Direct Observation of Long-strand DNA Stretching in Microchannel Flow

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This paper reports a direct observation method of macromolecules, such as long-strand DNA, in microchannel flow, and also demonstrates that coil-stretch transition of DNA strands.

Microchannel offers the laminar flow. In this unique flow, it is reported that the several different chemical behaviors from in bulk solution occur. For example, improvements of the enzymatic reaction,[1] PCR[2] or hybridization[3] are achieved successfully. However, these causes are not always made clear. We had assumed that these results had been brought by conformational changes of macromolecules in microchannel flow. For ascertaining this assumption, we studied the method of direct observation of macromolecules in microchannel flow.

Long-strand DNA molecules form entangled, coiled structures in bulk solution. Stretching of DNA macromolecules might enable selective and effective modifications of the molecule that are difficult in folded conformation. Several DNA stretching methods have been developed to create novel chemical and biochemical reaction apparatuses.[4–9] However, these methods utilize complicated techniques such as alternating-current electrical fields,[4] or laser manipulation;[5,6] they all require special apparatuses. And it is also reported that a DNA molecule can be stretched by elongational flow.[7–9] However, because a constrict channel part is required, stretched DNA cannot maintain a stretched state continuously. Therefore, it is difficult to generalize these methodologies for chemical reactions. Generally, DNA sensing methods for long-strand DNA, such as genomic DNA or long-strand PCR production, required DNA cleavage by restriction enzyme or other methods as pretreatments.

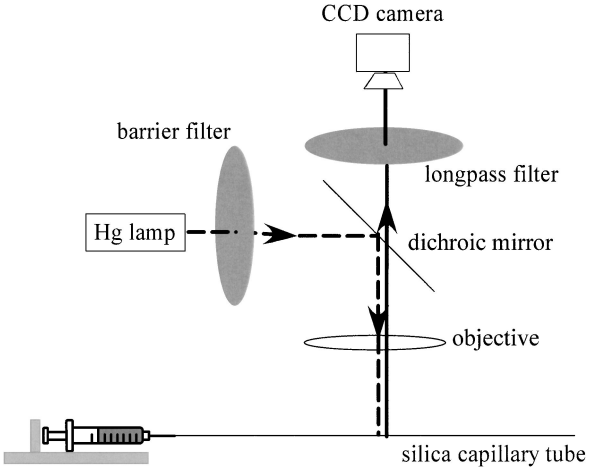

Figure 1. Schematic diagram of optics for direct observation of macromolecules in microchannel flow.

Earlier theoretical conformational studies of polymer molecules[10] speculated that a DNA molecule would be stretched when flowing within a narrower microchannel at a faster flow rate. This is a known theory as a coil-stretch transition of polymer molecules. If this is true, we can stretch DNA molecule just passing through the microchannel.

We prepared an aqueous solution (0.13 mM Tris-HCl buffer (pH 7.9) and 0.013 mM EDTA) of 10 μM (per base pair) T4 GT7 DNA (Nippon Gene Co., Ltd., Japan), 10 μM 4′,6-diamino-2-phenylindole (DAPI) (Dojindo Inc., Japan), and 4% (v/v) 2-mercaptoethanol (Wako Pure Chemical Inds., Ltd., Japan). This solution was injected to a fused silica capillary tube (i.d.: 530 μm, o.d.: 660 μm; GL Sciences, Inc., Japan) using a syringe pump (KDS230; KD Scientific Inc., USA). The capillary tube interior was observed using fluorescence microscopy (Eclipse TE2000-U; Nikon Inc., Japan) equipped with a 100× oil immersed objective lens (CFI Plan Fluor 100×H; Nikon Inc., Japan) and an ul-

