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Cationic Comb-type Copolymers Do Not Cause Collapse but Shrinkage of DNA Molecules

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A flow-stretching assay was employed to elucidate polycation/DNA interactions at the single-molecule level. Bacteriophage λ-DNA (48.5 kbp) was attached at one end to a PEG-modified glass surface and stretched by buffer flow. The stretched DNA had an approximate length of 12 μm. Upon injection of polylysine homopolymer, the DNA folded into a globule structure within a few seconds. Injection of a polylysine graft copolymer having hydrophilic dextran side chains also induced collapse of the stretched DNA. In comparison to these polymers, a copolymer with higher graft content did not cause DNA collapse but rather caused 25% shrinkage of the extended DNA. These results were compared with those observed with unstretched DNA.

Long DNA molecules can undergo discrete coil to globule transitions. Polycations like polylysine are effective inducers of the transition.[1] Upon complex formation with polycations, the DNA molecules collapse into compact globule conformations. The complexes subsequently aggregate and precipitate from the solution. We have been interested in soluble complex formation observed with cationic graft copolymers containing poly(L-lysine) and dextran, PLL-*g*-Dex (Figure 1); PLL-*g*-Dex has a polycation backbone and hydrophilic side chains.[2] The copolymer with a high degree of grafting forms totally soluble DNA complexes without inducing collapse.[3] We have shown that PLL-*g*-Dex increases stability of DNA hybrids,[2] accelerates DNA hybridization,[4] and activates DNA strand-exchange reactions.[5] However, interaction of the copolymer with DNA is not thoroughly understood and the influence of the copolymer grafting degree on conformation of DNA has not been assessed. Here, we employed a DNA flow-stretching assay to further evaluate DNA/polycation interactions. The DNA flow-stretching assay has high spatiotemporal resolution and has been used to study DNA/protein interactions.[6] The results of the flow-stretching assay were compared to that from assays with DNA and polycations in solution.

PLL-*g*-Dex copolymers (M_n of PLL: 4.0×10^4, M_n of Dex: 8.4×10^3, Dex grafts: 38, 61, and 91 wt %) were prepared as previously reported.[2] The nomenclature used for the copolymers reflects the degree of grafting: Polymers with grafts of 38, 61, and 91 wt % are referred to as 40K38D, 40K61D, and 40K91D, respectively. First, we observed the effect of these copolymers on conformation of bacteriophage λ-DNA (48.5 kbp) in aqueous buffer solution using a method described by Yoshikawa et al.[7] We observed images of cationic polymers in the same mixtures by employing a second fluorescent label. As shown in Figure 2,[11] DNA was visualized with SYBR Gold staining (488 nm excitation) and polymers were imaged (561 excitation) by TRITC labeling. The λ-DNA alone shows amorphous fluorescence images with 3–5 μm long axes on average. The DNA images wobbled due to Brownian motion, indicating an extended coil conformation of the DNA. When polylysine homopolymer (40K) was added to the DNA solution, bright DNA clots were observed, indicating that the 40K homopolymer caused condensation and subsequent aggregation of DNA molecules. Similar images were also observed when the complex was observed at 561 nm excitation, confirming involvement of 40K in the aggregates. When the graft copolymer 40K38D was added to the DNA, DNA images considerably smaller than those of DNA alone were observed at both 488 and 561 nm excitations. This indicated that 40K38D induced DNA condensation into globule particles but did not cause aggregation of the condensed particles. Dextran grafts of the copolymer likely reduced the secondary aggregation of the condensed DNA particles. 40K61D with intermediate graft content also condensed DNA as judged from the images at 488 nm. DNA images in the presence of 40K61D were smaller than those of DNA alone, and 40K61D/DNA images aquired at 561 nm excitation were smaller than images acruired at 488 nm. We propose that 40K61D induced formation of partial globule structures in the DNA. The partial globule structure was previously observed with poly(oxyethylene) derivatives having amino groups.[8] In agreement with a previous report,[3] the copolymer having the

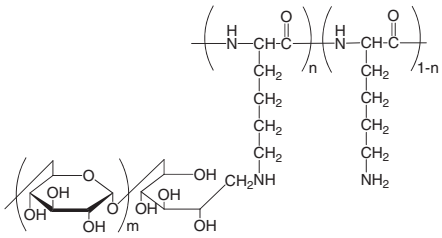

Figure 1. Structural formula of PLL-*g*-Dex.

