JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by FENG CHIA UNIV

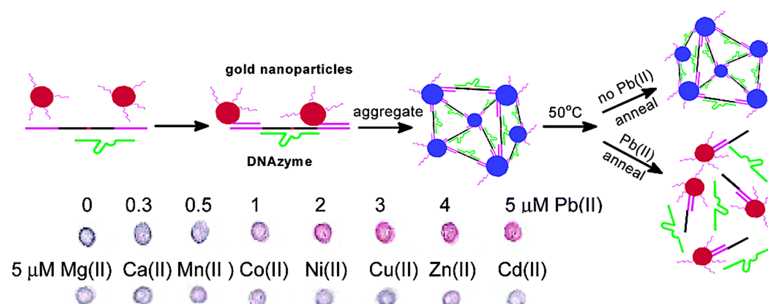
Communication

A Colorimetric Lead Biosensor Using DNAzyme-Directed Assembly of Gold Nanoparticles

Juewen Liu, and Yi Lu

J. Am. Chem. Soc., **2003**, 125 (22), 6642-6643• DOI: 10.1021/ja034775u • Publication Date (Web): 10 May 2003

Downloaded from http://pubs.acs.org on March 29, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 82 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 05/10/2003

A Colorimetric Lead Biosensor Using DNAzyme-Directed Assembly of Gold Nanoparticles

Juewen Liu and Yi Lu*
Department of Chemistry, University of Illinois at Urbana—Champaign, Urbana, Illinois 61801

Received February 20, 2003; E-mail: yi-lu@uiuc.edu

The design of metal ion sensors has long been a focus of research as it can provide on-site, real-time detection and quantification of beneficial and toxic metal ions in applications such as household and environmental monitoring, developmental biology, or clinical toxicology. While significant progress has been made in making fluorescent sensors,[1−6] including Pb(II) sensors,[7−10] few colorimetric metal sensors have been reported.[11] A simple colorimetric sensor could eliminate or minimize most costs associated with instrumentation and operation in fluorescence detection and thus can make on-site, real-time detection easier. Furthermore, a metal ion sensor with a tunable dynamic range is desirable for applications in widely different concentration ranges. However, few general strategies have been reported to vary the dynamic range without having to design new sensors. Here we describe a design of colorimetric metal sensors based on DNAzyme-directed assembly of gold nanoparticles and their use for sensitive and selective detection and quantification of metal ions, particularly lead in leaded paint. A novel strategy for tuning the dynamic range of the sensor using an inactive variant of the DNAzyme is also demonstrated.

We chose gold nanoparticles as the colorimetric reporter group because previous work has shown that different aggregation states of metal nanoparticles can result in distinctive color changes.[12−14] One remarkable example is the use of DNA-functionalized gold nanoparticles for selective colorimetric detection of DNA.[15] The sensitivity and selectivity of this method rivals other methods, such as those based on fluorescence. While this method is playing an increasingly important role in genomic research, it is desirable to expand this nanoparticle methodology beyond DNA detection to other analytes such as metal ions.

Among the metal ions, Pb(II) has received much attention,[9,10] largely because of the adverse health effects of lead exposure, especially in children.[16] One main source of lead is leaded paint in millions of old houses around the world. According to the U.S. Environmental Protection Agency, test kits currently available for leaded paint showed high rates of both false positive and false negative results when compared to laboratory analytical results.[17]

We chose DNAzymes (also called deoxyribozymes, catalytic DNA, or DNA enzymes) as the metal-binding moiety because DNAzymes that are highly specific for metal ions such as Pb(II),[18,19] Cu(II),[20,21] and Zn(II)[22,23] have been obtained through a combinatorial biology approach called in vitro selection.[24−26] Effector-activated RNAzymes (aptazymes) have also been shown to be able to have specific metal-ion-dependent activities.[27] A "8−17" DNAzyme[22,28,29] (Figure 1a) was chosen because it has shown high activity and specificity toward Pb(II).[8,10]

