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Rapid Aggregation of Gold Nanoparticles Induced by Non-Cross-Linking DNA Hybridization

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In this paper, we report a newly discovered aggregation phenomenon of DNA-functionalized gold nanoparticles induced by hybridization of target DNA which does not cross-link the nanoparticles.

In general, a colloidal solution of gold nanoparticles with diameters of 5−20 nm exhibits a red color, because such nanoparticles have an optical absorption peak around 520 nm caused by surface plasmon resonance.[1] Aggregation of the nanoparticles shifts the absorption peak toward longer wavelength and changes the color of the colloidal solution to purple.[2] Recently, this principle has been applied to analyses of various substances such as DNA,[3−6] lectin,[7] heavy metal ion,[8] potassium ion,[9] and protein A.[10] All of these methods are based on the cross-linking mechanism by the target molecules between the gold nanoparticles. For example, Mirkin et al.[3] invented a DNA analysis method by modifying two sets of gold nanoparticles with different single-stranded DNA probes and by mixing them with target DNA. If the target DNA has sequences complementary to both of the two probes, the target cross-links the nanoparticles by hybridization, and this results in particle aggregation. Detection of a single-base mismatch is possible using this system with appropriate temperature control.

On the other hand, aggregation of DNA-functionalized poly(*N*-isopropylacrylamide) (PNIPAAm) nanoparticles without the cross-linking mechanism has been reported by our group.[11] In this system, only one kind of single-stranded probe DNA is grafted on PNIPAAm, which spontaneously forms nanoparticles above 40 °C. When the target DNA is perfectly complementary to the probe, in sequence as well as in chain length, the nanoparticles aggregate together at considerably high salt concentration. Although the mechanism of this phenomenon is not fully understood at present, we suppose that conformational transition of the immobilized DNA plays an essential role. Formation of the probe-target duplex makes the conformation tighter and stiffer. This transition may reduce two parts of the repulsive interaction between nanoparticles: (1) electrostatic repulsion may be decreased by the screening effect, because the tight conformation raises the binding constant with counterions,[12] and (2) steric repulsion may be reduced, because the stiffening of the DNA lowers the entropic effect.

In this paper, we prove that the aggregation induced by non-cross-linking DNA hybridization is not a unique property to the PNIPAAm nanoparticles. As described below, this phenomenon also occurs with gold nanoparticles, which have three advantages as compared to PNIPAAm: (1) various sizes of gold nanoparticles are readily available at low cost, (2) surface modification with probe DNA is easier, and (3) the temperature control for the formation of nanoparticles is not necessary. We also demonstrate unusual sensitivity of this system for a single-base mismatch at the terminus opposite to the anchored side.

A colloidal solution containing gold nanoparticles with a diameter of 15 nm was purchased from British-Biocell. The particle concentration was 1.4×10^{12} mL^{-1} (=2.3 nM). The nanoparticles were functionalized with a single-stranded DNA by following the procedure described by Mirkin et al.[4,5] with a minor modification. Briefly, 3 nmol of HS-$(CH_2)_6$-5′-TAC GCC ACC AGC TCC-3′ (Takara Bio, Japan) was incubated with 1 mL of the gold nanoparticle solution at 50 °C for 16 h. The solution was changed into 0.1 M NaCl, 10 mM phosphate buffer (pH 7) by addition of the necessary salts and was kept at 50 °C for 40 h. To remove unreacted probes, the solution was centrifuged at 14 000 rpm for 25 min, and the supernatant was replaced by 1 mL of the same buffer. After another centrifugation under the same condition, the precipitate was redispersed into 0.5 mL of the same buffer to make a stock solution, which was used for the following experiments.

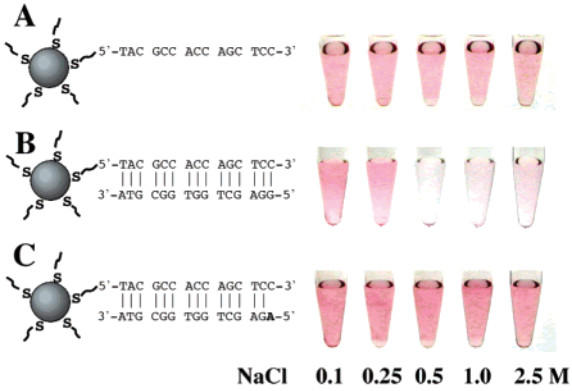

Figure 1. Aggregation behaviors of the DNA-gold nanoparticles at various NaCl concentrations at room temperature: (A) without a target DNA, (B) with the complementary target, and (C) with a target containing a single-base mismatch at its 5′ terminus. The final concentrations of the particle, the probe DNA, and the targets were 2.3, 500, and 500 nM, respectively.

To estimate the number of DNA probes immobilized on the gold nanoparticle surfaces, dithiothreitol was added (final concentration = 10 mM) to release the probes from the nanoparticles into solution.[5] Using the OliGreen ssDNA Quantitation Kit (Molecular Probes, Oregon), we measured the concentration of the released DNA to be about 1 M in the stock solution, which means about 200 DNA probes per one nanoparticle (surface coverage = 50 pmol cm^{-2}). This result is comparable to the literature value of 159 DNA probes per one nanoparticle (34 pmol cm^{-2}).[5]

With various NaCl concentrations, we investigated the aggregation behaviors of the gold nanoparticles by observing the solution colors and by monitoring the visible spectra. All of the experiments described below were carried out at room temperature (ca. 25 °C). Bare nanoparticles without probe DNA immediately aggregated at 0.1 M NaCl (data not shown). In contrast, the DNA-functionalized nanoparticles did not exhibit any visible change within the experimental range up to 2.5 M NaCl (Figure 1A). The surface-immobilized probe DNA stabilized the nanoparticle dispersion.

