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Short communication

Application of capillary gel electrophoresis to the diagnosis of the aldehyde dehydrogenase 2 genotype

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Abstract

This study dealt with the application of capillary gel electrophoresis (CGE) to diagnosis of the aldehyde dehydrogenase 2 (ALDH-2) genotype. Electrophoresis was performed on a low cross-linked polyacrylamide gel {3% T [g acrylamide + g Bis (N,N′-methylenebisacrylamide)], 0.5% C (g Bis/%T)} in 100 m*M* Tris–borate buffer (pH 8.3) at −10 kV with on-column UV detection (260 nm). During the PCR reaction, DNA from the wild-type allele generated a MboII restriction site, which is an amplification created restriction site. This did not occur, however, with DNA fragments from the mutant allele. Therefore, determination of the heterozygous genotype, the coexistence of wild-type and mutant alleles, was easily possible. Analysis of the MboII restriction digests of the PCR products was completed in less than 20 min, showing two peaks corresponding to fragments of 125 (cleaved) and 135 (uncleaved) base pairs (bp), respectively. On the other hand, determination of the homozygous genotype, wild-type or mutant, was difficult in one electrophoresis run. The CGE of the MboII restriction digests gave a single peak and the identification, cleaved or uncleaved, was difficult under our experimental conditions. However, the addition of aliquots of the PCR reaction mixture to the restriction digests, followed by re-electrophoresis, allowed successful diagnosis, yielding two peaks (cleaved and uncleaved) for the wild-type and one peak (uncleaved) for the mutant allele. This study demonstrated that CGE offers a high-speed, high-resolution analytical tool for determining genetic types, as compared with the conventional slab gel methodologies.

Keywords: Aldehyde dehydrogenase 2; Enzymes; DNA

1. Introduction

Examination of double-stranded DNA (dsDNA) fragments, such as PCR amplification products and their restricted DNA fragments, plays a crucial role in important areas of molecular biology such as human disease diagnosis, forensic identification and human genomic mapping. In the past decade, capillary electrophoresis (CE) has been the object of intensive research and development in analytical chemistry and, consequently, has found application in many analytical fields, including the separation and examination of biopolymers and particularly DNA fragments, in which CE undoubtedly is one of

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the most interesting and quickly advancing new instrumental devices. In reality, CE is a high-resolution tool which can provide an alternative to the conventional slab gel methods of DNA analysis [1–8].

Liver aldehyde dehydrogenase (ALDH) plays a major role in alcohol metabolism. In particular, mitochondrial aldehyde dehydrogenase (ALDH-2) has been reported to play a crucial role in acetaldehyde oxidation in vivo, suggested to be the cause of the existence of racial differences in sensitivity to alcohol intake [9–11]. A point mutation in the ALDH-2 gene, which resulted in two distinct alleles, has been characterized. The ALDH-2 genotype has been diagnosed using an allele-specific oligonucleotide probe after PCR amplification [12–14]. Recently, Harada and Zhang [15] reported a new technique for analyzing the ALDH-2 mutant by means of an amplification-created restriction site (ACRS). The small DNA sequences obtained have been separated conventionally by slab gel electrophoresis.

We report here an application of CE for the diagnosis of the ALDH-2 genotype using the ACRS technique that creates a MboII restriction site on the wild-type allele but not on the mutant one. The advantage of this method is the ability to carry out real-time detection and accurate identification using an on-line optical detection system. In addition, the data can be obtained in the short time of within 20 min for one electrophoresis run, after which they can be stored and examined in electropherograms. Other advantages are the very small sample volumes and multiple use of the capillary.

2. Experimental

2.1. Chemicals

γ-Methacryloxypropyltrimethoxysilane was purchased from Sigma (St. Louis, MO, USA). PhiX174 DNA-HaeIII digest (DNA M_r marker), acrylamide, N,N′methylenebisacrylamide (Bis), N,N,N′,N′-tetramethylenediamine (TEMED) and ammonium peroxodisulphate (APS) were obtained from Wako (Osaka, Japan). A fused-silica capillary tube (100 μm I.D.) was obtained from GC Sciences (Tokyo, Japan). A Gene Amp PCR reagent kit with Amplitaq DNA polymerase and a restriction enzyme, MboII, were obtained from Takara (Otzu, Japan). Other chemicals were of analytical-reagent grade.

