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Notes

Fast Separation of Oligonucleotide and Triplet Repeat DNA on a Microfabricated Capillary Electrophoresis Device

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Introduction

An extension for repeats of trinucleotide, like CTG, is known to cause some inherited neuromuscular diseases, called triplet repeat diseases.[1] Chastain *et al*. have reported that DNA fragments containing CTG repeats migrate up to 20% faster than expected in non-denaturing polyacrylamide gels.[2] This unusual electric behavior of triplet repeat DNA is expected to be used for the detection of an extension of the triplet repeat. We have studied the electrophoretic behavior of triplet repeat DNA fragments by means of conventional capillary electrophoresis, and found that even a large CNG (N: C, A, T, G) repeat DNA moves much faster than a small random-sequence DNA in a polymer solution.[3-5] In this work, we performed the fast separation of a triplet repeat DNA fragment on a μ-CE (microfabricated-capillary electrophoresis) device. The μ-CE is a kind of device based on the idea of μ-TAS (micro total analytical system) or Lab-on-Chip (laboratory on a chip).[6] In the future, we plan to integrate on a chip all of the analytical methods for DNA diagnosis and gene therapy for human diseases, like the triplet repeat diseases. Firstly, we have investigated the separation of triplet repeat with chip-base, and demonstrated faster separation compared to conventional capillary electrophoresis.

Experimental

Materials

We used two kinds of synthetic single-stranded DNA fragments labeled by FITC with PAGE-purification grade. One was 30mer of a triplet repeat DNA fragment, 10 repeats of CTG. Another was 20mer of the sequence 5′-GTTGGCTCTGACTGTACCAC-3′, which was used as a DNA marker. These DNA fragments were dissolved with TB buffer (50 mM Tris-borate, pH 8.2). Methylcellulose (4000 cP at 2% solution) was used as a separation polymer.

Apparatus

Figure 1 shows a schematic diagram of the μ-CE system. The microchip was made of two pieces of quartz plate (35 mm × 12 mm × 1 mm). The bottom plate had two orthogonal microchannels (50 μm wide and 20 μm deep), that ware generated by photolithography and chemical etching. The cover plate had four holes (1 mm in diameter) used as reservoirs (R1 – R4). The two plates were bonded by a treatment with a 1% hydrofluoric acid solution.[7] The inner wall of the channels was coated with linear polyacrylamide. The channels were filled

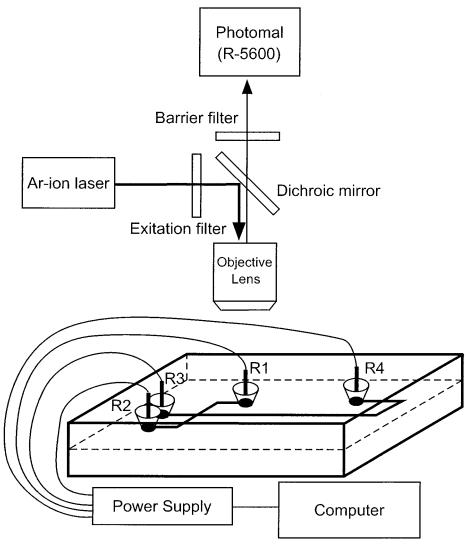

Fig. 1 Schematic diagram of the μ-CE system with a laser-induced fluorescence detector.

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with TB buffer including 1% methylcellulose. The channel lengths from each reservoir (R1, R2, R3, R4) to the intersection of the channels were 7 mm, 7 mm, 7 mm, and 32.5 mm, respectively. Pt wires were inserted into the reservoirs. DNA labeled with fluorescence dye (FITC) was exited by an Argon-ion laser, and was detected through an objective lens (40×) and a filter set (Nikon EX450/490, DM510, and Omega 530DF30) by a photomaltiplier (R-5600, Hamamatsu Photonics, Hamamatsu) at the point of 29.5 mm from the intersection.

