] C.B. Swearingen, D.P. Wernette, D.M. Cropek, Y. Lu, J.V. Sweedler, P.W. Bohn, Immobilization of a catalytic DNA molecular beacon on Au for Pb(II) detection, Anal. Chem. 77 (2005) 442–448.
[29] T. Fu, S. Ren, L. Gong, H. Meng, L. Cui, R.-M. Kong, X.-B. Zhang, W. Tan, A label-free DNAzyme fluorescence biosensor for amplified detection of Pb^{2+}-based on cleavage-induced G-quadruplex formation, Talanta 147 (2016) 302–306.
[30] M. Liu, X. Lou, J. Du, M. Guan, J. Wang, X. Ding, J. Zhao, DNAzyme-based fluorescent microarray for highly selective and sensitive detection of lead (II), Analyst 137 (2012) 70–72.
[31] P. Zuo, B.C. Yin, B.C. Ye, DNAzyme-based microarray for highly sensitive determination of metal ions, Biosens. Bioelectron. 25 (2009) 935–939.
[32] C.M. Niemeyer, Nanoparticles, proteins, and nucleic acids: biotechnology meets materials science, Angew. Chem. Int. Ed. 40 (2001) 4128–4158.
[33] P. Chen, R. Zhang, Q. Jiang, X. Xiong, S. Deng, Colorimetric detection of lead ion based on gold nanoparticles and lead-stabilized G-quartet formation, J. Biomed. Sci. Eng. 8 (2015) 451.
[34] J.J. Storhoff, C.A. Mirkin, Programmed materials synthesis with DNA, Chem. Rev. 99 (1999) 1849–1862.
[35] M.C. Daniel, D. Astruc, Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology, Chem. Rev. 104 (2004) 293–346.
[36] J. Liu, Y. Lu, A colorimetric lead biosensor using DNAzyme-directed assembly of gold nanoparticles, J. Am. Chem. Soc. 125 (2003) 6642–6643.
[37] J. Liu, Y. Lu, Accelerated color change of gold nanoparticles assembled by DNAzymes for simple and fast colorimetric Pb^{2+} detection, J. Am. Chem. Soc. 126 (2004) 12298–12305.
[38] W. Zhao, J.C. Lam, W. Chiuman, M.A. Brook, Y. Li, Enzymatic cleavage of nucleic acids on gold nanoparticles: a generic platform for facile colorimetric biosensors, Small 4 (2008) 810–816.
[39] P. Wang, Y. Wan, A. Ali, S. Deng, Y. Su, C. Fan, S. Yang, Aptamer-wrapped gold nanoparticles for the colorimetric detection of omethoate, SCIENCE CHINA Chem. 59 (2015) 237–242.
[40] H. Li, L. Rothberg, Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles, Proc. Natl. Acad. Sci. 101 (2004) 14036–14039.
[41] H. Wei, B. Li, J. Li, S. Dong, E. Wang, DNAzyme-based colorimetric sensing of lead (Pb^{2+}) using unmodified gold nanoparticle probes, Nanotechnology 19 (2008) 095501.
[42] W. Yun, D. Cai, J. Jiang, P. Zhao, Y. Huang, G. Sang, Enzyme-free and label-free ultrasensitive colorimetric detection of Pb^{2+} using molecular beacon and DNAzyme based amplification strategy, Biosens. Bioelectron. 80 (2016) 187–193.
[43] S. Liu, W. Wei, X. Sun, L. Wang, Ultrasensitive electrochemical DNAzyme sensor for lead ion based on cleavage-induced template-independent polymerization and alkaline phosphatase amplification, Biosens. Bioelectron. 83 (2016) 33–38.
[44] Q. Zhou, Y. Lin, Y. Lin, Q. Wei, G. Chen, D. Tang, Highly sensitive electrochemical sensing platform for lead ion based on synergetic catalysis of DNAzyme and Au–Pd porous bimetallic nanostructures, Biosens. Bioelectron. 78 (2016) 236–243.
[45] J. Zhang, Y. Tang, L. Teng, M. Lu, D. Tang, Low-cost and highly efficient DNA biosensor for heavy metal ion using specific DNAzyme-modified microplate and portable glucometer-based detection mode, Biosens. Bioelectron. 68 (2015) 232–238.
[46] F. Li, Y. Feng, C. Zhao, B. Tang, Crystal violet as a G-quadruplex-selective probe for sensitive amperometric sensing of lead, Chem. Commun. 47 (2011) 11909–11911.
[47] J. Zhuang, L. Fu, M. Xu, Q. Zhou, G. Chen, D. Tang, DNAzyme-based magneto-controlled electronic switch for picomolar detection of lead (II) coupling with DNA-based hybridization chain reaction, Biosens. Bioelectron. 45 (2013) 52–57.
[48] J. Gu, X. Lu, H. Ju, DNA sensor for recognition of native yeast DNA sequence with methylene blue as an electrochemical hybridization indicator, Electroanalysis 14 (2002) 949.
