Anal. Chem. **2004,** *76,* 1627−1632

Adenosine-Dependent Assembly of Aptazyme-Functionalized Gold Nanoparticles and Its Application as a Colorimetric Biosensor

Juewen Liu and Yi Lu*

Department of Chemistry, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801

Previous work has shown that DNAzyme-directed assembly of gold nanoparticles can be utilized to make effective colorimetric biosensors. However, the method is restricted to analytes that are directly involved in phosphodiester cleavage. To expand the methodology to a broader range of analytes, a colorimetric adenosine biosensor based on the aptazyme-directed assembly of gold nanoparticles is reported here. The aptazyme is based on the 8−17 DNAzyme with an adenosine aptamer motif that can modulate the DNAzyme activity through allosteric interactions depending on the presence of adenosine. In the absence of adenosine, the aptazyme is inactive and the substrate strands can serve as linkers to assemble DNA-functionalized 13-nm-diameter gold nanoparticles, resulting in a blue color. However, the presence of adenosine activates the aptazyme, which cleaves the substrate strand, disrupting the formation of nanoparticle aggregates. A red color of separated gold nanoparticles is observed. Concentrations of adenosine of up to 1 mM can be measured semiquantitatively by the degree of blue to red color changes or quantitatively by the extinction ratio at 520 and 700 nm. Under the same conditions, 5 mM guanosine, cytidine, or uridine resulted in a blue color only, indicating good selectivity of the sensor. The color difference can be clearly observed by the naked eye by spotting the resulting sensor solution onto an alumina TLC plate. Since aptamers that can target many classes of important analytes have already been selected, they can be adapted into aptazyme systems through rational design or further selection. Thus, colorimetric biosensors for many analytes of interest can be designed using the method presented here, regardless of whether the analytes are directly involved in the cleavage reaction or not.

Designing sensors has long been a focus of research as it can provide on-site, real-time detection and quantification of target analytes of interest for civilian, clinical, and military applications.[1−8] The research has resulted in many effective chemical and biological sensors, such as sensors for Ca(II)[1] and glucose,[9] whose practical applications have been demonstrated. However, the design strategy of these successful sensors has been applied to other sensor design in only limited cases.[10] Given the vast number of target analytes demanded for detection, it is desirable to use a strategy that is general enough so that it can be applied to the design of sensors for almost any chosen target analyte. Toward this goal, immunosensors,[8,11] based on antibody/antigen interaction, have been developed so that the chosen target analyte can be used as a surrogate antigen to obtain corresponding antibodies as target recognition elements. Inspired by the success of immunosensors, sensors based on imprinted polymers have also been developed, in which functional monomers and cross-link monomers are copolymerized to form a solid polymer network in the presence of a target analyte.[7]

In complement to sensors based on antibodies and imprinted polymers, sensors based on the nucleic acid platform are emerging,[2−5,12−19] as DNA and RNA that recognize specific target analytes can be obtained through combinatorial biology approaches such as systematic evolution of ligands by exponential enrichment (SELEX) or in vitro selection.[20−25] In a manner similar

* To whom correspondence should be addressed. Tel: (217) 333-2619. Fax: (217) 333-2685. E-mail: yi-lu@uiuc.edu.

