A sensitive fluorescence anisotropy method for detection of lead (II) ion by a G-quadruplex-inducible DNA aptamer

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HIGHLIGHTS

- A fluorescence anisotropy approach for detection of Pb²⁺ was developed.
- The strategy was based on binding-induced allosteric conformational change of aptamer probe.
- The sensing mechanism was established by testing the photoinduced electron transfer interaction.

GRAPHICAL ABSTRACT

ARTICLE INFO

Article history:

Received 20 August 2013

Received in revised form

10 December 2013

Accepted 24 December 2013

Available online 3 January 2014

Keywords:

Lead ion

G-quadruplex

Fluorescence anisotropy

Thrombin binding aptamer

ABSTRACT

Sensitive and selective detection of Pb²⁺ is of great importance to both human health and environmental protection. Here we propose a novel fluorescence anisotropy (FA) approach for sensing Pb²⁺ in homogeneous solution by a G-rich thrombin binding aptamer (TBA). The TBA labeled with 6-carboxytetramethylrhodamine (TMR) at the seventh thymine nucleotide was used as a fluorescent probe for signaling Pb²⁺. It was found that the aptamer probe had a high FA in the absence of Pb²⁺. This is because the rotation of TMR is restricted by intramolecular interaction with the adjacent guanine bases, which results in photoinduced electron transfer (PET). When the aptamer probe binds to Pb²⁺ to form G-quadruplex, the intramolecular interaction should be eliminated, resulting in faster rotation of the fluorophore TMR in solution. Therefore, FA of aptamer probe is expected to decrease significantly upon binding to Pb²⁺. Indeed, we observed a decrease in FA of aptamer probe upon Pb²⁺ binding. Circular dichroism, fluorescence spectra, and fluorescence lifetime measurement were used to verify the reliability and reasonability of the sensing mechanism. By monitoring the FA change of the aptamer probe, we were able to real-time detect binding between the TBA probe and Pb²⁺. Moreover, the aptamer probe was exploited as a recognition element for quantification of Pb²⁺ in homogeneous solution. The change in FA showed a linear response to Pb²⁺ from 10 nM to 2.0 μM, with 1.0 nM limit of detection. In addition, this sensing system exhibited good selectivity for Pb²⁺ over other metal ions. The method is simple, quick and inherits the advantages of aptamer and FA.

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1. Introduction

Lead ion (Pb²⁺), a heavy metal environmental pollutant, poses a severe risk to human health and the environment. For example, it can cause renal malfunction and impair brain development, especially to children [1]. Thus, monitoring Pb²⁺

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level in aqueous solution is an important task. Atomic absorption spectroscopy (AAS) [2], inductively coupled plasma optical emission spectroscopy (ICP-OES) [3], inductively coupled plasma mass spectrometry (ICP-MS) [4], anodic stripping voltammetry [5] and reversed-phase high-performance liquid chromatography (RP-HPLC) [6] are the most common techniques for the detection of Pb^{2+} . Nevertheless, these techniques are time-consuming, expensive, and require sophisticated equipment. Therefore, the development of a simple, inexpensive, sensitive, and on-site sensor for Pb^{2+} is very important for environmental and food monitoring.

Recently, functional DNA molecules have received great attention for Pb^{2+} detection, with the development of the investigation into the interaction between DNA and metal ions [7]. Two types of functional nucleic acid molecules are used to detect Pb^{2+} . One is Pb^{2+} -dependent DNAzyme, which is composed of an enzyme strand and a substrate strand [8,9]. The substrate contains a single RNA linkage (rA), which serves as the cleavage site. In the presence of Pb^{2+} , the enzyme cleaves the substrate strand at the rA position into two fragments. There are two types of Pb^{2+} -dependent DNAzymes, 8–17 DNAzyme [10] and GR-5 DNAzyme [11]. Of these, 8–17 DNAzyme has attracted much more attention. They have been extensively explored for Pb^{2+} detection via fluorescence [12–29], fluorescence anisotropy [30], colorimetry [31–34], electrochemistry [35–38], electrochemiluminescence [39], dynamic light scattering [40,41], and surface enhanced Raman scattering [42].

