analytical chemistry

Article

pubs.acs.org/ac

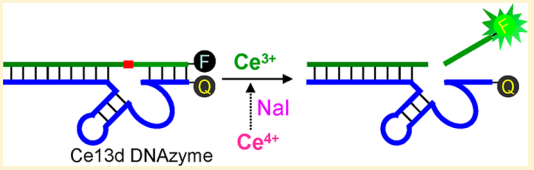
Ultrasensitive DNAzyme Beacon for Lanthanides and Metal Speciation

Po-Jung Jimmy Huang, Jenny Lin, Jing Cao, Mahsa Vazin, and Juewen Liu*

Department of Chemistry, Waterloo Institute for Nanotechnology , University of Waterloo 200 University Avenue West, Waterloo, Ontario N2L 3G1, Canada

Supporting Information

ABSTRACT: Metal-ion detection and speciation analysis is crucial for environmental monitoring. Despite the importance of lanthanides, few sensors are available for their detection. DNAzymes have been previously used to detect divalent metals, while no analytical work was carried out for trivalent and tetravalent ions. Herein, in vitro selection was performed using a Ce^{4+} salt as the target metal, and a new DNAzyme (named Ce13) with a bulged hairpin structure was isolated and characterized. Interestingly, Ce13 has almost no activity with Ce^{4+} but is highly active with all trivalent lanthanides and Y^{3+}, serving as a general probe for rare earth metals (omitting Sc). A DNAzyme beacon was engineered detecting down to 1.7 nM Ce^{3+} (240 parts per trillion), and other lanthanides showed similar sensitivity. The feasibility of metal speciation analysis was demonstrated by measuring the reduction of Ce^{4+} to Ce^{3+}.



INTRODUCTION

Lanthanides are extremely important for their catalytic, magnetic, optical, and electronic properties.[1,2] Well-known lanthanide-containing examples include superconductors, lasers, TV displays, luminescent probes, and imaging contrast agents. Their widespread applications underscore the importance of accurately measuring lanthanides to control pollution, monitor industrial processes, study reaction mechanisms, understand biodistribution, and find new mineral deposits.

Analysis of lanthanides is currently carried out using instrumentation methods such as inductively coupled plasma-mass spectrometry (ICP-MS), capillary electrophoresis, and vibration spectroscopy.[3] While these methods provide high sensitivity and detect a few metals at the same time, they often require multiple steps of sample pretreatment and sending samples to centralized laboratories at a high cost with a long turnaround time. It is more desirable to perform on-site and real-time detection. To this end, sensors provide a useful alternative. A number of ion-selective electrodes and fluorescent/colorimetic chelators have been reported for lanthanides.[4,5] Most of these small organic molecule-based probes require organic solvents with poor sensitivity. In addition, the current sensors detect a few lanthanides; no sensor can selectively detect each element. At the same time, no general sensors are available to detect all the lanthanides with the same sensitivity either. While lanthanide complexes have been used as a label for immunoassays, using the biosensor strategy for their detection has not been explored. Out of the many biomolecules, DNAzymes have emerged as a unique platform for designing metal biosensors.

DNAzymes are DNA-based biocatalysts. To date, all DNAzymes were isolated from in vitro selection. DNAzymes have found important applications for biosensor development, antiviral agents, and assembly of smart nanomaterials.[6−13] DNAzymes for RNA hydrolysis are particularly attractive due to excellent activity, high enzyme stability, and versatility in signal generation.[14−21] Almost exclusively DNAzymes require divalent metal ions as a cofactor.[22,23] A number of metal-specific DNAzymes have been isolated, showing high activity in the presence Pb^{2+},[24,25] Zn^{2+},[26] Cu^{2+},[27−29] UO_2^{2+},[30] and Hg^{2+}.[31,32] Many of these DNAzymes have been engineered into metal biosensors.[15] However, using DNAzymes for detecting trivalent and tetravalent metals has yet to be explored.

Trivalent lanthanides have been used in nucleic acids research (including DNAzymes) in a few systems. Luminescent lanthanides were employed to probe nucleic acids.[33−35] The Leadzyme is a small ribozyme, and lanthanides (in particular Nd^{3+}) enhance its activity in the presence of Pb^{2+}, although the enzyme is inactive with lanthanides alone.[36,37] Ce^{3+}, Eu^{3+}, and Yb^{3+} were used together with Zn^{2+} to select DNAzymes for DNA cleavage.[38] These lanthanides were essential for enzyme activity. Recently,[39] Tb^{3+} and Gd^{3+} were reported to accelerate a previously published divalent metal-dependent DNA ligase.[40] Tb^{3+} was also reported as an inhibitor for the 8−17 DNAzymes.[33] Lanthanides might be a good choice for RNA/DNA cleavage since many lanthanide complexes are efficient catalysts for nonspecific nucleic acid hydrolysis.[41,42]

To date, however, no selection was carried out using lanthanides as the sole metal cofactor to obtain RNA-cleaving DNAzymes. Most previous studies require coaddition of divalent metals, complicating downstream analytical applications. Herein, we report selection of an RNA-cleaving

Received: November 19, 2013
Accepted: January 3, 2014
Published: January 3, 2014

   dx.doi.org/10.1021/ac403762s | *Anal. Chem.* 2014, 86, 1816−1821

DNAzyme using Ce^{4+} as the intended metal cofactor, since it is highly efficient in assisting cleavage of phosphate diester bonds.[43] Interestingly, we obtained a new DNAzyme that is active with Ce^{3+} and other trivalent lanthanides. Conversion between these two oxidation states of cerium was also monitored, showing the feasibility of metal speciation analysis.