(1) Tsien, R. Y. In *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; ACS Symposium Series 538; American Chemical Society: Washington, DC, 1993; pp 130−146.
(2) Hesselberth, J.; Robertson, M. P.; Jhaveri, S.; Ellington, A. D. *Rev. Mol. Biotechnol.* **2000**, *74*, 15−25.
(3) Breaker, R. R. *Curr. Opin. Biotechnol.* **2002**, *13*, 31−39.
(4) Seetharaman, S.; Zivarts, M.; Sudarsan, N.; Breaker, R. R. *Nat. Biotechnol.* **2001**, *19*, 336−341.
(5) Lu, Y. *Chem. Eur. J.* **2002**, *8*, 4588−4596.
(6) Jayasena, S. D. *Clin. Chem.* **1999**, *45*, 1628−1650.
(7) Haupt, K.; Mosbach, K. *Chem. Rev.* **2000**, *100*, 2495−2504.
(8) Gizeli, E.; Lowe, C. R. *Curr. Opin. Biotechnol.* **1996**, *7*, 66−71.
(9) Turner, A. P. F. *Science* **2000**, *290*, 1315−1317.
(10) Burdette, S. C.; Walkup, G. K.; Spingler, B.; Tsien, R. Y.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 7831−7841.
(11) Blake, D. A.; Jones, R. M.; Blake, R. C.; Pavlov, A. R.; Darwish, I. A.; Yu, H. *Biosens. Bioelectron.* **2001**, *16*, 799−809.
(12) Sun, L. Q.; Cairns, M. J.; Saravolac, E. G.; Baker, A.; Gerlach, W. L. *Pharmacol. Rev.* **2000**, *52*, 325−347.
(13) Nutiu, R.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 4771−4778.
(14) Mei, S. H. J.; Liu, Z.; Brennan, J. D.; Li, Y. *J. Am. Chem. Soc.* **2003**, *125*, 412−420.
(15) Jhaveri, S. D.; Kirby, R.; Conrad, R.; Maglott, E. J.; Bowser, M.; Kennedy, R. T.; Glick, G.; Ellington, A. D. *J. Am. Chem. Soc.* **2000**, *122*, 2469−2473.
(16) Stojanovic, M. N.; Landry, D. W. *J. Am. Chem. Soc.* **2002**, *124*, 9678−9679.
(17) Li, J.; Lu, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10466−10467.
(18) Fahlman, R. P.; Sen, D. *J. Am. Chem. Soc.* **2002**, *124*, 4610−4616.
(19) Robertson, M. P.; Ellington, A. D. *Nat. Biotechnol.* **1999**, *17*, 62−66.
(20) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818−822.
(21) Gold, L.; Polisky, B.; Uhlenbeck, O.; Yarus, M. *Annu. Rev. Biochem.* **1995**, *64*, 763−797.
(22) Osborne, S. E.; Ellington, A. D. *Chem. Rev.* **1997**, *97*, 349−370.
(23) Breaker, R. R. *Chem. Rev.* **1997**, *97*, 371−390.

10.1021/ac0351769 CCC: $27.50
Published on Web 02/07/2004

to immunosensors, DNA or RNA that binds specifically to chosen target analytes can be isolated from large libraries (often up to 10^{15} different sequences) through an iterative process of selection, amplification, and mutation. The resulting DNA/RNA are called aptamers[6,20,24] if they recognize a target analyte or DNA/RNAzymes[5,26,27] if the resulting analyte recognition also helps to catalyze a chemical reaction. Furthermore, an interesting combination of aptamers and DNA/RNAzymes has resulted in a new nucleic acid enzyme system called aptazymes,[2,28−30] where the DNA/RNAzyme activity is regulated by the aptamer binding to the target analyte through allosteric interactions.

Many aptamers, DNA/RNAzymes, and aptazymes have been isolated to target different classes of analytes. For example, aptamers have been developed for the following uses: to target metal ions such as zinc(II) and Ni(II); nucleotides such as ATP; cofactors such as NAD and flavin; antibiotics such as viomycin and streptomycin; proteins such as HIV reverse transcriptase and coagulation factor IXa; toxins such as staphylococcal enterotoxin B; and bacterial spores such as anthrax spore.[6,24] DNA/RNAzymes[5,26,27] and aptazymes[2,28−30] that target metal ions such as Pb(II),[26,31] Cu(II),[32−34] and Zn(II)[35] and small molecules such as ATP[30] have also been obtained.

A number of studies have now shown that aptamers, DNA/RNAzymes, or aptazymes can often rival antibodies in the broad range of analytes they can recognize.[6] At least two features make them excellent platforms for making sensors.[13−15,17] First, the selection is in vitro and thus can be carried out in short time and with limited cost. Second, unlike antibodies, most aptamers, DNA/RNAzymes or aptazymes can be denatured and renatured many times without losing the binding ability or activity and, therefore, can be processed and stored under rather harsh conditions.

Obtaining analyte-specific aptamers, DNA/RNAzymes, or aptazymes is the first step in sensor design. A critical step is to transfer the target-specific recognition event into a physically detectable signal. Toward this goal, conductivity[18] and radioisotope[4] or fluorescence[13−15,17,36,37] labeling methods have been developed.[2,3,5,12] In many sensor applications, colorimetric detection can be advantageous because it can avoid difficulties associated with handling and disposing of radioisotopes, eliminate or minimize most costs associated with instrumentation and operation in fluorescence detection, and thus make on-site, real-time detection and quantification easier.[16,38]

We previously communicated the design of a colorimetric biosensor for Pb(II) based on the DNAzyme-directed assembly of gold nanoparticles.[39] In the presence of Pb(II), the DNAzyme cleaves the substrate strand, which acts as a linker to assemble gold nanoparticles. The assembled nanoparticles give a blue color. Upon cleavage, the substrate can no longer act as a linker and the aggregation of gold nanoparticles is disrupted, which results in a red color from dispersed gold nanoparticles. This strategy took advantage of recent progress in two emerging fields, the DNAzyme field as described above and the nanoscale science and technology field,[39−43] particularly the use of DNA-functionalized gold nanoparticles for detection of complementary DNA as demonstrated by Mirkin, Letsinger, and co-workers.[38,44] Because gold nanoparticles have extinction coefficients 3 orders of magnitude larger than those of organic dyes and have strong distance-dependent optical properties,[45] the sensitivity and selectivity of this method often rival fluorescent detection.