The other type of functional DNA is Pb^{2+} -induced allosteric G-quadruplex oligonucleotides. Pb^{2+} has high binding affinity to these oligonucleotides and induces the formation of G-quadruplexes. G-quadruplexes are highly-ordered structures of G-rich nucleic acid sequences, which form stacked arrays of G-quartets connected by Hoogsteen-type base pairing [43]. In addition, some of these G-quadruplexes, with hemin as a co-factor, exhibit superior peroxidase-like activity and can effectively catalyze the H_2O_2 -mediated oxidation of 2,2'-azino-Bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) or luminol [44]. Based on Pb^{2+} -induced allosteric G-quadruplex oligonucleotides and the effect of Pb^{2+} on peroxidase-mimetic activity, highly sensitive, and selective Pb^{2+} -sensors have utilized these properties to generate fluorescent [45–49], colorimetric [50,51], luminescent [52], electrochemical [53–55], and resonance scattering signals [56,57]. Compared with Pb^{2+} -dependent RNA-cleavage DNAzymes, G-quadruplexes have a relatively low cost of synthesis and high stability. However, less attention has been paid to G-quadruplexes for sensing Pb^{2+} .

Although various fluorescence methods based on functional DNA have been developed for detection of Pb^{2+} , to the best of our knowledge, there is no fluorescence anisotropy (FA) method for Pb^{2+} detection based on the induction of G-quadruplex formation. FA has the advantages of simplicity, rapid response, real-time detection, and single-fluorophore labeling. In addition, FA assay does not require use of radioactive compounds and separation step. Most importantly, FA is a ratiometric detection technique, so it is not susceptible to the photo-bleaching and the fluctuation of light source [58]. In general, FA is sensitive to the change in molecular size and increases with increasing molecular size. Therefore, FA can be used in direct assay for macromolecular targets such as proteins and even cells. However, to the small molecules, the binding of small molecules to the fluorescent probe only induce a small change in FA, so they are usually detected by indirect FA assay, e.g., competitive displacement and mass amplification [59,60].

Here we present a direct FA technique for sensitive and selective detection of Pb^{2+} based on the conformational change of thrombin binding aptamer (TBA) probe, which switches from the random-coil state to a highly-ordered G-quadruplex structure upon binding to Pb^{2+} . The probe was obtained by internal labeling of the TBA with a

single fluorophore (6-carboxytetramethylrhodamine (TMR)) at the seventh thymidine nucleotide. The sensing mechanism is probable that the intramolecular interaction between TMR and adjacent guanine bases via photoinduced electron transfer (PET) mechanism is eliminated upon Pb^{2+} -binding to the TBA probe to form G-quadruplex, which results in the increase in fluorescence lifetime of the fluorophore and the reduction in FA. Thus, Pb^{2+} can be quantitatively detected by monitoring the change in FA, resulting from the conformational change of TBA probe.

2. Material and methods

2.1. Reagents and materials

The thrombin binding aptamer probes (5'-GGT TGG TGT GGT TGG-3') and the mutant aptamer probes (see supporting information Table S1) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). These were fluorescently labeled at the specified position with a single TMR and purified by HPLC. The concentration of oligonucleotides is given as the single-stranded form, quantified by measuring absorbance at 260 nm using a Nanodrop 2000 (Thermo Scientific, Wilmington, DE). Lead acetate and acetate acid were purchased from National Pharmaceutical Group Chemical Reagent Company (Beijing, China). Tris(hydroxymethyl)aminomethane (Tris) was purchased from Amresco (Solon, OH). All reagents were used as received without further purification. Ultrapure water with an electrical resistivity of 18.2 M Ω ·cm was obtained from a Purelab Ultra Elga Lab water system (VWS Ltd., High Wycombe, Bucks HP14 3JH, UK). Because Pb^{2+} and some of the other heavy metal ions tested are highly toxic, they should be used with caution.