2.2. Apparatus

A Model 270A capillary electrophoresis system (Applied Biosystems, Division of Perkin-Elmer, Foster City, CA, USA) was used in the reversed-polarity mode (cathode at the injection side). A fused-silica capillary (50 cm total length; effective length 30 cm; 100 μm I.D.) was rinsed with distilled water for 20 min, after which a mixture of methanol and γ-methacryloxypropyltrimethoxysilane (1:1, v/v) was injected into the capillary and left to stand for 3 h. After washing the capillary column with distilled water for 5 min, a gel solution was introduced into the capillary quickly by a vacuum injection system for 20 min and left to stand overnight. The gel solution was prepared as follows. A stock solution consisting of 24% acrylamide and 0.12% Bis was prepared. Then 1 ml of the stock solution was added to 1.6 ml of 100 m*M* Tris–250 m*M* borate buffer (pH 7.8), and the mixture was diluted to 8 ml with distilled water. This solution was carefully degassed, after which 3.2 μl of TEMED and 80 μl of 10% (w/v) APS were added. Electrophoresis was performed using 100 m*M* Tris–borate (pH 8.3) as a running buffer at −10kV at 30°C. The separations were monitored on a column at 260 nm wavelength.

2.3. Sample preparation.

The genomic DNA template for PCR amplification was isolated from healthy human peripheral blood using a nucleic acid extraction kit (IsoQuick, Microprobe, USA).

Two oligonucleotide primers (5′-CAAAT-TACAGGGTCAAGGGCT-3′ and 5′-CCACAC-TCACAGTTTTCTCTT-3′) were synthesized by a DNA synthesizer (Applied Biosystems). ACRS was performed according to the method of Harada and Zhang [15]. A total of 30 cycles of amplification were carried out as follows; denaturation for 1.5 min at 94°C, annealing for 3 min at 58°C and extension for 1 min at 72°C. With this PCR technique, only the wild-type allele can acquire the MboII restriction site during the amplification step. Therefore, the PCR

products obtained from the wild-type allele can be digested by MboII into smaller fragments. In contrast, those from the mutant allele cannot be digested. After 30 amplification cycles, aliquots (10 μl) of the PCR reaction mixture were incubated with the restriction enzyme (MboII; 5 units) for 4 h at 37°C. The digested samples or the mixtures with the PCR-amplified products were introduced into the capillary column electrophoretically for 30 s at −5 kV.

2.4. Slab gel electrophoresis

The PCR-amplified products and their digested samples were precipitated by 70% ethanol and then were diluted in TE buffer (10 m*M* Tris–HCl, pH 8.0, 1 m*M* EDTA). Electrophoresis of both samples was performed on 10% polyacrylamide gel in a TBE running buffer (90 m*M* Tris–88 m*M* borate, pH 8.3, 2 m*M* EDTA). After electrophoresis (3 h), the bands on the gel were visualized by UV light after staining (20 min) with ethidium bromide at a concentration of 1 mg/ml.

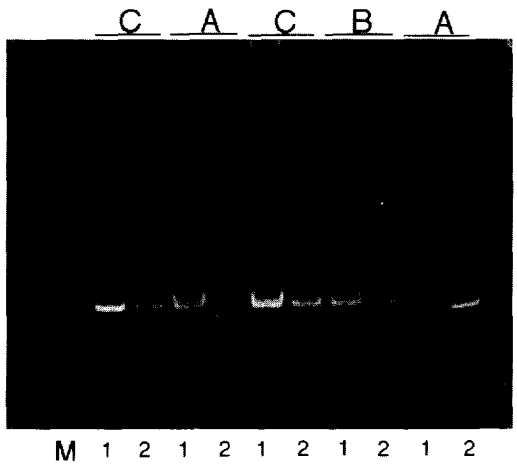

Fig. 2. Polyacrylamide (10%) gel electrophoresis of the PCR-amplified products (1) and the respective MboII restriction digest (2). A: Homozygous genotype of wild-type, B: Homozygous genotype of mutant, C: Heterozygous genotype, M: Marker.

