Affinity Capillary Electrophoresis in a Poly(dimethylsiloxane)-glass Hybrid Microchip

Toshiyuki Ito, Akira Inoue, Kae Sato, Kazuo Hosokawa, and Mizuo Maeda*
Bioengineering Laboratory, RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako, Saitama 351-0198

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Affinity capillary electrophoresis of DNA was conducted in a poly(dimethylsiloxane)-glass hybrid microchip with a 100 μm (width) × 130 μm (height) microchannel containing poly(*N*,*N*-dimethylacrylamide)-DNA conjugate. By this method, sample 12-mer oligonucleotide composed of c-K-*ras* codon 10-13 was separated from its single-base substituted mutant within 2 min.

Sequence-specific separation of DNA using electrophoresis is a promising candidate of rapid and reliable method for detection of gene point mutation and typing of single nucleotide polymorphism. Denaturing gradient capillary electrophoresis (DGCE) and single-strand conformation polymorphism (SSCP) are well-known methods for screening of point mutations in DNA.[1] On the other hand, affinity capillary electrophoresis (ACE) has unique advantages such as general applicability and high selectivity.[2–5] In the method, an affinity ligand DNA which is complementary to a sequence around suspected mutation point of the sample DNA is immobilized onto the inner surface of the capillary,[4] or is bound to polymer matrix such as polyacrylamide.[5]

Recently, miniaturization and integration of chemical analysis including capillary electrophoresis (CE) on a microfabricated device have attracted considerable attention as micro total analysis systems (μ-TAS).[6] For μ-TAS, "hard" materials such as silicon, glass, and quarts have been traditionally used. However, in these two or three years, a "soft" silicone elastmer-poly(dimethylsiloxane) (PDMS) is becoming one of the standard materials for μ-TAS because of its rapid fabrication process.[7] In both groups of materials, conventional electrophoresis of DNA based on its chain length has been well established, and power of miniaturization has been demonstrated as dramatic reduction of analysis time.[7] However, there have been only few reports on miniaturization of sequence-specific separation.

In this communication, we demonstrate ACE separation of 12-mer oligonucleotide from its single-base substituted mutant in a PDMS-glass hybrid microchip. To suppress electroosmotic flow,[8] we used poly(*N*,*N*-dimethylacrylamide) (PDMA) instead of polyacrylamide as the polymer support matrix to which 6-mer affinity ligand oligonucleotide is bound.

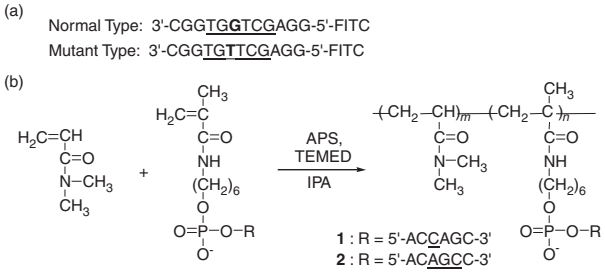

Figure 1. (a) Sequences of the sample 12-mer oligonucleotides. (b) Synthesis of PDMA-oligonucleotide conjugates. (APS, ammonium persulfate; TEMED, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; IPA, 2-propanol).

Two chemically synthesized oligonucleotides whose termini were labeled with fluorescein isothiocyanate (FITC) were used as samples (Figure 1a). One of the two has the same sequence as codon 10-13 of c-K-*ras* gene, while the other has a single-base substitution. The concentration of the samples was kept at 0.5 μM (1 M = 1 mol dm^{-3}) in all experiments. As shown in Figure 1b, two types of PDMA-oligonucleotide conjugates were synthesized: complementary sequence to the middle part of the normal type sample (**1**), and scrambled sequence (**2**). The synthetic procedure was similar to that previously described.[5] Thirty-one μL of *N*,*N*-dimethylacrylamide (Wako Pure Chemical Industries, Japan), 25 μL of 1 mM of methacryloyl-modified oligonucleotide (6-mer, Sigma Genosys Japan), 10 μL of 2-propanol (Junsei Chemical Co., Japan) and 263 μL of 50 mM Tris-borate buffer (pH 7.4) were mixed, and degassed by bubbling of nitrogen gas for 5 min. Copolymerization was initiated by adding 2 μL of 10 wt% ammonium persulfate (Wako Pure Chemical Industries, Japan) and 2 μL of 10 wt% *N*,*N*,*N*′,*N*′-tetramethyethylenediamine (Wako Pure Chemical Industries, Japan). Then the mixture was placed in an incubator for 1.5 h at 50 °C. After the polymerization mixture was diluted twice with the 50 mM Tris-borate buffer, the PDMA-oligonucleotide conjugate was used for the experiment described below without any purification. The final concentrations of the ligand oligonucleotide and PDMA were 38 μM and 5 wt%, respectively.

Design of the PDMS-glass hybrid microchip is shown in Figure 2. The microchip contains a cross-shaped microchannel and four reservoirs for sample loading and electrodes. The channel cross section is 100 μm in width and 130 μm in height. The separation length is 13 mm. The PDMS part with surface relief

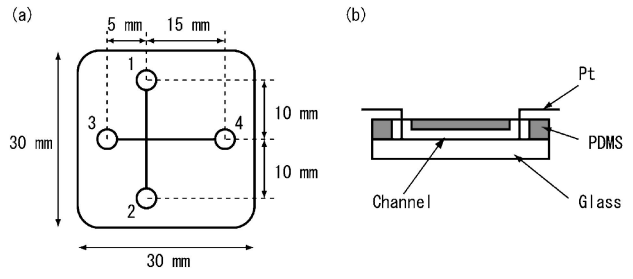

Figure 2. Schematic representation of the PDMS microchip used in this work. (a) Top view. The channel cross section is 100 μm (width) × 130 μm (height). The circles indicate reservoirs: (1) sample; (2) injection waste; (3) buffer; (4) waste. (b) Cross sectional view of the PDMS microchip attached on the glass plate. Solid lines indicate platinum electrodes (d = 0.5 mm) inserted into the reservoirs.

was prepared by the replica-molding technique according to the previous paper,[7] with some minor modifications. Four reservoirs ($d = 3$ mm) were punched through the bulk PDMS, and it was reversibly bonded to a flat glass plate.