METHODS

In Vitro Selection. For this in vitro selection experiment the initial DNA library was prepared by ligating two pieces of DNA (Lib-FAM and Lib-rA) with a splint DNA. Lib-FAM DNA (200 pmol) and Lib-rA DNA (300 pmol) were mixed with splint DNA (300 pmol) first in buffer A (50 mM pH 7.5 Tris-HCl, pH 7.5, 10 mM $MgCl_2$). The three strands of DNA were annealed at 95 °C for 1 min followed by slow cooling to room temperature. The T4 ligation protocol provided by New England Biolabs was followed for the ligation reaction. Ligated DNA product was purified with 10% denaturing polyacrylamide gel (dPAGE) at 650 V for 1 h, and DNA was extracted from the gel with buffer B (1 mM EDTA, 10 mM Tris-HCl, pH 7.0). The extracted DNA library was further concentrated via ethanol precipitation and resuspended in 60 μL of buffer C (50 mM MES, pH 6.0, 25 mM NaCl), which was the selection buffer. This DNA was used directly as the DNA library for the first round of selection. For each of the subsequent rounds the library was generated from PCR. For the in vitro selection experiment, the random DNA pool was incubated with freshly prepared Ce(IV) metal ion. Incubation time and concentration of metal salt are in Table S2, Supporting Information. After incubation, the reaction was quenched with 8 M urea and purified in 10% dPAGE. A fraction of the selected DNA was extracted from the gel and further purified with a Sep-Pak C18 column (Waters). The purified selected DNA was then dried in an Eppendorf Vacufuge at 30 °C overnight. Dried DNA was resuspended in 70 μL of 5 mM HEPES buffer (pH 7.5).

PCR. During the in vitro selection experiment three PCR reactions were carried out for each round. After the cleavage reaction, a real-time PCR (rt-PCR) was carried out to quantify the amount of cleaved DNA that was extracted from the gel. The 20 μL reaction mixture contains 1 μL of purified DNA template, 400 nM primer (P1 and P2), and 10 μL of SsoFast EvaGreen Supermix (Bio-Rad). The thermocycling steps provided by the vendor were followed (95 °C for 30 s, 95 °C for 5 s, and 55 °C for 5 s).

PCR1. A 50 μL PCR reaction mixture contained the following: 1 μL of DNA template, 200 nM each of P1 and P2, 200 μM dNTP mixture, 1 × Taq buffer, and 1.25 units of Taq DNA polymerase. The reaction was carried out for 15−20 cycles. DNA was amplified using the following cycling steps: 94 °C for 5 min, 94 °C for 30 s, 55 °C for 30 s, and 72 °C 30 s. A gel/PCR DNA fragment extraction kit (IBI Scientific) was used to purify the PCR1 product. Purified product was used as template for PCR2.

PCR2. One-tenth of the purified product was further amplified for 12 cycles using P3 and P4 as primers. A 200 μL PCR reaction mixture contains 4 μL of diluted template from PCR1, 250 nM each of P3 and P4, 200 μM dNTP mixture, 1 × Taq buffer, and 5 units of Taq DNA polymerase. The thermocycling steps mentioned above were also used here. Final PCR2 product was again purified in 10% dPAGE. Single-stranded FAM-labeled DNA was excised from the gel. After ethanol precipitation, it was used as the library pool for the subsequent round of selection.

Cloning and Sequencing. Selection was stopped at round 6, where the PCR1 product was cloned using the TA-TOPO Cloning Kit (Invitrogen) and Subcloning Efficiency DH5α competent cells (Invitrogen). The protocol provided by the vendor was followed. The plasmid DNA was extracted and purified by using a DirectPrep 96 miniPrep Kit (QIAGEN). Sample was then submitted to TCAG DNA Sequencing Facility (Toronto, ON) for analysis.

Activity Assays. For a typical gel-based activity assay, a final of 10 μM metal ions were incubated with 5 μL of 1 μM DNAzyme complex in buffer C for 1 h. The complex was formed by annealing the FAM-labeled substrate and the enzyme in buffer C. Samples were quenched with 8 M urea and run in 15% dPAGE at 120 V for 80 min. Gel images were taken with a Bio-Rad ChemiDoc MP imaging system.