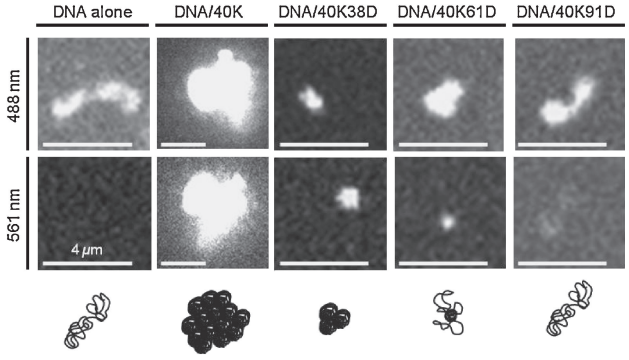

Figure 2. Confocal laser microscopic images of mixtures of λ-phage DNA and cationic polymers (50 nM). DNA was visualized with SYBR Gold staining and 488 nm excitation. TRITC-labeled polymers were observed with 561 nm excitation. Different view fields were imaged at 488 and 561 nm.

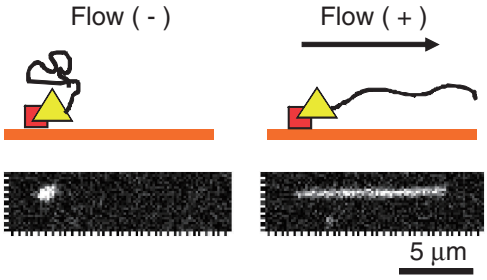

Figure 3. Fluorescent image of λ-phage DNA extended by buffer flow.

highest graft content, 40K91D, gave DNA images similar to DNA alone. The conclusion that DNA condensation was not induced by 40K91D was supported by the images at 561 nm: Complex images similar in size and mobility were observed with 40K91D visualization and DNA imaging.

We then examined effects of these polycations on DNA molecules stretched by flow. Biotinylated oligonucleotides were annealed to the 12-nucleotide overhang at one end of λ-DNA. The λ-DNA and the oligonucleotide were ligated using DNA ligase.[9] Cover glass surfaces were aminated with 3-aminopropyltriethoxysilane and treated with a 50:1 mixture of mPEG-NHS and biotin-PEG-NHS.[10] A flow cell was prepared using two cover glasses and parafilm as a spacer. The cell was injected with 1 µM neutravidin solution, followed by the biotinylated λ-DNA solution. To visualize DNA, SYBR Gold was dissolved in buffer (10 mM sodium phosphate, 150 mM NaCl, pH 7.2), and the buffer with or without cationic polymer was injected into the cell. Emission was observed with a fluorescence microscope equipped with a confocal scan unit (CSU-X1, Yokogawa Electric Corp., Tokyo, Japan).

Fluorescence images of the λ-phage DNA immobilized at one end are shown in Figure 3. In the absence of buffer flow, the DNA molecules adopted a coiled form (Figure 3, left). In accordance with a previous study,[9] the DNA molecules were stretched to the length of approximately 12 µm by buffer flow at 3.7 mm s^{-1} (Figure 3, right). Figure 4 shows the change in DNA images when cationic polymers were added to the buffer. With buffer containing 60 nM 40K PLL was injected, the extended DNA collapsed into a globule conformation in less than 4 s (two frames). Similar rapid changes, but to a larger particle, were observed when buffer contained 40K61D. No collapse of the DNA molecule was observed when 40K91D was injected. Of interest, 40K91D caused 25% shrinkage of the DNA length. This shrinkage was also rapid and was completed within 4 s. Since fluorescence intensity along the entire length of the DNA image decreased upon the polymer addition, we hypothesize that 40K91D binds homogeneously to the DNA molecule and competes with SYBR Gold dye. No collapse of the stretched DNA molecules was observed even when 40K91D concentration was increased from 60 to 1500 nM (Figure 4). Condensation of DNA/polycation complex is thought to be driven by hydrophobic interactions of the DNA/polycation inter-polyelectrolyte complex. We speculate that hydrophilic graft chains of the 40K91D copolymer surround the spine of the PLL/DNA complex and suppress the coil to globule transition.

