SHORT COMMUNICATIONS

Stability Change of DNA-Carrying Colloidal Particle Induced by Hybridization with Target DNA

Takeshi MORI[†] and Mizuo MAEDA^{*,††}

Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, 6–10–1 Hakozaki, Higashi-ku, Fukuoka 812–8581, Japan

^{*}The Institute of Physical and Chemical Research (RIKEN), 2–1 Hirosawa, Wako 351–0198, Japan

(Received April 18, 2002; Accepted July 15, 2002)

KEY WORDS Aggregation / Colloid / Salt Effect / DNA / Poly(*N*-isopropylacrylamide) /

A well-known example of a detection method utilizing the optical property change of a colloidal particle dispersion is the latex agglutination immunoassay.¹ In the assay, the antigen- or antibody-modified latex particle is crosslinked by the complementary antibody or antigen and the resulting particle aggregate is detected easily by the increase in turbidity or light scattering intensity of the dispersion. Recently, the DNA detection method using the aggregation phenomenon of colloidal particles was reported by Mirkin *et al.*² In their method, the single-stranded DNA-modified gold nanoparticle was used as a detection probe and the nanoparticle was crosslinked by the hybridization with the complementary DNA to form aggregates (Figure 1a). The aggregates were detected by the color change of the dispersion due to a red shift of the plasmon band.

We reported previously the colloidal particle formation of the graft copolymer **1**, which consists of poly(*N*-isopropylacrylamide) (PNIPAAm) as a main chain and single-stranded DNA as a graft chain (Scheme 1).³ Since PNIPAAm is known to have the phase transition temperature in an aqueous solution and become insoluble above the temperature,⁴ **1** formed a core-shell colloidal particle with a hydrophobic PNIPAAm core and a hydrophilic DNA shell above the phase transition temperature of PNIPAAm main chain.^{3,5} This particle aggregated in the presence of complementary DNA according to the crosslinking mechanism, the process being observed as a turbidity increase of the dispersion.

We report here the novel aggregation mechanism of this DNA-containing colloidal particle, that is, the stability decrease of the colloidal dispersion induced by the hybridization of the surface DNA with the comple-

a) crosslinking

b) hybridization-induced stability change

Figure 1. Two different aggregation mechanisms of DNA-carrying colloidal particle induced by the hybridization with complementary DNA.

Scheme 1. Chemical structure of poly(*N*-isopropylacrylamide)-graft-DNA.

[†]Present Address: Department of Chemical Science and Technology, Faculty of Engineering, The University of Tokushima, 2–1 Minamijosanjima, Tokushima 770–8506, Japan.

^{††}To whom correspondence should be addressed (Tel: +81-48-467-9311, Fax: +81-48-462-4658, E-mail: mizuo@riken.go.jp).

Table I. Sequences of target DNAs and melting temperatures of their duplex DNAs

target DNA	sequence (3' → 5')	$T_m(^{\circ}\text{C})^a$
I	CGGTGGTCG	53
II	CGGT A GTCG	29
III	TT CGGTGGTCG	55
IV	TTTTT CGGTGGTCG	52
V	CGGTGGTCG TT	57

^aMelting temperatures of duplex DNAs between each target DNA (I–V) and 5'-GCCACCAGC-3' in 500 mM NaCl/10 mM Tris-HCl buffer (pH 7.4). The duplex DNA concentration was 2.8 μM . Heating rate was 0.5 $^{\circ}\text{C min}^{-1}$.

mentary DNA (Figure 1b). The instability of the particle results from the stronger binding of metal cation of the solution to the duplex DNA on the particle surface.

EXPERIMENTAL

Synthesis of Copolymer 2

Graft copolymer **2** (Scheme 1) was obtained by radical copolymerization between *N*-isopropylacrylamide and DNA macromonomer as reported previously.^{3,6} The copolymer composition was determined by dry weight of copolymer and UV absorption at 260 nm due to DNA macromonomer unit using the molar extinction coefficient of 81100 $\text{M}^{-1} \text{cm}^{-1}$, which was calculated from the nearest-neighbor model.⁷

Stability Measurement of Colloidal Particle

Copolymer **2** was dissolved in 5 mM MgCl_2 / 10 mM Tris-HCl (pH 7.4) (buffer **a**) or 300 mM NaCl / 10 mM Tris-HCl (pH 7.4) (buffer **b**) to be the concentration of 0.1 mg mL^{-1} (2.8 μM in DNA unit concentration). These solutions were heated to 40 $^{\circ}\text{C}$ and placed for 30 min to give the colloidal particle, and then the target DNA (Table I) solution was added to be the concentration of 2.8 μM . The obtained particle dispersion was mixed with NaCl or MgCl_2 solution at 40 $^{\circ}\text{C}$, and placed for 30 min. The extent of aggregation of the particle was estimated from the absorbance of the dispersion at 500 nm.

