Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Colloids and Surfaces B: Biointerfaces 40 (2005) 123–126
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

Stretching of single DNA molecules complexed with restriction endonuclease by Langmuir–Blodgett method

Yasutaka Matsuo [a], Kuniharu Ijiro [a, b], Masatsugu Shimomura [c, *]

[a] *Precursory Research for Embryonic Science and Technology, Japan Science and Technology Corporation, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan*
[b] *Research Institute for Electronic Science, Hokkaido University, N12W6 Kita-ku, Sapporo, Hokkaido 060-0812, Japan*
[c] *Nanotechnology Research Center, Research Institute for Electronic Science, Hokkaido University, N12W6 Kita-ku, Sapporo, Hokkaido 060-0812, Japan*

Abstract

We have proposed a new technique for stretching single double-stranded DNA molecules on solid substrates by the Langmuir–Blodgett (LB) method. The polyion complex monolayer of a cationic amphiphile and DNA molecules formed at the air–water interface was transferred on a clean glass substrate. Vertical lifting up of the glass substrate provided the transferred monolayer consisting the stretched individual DNA molecules aligned parallel to the lifting direction on the glass. The DNA molecules complexed with the restriction endonuclease (*Eco*RI) were employed for stretching by using this method. Fluorescence images of the transferred monolayer showed that the *Eco*RI-binding DNA molecules could be stretched and immobilized on the glass substrate. A specific sequence of DNA recognized by *Eco*RI was detected as spatial positions of the stretched DNA molecules.
© 2004 Elsevier B.V. All rights reserved.

Keywords: DNA; Langmuir–Blodgett method; Polyion complex monolayer; Restriction enzyme

1. Introduction

The techniques of stretching of double-stranded DNA molecules have been developed for revealing chemical, physical and biological properties of the DNA molecules. In particular, stretching of DNA molecules plays an important role in physical mapping of genomes. For example, Schwartz et al. has developed the ordered optical restriction enzyme mapping method [1]. Restriction endonuclease digesting events on stretched DNA molecules were observed by fluorescence microscopy. Perfect stretching of the DNA molecules is required for the accurate mapping in this method. Recently, it has been reported that DNA molecules can be uniformly stretched by various approaches, e.g., molecular combing and electrophoresis for the genome analysis at single DNA molecule level [2–4]. However, these techniques require specific binding of one end of DNA molecules on a solid substrate. Therefore, specific surface modification of the solid substrates (usually glass coverslips) is necessary for binding of DNA molecules.

We have developed the new technique that double-stranded DNA molecules can be stretched and immobilized on a clean glass substrate by transferring a cationic monolayer complexed with the DNA molecules formed at the air–water interface by the Langmuir–Blodgett (LB) method [5,6]. The advantage of this technique is to stretch linear DNA without anchoring the end of DNA molecules on the surface of the substrate. Furthermore, various DNA molecules, which have a wide range of molecular weight (a few kilobase-pairs (kbp) to a few hundred kbp), are applicable.

In this report, we applied this method to stretching double-stranded DNA molecules complexed with restriction endonuclease. Although restriction endonuclease is an enzyme which can digest a certain base sequence of double-stranded DNA, it can bind to the site of the DNA molecules without di-

* Corresponding author. Tel.: +81 11 706 2997; fax: +81 11 706 4974.
E-mail address: shimo@poly.es.hokudai.ac.jp (M. Shimomura).

0927-7765/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfb.2004.10.025

gesting them under a specific condition. Fluorescence images of DNA molecules and restriction endonuclease stained by different fluorescent dyes in the polyion complex monolayer transferred on the glass substrate were observed by fluorescence microscopy.

2. Experimental

Double-stranded Lambda-phage DNA (Nippon Gene, Japan) and restriction endonuclease, *Eco*RI (Takara, Japan) were used without further purification. Fluorescence-labeled *Eco*RI was obtained as follows: *Eco*RI was biotinylated by EZ-link Sulfo-NHS-LC-Biotin (PIERCE, USA). Then Alexa Fluor®532-conjugated streptavidin (Molecular Probes, USA) was attached to *Eco*RI through the biotin–streptavidin interaction.