2.2. Fluorescence anisotropy analysis

Unless otherwise stated, the binding buffer for FA assay was 10 mM Tris-HAc, pH 7.5. The oligonucleotides and Pb^{2+} stock solution were prepared in sterile pure water at 100 μM and 1 M, which were stored at -20°C and room temperature, respectively. DNA solutions (20 nM) were diluted in 10 mM Tris-HAc buffer (pH = 7.5). Different concentrations of Pb^{2+} were added to the solution (final volume 200 μL), and the mixture incubated for 30 min at room temperature before FA measurements. FA was measured using a Fluoromax-4 spectrometer (Horiba Jobin Yvon, Edison, NJ). A 150 μL quartz cuvette was used for all measurements. The excitation and emission wavelengths were set at 555 and 580 nm, and their corresponding slit bandwidths were set at 5 and 10 nm, respectively. The integration time was 0.5 s. The background signal of the buffer solution was subtracted from the collected data. FA was measured using the L-format configuration. FA (r) was defined as:

$$r = \frac{(I_{VV} - G \times I_{VH})}{(I_{VV} + 2G \times I_{VH})} \quad (1)$$

$$G = \frac{I_{HV}}{I_{HH}} \quad (2)$$

where I and G are the measured fluorescence intensity and the grating factor of the spectrometer, respectively. G factor was measured according to the manufacturer's instructions using the free fluorophore. The subscripts V and H refer to vertical and horizontal orientation of the polarizer. The first subscript and the second subscript indicate the position of the excitation polarizer and emission polarizer, respectively.

To characterize the affinity between Pb^{2+} and the fluorophore-labeled aptamer probe, we used nonlinear fitting to model the

observed change in FA upon titration with Pb^{2+} . A simple model was assumed for aptamer binding to Pb^{2+} as follows [61,62]:

The dissociation constants (K_d) were determined as follows:

$$K_d = \frac{[\text{Apt}] \times [\text{Pb}^{2+}]}{[\text{Apt} \times \text{Pb}^{2+}]} = \frac{([\text{Apt}]_0 - [\text{Apt} \times \text{Pb}^{2+}])([\text{Pb}^{2+}]_0 - [\text{Apt} \times \text{Pb}^{2+}])}{[\text{Apt} \times \text{Pb}^{2+}]} \quad (4)$$

where $[\text{Apt}]_0$ and $[\text{Pb}^{2+}]_0$ are the total concentrations of aptamer and Pb^{2+} , respectively. $[\text{Apt} \times \text{Pb}^{2+}]$ is the concentration of the bound aptamer or Pb^{2+} .

Solving equation 4 for $[\text{Apt} \times \text{Pb}^{2+}]$ gives:

$$[\text{Apt} \times \text{Pb}^{2+}] = \frac{([\text{Apt}]_0 + [\text{Pb}^{2+}]_0 + K_d) - \sqrt{(-[\text{Apt}]_0 - [\text{Pb}^{2+}]_0 - K_d)^2 - 4[\text{Apt}]_0[\text{Pb}^{2+}]_0}}{2} \quad (5)$$

The relationship between the concentration of the bound aptamer and the steady-state FA can be determined as follows:

$$[\text{Apt} \times \text{Pb}^{2+}] = \frac{[\text{Apt}]_0(r - r_u)}{(r_b - r_u)} \quad (6)$$

where r is the steady-state FA, r_u is the FA of the aptamer probe in the absence of Pb^{2+} , and r_b is the FA in saturated Pb^{2+} . So the final fitting equation is:

$$\frac{[\text{Apt}]_0(r - r_u)}{(r_b - r_u)} = \frac{([\text{Apt}]_0 + [\text{Pb}^{2+}]_0 + K_d) - \sqrt{(-[\text{Apt}]_0 - [\text{Pb}^{2+}]_0 - K_d)^2 - 4[\text{Apt}]_0[\text{Pb}^{2+}]_0}}{2} \quad (7)$$

The dissociation constant, K_d , was determined by fitting the bound aptamer (correlating with the measured FA) as a function of the total concentration of titrated Pb^{2+} ($[\text{Pb}^{2+}]_0$) using the non-linear least square approach.

2.3. Fluorescence lifetime measurements

The TBA solution (2 μM) was prepared in 10 mM Tris-HAc (pH 7.5). This solution was added to 4 μM Pb^{2+} (final volume 200 μL), and the mixture was allowed to incubate at room temperature for 30 min. The fluorescence lifetime of the TMR fluorescent probe was measured using a home-built fluorescence detection system at room temperature, using time-correlated single photon counting (TCSPC) [63]. The fluorescence lifetime was obtained by fitting a biexponential fluorescence decay curve.