Fluorophore/Quencher-Based Assay. Kinetics studies were carried out using 96-well plates and monitored with a Molecular Device SpectraMax M3 microplate reader. Complex was formed by annealing the FAM-labeled substrate and the quencher-labeled enzyme in buffer C. A 100 μL amount of 50 nM FAM-Q DNAzyme in 50 mM pH 7.5 HEPES (pH 7.5) was used for each well. A 1 μL amount of target ions was added after 5 min of background reading. Samples were continuously monitored after addition for at least 30 min with a 10 s interval. Chemicals used in this work and additional methods are in the Supporting Information. For reaction between Ce^{4+} and NaI, the initial Ce^{4+} concentration was 10 mM and then diluted to 1 μM for detection, and the NaI concentration was 50 mM.

RESULTS AND DISCUSSION

DNAzyme Selection. The in vitro selection experiment was carried out using a DNA library containing a 50-nucleotide randomized region (N_{50}) as shown in Figure 1A (in blue). There is a single RNA linkage (rA) in the whole sequence to provide a scissile bond, since RNA is about one million fold less stable than DNA.[44] Our initial library contained ~10^{14} random DNA sequences. $(NH_4)_2Ce(NO_3)_6$ was used as the Ce^{4+} source because of its excellent solubility and stability. After incubating the library with the salt, the cleaved sequences were isolated using gel electrophoresis since they were 28-nucleotide shorter than the original full-length DNA. Two rounds of polymerase chain reactions (PCR) were carried out to amplify the cleaved DNA (primer sequences in Table S1, Supporting Information). In PCR1, the full-length library was regenerated, and in PCR2, two special primers were used. P3 has a FAM label on its 5′-end and a ribo-adenosine (rA) base near its 3′-end. P4 has a polymer spacer that can stop the polymerase reaction. As a result, two strands of unequal length were produced after PCR2, allowing isolation of the positive strand containing rA via gel electrophoresis to seed the next round of selection. The DNA sequence of the selection library is shown in Figure 1B. Selection was stopped at round 6, when the library was cloned and sequenced (see Table S2, Supporting Information, for selection conditions and progress).

DNAzyme Secondary Structure Analysis. We obtained 19 sequences with the correct DNA insertion (Table S3, Supporting Information). The final library was quite converged, and one-half of the sequences were almost identical. One of the representative clones, named Ce13, folds into the structure shown in Figure 1C, and the N_{50} region is shown in blue. Ce13 contains a hairpin-like structure (in black box), which has been confirmed based on mutation studies (Figure S1, Supporting Information). This hairpin plays only a structural role and can

dx.doi.org/10.1021/ac403762s | Anal. Chem. 2014, 86, 1816−1821

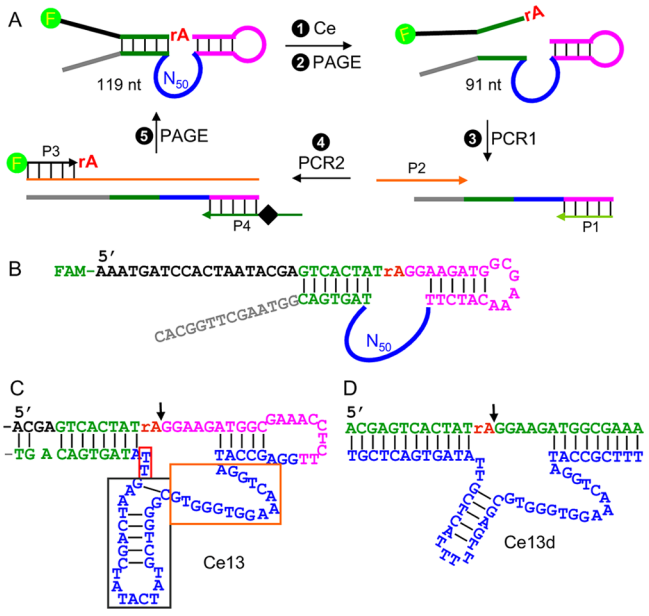


Figure 1. (A) Scheme of DNAzyme selection. Initial library contains N_{50} randomized region and a single RNA linkage (rA) as the cleavage site. Cleaved sequences in the presence of Ce (step 1) are isolated using gel electrophoresis (PAGE, step 2). Two rounds of PCR are carried out followed by another PAGE to regenerate the full-length single-stranded library. (B) Sequence of the library prior to the cleavage step. (C) Secondary structure of the original Ce13 DNAzyme with the N_{50} region in blue. Three regions of this enzyme (in the boxes) are assayed. (D) Optimized and truncated trans-cleaving DNAzyme, Ce13d.

