A facile method for the detection of DNA by using gold nanoparticle probes coupled with dynamic light scattering

Yang Zhang, Wei-Wei Fei and Neng-Qin Jia*

Abstract

In this paper, we present a simple and rapid method for deoxyribonucleic acid (DNA) detection using gold nanoparticle probes coupled with dynamic light scattering (DLS) analysis. The redox agent 1,4-dithio-DL-threitol cross-links the gold nanoparticles (AuNPs) to form clusters, while the monothiol DNA could terminate the formation and stabilize the assembled clusters by their negatively charge-based repulsions. By varying the concentration of DNA, the different sizes of DNA-AuNP clusters can be obtained. The sizes of the DNA-AuNP clusters were determined by DLS. A linear correlation was obtained between the sizes and the logarithm of DNA concentration from 2 nM to 5 μM with a detection limit of 1 nM (S/N = 3).

Keywords: DNA detection, Gold nanoclusters, Dynamic light scattering

Background

Gold nanoparticles (AuNPs), including spherical particles, nanorods, and nanoshells with sizes ranging from 10s to 100s nm, have attracted enormous attention in the recent years due to their unique properties, such as the quantum size effect, remarkably enhanced surface-to-volume ratio, and surface plasmon resonance [1-3].

The light-scattering cross section of gold nanoparticles (AuNPs) with a diameter of 60 nm is 4 to 5 orders of magnitude stronger than that of a strong fluorescence dye, e.g., fluorescein [4]. Plasmonic nanoparticles, such as AuNPs and silver nanoparticles, can be used for colorimetric detection, and its color change can be easily observed with the naked eye [5,6]. This results in a color change providing a simple, sensitive colorimetric method useful for many applications, such as metal cation [7,8], small molecule [9,10], protein [11,12], and cell imaging [13].

Dynamic light scattering (DLS), known as photon correlation spectroscopy, is a well-established noninvasive technique for measuring the size of particles in the range from 0.5 nm to 6 μm [14-18]. This technique is based on the Brownian motion of spherical particles which causes a Doppler shift of incident laser light. The diffusion constant of particles is measured, and the size of the particles is calculated according to the Stokes-Einstein relation [19]. DLS is a powerful tool for determining small changes in the size of particles. This technique couples the use of AuNPs probes as a light-scattering enhancer and DLS as a read-out system. The subsequent average particle size increase was then measured by DLS and correlated to the analyte concentration. Therefore, it is a potential analytical tool for quantitative immunoassay.

In this paper, we reported a facile and convenient method for the detection of deoxyribonucleic acid (DNA) based on the aggregation of AuNPs from a well-dispersed state. DNA functionalized AuNPs have been often used as colorimetric probes for DNA detection [20]. DLS is a sensitive method to determine small changes in the size of AuNPs, and the size distribution of Au nanoclusters has a linear response to DNA concentration in a wide range. Therefore, DLS can be a very sensitive technique for quantitative detection of DNA, and it opens many more possibilities in biomolecular analysis.

Methods

Materials

HAuCl₄·4H₂O and 1,4-dithio-DL-threitol (DTT) were obtained from Sinopharm Chemical Reagent Co., Ltd.
Tri-sodium citrate (C₆H₅Na₃O₇·2H₂O) was purchased from Shanghai Chemical Reagent Company (Shanghai, China). Single-stranded monothiol DNA (TAACAATAATCCCTC-SH) was obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Songjiang, Shanghai, China). All other chemical reagents were of analytical grade, and all the solutions were prepared with double-distilled water.

**Apparatus and measurements**

Transmission electron microscopy (TEM) was taken with a JEOL model JEM2100 (JEOL Ltd., Tokyo, Japan) transmission electron microscopy and operating at 200 kV. Field emission scanning electron microscopy (FESEM) image was performed using a JSM-840 field emission SEM system (JEOL Ltd., Tokyo, Japan). Ultra-violet (UV)-Vis absorption spectra were recorded by Thermo Multiskan spectrum (Thermo Fisher Scientific, Tai To-ku, Tokyo, Japan). Dynamic light scattering and surface potential were obtained from Malvern Zetasizer Nano ZS 90 (Malvern Instruments Ltd., Malvern, Worcestershire, UK).

**Preparation of AuNPs**

AuNPs were prepared by the citrate reduction of HAuCl₄ [21]. Briefly, a solution of tri-sodium citrate (1 mL, 1% (w/v)) was rapidly added to a vigorously stirred boiling aqueous solution of HAuCl₄ (50 mL, 0.01% (w/v)), which resulted in a change in solution color from deep red to purple. The solution was kept boiling for an additional 15 min. Then the solution was cooled to room temperature and filtered, the AuNPs were obtained and stored at 4°C for further use.

