DNA-Functionalized Polymers and Nanoparticles for Gene Sensing

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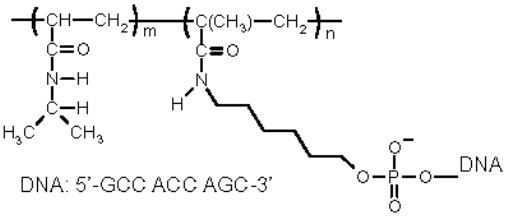
Gene diagnosis shows tremendous promise for medical, pharmaceutical, and other applications. The most reliable method for detection of single-nucleotide polymorphisms (SNPs) is direct sequencing. However, this approach suffers from high cost and low throughput. Instead of the direct sequencing, various assays have been developed. Especially, combination of the allele-specific hybridization and the fluorometric detection has provided a variety of assay techniques such as molecular beacons and DNA microarrays. These techniques usually require bulky and expensive instruments for the fluorometric detection. As an easy and inexpensive alternative, gene diagnosis using gold nanoparticles (GNPs) has been attracting considerable interests [1]. Aggregation of GNPs, accompanied by the surface plasmon shift, can be clearly recognized with the naked eye (color change from red to purple, and to black). Therefore, no specialized instrument is required. Most of the previously reported GNP aggregation systems rely on cross-linking of the GNPs by hybridization. In principle, these systems detect the difference between single-stranded (ss) DNA and double-stranded (ds) DNA. In this case, discrimination of SNPs requires strict control of the hybridization temperature depending on the sequence, because a single-base mismatch gives only little instability to the dsDNA. This limitation is common to all the assay methods based on the allele-specific hybridization.

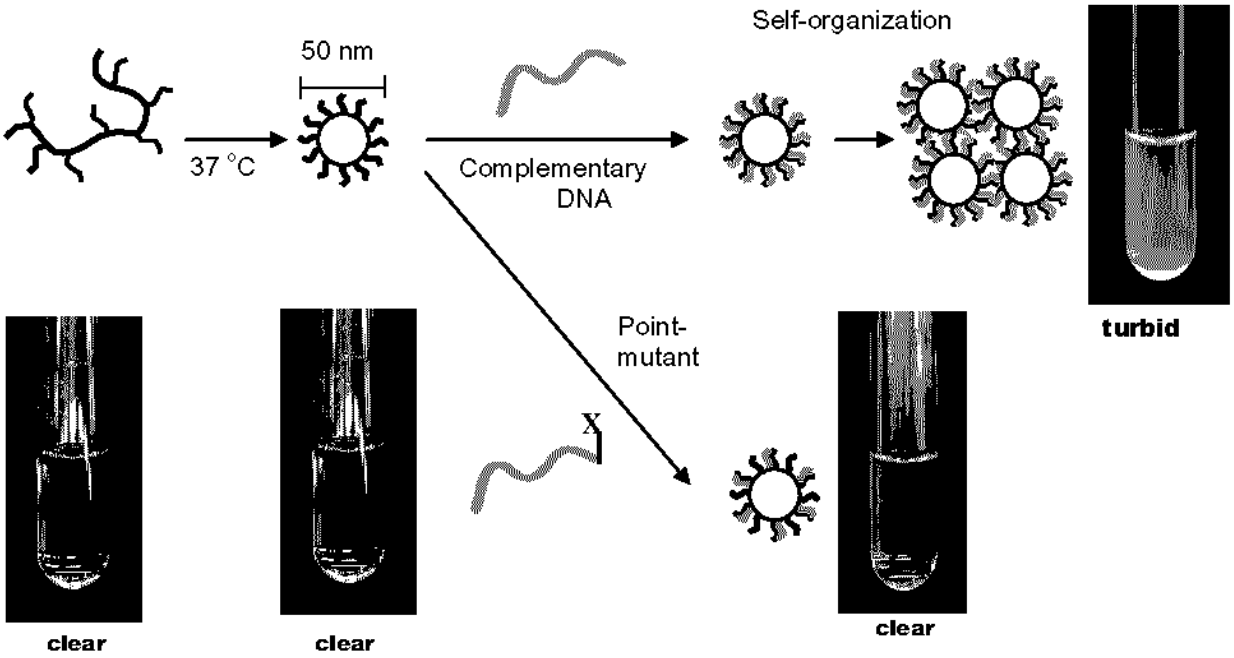
In contrast, we have prepared a series of DNA conjugate materials which can recognize DNA fragments with one-base specificity [2]. A conjugate consisting of water-soluble synthetic polymers and oligo DNA are used in its aqueous solution [3]. DNA conjugate materials are very much different from conventional solid materials on which DNA probe is immobilized. In addition to soluble DNA conjugates, dispersed colloidal nanoparticles carrying DNA are promising for biological and medical diagnosis [4].

