A One-Step Highly Sensitive Method for DNA Detection Using Dynamic Light Scattering

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · AUGUST 2008

Impact Factor: 12.11 · DOI: 10.1021/ja801947e · Source: PubMed

J|A|C|S
COMMUNICATIONS

Published on Web 06/10/2008

A One-Step Highly Sensitive Method for DNA Detection Using Dynamic Light Scattering

Qiu Dai, Xiong Liu, Janelle Coutts, Lauren Austin, and Qun Huo*

NanoScience Technology Center, Department of Chemistry, and Department of Mechanical, Materials and Aerospace Engineering, University of Central Florida, 12424 Research Parkway Suite 400, Orlando, Florida 32826

Received March 16, 2008; E-mail: qhuo@mail.ucf.edu

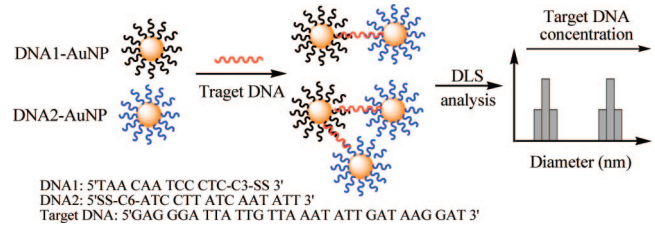
There is a considerable demand for rapid, low-cost, and sensitive detection of specific DNA sequences for the clinical diagnosis of genetic and pathogenic diseases.[1] Currently, fluorescent optical label-based DNA detection methods such as DNA microarray[2] and molecular beacons[3] are dominating the market. However, there are several limitations in these widely adopted methods. One problem is the relatively low signal amplification. Because one DNA probe is labeled with one or a few fluorophores, the fluorescence signal is rather weak when the target DNA concentration is low, leading to relatively poor sensitivity. A second problem is the poor photostability of typical fluorophores. Most organic dyes suffer from serious photobleaching, and this often leads to irreproducible results.

To solve some of these problems, several types of nanomaterials such as quantum dots, carbon nanotubes, silicon nanowires, and metallic nanoparticles have been explored as signaling probes for DNA detection.[4] Among them, gold nanoparticles (AuNPs) have been used in a variety of forms for detecting DNAs based on their unique size and distance-dependent optical properties. Mirkin and co-workers first developed a colorimetric detection of DNA hybridization in a homogeneous solution based on the formation of oligonucleotide-functionalized AuNP aggregates in the presence of target DNAs.[4d] The nanoparticles' aggregation can be detected directly by observing the color change of the solution from red to purple or monitored by UV−vis absorption spectroscopy. However, the main limitation of this approach is its low sensitivity (10 nM). To increase the sensitivity, DNA bar code amplification[5] and other optical signal amplification techniques such as scanometric method, and surface enhanced Raman spectroscopy[6] have been developed to improve the detection limit to femtomolar and attomolar range. However, all these amplification methods involve complicated multiple-step procedures that not only are time-consuming but also often cause problems in reproducibility.

In addition to their large light absorption cross section, AuNPs are known to have large light scattering cross section. The cross section of light scattering from an 80 nm AuNP is about a million-fold larger than the cross section of emission from typical fluorophores or even quantum dots.[7] Unlike fluorescent optical probes, the scattering light generated by AuNPs is not prone to quenching and does not photobleach with repeated or continuous exposure to light. Recently, both linear and nonlinear scattering properties of AuNPs have been introduced for DNA detection. Du et al. reported a one-step homogeneous detection of DNA hybridization using a linear light scattering technique.[8] The detection limit was reported to be around 0.1 pM. A single base pair mismatch hybridization can be detected at a DNA concentration around 60 pM. Using a hyper-Rayleigh scattering technique, Ray et al. also succeeded at detecting perfectly matched and single mismatched target DNA in homogeneous solution through a one-step process; however, the detection limit is only in the 10 nM range.[9]

Recently, we reported a homogeneous one-step immunoassay for protein target detection using gold nanoparticle probes coupled with a dynamic light scattering (DLS) technique.[10] This assay is based on the monitoring of antibody−antigen induced gold nanoparticle aggregation in a homogeneous solution through DLS. In this paper, we apply a similar concept for DNA detection. As illustrated in Scheme 1, two sets of single-stranded DNA probes are functionalized onto citrate-protected gold nanoparticles (DNA1−AuNP and DNA2−AuNP). When the two DNA-functionalized AuNP probes are mixed in a sample solution that contains complementary target DNAs, the hybridization of target DNA with two nanoparticle probes will cause nanoparticles to form dimers, trimers, and larger aggregates. This nanoparticle aggregation will increase the average diameter of the whole nanoparticle population, which can be detected by DLS analysis. The average diameter increase of the nanoparticles can then be correlated quantitatively to the target DNA concentration. A higher target DNA concentration should lead to more extensive nanoparticle aggregation and larger average nanoparticle size increase as shown in Scheme 1.