An aqueous DNA solution was mixed with a fluorescence-labeled *Eco*RI solution of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.8). The mixed solution was incubated for 3 h at room temperature. TE buffer solution contains EDTA for removal of Mg^{2+} which is an activator of an enzymatic reaction of *Eco*RI. Therefore, *Eco*RI cannot digest DNA but can bind to the specific sequence (GAATTC) of DNA [7]. A dilute solution of DNA/*Eco*RI was prepared at a concentration of 10 nM in base pair (bp) with TE buffer (pH 7.8). The DNA molecules were stained with 1 nM YOYO-1 (Molecular Probes, USA).

Cationic amphiphile, dimethyldioctadecylammonium bromided ($2C_{18}N^{+}2C_1$) (Sogo Pharmaceutical, Japan) was spread on the dilute solution of DNA/*Eco*RI from the chloroform solution (spectrophotometric grade, Wako pure chemical industries, Japan). The DNA molecules adsorbed to the cationic monolayer by electrostatic interaction to form the polyion complex monolayer at the air–water interface. After 5 min, the formed monolayer was compressed at the rate of 0.02 nm^2 per molecule/min by a film balance system FSD-50 (USI System, Japan). The surface pressure was measured with a Wilhelmy plate. The subphase temperature was maintained at $20 \pm 0.2\,^{\circ}C$. When the surface pressure reached at 5 mN/m, a clean glass substrate immersed under the subphase before spreading the monolayer was lifted up at a speed of 2 mm/min to transfer the polyion complex film on the substrate (Fig. 1). Fluorescence images of the polyion complex film on the glass substrate were acquired by a fluorescence microscope Eclipse E600 (Nikon, Japan) equipped with fluorescence filter sets, 100× (N.A. 1.3) oil immersion objective lens and a digital charge coupled device (CCD) camera ORCA-II-ER (HAMAMATSU PHOTONICS, Japan) or a color chilled 3CCD camera M-3204C (Olympus, Japan).

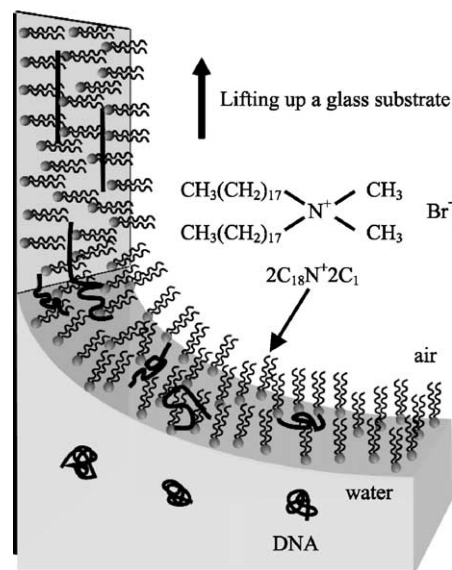

Fig. 1. Schematic illustration of a polyion complex film transferred on a glass substrate.

3. Results and discussion

Fig. 2 shows a fluorescence image of the polyion complex film transferred from the subphase without *Eco*RI by the vertical lifting method. Fluorescent lines aligned parallel to the lifting direction of the glass substrate were observed. The length histogram of the fluorescent lines except non-linear and inhomogeneous fluorescent lines was shown in

