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Structural study on gold nanoparticle functionalized with DNA and its non-cross-linking aggregation

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ABSTRACT

Hybridization of DNA tethered on colloidal nanoparticles with fully matched complementary one induces the aggregation of the particles in a non-cross-linking configuration. Here, we performed a structural study on DNA-functionalized gold nanoparticle and its non-cross-linking aggregation mainly using synchrotron radiation small-angle X-ray scattering. To understand the non-cross-linking aggregation, the nanoparticles with various DNA lengths and core sizes were used. In the aggregation, the surface distance between the gold nanoparticles increased with the length of DNA duplex, although the increment of the distance per base pair was not constant and showed the tendency to become small with increasing DNA length, meaning the interdigitation of DNA layers. The aggregation was also found to occur between the identical cores, without being affected by tethered DNA. Furthermore, it was proved that the relative increase in DNA length to core size leads to the increase in colloidal stability. Even the nanoparticles with full-matched DNA duplex were dispersed stably. These facts suggested that van der Waals interaction between core particles rather than end-to-end stacking between DNA duplexes is a dominant attractive interaction. The steric repulsion force arising from entropic loss of thermal fluctuation of DNA molecules might be a key factor to characterize the non-cross-linking aggregation.

1. Introduction

Colloidal materials are fundamental for new functional materials and widely used in several fields. The change in optical property of the colloidal system, resulting from, e.g., colloidal dispersion/aggregation, is useful for application as a sensor. Biomolecule-functionalized particle system is a representative example of the application to colorimetric diagnostics and prognostics. The use of gold nanoparticle as a core particle is advantageous for those sensors because the surface plasmon shift by dispersion/aggregation is visible. For example, DNA/gold nanoparticle materials have been drawn much attention toward a colorimetric detection method of single-base mutation such as single-nucleotide polymorphisms.

Mirkin and coworkers first reported a DNA sensing system using DNA/gold nanoparticles [1,2]. Two sets of single-stranded DNA (ssDNA)-functionalized gold nanoparticles assemble in the presence of a complementary target DNA, which hybridizes to both tethered ssDNA molecules on the gold nanoparticles, acting as a cross-linker. Using this system, the discrimination between fully complementary and mismatched targets is visible at an appropriate temperature because the dissociation temperatures of the duplexes are different. The cross-linking aggregation of DNA/gold nanoparticles assisted by hybridization has been widely applied to other detection methods such as metal ions and small molecules [3–5]. This technique has been extended to the methodology of programmable nanoassemblies directed by DNA [6,7].

Meanwhile, it was discovered that DNA-functionalized nanoparticles assemble by hybridization in a non-cross-linking configuration. When ssDNA molecules grafted on the nanoparticles hybridize to the fully complementary ones, the resulting nanoparticles, which are covered with double-stranded DNA (dsDNA), immediately assemble without molecular cross-linking at high salt concentrations, while the nanoparticles with ssDNA molecules remain stable [8–10]. This phenomenon called non-cross-linking aggregation occurs very rapidly. More remarkable point is that the dispersion/aggregation of the particles is very sensitive to the terminal base pair of DNA duplex. When the tethered ssDNA hybridizes with a single-base mismatched DNA, the nanoparticles still disperse stably at the high salt concentrations. Accordingly, the discrimination between fully complementary and mismatched targets is easy in short time without temperature control. The non-cross-linking

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aggregation was observed in poly(*N*-isopropylacrylamide)-DNA [8], gold nanoparticle-DNA [9], and polystyrene nanoparticle-DNA [10].

Therefore, the non-cross-linking aggregation system is also potentially useful for single-base mutation detection. Furthermore, this system has been applied to detections of metal ions, and small molecules, using the nanoparticles covered with functional nucleic acids such as DNA aptamer [11,12]. However, the nature of the non-cross-linking aggregation interaction has not been understood yet. The hybridization of ssDNA with its complementary one might bring about the change in electrostatic, steric repulsion potential, or both. On the other hand, the possibility of a specific interaction between the terminal base pairs of DNA duplexes, end-to-end stacking [13–17], has been supposed in order to explain the feature of the non-cross-linking aggregation interaction. Such interparticle interactions must be reflected in the configuration of the assembling particles. No structural information on DNA-functionalized nanoparticle and its assemble has been, however, provided so far. In this study, the non-cross-linking aggregation was explored from the structural point of view, using mainly small-angle X-ray scattering (SAXS). The structural characterization of the gold nanoparticles with ss- or dsDNA was performed, and the effects of DNA length, core size, and temperature on the non-cross-linking aggregation/dispersion were also investigated.