Light Scattering Measurement

Static light scattering (SLS) and dynamic light scattering (DLS) measurements were conducted with DLS-7000 instrument (Otsuka Electronics) according to the previous paper.³ The dn/dC of copolymer was calculated from the additive equation,^{5a} $dn/dC = w_{\text{PNIPAAm}}(dn/dC)_{\text{PNIPAAm}} + w_{\text{DNA}}(dn/dC)_{\text{DNA}}$, where w represents the weight fraction. The dn/dC of DNA macromonomer, $(dn/dC)_{\text{DNA}}$, was measured by DRM-1021 (Otsuka Electronics) at 488 nm and determined to

be 0.247 mL g^{-1} (25 $^{\circ}\text{C}$) and 0.226 mL g^{-1} (40 $^{\circ}\text{C}$). The literature values were used for the dn/dC of PNIPAAm, $(dn/dC)_{\text{PNIPAAm}}$.⁸

Turbidimetric DNA Detection

Copolymer **2** was dissolved in 500 mM NaCl/10 mM Tris-HCl (pH 7.4) to be the concentration of 0.1 mg mL^{-1} (2.8 μM in DNA unit concentration), and then the solution was heated to 40 $^{\circ}\text{C}$ and placed for 30 min to give the colloidal particle. To the particle dispersion, the target DNA (**I–III**, **V**) was added to be the concentration of 2.8 μM . The time-dependent absorbance increase of the dispersion was monitored at 500 nm.

RESULTS AND DISCUSSION

Copolymer **2** was obtained by the copolymerization between *N*-isopropylacrylamide monomer and vinyl group-introduced DNA (DNA macromonomer). The M_w of **2** was 4.0×10^5 and the mol fraction of DNA macromonomer unit in **2** was 0.34 mol%, indicating that **2** contained *ca.* 10 DNA molecules. Copolymer **2** was dissolved in 5 mM MgCl_2 / 10 mM Tris-HCl (pH 7.4) (buffer **a**) or 300 mM NaCl / 10 mM Tris-HCl (pH 7.4) (buffer **b**) to be the concentration of 0.1 mg mL^{-1} . To form the core-shell colloidal particle from **2**, the temperature of the solution was raised to 40 $^{\circ}\text{C}$, which was higher than the phase transition temperature of the copolymer.⁹ The hydrodynamic radius (R_h) of the colloidal particles was rather different depending on the solution conditions as follows; 22 nm in buffer **a** and 18 nm in buffer **b**.¹⁰ These R_h values did not change after the incubation of 6 h. The M_w of the colloidal particles were determined by static light scattering measurement to be 9.0×10^6 and 6.6×10^6 in buffer **a** and **b**, respectively. The surface area occupied by one DNA molecule, which was calculated from the R_h and M_w values of the particles, were 26 and 24 nm^2 in buffer **a** and **b**, respectively.¹¹

We studied the stability of these colloidal particles when changing the concentration of salt (MgCl_2 or NaCl). Generally, the colloidal particle in dispersion is known to aggregate at a certain salt concentration, called critical coagulation concentration. Figure 2a shows the absorbance of particle dispersion at 500 nm after the mixing with NaCl or MgCl_2 solution. The increase of absorbance resulting from the aggregation of colloidal particle was observed at 20 mM for MgCl_2 but not for NaCl in the experimental concentration range (300–1400 mM). The stability of the colloidal particle was also examined in the presence of complementary DNA **I** or one point-mismatching DNA **II** (Table I). As

Figure 2. Absorbance of the colloidal particle dispersion as a function of salt concentration a) in the absence and b) in the presence of complementary DNA (I) or one point-mismatching DNA (II) at 40°C. The colloidal particle concentration was 0.1 mg mL⁻¹, containing 2.8 μM of DNA unit. The target DNA concentration was 2.8 μM.

shown in Figure 2b, the absorbance of the particle dispersion in the presence of I increased steeply at 10 mM for MgCl_2 and 400 mM for NaCl . On the other hand, any change was not observed in the presence of II as compared with the DNA absence. These results suggested that the instability of the colloidal particle in the presence of I should be caused by the selective hybridization of the particle surface DNA with I.

