REAL-TIME OBSERVATION OF DNA COMFORMATIONAL TRANSITIONS AT A SINGLE-MOLECULE LEVEL BY MICROFLUIDIC DEVICES

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ABSTRACT

We have developed a precise fluid control and a solution exchange system by a combination of novel microchannel designs and electroosmotic pumps for real-time observation of DNA conformational transitions at a single-molecule level. By using the microfluidic devices, we revealed the stepwise conformational transitions induced by ethanol solution.

KEYWORDS: DNA, Conformational Transition, Single-Molecule Analysis

INTRODUCTION

Conformational transitions including B-form to A-form DNA and condensation play a key role in genome expression and maintenance, e.g. chromatin folding [1] and G-quartet formation of telomeres [2]. Several single-molecule approaches using microfluidic devices have been attempted to know the kinetics of DNA chromatin assembly [3,4] because microfluidic devices can easily provide stretched DNA molecules by laminar flow and exchange the solution quickly. In our previous reports, one end of DNA molecule was attached on a cover glass and elongated by laminar flow in microchannel, and then, the contraction process was observed by exchanging solutions from water to ethanol or histone [5]. Another approach was based on diffusive mixing of water and ethanol through a branched channel to observe the contraction process simultaneously under the different concentrations [6]. However, some problems, especially the uncertainty on time 0 at the solution exchange process, were associated with these microfluidic devices. In these kind of kinetic experiments, it is critical to know when a front of target solution approaches to target DNA molecules. Therefore, to address this problem, we have developed a new design of a microfluidic device that enables instantaneous exchange of solutions in the observation channel and accurate measurements of DNA conformational transition have been performed.

EXPERIMENTAL

The microfluidic device was fabricated by conventional softlithography techniques. This device consists of 5 microchannels: two input channels (W: 500 μm, H: 50 μm), two side channels (W: 250 μm, H: 50 μm), and an observation channel (W: 500 μm, H: 50 μm). Flow rate was precisely controlled by electro-osmotic pumps (Nano Fusion Technologies, Inc., Tokyo) in inlet reservoirs as shown in Figure 1. To confirm whether the interface of water and ethanol was clearly formed and flew in the observation channel without unexpected mixing, ethanol solution containing 1,8-ANS (1-anilinonaphthalene-8-sulfonic acid) which shows highly fluorescent signal in low polarity regions was injected from one of the inlet. The interface formation and the time for solution exchange in the observation channel were measured (Fig. 1).

For DNA observation, one adhesive end of λ-DNA (48.5 kbp) was ligated with complemental biotinylated oligonucleotide. A cover glass was bonded to the microfluidic channel made of PDMS and silicon tubes were fit to the five reservoirs. The cover glass inside the microfluidic channel was dynamically coated by streptavidin, and then, biotinylated λ-DNA was incubated for 10 min (Fig. 2). The DNA molecules were stretched by the laminar flow at 17 V (~6 μl/min), which generated 85 to 95% stretch of the theoretical length. The fluorescent images of DNA molecules were observed by total internal reflection fluorescence microscope (Olympus) and analyzed by an image analysis software AQUA COSMOS (Hamamatsu Photonics).

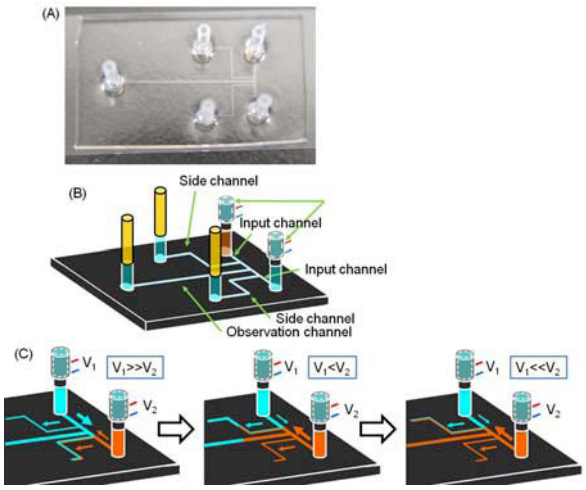

Figure 1: (A) A photo of a PDMS microfluidic device for real-time observation of DNA conformational transitions. (B) A schematic of the microfluidic device. EO pumps were used for DNA elongation and exchanging the solutions in the observation channel. (C) Schematics of flow control in the microchannel by EO pumps for instantaneous solution exchange. V1 and V2 are the applied voltages to the EO pumps.