While our reported method has expanded the detection range of the DNA-functionalized gold nanoparticle system to many analytes beyond nucleic acids, it is still restricted only to those analytes (e.g., metal ions) that are directly involved in the catalytic cleavage of a phosphodiester bond. Therefore, it is desirable to expand the above methodology to more analytes, regardless of whether they can cleave phosphodiester bonds.

Herein, we report a colorimetric biosensor for adenosine based on the adenosine aptazyme-directed assembly of gold nanoparticles. The design strategy described here can be applied to other aptazyme systems for colorimetric detection of many analytes of interest.

EXPERIMENTAL SECTION

Oligonucleotides and Reagents. All oligonucleotides were purchased from Integrated DNA Technology Inc. All oligonucleotides were purified by HPLC, except for the two thiol-modified 12-mer DNA, which were used as received. Adenosine and other nucleosides were purchased from Aldrich. Gold nanoparticles with 13-nm diameter were prepared following literature procedures.[44] The size of gold nanoparticles was verified by TEM (JEOL 2010). The 3′-and 5′-thiol-modified 12-mer DNA were attached to gold nanoparticles using standard methods.[44]

Adenosine Detection. In a typical experiment, in a 38-μL solution containing 3 μM substrate, 6 μM enzyme, and 9 μM regulator strands of DNA with 300 mM NaCl, 50 mM Tris-acetate, pH 7.2 buffer, 2 μL of metal ion stock solution (5 mM Pb(II) and 200 mM Mg(II)) was added to initiate the cleavage reaction. Thus, the metal ion concentration was 0.25 mM Pb(II) and 10 mM Mg(II) for the cleavage. After 30 min, a 2-μL aliquot was taken out from the reaction microcentrifuge tube and transferred into another tube containing 50 μL of gold nanoparticle solution (25 μL of both 5′-DNA_{Au} and 3′-DNA_{Au}, whose extinction at 522 nm

(24) Wilson, D. S.; Szostak, J. W. *Annu. Rev. Biochem.* **1999**, *68*, 611−647.
(25) 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.
(26) Breaker, R. R.; Joyce, G. F. *Chem. Biol.* **1994**, *1*, 223−229.
(27) Li, Y.; Breaker, R. R. *Curr. Opin. Struct. Biol.* **1999**, *9*, 315−323.
(28) Tang, J.; Breaker, R. R. *Chem. Biol.* **1997**, *4*, 453−459.
(29) Wang, D. Y.; Lai, B. H. Y.; Sen, D. *J. Mol. Biol.* **2002**, *318*, 33−43.
(30) Levy, M.; Ellington, A. D. *Chem. Biol.* **2002**, *9*, 417−426.
(31) Pan, T.; Uhlenbeck, O. C. *Nature* **1992**, *358*, 560−563.
(32) Cuenoud, B.; Szostak, J. W. *Nature* **1995**, *375*, 611−614.
(33) Carmi, N.; Shultz, L. A.; Breaker, R. R. *Chem. Biol.* **1996**, *3*, 1039−1046.
(34) Wang, W.; Billen, L. P.; Li, Y. *Chem. Biol.* **2002**, *9*, 507−517.
(35) Santoro, S. W.; Joyce, G. F.; Sakthivel, K.; Gramatikova, S.; Barbas, C. F., III. *J. Am. Chem. Soc.* **2000**, *122*, 2433−2439.
(36) Li, J. J.; Fang, X.; Tan, W. *Biochem. Biophys. Res. Commun.* **2002**, *292*, 31−40.
(37) Santra, S.; Zhang, P.; Wang, K.; Tapec, R.; Tan, W. *Anal. Chem.* **2001**, *73*, 4988−4993.
(38) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078−1080.
(39) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2003**, *125*, 6642−6643.
(40) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. *Nature* **1996**, *382*, 607−609.
(41) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P., Jr.; Schultz, P. G. *Nature* **1996**, *382*, 609−611.
(42) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849−1862.
(43) Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4128−4158.
(44) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959−1964.
(45) Storhoff, J. J.; Lazarides, A. A.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L.; Schatz, G. C. *J. Am. Chem. Soc.* **2000**, *122*, 4640−4650.