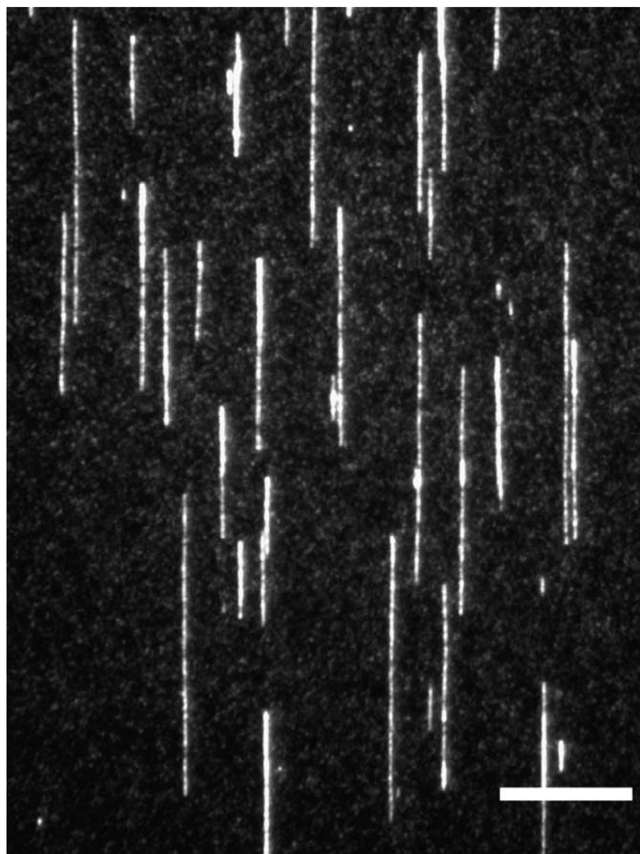

Fig. 2. Fluorescence image of Lambda DNA stained with YOYO-1. Scale bar is 10 μm.

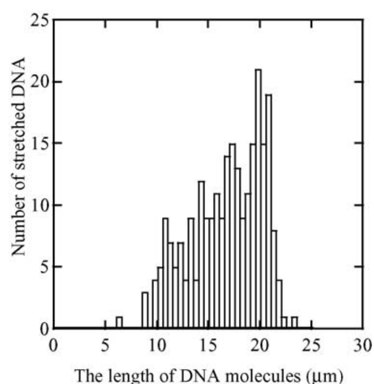

Fig. 3. Length histogram of the stretched DNA molecules.

Fig. 3. A length distribution is broad. However, a definite peak is existed at about 20 μm in the length histogram. The average length of the fluorescent lines was about 16 μm. The theoretical length of B-formed Lambda DNA (48.5 kbp) is 16.5 μm. Therefore, this result indicates that each fluorescent line could be attributed to the stretched single DNA molecule. The DNA molecules bound to the amphiphile monolayer were in random coil state at the air–water interface before the transfer on the substrate (data not shown). This indicates that the DNA molecules are stretched during the lifting process.

Michalet et al. have stretched DNA molecules by lifting up of a silanized glass substrate from an aqueous DNA solution in which is called dynamic molecular combing (DMC) method [3]. They used a DNA solution with the DNA concentration of around 1 μg/ml. In contrast, we require the much lower DNA concentration (about only a several ng/ml) than that in their method, because the electrostatic interaction between the cationic amphiphile and DNA at the air–water interface is strong enough to condense the DNA molecules on the cationic monolayer. Therefore, the LB method is more efficient for immobilization of DNA from a diluted DNA solution than the DMC method.

Stretching mechanism of DNA in our method may differ from that in the DMC method because of the existence of anchoring of the end of DNA onto the substrate [6]. It has been reported that rod like polymers can be aligned by the surface flow during the deposition process [8,9]. In the LB method, DNA molecules are stretched at the meniscus during the lifting process as same as the DMC. Stretching of DNA depend on the phase condition of the polyion complex monolayer and the speed of lifting the substrate. When the polyion complex monolayer is in the solid state or lifting speed is higher than about 5 mm/min, DNA molecules are not stretched. Therefore, the monolayer flow or water flow at the meniscus plays an important role for stretching of DNA. However, the stretching mechanism in our method has been unsolved in details.

We investigated the transfer of the polyion complex monolayer from the aqueous DNA subphase mixed with the fluorescence-labeled *Eco*RI. Fig. 4 shows a fluorescence image of the polyion complex monolayer transferred on the glass substrate by the LB method. Stretched DNA molecules were observed as green emitted lines by a fluorescence microscope equipped with a color CCD camera. In addition, orange fluorescence spots were observed on the stretched DNA molecules. Science the emission peak of Alexa Fluor®532 conjugated with *Eco*RI is 554 nm, the orange fluorescence spots are ascribed to Alexa Fluor®532 molecules on the green fluorescent DNA. On the other hand, when the polyion complex monolayer was transferred from the aqueous Lambda DNA solution mixed with the Alexa Fluor®532-labeled streptavidin without biotinylated *Eco*RI, no fluorescence of Alexa Fluor®532 was observed on the stretched DNA molecules. Therefore, fluorescent-labeled streptavidin did not bind to DNA molecules specifically, indicating that the orange fluorescence spots on the stretched DNA molecules were at-