Final goal of this work is to apply the microfluidic device for chromatin assembly process. As an initial step, in this experiment, we adopted ethanol as a source of DNA conformational transition because it is widely used for DNA precipitation and is known to induce reversible conformational transition.

RESULTS AND DISCUSSION

Because the power of electro-osmotic pumps (EO pumps) depends on the conductivity of the working solution, calibration curves were constructed in two working solutions, 99.5%(v/v) ethanol and distilled water. Both working solutions showed excellent linearity between the applied voltage and the flow rate ($R^2 > 0.997$). To produce stable flow, appropriate back pressure was applied by fitting PEEK tubes in the three outlet reservoirs as shown in Figure 1. Actually, it was difficult to produce stable and reproducible flow with the back pressure by EO pumps or without the PEEK tubes. So simple but reliable PEEK tubing method was applied for the following experiments. Contour lengths of stretched DNA molecules were gradually increased according to the flow rate and reached a plateau value around 12-14 μm over 5μl/min. Considering the extension of DNA molecules by the intercalation of YOYO-1 at a ratio of 1 dye to 20 base pairs, almost full stretch (>85%) was achieved under this flow condition. Figure 3 shows time series of the fluorescence intensity of 1,8-ANS dissolved in 99.5%(v/v) ethanol solution during the solution exchange in the observation channel. Both processes, from water to ethanol and ethanol to water, were quickly finished within 0.5 s. This means that the interface of ethanol and water could approach the immobilized DNA molecules on the cover slip with maintaining the initial concentration gap without the mixing by passive diffusion. This solution exchange system by a combination of the novel microchannel design and the electroosmotic pumps was proved to work as a real-time observation system for DNA conformational transitions at a single-molecule level to the exclusion of the uncertainty on reaction time 0 in the solution exchange process.

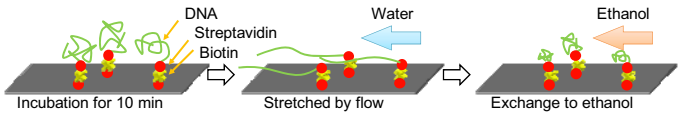

Figure 2: One end of λ-DNA molecules were attached on a cover glass through biotin-streptavidin interaction inside the observation channel.

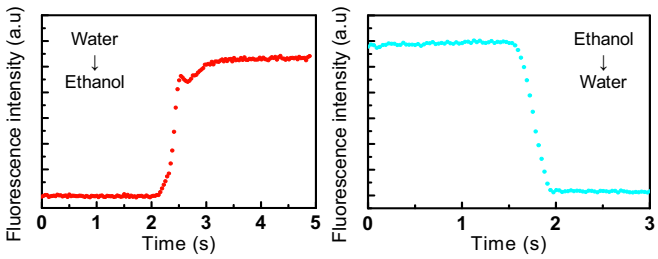

Figure 3: Time series of the fluorescence intensity of ANS dissolved in ethanol at the observation channel. When the solution in the observation channel was exchanged to ethanol, the fluorescence intensity suddenly increased within 0.5 s, and vice versa.