A typical trans-cleaving DNAzyme system consists of an enzyme strand and a substrate strand (Figure 1a). In the presence of an analyte, the enzyme carries out catalytic reactions, such as hydrolytic cleavage of the substrate strand at the scissile ribonucleic acid adenosine (rA) (Figure 1b). The substrate can be extended on both the 3′ and 5′ ends as long as the enzyme recognition portion is maintained. The design of the lead biosensor is shown in Figure

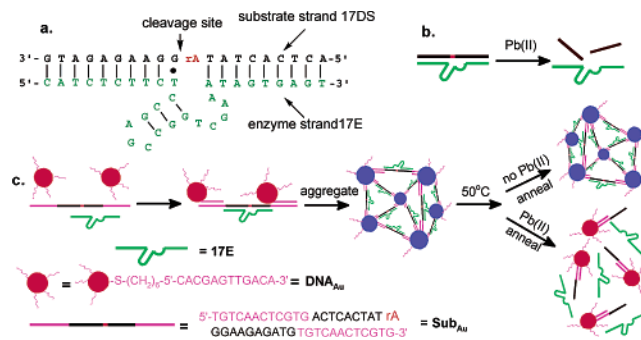


Figure 1. (a) Secondary structure of the "8−17" DNAzyme system that consists of an enzyme strand (17E) and a substrate strand (17DS). The cleavage site is indicated by a black arrow. Except for a ribonucleoside adenosine at the cleavage site (rA), all other nucleosides are deoxyribonucleosides. (b) Cleavage of 17DS by 17E in the presence of Pb(II). (c) Schematics of DNAzyme-directed assembly of gold nanoparticles and their application as biosensors for metal ions such as Pb(II). In this system, the 17DS has been extended on both the 3′ and 5′ ends for 12 bases, which are complementary to the 12-mer DNA attached to the 13-nm gold nanoparticles (DNA_{Au}).

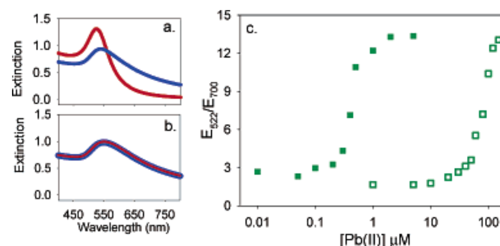


Figure 2. UV−vis extinction spectra of an active 17E DNAzyme−nanoparticle sensor (a) and an inactive 17Ec DNAzyme−nanoparticle sensor (b) in the absence (blue curve) or in the presence (red curve) of 5 μM Pb(II). (c) Pb(II) detection level of the sensor. When the enzyme strand is the active 17E only, the Pb(II) detection range is 0.1−4 μM (solid green squares). When the ratio of 17E and 17Ec is 1:20, the Pb(II) detection range is 10−200 μM (open green squares).

1c. It consists of 5′-thio-modified 12-mer DNA attached to 13-nm-diameter gold nanoparticles (called DNA_{Au}), a DNAzyme (called 17E), and its substrate (called Sub_{Au}). The sequence of the Sub_{Au} is designed so that it can hybridize specifically to a DNA_{Au} on each end, while maintaining the 17E recognition portion. These hybridizations cause aggregation of gold nanoparticles and result in a blue color. However, in the presence of Pb(II), the 17E catalyzes hydrolytic cleavage of Sub_{Au} and prevents the formation of nanoparticle aggregates. A red color appears as a result.

The color change of the sensor induced by Pb(II) can be monitored by UV−vis spectroscopy. Typical UV−vis spectra of the DNAzyme−gold nanoparticle sensor in the absence and in the presence of 5 μM Pb(II) are shown in Figure 2a. The color change is attributable to the cleavage of Sub_{Au} by 17E in the presence of Pb(II), which prevents the DNA_{Au} from aggregating effectively. Two control experiments supported this conclusion. First, in the absence of Pb(II), only blue precipitates were observed. Second,