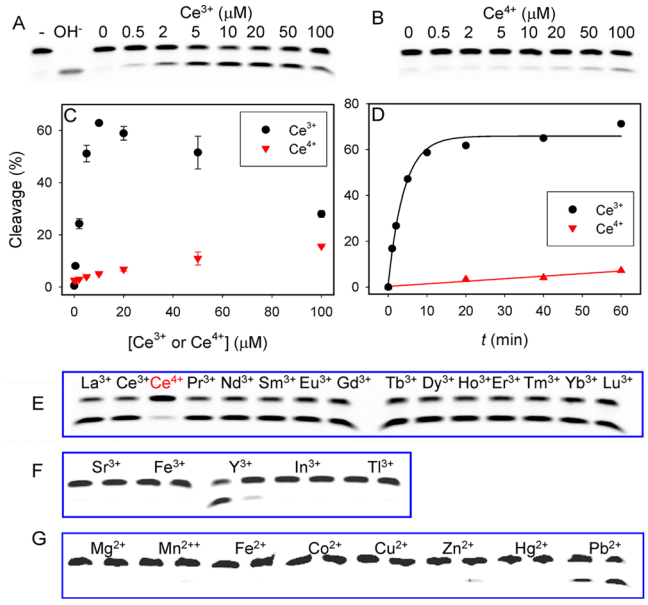


Figure 2. Gel image of Ce13d assay after incubating with various concentrations of Ce^{3+} (A) or Ce^{4+} (B) for 1 h. (A) First lane is the substrate alone (no Ce13d, negative control); second lane is the substrate treated with NaOH (positive control). (C) Quantification of the gel data in A and B. (D) Kinetics of Ce13d cleavage with 10 μM Ce^{3+} or Ce^{4+}. Gel images with 10 μM various lanthanides (E) with 10 and 100 μM trivalent metals (F) or divalent metals (G). (F, G) Below each metal label the left lane is 10 μM and the right lane is 100 μM of the metal. All assays are performed in 50 mM MES buffer (pH 6) with 25 mM NaCl.

be replaced by other hairpin sequences, allowing us to design an optimized and truncated trans-cleaving version of the enzyme named Ce13d (Figure 1D). To further understand this enzyme, additional mutants were tested. There is a TT sequence right next to the enzyme hairpin (in the red box of Figure 1C). Deleting one or both of the thymines only slightly decreased the activity (see Figure S2, Supporting Information). On the other hand, changes to the nucleotides in the orange box often completely abolish the activity. Therefore, these nucleotides appear to be critical for catalysis. Additional enzyme structural analysis is presented in Figures S3 and S4, Supporting Information. Overall, Ce13d is an optimal construct and used for subsequent studies.

Metal Specificity Test. An interesting question that was not previously answered is DNAzyme activity with metals of different oxidation states. Since both Ce^{3+} and Ce^{4+} are stable, this DNAzyme allows us to test it. An assay was performed as a function of Ce^{3+} and Ce^{4+} concentration (Figure 2A). With increasing Ce^{3+} concentration, the cleavage product initially increased followed by an inhibition effect. Optimal activity was obtained at 10 μM Ce^{3+} (Figure 2C, black dots). To our surprise, little activity was observed in the presence of Ce^{4+}, although selection was originally carried out using Ce^{4+} as the intended metal cofactor. Moderate cleavage (~10%) was observed only with ~100 μM Ce^{4+} (Figure 2B and Figure 2C, red dots). Since we used the nitrate salt for Ce^{4+} and the chloride salt for Ce^{3+}, to test whether the activity difference is related to the different salt anions, the enzyme was assayed in the presence of $NaNO_3$. The enzyme was still active with Ce^{3+}, even with additional nitrate (Figure S5, Supporting Information). We further confirmed that the Ce^{4+} salt is not an enzyme inhibitor (Figure S6, Supporting Information).

On the basis of the above data, we reason that there might be a small fraction of Ce^{3+} in the Ce^{4+} salt, which is possible since Ce^{4+} is a strong oxidant and could be converted into Ce^{3+}. The amount of Ce^{3+} is estimated to be ~1% if assuming Ce^{4+} to be completely inactive. During the selection process the Ce^{3+}-catalyzed reactions are faster than those by Ce^{4+}, guiding the library toward Ce^{3+}-dependent DNAzymes. Nevertheless, this enzyme can still distinguish between the two oxidation states of cerium, although in an opposite way as we originally designed. Ce13d shows a cleavage rate of 0.25 min^{-1} with 10 μM Ce^{3+} (Figure 2D). This rate is comparable with many divalent metal-dependent DNAzymes that are selected or assayed at pH ≈ 6.[30,45] The enzyme was active from pH 5 to 8.5, and the maximal rate was at pH 6–7.5 (Figure S7, Supporting Information), which is useful for detection in water samples.

Since divalent metal ions are much more efficient than monovalent ions for DNAzyme catalysis, one may deduce that higher valent metal ions might be even more efficient. From the inorganic chemistry standpoint, a higher oxidation state corresponds to a higher positive charge density and possibly different coordination geometry. The pK_a value of the bound water may also be affected, all of which are important parameters to influence DNA catalysis. However, despite the fact that Ce^{4+} was initially used, the selected enzyme was more active with Ce^{3+}. In addition, the rate for this Ce^{3+}-dependent DNAzyme does not appear to be superior to other DNAzymes using divalent metal cofactors. It is possible that with increasing positive charge density higher valent cations display stronger nonspecific interactions with DNA, making it more difficult to form efficient enzyme structures. Since combining divalent

