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Low-cost and highly efficient DNA biosensor for heavy metal ion using specific DNAzyme-modified microplate and portable glucometer-based detection mode

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ABSTRACT

A simple and low-cost DNA sensing platform based on Pb^{2+}-specific DNAzyme-modified microplate was successfully developed for highly sensitive monitoring of lead ion (Pb^{2+}, one kind of toxic heavy metal ion) in the environmental samples coupling with a portable personal glucometer (PGM)-based detection mode. The detection cell was first prepared simply by means of immobilizing the DNAzyme on the streptavidin-modified microplate. Gold nanoparticle labeled with single-stranded DNA and invertase (Enz-AuNP-DNA) was utilized as the signal-transduction tag to produce PGM substrate (glucose). Upon addition of lead ion into the microplate, the substrate strand of the immobilized DNAzyme was catalytically cleaved by target Pb^{2+}, and the newly generated single-strand DNA in the microplate could hybridize again with the single-stranded DNA on the Enz-AuNP-DNA. Accompanying with the Enz-AuNP-DNA, the carried invertase could convert sucrose into glucose. The as-produced glucose could be monitored by using a widely accessible PGM for *in situ* amplified digital readout. Based on Enz-AuNP-DNA amplification strategy, as low as 1.0 pM Pb^{2+} could be detected under the optimal conditions. Moreover, the methodology also showed good reproducibility and high selectivity toward target Pb^{2+} against other metal ions because of highly specific Pb^{2+}-dependent DNAzyme, and was applicable for monitoring Pb^{2+} in the naturally contaminated sewage and spiked drinking water samples.

1. Introduction

Heavy metal ions, as the serious source to pollute the biosphere throughout the world, are very toxic and can exert direct impact on human body resulting in many healthy and physiological diseases because of their difficult biodegradation and easy accumulation in soft tissues through the food chain (Cui et al., 2015; Gherasim et al., 2014). Among these heavy metal ions, lead ion (Pb^{2+}, one of the most common metal pollutants) has tremendous effects on human biological systems including reproduction, immune, nerve and cardiovascular (Li et al., 2014a, 2014b; Lien et al., 2014). Accordingly, US Environmental Protection Agency (EPA) in 2013 has set the legal limit to 15 parts per billion for lead in drinking water, and to the pM level (10–80 pM) in the uncontaminated seawater. Common techniques used for the detection of lead ion mainly include chromatography, spectroscopy, and electrochemical method (Dong et al., 2014; Mandappa et al., 2014; Su et al., 2013; Aboufazeli et al., 2013; Wang et al., 2008). Despite acceptable sensitivity and accuracy in this field, they often involve expensive sophisticated instruments, complicated sample pretreatments, rather cumbersome operating conditions, thereby limiting their widespread application. Therefore, it would be valuable to develop new methods and strategies for monitoring lead ion at a low level in the environmental samples from an ecotoxicological point of view.

DNAzymes, that are *in vitro* selected DNA molecules with enzyme-like catalytic activities, are a series of synthetic DNA oligonucleotides with some merits including simple synthesis, easy labeling, good stability, and design flexibility (Ge et al., 2014, Cheng et al., 2014). '8–17' DNAzyme consisting of a substrate strand and a catalytic strand has a specific cleavage site of Pb^{2+}, which has been widely used for the detection of lead ion based on different signal-transduction principles (Lu et al., 2015; Zhou et al.,

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2014; Mazumdar et al., 2009). The Lu's group designed a series of fluorescent/colorimetric sensors for the monitoring of lead ions by using Pb^{2+}-specific DNAzyme owing to their easy-to-use and small-in-size (He and Lu, 2011; Xiang and Lu, 2014; Lan et al., 2010). The lead-dependent DNAzyme with a two-step mechanism was described in detail in this work (Brown et al., 2003). Tang et al. developed an enzyme-free electrochemical Pb^{2+} sensor by using '8–17' DNAzyme-assisted hybridization chain reaction with the quantum dots for signal amplification (Tang et al., 2014). Zhang et al. reported a ZnO nanoflower-based photoelectrochemical DNAzyme sensor for the detection of Pb^{2+}, using Pb^{2+}-dependent DNAzyme as the recognition unit and a double-stranded intercalator as the signal reporter (Zhang et al., 2014). Li et al. also constructed a Pb^{2+} fluorescence sensor by coupling Pb^{2+}-specific DNAzyme with isothermal strand displacement reaction (Li et al., 2014a, 2014b). As expected, our strategy in this work would like to utilize '8–17' DNAzyme as the recognition element for the design of Pb^{2+} sensor.

