An Effect of Protruding Ends of λ-DNA on its Adsorption onto Hydrophobic Solid Surfaces during Molecular Combing

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We investigated an effect of difference in extremities of DNA on its adsorption onto chemically modified cover glass slips. Two kinds of DNA were used. These are λ-DNA and λ-DNA treated with T4 DNA polymerase, which have protruding ends and blunt ends, respectively. The glass substrate surfaces were silanized with 3-aminopropyltrimethoxysilane (APS) or octadecyltrimethoxysilane (OTS). Both DNA molecules were adsorbed on the APS-treated surface due to electrostatic interaction between the positively charged ammonium groups and the negatively charged phosphate groups along the DNA chains, while only untreated λ-DNA was adsorbed on the OTS-treated surface. The latter result is attributable to hydrophobic interaction between the protruding ends and the OTS-treated surface.

A molecular combing method is a way to immobilize and linearly stretch DNA on solid substrate surfaces. Since the first report by Bensimon et al. in 1994,[1] this method has been widely used for optical mapping[2] and nanowire fabrication.[3,4] The detailed procedures have a few differences depending on each research group,[3] but these can be classified roughly into two types: i) a droplet of DNA aqueous solution is deposited and dragged on the substrate surface and ii) the substrate is dipped into the DNA solution and then lifted up. In short, movement of a water meniscus is needed for the molecular combing. The detailed stretching mechanism of the molecular combing has, however, not been clarified yet.[5,6]

It can be supposed that the extremity (or the extremities) of DNA needs to be attached onto the surface at first in the solution in order for DNA to be stretched subsequently. This hypothesis was corroborated by the fact that some DNA were immobilized in U-shapes.[5] Why does DNA attach to the surface with its extremity in preference to its mid segment? We have investigated the molecular combing using glass substrates covered with a mixed monolayer containing amino and methyl terminal groups.[7] It was found that the amount of the attached DNA molecules was increased with an increase in the surface amino groups. It was also found that the attached DNA molecules were not stretched linearly but immobilized in wound forms, as the amount of the amino groups was increased. This can be due to multiple pinning of a DNA molecule in a coil state (the entropy effect) in the solution through a lot of ionic bonds between the positively charged ammonium groups and the negatively charged phosphate groups of DNA.

DNA was also found to adsorb on the surface containing only methyl groups although the amount was much less than that observed on the surface containing only amino groups.[7] On the surface only with methyl groups, DNA was stretched linearly. Therefore, we thought that DNA was attached by its extremity (or extremities) rather than by its mid segment on the electrically neutral surfaces, as proposed previously.[8] Because the neutral surfaces with methyl terminals are hydrophobic, the interaction between the surface and the extremity can be hydrophobic interaction. The hydrophobic site of DNA can be the internal core (nucleobases) of the double helix.[8] Namely, the interaction of the hydrophobic surface with single-stranded DNA is considered to be stronger than that with double-stranded DNA.

In order to expose the internal core of the double helix, it needs to be "unzipped" into single strands so that the internal core can interact with the hydrophobic surface. The tendency for DNA to unzip near its extremity is stronger than that in its mid region. Allemand et al. reported that unzipping at the extremities was enhanced at lower pH (ca. 5.5).[8]

There is another case where the internal core is exposed regardless of unzipping, i.e., protruding ends. The protruding end is a single strand extending beyond the complementary region in DNA. Therefore, if the adsorbed amount of DNA with such protruding ends is larger than that without the protruding ends, this will support the hypothesis that the adsorption of DNA on the hydrophobic surface is due to the internal core of DNA. The extremity terminated with double strand is called blunt ends. λ-DNA (48.5 kbp) has a pair of 12-base protruding ends being complementary each other.[9] T4 DNA polymerase is one of the DNA polymerase, which can synthesize double-stranded ends (i.e., the blunt ends) from the protruding ends of λ-DNA.[9]

In this study, we investigated the effect of the protruding ends on the adsorption using λ-DNA and λ-DNA treated with T4 DNA polymerase in order to clarify the adsorption mechanism of the DNA molecules onto the hydrophobic surface. To our knowledge, there have been no previous reports of the comparison between the protruding ends and the blunt ends in connection with DNA adsorption onto hydrophobic surfaces by the molecular combing.