dx.doi.org/10.1021/ac403762s | Anal. Chem. 2014, 86, 1816–1821

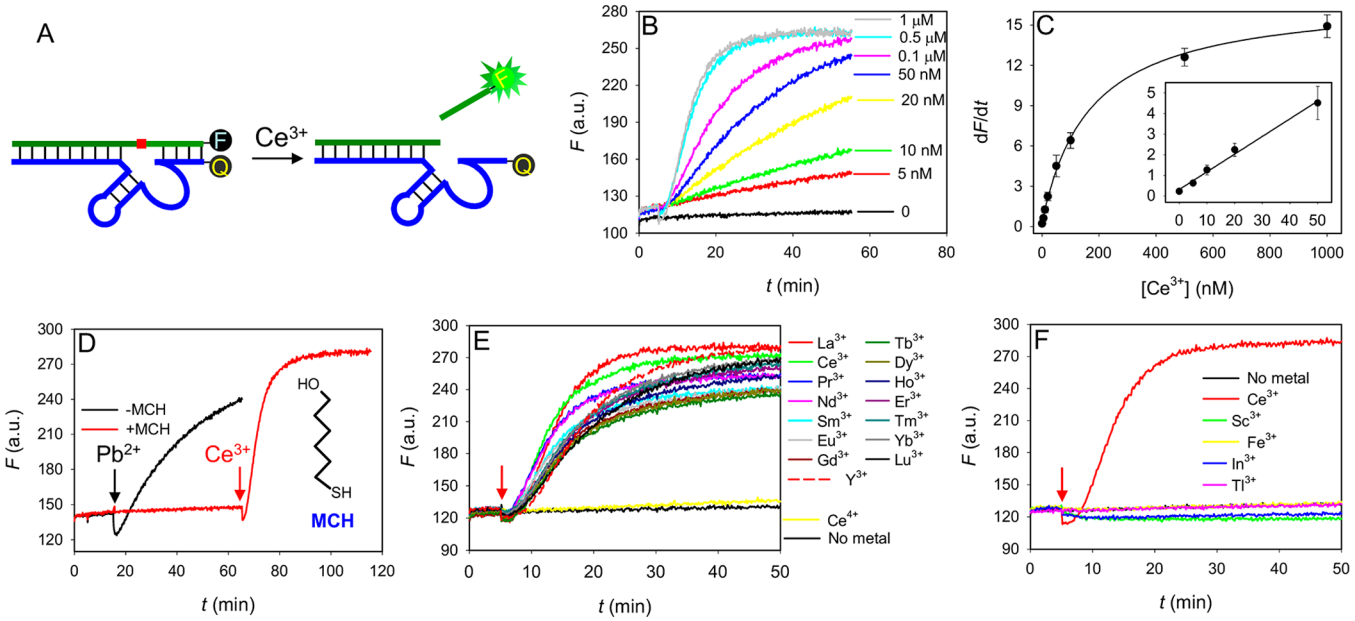


Figure 3. (A) Schematic representation of the DNAzyme beacon. (B) Sensor signaling kinetics to various concentrations of Ce^{3+}. DNAzyme concentration = 50 nM in 50 mM HEPES buffer (pH 7.6). (C) Quantification of Ce^{3+} based on the initial rate of fluorescence enhancement. (Inset) Linear region at low Ce^{3+} concentrations. (D) Masking the 10 μM Pb^{2+} response by 1 mM MCH. Sensor response to 1 μM various lanthanides and Y^{3+} (E) and to other trivalent metal ions (F).

metals and lanthanides has been shown to accelerate DNAzyme catalysis,[36−38] further studies are needed to fully understand the use of lanthanide alone as the metal cofactor. For example, it is possible that selection needs to be carried out with very low metal concentrations to produce DNAzymes active in the presence of Ce^{4+} (e.g., in this selection we used 500 μM Ce^{4+}).

We tested metal specificity next. Interestingly, Ce13d can be cleaved by all the trivalent lanthanides with a similar activity (Figure 2E), reaching ∼70% cleavage in 1 h with 10 μM metal ions. The cleavage rate of a few lanthanides was also measured (Figure S8, Supporting Information), where the activity was similar to that in the presence of Ce^{3+} (within 2-fold). Other trivalent metal ions were also tested (Figure 2F), including Fe^{3+}, Cr^{3+}, In^{3+}, Sc^{3+}, Y^{3+}, and Tl^{3+}. Only Y^{3+} showed cleavage activity; Y is known to be very similar to lanthanides (e.g., ionic radius similar to Ho^{3+}) and classified also as one of the rare earth metals together with the lanthanides. Sc^{3+} showed no activity, although it is in the same row, possibility due to its smaller size. None of the other trivalent metals showed activity, despite the size of Tl^{3+} it is also similar to that of Y^{3+}. Therefore, in addition to size and charge (see Table S4, Supporting Information), metal coordination chemistry must also play an important role. For divalent metal ions Pb^{2+} is the only one that showed moderate activity at 10 μM concentration, while Zn^{2+} showed a very small amount of cleavage at 100 μM (Figure 2G). On the basis of the cleavage assay, the selectivity for lanthanides over Pb^{2+} is more than 20 fold and for other divalent ions is more than 500 fold. Since Pb^{2+} is a thiophilic soft cation while lanthanides are hard Lewis acids with low affinity toward thiol, interference from Pb^{2+} can be masked (vide infra). Overall, this enzyme may serve as a general probe for rare earth metals, which has not been achieved previously.