As a source inducing DNA conformational transition, various concentration of ethanol solution, from 30 to 70%(v/v), was applied. Although full DNA contraction to the globular state was observed above 50% ethanol, no outstanding change was observed in the concentration below 40% during 15 minutes observation. When the DNA molecules were exposed ethanol solution above 50%, they gradually shrunk to 80 to 85% of their initial lengths and then contracted to the condensed structures with 1 to 2 μm diameter as shown in Figure 4. In more detailed observation, it was revealed that the DNA conformational transition passed through the following three steps regardless of the concentration of ethanol, (i) slow and constant-velocity contraction, (ii) accelerating contraction, and (iii) decelerating contraction, as shown in Figure 4. Based on the difference in the rise per base pair of 0.34 nm for B-form and 0.26 nm for A-form DNA, the first slow contraction until 80 to 85% of the initial length might correspond to conformational transition from B-form to A-form by gradual dehydration. In this process, the uniform increase of the fluorescence intensity of the whole DNA molecule also supported this assumption. The second process might be due to coil-globule transition since the free end of DNA molecule started contraction. The velocity of this contraction process is determined by the balance of a DNA contraction force and a hydrodynamic drag force especially on the coiled part. This could explain why the contraction velocity slowed down at the final stage of the contraction.

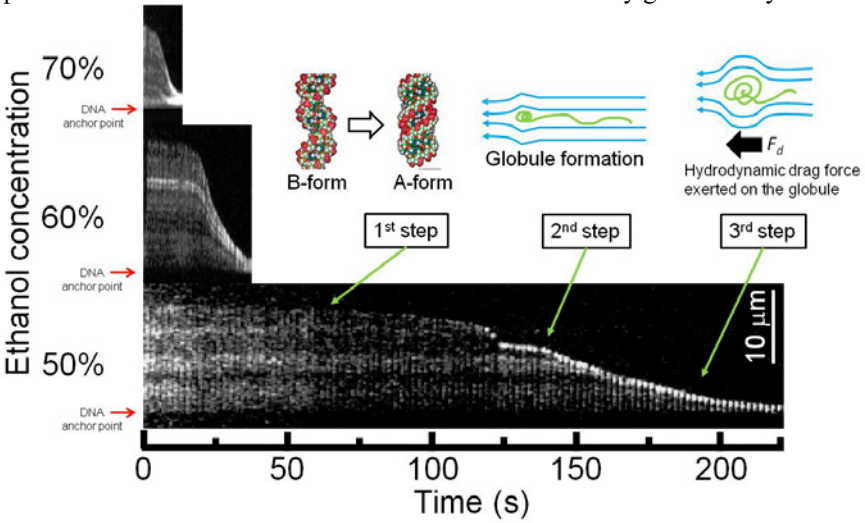

Figure 4: Time series of DNA fluorescence images after the ethanol injection. At 0 s, the front of ethanol solution at the indicated concentration was reached.

At a concentration of 30 and 40%(v/v), no contraction was observed over 15 minutes. These results seems to be different from the previous report by Y. Fang et al [8]. By using AFM, they observed B-

form to A-form transition over 25% ethanol solution and also several higher order structures including flower shaped condensates and toroids. Unfortunately, our optical microscope system has optical resolution limit around ~200 nm, and hence such flower shaped condensates and toroids could not be observed. The difference of critical ethanol concentrations which induce DNA conformational transition might attribute to the observation system. In the AFM observation, because they dried DNA samples on mica substrate, the evaporation process might generate spatial nonuniformity of the ethanol concentration and induce additional influences on DNA conformation. We think that more quantitatively accurate data could be obtained in our microfluidics-based system because whole observation process of DNA conformational transition was performed in solution.

CONCLUSIONS

Our microfluidic device could easily generate and control the interface of ethanol and water in the observation channel. Since this device does not require valve structures, it could be easily applied for various microfluidic devices which require quick solution exchange. As a model system of DNA conformational transition, ethanol-induced conformational transition was observed by the microfluidic device and succeeded to measure step-wise conformational transition. Our real-time observation system is expected to contribute the kinetic analysis of DNA conformational transition such as kinetics of chromatin assembly.

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