The experimental procedure was as follows. λ-DNA treated with T4 DNA polymerase was called below T4t-DNA. λ-DNA was purchased from Takara Bio and used without further purification. T4t-DNA was obtained from λ-DNA by treating with T4 DNA polymerase followed by purification.[10] Glass cover slips ($18 \times 24\,mm^2$, Matsunami Glass) silanized with 3-aminopropyltrimethoxysilane and octadecyltrimethoxysilane (Tokyo Chemical Industry) were used as substrates, and their surfaces were called below APS surface and OTS surface, respectively. The detailed silanization procedure was described previously.[7] λ-DNA and T4t-DNA were stained with YOYO-1 (Molecular Probes) at a ratio of 1 dye molecule to 20 base pairs. The stained DNA was diluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) (Dojindo). The final concentrations are listed in Table 1. The silanized substrate was vertically dipped into the solution at 3.4 mm/s using a mechanical apparatus.

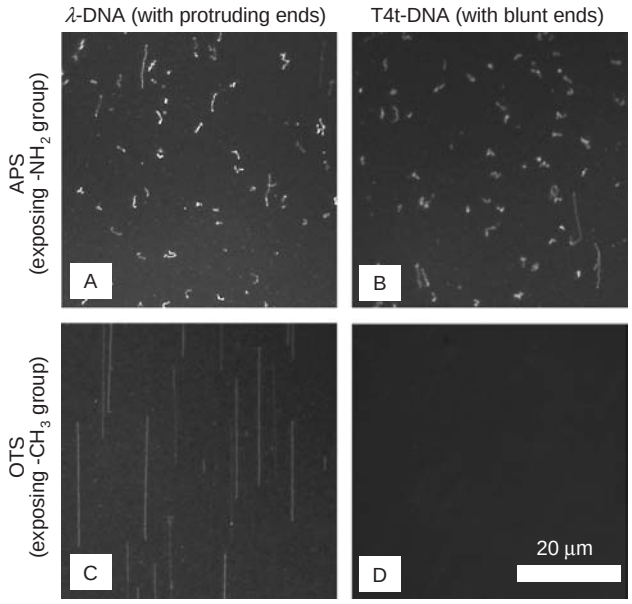

Figure 1. Typical fluorescence images of (A), (C) λ-DNA and (B), (D) T4t-DNA immobilized on glass surfaces silanized with (A), (B) APS and (C), (D) OTS. Concentrations of the DNA solution used for the immobilization were (A), (B) $0.18\,\mu g/cm^3$ and (C), (D) $18\,\mu g/cm^3$, respectively.

The substrate was immediately lifted up from the solution at a constant speed of 0.3 mm/s. Immobilized DNA molecules were observed with a fluorescence microscope (Diaphoto 300, Nikon), and the images were taken with a CCD camera (VB-7000, Keyence).

Figure 1 shows typical fluorescence images of λ-DNA and T4t-DNA molecules immobilized on the APS and the OTS surface by the molecular combing. On the APS surface, both λ-DNA and T4t-DNA were adsorbed but not stretched,[11] as shown in Figures 1A and 1B. Namely, the adsorption on the APS surfaces was not affected by the difference between the protruding ends and the blunt ends. This was due to the electrostatic interaction as was expected from the previous studies.[7] Because the negatively charged phosphate groups exist along a DNA chain, the whole chain of DNA is attracted to the positively charged surface. Therefore, DNA was adsorbed regardless of the type of the extremities. Since the shape of a DNA molecule in solution is considered to be a coil due to the entropic effect, the DNA images in Figures 1A and 1B suggest that DNA molecules adsorb as they are in the solution in the initial stage of the molecular combing and thus they are not stretched. On the OTS surface, only λ-DNA with the protruding ends was adsorbed, as shown in Figures 1C and 1D. This result supports the assumption that λ-DNA can be pinned onto the hydrophobic surface only at the protruding ends. Thus, the protruding ends enhanced DNA adsorption through the hydrophobic interaction.