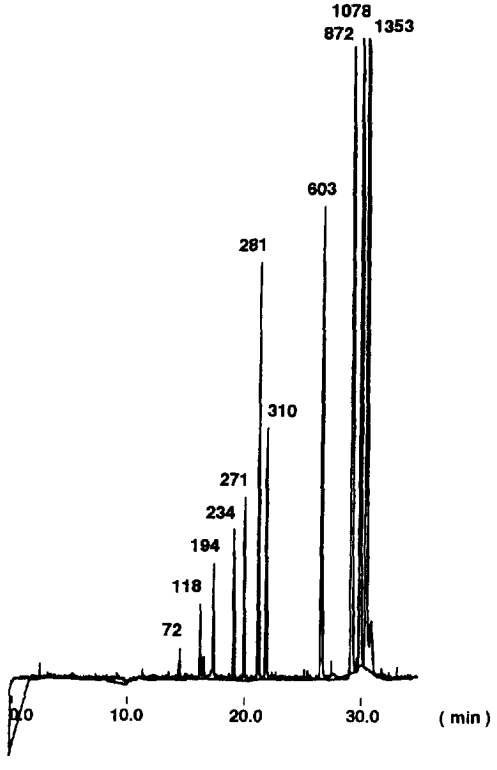

Fig. 1. Electropherogram of PhiX174DNA-HaeIII digest (DNA M_r marker) at 3% T, 0.5% C.

3. Results and discussion

Two approaches have been employed for the analysis of DNA fragments by CE: dynamic sieving electrophoresis and capillary gel electrophoresis (CGE) [16–26]. Others have explored the use of different polymers for better separation of DNA molecules, including glucomannan [27], galactomannan [28], poly(vinyl alcohol) [17,18,28], poly-

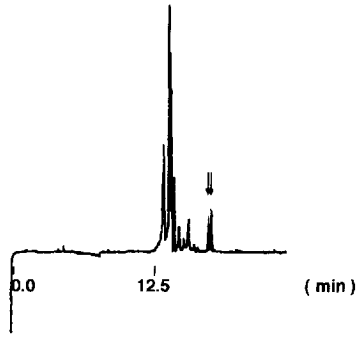

Fig. 3. Electropherogram of the MboII restriction digest of PCR-amplified products from the heterozygous genotype of ALDH-2. Two peaks are shown (arrows).

(ethylene glycol) [28], polyethylene oxide [29] and liquified agarose [17].

CGE, which uses covalently bonded and cross-linked linear polyacrylamide as the sieving mechanism, has been found to provide one of the highest level of resolution for DNA analysis of PCR-amplified products. The weight percentage of monomer plus cross-linker in the polymer is denoted by %T. The mole fraction of cross-linker to monomer is denoted by %C. By varying the concentration of the monomer and the degree of cross-linking, it is possible to create stable and adequate gels to perform sieving electrophoretic separations. Arakawa et al. [30] successfully applied CGE to the detection of single base substitutions in genome genes. Comparing 3% T–0.5% C and 8% T–0% C polymer, these