Procedure

DNA sample (0.8 μl) was introduced into reservoir R1, and R2 – R4 reservoirs were filled with 1 μl of TB buffer. The DNA sample was loaded to the intersection while maintaining the following potentials for 20 s: R1, –0.40 kV; R2, GND; R3, –0.55 kV; R4, –0.95 kV. After that, for injecting the sample plug into a separation channel, the potentials were changed to: R1, –0.40 kV; R2, –0.4 kV; R3, –1.4 kV; R4, GND. The electric field in a separation channel (from R3 to R4) was 1.4 kV/(7 mm + 32.5 mm) = 354 V/cm. All experiments were performed at 25˚C.

Results and Discussion

Figure 2 shows electropherograms detected at a point of 29.5 mm effective length. The separation was achieved within 35 s, as shown in Fig. 2a (final concentration: 6.5 μM $(CTG)_{10}$, 3.9 μM 20mer random sequence DNA). In order to identify each peak, we added small amounts of each fragment to the sample solution. Figure 2b shows the result after adding $(CTG)_{10}$ to the mixture sample. The inserted arrow in Fig. 2b indicates that the intensity of the first peak became higher relative to the intensity of the second peak than in Fig. 2a. On the other hand, Fig. 2c shows the result after adding a random-sequence fragment of 20mer. The inserted arrow in Fig. 2c indicates that the intensity of the second peak became higher than the intensity of the first peak. From the results of Figs. 2a, b and c, we can conclude that a longer triplet repeat DNA fragment migrates faster than a shorter random-sequence DNA fragment. The speed achieved by the μ-CE device in this study is at least 6 times faster than that of conventional capillary electrophoresis,[4] because, in the μ-CE device, we can choose the effective length with the wide range from zero to the total length. Further research is now in progress to investigate the experimental conditions for the rapid separation of more extended (CNG) repeat DNA, and to understand the mechanism concerning the unusual electrophoresis behavior of triplet repeat DNA.

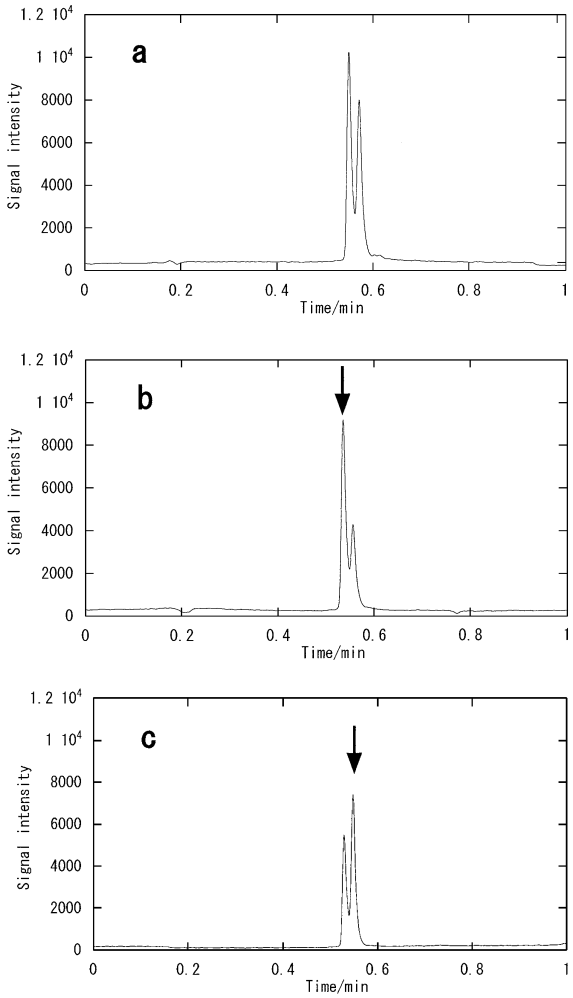

Fig. 2 Electropherograms for 20 bases random-sequence and $(CTG)_{10}$. The inserted arrows in b and c indicate the peak with increased intensity. In pherograms a, b and c, only relative intensity between the first and the second peaks is meaningful. We did not adjust the size of the laser spot in the individual experiments because of the experimental simplicity.

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