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Fast high-throughput screening of angiotensin-converting enzyme insertion/deletion polymorphism by variable programmed electric field strength-based microchip electrophoresis

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ABSTRACT

An insertion (I)/deletion (D) polymorphism in angiotensin-converting enzyme (ACE) has been associated with susceptibility to various diseases in numerous studies. Traditionally, slab gel electrophoresis (SGE) after polymerase chain reaction (PCR) has been used to genotype this ACE I/D polymorphism. In this study, single- and multi-channel microchip electrophoresis (ME) methods based on variable programmed electric field strength (PEFS) (i.e., low constant, high constant, (+)/(−) staircase, and random electric field strengths) were developed for fast high-throughput screening of this specific polymorphism. The optimum PEFS conditions were set as 470 V/cm for 0–9 s, 129 V/cm for 9–13 s, 470 V/cm for 13–13.9 s, 294 V/cm for 13.9–16 s, and 470 V/cm for 16–20 s for single-channel ME, and 615 V/cm for 0–22.5 s, 231 V/cm for 22.5–28.5 s, and 615 V/cm for 28.5–40 s for multi-channel ME, respectively. In the multi-channel PEFS-ME, target ACE I/D polymorphism DNA fragments (D = 190 bp and I = 490 bp) were identified within 25 s without loss of resolving power, which was ∼300 times faster than conventional SGE. In addition, PCR products of the ACE gene from human blood samples were detected after only 10 cycles by multi-channel PEFS-ME, but not by SGE. This parallel detection multichannel-based PEFS-ME method offers a powerful tool for fast high-throughput ACE I/D polymorphism screening with high sensitivity.

1. Introduction

Angiotensin-converting enzyme (ACE) is a part of the renin-angiotensin system (RAS), a hormone or hormonal system that regulates blood pressure and body fluids [1]. ACE mainly plays an important role in RAS and an ACE insertion (I)/deletion (D) polymorphism is associated with high blood pressure. The ACE gene, located on chromosome 17, consists of 26 exons and 25 introns and is involved in the metabolism of two major vasoactive peptides, converting angiotensin (Ang) I into the vasoconstricting peptide Ang II and inactivating the vasodilatory peptide bradykinin [2,3]. Many researchers suggest that approximately 50% of the interindividual variability in plasma ACE is related to a major gene polymorphism [4,5]. The ACE I/D polymorphism refers to the presence (I) or absence (D) of Alu elements in intron 16 that are 287 bp long leading to three genotypes: I/I, D/D, and I/D (Fig. 1A). This polymorphism is associated with various diseases such as high blood pressure [6–9], myocardial infarction [10–14], and Alzheimer disease [15,16]. However, it is not easy to directly differentiate between blood with different ACE I/D polymorphisms and wild-type blood samples. For practical clinical application of ACE I/D polymorphism markers, a fast and highly accurate diagnostic method is needed.

Until now, slab gel electrophoresis (SGE) after polymerase chain reaction (PCR) has typically been used to genotype the ACE I/D polymorphism. However, conventional SGE methods are time-consuming with off-line detection and need a large sample volume. In addition, the applied electric field typically ranges from 5 to

Abbreviations: ACE, angiotensin-converting enzyme; Ang, angiotensin; D, deletion; EtBr, ethidium bromide; HCEFS, high constant electric field strength; HVPS, high-voltage power supply; I, insertion; LCEFS, low constant electric field strength; ME, microchip electrophoresis; OL, objective lens; PEFS, programmed electric field strength; PEO, poly(ethylene oxide); PVP, poly(vinylpyrrolidone); R, resolution; RAS, renin-angiotensin system; SGE, slab gel electrophoresis; TBE, tris-Borate-EDTA.

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(A) CTG GAG AGC CAC TCC CAT CCT TTC TCC CAT TTC TCT AGA CCT GCT GCC TAT ACA GTC ACT TTT **TTT TTT TTT TTG AGA CGG AGT CTC GCT CTG TCG CCC AGG CTG GAG TGC AGT GGC GGG ATC TCG GCT CAC TGC AAG CTC CGC CTC CCG GGT TCA CGC CAT TCT CCT GCC TCA GCC TCC CAA GTA GCT GGG ACC ACA GGC GCC CGC CAC TAC GCC CGG CTA ATT TTT TGT ATT TTT AGT AGA GAC GGG GTT TCA CCG TTT TAG CCG GGA TGG TCT CGA TCT CCT GAC CTC GTG ATC CGC CCG CCT CGG CCT CCC AAA GTG CTG GGA TTA CAG GCG TGA TAC AGT CAC TTT T**AT GTG GTT TCG CCA ATT TTA TTC CAG CTC TGA AAT TCT CTG AGC TCC CCT TAC AAG CAG AGG TGA GCT AAG GGC TGG AGC TCA AGS CAT TCA AMC CCC TAC CAG ATC TGA CGA ATG TGA TGG CCA CRT C

(B)

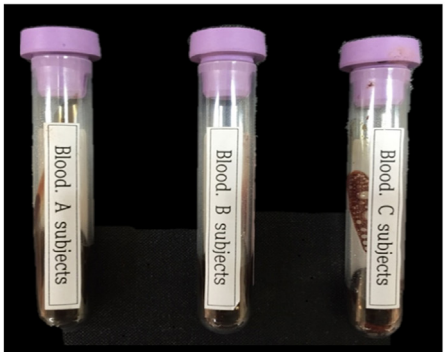

Fig. 1. (A) PCR-amplified DNA sequences of insertion polymorphism. The 1–63 and 353–481 regions of the sequence are unknown. The bold region (64–352) represents the Alu element [7]. The presence or absence of the Alu element in the DNA sequence indicates a genotype of I (490 bp) or D (190 bp). (B) Three human blood samples (a–c) with different ACE I/D genotypes.