10.1021/ja034775u CCC: $25.00 © 2003 American Chemical Society

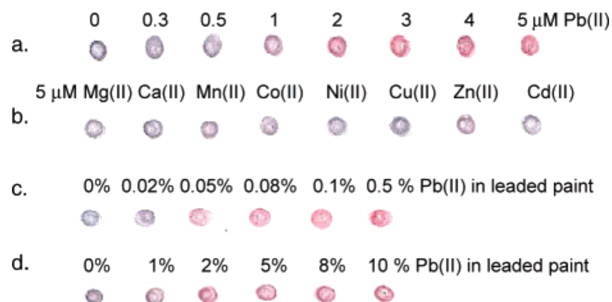


Figure 3. The color of the sensor developed on an alumina TLC plate with different Pb(II) concentrations (a) and with 5 μM concentrations of eight other divalent metal ions (b). The reactions in both (a) and (b) are carried out in 25 mM Tris−acetate buffer, pH 7.2, containing 300 mM NaCl. Colorimetric detection and quantification of lead in leaded paint. The color developed on a TLC plate by the sensor after reacting with 360- (c) and 15 000-fold (d) dilution of the soaking solution for the leaded paint. The pictures were acquired with an EPSON Perfection 1200S scanner.

the G·T wobble pair in the DNAzyme (highlighted in Figure 1a by a black dot) has been shown to be essential for the DNAzyme activity.[22,28,29] When the G·T wobble pair was changed to a GC canonical base pair (by changing the 10th nucleotide in 17E from a thymine to a cytosine and keeping other bases the same), the resulting mutant DNAzyme (called 17Ec) lost its activity completely.[22,28,29] Although 17Ec was as capable of forming blue nanoparticle aggregates as 17E, no color change was observed in the presence of Pb(II) (Figure 2b). These results indicate that Pb(II)-assisted DNAzyme cleavage of Sub_{Au} is responsible for the color change and thus for the metal ion detection.

For quantitative analysis of Pb(II), the ratio of extinction at 522 and 700 nm was used. These two wavelengths were chosen to represent the relative amount of free and aggregated gold nanoparticles, respectively. The ratiometric method allows the determination of metal ion concentration independent of sampling conditions. A plot of this extinction ratio versus Pb(II) concentration is shown in Figure 2c (solid squares), which shows that this unoptimized sensor is capable of detecting Pb(II) between 100 nM and 4 μM, and thus it is well suited for household and environmental monitoring since 480 nM is considered the toxic level for human beings.[30] Although the melting temperature of the nanoparticle assembly has been shown to be highly reproducible,[15] the exact extinction ratio may depend on the preparation of a particular assembly used in the sensing. Therefore, a calibration curve such as that shown in Figure 2c is necessary for quantitative analysis.

An important attribute of the sensor is that the Pb(II) detection range can be tuned over several orders of magnitude by varying the relative concentrations of 17E over 17Ec. Because of the similar base-pairing properties, the optical properties of the gold nanoparticle aggregates linked by 17E and 17Ec are very similar. When the enzyme strand is 17E only, a Pb(II) detection range of 100 nM to 4 μM is obtained (solid squares, Figure 2c). However, when the ratio is 1:20, the detection region shifts to the range of 10−200 μM (open squares, Figure 2c). The ability to vary the detection range without having to develop new sensors is a unique aspect of the DNAzyme−nanoparticle system. It allows accurate analyte quantification suitable for desirable applications involving different concentration ranges of analytes.

For qualitative or semiquantitative analysis of Pb(II), the color signal can be displayed by spotting the resulting solution on a solid surface, such as an alumina TLC plate (Analtech, Inc.). A clear color progression from blue to purple to red with increasing concentration of Pb(II) can be observed (Figure 3a). At the same time, the color remains blue in the presence of 5 μM Mg(II), Ca(II), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), or Cd(II) (Figure 3b). Other divalent metal ions, based on enzyme activity assays, were even less active than the eight metal ions tested in this experiment. Therefore, the Pb(II) sensor is highly selective.

Given the high sensitivity and selectivity of the sensor, we tested its ability to detect and quantify lead in leaded paint. As shown in Figure 3c,d, different percentages of lead in leaded paint can be easily detected and quantified, including the sample with 0.5% lead, the U.S. Federal threshold for leaded paint, defined by the U.S. EPA.[17]

In summary, the DNAzyme−gold nanoparticle sensor described here is highly sensitive and selective for lead. The novel approach of introducing inactive DNAzymes allows tuning the detection level over several orders of magnitude without selection of new sensors. Since in vitro selection can be used to select DNAzymes specific for a desired analyte, the design principle in this work can be generally applied to the creation of colorimetric sensors for many analytes of choice.

