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Notes

Effect of DNA Methylation on the Velocity of DNA Translocation through a Nanochannel

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Here, we report the effect of DNA methylation on the velocity of DNA translocation through a nanochannel, as determined by measuring differences in translocation velocities between methylated and non-methylated DNA molecules. We found that the velocity of translocation of methylated DNA was faster than that of non-methylated DNA, which we attributed to variation in the coefficients of diffusion and friction with the nanochannel wall, due to the increased molecular weight and stiffness, respectively, of methylated DNA.

Keywords DNA methylation, single DNA molecule, DNA translocation velocity, nanochannel

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Introduction

The effect of DNA methylation on cellular processes has been investigated for decades to determine its biological function.[1] It is well known that DNA methylation is an important regulator of epigenetic alterations, and increased methylation has been detected in patients with cancers.[2,3] Therefore, a better understanding of DNA methylation is desirable for improving the diagnosis and prognosis of cancer and prediction of responses to many kinds of cancer therapies.[4]

Although understanding how DNA methylation affects DNA flexibility is important for understanding the role of DNA methylation in regulating chromosome packaging and gene expression at the molecular scale, the effect of DNA methylation on the physical properties of DNA remains poorly understood.[5] An improved understanding of how DNA methylation affects the physical properties of DNA could improve our understanding of methylation characteristics. Recently, nanofluidic devices have been developed to investigate the physical properties of DNA in a confined environment,[6] facilitating the study of how DNA methylation affects the physical properties of DNA.

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In this study, we measured differences in translocation velocity between single-methylated and non-methylated DNA through a nanochannel. A schematic of the nanochannel device is shown in Fig. 1. Two microchannels were connected by a single nanochannel that was 250 μm long, 300 nm deep, and 300 nm wide.[7] To measure the DNA translocation velocities, methylated and non-methylated DNA molecules[7] were introduced into the nanochannel by application of an electric voltage.[7] The concept graph (Fig. 2) shows DNA translocation inside the nanochannel under the applied electric voltage. DNA translocation time and distance inside the nanochannel were obtained by analyzing their fluorescence video with software. Finally, DNA translocation velocity inside the nanochannel was calculated using translocation time and distance.

Experimental

Nanochannel fabrication

A 10-nm Cr film was deposited on the quartz glass substrate using a sputter coating apparatus (Model SVC-700LRF, Sanyu Electron Co., Tokyo, Japan) to pattern the two microchannels. Then a positive photoresist (TSMR-V50 EL, Tokyo Ohka Kogyo Co., Ltd., Tokyo, Japan) was spin-coated on the glass slide. Then, the exposed microchannels were removed with AZ 300

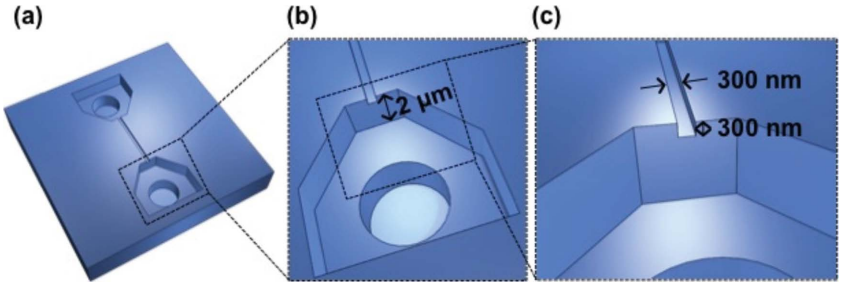

Fig. 1 Schematic of the top view of the device layout: two microchannels are connected by a single nanochannel.

