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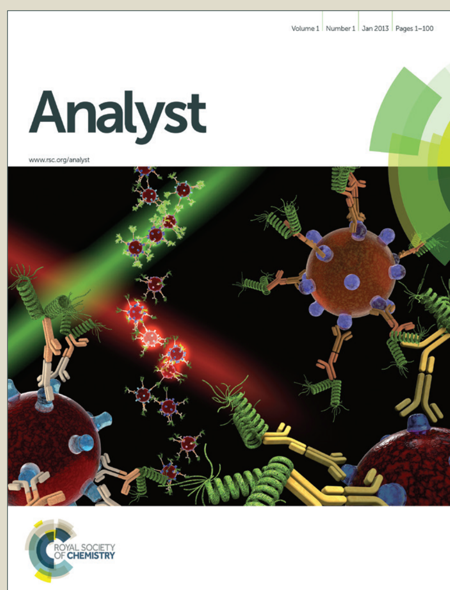

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ARTICLE TYPE

Highly sensitive chemiluminescent detection of lead ion based on its displacement of potassium in G-Quadruplex DNAzyme

Hong Wang, [a] **Dong Mei Wang,** [a] **Cheng Zhi Huang*** [a,b]

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A simple highly sensitive method for detecting lead ion (Pb^{2+}) in biosamples was developed based on its displacement of potassium in G-Quadruplex DNAzyme that can catalyze the luminol-H_2O_2 chemiluminescence (CL) reaction. By introducing a G-rich DNA sequence, PS2.M, which can fold into G-quadruplex when binding with hemin in the presence of K^+ and act as a superior horseradish peroxidase (HRP) mimicking-enzyme, we found this DNAzyme can effectively catalyze the H_2O_2-mediated oxidation of luminol, resulting in strong CL emission. The K^+-stabilized G-quadruplex, upon the addition of Pb^{2+}, is transformed into Pb^{2+}-stabilized G-quadruplex with higher stability but poor DNAzyme activity, sharply declining CL readout signal. With that, a simple and sensitive detection method of Pb^{2+} in biosamples such as human hairs was developed with a linear range of 0.4-10 nM Pb^{2+} and a limit of detection (3σ) of 0.06 nM. Owing to the introduction of G-quadruplex DNAzyme which was employed not only as sensing unit but also as a catalyst in the chemiluminescent assay, this method holds a great potential for clinical plumbism diagnosis by testing hair.

1. Introduction

Lead, one of the well-known toxic heavy metal elements and being widely used in daily life, such as batteries, pipes, gasoline, paint pigments, leads to an increasingly severe environmental problem for lead contamination.[1] Through food chain, lead ions accumalate in human body and cause a serious risk for human health because of their non-biodegradability.[2-4] A certain concentration of Pb^{2+} could prevent the circulation and production of blood, triggering headaches, dizziness, fatigue, memory loss, irratability or even mental ratardation, especially for children.[5, 6] Thus, to design accurate, ultrasensitive, specific detection methods of Pb^{2+} becomes a task of emergency in environmental and food tracking, as well as in clinical diagnosis and toxicology for analytical workers.[5] According to clinical medicine, Pb^{2+} are mainly ditributed in the blood, soft tissue and bone,[7] and thus clinicians generally test blood lead (BPb) in hospital to make certain whether there is saturnism or not.[8] However, BPb only reflects a short-term intake of lead. On the opposite, hair lead (HPb) can record a long-term intake of lead. In this work, we developed a detection method of lead in human hair by using Pb^{2+} in hair directly, which can induce an allosteric conformation of K^+-stabilized G-quadruplex DNAzyme, to test the practical applicability of the proposed protocol.

