Interaction of Self-assembled Cationic Nanogels with Oligo-DNA and Function as Artificial Nucleic Acid Chaperone

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Cationic self-assembled nanogels with ethylenediamine or dipropylenetriamine groups formed complex nanoparticles with double-stranded oligo-DNA and accelerated the rate of strand exchange reaction as artificial nucleic acid chaperones.

Nanogels have been given considerable attention in pharmaceutical and biomedical applications.[1] We developed physically crosslinked nanogels (self-assembled nanogels) of hydrophobically modified water-soluble polymers.[2] In particular, cholesterol-bearing pullulan (CHP) nanogels form complexes with various proteins[3] and show chaperone-like activity against chemically denatured proteins.[4] Cationic CHP nanogels showed high activity for the intercellular delivery of various proteins.[5] We report here the interaction of cationic nanogels with double-stranded oligo-DNA and evaluate it as a function of artificial nucleic acid chaperones. This is the first report of cationic nanogels that show nucleic acid chaperone-like activity.

Two types of cationic CHP were prepared by the substitution of ethylenediamine ($CHPNH_2$) or dipropylenetriamine-[*N*-(3-aminopropyl)-1,3-propanediamine, (CHPdpt)] with CHP by the activation method previously described[5] using 1,1′-carbonyldiimidazole (Figure 1). The molecular weight (Mw) of pullulan was 1.0×10^5 and the degree of substitution of cholesterol was 1.4 per 100 glucose units in pullulan. Also, the degrees of substitution of cationic groups were 17 ($CHPNH_2$) and 16 (CHPdpt) in 100 glucose units in pullulan. The hydrodynamic radius and zeta-potentials of $CHPNH_2$ and CHPdpt in 10 mM phosphate buffered saline (PBS) containing 100 mM NaCl were 33.9 ± 0.3 nm, $+6.1$ mV ($CHPNH_2$) and 59.4 ± 0.2 nm, $+7.2$ mV (CHPdpt), respectively. Both cationic CHPs self-associated and formed stable nanogels with positively charged surfaces.

To examine the interaction between cationic nanogels and double-stranded oligo-DNA (dsDNA), gel electrophoreses of mixtures of cationic nanogels and dsDNA at various C/P ratios (number of cationic groups/number of phosphate groups) were carried out by using FITC-labeled oligo-DNA (20 mer). The sequences were as follows:

5′-TCA TAA TCA GCC ATA CCA CA-3′-FITC (F-ss) (1)

3′-AGT ATT AGT CGG TAT GGT GT-5′ (n-ss) (2)

For $CHPNH_2$ nanogels, the free dsDNA band disappeared at C/P = 10, indicating that formation of a complex was completed at C/P = 5 to 10. In contrast, CHPdpt nanogels formed a complex with dsDNA at C/P = 2.5 to 5 (Figure 2). CHPdpt nanogels effectively interacted with dsDNA compared with that of the $CHPNH_2$ nanogels due to the divalent cationic character of dipropylenetriamine. At the experimental pH, the secondary amine of dipropylenetriamine is protonated.

Next, the melting temperatures (T_m) of the duplex of oligo-DNA in the presence of nanogels were examined (Figure 3). Dissociation of the double strands was followed by a change in UV absorption at 260 nm as a function of temperature. The T_m of oligo-DNA was 61.3 °C in the absence of nanogels and increased as the concentration of nanogels was increased. Complexation with the nanogels stabilized the double-stranded DNA. The increase in T_m value reached maximum at C/P ratio ≈10 in $CHPNH_2$ system and at C/P ratio ≈5 in CHPdpt system. The C/P ratios were comparable to the values in the formations of electrostatically stoichiometric complexes with DNA (Figure 2). In the presence of an excess amount of nanogels, the values remained constant. The sizes of the cationic nanogels/dsDNA complexes were estimated by dynamic light scattering measurements at various C/P ratios. In the case of the $CHPNH_2$ nanogels/dsDNA complex, the sizes were almost the same as the nanogels at all C/P ratios (30–35 nm in diameter). This means that the nanogels stably incorporated oligo-DNA inside the gel network. In contrast, in the CHPdpt/oligo-DNA system at low C/P ratios of 0.25 and 1.0, the particle sizes increased to

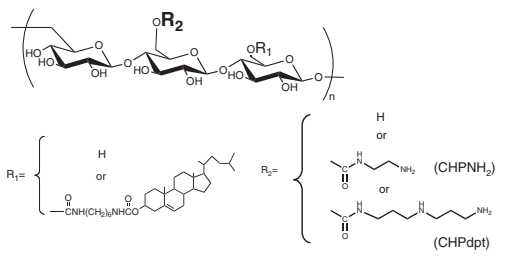

Figure 1. Chemical structure of $CHPNH_2$ and CHPdpt.

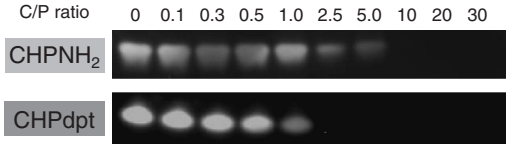

Figure 2. Gel electrophoresis of cationic nanogels/oligo-DNA complex. Cationic nanogels and double-stranded DNA (dsDNA) were mixed at C/P ratios of 0, 0.1, 0.3, 0.5, 1.0, 2.5, 5.0, 10, 20, and 30 (final concentration of DNA: 1.0 μM). After incubation for 15 min at room temperature, samples were applied onto 15% polyacrylamide gel. Gel electrophoresis was performed in Tris-borate-EDTA (TBE) buffer at 100 V.