Another important concern for the development of Pb^{2+} sensor lies in the construction of detection cell and usage of signal-transduction method. Microtiter plate (MTP) is a flat plate with multiple "wells" used as small test tubes, which has become a standard tool in analytical research and clinical diagnostic testing laboratories (Duetz, 2007). Since the high-binding MTP is usually modified using an oxygen plasma discharge to make their surfaces more hydrophilic, it is easier for adherent proteins, *e.g.*, antibody, cell and streptavidin (Harink et al., 2014; Wilming et al., 2014; Sabaeifard et al., 2014). Through modifying nucleic acids with the proteins, the oligonucleotides can be immobilized onto the MTP. Typically, the MTP-based sensing platforms are often monitored on the plate readers by coupling with fluorescence, chemiluminescence, UV–vis spectroscopy and colorimetric assay. Certainly, the MTP-based colorimetric assay can acquire a rapid detection within a short time by using bare-eyes. Unfavorably, it often gives a qualitative result. Glucometer, one of the most widely used diagnostic devices, has utilized as a portable tool for the biochemical analyses owing to its easy operation, low cost and reliable quantitative results (Cha et al., 2014; Yan et al., 2013). Recently, our group designed several glucometer-based detection methods for quantitative monitoring of adenosine triphosphate, brevetoxin B and lead ion (Fu et al., 2013; Gao et al., 2014; Hou et al., 2014). To this end, our motivation is to construct a low-cost and highly efficient DNA biosensor for screening lead ion in the environmental sample using DNAzyme-modified microplate and portable glucometer-based detection mode.

Herein we initially design a simple and feasible Pb^{2+} sensing platform by immobilizing Pb^{2+}-dependent DNAzyme onto a streptavidin-functionalized microtiter plate based on the avidin–biotin chemistry. Nanogold particles, labeled with invertase and single-stranded DNA, are utilized as the signal-production tags. Upon target Pb^{2+} introduction into the microplate, target analyte catalytically cleaves the substrate strand of the DNAzyme, therefore resulting in the detachment of catalytic strand from the microplate. The newly generated single-stranded DNA on the microplate hybridizes with the oligonucleotides on the gold nanoparticles. The carried invertase accompanying nanogold particles hydrolyzes sucrose to glucose, which can be quantitatively monitored by using a portable personal glucometer. The main purpose of this work is to design a simple, user-friendly and sensitive monitoring platform for lead ion in the environment by coupling a low-cost microplate with a portable glucometer.

2. Experimental

2.1. Material and reagent

Streptavidin (Amyjet Scientific Inc., Wuhan, China), $HAuCl_4 \cdot H_2O$ (Sigma-Aldrich, St. Louis, MO, USA), invertase from baker's yeast (Sigma-Aldrich), and tris(2-carboxyethy) phosphine hydrochloride (TCEP, Sigma-Aldrich) were employed in this work. Colloidal gold nanoparticles (AuNPs, $\Phi = 16$ nm) were synthesized and characterized, as described in detail in our previous report (Zhang et al., 2012). High-binding polystyrene 96-well microplate (Greiner Bio-one, Frickenhausen, cat# 705071, Germany) and personal glucometer (PGM, Roche, Accu-Chem® Active, Malaysia) were used in all runs. The buffer for PGM measurement (pH 7.3, 72.9 mM Na_2HPO_4 + 27.1 mM NaH_2PO_4 + 50 mM NaCl + 5 mM $MgCl_2$) was prepared by adding the chemicals into 1000 mL distilled water. The washing and blocking buffers were achieved by throwing 0.05% Tween 20 (v/v) (Genview, USA) and 1.0% (w/v) bovine serum albumin (BSA) in above-mentioned buffer. Pb $(OAc)_2 \cdot H_2O$, BSA, and sucrose were purchased from Sinopharm Chem. Re. Inc. (Shanghai, China). The catalytic strand and substrate strand of Pb^{2+}-dependent DNAzyme were purchased from Sangon Biotech. Inc (Shanghai, China) and TaKaRa Biotech. Inc (Dalian, China), respectively. The sequences of the oligonucleotides including biotinylated substrate strand were listed as follows:

Catalytic strand: 5′-CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-3′
Substrate strand: 5′-bio-CGATAACTCACTAT**rA**GGAAGAGATG-3′
Complementary DNA: 5′-SH-ATAGTGAGTTATCG-3′

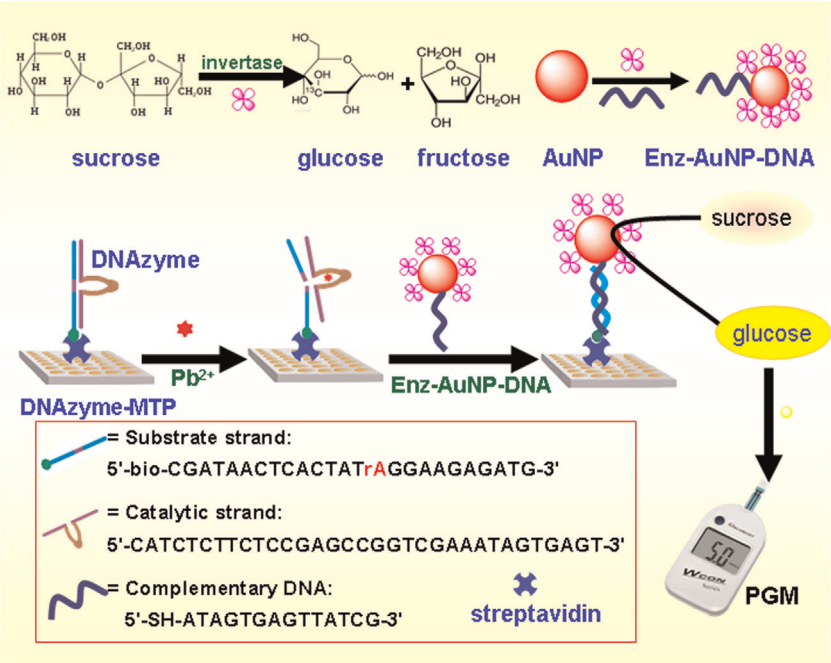
The oligonucleotide stock solutions were prepared by using the detection buffer. The standards of Pb^{2+} ions with different concentrations were prepared in 10 wt% HOAc, and diluted with 50 mM of pH 8.2 Tris–acetate including 0.5 M NaCl. All other reagents were of analytical grade. Ultrapure water based on Millipore purification system (18.2 MΩ cm^{-2}, Milli-Q, Millipore) was used in all runs.