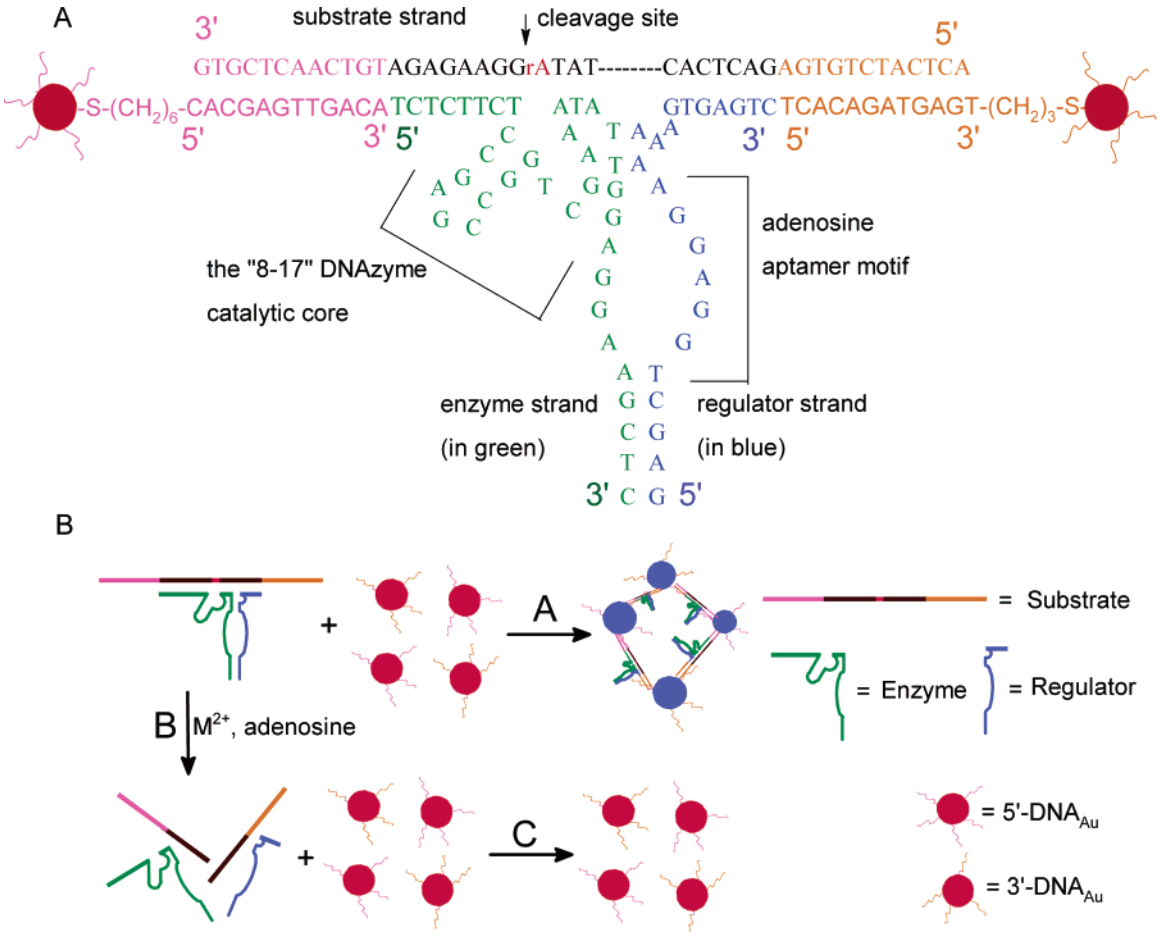

Figure 1. (A) The primary and the proposed secondary structure of the adenosine aptazyme built on the 8−17 DNAzyme platform. Shown in the figure are four components. See the text for descriptions. The red balls with pink or orange strings are 12-mer DNA-functionalized 13-nm-diameter gold nanoparticles. (B) Schematic representation of the colorimetric detection of adenosine. The sequences of the substrate, enzyme, and regulator strands are given in (A). The substrate strands (both free substrate and substrate hybridized with the enzyme and regulator strands) can act as linkers for the DNA-functionalized gold nanoparticles to form aggregates, which have a blue color (reaction A). In the presence of adenosine and metal ions, the substrate can be cleaved (reaction B). The cleaved substrate can no longer act as linker for nanoparticles and the color remains red (reaction C). The nanoparticles and DNA strands are not drawn to scale.

was 2.2) with 10 μM EDTA. The 10 μM EDTA is assumed to specifically chelate Pb(II) (5 μM) in solution, even in the presence of 0.4 mM Mg(II), because the formation constant of EDTA for Pb(II) is ~10 orders of magnitude higher than that for Mg(II).[46] In the presence of 0.4 mM Mg(II) only, the cleavage rate for the aptazyme is insignificant. Thus, the cleavage reaction could be considered quenched when the reaction solution was transferred into the gold nanoparticle solution. The 2-μL aliquot gives a 120 nM substrate concentration in the 50-μL volume if no cleavage occurs, which is the threshold value determined in the text. The cleavage can decrease the concentration of substrate, which is reflected in the degree of aggregation of gold nanoparticles.