DNAzyme Beacon Sensor. We next aim to engineer this DNAzyme into a biosensor (Figure 3A). The 5′-end of the substrate strand was extended by 3 nucleotides to form a 15-mer duplex with the enzyme. The 3′-end of the substrate is labeled with a FAM, and it still forms 9 base pairs to allow fluorophore release after cleavage (see Figure S9A, Supporting Information, for the sequences). The enzyme strand was labeled with a quencher on the 5′-end. In the presence of Ce^{3+}, the cleavage reaction may facilitate release of the FAM-labeled fragment to enhance fluorescence (Figure 3A).[25] Cleavage of this sensor system was confirmed by gel electrophoresis (Figure S9B, Supporting Information). With 50 nM of the sensor complex, we observed stable fluorescence in the absence of Ce^{3+}, and the rate of fluorescence enhancement was progressively faster with increasing Ce^{3+} concentration (Figure 3B). The initial slopes of the fluorescence traces were compared to quantify Ce^{3+} up to 500 nM (Figure 3C). A detection limit of 1.7 nM Ce^{3+} (240 parts per trillion) was obtained based on signal greater than 3 times of background variation (Figure 3C, inset). Figure 3E confirms that the sensor works for all lanthanides and Y^{3+} with a similar sensitivity (slope difference within 1 fold). Therefore, the detection limits for all these elements are better than 4 nM. This represents the most sensitive biosensor for lanthanides. Note that this study does not include promethium since it is radioactive, but it is reasonable to believe that Pm^{3+} should also be active based on the chemical trend. The previous gel-based assays indicated that Pb^{2+} might be the main interfering ion. Indeed, 10 μM Pb^{2+} produced a strong signal (Figure 3D, black trace). With 1 mM mercaptohexanol (MCH), the Pb^{2+} response was completely masked while 1 μM Ce^{3+} still produced a strong fluorescence increase (red trace). The sensor has no response to other trivalent metal ions (Figure 3F), consistent with previous gel-based assays.

Ce^{4+}/Ce^{3+} Conversion. To have a complete analysis of metal ions in the environment, not only total metal concentration but also metal speciation information is needed.[46,47] Most analytical instruments such as ICP-MS only measure the total metal concentration. The DNAzyme platform

dx.doi.org/10.1021/ac403762s | Anal. Chem. 2014, 86, 1816−1821

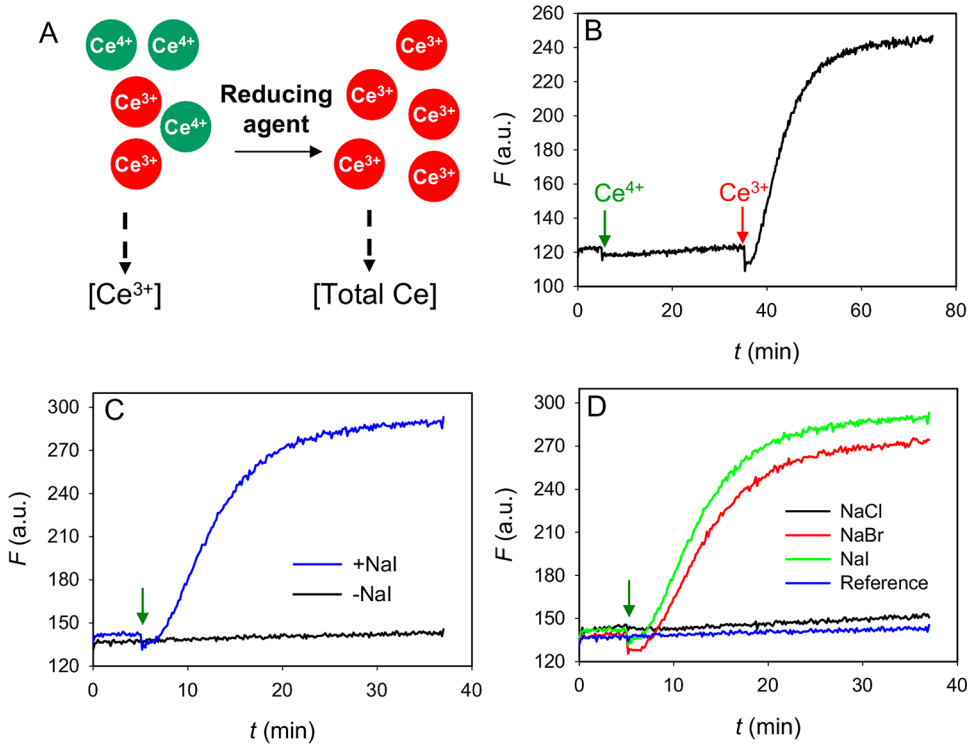


Figure 4. (A) Scheme illustrating analysis of metal speciation using the DNAzyme sensor for different oxidation states. (B) Sensor response to 1 μM Ce^{4+} and then 1 μM Ce^{3+}. (C) Sensor response to Ce^{3+} generated by reacting Ce^{4+} with 50 mM NaI. (D) Sensor response to Ce^{3+} generated by reacting Ce^{4+} with various halide salts, all at 50 mM concentration. Final Ce concentration in the sensor was 1 μM.