Acknowledgment. This material is based upon work supported by the U.S. Department of Energy (NABIR program, Office of Biological and Environmental Research, DEFG02-01-ER63179) and by the Nanoscale Science and Engineering Initiative of the National Science Foundation (DMR-0117792).

Supporting Information Available: Experimental procedures for sample preparation and sensing (PDF). This material is available free of charge via Internet at http://pubs.acs.org.

References

(1) Tsien, R. Y. In *Fluorescenct Chemosensors for Ion and Molecule Recognization*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993; Vol. 538, pp 130−146.
(2) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423−428.
(3) Imperiali, B.; Pearce, D. A.; Sohna Sohna, J.-E.; Walkup, G.; Torrado, A. *Proc. SPIE-Int. Soc. Opt. Eng.* **1999**, *3858*, 135−143.
(4) Rogers, C. W.; Wolf, M. O. *Coord. Chem. Rev.* **2002**, *233−234*, 341−350.
(5) Fahrni, C. J.; O'Halloran, T. V. *J. Am. Chem. Soc.* **1999**, *121*, 11448−11458.
(6) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831−7841.
(7) Deo, S.; Godwin, H. A. *J. Am. Chem. Soc.* **2000**, *122*, 174−175.
(8) Li, J.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10466−10467.
(9) Godwin, H. A. *Curr. Opin. Chem. Biol.* **2001**, *5*, 223−227.
(10) Lu, Y. *Chem. Eur. J.* **2002**, *8*, 4588−4596.
(11) Oehme, I.; Wolfbeis, O. S. *Mikrochim. Acta* **1997**, *126*, 177−192.
(12) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849−1862.
(13) Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4128−4158.
(14) Hilliard, L. R.; Zhao, X.; Tan, W. *Anal. Chim. Acta* **2002**, *470*, 51−56.
(15) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078−1080.
(16) Needleman, H. L. *Human Lead Exposure*; CRC Press: Boca Raton, FL, 1992.
(17) Schmehl, R. L.; Cox, D. C.; Dewalt, F. G.; Haugen, M. M.; Koyak, R. A.; Schwemberger, J. G., Jr.; Scalera, J. V. *Am. Ind. Hyg. Assoc. J.* **1999**, *60*, 444−451.
(18) Pan, T.; Uhlenbeck, O. C. *Nature* **1992**, *358*, 560−563.
(19) Breaker, R. R.; Joyce, G. F. *Chem. Biol.* **1994**, *1*, 223−229.
(20) Cuenoud, B.; Szostak, J. W. *Nature* **1995**, *375*, 611−614.
(21) Carmi, N.; Shultz, L. A.; Breaker, R. R. *Chem. Biol.* **1996**, *3*, 1039−1046.
(22) Li, J.; Zheng, W.; Kwon, A. H.; Lu, Y. *Nucleic Acids Res.* **2000**, *28*, 481−488.
(23) Santoro, S. W.; Joyce, G. F.; Sakthivel, K.; Gramatikova, S.; Barbas, C. F., III. *J. Am. Chem. Soc.* **2000**, *122*, 2433−2439.
(24) Breaker, R. R. *Curr. Opin. Biotechnol.* **2002**, *13*, 31−39.
(25) Breaker, R. R. *Chem. Rev.* **1997**, *97*, 371−390.
(26) Joyce, G. F. In *The RNA World*, 2nd ed.; Gesteland, R. F., Cech, T. R., Atkins, J. F., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1999; Vol. 37, pp 687−689.
(27) Seetharaman, S.; Zivarts, M.; Sudarsan, N.; Breaker, R. R. *Nat. Biotechnol.* **2001**, *19*, 336−341.
(28) Santoro, S. W.; Joyce, G. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4262−4266.
(29) Faulhammer, D.; Famulok, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2837−2841.
(30) *Preventing lead poisoning in young children*; U.S. Department of Health and Human Services, U.S. GPO: Washington, DC, 1991.

JA034775U