The ACE separation was carried out as follows. The PDMA-oligonucleotide conjugate was introduced into the microchannel. The sample solution was pipetted into one of the reservoirs, and platinum electrodes were dipped into all reservoirs. Two-step voltage control for each electrode was carried out as follows. For injection step, reservoirs 1–4 were kept at −350 V, +150 V, 0 V, and 0 V, respectively for 90 s. After the channel intersection volume was filled with sample solution, separation step was started by applying electric voltages of 0 V, 0 V, −500 V, and 0 V to reservoirs 1–4, respectively (separation field = 250 V/cm). All experiments were carried out at room temperature without specific temperature control of the microchannel. Fluorescence of the sample was detected at the end point of the separation channel using a fluorescence microscope (ECLIPSE TE2000-U, Nikon Co., Japan) and a cooled CCD camera (C5985, Hamamatsu Photonics Co., Japan). The electrophoresis image was recorded by a digital video recorder, and the electropherogram was obtained using an image analysis software (ImageJ 1.28U).

Figures 3a and 3b show the electropherograms of the mixed solution of the normal type and mutant type using the complementary affinity ligand (**1**). Without Mg^{2+} (Figure 3a), the two types of oligonucleotides migrated together, and appeared as a single major peak at 32 s. Minor peaks at 40 and 50 s should be impurities such as fluorescent dye. In the absence of Mg^{2+}, interaction between the affinity ligand and sample oligonucleotides is considered to be too small for separation.

In contrast, with 10 mM Mg^{2+} (Figure 3b), the two types of oligonucleotides were clearly separated. The presence of Mg^{2+} reduced the electrostatic repulsion between the ligand and the samples, and enhanced the interaction between them. The assignments of the two major peaks shown in Figure 3b were confirmed by comparison with control experiments for pure samples shown in Figures 3c and 3d. Because the normal type has stronger interaction with the ligand than the mutant type does, the former migrated slower than the latter. The band-broadening also reflects the strength of interaction. In addition, it should be noted that Figure 3b seems the simple superimpose of Figures 3c and 3d. This means that the two samples behaved independently even in the mixture.

Figure 3e shows the electropherogram of PDMA-oligonucleotide conjugate of the scrambled ligand (**2**). Since both the normal and the mutant types have almost no interaction with the ligand (**2**), the electropherogram shows only a single peak. In comparison with this case, even the mutant type has some affinity with the complementary ligand (**1**), and appeared as the slightly retarded peaks in Figures 3b and 3c.

In conclusion, we demonstrated the separation of 12-mer oligonucleotide which has c-K-*ras* codon 10-13 and its single-base substituted mutant in a PDMS-glass hybrid microchip filled with a PDMA-oligonucleotide conjugate within 2 min. Compared to the conventional CE apparatus,[5] over 10 times faster separation has been achieved by the miniaturization. These results demonstrate that microchip ACE is an efficient and accurate technique for the gene mutation assay. Application for longer DNA samples such as amplified by polymerase chain reaction is now in progress.

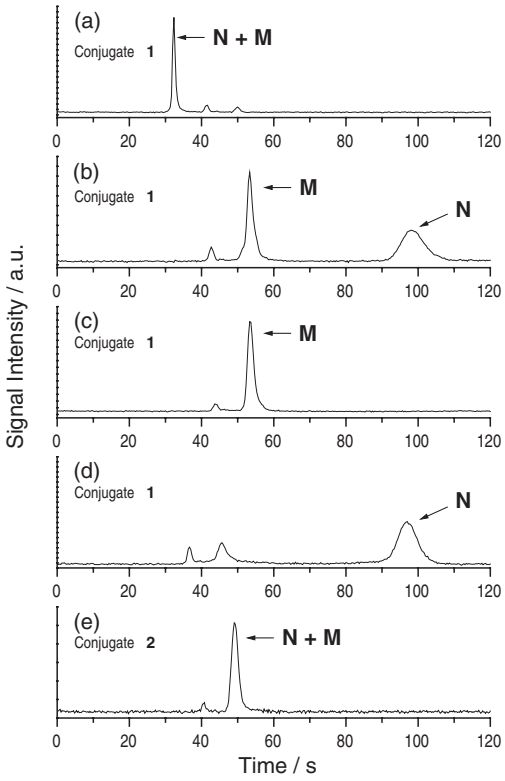

Figure 3. Electropherograms of 12-mer oligonucleotides (N, normal type; M, mutant type) by the use of conjugate **1** (a-d), and conjugate **2** (e), in 50 mM Tris-borate buffer. The separation field was 250 V/cm. Separation length was 13 mm. (a) Mixed sample without Mg^{2+}. (b) Mixed sample with 10 mM Mg^{2+}. (c) Control, mutant type with 10 mM Mg^{2+}. (d) Control, normal type with 10 mM Mg^{2+}. (e) Mixed sample with 10 mM Mg^{2+}.

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