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An invasive DNA approach toward a general method for portable quantification of metal ions using a personal glucose meter†,‡

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Abstract

We report herein a general methodology for metal ion detection using low-cost, simple, and widely accessible personal glucose meters through an invasive DNA approach.

Metal ions can be beneficial or hazardous to human health and the environment. Of particular concern are toxic metal ions such as lead (Pb^{2+}), which can interfere with a variety of physiological processes and cause damage to many organs, leading to several disorders in permanent learning and behavior in children,^{1,2} and uranium (UO_2^{2+}), which is a radioactive and potentially carcinogenic species.³ To prevent potential human exposure to these hazardous metal ions, methods to quantify them are in high demand. Traditional techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), are sensitive but require sophisticated instruments and skilled personnel. In contrast, methods based on chemosensors and biosensors are simpler in instrumentation and operation,^{4–15} and are therefore more suitable for on site and real time detection with limited resources.

One challenge in developing such sensors is the establishment of a general platform for designing sensors for a broad range of target metal ions, while avoiding the time-consuming trials and errors in designing sensors for each type of metal ion. One way to meet this challenge is to employ DNAzymes^{16,17} that are specific to a variety of metal ions, such as Mg^{2+} ,¹⁷ Pb^{2+} ,^{17,18} UO_2^{2+} ,¹⁹ Cu^{2+} ,^{20,21} Zn^{2+} ,¹⁷ and Hg^{2+} .^{22,23} Many analytical techniques have been utilized to transform the target-specific activity of DNAzymes into physically detectable signals for the detection of metal ions,^{24–28} including colorimetry,^{12,29–34} fluorescence,^{13,19,23,35–38} and electrochemistry.^{39–42} Most of these methods, however, require laboratory-based or customized instruments that are not cost effective or commercially available to the general public. Although some colorimetric or fluorescent tests, including dipsticks,^{34,43} can detect metal ions without any instruments, they can only achieve qualitative or semi-quantitative detection based on color observation, whose accuracy may vary among different observers or can change under different light conditions.

To meet this challenge, we propose the use of low-cost, simple, and widely accessible personal glucose meters (PGMs) to quantify hazardous metal ions. PGM is currently one of the most widely used diagnostic devices in the world, as a result of the more than 30 years of development.^{44,45} The recent integration of PGMs into mobile phones can further facilitate their use by the public.⁴⁶ However, the state-of-art PGMs are currently only used for blood glucose monitoring by diabetic patients. Recently, we have successfully developed a method to use PGMs to detect a broad range of non-glucose targets.^{47–49} The method was applied to

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UO₂²⁺ detection using a UO₂²⁺-specific DNAzyme¹⁹ and showed promising results.⁴⁷ However, we found that the performance of the above method, such as the sensitivity, significantly decreased when the method was applied to detect other heavy metal ions, such as Pb²⁺, probably because the proteins used in the design (including streptavidin, invertase and blocking proteins on the magnetic beads (MB)) could compete with DNAzymes in binding metal ions through accessible amino acid residues such as on those proteins. To make the methodology more generally applicable to different metal ions, including thiophilic ones like Pb²⁺,^{29,35,37} we report here a new approach to separate the metal-ion-induced cleavage of DNAzymes' substrates from the target-induced release of invertase-DNA conjugates.

The schematic diagram of the methodology is shown in Fig. 1. To detect Pb²⁺, the DNA sequence of the original 8–17 DNAzyme^{17,18} was used with minor base changes in the arm (shown in green), while the substrate (in black and red) was extended by 9 nucleotides (nt) in the 5' arm to reduce the amount of non-hybridized substrates (Fig. 1a). To demonstrate the generality of the method, we also chose a UO₂²⁺-specific 39E DNAzyme^{19,31,50} and made similar minor changes to its sequences (Fig. 1b). One remarkable feature of DNAzymes is that the sequences in the substrate binding arms at both ends can be varied with little effect on the enzymatic activity or metal ion selectivity.^{27,28,51,52} Upon addition of the respective metal ions, *i.e.*, Pb²⁺ for the 8–17 DNAzyme, or UO₂²⁺ for the 39E DNAzyme, the DNAzymes catalyzed the cleavage of the DNA substrates at the 3' phosphoester bond of the ribonucleotide A (rA). The right arm (red) of the cleaved substrates could no longer form a stable duplex with the DNAzymes due to the decrease of base pair number from 23 to 9, and thus was released as a 19-nt oligonucleotide (called invasive DNA, Fig. 1c) into the solution. In a separate solution, the invertase was conjugated to a 24-nt DNA (in green, containing a A₁₂ linker, as in Fig. 1c)⁴⁷ that could hybridize to a biotinylated DNA (in black) that bound to streptavidin-coated MBs. Because the released 19-nt oligo-nucleotide from the cleaved substrate is longer in matched base pairs with the biotinylated DNA than the 24-nt DNA on the invertase, the former could serve as an invasive DNA to compete with the DNA–invertase conjugates in hybridizing with the biotinylated DNA on the MBs, and induce the release of the DNA–invertase conjugates. Finally, the released DNA–invertase conjugates were collected by magnetic separation and used to transform sucrose into glucose, which was measured by a PGM to determine the Pb²⁺ or UO₂²⁺ concentrations in the samples (Fig. 1d). In contrast, in the absence of Pb²⁺ or UO₂²⁺, the un-cleaved substrates remained hybridized to the DNAzymes to form a stable duplex and could not cause the release of DNA–invertase conjugates, resulting in no glucose production for PGM measurement. By separating the DNAzyme-catalyzed reaction in the presence of metal ions shown in Fig. 1a and b from the solution containing the invertase conjugates and MBs for DNA invasion (Fig. 1c and d), we can avoid the interferences of the proteins associated in the sensor system in binding to the target heavy metal ions.