Existing conventional detection methods of Pb^{2+} generally require sophisticated operation or precise instruments, such as atomic absorption spectrometry (AAS),[9] X-ray fluorescence spectroscopy,[10, 11] inductively coupled plasma atomic emission spectrometry (ICP-AES),[12, 13] surface plasmon resonance (SPR) spectroscopy,[14] and so on. Chemiluminescence (CL) technique, on the other hand, has received a soaring attention due to its simplicity, rapidness, low background, low-cost, remarkably high sensitivity and strong operability.[15] Of reported CL systems, horseradish peroxidase (HRP), a natural enzyme, has been commonly employed as a catalyst of H_2O_2-mediated Fenton-like CL reaction system, which is sensitive to the environmental conditions, such as temperature, pressure, acidity, inhibitors, salts and solvent.[16-18] In comparison to HRP's uncontrollability, low stability on account of their denaturation and difficulties in preparation, hemin/G-quadruplex DNAzyme, as an artifical enzyme, has attracted an increasingly significant focus for researchers because of its thermodynamic stability under near-physiological conditions.[19-21]

It has been known that G-quadruplexes have special secondary structures with four-strand helix,[22, 23] which are usually stabilized by coordination alkline cations, especially K^+ and Na^+.[24-29] Interestingly, a few K^+-induced G-quadruplexes binding with hemin exhibit superior HRP-like activity and effectively catalyze the H_2O_2-mediated oxidation of tetramethyl benzidine (TMB), 2,2'-amino-di (2-ethyl-benzothiazoline sulphonic acid-6) ammonium ($ABTS^{2-}$) or luminol.[30-32] By utilization of these G-quadruplex DNAzymes, a series of colorimetric or CL assays have been developed for the detection of protien,[31, 32] small molecules,[31, 32] DNA[15, 33-35] and metal ions.[26, 29, 30, 36] Considering that Pb^{2+} can induce PS2.M, a G-rich oligonucleotide, to form more compact quadruplex structures than K^+, and the efficiency of Pb^{2+} to stabilize the G-quadruplex is much higher than that of

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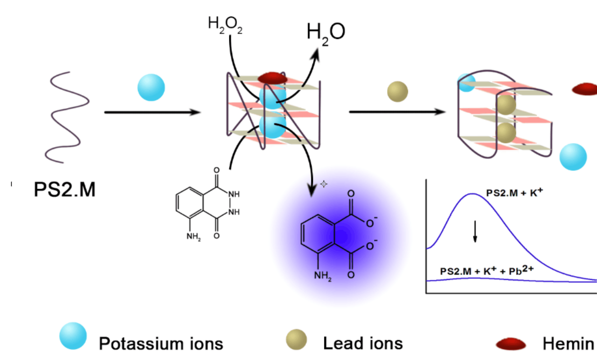

Scheme 1 Schematic illustration by Pb^{2+}-induced allosteric G-quadruplex DNAzyme, PS2.M is chosen as the sensing element for CL detection of Pb^{2+}.

K^+,[26, 37] herein we take the advantages of these structural features and develop a detection method of Pb^{2+} in human hairs based on the Pb^{2+}-induced allosteric K^+-stabilized G-quadruplex DNAzyme to catalyze luminol-H_2O_2 CL reaction system.

2. Experimental section

2.1 Materials

The purified G-rich oligonucleotide (PS2.M: GTGGGTAGGG-CGGGTTGG) were bought from Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China). Luminol and hemin were purchased from Sigma-Aldrich (St. Louis, MO, USA). 30% H_2O_2 and Triton X-100 were provided by Chengdu Kelong Chemical Reagent (Chengdu, China). Tris(hydroxymethyl)amino-methane (Tris) was obtained from Ningbo Dachuan Fine Chemical Co., Ltd (Ningbo, China). Dimethylsulfoxide (DMSO) was supplied by Chongqing Chuandong Chemical Co., Ltd. The stock solution of luminol (10 mM) was prepared in 0.1 M NaOH and stored in the dark.[15] The stock solution of hemin (2.38 mM) was prepared in DMSO, and stored in the dark at -20 °C. Other chemicals were of analytical reagent grade. Milli-Q purified water (18.2 MΩ) was used in all experiments.