For the kinetics assay using gold nanoparticle aggregation as the detection method, at each predetermined time point, 2-μL aliquots from the aptazyme reaction tube were transferred to a gold nanoparticle solution for detection, with all other reaction conditions the same as described above. For biochemical assays, 4 μM substrate, 10 μM enzyme, and 20 μM regulator strand DNA were dissolved in 20 mM $MgCl_2$ and 150 mM NaCl in 50 mM TA buffer, pH 7.2. Adenosine (5 mM) and Pb(II) (500 μM) were added to initiate the cleavage reaction. The reaction was quenched after 5 min by adding EDTA and 8 M urea. The samples were loaded into a 20% denaturing polyacrylamide gel, and the gel was stained by ethidium bromide.

Gold Nanoparticle Aggregation. The nanoparticle solution mixed with a 2-μL aliquot mentioned above was incubated in a water bath at 70 °C for 3 min and then allowed to cool slowly in the water bath to room temperature (over ~2 h). The resulting solution was assayed using UV−visible spectroscopy or was spotted onto a TLC plate.

RESULTS AND DISCUSSION

Design of a Colorimetric Biosensor for Adenosine. To illustrate the general design strategy for colorimetric biosensors based on the aptazyme platform, we chose a well-characterized adenosine-specific aptazyme reported previously[29,47,48] and made appropriate modifications as shown in Figure 1A. The system is

(46) Martell, A. E. *Stability constants of metal-ion complexes*; Special Publication 17; Royal Society of Chemistry: London, 1964.

(47) Wang, D. Y.; Sen, D. *J. Mol. Biol.* **2001**, *310*, 723−734.

(48) Wang, D. Y.; Lai, B. H. Y.; Feldman, A. R.; Sen, D. *Nucleic Acids Res.* **2002**, *30*, 1735−1742.

composed of four components. The first component is the 5′- and 3′-thiol-modified 12-mer DNA-functionalized gold nanoparticles (5′-DNA_{Au} and 3′-DNA_{Au}), which are designed to hybridize to the two ends of the substrate strand. The second component is the substrate strand, which is a DNA/RNA chimer with a single RNA linkage (in red) that serves as the cleavage site. The substrate strand is flanked with two 12-mer overhangs (in pink and orange), which are designed to hybridize with 3′-DNA_{Au} and 5′-DNA_{Au}. The third component is the catalytic part of the aptazyme, which is adapted from the 8−17 DNAzyme[49−51] (in green) and has been optimized for high activity in the presence of Pb(II).[17,52] The 3′-end of the DNAzyme is hybridized with the fourth component of the aptazyme (in blue) to form the adenosine aptamer motif. This adenosine aptamer motif was obtained through in vitro selection[53] and was adapted into an aptazyme by Sen and co-workers.[29,47,48] Therefore, the presence of adenosine can promote formation of the active tertiary DNAzyme structure. This complex then promotes cleavage of the substrate strand at the single riboadenosine position (in red). Without adenosine, even though the four components may still be able to come together by Watson−Crick base pairing, the cleavage activity is dramatically reduced.[29,47,48]

A schematic representation of the colorimetric detection of adenosine is shown in Figure 1B. In the absence of adenosine, the substrate strand in this aptazyme is used as a linker to assemble DNA-functionalized gold nanoparticles through DNA hybridization (reaction A). Since the color of gold nanoparticles changes, from red for separated nanoparticles to blue for aggregated nanoparticles,[38] the hybridization is expected to result in a blue color. However, the presence of adenosine can activate the cleavage activity of the aptazyme and the substrate strand can be cleaved (reaction B). The cleaved substrate can no longer be used to assemble gold nanoparticles to form aggregates; thus, a red color from separated nanoparticles should be observed (reaction C). If only a fraction of the substrate strand is cleaved, the system will be made of a mixture of nanoparticle aggregates and separated nanoparticles, which gives a purple color. Since the rate of the cleavage reaction can be modulated by the concentration of adenosine, the fraction of the substrate cleaved at a set time should be dependent on the concentration of adenosine. Different ratios of the cleaved to noncleaved substrate will then result in different extents of gold nanoparticle aggregation and thus different colors from blue to red. Therefore, the concentration of adenosine can be quantified from the colors displayed by the resulting aptazyme−gold nanoparticle system.