Scheme 1. Schematic Illustration of a Homogeneous Detection of DNA Using Gold Nanoparticle Probes and Dynamic Light Scattering



For this study, we synthesized a citrate-stabilized gold nanoparticle with a core diameter of 30 nm according to a reported procedure.[11] Two DNA probes as previously used in the work by Mirkin et al. are conjugated to the gold nanoparticles (see Supporting Information). Figure 1a is the UV−vis absorption spectra of AuNP probes before and after conjugating with DNA probes. The AuNP solution was initially pinkish-red with a SPR band at 520 nm. Upon functionalization of single-stranded DNA, the SPR band remained at 520 nm, indicating no particle aggregation due to an increased repulsive interaction

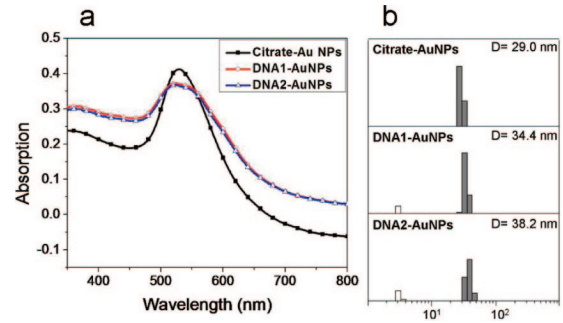


Figure 1. UV−vis absorption spectra (a) and size distribution (b) of AuNPs before and after DNA functionalization.

10.1021/ja801947e CCC: $40.75

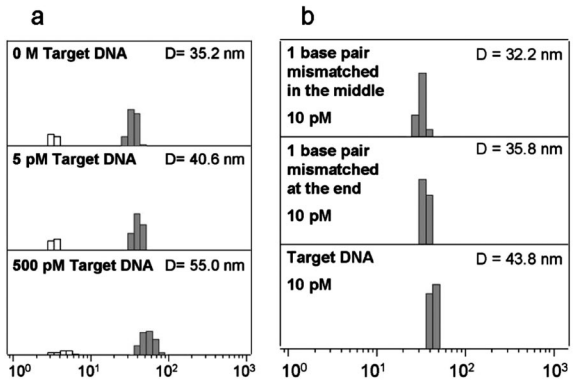


Figure 2. The size and size distribution (in diameter, nm) of DNA−AuNP assay solutions in the presence of perfectly matched target DNAs (a), and single base pair mismatched DNAs at a concentration of 10 pM (b).

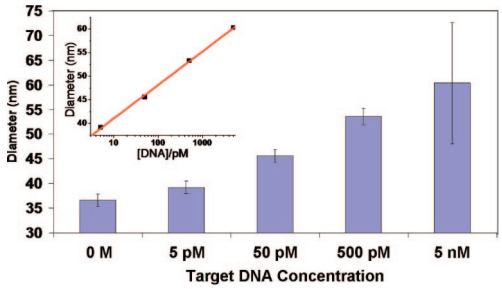


Figure 3. The average diameters of AuNPs as determined from DLS measurement and plotted against the target DNA concentration.

between nanoparticles after conjugating with single-stranded DNAs. DLS measurement was used to monitor the size change of AuNPs before and after conjugating with the two DNA probes. As shown in Figure 1b, the hydrodynamic diameter of the nanoparticles increased slightly from 29.0 to 34.4 nm for DNA1−AuNP and 38.2 nm for DNA2−AuNP. This hydrodynamic diameter change is caused by the addition of a DNA layer on the nanoparticle surface. The DLS data also revealed a very narrow size distribution of AuNPs before and after DNA modification. Both UV−vis absorption and DLS measurement indicate that the DNA−AuNP probes remained to be individually dispersed in the solution.