[49] Y. Xiao, A.A. Rowe, K.W. Plaxco, Electrochemical detection of parts-per-billion lead via an electrode-bound DNAzyme assembly, J. Am. Chem. Soc. 129 (2007) 262–263.
[50] S. Tang, P. Tong, X. You, W. Lu, J. Chen, G. Li, L. Zhang, Label free electrochemical sensor for Pb^{2+} based on graphene oxide mediated deposition of silver nanoparticles, Electrochim. Acta 187 (2016) 286–292.
[51] L. Shen, Z. Chen, Y. Li, S. He, S. Xie, X. Xu, Z. Liang, X. Meng, Q. Li, Z. Zhu, Electrochemical DNAzyme sensor for lead based on amplification of DNA-Au bio-bar codes, Anal. Chem. 80 (2008) 6323–6328.
[52] X. Yang, J. Xu, X. Tang, H. Liu, D. Tian, A novel electrochemical DNAzyme sensor for the amplified detection of Pb^{2+} ions, Chem. Commun. 46 (2010) 3107–3109.
[53] H.X. Zhang, B.Y. Jiang, Y. Xiang, J. Su, Y.Q. Chai, R. Yuan, DNAzyme-based highly sensitive electronic detection of lead via quantum dot-assembled amplification labels, Biosens. Bioelectron. 28 (2011) 135–138.
[54] S. Tang, W. Lu, F. Gu, P. Tong, Z. Yan, L. Zhang, A novel electrochemical sensor for lead ion based on cascade DNA and quantum dots amplification, Electrochim. Acta 134 (2014) 1–7.
[55] S. Ge, K. Wu, Y. Zhang, M. Yan, J. Yu, Paper-based biosensor relying on flower-like reduced graphene guided enzymatically deposition of polyaniline for Pb^{2+} detection, Biosens. Bioelectron. 80 (2016) 215–221.
[56] L. Luo, Z. Zhang, Y. Ding, D. Deng, X. Zhu, Z. Wang, Label-free electrochemical impedance genosensor based on L-aminopyrene/graphene hybrids, Nanoscale 5 (2013) 5833–5840.
[57] Y. Zhou, L. Tang, G. Zeng, C. Zhang, X. Xie, Y. Liu, J. Wang, J. Tang, Y. Zhang, Y. Deng, Label free detection of lead using impedimetric sensor based on ordered mesoporous carbon–gold nanoparticles and DNAzyme catalytic beacons, Talanta 146 (2016) 641–647.
[58] L. Shi, G. Liang, X.H. Li, X.H. Liu, D.N.A. Impedimetric, Sensor for detection of Hg^{2+} and Pb^{2+}, Anal. Methods 4 (2012) 1036–1040.
[59] Y. Tan, J. Qiu, M. Cui, X. Wei, M. Zhao, B. Qiu, G. Chen, An immobilization free DNAzyme based electrochemical biosensor for lead determination, Analyst 141 (2016) 1121–1126.
[60] G.C. Han, X.Z. Feng, Z.C. Chen, Hemin/G-quadruplex DNAzyme for designing of electrochemical sensors, Int. J. Electrochem. Sci. 10 (2015) 3897–3913.
[61] J. Kosman, B. Juskowiak, Peroxidase-mimicking DNAzymes for biosensing applications: a review, Anal. Chim. Acta 707 (2011) 7–17.
[62] B. Shen, Q. Wang, D. Zhu, J. Luo, G. Cheng, P. He, Y. Fang, G-quadruplex-based DNAzymes aptasensor for the amplified electrochemical detection of thrombin, Electroanalysis 22 (2010) 2985–2990.
[63] L. Guo, D. Nie, C. Qiu, Q. Zheng, H. Wu, P. Ye, Y. Hao, F.F. Fu, G. Chen, A G-quadruplex based label-free fluorescent biosensor for lead ion, Biosens. Bioelectron. 35 (2012) 123–127.
[64] Q. Long, H. Li, Y. Zhang, S. Yao, Upconversion nanoparticle-based fluorescence resonance energy transfer assay for organophosphorus pesticides, Biosens. Bioelectron. 68 (2015) 168–174.
[65] Y. Zhao, Q. Zhang, W. Wang, Y. Jin, Input-dependent induction of G-quadruplex formation for detection of lead (II) by fluorescent ion logic gate, Biosens. Bioelectron. 43 (2013) 231–236.
[66] W. Wang, Y. Jin, Y. Zhao, X. Yue, C. Zhang, Single-labeled hairpin probe for highly specific and sensitive detection of lead (II) based on the fluorescence quenching of deoxyguanosine and G-quartet, Biosens. Bioelectron. 41 (2013) 137–142.
[67] Y. Zang, J. Lei, Q. Hao, H. Ju, "Signal-on" photoelectrochemical sensing strategy based on target-dependent aptamer conformational conversion for selective detection of lead(II) ion, Appl. Mater. Interfaces 6 (2014) 15991–15997.