2.4. Circular dichroism (CD) analysis

CD spectra were obtained on a JASCO J-815 spectrometer (Tokyo, Japan) at room temperature. DNA solutions (2.5 μM) were prepared in 10 mM Tris-HAc buffer (pH 7.5). 5 μM Pb^{2+} was added (final volume 300 μL), and then the mixture was allowed to incubate at room temperature for 30 min. CD spectra were recorded between 220 and 350 nm with a 10 mm path length quartz cuvette. The scan rate was set at 100 nm/min with a response time of 1 s and a bandwidth of 0.5 nm. The spectra were averaged over 3 scans. The background signal of the 10 mM Tris-HAc (pH 7.5) buffer was subtracted from all CD data.

3. Results and discussion

3.1. Principle of sensing Pb^{2+} by FA

Pb^{2+} can effectively stabilize G-rich oligonucleotides, forming a more compact G-quadruplex than that stabilized by K^+ [64,65].

Thus, G-rich oligonucleotides undergo conformational changes upon addition of Pb^{2+} . Based on our recent finding that the FA of a G-rich oligonucleotide probe dramatically decreased upon formation of a G-quadruplex [66], we hypothesize that if the reduction in FA is dependent on Pb^{2+} , a novel FA method for Pb^{2+} detection might be possible. Here TBA, a G-quadruplex oligonucleotide, was chosen as the recognition element for sensing aqueous Pb^{2+} by measuring the reduction in FA. Scheme 1 shows the FA-based Pb^{2+} sensing strategy using a TBA probe. A fluorophore (TMR) is internally labeled at the seventh thymine nucleotide of TBA (T7-TMR-TA15), which could be used for sensitive FA detection. The TBA has a random-coil structure in the absence of Pb^{2+} . Because the internally labeled TMR can intramolecularly interact with the adjacent guanine bases by photo-induced electron transfer (PET) mechanism due to its good electron donating property [67–71], the

rotation of TMR is firmly restricted, which results in the aptamer probe having large FA value. In the presence of Pb^{2+} , because Pb^{2+} can interact with aptamer probe and induce conformational change from a random-coil structure into a highly-ordered G-quadruplex [65,72], this would weaken or eliminate the intramolecular interaction between the TMR and the adjacent guanine bases involved in formation of the G-quadruplex. Thus, the fluorophore TMR

has more freedom and rotates faster. FA is a measure of the diffusional rotation of the fluorophore and is inversely proportional to the rate of diffusional rotation, so this results in a sharp reduction in FA of the TBA probe. It was really observed that the FA of T7-TMR-TA15 significantly decreased from $r=0.191$ to $r=0.133$ upon addition of 500 nM Pb^{2+} ($\Delta r=-0.058$, see Fig. 1A).

To test whether the reduction in FA of T7-TMR-TA15 was due to the conformational change, CD was used to confirm the formation of a G-quadruplex in the presence of Pb^{2+} , because G-quadruplexes have the characteristic CD spectra [73]. As shown in Fig. 1B, the CD spectrum of T7-TMR-TA15 is of relatively low amplitude in the absence of Pb^{2+} , which suggests that the aptamer probe possesses little or no G-quadruplex structure in the absence of Pb^{2+} . When the probe incubated with Pb^{2+} , the spectrum displayed a positive peak at longer wavelength near 310 nm and a negative peak near 268 nm, which is consistent with the Pb^{2+} -stabilized anti-parallel G-quadruplex structure [64,72]. To further confirm that the large reduction in FA resulted from Pb^{2+} -inducing G-quadruplex formation, we examined a mutant aptamer probe (T7-TMR-TA15-G1T) obtained by only substitution of the first G with T. The CD spectrum of this mutant aptamer probe showed no conformation change and no characteristics of G-quadruplex structure in the presence of Pb^{2+} (see Fig. 1B), and the FA of this mutant aptamer showed nearly no change ($\Delta r=0.003$, see Fig. 1A).