2.2. Preparation of invertase-AuNP-complementary DNA conjugates (Enz-AuNP-DNA)

Invertase and complementary DNA-conjugated gold nanoparticle (designated as Enz-AuNP-DNA) were prepared according to our previous report with minor modification (Tang et al., 2013). The preparation process could be summarized as the following steps: (i) pH of the as-prepared gold colloids (gold conc. = 24 nM) was adjusted to 9.0 by using 0.1 M Na_2CO_3 aqueous solution; (ii) 200 μL of 100,000 units L^{-1} invertase and 20 μL of 100 μM complementary DNA were injected into 5 mL of the above-prepared gold colloids and reacted overnight at 4 °C; (iii) the resulting suspension was centrifuged at 13,000*g* for 20 min at 4 °C; and (iv) the deposited particle (*i.e.*, Enz-AuNP-DNA, gold conc. = 120 nM) was dispersed into 1.0-mL detection buffer including 1.0 wt% BSA for further usage.

2.3. Preparation of DNAzyme-functionalized microtiter plate (DNAzyme-MTP)

Initially, 50 μL of streptavidin (per well, in detection buffer) was added into a new microwell plate, and incubated for 12 h at 37 °C (*Note*: To avoid the evaporation of solution, the microplate should be sealed with an adhesive plastics film during the incubation process). After washing two times with the washing buffer, the resulting microplate was blocked for 60 min at 37 °C with the blocking buffer (300 μL per well). Subsequently, 50 μL (per well) of 1.0 μM biotinylated substrate strand was added into



Scheme 1. Schematic illustration of low-cost and highly efficient DNA biosensor for Pb^{2+} detection using specific DNAzyme-modified microplate and portable glucometer-based detection mode.

the microplate, and reacted for 4 h at 37 °C. During this process, the biotinylated substrate strand was immobilized onto the microplate by the biotin–avidin reaction. After washing again, the resultant microplate was incubated with the catalytic strand (50 μL per well, 1.0 μM) for 8 h at 37 °C to form the Pb^{2+}-specific DNAzyme. Finally, the DNAzyme-modified microplate (designed as DNAzyme-MTP) was stored at 4 °C until use.

2.4. PGM measurement toward target Pb^{2+}

Scheme 1 represents the assay principle of the designed sensing platform toward target Pb^{2+}. Initially, Pb^{2+} standards or samples with different concentrations (50 μL per well) were added into the DNAzyme-MTP, and reacted for 45 min at 37 °C on an end-over-end shaker (*Note*: The substrate strand of the immobilized DNAzyme on the MTP was cleaved by lead ion at this step). After washing with the washing buffer, the Enz-AuNP-DNA suspension prepared above (50 μL per well) was injected into the cell, and incubated for 70 min at 37 °C (*Note*: The newly formed single-stranded DNA hybridized with complementary DNA on the AuNPs during this process). Following that (washing), the sucrose solution (50 μL per well, 0.5 M) was added to the cell, and allowed to react at 37 °C for 30 min (*Note*: At this step the added sucrose was hydrolyzed to glucose by the labeled invertase on the AuNPs). The enzymatically produced glucose was finally monitored by the glucometer.

2.5. Real sample pretreatment

Water samples were collected in polyethylene bottles and, as far as possible, analyzed within 24 h or stored at 4 °C for less than 2 days. Sewage samples from water fish ponds were analyzed as soon as possible to avoid further microbial degradation. Initially, sewage samples were immediately filtered through a cellulose filter membrane (pore size 0.45 μm), and were then acidified to pH 2.0 for storage. Tap water samples were taken from our research laboratory without pretreatment. Before analysis, these water samples were adjusted to pH 5.0.