[68] C.L. Li, K.T. Liu, Y.W. Lin, H.T. Chang, Fluorescence detection of lead(II) ions through their induced catalytic activity of DNAzymes, Anal. Chem. 83 (2010) 225–230.
[69] C.L. Li, C.C. Huang, W.H. Chen, C.K. Chiang, H.T. Chang, Peroxidase mimicking DNA-gold nanoparticles for fluorescence detection of the lead ions in blood, Analyst 137 (2012) 5222–5228.
[70] X. Chen, N. Zhai, J.H. Snyder, Q. Chen, P. Liu, L. Jin, Q. Zheng, F. Lin, J. Hu, H. Zhou, Colorimetric detection of Hg^{2+} and Pb^{2+} based on peroxidase-like activity of graphene oxide–gold nanohybrids, Anal. Methods 7 (2015) 1951–1957.
[71] B.Q. Liu, B. Zhang, G.N. Chen, D.P. Tang, An omega-like DNA nanostructure utilized for small molecule introduction to stimulate formation of DNAzyme–aptamer conjugates, Chem. Commun. 50 (2014) 1900–1902.
[72] H. Sun, X. Li, Y. Li, L. Fan, H.-B. Kraatz, A novel colorimetric potassium sensor based on the substitution of lead from G-quadruplex, Analyst 138 (2012) 856–862.
[73] T. Li, E.K. Wang, S.J. Dong, Lead (II)-induced allosteric G-quadruplex DNAzyme as a colorimetric and chemiluminescence sensor for highly sensitive and selective Pb^{2+} detection, Anal. Chem. 82 (2010) 1515–1520.
[74] T. Li, E.K. Wang, S.J. Dong, Potassium-lead-switched G-quadruplexes: a new class of DNA logic gates, J. Am. Chem. Soc. 131 (2009) 15082–15083.
[75] Y. Wang, J. Wang, F. Yang, X. Yang, Spectrophotometric detection of lead (II) ion using unimolecular peroxidase-like deoxyribozyme, Microchim. Acta 171 (2010) 195–201.
[76] J. Elbaz, B. Shlyahovsky, I. Willner, A. DNAzyme, Cascade for the amplified detection of Pb^{2+} ions or L-histidine, Chem. Commun. (2008) 1569–1571.
[77] C. Teller, S. Shimron, I. Willner, Aptamer–DNAzyme hairpins for amplified biosensing, Anal. Chem. 81 (2009) 9114–9119.
[78] X. Zhu, X. Gao, Q. Liu, Z. Lin, B. Qiu, G. Chen, Pb^{2+}-introduced activation of horseradish peroxidase (HRP)-mimicking DNAzyme, Chem. Commun. 47 (2011) 7437–7439.
[79] Q. Zhang, Y. Cai, H. Li, D.M. Kong, H.X. Shen, Sensitive dual DNAzymes-based sensors designed by grafting self-blocked G-quadruplex DNAzymes to the substrates of metal ion-triggered DNA/RNA-cleaving DNAzymes, Biosens. Bioelectron. 38 (2012) 331–336.
[80] T. Liu, G. Nie, X. Zhang, W. Liu, W. Zhang, Y. Wang, D. Zhang, J. Wang, Development of a detection kit based on G-quadruplex DNAzyme for detection of lead(II) ion in food samples, Food Anal. Methods 8 (2015) 1133–1140.
[81] A. Mehdinia, S.H. Kazemi, S.Z. Bathaie, A. Alizadeh, M. Shamsipur, M.F. Mousavi, D.N.A. Electrochemical, Nano-biosensor for the study of spermidine–DNA interaction, J. Pharm. Biomed. Anal. 49 (2009) 587–593.
[82] Q. Sheng, J. Zheng, Bienzyme system for the biocatalyzed deposition of polyaniline templated by multiwalled carbon nanotubes: a biosensor design, Biosens. Bioelectron. 24 (2009) 1621–1628.
[83] K. Amano, H. Ishikawa, A. Kobayashi, M. Satoh, E. Hasegawa, Thermal stability of chemically synthesized polyaniline, Synth. Met. 62 (1994) 229–232.
[84] F. Li, L. Yang, M. Chen, Y. Qian, B. Tang, A novel and versatile sensing platform based on HRP-mimicking DNAzyme-catalyzed template-guided deposition of polyaniline, Biosens. Bioelectron. 41 (2012) 903–906.
[85] B. Zhang, J. Chen, B. Liu, D. Tang, Amplified electrochemical sensing of lead ion based on DNA-mediated self-assembly-catalyzed polymerization, Biosens. Bioelectron. 69 (2015) 230–234.
[86] G. Liang, X.H. Liu, G-quadruplex based impedimetric 2-hydroxyfluorene biosensor using hemin as a peroxidase enzyme mimic, Microchim. Acta 182 (2015) 2233–2240.
[87] G. Liang, X.H. Liu, X.H. Li, Highly sensitive detection of α-naphthol based on G-DNA modified gold electrode by electrochemical impedance spectroscopy, Biosens. Bioelectron. 45 (2013) 46–51.