2.2 Apparatus

The CL spectral measurements were conducted with a BPCL ultraweak luminescence analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) with a series of high-energy optical filters of 230, 260, 290, 320, 350, 380, 400, 425, 440, 460, 490, 535, 555, 575, 620, and 640 nm between the CL flow cell and PMT, as described in ref 24 and 38. A model JASCO-810 spectropolarimeter (Hitachi, Tokyo, Japan) was utilized to record circular dichroism (CD) spectrum. Absorption spectra were measured on a UV-3600 spectrometer (Hitachi, Tokyo, Japan). All kinds of solutions were blent through a QL-901 vortex mixer (Haimen, China).

2.3 Preparation of K^+- or Pb^{2+}-stabilized hemin/G quadruplexes

Following the literature protocal,[30] DNA solution was prepared in the pH 7.4 10 mM Tris-HAc buffer and heated at 88 °C for 8 min to dissociate the interaction of intermolecules, and gradually cooled to room temperature. Then, KAc or $Pb(NO_3)_2$ in appropriate concentratrion was added into 5 μM DNA, allowing the PS2.M to fold for 40 min to form G-quadruplexes stabilized by K^+ or Pb^{2+} at room temperature. Finally, an equal volume of 10 μM hemin was incubated with the G-quadruplexs for over 1h in 10 mM Tris-Ac buffer (pH 7.4) containing 10 mM KAc,[26] 1% (v / v) DMSO and 0.05% (w / v) Triton X-100. It would allow the formation of hemin/G-quadruplex complexes.

2.4 CL analysis of Pb^{2+}

CL detection Pb^{2+} was performed in the luminol-H_2O_2 reaction system catalyzed by hemin/G-quadruplex DNAzyme at room temperature. Briefly, 40 μL of DNAzyme solution that contained 10 mM KAc, $Pb(NO_3)_2$ in different concentration, 50 μL of 1 mM luminol and 160 μL reaction buffer (50 mM Tris-Ac, 1% DMSO, 0.05% Triton X-100, pH 8.2) were added to a cuvette. Then, the measurement was started and 250 μL of 300 mM H_2O_2 was injected later. The CL emission was collected by the BPCL system with the voltage of PMT at 750 V.

2.5 DNAzyme melting experiments

The UV melting curves of K^+- or Pb^{2+}-stabilized G-quadruplex DNAs were obtained by a UV-vis-NIR spectrophotometer equipped with a temperature-controlled water bath. The absorbance at 295 nm was recorded, which is the characteristic absorption of the quadruplex structures.[23,35] In a characteristic experiment, 400 μL of G-quadruplex solution, containing 2.5 μM PS2.M, 10 mM KAc or 50 n M $Pb(NO_3)_2$ was added into a 0.5 cm path length quartz cuvette, and then coverd with a layer of paraffin oil to impede evaporation. The solution was held at different temperatures for 2 min, then scanned the absorption spectra respectively.

2.5 Pretreatment of real sample of hair

Because of liquid phase reaction, hair samples must undergo pretreatment. The processing steps of pretreatment are as follows: (1) The uncontaminated hair samples obtained from volunteers were washed, dried and weighted respectively. (2) Heat the samples in the crucible on electric stove, till they were hardened. This step was conducted in fuming cupboard. (3) Calcine the samples in muffle furnace for 4 hours until they were became lime and weigh them again respectively. (4) Dissolve the lime in

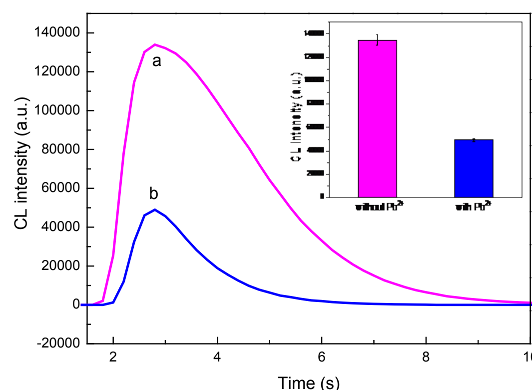

Fig. 1 Kinetic monitoring of luminol-H_2O_2 reactions catalyzed by hemin-PS2.M complex in the presence of 10 mM K^+ (curve a) and 10 mM K^+ + 50 nM Pb^{2+} (curve b) The inset shows the CL intensities of a and b. Concetrations: luminol, 0.1 mM; H_2O_2, 150 mM; DNA, 200 nM; hemin, 200 nM .