Determination of Optimal Detection Time. The detection and quantification of adenosine relies on the cleavage of the substrate strand, which requires the presence of both divalent metal ions and adenosine. Although binding of adenosine to the aptamer motif promotes formation of the active aptazyme tertiary structure, metal ions are required as a cofactor for the enzyme activity. An effective sensor should possess a fast response time. In the presence of Mg(II)only, which was the typical condition reported previously,[29] the cleavage rate constant of the aptazyme was 3.5×10^{-3} min^{-1} in the presence of 5 mM adenosine. Thus, ~200 min was needed for 50% substrate cleavage. We found that the 8−17 DNAzyme was at least 5000-fold more active in the presence of Pb(II) than Mg(II) under the same conditions.[17,51,52] Therefore, Pb(II) was used in the current system in addition to Mg(II) to accelerate the speed of detection.

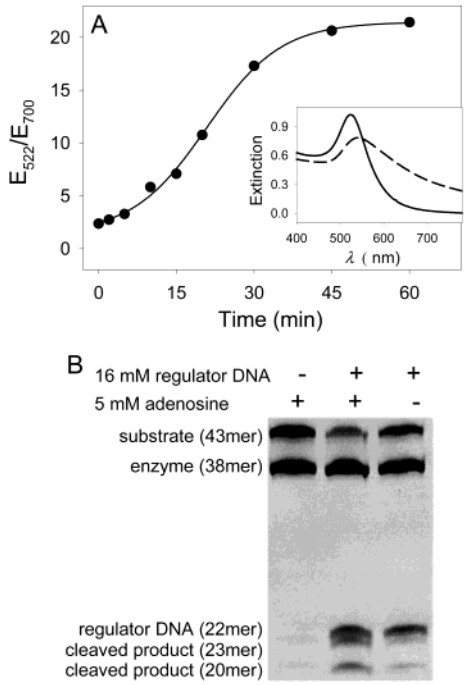

Figure 2. (A) Kinetics of the color change of the colorimetric adenosine sensor in the presence of 5 mM adenosine. A high extinction ratio between 522 and 700 nm indicates a red color, and a low ratio indicates a blue color. The curve was not obtained in real time, and the procedures for the kinetics assay were described in the Experimental Section. The data can be fit to a sigmoidal curve. Inset shows the extinction spectra of separated 13-nm-diameter gold nanoparticles (solid line) and gold nanoparticles aggregated by the aptazyme (dashed line) in the visible region. (B) Cleavage of the substrate by the aptazyme in the presence of 5 mM adenosine (middle lane) and in the absence of adenosine (right lane). If the regulator DNA was absent, no substrate cleavage was observed even with 5 mM adenosine present (left lane).

To provide a better understanding of the system, a kinetic assay of the aptazyme-catalyzed cleavage reaction was performed. Usually, a nucleic acid cleavage reaction is monitored by radioisotope labeling of the substrate strand and separating the cleaved substrate from the uncleaved by gel electrophoresis or by labeling the substrate strand with a fluorophore and monitoring the cleavage either in real time or by gel electrophoresis. We, for the first time, monitored the kinetics of the cleavage reaction colorimetrically by the assembly of gold nanoparticles using UV−visible extinction spectroscopy. Typical extinction spectra of separated gold nanoparticles and gold nanoparticle aggregates linked by aptazymes are presented in the inset of Figure 2A. Separated gold nanoparticles have an extinction peak at 522 nm. Upon aggregation, the intensity of the 522-nm peak decreases and shifts to longer wavelength, while the intensity of the spectrum at 700 nm increases. The extinction ratio between 522 and 700 nm, chosen

(49) Santoro, S. W.; Joyce, G. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4262−4266.
(50) Faulhammer, D.; Famulok, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2837−2841.
(51) Li, J.; Zheng, W.; Kwon, A. H.; Lu, Y. *Nucleic Acids Res.* **2000**, *28*, 481−488.
(52) Brown, A. K.; Li, J.; Pavot, C. M. B.; Lu, Y. *Biochemistry* **2003**, *42*, 7152−7161.
(53) Huizenga, D. E.; Szostak, J. W. *Biochemistry* **1995**, *34*, 656−665.

to represent the relative quantity of separated and aggregated nanoparticles, respectively, was used to assess the degree of aggregation.[39] This ratio is also a measure of color change from red to blue, where a high ratio is associated with a red color and a low ratio is associated with a blue color. Figure 2A shows the kinetics of the color changes of the system, monitored by the extinction ratio between 522 and 700 nm, in the presence of 5 mM adenosine. The kinetics data can be fit to a sigmoidal curve, from which it can be concluded that the reaction progressed to a substantial extent in 30 min and almost completely finished in 60 min. Furthermore, the results showed that small errors in timing (e.g., less than 1 min) during the detection would not induce large errors in the observed nanoparticle aggregation and thus the color change. From the above kinetics study, 30 min was chosen as the assay time in a compromise between the speed and the sensitivity of the sensor.

