A Highly Sensitive and Selective Catalytic DNA Biosensor for Lead Ions

Jing Li[†] and Yi Lu*[,†,‡]

Departments of Chemistry and Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

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The recent discovery of catalytically active DNAs (deoxyribozymes)[1] has led to a wide-spread interest in their use as simple, stable, and cost-effective alternatives to proteins and ribozymes in biochemical and pharmaceutical applications.[2,3] We report here a new application for catalytic DNAs as a unique class of biosensors for metal ions, specifically Pb^{2+}, with a quantifiable detection range from 10 nM to 4 μM and a selectivity of >80-fold for Pb^{2+} over other metal ions.

Lead is a common environmental contaminant. Low-level lead exposure can lead to a number of adverse health effects.[4] The lead level in the blood is considered toxic when it is ≥480 nM.[5] Current methods for lead detection, such as atomic absorption spectrometry,[6] inductively coupled plasma mass spectrometry,[7] and anodic stripping voltammetry,[8] often require sophisticated equipment or sample treatment. Simple and inexpensive methods that permit real-time sampling of Pb^{2+} are important in the fields of environmental monitoring, clinical toxicology, wastewater treatment, and industrial process monitoring.

Fluorosensors based on fluorescently labeled organic chelators,[9,10] proteins,[11−13] or peptides[14,15] have emerged as powerful tools toward achieving the above goals.[16] While remarkable progress has been made in developing fluorosensors for metal ions such as Ca^{2+} [9,12] and Zn^{2+},[13,14] designing and synthesizing sensitive and selective metal ion fluorosensors remains a significant challenge. Perhaps the biggest challenge in fluorosensor research is the design and synthesis of a sensor capable of specific and strong metal-binding. Since our knowledge about the construction of metal-binding sites is limited, searching for sensors in a combinatorial way is of significant value. In this regard, *in vitro* selection of DNA/RNA from a library of $10^{14}-10^{15}$ random DNA/RNA sequences offers considerable opportunity.[1,3] Compared with combinatorial searches of chemosensors and peptidyl sensors, *in vitro* selection of DNA/RNA is capable of sampling a larger pool of sequences, amplifying the desired sequences by the polymerase chain reaction (PCR), and introducing mutations to improve performance by mutagenic PCR. For example, the *in vitro* selection method has been used to obtain DNA/RNA aptamers[17,18] and aptazymes[19] that are responsive to small organic molecules. Similarly, ribozymes/deoxyribozymes that are highly specific for Pb^{2+},[1,20] Cu^{2+},[21] and Zn^{2+} [22,23] have been obtained. The use of DNA/RNA aptamers to transduce the molecular recognition of small organic molecules to a change in fluorescence intensity has been demonstrated recently.[18] A sensitive deoxyribozyme/fluorophore system was also designed to detect and quantify nucleic acids in clinical specimens.[24] These results set the stage for the utilization of deoxyribozymes with hydrolytic cleavage activity for detection of metal ions. A deoxyribozyme is chosen for this study because it is capable of transducing signals through both molecular recognition of metal ions and metal-ion-dependent hydrolytic cleavage activity, resulting in a potentially wide detection range and high sensitivity.

An *in vitro*-selected deoxyribozyme (termed 17E) that is capable of cleaving a single RNA linkage within a DNA substrate (termed 17DS)[22] (Figure 1a) was chosen for this study. The same 17E sequence motif was obtained from three different *in vitro* selection processes involving 10 mM Mg^{2+},[2] 0.5 mM Mg^{2+}/50 mM histidine,[25] or 100 μM Zn^{2+},[22] with activity in the order of $Zn^{2+} \gg Ca^{2+} > Mg^{2+}$ under similar conditions.[22,26] Further assays of this enzyme indicate a highly Pb^{2+}-dependent activity with $k_{obs} = 6.5\ min^{-1}$ at pH 6.0.[27] The apparent K_d values for Pb^{2+}, Zn^{2+}, and Mg^{2+} are 13.5 μM (at pH 6.0), 0.97 mM (at pH 6.0), and 10.5 mM (at pH 7.0), respectively.[27] The fluorosensor was constructed by labeling the 5′-end of the substrate with the fluorophore 6-carboxytetramethylrhodamine (TAMRA) and the 3′-end of the enzyme strand with a fluorescence quencher, 4-(4′-dimethylaminophenylazo)benzoic acid (Dabcyl).[28] Steady-state fluorescence spectra were obtained by exciting the sample at 560 nm and scanning its emission from 570 to 700 nm.[29] When the substrate (Rh-17DS) was hybridized to the enzyme strand (17E-Dy), the fluorescence of TAMRA was quenched by the nearby Dabcyl (Figure 1b). Upon addition of Pb^{2+}, this quenching was

[†] Department of Chemistry.
[‡] Department of Biochemistry.

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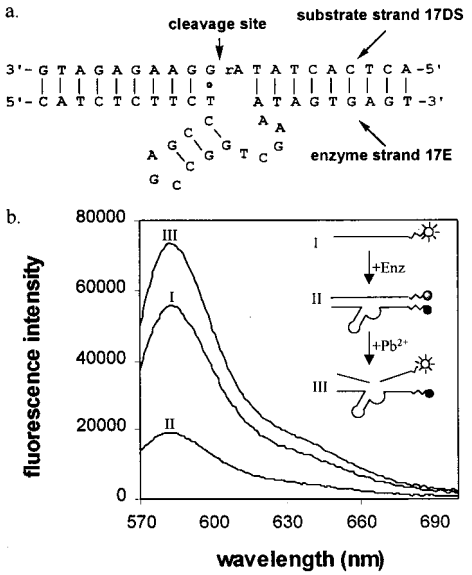

Figure 1. (a) Sequence and proposed secondary structure of the deoxyribozyme/substrate complex. The cleavable substrate (Rh-17DS) is a DNA/RNA chimera in which rA represents a ribonucleotide adenosine. (b) Steady-state fluorescence spectra of the substrate (Rh-17DS) alone (I), after annealing to the deoxyribozyme (17E-Dy) (II), and 15 min after adding 500 nM $Pb(OAc)_2$ (III).[29]

eliminated and the fluorescence of TAMRA increased by ~400%. Little change in the fluorescence signal occurred with addition of Pb^{2+} to the substrate alone or to the complex of the enzyme and a noncleavable all-DNA substrate with identical sequence to 17DS. These findings suggest strongly that the change in fluorescent signal with Rh-17DS/17E-Dy results from a deoxyribozyme-catalyzed cleavage, followed by product release.