2. Experimental

2.1. Materials

Gold nanoparticles with diameters of ca. 5, 15, and 40 nm (Au5, Au15, and Au40, respectively, hereafter) were purchased from British-Biocell, UK. The gold nanoparticles were functionalized with ssDNA according to the method reported in Ref. [9]. In this study, the DNAs with seven different lengths (15, 20, 25, 30, 35, 40, and 45 mer) (Operon Biotechnologies, Japan) were used. The ssDNA has a C6 thiol linker at the 5′-end. The sequences of probe DNAs are summarized in Table 1. The functionalized gold nanoparticles were suspended in 10 mM phosphate buffer (PB) (pH 7.0) containing 0.1 M NaCl. The graft number of probe DNA on gold nanoparticle was evaluated according to the procedure reported in Ref. [9]. Unless otherwise noted, the concentration of the gold nanoparticle was prepared at around 1 OD at 520 nm (5.0×10^{10} particles/μL for Au5, 1.4×10^{9} particles/μL for Au15 and 9.0×10^{7} particles/μL for Au40). For hybridization of DNA, the complementary DNA (Operon Biotechnologies, Japan) was added to the suspension (final concentration of 0.5 μM) and, for aggregation experiments, the NaCl was further added to it (final concentration of 1 M).

2.2. Characterization

The dynamic light scattering (DLS) measurements were conducted on a Zetasizer Nano ZS ZEN3600 with a He–Ne laser (633 nm) (Malvern Instrument Limited, UK). According to cumulants analysis, z-averaged size and variance (hydrodynamic radius (R_h), and polydispersity index (PDI)) of the nanoparticles dispersed in 10 mM PB (pH 7.0) containing 0.1 M NaClat 25 °C were evaluated. UV–vis spectra were measured with a Cary 50 Bio UV–Visible Spectrometer (VARIAN, Inc., CA, USA) in order to confirm the dispersion/aggregation of the nanoparticles at 25 °C. The UV–vis spectra at various temperatures were taken using a UV-2550 UV–Vis spectrophotometer (Shimadzu Corporation, Japan). Circular dichroism (CD) spectra were measured with a JASCO J-720WI Spectropolarimeter (JASCO Corporation, Japan) at 25 °C. In CD, the spectra of hybridized DNA (5 μM) without gold nanoparticles (10 mM PB containing 0.1 M or 1.0 M NaCl) were also observed. For gold nanoparticles with dsDNA, the samples concentrated to more than 8 times (10 mM PB containing 0.1 M or 1.0 M NaCl, target DNA: 4–5 μM) were used for CD measurements.

2.3. Solution small-angle X-ray scattering

Solution SAXS measurements were carried out at the BL45XU RIKEN Structural Biology Beamline I (wavelength, $\lambda = 0.09$ nm) of the SPring-8, Harima, Japan [18]. The camera length was set to be about 2.5 m and calibrated using a silver behenate. Two-dimensional (2-D) SAXS images were recorded with a CCD camera (C4880-10-14A, Hamamatsu Photonics, Japan) coupled with an X-ray image intensifier (V5445P MOD, Hamamatsu Photonics, Japan). The pixel size of CCD camera was about 0.15 mm × 0.15 mm. The 2-D SAXS images were converted into one-dimensional profiles by circular averaging. The sample solution (ca. 50 μL) was placed in a sample cell and thermostatted at 25 °C except for temperature-controlled experiments. For aggregation experiments, the SAXS images were acquired about 3 min after the complementary DNA and then NaCl was added to the suspension.

3. Results and discussion

In this study, the gold nanoparticles with the diameters of 15 and 40 nm were mainly used as core particles, and their DNA-functionalized gold particles were first characterized. The values of DNA graft density Γ evaluated for the samples subjected to SAXS experiments are summarized in Table 2. The graft density was dependent on the core size as reported previously [19]and evaluated at about 0.25 strands/nm^2 for Au15 and 0.15 strands/nm^2 for Au 40, respectively, although the values somewhat varied with the length of DNA. The hydrodynamic radii of the DNA-functionalized gold particles measured by DLS are given in Table 3. The values of R_h strongly depended on DNA length. There was little difference of R_h between ss- and dsDNA-functionalized particles. This is likely because R_h results from the slow motion of particle diffusion and the difference in rapid molecular motion of DNA between single- and double-stranded structures might little affect the overall R_h. Although some particles showed somewhat high

Table 1
Sequences of probe DNA used in this study.

Length (bases)	Sequence (5′–3′)
15	TAC GCC ACC AGC TCC
20	TAC TCT ACG CCA CCA GCT CC
25	TAC TCC TTA TTA CGC CAC CAG CTC C
30	TAC TCC TTA TTC TTT TAC GCC ACC AGC TCC
35	TAC TTT TCT CCT TAT TCT TTT ACG CCA CCA GCT CC
40	TAC TTT TCT TTT CTC CTT ATT CTT TTA CGC CAC CAG CTC C
45	TAC TTT TCT TTT TTC TAC TCC TTA TTC TTT TAC GCC ACC AGC TCC

Table 2
Graft density of probe DNA.

Length (bases)	Γ (strands/nm^2)	
	Au15	Au40
15	0.25	0.14
20	0.17	0.10
25	0.21	0.18
30	0.20	0.11
35	0.27	0.17
40	0.17	0.12
45	0.13	0.054