To confirm that the above changes of the optical properties of gold nanoparticles were due to cleavage of the substrate strand, a biochemical assay on the aptazyme system was carried out. Gel electrophoresis was used for DNA separation, and the detection of cleavage was by ethidium bromide staining. Since the aptazyme system was composed of three strands (substrate, enzyme, and regulator strand, Figure 1A), together with the two cleaved products, a total of five bands should be observed in each lane if cleavage occurred. As shown in Figure 2B, five bands can be observed in the middle and the right lanes of the gel. Adenosine (5 mM) was added to the sample loaded into the middle lane, and a larger extent of cleavage can be observed in this lane as compared to that in the right lane, for which no adenosine was added. As a control, no regulator DNA strand was added to the sample for the left lane. Even when adenosine was added, little cleavage was observed. With this experiment, it can be concluded that the substrate strand can be cleaved in the aptazyme system and the reduction in the quantity of substrate can affect the aggregation of gold nanoparticles and the resulting color changes.

Optimization of Other Detection Conditions. It is important to find an optimal ratio between the substrate strand and DNA-functionalized gold nanoparticles so that any decrease in the quantity of the substrate induced by cleavage will be reflected in the optical properties of the resulting nanoparticle aggregates. If the substrate is in excess, only a small fraction of substrate can be cleaved at a set time, resulting in decreased sensitivity.

To determine the optimal ratio, to a mixture of 25 μL of 5′-DNA_{Au} and 25 μL of 3′-DNA_{Au}, each with an extinction of 2.2 at 522 nm (corresponding to a concentration of ~8 nM by taking 2.7×10^8 M^{-1} cm^{-1} as the extinction coefficient), different quantities of the substrate strand were added, so that a series of samples with different substrate concentrations were prepared. The amount of the enzyme strand and the regulator strand were added in excess. After incubating at 70 °C for 3 min followed by cooling to room temperature, the extinction property of the resulting solutions was measured by UV−visible extinction spectroscopy.

The extinction ratio at 522 and 700 nm decreased exponentially with an increasing concentration of the substrate strand (Figure 3), suggesting that the addition of the substrate increased nanoparticle aggregation. When the substrate concentration was higher than 120 nM, the degree of aggregation was no longer sensitive to further increases in the substrate concentration. Therefore, 120 nM substrate was chosen as a starting point for the current system. Any cleavage of the substrate can disturb formation of the nanoparticle aggregates and increase the extinction ratio. As a result, a color change can be observed.

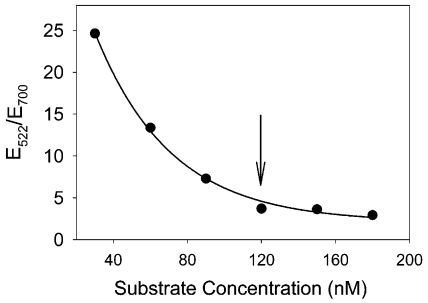

Figure 3. Relationship between the substrate concentration and the degree of gold nanoparticle aggregation. The data can be fit to an exponentially decreasing curve. For substrate concentration higher than 120 nM (indicated by an arrow), further increase of substrate does not significantly increase the degree of aggregation, while decrease of substrate concentration from 120 nM gives relatively sharp changes in the extinction ratio.

In the previously reported DNAzyme-based colorimetric Pb(II) sensor,[39] the correct ratio between the substrate strand (acts as a linker) and gold nanoparticles was achieved by preforming substrate/nanoparticle aggregates. Thus, the substrate strand with the appropriate stoichiometry is embedded into the aggregate, and any decrease of the substrate quantity reduces the degree of nanoparticle aggregation. The DNAzyme-assembled nanoparticle aggregates were heated to above 50 °C and then allowed to cool to room temperature slowly. In the cooling process, if Pb(II) was present, the linking substrate was cleaved and the aggregation of nanoparticles was inhibited. As a result, the color changed from blue for nanoparticle aggregates to red for separated nanoparticles. Therefore, the signaling of the sensor was synchronized with the cleavage reaction, and the detection was a one-pot process.[39] However, this detection method cannot be used for the adenosine aptazyme system for the following reasons. First, to combine the cleavage reaction and detection in the same process, the rate of cleavage should be comparable with the rate of nanoparticle aggregation. However, the rate of cleavage is much slower for the adenosine aptazyme as compared to that of the 8−17 DNAzyme.[29,52] Second, the cleavage reaction requires a high concentration of Mg^{2+} (10 mM) and Pb^{2+} (0.25 mM), and the stability of DNA-functionalized gold nanoparticles is significantly lower in the presence of high concentrations of divalent metal ions, especially during heating, when nanoparticles tend to aggregate irreversibly.[54] Therefore, a two-step detection method has been adopted.

