ABSTRACT

We demonstrated methylation mapping of DNA molecules specifically in nanoslit devices by using the quantum dots (QDs) combined with methyl-CpG-binding domain protein (MBDp) at a single molecule level. We fabricated nanoslit devices for fully elongation of DNA molecules. We measured the methylation sites using QDs-MBDp complexes in the nanoslit devices at a single molecule level. We found that these methylation sites showed a good agreement with the enzymatic sites of the methyltransferase.

KEYWORDS: Quantum Dots (QDs), Methylation Mapping, Nanoslit Devices, DNA

INTRODUCTION

Diagnosis and pathogenic mechanism elucidation for cancer and lifestyle-related diseases have been extensively studied in congenital genomic alterations, such as single nucleotide polymorphisms. In recent years, the importance of epigenomic analysis which is acquired genomic alteration without sequence changes such as DNA methylation has been noted. The methylation mapping of DNA molecules is the central field for epigenomic analysis. The conventional approach for the epigenomic analysis has some disadvantages; taking a long time; wasting a great deal of money; requiring troublesome manipulations such as genomic extraction, DNA fragmentation, PCR amplification and DNA sequencing. To overcome these disadvantages, observation of DNA molecules at a single DNA level was decided to use for the epigenomic analysis. However, it is difficult to observe DNA molecules in a free-solution because DNA molecules have random coil structure in the free-solution. It was reported that the 50 nm nanospace would be required in order for fully elongation of DNA molecules[1]. Therefore, we noted that mapping of DNA methylation using the nanoslit devices[2, 3].

Development of techniques to visualize and manipulate target molecules at a single DNA molecule level is useful to probe heterogeneities in such an ensemble of molecules and to analyze biological reactions. In particular, the technique has been applied extensively to analysis for DNA molecules, such as optical tweezers, microfibers, magnetic tweezers and atomic force microscopy. These techniques were first used to study the mechanical properties of a single DNA molecule. The conventional approach for the single molecule analysis still has a disadvantage photobleaching of organic dye or fluorescence proteins to use as fluorescence probe of labeled DNA. Therefore, to observe individual methylation sites of DNA, we noted that the intensity of QDs is very high and utilized strong bond of streptavidin and biotin. In this paper, we decided epigenomic analysis observed at a single molecule level. So, we fabricated the nanoslit devices (150 and 120 nm in width and depth, respectively) for elongation of DNA molecules to measure the methylation sites using QDs-MBDp complexes at single molecule level.

EXPERIMENTAL

We prepared methylated lambda DNA using methyltransferase. Methylated lambda DNA were dyed by YOYO-1 at dye-to-base pair ratio of 1:5. To measure the methylation sites at a single molecule level, methylation sites on methylated DNA molecules were detected QDs-MBDp complexes inside the nanoslit devices (Figure 1a). The QDs-MBDp complexes were combined QDs with MBDp via streptavidin-biotin bond (Figure 1b). The methylated DNA with QDs-MBDp complexes is injected inlet (left hole in Figure 1c), and then the methylated DNA is migrated in the direction of yellow arrow with applied voltages as shown in Figure 1c. When the methylated DNA comes into nanoslit structure, the methylated DNA molecules with QDs-MBDp were elongated and the methylation sites were measured. With this method, taking a long time, wasting a great deal of money, and requiring troublesome manipulations would be eliminated.

The nanoslit devices were fabricated on quartz substrates. First, Cr layer was deposited on the substrates by sputtering (Figure 2a), then the microchannel pattern was formed by photolithography (Figure 2b). After development of the resist, the patterned area of Cr was etched by immersing in Cr etchant for 5 minutes (Figure 2c). The microchannel was formed by reactive ion etching (RIE) (Figure 2d). The Cr layer of the pattern was removed by Cr etchant (Figure 2e). We also patterned the nanoslit inside the microchannel. A 20 nm thick Cr layer was deposited inside the microchannel and surface of substrates (Figure 2f). EB resist was coated on the microchannel and surface of substrates by spin-coating (Figure 2g), and then the nanoslit pattern was drawn by electron beam lithography (Figure 2h). The nanoslit channel was formed by reactive ion etching (RIE) (Figure 2i). After developing the resist, the Cr layer of the pattern was removed by Cr etchant (Figure 2j). Finally, the microchannel was sealed using quartz cover plates (Figure 2k).
RESULTS AND DISCUSSION

Fabricated nanoslit devices have some parameter as shown Figure 3a. We applied the voltage at both sides after injecting methylated DNA samples as shown in Figure 1c. After applying voltages, we could observe that the methylated DNA is migrated in the direction yellow arrow (Figure 1c), as expected. In addition, we could observe elongated lambda DNA molecules with 20 µm length in the nanoslit devices. We showed the methylation mapping of lambda DNA molecules using the QDs-MBDp complexes in the nanoslit devices in Figures 3b-3d. Inside the nanoslit devices, we measured fluorescent intensities onto the methylated lambda DNA molecules and confirmed five methylation sites of the methylated DNA molecules (Figure 3b-3d). These methylation sites showed a good agreement with the enzymatic sites of the methyltransferase (Figure 3e). Thus, we demonstrated simple and rapid technique of methylation mapping of lambda DNA molecules using the QDs-MBDp complexes and the nanoslit devices.

CONCLUSION

In summary, we have demonstrated methylation mapping of methylated lambda DNA molecules specifically in the nanoslit devices by using QDs combined with MBDp at a single molecule level. In this device, we tried to measure the methylation sites using QDs-MBDp complexes at single molecule level. We measured fluorescent intensities on the methylated lambda DNA molecules and confirmed five methylation sites derived from methyltransferase. We found that these methylation sites showed a good agreement with the enzymatic sites of the methyltransferase.

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Figure 3  (a) Illustration of the nanoslit devices. This structure have 42 straight slits and 150 nm slit in width, \( w_1 \), 500 nm slit in spacing, \( g \), 120 nm slit in depth, \( d \), and 25 \( \mu \text{m} \) channel in width, \( w_2 \). (b) Methylation mapping of methylated lambda DNA molecules using the QDs-MBDp complexes in the nanoslit devices. (c) Magnified image of a white dotted circle in (b). (d) Fluorescent intensities for the methylated lambda DNA molecules. (e) The enzymatic sites of the methyltransferase.

REFERENCES

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