To confirm this conjecture, we investigated the temperature effect on the particle aggregate, which was formed in the presence of I. As the temperature increased, the absorbance of the dispersion decreased steeply in the narrow temperature range and then reached almost zero, as shown in Figure 3a. The R_h of colloidal particle at 55°C was 22 nm, which agreed well with the R_h of particle at that temperature without the addition of DNA, indicating that the aggregate dissociated into the individual particle after the abrupt decrease of absorbance. Since the temperature range of this aggregate dissociation process was included in the temperature range of the dissociation of free duplex DNA (38–63°C) (Figure 3b), the aggregate dissociation should be attributed to the dissociation of DNA duplex in the particle surface.

These results suggest that the stability of colloidal particle decreases when the particle surface DNA forms duplex in spite of the anionic charge increase. This may be explained by a so-called salting out effect, since it is known that the binding constant of Mg^{2+} (phosphate anion basis) to DNA or RNA duplex is larger than to single strand because of the higher anionic charge density of duplex.¹² Thus, in the duplex state, the particle would become more neutral by the stronger cation

Figure 3. a) Temperature dependence of absorbance of the particle aggregate formed in the presence of I. The concentrations of the colloidal particle and I were the same with Figure 2. b) Melting curve of free duplex DNA in 500 mM NaCl/10 mM Tris-HCl buffer (pH 7.4). The free duplex DNA concentration was 2.8 μM. Heating rate was 0.5 °C min⁻¹.

binding to the duplex and susceptible to aggregation.

The dissociation of aggregate took place in the very narrow temperature range in comparison with the melting of free duplex DNA (Figure 3). Similar phenomenon was observed for the aggregate from DNA modified-gold nanoparticles which were crosslinked by the complementary DNA.² This property is important for the discriminative detection of DNA, when the method is based on the difference in the melting temperature of duplex DNAs formed from probe DNAs and target DNAs.

We presumed that the discrimination of the target DNA in the chain length would be possible according to the above mentioned aggregation mechanism: the hybridization of particle surface DNA with the longer DNA (III–V) should result in the appearance of the protruding single-stranded region at the end of duplex DNA, so that the colloidal particles would become more stable than when the particle hybridized with the same length DNA I. Therefore, we investigated the colloidal stability to the increasing concentration of salt

Figure 4. Absorbance of the colloidal particle dispersion as a function of a) MgCl_2 and b) NaCl concentration in the presence of complementary DNA (I) or complementary but the different length DNA (III–V) at 40°C . The concentrations of colloidal particle and the target DNA were the same with Figure 2.

(MgCl_2 or NaCl) in the presence of the longer DNA (III–V). Figure 4a shows the stability of the particle to MgCl_2 . The particle was clearly demonstrated more stable in the presence of III–V than in the presence of I as we expected. In the presence of V having the additional T_2 at its 5'-terminus, the particle became more stable than in the presence of III or IV having the additional T_2 or T_5 at its 3'-terminus, respectively. Since the hybridization of V with the particle surface DNA should result in the exposure of the protruding single-stranded region to the exterior solvent, the property of single-stranded region, namely the less affinity to Mg^{2+} , remarkably appeared in this case. The comparison between III and IV indicates that the particle is more stable in the presence of longer DNA (IV). As for NaCl (Figure 4b), the critical concentration was not observed for V in the experimental concentration range, but the order of the effect of each DNA on the colloidal stability was in accordance with the case of MgCl_2 . These results suggest that the particle aggregation according to the proposed mechanism would be applicable to the chain length-selective DNA detection. It was reported that a few base deletion in a certain region of genome is closely related to being a cancer,¹³ and this method can be applicable to the diagnosis for such a case.