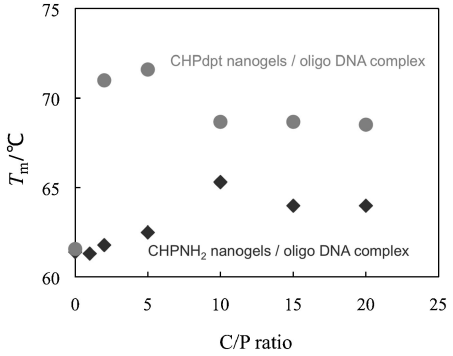

Figure 3. UV-T_m values of dsDNA in the presence or absence of cationic nanogels. The differential absorbance ($\Delta A = A_{260} - A_{340}$) was plotted and the melting temperature (T_m) of DNA was defined as the point where 50% of the single strands dissociate from its duplex.

≈360 (C/P = 0.25) and ≈650 nm (C/P = 1.0), respectively. Then, with a further increase in the C/P ratio, the complex sizes were constant, at about 60 nm, which was almost the same as the nanogels. The molar ratio of nanogel and oligo-DNA is about 1:1 when C/P is 10. Since excess oligo-DNA exists below C/P = 1.0, the CHPdpt nanogels with longer and divalent cationic side chain were effectively cross-linked by DNA, and resulted in the formation of a large aggregate.

Finally, we investigated DNA strand exchange reaction in the presence of nanogels to determine whether the nanogels acted as nucleic acid chaperones. The strand exchange reaction was performed on cationic nanogels/FITC-labeled dsDNA (F-ds) by the addition of 5 fold complementary nonfluorescence-labeled single strand 5′-TCA TAA TCA GCC ATA CCA CA-3′ (n-ss). The reaction rate was calculated by the following equation:

$$\text{Degree of exchange (\%)} = \frac{\text{Exchanged F-ss band intensity at } t = x}{\text{Band intensity of F-ss}} \times 100 \quad (3)$$

Figure 4 shows the time course of the strand exchange reaction of F-ds in the presence of cationic nanogels when the C/P ratio was applied to 10. In the absence of cationic nanogels (DNA only), the strand exchange was very slow (exchange rate of 6.0% after 30 min). However, in the presence of cationic nanogels, the reaction rates markedly increased. The exchange ratio reached about 80% after 30 min. Especially in the case of the CHPdpt nanogels, the exchange reaction was equilibrated in about 5 min with an increase in the C/P ratio; the reaction rates also increased. The degree of exchange after 10 min was 60.5% (C/P = 3.0) and 48.1% (C/P = 1.0), respectively. In the case of the CHPNH$_2$ nanogels, the degree of exchange was 38.2% (C/P = 3.0) and 9.3% (C/P = 1.0) after 10 min. The CHPdpt nanogels showed a higher activity rate than did that of the CHPNH$_2$ nanogels.

Maruyama *et al.* reported that dextran-grafted poly(L-lysine) (DL) showed high chaperone-like activity in a DNA strand exchange reaction.[6] The acceleration of the reaction was interpreted as the stabilization effect of the transition state consisting of the parent duplex and the single strand. The high anionic environment of the transition state were stabilized by the polyvalent cations of DL without aggregation. The grafted dextran chains prevent aggregation of the polyion complex. The chaperone-like activity of the cationic nanogels in this study was comparable to that of DL. DL exists in the positive charges on the main backbone of a polymer. Cationic nanogels, which have cationic groups in the side chains of the polymer, are a new type of artificial nucleic acid chaperone. Positive charges surrounding the nanogels probably stabilize the transition state and accelerate the exchange reaction. The nanogels have the benefit of additional functions such as the high ability of protein to bind and intercellular uptake.

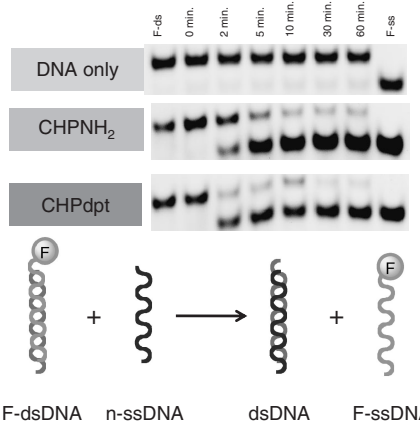

Figure 4. Strand exchange reaction between F-ds DNA and nF-ss DNA in the absence or presence of cationic nanogels. Cationic nanogels and FITC-labeled double-stranded DNA (F-ds) were mixed at C/P = 10 (final concentration of F-ds: 0.33 μM) and incubated with its complementary single strand, nF-ss at 37.0 °C for reaction time (t) = 0, 2, 5, 10, 30, and 60 min. After the reaction was stopped by adding 2.0 wt % solution of anionic poly(vinylsulfonic acid), the reaction mixture was applied to 13% polyacrylamide gel.

In summary, complex nanoparticles were formed between self-assembled cationic nanogels and double-stranded oligo-DNA. The nanogels with dipropylenetriamine groups effectively formed complex, stabilized double-stranded oligo-DNA and showed effective nucleic acid chaperone-like activity for DNA strand exchange reactions. These results open the door to new applications of self-assembled nanogels.

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