It is known that FA is sensitive to the change of the rotation of the fluorophore, which depends on molecular volume, fluorescence lifetime, temperature, and solution viscosity. If the solution temperature and viscosity are constant, FA is related to molecular volume and fluorescence lifetime. Here, an increase in molecular volume is excluded to produce reduction in FA. Firstly, it is not consistent with the traditional concept that the FA increases with the molecular size of the fluorescent probe. In addition, the molecular weight of Pb^{2+} (MW = 207) is insignificant relative to T7-TMR-TA15 (MW = 5291). Therefore, we subsequently determined the fluorescence lifetime of T7-TMR-TA15 in the presence or absence of Pb^{2+} .

Scheme 1. Schematic illustration of the use of a thrombin binding aptamer (TBA) probe for fluorescence anisotropy (FA) detection of Pb²⁺.

As shown in Fig. 2, the average fluorescence lifetime (τ) of the T7-TMR-TA15 probe increased from 2.07 to 2.96 ns upon it binding to Pb²⁺. According to the Perrin equation [74], FA is inversely proportional to the fluorescence lifetime (τ), so the FA of T7-TMR-TA15 would decrease upon it binding to Pb²⁺. In addition, we observed that the aptamer probe exhibited about 2.5-fold fluorescence enhancement upon binding to Pb²⁺ to form stabilized G-quadruplex (see supporting information Fig. S1). The increase in fluorescence lifetime and the enhancement of fluorescence intensity are consistent with PET mechanism reported by others and

our previous studies [63,66,70,71,75]. These results suggest that the intramolecular interaction between the labeled TMR and adjacent guanine bases in the aptamer probe gives rise to PET, which is eliminated upon binding to Pb²⁺, resulting in the formation of a G-quadruplex.

3.2. Optimization of sensing conditions

FA was first studied as a function of incubation time to investigate the kinetics of formation of the Pb²⁺-G-quadruplex complex. As shown in Fig. 3A, the FA of the aptamer probe T7-TMR-TA15 dramatically decreased from 0.192 to 0.128 ($\Delta r = -0.064$) in the presence of 1 μM Pb²⁺. The reaction reached equilibrium within 1 min (20 s for mixing the solution and 40 s for three measurements) and maintained that equilibrium at least for 30 min. The results suggest that the aptamer probe binding to Pb²⁺ is rapid, occurring within 1 min to form a stable complex. Thus, FA measurements were performed within an incubation time of 30 min.

It is known that the position of the fluorophore influences the change in FA [76,77], so a further study was performed to investigate the effect of the labeling position on FA response by these TBA probes. The TMR dye was conjugated by a flexible linker to the 5'-terminal or third, fourth, seventh, ninth, twelfth or thirteenth thymine nucleotides (5'-, T3, T4, T7, T9, T12 and T13) of TBA [78]. As shown in Fig. 3B, these aptamer probes exhibited different changes in FA (Δr) upon the addition of 500 nM Pb²⁺. It is observed that T7-TMR-TA15 exhibited the largest change in FA ($\Delta r = -0.059$) upon binding to Pb²⁺, so T7-TMR-TA15 was chosen as the best Pb²⁺ sensing probe. The results suggest that the FA change of aptamer upon binding to Pb²⁺ is influenced by the local environment of fluorophore TMR. Because the FA value typically reflects

Fig. 1. (A) Fluorescence anisotropy (FA) of 20 nM T7-TMR-TA15 and T7-TMR-TA15-G1T in the absence (black bars) and presence (gray bars) of 500 nM Pb²⁺, and the fluorescence anisotropy change (Δr , white bars) of the corresponding aptamers. (B) Circular dichroism of 2.5 μM T7-TMR-TA15 (square) and T7-TMR-TA15-G1T (circle) in the absence (solid) and presence (open) of 5 μM Pb²⁺. A 10 mM Tris-HAc (pH 7.5) buffer was used. The results represent the mean of 3 measurements, and the error bars represent the standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Fluorescence decay time profiles of 2 μM T7-TMR-TA15 in the absence (square) and presence (circle) of 4 μM Pb²⁺. A 10 mM Tris-HAc (pH 7.5) buffer was used.