Quite recently, we discovered a simple method for detecting single nucleotide mutation by using reversible aggregation of a DNA-carrying colloidal nanoparticle, which was composed of a hydrophobic core from poly(N-isopropylacrylamide) (NIPAAm) and a hydrophilic shell from DNA. The particle with the diameter of 50 nm was found to form through the self-assembly of polyNIPAAm-graft-DNA copolymers above the phase transition temperature of polyNIPAAm part. A typical preparation procedure of polyNIPAAm-*graft*-DNA copolymer is as follows: the DNA macromonomer was synthesized by the coupling reaction between methacryloyloxysuccinimide and amino-linked DNA and was purified by reversed-phase high-performance liquid chromatography. The graft copolymer was prepared by radical copolymerization between NIPAAm and the DNA macromonomer. The DNA-carrying nanoparticle with the diameter of 50 nm was formed through the self-assembly of the graft copolymers above the phase transition temperature of polyNIPAAm part (ca. 37℃) [4]. The typical sequence of anchoring DNA examined was 5'-GCCACCAGC-3' which is a part of oncogene, *ras*.



When adding the complementary DNA into the DNA-carrying colloidal nanoparticle dispersion at 40℃, the particles were found to aggregate rapidly in 10 mM Tris-HCl buffer (pH 7.4) containing 500 mM NaCl. Since we added here the exact match DNA (3'-CGGTGGTCG-5') in terms of not only the sequence but also the length, the aggregation is considered to take place by a non-crosslinking mechanism [4]. In contrast, the colloidal system kept dispersing in the presence of the point-mutated DNA (3'-CGGTAGTCG-5') under the identical conditions. Other mismatched DNAs including 3'-AGGTGGTCG-5' and 3'-CGGTGGTCG-5' did not induce the aggregation either. Interestingly, the complementary DNA but having one protruding nucleotide (3'-CGGTGGTCGA-5') did not trigger any aggregation. This was really surprising since the DNA should form stable double helical structure as well as the exact match DNA.

In the presence of the complementary DNA, the DNA-carrying colloidal nanoparticle dispersion aggregates rapidly in the conditions of relatively high salt concentration by a non-crosslinking mechanism. In contrast, they kept dispersing in the presence of the point-mutated DNA under the identical conditions. Thus we found a novel behavior; at certain critical salt concentrations the stability of the colloidal particle is destabilized by the hybridization of the surface DNA with the complementary DNA in solution. This system is very



sensitive to exact matches in DNA sequence between the immobilized DNA on the colloid and DNA fragment in the suspending fluid. The phenomenon appears to depend on a delicate balance between electrostatic forces between particles that promote dispersion, and hydrophobic interaction of the polymer cores that favor aggregation. Using this method, the single-nucleotide mutation of 39 mer DNA was successfully detected by turbidity change.