50 V/cm due to the heat generated, which leads to a long analysis time as well as poor separation efficiency.

Microchip electrophoresis (ME) has been used typically to micro-analysis due to its short analysis time, high efficiency, and small volume required [17]. Moreover, ME techniques allow DNA analysis using sub-microliter (<μL) volumes and high electric fields with on-capillary detection leading to shorter analysis time, higher resolution, and greater precision than SGE, which commonly needs 2–3 h of analysis time. Thus, the application of ME to biological research on DNA [18–21], proteins [22,23], neurotransmitters [24–26], and nerve agent detections [27] has been widely reported. In particular, since programmed electric field strength (PEFS) in ME is a fast and simple-to-use analysis technique without loss of resolving power by changing the electric field for analysis of target DNA molecules with a specific length, many applications of PEFS have been reported [28–35].

In this study, we provide a fast detection method for the ACE gene using a lab-built ME system with a laser-induced fluorescence detector based on various PEFS parameters. PCR-amplified specific DNA fragments (i.e., D = 190 bp and I = 490 bp) from human blood samples were analyzed in only 25 s using ~nL sample volumes with high sensitivity. The parallel detection-based PEFS-ME method was also applied for fast high-throughput screening of the ACE I/D polymorphism. We believe this method has the potential to be a convenient tool for fast high-throughput screening of various gene-related diseases.

2. Materials and methods

2.1. Materials

1 × TBE buffer (0.089 M Tris, 0.089 M Borate, 0.002 M EDTA, pH 8.44) was made by dissolving a pre-mixed powder (Amresco, Solon, OH, USA) in deionized water. A dynamic coating gel was made by dissolving 1% w/v poly(vinylpyrrolidone) (PVP, M_r = 1 000 000; Polyscience, Warrington, England) in 1 × TBE buffer containing 0.5 μg/mL ethidium bromide (EtBr, Molecular Probes, Eugene, OR, USA). The sieving matrix was made by dissolving 0.5% w/v poly(ethylene oxide) (PEO, M_r = 8 000 000; Sigma, St. Louis, MO, USA) in 1 × TBE buffer containing 0.5 μg/mL EtBr. A 100-bp DNA ladder (3.6 ng/μL) (Bionics, Korea) was used as a reference mark.

2.2. Preparation of PCR products

Peripheral blood samples from each subject were collected in EDTA-coated tubes and stored in a −20 °C freezer (Fig. 1B). Genomic DNA was prepared from peripheral blood using a genomic DNA isolation reagent kit (High Pure PCR template preparation kit; Roche, USA). Genotyping for the ACE I/D polymorphism was performed by PCR, using the forward primer 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and reverse primer 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′ [36]. The PCR was conducted using a commercial PCR mastermix (NanoHelix, Daejeon, Korea). PCR conditions were as follows: initial denaturation at 94 °C for 5 min; annealing at 94 °C for 30 s, 58 °C for 40 s, and 72 °C for 1 min in 10 cycles, 20 cycles, or 30 cycles increments; elongation at 72 °C for 10 min.

2.3. Slab gel electrophoresis

Amplified DNA molecules were identified by SGE in a 1.8% agarose gel (Agarose A, BIO BASIC, Canada) using 0.5 × TBE buffer, and run at 100 V on an MR-100 system (Core Bio System Co., Ltd, Korea). After each electrophoresis, the gel was stained with EtBr, and then the samples were photographed under UV light using a gel imaging system (GelDoc, Core Bio System, Korea). The sizes of the DNA products were determined relative to a 100-bp DNA ladder.

2.4. Single- and multi-channel ME systems

The single-channel ME system was performed using a DBCE-100 Microchip system (Digital Bio Technology, Suwon, South Korea) with a diode-pumped solid-state laser (λ_{ex} = 532 nm) and a high-voltage power supply (HVPS) (DBHV-100, Digital Bio Technology, Suwon, Korea) (Fig. 2A and B). A dichroic mirror and an objective lens (OL) focused the laser on microchip and allowed the excitation fluorescence entering the photomultiplier tube (PMT). A PC (2.80 GHz Intel Pentium® 4) with DBMA-100 software was used to control the HVPS and the PMT. The glass single-channel microchip (MC-BF4-TT100, Micralyne, USA) was 50 μm wide and 20 μm deep (Fig. 2C). The reservoirs were 2 mm in diameter and 1 mm deep. The introduction channel had a double-T structure with a 100 μm offset. The introduction channel was 8.0 mm long, the separation channel was 85 mm long and the detection was performed at 13 mm from the injection point of the double-T channel.

The lab-built multi-channel ME system was equipped with a diode-pumped solid-state laser (model GL-532T-030, λ_{ex} = 532 nm, 30 mW; Shanghai laser & Optics Century Co., Shanghai, China) which was applied for laser excitation (Fig. 2D and E). A charge-coupled device (CCD) (model 01-EXI-BLU-R-F-M-14-C, QImaging Co., Surrey, BC, Canada) was used to monitor fluorescence signals