3. Results and discussion

3.1. Design and characteristics of PGM-based Pb^{2+} sensing platform

To meet the requirement of environmental monitoring toward majority users, a simple and sensitive assay protocol would be advantageous. In the present work, the analyte was monitored through target-induced Pb^{2+}-specific DNAzyme cleavage accompanying bioactive enzymatic assembly on the microplate with glucometer readout. The biotinylated DNAzyme strands were immobilized on the microplate based on the streptavidin–biotin reaction. Invertase and the oligonucleotides were conjugated to the gold nanoparticles possibly because of the reaction between cysteine or NH_3^+-lysine residues of invertase or –SH group and gold nanoparticles. The major aim of gold nanoparticles heavily functionalized with invertase and complementary DNA was to enhance their hydrolyzed efficiency toward sucrose. With the increasing target Pb^{2+} in the sample, the newly formed single-stranded DNA after cleavage increased, which resulted in the assembly of numerous Enz-AuNP-DNA conjugates. Therefore, the PGM signal increased with the increment of Pb^{2+} ion. By monitoring the change in PGM signal, we could calculate the amount of Pb^{2+} in the sample.

As described above, the Enz-AuNP-DNA conjugates were immobilized onto the microplate, following target-triggered DNAzyme cleavage. To realize this strategy, one very important precondition was whether target Pb^{2+} could induce the successful cleavage of DNAzyme. To this end, we first used gel electrophoresis to investigate the DNAzyme in the presence of target Pb^{2+} (Fig. 1A). As shown from lane 2 and lane 3, the base numbers of catalytic strand and substrate strand were close to 35 nt and 25 nt, respectively, which was in accordance with our design. The mixture including 0.1 μM catalytic strand and 0.1 μM substrate strand could cause the increase of the oligonucleotide base (~60 nt, lane 4), indicating that the DNAzyme could be formed. When target Pb^{2+} (50 pM) was added to the formed DNAzyme suspension, significantly, two new bands were appeared (lane 5). The base number of the middle band was almost the same as that of lane 2,

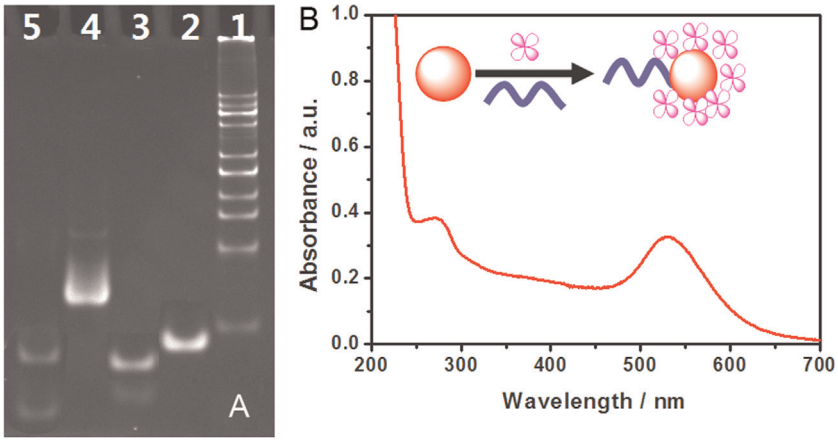


Fig. 1. (A) Gel electrophoresis (lane 1: DNA ladder; lane 2: catalytic strand; lane 3: substrate strand; lane 4: catalytic strand+substrate strand; land 5: catalytic strand+substrate strand+Pd^{2+}), and (B) UV–vis absorption spectroscope of Enz-AuNP-DNA.