Finally, we demonstrated the turbidimetric detection of DNA with sequence and chain length selectivity. After the addition of DNA (I–III, V), the time-dependent absorbance change of the particle dispersion containing 500 mM NaCl was monitored. The absorbance did not change at all by the addition of either one point-mismatching DNA (II) or longer DNA (III, V), while the absorbance increased steeply by the addition of complementary DNA I and the increasing was satu-

rated within 5 min. The absorbance of the aggregate dispersion after 5 min incubation was 0.26 and the dispersion could be seen as turbid by naked eyes.

In conclusion, we proposed here the novel aggregation mechanism of DNA-containing colloidal particle, being based on the stability decrease of colloidal particle accompanied by the duplex formation of the surface DNA with the complementary DNA. Using the particle aggregation assay according to the present mechanism, the sequence- and the chain length-selective DNA detection was attained. Further studies about the generality and limitations of the method on various kinds of DNAs (both probe and target) and the core particles for the immobilization of DNA continue in our group.

Acknowledgment. We thank Dr. Tohru Takarada for the valuable discussion and Mr. Zhonglan Tang for the melting temperature measurement. This work was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science and Technology of Japan, and by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

REFERENCES

1. A recent review on latex agglutination immunoassay: F. J. Gella, J. Serra, and J. Gener, *Pure Appl. Chem.*, **63**, 1131 (1991).
2. a) C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature*, **382**, 607 (1996).
b) R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, and C. A. Mirkin, *Science*, **277**, 1078 (1997).
c) J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, and R. L. Letsinger, *J. Am. Chem. Soc.*, **120**, 1959 (1998).
d) R. A. Reynolds III, C. A. Mirkin, and R. L. Letsinger, *J. Am. Chem. Soc.*, **122**, 3795 (2000).
e) J. J. Storhoff, A. A. Lazarides, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, and G. C. Schatz, *J. Am. Chem. Soc.*, **122**, 4640 (2000).
3. T. Mori and M. Maeda, *Polym. J.*, **33**, 830 (2001).
4. H. G. Schild, *Prog. Polym. Sci.*, **17**, 163, (1992).
5. The first reports about temperature-responsive formation of core-shell colloidal particle from PNIPAAm containing copolymer were
a) X. Qiu and C. Wu, *Macromolecules*, **30**, 7921 (1997).
b) M. D. C. Topp, P. J. Dijkstra, H. Talsma, and J. Feijen, *Macromolecules*, **30**, 8518 (1997).
6. T. Mori, D. Umeno, and M. Maeda, *Biotechnol. Bioeng.*, **72**, 261 (2001).
7. C. R. Cantor, M. M. Warshaw, and H. Shapiro, *Biopolymers*, **9**, 1059 (1970).
8. X. Qiu, C. M. S. Kwan, and C. Wu, *Macromolecules*, **30**, 6090 (1997).
9. Phase transition temperatures of **2** in buffer **a** and **b** were 35.9 and 31.8°C , respectively.

10. The anionic charge of DNA part of **2** is shielded to some extent depending on the kind and the concentration of coexisting cation (Na^+ or Mg^{2+}). This shielding effect would affect the particle radius. This kind of phenomenon was observed in the colloidal particle formed from the copolymer between NIPAAm and diallyldimethylammonium chrolide:
Y. Deng and R. Pelton, *Macromolecules*, **28**, 4617 (1995).
11. The surface area occupied by one DNA molecule was sufficiently larger than that of cross sectional area of 9 base-pair B-form duplex DNA ($4.4\text{--}7.3\text{ nm}^2$), suggesting that the hybridization with complementary DNA should take place in the surface DNA.
12. a) H. Krakauer, *Biopolymers*, **10**, 2459 (1971).
b) C. Sander and P. O. P. Ts'o, *J. Mol. Biol.*, **55**, 1 (1971).
c) G. Strunk and H. Diebler, *J. Chem. Soc. Dalton Trans.*, 1929 (1994).
d) B. I. Kankia, L. A. Marky, *J. Phys. Chem. B*, **103**, 8759 (1999).
13. Y. Akiyama, R. Iwanaga, K. Saitoh, K. Shibata, K. Ushio, E. Ikeda, T. Iwama, T. Nomizu, and Y. Yuasa, *Gastroenterology*, **112**, 33 (1997).