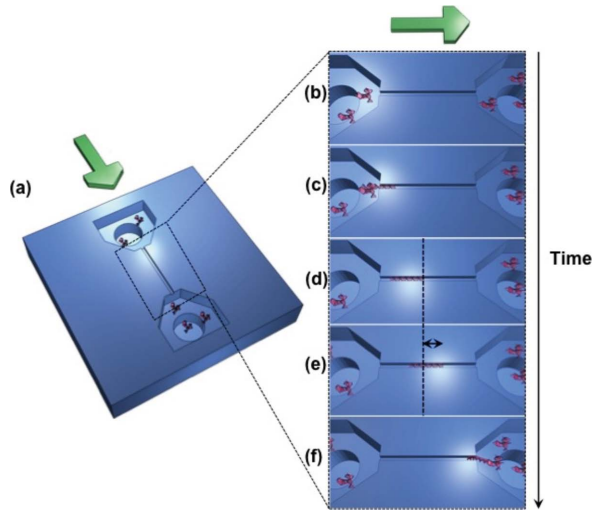

Fig. 2 Schematic illustration of DNA translocation progress in a nanochannel. The green arrow shows the direction of DNA electrophoresis; the black arrow shows translocation distance of DNA inside the nanochannel.

MIF developer (AZ Electronic Materials Plt., Tokyo, Japan), and the Cr film was removed from the two microchannels using a Cr etchant solution. Two microchannels were etched to a depth of 2 μm with reactive ion etching (RIE; RIE-10NR, Samco Co., Kyoto, Japan). The remaining Cr film was removed using the Cr etchant solution. A new Cr film was deposited on the quartz glass substrate to pattern the nanochannel. A positive electron beam (EB) resist (ZEP520A-7, Zeon Corp., Tokyo, Japan) was spin-coated on the Cr film. The nanochannel was patterned by EB lithography (EBL; SPG-724, Sanyu Electron Co.), and removed with EB developer solution (ZED-N50, Zeon Corp.). The new Cr film was removed from the nanochannel with Cr etchant solution. A 300 nm × 300 nm × 250 μm (depth × width × length) nanochannel was etched using RIE. The remaining Cr layer was removed using Cr etchant solution. Finally, a cover glass (Crystal Base Co., Osaka, Japan) was used to bond the nanochannel with a chemical bonding method. The nanochannel fabrication process has previously been described in detail.[7]

DNA sample preparation

T4 DNA molecules (166 kbp, T4GT7 DNA, Nippon Gene Co., Ltd., Tokyo, Japan) and λ DNA molecules (48.5 kbp, Nippon Gene Co., Ltd.) were used to measure the DNA translocation velocity through the nanochannel by applying an electric field. For fluorescence observation, the fluorescent dye YOYO-1 (Invitrogen, Carlsbad, USA) was used to stain methylated and non-methylated T4 DNA molecules at a dye:base ratio of 1:5. Stained T4 DNA molecule samples were diluted to 5 ng mL^{-1} for observation of single DNA molecules under a microscope. To prepare methylated T4 DNA molecules, T4 DNA molecules (166 kbp, T4GT7 DNA, Nippon Gene Co., Ltd.) were mixed with CpG methyltransferase (M.SssI) (New England Biolabs Inc., Tokyo, Japan), and the mixed samples were incubated at 37°C for 1 h, and then heated at 65°C for 20 min to inactivate the methyltransferase. To verify that the T4 DNA samples had been methylated, methylated T4 DNA molecules were incubated with the restriction enzyme SalI (New England Biolabs Inc.), which digests the methylated region of T4 DNA molecules. Finally, the methylated T4 DNA molecules were stained with YOYO-1 overnight at 4°C before use.[7] λ DNA molecules were stained and methylated by using the same method as for T4 DNA molecules. To verify that the λ DNA samples had been methylated, methylated λ DNA molecules were incubated with the restriction enzyme AvaI (New England Biolabs Inc.), which digests the methylated region of λ DNA molecules. After gel electrophoresis, methylated λ DNA molecules had one band, while the non-methylated λ DNA molecules had several bands (Fig. S1, Supporting Information).