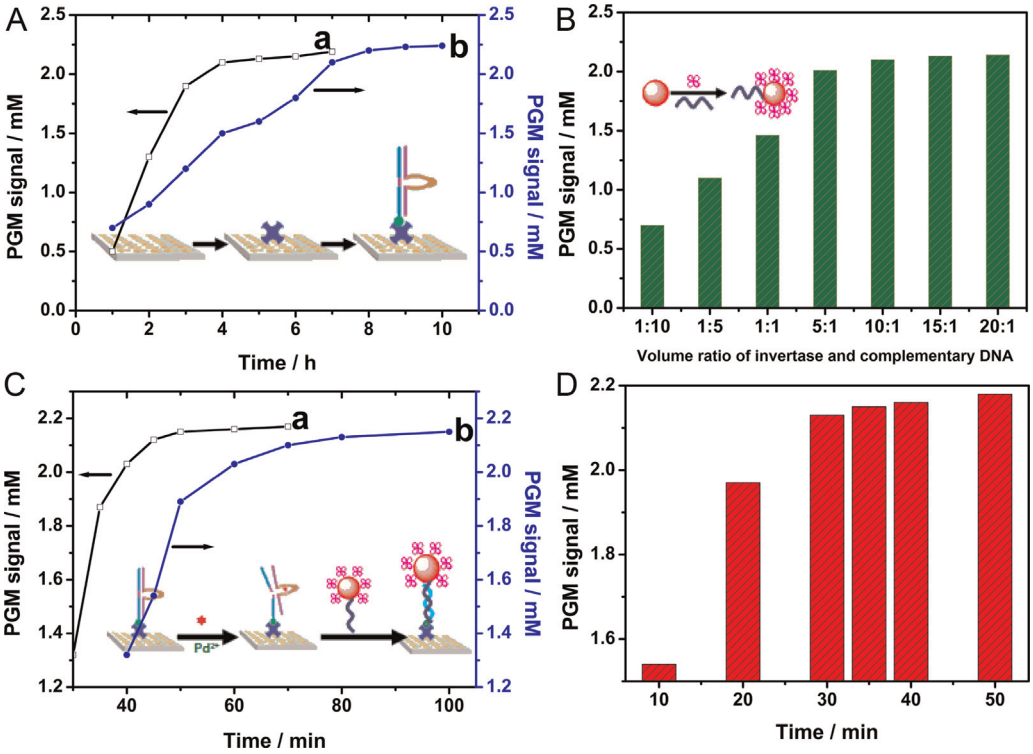


Fig. 2. The effects of (A) reaction time between streptavidin and biotinylated substrate strand (a), and hybridization time between catalytic strand with substrate strand (b), (B) the volume ratio between 100,000 units L^{-1} invertase and 100 μM complementary DNA, (C) the cleavage time of target Pb^{2+} toward DNAzyme (a) and hybridization time for Enz-AuNP-DNA (b), and (D) hydrolysis time of invertase on the signal of PGM-based sensing platform (50 pM Pd^{2+} used in this case).

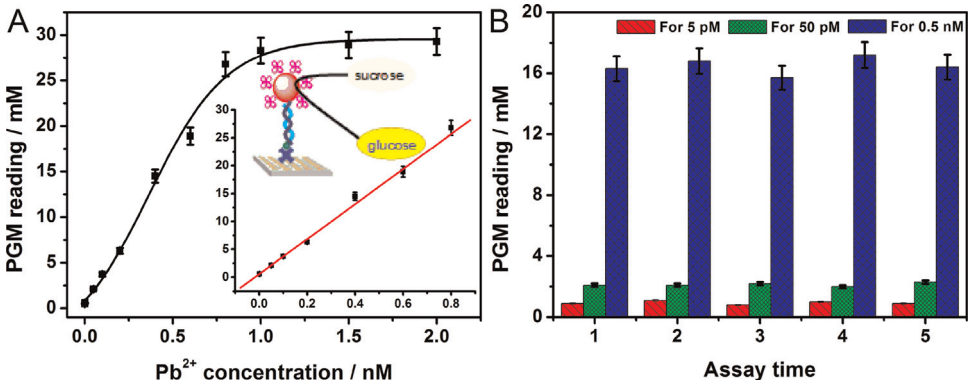


Fig. 3. (A) The corresponding glucometer readout of PGM-based sensing platform toward Pd^{2+} standards with different concentrations (inset: calibration curve), and (B) the reproducibility of PGM-based sensing platform toward 5 pM, 50 pM and 0.5 nM Pd^{2+} within five assays.

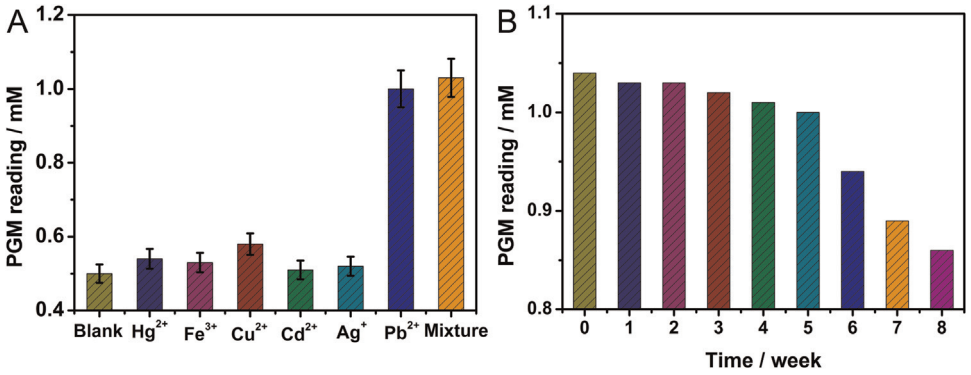


Fig. 4. (A) The specificity of PGM-based sensing system against Hg^{2+} (5 nM), Fe^{3+} (5 nM), Cu^{2+} (5 nM), Cd^{2+} (5 nM), Ag^{+} (5 nM) and target Pb^{2+} (5 pM), and (B) the stability of PGM-based sensing platform.

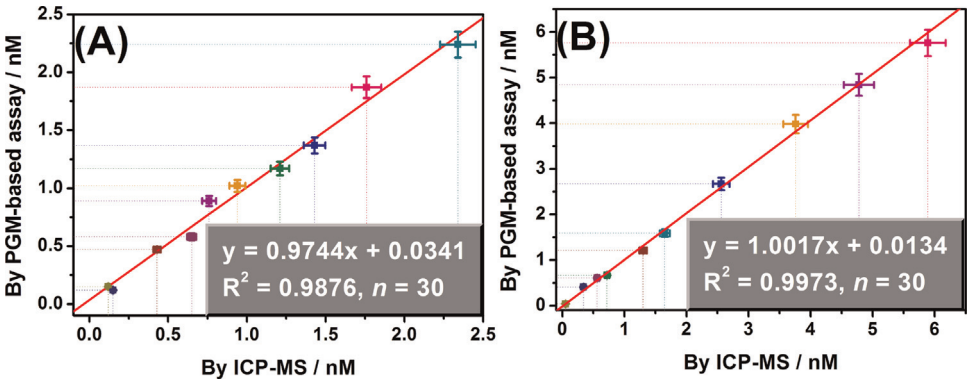


Fig. 5. Comparison of the assayed results for (A) naturally contaminated sewage and (B) spiked drinking water samples using PGM-based sensing platform and the referenced inductively coupled plasma mass spectrometry (ICP-MS).