might offer a solution to this problem as shown in Figure 4A. Although Ce^{4+} is insensitive to the sensor, its concentration can be obtained by measuring the Ce^{3+} concentration before and after a reducing reaction. To test this idea, the sensor was first exposed to 1 μM Ce^{4+} and the signal remained quite stable (Figure 4B). Addition of Ce^{3+} resulted in a quick fluorescence increase. This excellent selectivity between Ce^{3+} and Ce^{4+} makes it possible to monitor conversion between these two species. Next, we used NaI as the reducing agent to convert Ce^{4+}. The reaction product was added to the DNAzyme sensor, and immediate fluorescence enhancement was observed (Figure 4C), suggesting that Ce^{4+} was reduced to Ce^{3+}. We further tested reaction of Ce^{4+} with different halide anions, where reaction with NaI was the fastest followed by NaBr, while NaCl had no reaction (Figure 4D). This is consistent with the redox trends of these anions, indicating that NaI is the most efficient reagent for this reaction. Aside from its environmental application, this work represents the first example of monitoring metal redox reaction using a DNAzyme. Previously, conversion of protein enzyme-catalyzed chemical transformation has been monitored using aptamers,[48,49] and this ability is useful for understanding reaction mechanisms and screening for reaction inhibitors.

CONCLUSIONS

In this work, we made a number of important observations. (1) We selected the first RNA-cleaving DNAzyme using a lanthanide ion as the sole metal cofactor, and a new DNAzyme was obtained. This work has expanded the range of metal cofactors from divalent to trivalent. This DNAzyme has roughly the same sensitivity to all trivalent lanthanides plus Y^{3+}, and its activity requires no divalent metals. Our engineered DNAzyme beacon has remarkable sensitivity and represents the first general probe for rare earth metals. (2) This is the first example of using DNAzymes to distinguish the different oxidation states of the same metal ions, and monitoring conversion of oxidation states was further demonstrated. This work shows the possibility of using the DNAzyme technology to obtain metal speciation information, which is important for environmental and water quality monitoring. (3) This beacon is a general probe and can detect each lanthanide with excellent sensitivity. At the same time, the total metal information can be obtained (omitting Sc). More stringent selection conditions are needed to isolate for more specific DNAzyme probes. Even with this general probe specific analysis is still possible for certain applications. For example, many applications often involve specific metals (e.g., Gd for MRI and Ce for making antibacterial agents), where this probe can be used. Taken together, while lanthanides are unlikely to interact with DNA in biological systems, our chemical evolution method has made a linkage between them, allowing new analytical applications and offering insights into bioinorganic chemistry.

ASSOCIATED CONTENT

Supporting Information

Selection progress, DNAzyme secondary structure analysis, pH-dependent activity, and control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Fax: (+1) 519-746-0435. E-mail: liujw@uwaterloo.ca.

Notes

The authors declare no competing financial interest.

dx.doi.org/10.1021/ac403762s | *Anal. Chem.* 2014, 86, 1816−1821

■ ACKNOWLEDGMENTS

Funding for this work is from the University of Waterloo, the Canadian Foundation for Innovation, the NSERC of Canada, and the Early Researcher Award from the Ontario Ministry of Research and Innovation.