The substrate cleavage reaction was monitored in real time with fluorescence spectroscopy. Like the ratiometric, anisotropy, or lifetime-based method, kinetic fluorescence measurement is independent of sampling conditions such as illumination intensity and sample thickness.[16] To test the selectivity of the catalytic DNA sensor, we monitored the fluorescence change (λ_{em} = 580 nm, λ_{ex} = 560 nm) of Rh-17DS/17E-Dy upon addition of nine different divalent metal ions that are known to be active toward DNA/RNA cleavage (Figure 2, insert). At the same concentration (500 nM), Pb^{2+} caused the most rapid change in fluorescence with a rate of 380 counts s^{-1}. The sensitivity toward Pb^{2+} was >80 times higher than for other divalent metal ions (Figure 2). This trend of selectivity was maintained even under simulated physiological conditions containing 100 mM NaCl, 1 mM Mg^{2+}, and 1 mM Ca^{2+} (see Supporting Information). Furthermore, the signal response to Pb^{2+} was not affected by the presence of equal amounts of each of these divalent metal ions. Therefore, this deoxyribozyme sensor is well suited for selectively monitoring Pb^{2+} in the presence of other metal ions.

In addition to the selectivity, the range of Pb^{2+} concentrations which give rise to a fluorescent response is also important. As shown in Figure 3, the rate of fluorescence change increased with Pb^{2+} concentration up to 4 μM, with a detection limit of ~10 nM.

(29) The enzyme−substrate complex was prepared with 50 nM each of 17E-Dy and Rh-17DS in 50 mM NaCl, 50 mM HEPES (pH 7.5). The sample was heated at 90 °C for 2 min and cooled to 5 °C over 15 min to anneal the enzyme and substrate strands together. Steady-state and kinetic fluorescence spectra were collected with an SLM 8000S photon-counting fluorometer. Polarization artifacts were avoided by using "magic angle" conditions. The time-dependent DNA enzyme-catalyzed substrate cleavage was monitored at 580 nm at 2-s intervals. To initiate the reaction, 1−2 μL of concentrated divalent metal ion solution was injected into the cuvette using a 10-μL syringe while the DNA sample (600 μL) in the cuvette was constantly stirred.

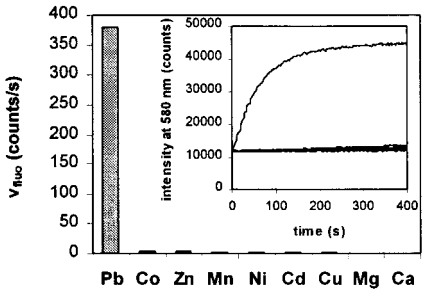

Figure 2. Fluorescence response rate (v_{fluo}) of Rh-17EDS-Dy in the presence of 500 nM of different divalent metal ions in 50 mM HEPES (pH 7.5).[29] The inset shows the change of fluorescence intensity at 580 nm in response to the addition of M^{2+}. The curve with dramatic change was collected in Pb^{2+}; the other curves were collected in one of the other eight divalent metal ions.

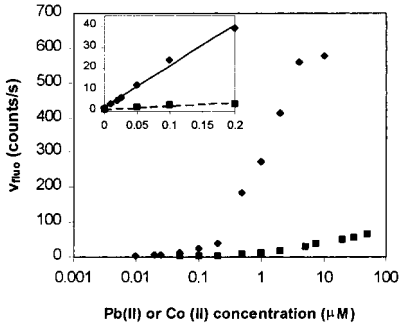

Figure 3. Variation of initial rate, v_{fluo}, with the concentration of Pb^{2+} (◆) or Co^{2+} (■). The inset presents the linear range in submicromolar concentrations of Pb^{2+} or Co^{2+}.

The system described here represents a new class of metal ion sensors and is the first example of a deoxyribozyme-based biosensor for metal ions. It combines the high selectivity of deoxyribozymes (>80-fold for Pb^{2+} over other divalent metal ions) with the high sensitivity of fluorescence detection (>400% signal increase), and can be applied to the quantitative detection of Pb^{2+} over a concentration range of 3 orders of magnitude. Since the fluorescence domain is decoupled from the metal-recognition/catalysis domain, the sensitivity and selectivity of this system can be further improved by a careful choice of fluorophores and by performing further *in vitro* selection of metal-binding domains to not only keep sequences reactive with Pb^{2+} but also remove sequences that also respond to other metal ions. In addition, DNA is stable, cost-effective, and easily adaptable to optical fiber and chip technology for device manufacture. The attachment of DNA enzymes to optical fibers or chips will also allow the regeneration of the sensors by washing away the cleavage products and adding new substrates. Finally, sequences specific for other metal ions and with various detection ranges can be isolated by varying the selection conditions, making this sensor one of the most promising fluorosensor systems.

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Supporting Information Available: Figure showing the fluorescence response rate in the presence of 500 nM of different divalent metal ions under simulated physiological conditions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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