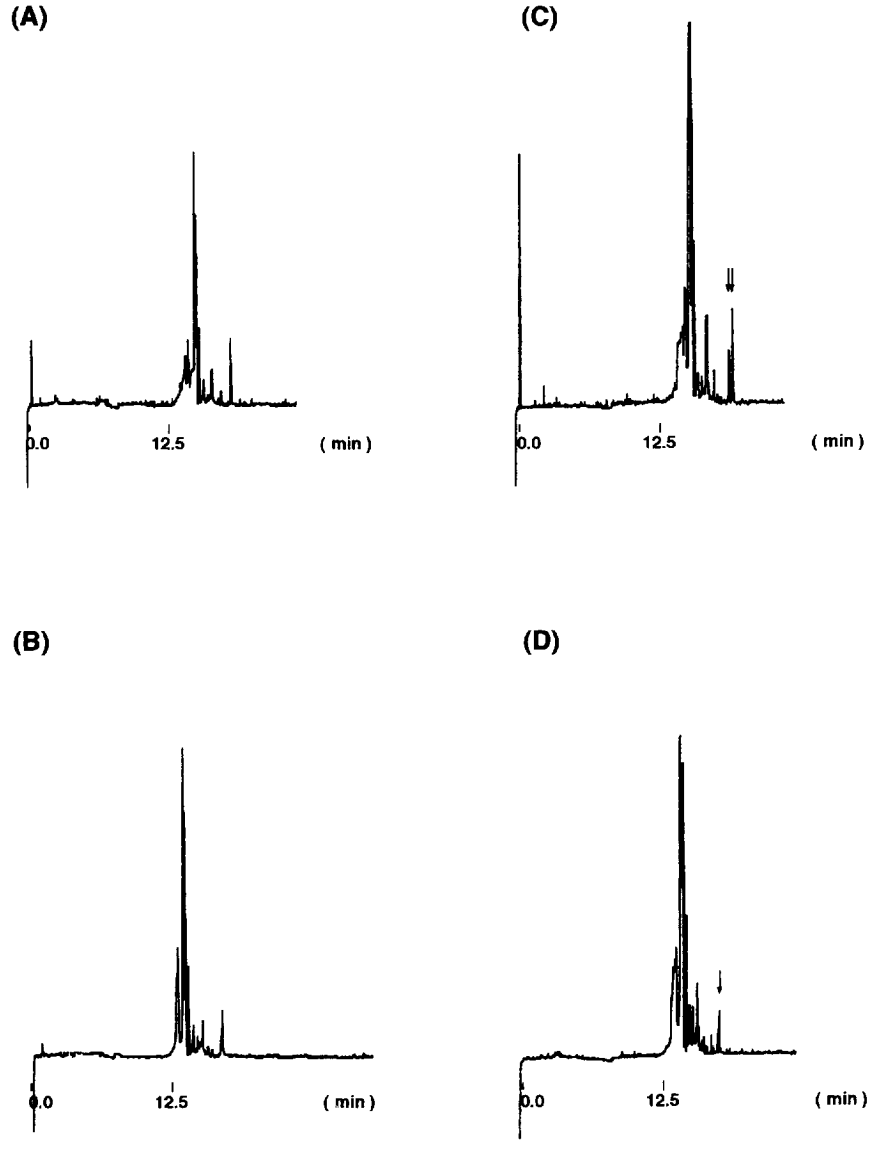

Fig. 4. Electropherogram of the MboII restriction digest of PCR-amplified products from the homozygous genotype of ALDH-2; (A) wild-type and (B) mutant. By adding aliquots of the PCR reaction mixture to the digested samples, two peaks were obtained for the wild-type (C), whereas one peak was obtained for the mutant (D). See the text for details.

researchers concluded that the former condition was superior to the latter in terms of analysis time (migration time) and precision for migration times. Therefore, we used a low cross-linked polyacrylamide gel (3% T–0.5% C) in our experiment. A typical electropherogram obtained from a PhiX174 DNA-HaeIII digest with 3% T–0.5% C cross-linked acrylamide is shown in Fig. 1. We obtained good correlation between migration time and DNA molecular size. The life of this capillary gel was about 60 separations.

For diagnosis of the ALDH-2 genotype, the PCR reaction was carried out according to the method of Harada and Zhang [15], and the amplification product was directly digested by MboII. We analyzed both the restriction fragments and the PCR-amplified products using CGE as well as conventional slab gel electrophoresis. Fig. 2 shows the gel band pattern obtained by slab gel electrophoresis. The ALDH-2 allele was successfully amplified, giving a 135 base pairs (bp) fragment. As shown in Fig. 2, homozygous alleles from the wild-type allele were digested by MboII, resulting in one band that was 10 bp less than the PCR products, whereas homozygous alleles from the mutant allele were resistant, showing no change in the band from the PCR products. Accordingly, after digestion by the enzyme, the heterozygous alleles showed two DNA fragments, which were 125 and 135 bp, respectively.

Fig. 3 shows a CGE electropherogram of the MboII digestion fragment of the PCR product from the heterozygous allele of ALDH-2. The heterozygous genotype, consisting of wild type and mutant alleles, showed two peaks after treatment with MboII (arrows), the analysis of which required less than 20 min. However, as shown in Fig. 4A and B, digested fragments of the PCR products from the homozygous allele showed one peak, which was difficult to identify as a wild-type or mutant allele with a single electrophoresis run. Therefore, we added aliquots of the PCR reaction mixture to the digested fragments and then conducted electrophoresis once more. As a result, two peaks appeared with the homozygous genotype of the wild-type (Fig. 4C; arrow), whereas the mutant gave a single peak (Fig. 4D; arrow). These results demonstrated that CGE analysis of ACRS is a useful and simple method for determining point mutations in a gene.

In summary, we could identify the ALDH-2 genotype from the number of peaks obtained by CGE. The heterozygous genotype showed two peaks with a single run. On the other hand, determination of the homozygous genotype necessitated re-electrophoresis. Using samples consisting of the PCR reaction mixture and the restriction digests, the genotype respectively showed two peaks and one peak for wild and mutant alleles. Thus, CE was shown to be a high resolution tool which can serve as an alternative to conventional slab gel methods.

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