■ REFERENCES

(1) Kobayashi, S.; Sugiura, M.; Kitagawa, H.; Lam, W. W. L. *Chem. Rev.* **2002**, *102*, 2227−2302.
(2) Wang, F.; Han, Y.; Lim, C. S.; Lu, Y. H.; Wang, J.; Xu, J.; Chen, H. Y.; Zhang, C.; Hong, M. H.; Liu, X. G. *Nature* **2010**, *463*, 1061−1065.
(3) Houk, R. S.; Fassel, V. A.; Flesch, G. D.; Svec, H. J.; Gray, A. L.; Taylor, C. E. *Anal. Chem.* **1980**, *52*, 2283−2289.
(4) Das, P.; Ghosh, A.; Das, A. *Inorg. Chem.* **2010**, *49*, 6909−6916.
(5) Zapata, F.; Caballero, A.; Espinosa, A.; Tarraga, A.; Molina, P. *Eur. J. Inorg. Chem.* **2010**, 697−703.
(6) Santoro, S. W.; Joyce, G. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4262−4266.
(7) Navani, N. K.; Li, Y. *Curr. Opin. Chem. Biol.* **2006**, *10*, 272−281.
(8) Sefah, K.; Phillips, J. A.; Xiong, X. L.; Meng, L.; Van Simaeys, D.; Chen, H.; Martin, J.; Tan, W. H. *Analyst* **2009**, *134*, 1765−1775.
(9) Willner, I.; Shlyahovsky, B.; Zayats, M.; Willner, B. *Chem. Soc. Rev.* **2008**, *37*, 1153−1165.
(10) Lu, Y.; Liu, J. *Acc. Chem. Res.* **2007**, *40*, 315−323.
(11) Du, Y.; Li, B. L.; Wang, E. K. *Acc. Chem. Res.* **2013**, *46*, 203−213.
(12) Chinnapen, D. J. F.; Sen, D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 65−69.
(13) Silverman, S. K. *Nucleic Acids Res.* **2005**, *33*, 6151−6163.
(14) Liu, J.; Cao, Z.; Lu, Y. *Chem. Rev.* **2009**, *109*, 1948−1998.
(15) Zhang, X.-B.; Kong, R.-M.; Lu, Y. *Annu. Rev. Anal. Chem.* **2011**, *4*, 105−128.
(16) Li, D.; Song, S. P.; Fan, C. H. *Acc. Chem. Res.* **2010**, *43*, 631−641.
(17) Schlosser, K.; Li, Y. F. *Chem. Biol.* **2009**, *16*, 311−322.
(18) Wang, H.; Kim, Y.; Liu, H.; Zhu, Z.; Bamrungsap, S.; Tan, W. *J. Am. Chem. Soc.* **2009**, *131*, 8221−8226.
(19) Liu, J. W.; Lu, Y. *J. Am. Chem. Soc.* **2003**, *125*, 6642−6643.
(20) Xiao, Y.; Rowe, A. A.; Plaxco, K. W. *J. Am. Chem. Soc.* **2007**, *129*, 262.
(21) Mei, S. H. J.; Liu, Z.; Brennan, J. D.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 412−420.
(22) Lu, Y. *Chem.—Eur. J.* **2002**, *8*, 4588−4596.
(23) Takagi, Y.; Warashina, M.; Stec, W. J.; Yoshinari, K.; Taira, K. *Nucleic Acids Res.* **2001**, *29*, 1815−1834.
(24) Breaker, R. R.; Joyce, G. F. *Chem. Biol.* **1994**, *1*, 223−229.
(25) Li, J.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10466−10467.
(26) Li, J.; Zheng, W.; Kwon, A. H.; Lu, Y. *Nucleic Acids Res.* **2000**, *28*, 481−488.
(27) Cuenoud, B.; Szostak, J. W. *Nature* **1995**, *375*, 611−614.
(28) Carmi, N.; Balkhi, H. R.; Breaker, R. R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2233−2237.
(29) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2007**, *129*, 9838−9839.
(30) Liu, J.; Brown, A. K.; Meng, X.; Cropek, D. M.; Istok, J. D.; Watson, D. B.; Lu, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2056−2061.
(31) Liu, J.; Lu, Y. *Angew. Chem., Int. Ed.* **2007**, *46*, 7587−7590.
(32) Hollenstein, M.; Hipolito, C.; Lam, C.; Dietrich, D.; Perrin, D. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 4346−4350.
(33) Kim, H.-K.; Li, J.; Nagraj, N.; Lu, Y. *Chem.—Eur. J.* **2008**, *14*, 8696−8703.
(34) Feig, A. L.; Panek, M.; Horrocks, W. D., Jr; Uhlenbeck, O. C. *Chem. Biol.* **1999**, *6*, 801−810.
(35) Zhang, M.; Le, H.-N.; Jiang, X.-Q.; Yin, B.-C.; Ye, B.-C. *Anal. Chem.* **2013**, *85*, 11665−11674.
(36) Sugimoto, N.; Ohmichi, T. *FEBS Lett.* **1996**, *393*, 97−100.
(37) Ohmichi, T.; Sugimoto, N. *Biochemistry* **1997**, *36*, 3514−3521.
(38) Dokukin, V.; Silverman, S. K. *Chem. Sci.* **2012**, *3*, 1707−1714.
(39) Javadi-Zarnaghi, F.; Hobartner, C. *J. Am. Chem. Soc.* **2013**, *135*, 12839−12848.
(40) Wang, Y.; Silverman, S. K. *J. Am. Chem. Soc.* **2003**, *125*, 6880−6881.
(41) Komiyama, M.; Takeda, N.; Shigekawa, H. *Chem. Commun.* **1999**, 1443−1451.
(42) Mancin, F.; Scrimin, P.; Tecilla, P.; Tonellato, U. *Chem. Commun.* **2005**, 2540−2548.
(43) Komiyama, M.; Aiba, Y.; Yamamoto, Y.; Sumaoka, J. *Nat. Protoc.* **2008**, *3*, 655−662.
(44) Li, Y.; Breaker, R. R. *J. Am. Chem. Soc.* **1999**, *121*, 5364−5372.
(45) Liu, Z.; Mei, S. H. J.; Brennan, J. D.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 7539−7545.
(46) Feldmann, J.; Salaun, P.; Lombi, E. *Environ. Chem.* **2009**, *6*, 275−289.
(47) Baun, D. L.; Christensen, T. H. *Waste Manage Res.* **2004**, *22*, 3−23.
(48) Nutiu, R.; Yu, J. M. Y.; Li, Y. *ChemBioChem* **2004**, *5*, 1139−1144.
(49) Rupcich, N.; Nutiu, R.; Li, Y.; Brennan, J. D. *Angew. Chem., Int. Ed.* **2006**, *45*, 3295−3299.