Measurement and analysis of DNA translocation velocity

Under an applied electrical potential difference of 3 V between two microchannels (Model 236, Keithley, Cleveland, USA), negatively charged DNA molecules were forced into the nanochannel. To prevent entropic interference at the nanochannel entrance, DNA translocation velocities were measured after the DNA molecules had fully entered the nanochannel. Once inside the nanochannel, the DNA molecules were observed using an inverted fluorescence microscope (ECLIPSE TE300, Nikon, Tokyo, Japan) equipped with a CCD camera (C7190-43, Hamamatsu Photonics K. K., Hamamatsu, Japan) through a 100×/1.40 NA objective lens. The fluorescently stained DNA molecules were observed under 488-nm laser irradiation (FLS-448-20, Sigma Koki Co., Ltd., Tokyo, Japan). The entire DNA translocation process was recorded with a DV tape (Sony DV 180 ME Digital Video Cassette, Sony Corp., Tokyo, Japan). DNA translocation time and distance inside the nanochannel were analyzed using image-processing software (Cosmos 32, Library, Tokyo, Japan). Finally, DNA translocation velocity inside the nanochannel was calculated using translocation time and distance. The translocation velocity was measured for 200 molecules, and a histogram was constructed to determine the distribution of translocation velocities, and to determine whether methylated and non-methylated DNA molecules differed significantly in translocation velocity.

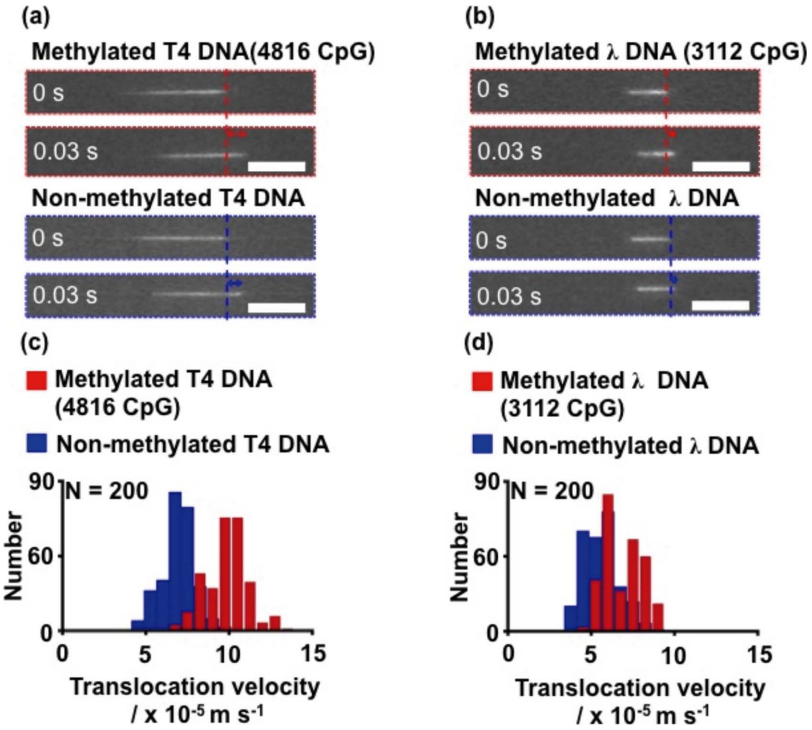

Fig. 3 Measurement of the velocity of translocation of methylated and non-methylated DNA inside the nanochannel. Arrows show the translocation distance of DNA in 0.03 s. (a) Fluorescence images of translocation of methylated and non-methylated T4 DNA inside the nanochannel. Scale bars are 10 μm. (b) λ DNA. Scale bars are 10 μm. (c) Histogram of translocation velocities of methylated and non-methylated T4 DNA molecules (n = 200). (d) λ DNA.

Results and Discussion

Methylated and non-methylated DNA molecules differed in translocation velocity through the nanochannel, as shown in Fig. 3, indicating that methylation affects translocation velocity. Fluorescence images of methylated and non-methylated T4 and λ DNA translocation are shown in Figs. 3a and 3b. After analyzing the translocation velocities of 200 molecules, we found that methylated DNA molecules were translocated inside nanochannel faster than non-methylated DNA molecules (Figs. 3c and 3d). In the nanochannel, electrophoretic mobility of the methylated and non-methylated T4 DNA was calculated as 8.54×10^{-9} m^2 V^{-1} s^{-1} and 6.11×10^{-9} m^2 V^{-1} s^{-1}, respectively. For λ DNA, electrophoretic mobility of the methylated and non-methylated λ DNA was calculated as 5.46×10^{-9} m^2 V^{-1} s^{-1} and 4.42×10^{-9} m^2 V^{-1} s^{-1}, respectively. The difference of electrophoretic mobility inside the nanochannel supports complete changes of translocation velocity of the methylated and non-methylated DNA molecules.