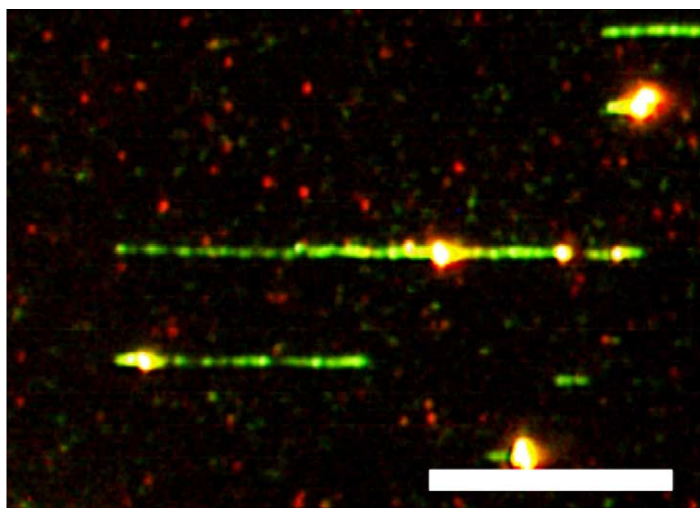

Fig. 4. Fluorescence image of Lambda DNA complexed with fluorescence-labeled *Eco*RI. Scale bar is 10 μm.

tributed to *Eco*RI complexed with Alexa Fluor®532 streptavidin. Stretching and immobilization of the *Eco*RI-binding DNA molecules onto the substrate was accomplished by our LB method. There are five binding sites of *Eco*RI on Lambda DNA employed in this experiment. Therefore, the specific sequences recognized by *Eco*RI are expected to be detected as the spatial positions of stretched single DNA molecules. In our experiment, however, the stretched single DNA molecules whose five binding sites were fully occupied by *Eco*RI were not observed. Some of five *Eco*RI proteins bound to the Lambda DNA molecule may be released from the DNA molecule during the lifting process or the binding constant of *Eco*RI bound to the DNA molecules may be insufficient.

4. Conclusion

We showed that the individual single DNA molecules could be stretched and immobilized on the glass substrate by the LB method. Stretching of DNA complexed with restriction endonuclease (*Eco*RI) could be achieved. One of benefits of this method is that clean glass substrates can be used without chemical treatments required for the DMC method. It is possible to achieve efficient stretching and immobilization of the DNA molecules at the low concentration. Our method is a powerful tool for analysis of specific sequences of a single DNA molecule.

Acknowledgments

This work was financially supported by the Precursory Research for Embryonic Science and Technology project (PRESTO) (Conversion and Control by Advanced Chemistry) of Japan Science and Technology Corporation (JST) and also by a Grant-in-Aid on Scientific Research from the Ministry of Education, Science, Sport, and Culture of Japan.

References

[1] W. Cai, H. Aburatani, V.P. Stanton, D.E. Housman, Y. Wang, D.C. Schwartz, Proc. Natl. Acad. Sci. 92 (1995) 5164.
[2] A. Bensimon, A. Simon, A. Chiffaudel, V. Croquette, F. Heslot, D. Bensimon, Science 265 (1994) 2096.
[3] X. Michalet, R. Ekong, F. Fougerousse, S. Rousseaux, C. Schurra, N. Hornigold, M. Slegtenhorst, J. Wolfe, S. Povey, J.S. Beckmann, A. Bensimon, Science 277 (1977) 1518.
[4] M. Ueda, K. Yoshikawa, M. Doi, Polym. J. 12 (1997) 1040.
[5] M. Shimomura, R. Mitamura, J. Matsumoto, K. Ijiro, Synth. Met. 133–134 (2003) 473.
[6] Y. Matsuo, K. Ijiro, M. Shimomura, Int. J. Nanosci. 1 (2002) 695.
[7] J.A. MaClarin, C.A. Frederick, B.C. Wang, P. Greene, H.W. Boyer, J. Grable, J.M. Rosenberg, Science 234 (1986) 1526.
[8] T. Maruyama, M. Friedenberg, G.G. Fuller, C.W. Frank, C.R. Robertson, A. Ferencz, G. Wegner, Thin Solid Films 273 (1996) 76.
[9] N. Minari, K. Ikegami, S. Kuroda, K. Saito, M. Saito, M. Sugi, J. Phys. Soc. Jpn. 58 (1989) 222.