which might stem from the detached catalytic strand. The base of the bottom band was ~15 nt, which might derive from two short oligonucleotides of the cleaved substrate strand. These results also revealed that the designed catalytic strand and substrate strand could be used for the formation of Pb^{2+}-specific DNAzyme.

Further, we used UV–vis absorption spectroscopy to investigate the Enz-AuNP-DNA (Fig. 1B). As is well known, the characteristic absorption peaks of gold colloids, bioactive enzyme and oligonucleotide were ~518 nm, ~278 nm and ~260 nm, respectively (Tang et al., 2008, 2013; Tang and Ren, 2008). As seen from Fig. 1B, the as-synthesized Enz-AuNP-DNA had two absorption peaks at 518 and 278 nm. However, we did not observe the characteristic peak of the oligonucleotide. The reason might be most likely as a consequence of the fact that the amount of the labeled oligonucleotide was relatively less. The high-carried invertase molecules on the AuNPs could provide a precondition for the development of highly sensitive Pb^{2+} sensor.

3.2. Optimization of preparation and assay conditions

For the preparation of DNAzyme-functionalized MTP, two possible conditions would affect the analytical properties of PGM-based Pb^{2+} sensing strategy toward the fixed streptavidin concentration: reaction time of substrate strand with streptavidin and hybridization time of catalytic strand with substrate strand. As shown from curve 'a' in Fig. 2A, the PGM signal increased with the increasing reaction time between streptavidin and substrate strand, and reached to a maximum response after 4 h (0.05 nM Pd^{2+} used in this case). In contrast with hybridization time of catalytic strand with substrate strand, the maximum PGM signal was obtained after 8 h toward the same-concentration target Pb^{2+} (curve 'b' in Fig. 2A). The reason might be the fact that the reaction between streptavidin and biotin based on the biotin–avidin chemistry was relatively faster than that of DNA-based hybridization chain reaction. In this regards, therefore, we used 4 h for the reaction between streptavidin and biotinylated substrate strand, and 8 h for the formation of Pb^{2+}-specific DNAzyme.

For the signal-transduction tag (Enz-AuNP-DNA), high-concentration complementary DNA on the AuNPs could increase the possibility of DNA hybridization, but it might decrease the PGM signal because many sites on the AuNPs were occupied by the oligonucleotides. In contrast, too a few of oligonucleotides might decrease the reaction of Enz-AuNP-DNA, thus resulting in a weak PGM signal. Fig. 2B displays the effect of the volume ratio between 100,000 units L^{-1} invertase and 100 μM complementary DNA on the signal of PGM-based sensing platform. Experimental results indicated that the high PGM signal could be acquired at 200 μL of 100,000 units L^{-1} invertase and 20 μL of 100 μM complementary DNA (*i.e.*, 10:1, v/v) for the preparation of Enz-AuNP-DNA.

During the measurement, the as-prepared Enz-AuNP-DNA was conjugated to the MTP after the cleavage of target-induced Pb^{2+}-dependent DNAzyme. To achieve a high PGM signal, the added target Pb^{2+} should be adequately reacted with the immobilized DNAzyme on the MTP. A short cleavage time would decrease the amount of the exposed DNA initial strand, thus indirectly affecting the PGM signal. As seen from curve 'a' in Fig. 2C, the maximum PGM signal was achieved when the cleavage time between target Pb^{2+} and the DNAzyme was more than 45 min. Of course, the hybridization time between Enz-AuNP-DNA and the newly formed signal-stranded DNA on the MTP would also affect the detectable PGM signal. As analyzed from curve 'b' in Fig. 2C, the optimal PGM signal could be complemented after 70 min. The long-time hybridization did not significantly cause the increase in the PGM signal. To save the assay time, 45 min and 70 min were selected as

the cleavage time for Pb^{2+}-specific DNAzyme and hybridization time for Enz-AuNP-DNA during the measurement, respectively.

Another important issue for the detectable PGM signal was the hydrolysis time of invertase toward the substrate sucrose. Usually, it takes some time for the bioactive enzyme to catalyze the corresponding substrate. As shown from Fig. 2D, a relatively strong PGM signal could be registered after 30 min, indicating that the hydrolysis reaction of invertase toward the sucrose tended to the equilibrium. So, 30 min was chosen for the production of glucose at the phase of signal collection.