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a certain amount of mixed acid of HCl and HNO_3 (v / v = 1:1) and heat stir on electric stove at the same time. (5) Add purified water to make volumn constant.

2.6 Safety consideration

Because of the highly toxic and the severe adverse effects on human health of Pb^{2+} and other heavy-metal ions, all the experiments involving in heavy-metal ions should be performed with protective gloves. In order to avoid polluting the environment, the reclamation of the waste solutions containing heavy-metal ions is necessary.

3. Results and discussion

3.1 Principle of sensing Pb^{2+} via G-quadruplex DNAzyme

As Scheme 1 depicts, the K^+-stabilized G-quadruplex DNAzyme (with hemin as a confactor) can effecttively catalyze the oxidation of luminol by H_2O_2, giving rise to the signal of CL. In contrast, the addition of Pb^{2+} can cause the DNAzyme deactived and the CL intensity decreased through the substitution of K^+ in DNAzyme by Pb^{2+}. (see in Fig. 1). In this systerm, the G-quadruplex DNAzyme was employed not only as the sensing element, but also as a catalyst in the chemiluminescent assay.

The catalysis on luminol-H_2O_2 CL reaction by K^+-stabilized hemin/G-quadruplex complex in the presence or absence of Pb^{2+} is investigated. As Fig. 1 shows, without Pb^{2+}, the K^+-stabilized DNAzyme gives great rise of CL intensity, while the CL intensity decreases shaply in the presence of 4 nM Pb^{2+}. It is apparent that CL intensity is reduced to about one third upon the introduction of Pb^{2+} (Fig. 1 inset). These results can be comprehended by the fact that Pb^{2+}-stabilized G-quadruplex cannot bind hemin,[26] and thereby the catalytic activity of G-quadruplex DNAzyme is weakened upon the addition of Pb^{2+}.

Importantly, Pb^{2+} induces K^+-stabilized G-quadruplex to undergo conformational transformation, accompanying by a deactived DNAzyme, which is demonstrated in the CD spectrum (Fig. 2A). The CD spectrum of PS2.M presents a strong positive band near 295 nm (curve 1). Then, upon the addition of Pb^{2+}, a positive peak gradually emerges at ~312 nm, which coincides with the typical characteristic peak of Pb^{2+}-stabilized antiparallel G-quadruplex structure.[26] The peak near 312 nm becomes stronger when the concentration of Pb^{2+} increased, whereas that near 295 nm fades away, demonstrating that PS2.M experiences a conformational transition induced by Pb^{2+}. This indicates Pb^{2+} binds with PS2.M strongly enough to effectively compete against K^+, forming shorter Pb^{2+}-O and O-O bands ~~rather~~ than K^+-O and O-O bands in chelation.[26] Therefore, the Pb^{2+}-stabilized G-quadruplex is usually more compact and stable than K^+-stabilized G-quadruplex, which is reflected in the melting curves. Fig. 2B shows that after incubating with Pb^{2+}, the T_m of PS2.M grows by about 10 °C, which may legitimately explain why Pb^{2+} can competitively bind to PS2.M.