For target DNA detection, a 1:1 mixture solution of the two DNA−AuNP probes at a concentration of 100 pM was added to a set of target DNA solutions with a concentration ranging from 5 pM to 5 nM. The concentration of DNA−AuNP probes was determined using UV−vis absorption spectroscopy. The mixed solution was incubated for 5 min at 70 °C and then allowed to cool down to room temperature and set for 2 h. The solution was then diluted 100-fold for DLS measurement (the DLS sample cell requires a 1−1.5 mL sample solution). As shown in Figure 2a, the average size of gold nanoparticles in the control sample with 0 M target DNA in solution is around 35.2 nm. In a sample solution containing 5 pM target DNAs, the average nanoparticle size increased to 40.6 nm. The whole nanoparticle population now contains the individually dispersed DNA−AuNP probes, nanoparticle dimers, trimers, and oligomers formed due to hybridization between DNA targets and DNA probes. With increased concentration of target DNAs, the average nanoparticle hydrodynamic diameter increased accordingly. Figure 3 is a plot of the particle size over the target DNA concentration from 5 pM to 5 nM. The detection limit is estimated to be around 1 pM. Without any optimization, this detection limit is already 4 orders of magnitude higher than absorption-based methods, as reported previously.[4d] The assay exhibits excellent reproducibility, as judged from the small standard deviation of each concentration (three samples were run for each concentration) and a good linearity of the assay (see inset in Figure 3). At the highest concentration of target DNA, 5 nM, the deviation is significant. This is because the ratio of target DNA exceeded the probe DNA substantially, approximately 50:1, leading to extensive formation of large aggregates.

To examine the selectivity of the new assay, we conducted a comparison study on single base pair mismatched DNAs from perfectly matched DNA targets. Two types of mismatched DNA targets were studied: one with a mismatched pair located at the end and one with a mismatched pair in the middle of the target DNA sequence (see Supporting Information for the mismatched sequences). Under the exact same assay conditions, DLS analysis revealed a much less degree of nanoparticle aggregation when the target DNA has a single mismatched base pair, judging from the hydrodynamic diameter of the nanoparticles as shown in Figure 2b. This single base pair mismatch study was conducted at a target DNA concentration of 10 pM. The result demonstrates the capability of our new assay to discriminate single base pair mismatched DNAs from perfectly matched target DNAs, without using the melting transition of DNA−nanoparticle aggregates as required by a previously reported method.[4d]

Although Du et al.[8] demonstrated a similar level of sensitivity and selectivity for DNA detection as our assay, one problem may arise is that, when their method is applied to biological fluids such as human blood serum or plasma, the strong background scattering from the sample will contribute significantly to the absolute scattering light intensity, making the assay unsuitable for biological fluid analysis. In contrast, the dynamic light scattering used in our assay measures the diffusion coefficient of each group of particles not affected by the absolute scattering intensity. We also should mention that the detection limit of our assay may be improved by orders of magnitude through many possible approaches such as using nanoparticles with larger scattering cross sections[7] or more sensitive DLS detection methods.

In conclusion, we developed a one-step homogeneous hybridization assay for DNA detection. This assay is extremely easy to conduct and provides much higher sensitivity compared to absorption-based methods, however, without any signal amplification process. Single base pair mismatched DNAs can be readily discriminated from perfectly cDNAs directly from the DLS analysis under ambient conditions.

Acknowledgment. This work is supported by National Science Foundation Grants 0552295 and 0506531.

Supporting Information Available: Experimental details and DLS analysis of target DNA assay solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Rosi, N. L.; Mirkin, C. A. *Chem. Rev.* **2005**, *105*, 1547. (b) Alivisatos, P. *Nat. Biotechnol.* **2004**, *22*, 47.
(2) (a) Duggan, D. J.; Bittner, M.; Chen, Y.; Meltzer, P.; Trent, J. M. *Nat. Genet.* **1999**, *21*, 10.
(3) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303.
(4) (a) Gerion, D.; Chen, F. Q.; Kannan, B.; Fu, A. H.; Parak, W. J.; Chen, D. J.; Majumdar, A.; Alivisatos, A. P. *Anal. Chem.* **2003**, *75*, 4766. (b) Wang, J.; Liu, G.; Jan, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 3010. (c) Hahm, J.; Lieber, C. M. *Nano Lett.* **2004**, *4*, 51. (d) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078.
(5) Nam, J.; Stoeva, S.; Mirkin, C. *J. Am. Chem. Soc.* **2004**, *126*, 5932.
(6) (a) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757. (b) Cao, Y. W.; Jin, R. C.; Mirkin, C. A. *Science* **2002**, *297*, 1536.
(7) Yguerabide, J.; Yguerabide, E. E. *Anal. Biochem.* **1998**, *262*, 137.
(8) Du, B.; Li, Z.; Liu, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 8022.
(9) Ray, P. *Angew. Chem., Int. Ed.* **2006**, *45*, 1151.
(10) Liu, X.; Dai, Q.; Austin, L.; Coutts, J.; Knowles, G.; Zou, J.; Chen, H; Huo, Q. *J. Am. Chem. Soc.* **2008**, *130*, 2780.
(11) Turkevich, J.; Stevenson, P. C.; Hillier, J. *Discuss. Faraday Soc.* **1951**, *11*, 55.

JA801947E