3.3. Dose response of PGM-based sensing platform toward target Pb^{2+}

To quantitatively monitor target analyte, Pb^{2+} standards with different concentrations were measured on the DNAzyme-functionalized MTP by using the as-prepared Enz-AuNP-DNA as the signal-transduction tag with the glucometer readout. As displayed in Fig. 3A, we could clearly observe that with the increment of Pb^{2+} concentration in the sample the digital signal of the glucometer increased accordingly in the range of 0–2 nM and almost tended to level off thereafter. Under optimal conditions, a good linear relationship [PGM signal (mM) *vs.* Pb^{2+} concentration (pM)] in the dynamic working range of 1.0 pM–0.8 nM was obtained, and as low as 1.0 pM Pb^{2+} could be unambiguously monitored. The detection limit (0.8 pM) was comparable to some existed detection schemes, *e.g.*, electrochemical oligonucleotide-based biosensor (LOD: 34.7 nM) (Jarczewska et al., 2015), fluorescent sensor (LOD: 3.79 ppb) (Zhan et al., 2014), colorimetric strategy (LOD: 100 pM) (Li et al., 2014a, 2014b), DNAzyme-based isothermal strand displacement signal amplification (LOD: 200 pM) (Li et al., 2014a, 2014b), and graphene quantum dot-based electrochemiluminescence system (LOD: 70 nM) (Dong et al., 2014). Such a low detection limit was possibly due to the signal amplification by the massive invertase on the AuNPs and the inherent high enzymatic turnover of invertase.

3.4. Reproducibility, selectivity and stability

To investigate the reproducibility and precision of PGM-based sensing platform, we used the newly prepared Enz-AuNP-DNA and DNAzyme-MTP for the detection of three Pb^{2+} levels, *i.e.* 5 pM, 50 pM and 0.5 nM. As shown in Fig. 3B, the relative standard deviations (RSD) toward the mentioned-above concentration were 12.1%, 5.3% and 3.4% ($n=5$), respectively. So, the reproducibility and precision of PGM-based sensing platform were satisfactory.

Next, the selectivity of PGM-based Pb^{2+} sensing system was evaluated by monitoring other metal ions, *e.g.* Hg^{2+} (5 nM), Fe^{3+} (5 nM), Cu^{2+} (5 nM), Cd^{2+} (5 nM), and Ag^{+} (5 nM), with target Pb^{2+} (5 pM). Initially, these ions were assayed alone, and then the mixtures containing Pb^{2+} standard were monitored, spectively. As shown in Fig. 4A, the PGM-based sensing system could be only reponsed with target Pb^{2+}. Moreover, other interferring ions did not cause the significant change of PGM-based Pb^{2+} sensing system in the PGM signal. Thus, the PGM-based Pb^{2+} sensing system could be used for specific determination of target Pb^{2+}.

The stability of the as-prepared DNAzyme-MTP and Enz-AuNP-DNA was investigated on an 8-week period (*Note*: DNAzyme-MTP and Enz-AuNP-DNA were stored at 4 °C when not in use). As indicated in Fig. 4B, the PGM signals were not almost changed within the initial five weeks toward 5 pM Pb^{2+}. However, the PGM signals at the 6th, 7th and 8th week could maintain 93.1%, 88.4% and 83.6% of its initial signal, respectively. Good long-term stability might be attributed to the strong reaction between streptavidin and biotinylated DNAzyme, and between gold nanoparticles and biomolecules.

3.5. Evaluation of environmental real samples

The method trueness of PGM-based sensing system was evaluated by monitoring real samples, *e.g.* naturally contaminated sewage and spiked drinking water, with the referenced inductively coupled plasma mass spectrometry (ICP-MS) (*Note*: The high-concentration samples were diluted with detection buffer). As seen from Fig. 5A and B, the slope and intercept of the regression equations between two methods were close to '1' and '0', respectively, regardless of naturally contaminated sewage or spiked drinking water. The results indicated that these two methods displayed a good correlation for the analysis of real samples. Thus, Pb^{2+}-specific DNAzyme-modified microplate could be preliminarily employed for monitoring lead ion in the environmental samples by coupling with PGM and the amplification efficiency of invertase on the Enz-AuNP-DNA.

4. Conclusion

In summary, we design a low-cost and highly efficient DNA biosensor for monitoring lead ion in the environmental samples by coupling with specific DNAzyme-modified microplate and portable glucometer-based detection mode. Use of Pb^{2+}-specific DNAzyme strengthens the selectivity of PGM-based sensing system, whilst utilization of Enz-AuNP-DNA enhances the sensitivity. Specifically, we employ DNAzyme-associated microplate as the functional unit for sensing target analyte with a relatively low cost. Target-responsive DNAzyme cleavage accompanying the assembly of Enz-AuNP-DNA can cause the hydrolysis of sucrose into glucose, which can be monitoring by using an external PGM. The assayed results are comparable with a commercialized available ICP-MS method. Importantly, the PGM-based sensing platform can be widely used in the developing countries without the requirement of sophisticated equipment. We suspect that such a PGM-based sensing system can be extended to monitoring other heavy metal ions by changing the immobilized DNA molecules.