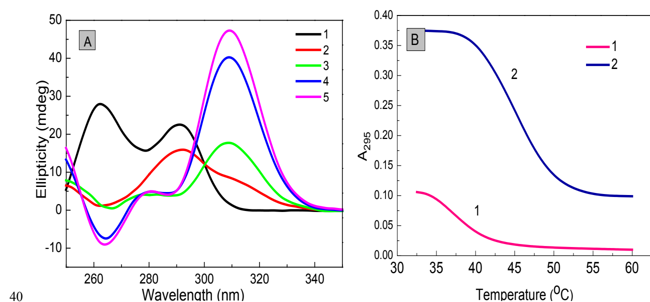

Fig. 2 Properties and structures of PS2.M stabilized by K^+ and Pb^{2+}. (A) Conformational transformation of PS2.M (stabilized by 10 mM K^+) upon addition of Pb^{2+}: 1→5, 0, 0.25, 0.625, 2.50, 6.25 μM. Concentration: DNA, 10 μM, K^+, 10 mM. (B) UV melting curves of PS2.M: 10 mM K^+ (curve 1) and 10 mM K^+ + 625 nM Pb^{2+} (curve 2).

The above observations clearly demonstrate the ability of Pb^{2+} to deactive the DNAzyme and to induce allosterism. It suggests a new avenue to quantify Pb^{2+} by applying K^+-stabilized DNAzyme in CL detection system.

3.2 Optimization of the chemiluminescent assay conditions

Several pivotal experimental conditions that have effects on the chemiluminescent assay of Pb^{2+}, such as the pH of the reaction buffer and the concentration of H_2O_2, luminol and DNAzyme, are optimized. To the best of our knowledge, CL of luminol-H_2O_2 reaction system is seriously pH-dependent. Firstly, we investigate the effect of the working buffer on the CL intensity of luminol-H_2O_2 reaction system catalyzed by G-quadruplex DNAzyme. As shown in Fig. 3A, CL intensity is most sensitive tend towards stability in the reaction buffer ≥ 8.0. Therefore, pH 8.2 Tris-Ac^- (50 mM Tris, v/w 1% DMSO, w/w 0.05% Triton X-100) is chosen as the optimal pH for the chemiluminescent assay.

The CL intensity rests with the concentration of hemin/G-quadruplex DNAzyme. Both CL signals of control and experiment increase along with the addition of DNAzyme. The

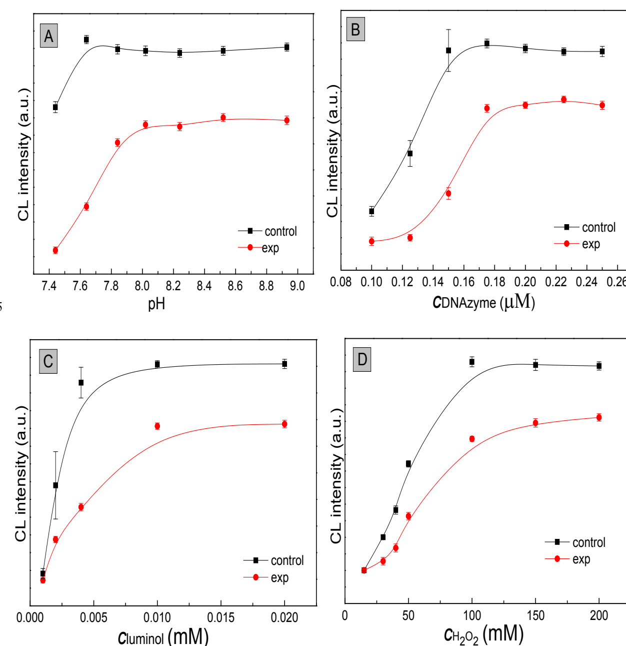

Fig. 3 Optimization of experimental conditions (A) CL intensity *vs*. pH of reaction buffer. (B) CL intensity *vs*. concentration of DNAzyme. (C) CL intensity *vs*. concentration of luminol. (D) CL intensity *vs*. concentration of H_2O_2. Concentrations: DNA, 200 nM; hemin, 200 nM; K^+, 10 mM; Pb^{2+}, 4 nM (without Pb^{2+} in control); luminol, 0.1mM; H_2O_2, 150 mM, pH 8.2. The red symbols refer experimental data, and the black symbols refer control.

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signals stop to enhance and keep constant after the concentration of DNAzyme reaches 0.18 μM. Accordingly, 0.20 μM is selected as the proper concentration in all experiments (Fig. 3B).