Performance of the Colorimetric Adenosine Biosensor. Figure 4A shows the adenosine-dependent changes of the red and blue extinction ratios. Under the conditions used in this study, adenosine can be quantified from 100 μM to 1 mM. To determine the selectivity of the aptazyme−gold nanoparticle sensor, 5 mM guanosine, cytidine, or uridine was used in the assay. Under the same conditions, all three nucleosides resulted in a blue color, which was the same as the background signal. These results

(54) Kim, Y.; Johnson, R. C.; Hupp, J. T. *Nano Lett.* **2001**, *1*, 165−167.

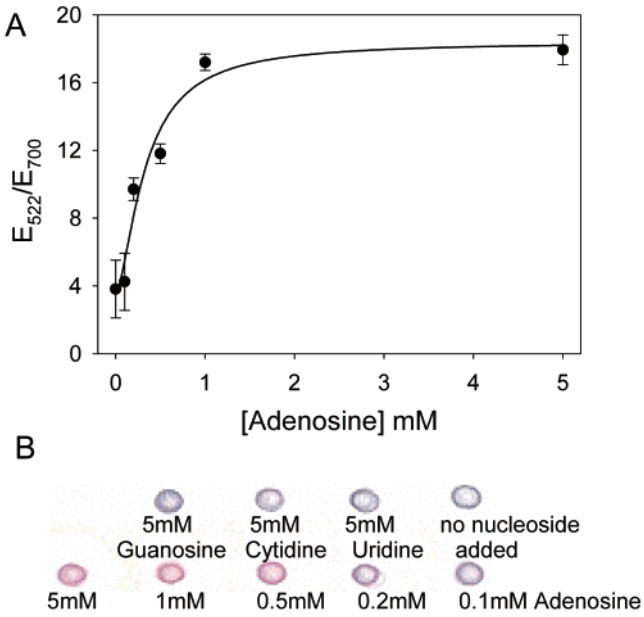

Figure 4. (A) Quantitative analysis of the adenosine concentration using UV−visible spectroscopy. The spectra were obtained on a Hewlett-Packard 8453 spectrophotometer. (B) Color of the adenosine sensor developed on an alumina TLC plate (Analtech, Inc.) in the presence of different analytes. The image was obtained using a scanner (Epson Perfection 1200S).

indicate that the high selectivity of the original aptazyme toward adenosine is maintained in this aptazyme−nanoparticle system. This is not surprising since there are no external labeling groups close to the adenosine aptamer motif in the cleavage step. Therefore, the high selectivity of the aptamer should be maintained.

The color changes can be conveniently monitored by spotting the resulting nanoparticle aggregates onto a solid substrate, such as an alumina TLC plate. As shown in Figure 4B, the color progression from blue to purple to red can be observed with increasing concentrations of adenosine. On the other hand, assays using 5 mM guanosine, cytidine, or uridine resulted in only the same blue color as observed in assays without any nucleosides added.

CONCLUSIONS

We reported a colorimetric sensor for detection and quantification of adenosine. This method combines the highly specific target recognition property of aptazymes with the simple yet powerful colorimetric detection of gold nanoparticle assembly. The work described here is a proof of the concept that the gold nanoparticle assembly-based detection method can be extended to analytes not directly involved in a cleavage reaction. Aptazymes based on other DNA/RNAzymes such as the 10−23 DNAzyme and the hammerhead ribozyme have been successfully designed and demonstrated.[29] In addition, in vitro selection of aptazymes offers another opportunity for those analytes whose aptazyme cannot be obtained using the rational design method and can be used to improve performance of existing aptazymes.[4,30] Therefore, using these aptazymes for directed assembly of nanoparticles, the colorimetric detection method can now be expanded to detect an even broader range of analytes.

ACKNOWLEDGMENT

This material is based upon work supported by the U.S. Department of Energy (DEFG02-01-ER63179) and the U.S. National Science Foundation (DMR-0117792 and CTS-0120978).

Received for review October 3, 2003. Accepted December 15, 2003.

AC0351769