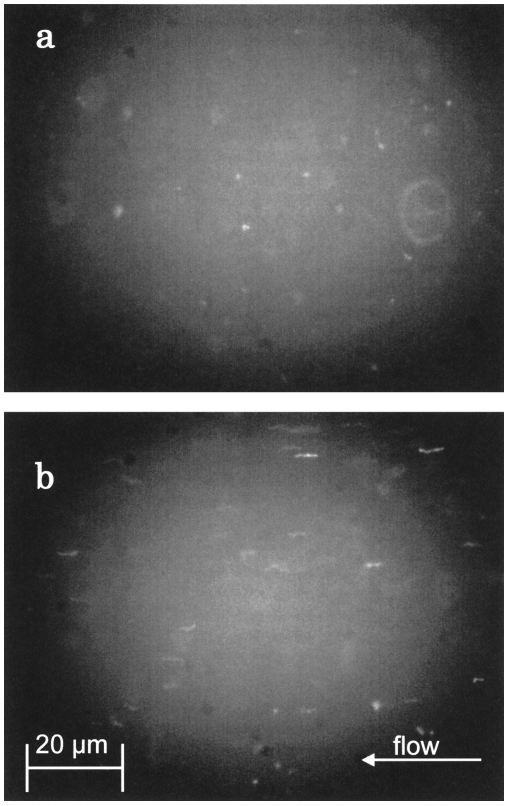

Figure 2. Long-strand DNA structures in a microchannel. All observations were made at room temperature, *ca.* 298 K. DNA molecules at non-flow state (a) and 10 μL/min (b) flow. Actual flow speed of (b) at the focus position is *ca.* 100 μm/s.

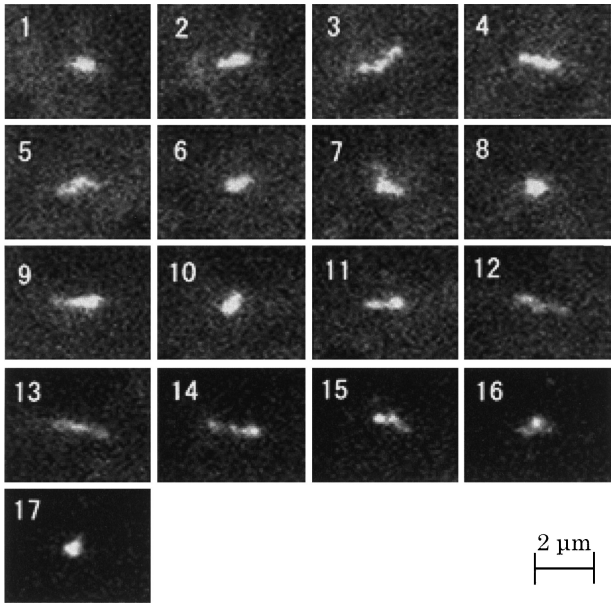

Figure 3. Sequential observation of DNA molecules. These are 61 ms step time-sequence photographs of one DNA molecule at a 2 μL/min average flow rate.

tra-high sensitive cooled CCD camera (ORCA-ER-1394; Hamamatsu Photonics K.K., Japan). A high-pressure mercury lamp was used with a color filter set (UV-1A; Nikon Inc., Japan) which transmitted only ca. 360-nm wavelength excitation light. (Figure 1) The focus of microscopy was adjusted to a 10-μm inner position from the inner wall.

First, we evaluated this speculation by monitoring DNA molecule movement within microfluidics. Long-strand DNA molecules in a microchannel were monitored using fluorescence microscopy and an ultra-high sensitivity cooled CCD camera.[11] Figure 2 shows photographs of T4 GT7 DNA stained with DAPI fluorescent dye. These DNA resembled coils in a non-flowing state (Figure 2a). However, these DNA stretched and flowed as though swimming. They oriented in the same direction in a flowing state (Figure 2b). Microchannel laminar flow stretched these DNA. According to theoretical studies,[7] the degree of stretching and shrinking of polymer chains depends on the microchannel size, polymer chain length, flow speed, and the solution's viscosity, density, and temperature. Figure 3 shows time-sequence photographs of one DNA molecule at an average flow rate of 2 μL/min. At such a slower flow, DNA strands formed equilibrium states of coiled and stretched states. Thus, DNA molecules changed conformation depending on flow. A DNA strand altered its shape incrementally when not flowing. At slower flows, such as that in Figure 3, DNA molecules moved while expanding and contracting, as 'inchworms' do. A DNA molecule stretched and undulated, as though swimming, at higher flow rates (*ca.* > 5 μL/min). We confirmed apparent lengths of DNA molecules in different viscosity or temperature conditions. The DNA strands became shorter in viscous conditions and longer in higher temperature conditions. (data not shown) These results conform to theoretical studies of coil-stretch transition[10] and relaxation time[12] of polymer molecules.

We confirmed that the traces of these stretching DNA images do not remained, as a "shooting star," by taking images of latex particles in similar conditions and calculating moving distance per frame at the microscopy focus position.

In conclusion, we have studied the direct observation method of macromolecules in microchannel, and conclude that DNA strands are stretched and oriented simply using microfluidics. We believe that this direct observation method for macromolecules is useful for elucidation of unique chemical behavior in microchannel flow.

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