The concentration of H_2O_2 and luminol play key roles in guaranteeing the reliability of data, high sensitivity and good selectivity in chemiluminescent assay. We carried out the following optimized experiments respectively under the optimal conditions ascertain the concentration of H_2O_2 and luminol. As a result, the optimal concentration of H_2O_2 and luminol are 150 mM (Fig. 3C) and 0.1 mM (Fig. 3D), respecitvely.

3.3 Sensitivity and selectivity for Pb^{2+} detection

Pb^{2+} solutions of different concentrations were conducted to explore the sensitivity of the proposed strategy. As shown in Fig. 4A, the CL intensity was decreased gradually as the addition of Pb^{2+} in the sensing system. Fig. 4B renders the relationship between ΔI (deviations of I_0 and I, wherein I_0 and I are the CL intensity in the absence and presence of Pb^{2+}, respectively) and the concentration of Pb^{2+}. The diagram illustrates that ΔI is linearly dependent on the concentration from 0.4 to 10 nM. It also shows that the rate of decline assumes a trend of slowing down beyond the line range. From the inset of Fig. 4B, the equation $\Delta I = 69339\ \lg[Pb^{2+}] + 665329$, $R^2 = 0.994$ is obtained and the detection limit (3σ) is calculated to be 0.06 nM. This suggests that this CL strategy can serve as an excellent alternative knob for Pb^{2+} assay in human body.

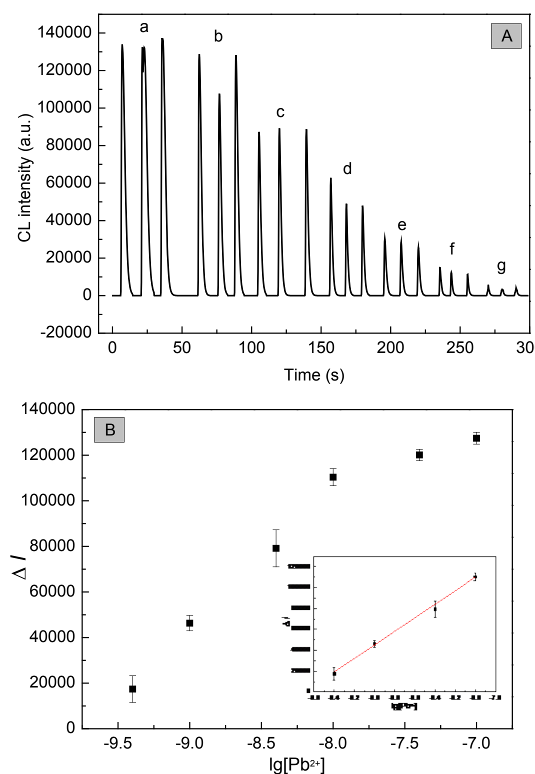

Fig. 4 Investigation of sensitivity of Pb^{2+} (A) Kinetic monitoring on the CL of luminol-H_2O_2 system upon addition of different concentrations of Pb^{2+}. (B) Plots of CL changes as a function of Pb^{2+} concentration. Concentrations: a→g, 0, 0.4, 1.0, 4.0, 10.0, 40.0, 100.0 nM; luminol, 0.1 mM; H_2O_2, 150 mM; DNA, 200 nM; hemin, 200 nM; working buffer, pH 8.2.