**Preparation of DNA-AuNP clusters**

The DNA-AuNP clusters were prepared according to the literatures [22,23]. Briefly, 50 μL of the DNA solution with different concentrations was combined with 4 μL of aqueous DTT solution (800 μM) and added to 1 mL of AuNPs solution. This mixture of solution was incubated at 37°C for 90 min and then the unconjugated free DNA was removed by the centrifugation. The different sizes of DNA-AuNP clusters were obtained and stored at 4°C for further use.

According to the literature [22], when DTT and DNA were added to an AuNPs solution, the dithiol molecule,
DTT, cross-links the AuNPs to form clusters; while the monothiol DNA was immobilized onto the AuNPs, which could terminate the formation and stabilize the assembled clusters by their negatively charge-based repulsions. The schematic illustration of the synthesis of DNA-AuNP cluster conjugates was shown in our previously reported literature [23]. Therefore, by fixing the concentration of DTT and varying the concentration of DNA, the different sizes of DNA-AuNP clusters can be obtained. The size of the DNA-AuNP clusters was determined by DLS.

Results and discussion

Characterization of AuNPs

The size of the prepared AuNPs was determined by DLS and TEM. As can be seen from Figure 1A, the hydrodynamic diameter of the AuNPs was estimated to be approximately 27 nm, while the TEM image of the AuNPs was shown in Figure 1B, which agrees well with the DLS analysis results.

UV-visible absorption spectra and colorimetric detection of DNA-AuNP clusters

UV-Vis absorption spectroscopy has been used as a common tool to monitor AuNPs aggregation, because the aggregation causes a shift or broadening of the surface plasmon resonance of the AuNPs [24]. The AuNPs with 27 nm in diameter have an intense plasmon absorption at approximately 520 nm (data not shown). As can be seen from Figure 2A, by increasing the concentrations of DNA (from a to e) at a constant DTT concentration, the absorption band at 524 nm gradually increased and meanwhile, the shoulder absorption band at 600 nm gradually decreased. The absorption coefficients at 524 and 600 nm are related to the quantities of dispersed and aggregated gold nanoparticles, respectively. The change in plasmon absorption is attributable to electronic dipole-dipole interaction and coupling between plasmons of neighboring particles in the aggregates. The corresponding color change is shown in Figure 2B; as the concentration of DNA increased, the color of the mixed solution changed from purple gradually to red. The color
varied sensitively, which offered a possibility for visual observation. Meanwhile, representative FESEM image (inset of Figure 2A) also clearly showed the formation of the aggregates based on DNA-AuNP clusters conjugates, which agrees well with the UV-visible absorption spectra and the following DLS analysis results.

Detection of DNA by dynamic light scattering
DLS measurement was used to monitor the size change of AuNPs after conjugating with DNA. As shown in Figure 3A, the AuNPs assembled to form aggregates by adding DTT; the average hydrodynamic diameter of nanoparticle decreased as the concentration of DNA increased. The diameter of the DNA-AuNP clusters was determined to be 178.3 nm when the concentration of DNA was 2 nM, while the diameter was determined to be 109 nm when the concentration of DNA increased to 5 μM. Due to the repulsions induced by the charge of DNA, it can effectively stabilize the formed clusters and prevent them against larger aggregate formation.

As can be seen from Figure 3B, a linear correlation was obtained between the sizes and the logarithm of DNA concentration from 2 nM to 5 μM \((R^2 = 0.994)\), and a detection limit of 1 nM was obtained using the method of 3σ. The obtained results showed that DLS measurement could be used for DNA detection.

Conclusions
In conclusion, by taking advantage of the large scattering cross section of AuNPs and the high sensitivity of DLS measurement, we demonstrated that DLS can be used very conveniently to quantitative studies of DNA. The size distribution of Au nanoclusters has a linear response to DNA concentration in a wide range. Our experimental results reported here offer a good platform for rapid, easy, and reliable detection of DNA. Therefore, DLS measurement opens up a new possibility for further study of other biomolecules.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
YZ drafted the manuscript. WWF carried out the biological studies. NQJ conceived the study and participated in its design and coordination and helped draft and revise the manuscript. All authors read and approved the final manuscript.

Acknowledgments
This work was supported by Shanghai Shuguang Project (09SG43), National 973 Project (2010CB933901), Shanghai Science & Technology Committee (11JC1408602), Shanghai Key Laboratory of Rare Earth Functional Materials, Shanghai Normal University (DZL806).

Received: 28 July 2012 Accepted: 26 September 2012 Published: 10 October 2012
References

1. Nehl CL, Hafner JH: Shape-dependent plasmon resonances of gold nanoparticles. J Mater Chem 2008, 18:2415–2419.

2. Jennings T, Strouse G: Past, present, and future of gold nanoparticles. In Bio-Applications of Nanoparticles. Edited by Chan WCW. Berlin: Springer-Verlag; 2007:34–47.