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References

Aboufazeli, F., Zhad, H., Sadeghi, O., Karimi, M., Najafi, E., 2013. Food Chem. 141, 3459–3465.
Brown, A., Li, J., Pavot, C., Lu, Y., 2003. Biochemistry 42, 7152–7161.
Cha, K., Jensen, G., Balijepalli, A., Cohan, B., Meyerhoff, M., 2014. Anal. Chem. 86, 1902–1906.
Cheng, Y., Huang, Y., Lei, J., Zhang, L., Ju, H., 2014. Anal. Chem. 86, 5158–5163.
Cui, L., Wu, J., Ju, H., 2015. Biosens. Bioelectron. 63, 276–286.
Dong, Y., Tian, W., Ren, S., Dai, R., Chi, Y., Chen, G., 2014. ACS Appl. Mater. Interface 6, 1646–1651.
Duetz, W., 2007. Trend Microbiol. 15, 469–475.
Fu, L., Zhuang, J., Lai, W., Que, X., Lu, M., Tang, D., 2013. J. Mater. Chem. B 1, 6123–6128.
Gao, Z., Tang, D., Xu, M., Chen, G., Yang, H., 2014. Chem. Commun. 50, 6256–6258.
Ge, C., Luo, Q., Wang, D., Zhao, S., Liang, X., Yu, L., Xing, X., Zeng, L., 2014. Anal. Chem. 86, 6387–6392.
Gherasim, C., Krivik, J., Mikulasek, P., 2014. Chem. Eng. J. 256, 324–334.
Harink, B., Le Gac, S., Barata, D., Van Blitterswijk, C., Habibovic, P., 2014. Lab Chip 14, 1816–1820.
He, Y., Lu, Y., 2011. Chem. Eur. J. 17, 13732–13742.
Hou, L., Zhu, C., Wu, X., Chen, G., Tang, D., 2014. Chem. Commun. 50, 1441–1443.

Jarczewska, M., Kierzkowska, E., Ziolkowski, R., Gorski, T., Malinowska, E., 2015. Bioelectrochemistry 101, 35–41.
Lan, T., Furuya, K., Lu, Y., 2010. Chem. Commun. 46, 3896–3898.
Li, C., Wei, L., Liu, X., Lei, L., Li, G., 2014a. Anal. Chim. Acta 831, 60–64.
Li, W., Yang, Y., Chen, J., Zhang, Q., Wang, Y., Wang, F., Yu, C., 2014b. Biosens. Bioelectron. 53, 245–249.
Lien, C., Tseng, Y., Huang, C., Chang, H., 2014. Anal. Chem. 86, 2065–2072.
Lu, L., Si, J., Gao, Z., Zhang, Y., Lei, J., Luo, H., Li, N., 2015. Biosens. Bioelectron. 63, 14–20.
Mandappa, I., Ranjini, A., Haware, D., Manonmani, H., 2014. J. Immunoass. Immunochem. 35, 1–11.
Mazumdar, D., Nagraj, N., Kim, H., Meng, X., Brown, A., Sun, Q., Li, W., Lu, Y., 2009. J. Am. Chem. Soc. 131, 5506–5515.
Sabaeifard, P., Abdi-Ali, A., Soudi, M., Dinarvand, R., 2014. J. Microbiol. Method 105, 134–140.
Su, W., Cho, M., Nam, J., Choe, W., Lee, Y., 2013. Biosens. Bioelectron. 48, 263–269.
Tang, D., Liu, B., Niessner, R., Li, P., Knopp, D., 2013. Anal. Chem. 85, 10589–10596.
Tang, D., Ren, J., 2008. Anal. Chem. 80, 8064–8070.
Tang, D., Yuan, R., Chai, Y., 2008. Anal. Chem. 80, 1582–1588.
Tang, S., Lu, W., Gu, F., Tong, P., Yan, Z., Zhang, L., 2014. Electrochim. Acta 134, 1–7.
Wang, Z., Lee, J., Lu, Y., 2008. Adv. Mater. 20, 3263–3267.
Wilming, A., Bahr, C., Kamerke, C., Buchs, J., 2014. J. Ind. Microbiol. Biotechnol. 41, 513–525.
Xiang, Y., Lu, Y., 2014. Inorg. Chem. 53, 1925–1942.
Yan, L., Zhu, Z., Zou, Y., Huang, Y., Liu, D., Jia, S., Xu, D., Wu, M., Zhou, Y., Zhou, S., Yang, C., 2013. J. Am. Chem. Soc. 135, 3748–3751.
Zhan, S., Wu, Y., Luo, Y., Liu, L., He, L., Xing, H., Zhou, P., 2014. Anal. Biochem. 462, 19–25.
Zhang, B., Liu, B., Tang, D., Niessner, R., Chen, G., Kopp, D., 2012. Anal. Chem. 84, 5392–5399.
Zhang, B., Lu, L., Hu, Q., Huang, F., Lin, Z., 2014. Biosens. Bioelectron. 56, 243–249.
Zhou, Z., Yu, Y., Zhang, M., Chen, J., Ren, Q., Song, J., Liu, B., Ma, Z., Zhao, Y., 2014. Sens. Actuators B 201, 496–502.