In 100 µL buffer A containing 50 mM HEPES (pH 7.1) and 0.15 M NaCl, 2 µM DNA substrate and 3 µM DNAzyme for Pb²⁺ detection were mixed with different concentrations of Pb²⁺ to initiate the cleavage reaction (Fig. 1a). After quenching the reaction with 2 µL 100 mM EDTA, the solution was mixed with 100 µL buffer B containing 0.1 M sodium phosphate (pH 7.2), 0.2 M NaCl, and 0.05% Tween-20. An aliquot of 150 µL of the resulting solution was then transferred to the MB residues containing DNA–invertase conjugates to induce release. After removal of the MBs by a magnet, 40 µL of the supernatant solution was mixed with 40 µL of 1 M sucrose in buffer B to generate glucose. Finally, the solution was tested by a PGM and the signal readouts were used to calculate the Pb²⁺ concentration in the samples.

As shown in Fig. 2a, when the concentrations of Pb^{2+} present in the samples increased from 0 to 1000 nM, the final readout in the PGM increased accordingly until reaching a plateau. The detection limit was found to be 16 nM, as defined by $3\sigma_b/\text{slope}$ (σ_b is the standard deviation of five blank samples), with a dynamic range of at least 16–1000 nM. This detection limit is more than 4-fold lower than the US EPA regulated level (72 nM) in drinking water, and comparable or even better than some other reported methods based on similar DNAzymes.^{18,29,32,37} In addition, other metal ions showed very low interference to the Pb^{2+} detection (Fig. 2b), suggesting that the high selectivity of the DNAzyme^{17,18} was well preserved in the sensor design. The effect of pH and ionic strength on the performance of the method was investigated and shown in Fig. S1 in ESI.† The method was also successfully applied to the detection of Pb^{2+} in drinking water and table dust, where the Pb^{2+} concentrations were dependent on the glucose signal readout *via* the PGM measurements (Fig. S2 in ESI†). The Pb^{2+} detection in drinking water, using two different batches of the DNA–invertase immobilized MBs, gave similar calibration curves (Fig. S3 in ESI†), suggesting that the batch-to-batch difference is small.

To demonstrate that our methodology is generally applicable to other metal ions, we have also employed a similar design using a UO_2^{2+} -dependent DNAzyme in buffer C containing 50 mM MES (pH 5.5) and 0.3 M NaCl (Fig. 1b).^{19,31} Because the invasive DNA here (Fig. 1b) has an identical sequence to that in the Pb^{2+} detection (Fig. 1a), the same DNA–invertase conjugate and MBs can be used for the detection of both Pb^{2+} and UO_2^{2+} in order to avoid the complicated procedures of preparing new DNA–invertase conjugates and MBs for detecting each metal ion. With a similar mechanism, the invasive DNA was produced only in the presence of UO_2^{2+} (Fig. 1b), and caused the release of DNA–invertase conjugates from the MBs *via* DNA invasion (Fig. 1c). Finally, the released DNA–invertase conjugates catalyzed the hydrolysis of sucrose into glucose, which was quantified by a PGM. More glucose production was detected by the PGM when an increasing amount of UO_2^{2+} was present in the samples until signal saturation, where all the DNA–invertase conjugates were released by the invasive ssDNA (Fig. 3a). The detection limit, according to the definition of $3\sigma_b/\text{slope}$, was estimated to be 5.0 nM, which is more than 20-fold lower than the US EPA regulated level (126 nM). Like the Pb^{2+} detection above, the UO_2^{2+} detection here was also very selective to UO_2^{2+} over other metal ions because of the high selectivity of the DNAzyme (Fig. 3b).

In summary, we have developed a general methodology using PGMs to detect metal ions, including thiophilic and nonthiophilic heavy metal ions, by separating the metal-dependent DNAzyme reactions from the release of DNA–invertase conjugates. The invasive DNA yielded from the DNAzyme-catalyzed reactions could release the DNA–invertase conjugates from the MBs, resulting in hydrolysis of sucrose to produce glucose for PGM measurement. Using this method, both Pb^{2+} and UO_2^{2+} were successfully quantified by PGMs, with detection limits (16 and 5.0 nM for Pb^{2+} and UO_2^{2+} , respectively) below the maximum contamination levels defined by the US EPA in drinking water (72 and 126 nM for Pb^{2+} and UO_2^{2+} , respectively), and with excellent selectivity. The methodology should also be applicable for the quantification of other metal ions, if the proper metal-specific DNAzymes and substrates are used. Because of the simple operation, low cost, and wide availability of PGMs, the method demonstrated in this work can be used by the public for quantitative detection of hazardous metal ions in drinking water and other samples at any point of interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
 (a) Pb^{2+} -induced cleavage of the DNA substrate by the Pb^{2+} -dependent 8-17 DNAzyme. (b) UO_2^{2+} -induced cleavage of the DNA substrate by the UO_2^{2+} -dependent 39E DNAzyme. Both reactions yield the cleaved ssDNA product (red) as the invasive DNA. (c) Release of DNA-invertase conjugates from MBs by the invasive DNA. (d) The invasion and release steps, followed by the conversion of sucrose into glucose by the released DNA-invertase conjugates for PGM measurement.

Fig. 2.

(a) Detection of Pb^{2+} using a PGM. (b) The selectivity of the Pb^{2+} detection over other divalent metal ions using a PGM.

Fig. 3.
(a) Detection of UO_2^{2+} using a PGM. (b) The selectivity of the UO_2^{2+} detection over other divalent metal ions using a PGM.