Fig. 3. (A) Fluorescence anisotropy change of 20 nM T7-TMR-TA15 upon binding to 1 μ M Pb^{2+} . Pb^{2+} solution was added at 0 min. (B) Fluorescence anisotropy change (Δr) of a TBA probe labeled with TMR at 5', T3, T4, T7, T9, T12, or T13 upon addition of 500 nM Pb^{2+} . A 10 mM Tris-HAc buffer was used. The results represent the mean of 3 measurements, and the error bars represent the standard deviation.

the integral rotation of the molecule, which contributes from the local rotational freedom of the fluorescent reporter and the global rotational diffusion of the entire species [76]. No matter what the TBA is present in a random-coil structure or a highly-ordered G-quadruplex structure, the contribution of the global rotational diffusion of the entire species is equal to these different positional labeling aptamer probes. So the different FA and FA change of these TBA probes result from the different local environment of TMR in the aptamer.

Next, another study was performed to investigate the pH-dependence of FA change (Δr) of T7-TMR-TA15 in the absence and presence of 1 μ M Pb^{2+} . As illustrated in Fig. S2A (see supporting information), the FA change (Δr) slowly decreased from pH 5.0 ($\Delta r = -0.054$) to pH 7.5 ($\Delta r = -0.066$), and then dramatically increased when the pH of solution was greater than 7.5. Accordingly, a buffer solution of pH 7.5 was selected as binding buffer.

Furthermore, we investigated the effect of ion strength on the FA change (Δr) of T7-TMR-TA15 upon binding to Pb^{2+} . The FA change increased promptly as the Na^+ concentration was increased (see Fig. S2B in supporting information). The results indicate that it is unhelpful for T7-TMR-TA15 binding to Pb^{2+} to form stable G-quadruplex in the presence of Na^+ . Therefore, buffer without containing Na^+ was used to obtain a good response for Pb^{2+} detection.

Fig. 4. (A) Fluorescence anisotropy change (Δr) of 20 nM T7-TMR-TA15 titrated with increasing concentrations of Pb^{2+} . The insert shows the linear relationship between the change in fluorescence anisotropy (Δr) and the logarithm of Pb^{2+} concentration. A 10 mM Tris-HAc (pH 7.5) buffer was used. The results represent the mean of 3 measurements, and the error bars represent the standard deviation. (B) Binding curve showing the bound aptamer probe as a function of Pb^{2+} concentration. The solid squares show experimental data; the line shows a nonlinear fit of the data to a single-component binding equation.

3.3. FA detection of Pb^{2+}

Based on this Pb^{2+} -stabilized G-quadruplex system, the concentration of Pb^{2+} can be determined by monitoring the change in FA of T7-TMR-TA15 under the optimal conditions. As shown in Fig. 4A, the FA change (Δr) of T7-TMR-TA15 gradually decreases with increasing concentrations of Pb^{2+} . The insertion in Fig. 4 reveals a good linear response to the logarithm of Pb^{2+} concentration range from 10 to 2000 nM ($R^2 = 0.981$). The limit of detection (LOD) for Pb^{2+} was 1 nM based on a signal to noise (S/N) ratio of 3. Furthermore, this method displayed comparable or better performance than some previous oligonucleotide-based biosensors (see supporting information Table S2). The results indicated that the proposed Pb^{2+} biosensor could potentially be used to monitor the level of Pb^{2+} in environmental and food samples, because the U.S. Environmental Protection Agency permits a maximum level of Pb^{2+} in drinking water to be 72 nM [79].

In addition, the affinity between T7-TMR-TA15 and Pb^{2+} could be determined by analyzing the FA of this aptamer probe with increasing concentrations of Pb^{2+} . As described in the experimental section, one-to-one (1:1) binding stoichiometry was assumed for calculating the dissociation constant (K_d). The dissociation constant (K_d) for T7-TMR-TA15 and Pb^{2+} was estimated to be $K_d = 96 \pm 6.1$ nM (see Fig. 4B), which was in agreement with the binding constant

Fig. 5. (A) Fluorescence anisotropy change (Δr) of 20 nM T7-TMR-TA15 in the presence of 500 nM Pb^{2+} or other metal ions (Mn^{2+} , Co^{2+} , Cr^{3+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Ti^{2+} , Cd^{2+} , Na^{+} , Ca^{2+} , Mg^{2+} , K^{+}). (B) Fluorescence anisotropy change (Δr) of 20 nM aptamer probe (T7-TMR-TA15) and base-substituted probes (T7-TMR-TA15-G1T, T7-TMR-TA15-G1T/G14T, T7-TMR-TA15-G2T/G15T, T7-TMR-TA15-G10T/G14T, T7-TMR-TA15-G11T/G15T, T7-TMR-PT15) in the presence of 500 nM Pb^{2+} . A 10 mM Tris-HAc (pH 7.5) buffer was used. The results represent the mean of 3 measurements, and the error bars represent the standard deviation.