The core material is not restricted to polyNIPAAm; DNA-functionalized gold nanoparticle was found to show a similar aggregation behavior induced by the fully-complementary DNA which does not crosslink the nanoparticles [5]. At high NaCl concentration (= 0.5M), hybridization with complementary target DNA induces nanoparticle aggregation. The aggregation can be detected by colorimetric change of the colloidal solution within 3 min. Furthermore, unusual sensitivity of this system for single-base mismatch at the terminus opposite to the anchored side has been discovered. In fact, target DNA with such kind of mismatch does not induce the colorimetric change at all. (Figure 1)

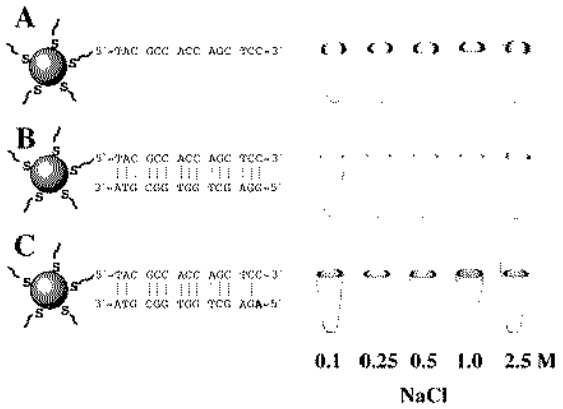


Fig. 1 Aggregation behavior of the DNA-gold nanoparticles at various NaCl concentrations at room temperature: (A) without a target DNA, (B) with the complementary target, and (C) with a target containing a single-base mismatch at its 5' terminus.

Thus, we have been developing a GNP-based SNPs typing method which does not depend on the specificity of dsDNA formation Instead, this method detects difference between dsDNAs with and without a mismatch. We call this method non-cross-linking method; because the aggregation of GNPs is induced not by the cross-linking mechanism, but by the formation of fully complementary dsDNA on the GNP surfaces. We have reported that; (1) the NCL aggregation occurs at relatively high salt concentrations [5]; (2) single-base mismatches at the distal ends greatly stabilize the colloidal dispersion [5,6]; and (3) the NCL method can be applied to products from the single-base primer extension reaction for SNPs typing [6].

In general, a single medical decision requires information of multiple SNPs of an individual patient. For this purpose, a low detection limit is desirable to minimize the blood sample volume. We discovered that larger GNPs enable lower detection limits in the NCL method. However, this effect saturates around 40-nm in diameter. Taking into account the gravitational sedimentation, we decided that 40-nm is the optimal diameter for SNPs typing [7]. Selectivity of the 40-nm GNPs was confirmed with the primer extension products which were ten times as dilute as those in our previous report using 15-nm ones.

On the other hand, efforts for reduction of sample volume and for multiplexing using microfluidic devices are now in progress [8]. We believe that the NCL method has a great potential for point-of-care SNPs typing.

References

[1] N.L. Rosi and C.A. Mirkin, Chem. Rev. **2005**, *105*, 1547-1562.
[2] M. Maeda, T. Mori, D. Umeno and Y. Katayama in Biomedical Diagnostic Science and Technology, W.T. Law, N. Akmal and A.M. Usmani, Eds., Marcel Dekker., New York, 2002, pp.215-226.
[3] T. Anada, T. Arisawa, Y. Ozaki, T. Takarada, Y. Katayama, and M. Maeda, Electrophoresis **2002**, *23*, 2267-2273.
[4] T. Mori and M. Maeda, Polym. J. **2002**, *34*, 624-628.
[5] K. Sato, K. Hosokawa, and M. Maeda, J. Am. Chem. Soc. **2003**, *125*, 8102-8103.
[6] K. Sato, K. Hosokawa, and M. Maeda, Nucleic Acids Res. **2005**, *33*, e4.
[7] K. Sato, M. Onoguchi, Y. Sato, K. Hosokawa, and M. Maeda, Anal. Biochem. **2006**, *350*, 162-164.
[8] K. Hosokawa, K. Sato, N. Ichikawa, and M. Maeda, Lab Chip **2004**, *3*, 181-185.