Table 1 shows average densities and their standard deviations of λ-DNA and T4t-DNA adsorbed on the two types of silanized surfaces from solutions with various DNA concentrations. We measured the average densities and the standard deviations from 10–15 fluorescence images. The fluorescence images used for the density measurements were taken around the horizontal center line of the dipped area, because the contact time of the surface with the solution increased from draft to bottom end (ca. 20 mm). The data are listed not only for the cases shown in Figure 1 but also for other adsorption conditions (λ-DNA of $81\,\mu g/cm^3$ and T4t-DNA of $90\,\mu g/cm^3$) on the OTS surface. On the OTS surface, it was confirmed that λ-DNA was adsorbed, while T4t-DNA was not adsorbed as pointed out above. On the APS surface, both DNA were adsorbed a lot. These densities (174 and $133/(10^4\,\mu m^2)$) were 5–6 times larger than that of λ-DNA on the OTS ($27/(10^4\,\mu m^2)$) even though the concentration of the solutions was 100 times smaller. More quantitative investigation about the relationship between the concentration and the adsorbed amount will be reported later.

Table 1. Density of the adsorbed DNA

DNA	Surface	Concentration of solution ($\mu g/cm^3$)	Density (molecules/($10^4\,\mu m^2$))
λ-DNA	APS	0.18	174 ± 14
	OTS	18	27 ± 4
		81	48 ± 5
T4t-DNA	APS	0.18	133 ± 22
	OTS	18	0
		90	0

In conclusion, we directly confirmed that protruding ends strongly enhance adsorption of DNA on an electrically neutral hydrophobic surface. This corroborated that DNA can be adsorbed on an electrically neutral hydrophobic surface only at the protruding ends. The result described here is very important to understand the initial stage of the molecular combing process. Furthermore, the present adsorption study of a hydrophobic surface and λ-DNA will be a useful model for polymer chains adsorbed on a surface only with their ends.[12]

References and Notes

1 A. Bensimon, A. Simon, A. Chiffaudel, V. Croquette, F. Heslot, D. Bensimon, *Science* **1994**, *265*, 2096.
2 X. Michalet, R. Ekong, F. Fougerousse, S. Rousseaux, C. Schurra, N. Hornigold, M. van Slegtenhorst, J. Wolfe, S. Povey, J. S. Beckmann, A. Bensimon, *Science* **1997**, *277*, 1518.
3 Q. Gu, C. Cheng, R. Gonela, S. Suryanarayanan, S. Anabathula, K. Dai, D. T. Haynie, *Nanotechnology* **2006**, *17*, R14, and references therein.
4 H. Kudo, M. Fujihira, *IEEE Trans. Nanotechnol.* **2006**, *5*, 90, and references therein.
5 D. Bensimon, A. J. Simon, V. Croquette, A. Bensimon, *Phys. Rev. Lett.* **1995**, *74*, 4754.
6 H. Kudo, K. Suga, M. Fujihira, submitted.
7 S. Yoda, S. P. Han, H. Kudo, K. J. Kwak, M. Fujihira, *Jpn. J. Appl. Phys.* **2004**, *43*, 6297.
8 J. F. Allemand, D. Bensimon, L. Jullien, A. Bensimon, V. Croquette, *Biophys. J.* **1997**, *73*, 2064.
9 *Genomes*, 2nd ed., ed. by T. A. Brown, BIOS Scientific Publishers, Oxford, **2002**.
10 For blunting, "Blunting-Convenience Kit" (Nippon Gene) was used. For purification, "QIAEX II Gel Extraction Kit" (Qiagen) was used.
11 In Reference 8, DNA was linearly stretched on a surface coated with amino-terminated silane. The disagreement with the present results might arise from the difference in the combing methods or the difference in the pH values.
12 a) A. M. Skvortsov, I. V. Pavlushkov, A. A. Gorbunov, E. B. Zhulina, *J. Chem. Phys.* **1996**, *105*, 2119. b) D. C. Driscoll, H. S. Gulati, R. J. Spontak, C. K. Hall, *Polymer* **1999**, *40*, 5207. c) J. Huang, W. Jiang, S. Han, *Macromol. Theory Simul.* **2001**, *10*, 339.

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