DNA Hybridization Analysis of PCR Products by Non-gel Sieving Capillary Electrophoresis

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Capillary electrophoresis (CE), using narrow-bore (50–100 μm i.d.) capillaries of fused silica tubing, can rapidly resolve complex mixture of biopolymers in high electrical field (100–500 V/cm).⁽¹⁾ In contrast to gel electrophoresis, CE has been reported as a promising technique for performing rapid and reproducible separation of double-stranded (ds) DNA as well as single-stranded (ss) DNA and as being feasible for automated instruments.⁽²⁾ CE has been used for analysis of DNA fragments amplified with PCR.⁽³⁾ Capillary electrophoretic analysis of hybrid DNA formed with the purified sense and antisense ssDNA has been reported.⁽⁴⁾ However, CE analysis of hybridization using dsDNA, which is generally used in Southern blot analysis, has not been described so far.

In this study we report that two different dsDNA fragments of a tumor suppressor gene p53 were amplified with PCR technique, and liquid-hybridized DNA of each denatured PCR product could be detected by non-gel sieving CE using capillary filled with the buffer containing polymer additives. These data demonstrate that this method might replace Southern blot hybridization.

MATERIALS AND METHODS

DNA Amplification with PCR

The specific primers for amplification of p53 exons 5 and 6 are demonstrated in Figure 1A. Two DNA fragments (325 and 73 bp) were obtained by amplification of human lymphocyte DNA (500 ng) with PCR technique using 0.5 μM oligonucleotides as specific primers, 20 μM dNTP, and 2.5 units of *Taq* DNA polymerase in 100 mM Tris-HCl (pH 8.3) containing 2 mM MgCl_2 , and 100 mM KCl. Forty cycles of the reaction, at 94°C for 1 min, 60°C for 2 min, and 72°C for 1 min, were carried out. Digoxigenin (DIG)-labeled PCR products were produced as the same condition above, except using 20 μM dATP, 20 μM dGTP, 20 μM dCTP, 13 μM dTTP, and 7 μM DIG-11-dUTP (Boehringer Mannheim) in place of 20 μM dNTP.

Liquid Hybridization

Prior to hybridization, 50 ng of each PCR product (325 and 73 bp) was mixed and dissolved in 20 μl of $2\times$ SSC [30 mM sodium citrate (pH 7.0), 300 mM NaCl] containing 1 mM EDTA. The mixture of PCR

products (20 μl) was denatured for 5 min at 95°C, chilled on ice, and hybridized for 15 min at 50°C.

Capillary Electrophoretic Conditions

CE was performed on the Bio-Focus 3000 (Bio-Rad Laboratories). The polyacrylamide-coated capillary ($\Phi 50\ \mu\text{m} \times 36\ \text{cm}$) (Bio-Rad Laboratories) was conditioned daily by rinsing a 5-column volume of TBE buffer (0.267 M Tris-borate at pH 8.3, 1 mM EDTA) containing polymer additives (Bio-Rad Laboratories). The buffer was filtered and degassed by centrifugation. Prior to the sample migration, pressurized injection at 60 psi/sec was performed to introduce the DNA sample ($\sim 25\ \text{pg}/10\ \text{nl}$) for analysis. The capillary electrophoretic conditions were set to 222 V/cm at 30°C. Ultraviolet absorbance was monitored at 260 nm to detect DNA fragments. Postrun analysis of data was performed using the Bio-Focus 3000 software (version 3.0).

RESULTS AND DISCUSSION

Hybridized DNA was analyzed by CE and conventional agarose gel electrophoresis. The 325- and 73-bp fragments were separated as different bands, and hybrid DNA band might be expected to be located between them on agarose gel electrophoresis (Fig. 1B). We analyzed DIG-labeled PCR product to confirm the hybrid DNA band. The DIG-labeled 325- and 73-bp fragments were hybridized to a nonlabeled counterpart, respectively. Each hybrid DNA band was found at the same position between two dsDNAs (Fig. 1C).

To evaluate the migration time of hybrid DNA in CE, the hybrid band was purified from the gel with the glass beads methods⁽⁵⁾ and analyzed with CE (Fig. 2A). The migration time of the hybrid DNA was determined to be 8.77 min (Fig. 2A). The CE profile of the whole liquid hybridization reaction mixture shown in Figure 2B suggested that the hybrid DNA was detected between the 325- and 73-bp fragments as in gel electrophoresis (Fig. 1B). In CE analysis, hybrid DNA migrated very close to the 73-bp fragment. The mobility of the heterogeneous hybrid DNA might be different from that of homogeneous dsDNA in the buffer containing polymer additives. Thus, we have established the detection method of hybrid DNA with

FIGURE 1 (A) Specific primer sequences of p53 for amplification with PCR. To be hybridized with each other, the 73-bp fragment was designed inside of the 325-bp fragment. (B) Four percent agarose gel electrophoretic analysis of hybrid DNA. (Lane 1) The 73- and 325-bp fragment mixture without denaturation; (lane 2) DNA reaction mixture after liquid hybridization. (C) Four percent agarose gel electrophoretic analysis of DIG-labeled hybrid DNA. (Lane 1) The DIG-labeled 73-bp fragment; (lane 2) hybridized DNA with the DIG-labeled 73-bp and nonlabeled 325-bp fragments; (lane 3) hybridized DNA with the DIG-labeled 325-bp and nonlabeled 73-bp fragments; (lane 4) the DIG-labeled 325-bp fragments. After DNA was transferred from the agarose gel to a nylon membrane (Biodyne B; Pall), the DIG-labeled DNA was detected using a chemiluminescent detection kit (Boehringer Mannheim).

CE using dsDNA fragments. In the method described here, we used two denatured DNA fragments but ssDNA could not be found (Fig. 1C). That may be because the velocity of renaturation or hy-

bridization might be expected to be fast due to the very high concentration of DNA.

To analyze the specific sequences of PCR products, Southern blot hybridization is a useful and indispensable technique. However, it needs multistep experiments and a long time period (>2 days) involving gel electrophoresis, hybridization on nylon membrane, and autoradiography. The detection of the specific sequences of PCR products by hybridization using CE would be easier and more useful than Southern blot hybridization. Using the capillary array system reported previously,⁽⁶⁾ this technique might be able to analyze many DNA samples simultaneously and apply to the routine analysis in place of Southern blot hybridization. Moreover, it will be possible to fractionate the analyzed DNA using CE with the apparatus for fractionation. Therefore, this method might be applied to perform the direct sequencing analysis of hybridized DNA and detect the sequence including a point mutation.

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FIGURE 2 (A) Capillary electrophoretic profile of purified hybrid DNA; (B) capillary electrophoretic profile of the whole liquid hybridization reaction mixture.

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