To point out the specificity of Pb^{2+} for this detecting system, other common positive ions substituting Pb^{2+} were introduced into K^+-stabilized G-quadruplex DNAzyme catalyzing luminol-H_2O_2 reaction. In our previous study,[24] K^+-stabilized DNAzyme has been proved to improve CL intensity remarkably due to its HRP-like activity, which is consistent with control. The results reveal that only the addition of Pb^{2+} can obviously weaken DNAzyme activity while other metal ions (except Hg^{2+}) cannot interfere DNAzyme activity even at a ~50 fold higher concentration than that of Pb^{2+} (see in Fig. 5). Considering in Hg^{2+} system, the much weaker reduction in DNAzyme activity of T-containing G-quadruplexes due to the formation of T-Hg^{2+}-T base pairs.[26] Moreover, it is well known that Cu^{2+} can catalyze the CL reaction of luminol-H_2O_2, but the amount of Cu^{2+} was not enough to be on a par with the DNAzyme in this CL detection system at 750 V voltage of PMT, so the catalysis of Cu^{2+} can be nearly ignored here.[39] It is safe to conclude that our ~~being~~ reported sensing platform exhibits stronge specificity for Pb^{2+} over other metal ions. So we can generalize this strategy for Pb^{2+} detection in water or hair.

We collected 4 hair samples with weight of 0.3770, 0.7711, 1.0785 and 0.8012 g respectively. Then the samples were prepared in 10 mL water solution after a series of pretreatment. The results (see in Table 1) agreed with those obtained from AAS, indicating that our sensing platform can be applied to hair samples.

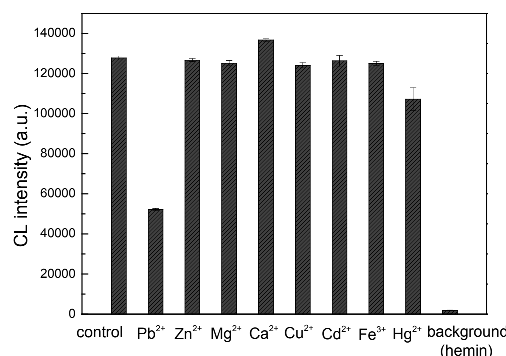

Fig. 5 Selectivity assays based on the catalytic to luminol-H_2O_2 system. Concentrations: luminol, 0.1 mM; H_2O_2, 150 mM; DNA, 200 nM; hemin, 200 nM; K^+,10 mM; Pb^{2+}, 4 nM; other metal ions, 200 nM. working buffer, pH 8.2.

Table 1 Analytical results of Pb^{2+} in hair [a]

Hair sample	Developed method		Total cont. (μg/g)	AAS (μg/mL)
	Cont. (μg/mL)	RSD (%) (n = 3)		
S1	0.341	1.53	9.17	0.349
S2	0.223	1.76	2.79	0.218
S3	0.420	2.43	3.80	0.414
S4	0.413	0.97	5.15	0.402

[a] Experimental conditions: concentrations, H_2O_2, 150 mM; luminol, 0.1 mM; DNA, 200 nM; hemin, 200 nM. reaction buffer, pH 8.2.

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4. Conclusion

In conclusion, we have introduced a highly sensitive chemiluminescent method for Pb^{2+} detection in real samples based on Pb^{2+}-induced allosteric G-quadruplex DNAzyme acting as a lable-free sensing element used in luminol-H_2O_2 reaction system. K^+-stabilized G-quadruplex DNAzyme can significantly enhance the CL, but upon the addition of Pb^{2+}, allosterism of G-quadruplex takes place, accompanied by a deactived DNAzyme. Pb^{2+} can be detected as low as 0.06 nM, which is lower than that of reported methods.

In comparison with other reported assays, this study has important characteristcs: (1) It provides a superhighly sensitive strategy for Pb^{2+} assay and successfully applies this strategy in hair lead detection, holding a great potential for pioneering a new iterm for plumbism diagnosis in hosipital; (2) By empolying DNAzyme not only as a sensing elment, but also as a catalyst, this assay exhibit excellent properties of simple, low-cost, isothermal and label-free.

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Notes and references

[a] Education Ministry Key Laboratory on Luminescence and Real-Time Analytical Chemistry, College of Chemistry and Chemical Engineering Southwest University, Chongqing 400715, China.
[b] College of Pharmaceutical Sciences, Southwest University, Chongqing, PR China. Fax: (+86) 23 68866796; Tel: (+86) 23 68254659; E-mail: chengzhi@swu.edu.cn

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