3. Iuchi I, Yamasaki S: Photochemical and analytical applications of gold nanoparticles and nanorods utilizing surface-plasmon resonance. Anal Bioanal Chem 2008, 391:2411–2421.

4. Jain PK, Lee KS, El-Sayed IH, El-Sayed MA: Photochemical and analytical applications of gold nanoparticles and nanorods utilizing surface-plasmon resonance. Anal Bioanal Chem 2008, 391:2411–2421.

5. Zhao W, Brook MA, Li Y: Design of gold nanoparticle-based colorimetric biosensing assays. Chem BioChem 2008, 9:2363–2371.

6. Namasivayam SKR, Gnanendra KKE, Reepika R: Synthesis of silver nanoparticles by Lactobacillus acidophilus 01 strain and evaluation of its in vitro genomic DNA toxicity. Nano-Micro Lett 2010, 2:160–163.

7. Li L, Li B, Qi Y, Jin Y: Label-free aptamer-based colorimetric detection of mercury ions in aqueous media using unmodified gold nanoparticles as colorimetric probe. Anal Bioanal Chem 2009, 393:2051–2057.

8. Si S, Koral A, Mandal TK: One-dimensional assembly of peptide-functionalized gold nanoparticles: an approach toward mercury ion sensing. J Phys Chem C 2007, 111:1248–1255.

9. Liu SP, Chen YH, Liu ZF, Hu XL, Wang F: A highly sensitive resonance Rayleigh scattering method for the determination of vitamin B1 with gold nanoparticles probe. Mikrochim Acta 2006, 154:87–93.

10. Chen Z, Luo SL, Liu CB: Simple and sensitive colorimetric detection of cysteine based on ssDNA-stabilized gold nanoparticles. Anal Bioanal Chem 2009, 395:489–494.

11. Xu Y, Wang J, Cao Y, Li G: Gold nanoparticles based colorimetric assay of protein poly(ADP-ribosylation). Analyst 2011, 136:2044–2046.

12. Shrivastava S, Dash D: Label-free colorimetric estimation of proteins using nanoparticles of silver. Nano-Micro Lett 2010, 2:164–168.

13. Huang Y, Lin YW, Lin ZH, Chang HT: Aptamer-modified gold nanoparticles for targeting breast cancer cells through light scattering. J Nanopart Res 2009, 11:775–783.

14. Du BA, Li ZP, Liu CH: One-step homogeneous detection of DNA hybridization with gold nanoparticle probes by using a linear light-scattering technique. Angew Chem Int Ed 2006, 118:8190–8193.

15. Du BA, Li ZP, Liu CH: One-step homogeneous detection of DNA hybridization with gold nanoparticle probes by using a linear light-scattering technique. Angew Chem Int Ed 2006, 45:8022–8025.

16. Liu X, Dai Q, Austin L, Courtts J, Knowles G, Zou J, Chen H, Huo Q: A one-step homogeneous immunassay for cancer biomarker detection using gold nanoparticle probes coupled with dynamic light scattering. J Am Chem Soc 2008, 130:2780–2782.

17. Raschke G, Kawarki S, Frani T, Klar TA, Feldmann J, Nieth A, Kurzinger K: Biomolecular recognition based on single gold nanoparticle light scattering. Nano Lett 2003, 3:935–938.

18. Ipe BI, Shukla A, Liu H, Zou B, Rehage H, Nieneyer CM: Dynamic light-scattering analysis of the electrostatic interaction of hexahistidine-tagged cytochrome P450 enzyme with semiconductor quantum dots. Chem Phys Chem 2006, 7:1112–1118.

19. Berne BJ, Pecora R: Dynamic Light Scattering: with Applications to Chemistry, Biology, and Physics. New York: Wiley; 1976.

20. Fan Q, Zhao J, Li H, Zhu L, Li G: Exonuclease III-based and gold nanoparticle-assisted DNA detection with dual signal amplification. Biosens Bioelectron 2012, 33:211–215.

21. Storhoff JJ, Elghanian R, Mucic RC, Merin CA, Letsinger RL: One-pot colorimetric differentiation of polynucleotides with single base imperfections using gold nanoparticle probes. J Am Chem Soc 1998, 120:1959–1964.

22. Kim JY, Lee JS: Synthesis and thermally reversible assembly of DNA gold nanoparticle cluster conjugates. Nano Lett 2009, 9:4564–4569.

23. Fei WW, Zhang Y, Sun XM, Zhang Y, Cao HM, Shen HB, Jia NQ: Direct electrochemistry and electrocatalysis of myoglobin immobilized on DNA-gold nanoparticle clusters composite film. J Electroanal Chem 2012, 6755–10.

24. Jani H, Liu X, Austin L, Maes G, Huo Q: Dynamic light scattering as a powerful tool for gold nanoparticle bioconjugation and biomolecular binding studies. Anal Chem 2009, 81:3945–3952.