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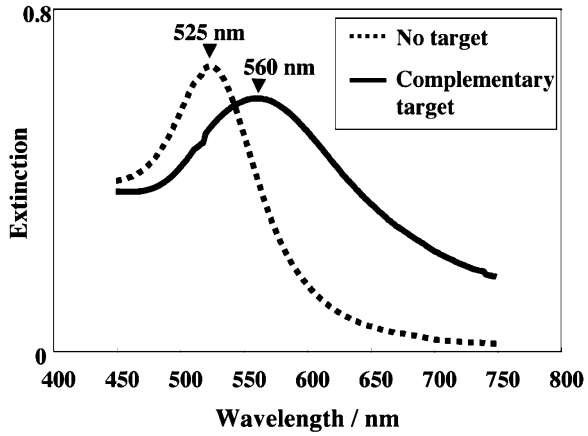

Figure 2. Visible spectra corresponding to Figure 1A (dotted line, no target) and 1B (solid line, complementary target) at 0.5 M NaCl.

Next, we added a target DNA with complementary sequence to the probe DNA, so that the amounts of the two DNAs should be balanced (Figure 1B). When the NaCl concentration was higher than 0.5 M, a clear colorimetric change into purple—representing the particle aggregation (see Figure S1 in the Supporting Information)—was immediately observed (<3 min). The aggregation process of this non-cross-linking system (see Figure S2 in the Supporting Information) is much more rapid than that of the cross-linking systems, which takes several tens of minutes to hours at room temperature.[6] We suppose that this difference originates from the different aggregation mechanisms. In this non-cross-linking system, the aggregation is driven by the London−van der Waals attractive force between the nanoparticles, when the repulsive interaction is greatly reduced by formation of duplexes on their surfaces. The attractive force works from a distance and leads to the rapid aggregation. On the other hand, in the cross-linking system, the kinetics of the aggregation is dominated by random collisions between the nanoparticles with relatively slow Brownian motion. The visible spectra corresponding to Figure 1A and 1B are shown in Figure 2. In the presence of 0.5 M NaCl, the extinction peak was shifted from 525 to 560 nm by the addition of the complementary target DNA.

More striking results were derived from experiments for another target DNA with a single-base mismatch at the 5′ terminus. As shown in Figure 1C, addition of this target DNA to the nanoparticle solution did not cause any colorimetric change even at high NaCl concentration. The visible spectrum also did not change except for the dilution effect caused by the target DNA solution (see Figure S3 in the Supporting Information). This behavior depends only on the position of the mismatch, and not on the combination: two other targets with different terminal mismatches, C−C and T−C, were also confirmed to keep the dispersion (see Figure S4 in the Supporting Information). In contrast, a single-base mismatch at the middle of the target DNA brought a colorimetric change similar to that in Figure 1B (see Figure S5 in the Supporting Information). Although interpretation of this unusual sensitivity for the terminal mismatch is difficult, we presume that even one base works as a single strand to produce a repulsive interaction when it is located at the outermost position of the DNA-nanoparticle conjugate. In addition, this terminal-mismatch sensitivity was also discovered in the non-cross-linking DNA-PNIPAAm system.[13] We suppose that the terminal-mismatch sensitivity is a common property to these non-cross-linking systems.

The aggregation phenomena reported here open up a new possibility of rapid, easy, and reliable genetic diagnosis because of three advantages: (1) the aggregation process is rapid and clearly visible, (2) colloidal gold is a common material and its functionalizing procedure is well established, and (3) single-base mismatch at the terminus opposite to the anchored side can be sensitively detected at room temperature. The last advantage is particularly significant if this system can be combined with the primer extension method[14] for analysis of single nucleotide polymorphisms. To do this, at least two issues should be examined. One is rather simple: the direction of the probe DNA, which was first determined for some inessential, technical reasons in the current system, must be inverted, because the primer extension produces a copy of the assumed polymorphism at the 3′ terminus. The other issue is the reliability of this system for a mixture of different target DNA, because an actual sample is usually a mixture including unreacted primer and products from heterogeneous alleles. Preferably, the nanoparticle system should correctly respond to a certain amount of the complementary target DNA regardless of the presence of other analogous DNA. We will investigate the above issues in our next step.

A conceivable disadvantage of the non-cross-linking system as compared to the cross-linking system is target DNA consumption. In principle, a single DNA molecule is sufficient to cross-link two nanoparticles together, whereas our current system requires about 200 DNA molecules per one nanoparticle to induce the aggregation. In reality, we used target DNA with an 8-fold greater concentration as compared to that of the previously reported cross-linking system:[4] 500 nM to 60 nM. However, a 500 nM DNA sample can be obtained through standard PCR protocol; therefore, we do not believe that this aspect will impose a severe limitation on our system for practical applications.

Supporting Information Available: Transmission electron microscope image, time courses of extinction, visible spectra, and results for other terminal mismatches (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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