($10^7 M^{-1}$) for Pb^{2+} to the unlabeled thrombin binding aptamer estimated by CD spectra [64].

3.4. Selectivity of Pb^{2+} sensor

Selectivity is another key parameter in evaluating the performance of an analytical method. To test the selectivity of this Pb^{2+} detection system, we first investigated the FA change (Δr) of the aptamer probe T7-TMR-TA15 in the presence of metal ions other than Pb^{2+} . As shown in Fig. 5A, only Pb^{2+} caused a substantial reduction in FA. We further investigated the FA response to Pb^{2+} of some base-substituted probes, which was obtained by substitution of T for one or two G nucleotides formed for G-quadruplex. As shown in Fig. 5B, these base-substituted probes showed no apparent change in FA, unlike the normal aptamer probe T7-TMR-TA15. These results suggest that this biosensor is highly selective for Pb^{2+} detection, which probably results from G-quadruplex formation induced by Pb^{2+} and high affinity for Pb^{2+} binding to the aptamer probe.

3.5. Application

To test the potential application of our developed approach in environmental analysis, we used a standard addition method to analyze freshwater samples (lake water). The results are shown in Fig. S3 (see supporting information). Comparing with the FA response in the binding buffer, it was found that there was some

interference to the FA response of aptamer probe T7-TMR-TA15 in the sample, and the probe showed large FA response to Pb^{2+} at concentration higher than 100 nM. The main reason may be that the real samples contain Mg^{2+} , Ca^{2+} and K^{+} around mM, which can non-specifically interact with aptamer probe to form G-quadruplex [80]. We have performed the experiment to analyze the Pb^{2+} in the presence of 2 mM Mg^{2+} , Ca^{2+} and K^{+} , respectively. As shown in Fig. S4 (see supporting information), the FA response of T7-TMR-TA15 in the presence of these metal ions is less sensitive to the titrated Pb^{2+} than that of T7-TMR-TA15 in the absence of metal ions. However, Pb^{2+} ranging from 10 nM to 10 μM was successfully detected in the 2-fold diluted lake water sample (see supporting information Fig. S3). The result indicates that our proposed G-quadruplex-based method could be applied to analyze Pb^{2+} in environmental samples, though the obtained sensitivity is lower than that in binding buffer. We will put our more effort in further research to find a good masking agent, which may enhance the sensitivity for Pb^{2+} in real sample.

4. Conclusions

In summary, we have introduced a novel FA method for real-time detection of Pb^{2+} in homogeneous solution with high sensitivity and selectivity. A significant reduction in FA was attributed to the conformational change of aptamer and the elimination of intramolecular interaction between the labeled TMR and the adjacent G nucleotide via PET, leading to an increase in the fluorescence lifetime of the aptamer probe. FA change permits real-time quantification of Pb^{2+} within 1 min. The assay is highly sensitive to detect Pb^{2+} down to 1 nM, and selective for Pb^{2+} over other metal ions. In addition, the affinity between the aptamer probe and Pb^{2+} was estimated to be approximately 96 nM. This FA assay has several following advantages. Firstly, FA can be used for sensitive detection of small molecules (metal ions such as Pb^{2+} in this case) by inducing conformational change, which resulted in altering the PET process occurred in between the TMR and the adjacent guanine. Secondly, compared with RNA-cleaving DNAzyme, the G-quadruplex DNA aptamer is cheaper and more stable. Thirdly, the assay can be performed by simply mixing the dye-labeled aptamer probe and the analyte, which is a simple and fast procedure. Finally, this FA reduction sensing strategy may potentially be used as a detection platform for analysis of a variety of other small and large molecules.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (21305153, 21077129, 21125523, and 20921063) and the National Basic Research Program of China (2011CB936001 and 2009CB421065).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aca.2013.12.029>.

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