In summary, comparative observation of free and flow-stretched DNA molecules at the single-molecule level provided

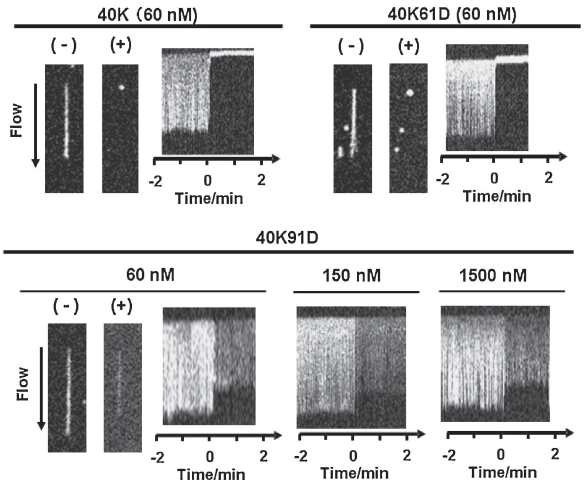

Figure 4. Conformation changes of flow-stretched DNA by PLL or PLL-*g*-Dex injections. Fluorescence images and kymographs acquired at 2 s/frame before and after polycation injection.

insight into the polycation-induced conformation changes of DNA molecules. Spatiotemporal changes in DNA conformation were characterized by employing the flow stretching assay. It was clearly demonstrated that the cationic comb-type copolymers with the highest graft content evaluated did not induce DNA collapse but shrinkage of stretched DNA molecules.

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References and Notes

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1 S. C. De Smedt, J. Demeester, W. E. Hennink, *Pharm. Res.* **2000**, *17*, 113.
2 A. Maruyama, M. Katoh, T. Ishihara, T. Akaike, *Bioconjugate Chem.* **1997**, *8*, 3; A. Maruyama, H. Watanabe, A. Ferdous, M. Katoh, T. Ishihara, T. Akaike, *Bioconjugate Chem.* **1998**, *9*, 292.
3 Y. Sato, Y. Kobayashi, T. Kamiya, H. Watanabe, T. Akaike, K. Yoshikawa, A. Maruyama, *Biomaterials* **2005**, *26*, 703.
4 H. Torigoe, A. Ferdous, H. Watanabe, T. Akaike, A. Maruyama, *J. Biol. Chem.* **1999**, *274*, 6161; L. Wu, N. Shimada, A. Kano, A. Maruyama, *Soft Matter* **2008**, *4*, 744.
5 W. J. Kim, T. Ishihara, T. Akaike, A. Maruyama, *Chem.—Eur. J.* **2001**, *7*, 176; W. J. Kim, T. Akaike, A. Maruyama, *J. Am. Chem. Soc.* **2002**, *124*, 12676; S. W. Choi, A. Kano, A. Maruyama, *Nucleic Acids Res.* **2008**, *36*, 342.
6 R. Roy, S. Hohng, T. Ha, *Nat. Methods* **2008**, *5*, 507; J. van Mameren, E. J. G. Peterman, G. J. L. Wuite, *Nucleic Acids Res.* **2008**, *36*, 4381.
7 K. Minagawa, Y. Matsuzawa, K. Yoshikawa, A. R. Khokhlov, M. Doi, *Biopolymers* **1994**, *34*, 555.
8 K. Yoshikawa, Y. Yoshikawa, Y. Koyama, T. Kanbe, *J. Am. Chem. Soc.* **1997**, *119*, 6473.
9 A. Granéli, C. Yeykal, T. K. Prasad, E. C. Greene, *Langmuir* **2006**, *22*, 292.
10 T. Ha, I. Rasnik, W. Cheng, H. P. Babcock, G. H. Gauss, T. M. Lohman, S. Chu, *Nature* **2002**, *419*, 638.
11 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