In general, DNA mobility is independent of molecular size above 400 bp in free solution.[8] In gel electrophoresis for DNA separation, it is difficult to analyze several kbp-long DNA molecules since the ends of longer DNA molecules become entangled in gel.[9] Although the electrophoretic mobilities in free solution and nanochannel cannot be directly compared because confinement in the nanochannel limits DNA behavior as a flexible polymer in the nanochannel, the difference in translocation velocities observed between methylated and non-methylated DNA can be attributed to two reasons. One possible reason is that diffusion of methylated and non-methylated DNA through the nanochannel differs due to variation in molecular weight. The molecular weight of DNA can be calculated as $M = 660N$,[10] where N is the number of base pairs in the DNA molecule. The molecular weight of methylated DNA is greater than that of non-methylated DNA, due to methyl group insertion. We estimated the molecular weight of the methylated and non-methylated T4 DNA molecules to be 1.094×10^8 and 1.093×10^8, respectively. For λ DNA molecules, the molecular weight of the methylated and non-methylated DNA molecules were estimated to be 3.201×10^7 and 3.205×10^7, respectively. The diffusion coefficient of DNA in free solution can be calculated using $D \approx 1/M^{0.5}$,[11] where D is the diffusion coefficient and M is the molecular weight of the DNA molecules. Based on this equation, we hypothesize that methylated T4 and λ DNA diffuses about 1.001 times slower than non-methylated T4 and λ DNA. As described previously, larger DNA molecules with a lower diffusion coefficient can show lower lateral dispersion in the direction of the width and depth of a nanoslit, and this lateral dispersion can affect DNA translocation velocity in the direction of the length of the nanoslit.[12] The nanochannel used here was 300 nm wide × 300 nm high, and the DNA could freely diffuse in the directions of the width and the depth of the nanochannel. Methylated DNA, with a lower diffusion coefficient, was assumed to have smaller lateral displacement fluctuation than non-methylated DNA, across the width and depth of the nanochannel. Consequently, methylated DNA was expected to translocate the length of the nanochannel faster than non-methylated DNA.

Rather than the diffusion, the more likely explanation is that methylated and non-methylated DNA molecules have different coefficients of friction with the nanochannel wall, due to differences in stiffness which is an index of flexibility as a polymer chain and reflects contraction probability inside a

nanochannel. It has been reported that methyl groups can increase DNA stiffness because of steric hindrance.[13] In addition, the persistence length of methylated DNA is increased by 1.30-fold with a methylation rate of 20% compared to non-methylated DNA, owing to insertion of methyl groups into the DNA.[14] In the present study, T4 and λ DNA molecules containing 31034 and 11362 cytosine residues were methylated at 4816 and 3112 CpG sites using CpG methyltransferase, respectively. The methylation rate of T4 and λ DNA molecules were approximately 15 and 27%, and we calculated that persistence length of T4 and λ DNA molecules would be increased by approximately 1.23-fold and 1.41-fold. This indicates that methylated DNA is stiffer than non-methylated DNA. During DNA translocation, the DNA was contracted inside the nanochannel, and stiff methylated DNA, with slower contraction, may have had a lower coefficient of friction with the nanochannel wall than non-methylated DNA. We hypothesize that the coefficient of friction of methylated DNA was lower than that of non-methylated DNA, allowing methylated DNA to transit the nanochannel faster than non-methylated DNA.

Conclusion

To the best of our knowledge, this is the first description of the effect of DNA methylation on DNA translocation velocity inside a nanochannel. The translocation velocity of methylated DNA was faster than that of non-methylated DNA through a nanochannel. We attribute this to variation in the diffusion coefficient owing to differences in molecular weight and variation in the coefficient of friction with the nanochannel wall owing to differences in stiffness. We hypothesize that this new approach will contribute to the further study of methylation-based epigenetics.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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