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Short communication

On-line capillary electrophoresis for enhanced detection sensitivity of *feline panleukopenia* virus

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ABSTRACT

A rapid on-line capillary electrophoresis (CE) method for highly sensitive detection of DNA molecules with specific lengths was developed based on the combination of base stacking (BS) and programmed field strength gradients (PFSG). The BS method has been performed for on-column concentration to improve detection sensitivity without any modification of the CE system. PFSG increased the electrophoretic velocity of DNA molecules, which effectively decreased analysis time. Using the BS and PFSG combination, the amplified PCR product (340-bp DNA) of cats infected with *feline panleukopenia* virus was detected within 6.5 min. Detection sensitivity (~10-fold) was enhanced compared to conventional CE analysis. The combined on-line CE/BS-PFSG methodology could be an effectively rapid analysis technique for the highly sensitive detection of disease-related specific DNA molecules.

1. Introduction

Feline panleukopenia (FP), caused by single-stranded DNA-containing *feline parvovirus*, is a highly contagious disease affecting all members of the biological family *Felidae* [1–3]. The clinical disease ranges from subclinical infection to peracute syndrome with sudden death. In most cases, the first signs are depression, fever, anorexia, and vomiting, particularly in the early stages. Diarrhea then develops and cats may become dehydrated [1]. Clinical manifestations of *feline panleukopenia* virus (FPV) infection are enteritis, panleukopenia, cerebellar hypoplasia, and fetal death.

FPV analytical methods include enzyme-linked immunosorbent assays [4] and rapid immunomigration tests for detecting viral antigen and polymerase chain reaction (PCR) tests for detecting viral DNA in samples [2,3]. However, these methods have some flaws, such as no reliable result (38.9–100% predictable value) [3], expensive reagents and time-consuming processes. Simple and accurate methods for fast diagnosis have been developed to overcome these problems.

Capillary electrophoresis (CE) has become one of the most powerful tools for DNA fragment analysis because it is easy to detect the specific amplification of PCR products. CE also has potential for use in clinical diagnosis methods [5–8]. Many studies have reported on detecting disease-related DNA fragments accurately and rapidly using CE [8]. However, for detecting clinical samples, conventional CE showed low detection sensitivity due to a small sample volume [9,10]. Recently, on-column concentration techniques such as base stacking (BS) have been developed and applied to improve the detection sensitivity of DNA fragments [5,9–12]. BS was achieved by an electrokinetic injection of hydroxide ion (OH^-) before injecting the DNA sample without chemical derivatization. BS showed some advantages, including short analysis time, no complications of procedure, and efficient method. In addition, the device or instrument did not require modification [5].

To reduce analysis time, a programmed field strength gradient (PFSG) technique has been performed [13–17]. PFSG can control the electrophoretic velocity of DNA molecules by changing electric fields. By altering the electric field strength, target DNA molecules of a specific length can be rapidly separated without losing resolving power.

In this study, the BS and PFSG methods were combined with CE to enhance detection sensitivity and decrease analysis time. The combined on-line CE/BS-PFSG method was applied to detect FPV as an infection disease model for fast and enhanced detection sensitivity. The on-line CE technique combined with BS and PFSG is expected to be an effective analysis method with rapid and highly sensitive detection of disease-related specific DNA molecules.

2. Materials and methods

2.1. Materials

The 1 × TE (50 mM Tris–HCl, 2 mM EDTA disodium, pH 8.0) buffer was prepared by dissolving Tris–base, Tris–HCl, (Amresco, Solon,

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OH, USA), and EDTA disodium salt (Sigma, St. Louis, MO, USA) in deionized water (18 MΩ). The capillary sieving matrix was made with 2.0% (w/v) poly(vinylpyrrolidone) (PVP, M_r = 1 000 000) (Polyscience, Warrington, England) diluted in 1× TE buffer containing 0.5 μg/mL ethidium bromide (EtBr, Molecular Probes, Eugene, OR, USA). The mixture was shaken for 1 min and left to stand for 2 h to release bubbles. The 100-bp DNA ladder was purchased from Genepia (Seoul, Korea). All DNA samples were diluted with 1× TE buffer prior to use.

2.2. Clinical sample preparation

The FPV sample was acquired from the Neodin Veterinary Science Institute (Seoul, Korea). A 2-year-old neutered male Domestic Short Hair had a history of vomiting and diarrhea. A 2 mL sample of peripheral blood was obtained by venipuncture and collected into an EDTA anticoagulant tube for viral DNA extraction by PCR.

2.3. Viral DNA extraction and PCR amplification of the VP2 gene

Viral DNA was extracted from specimens using a Viral Nucleic Acid Extraction Kit II (Geneaid, Taiwan) according to the manufacturer's instructions. To amplify the VP2 gene of FPV, forward (5′-CAGAATCTGCTACTCAGCCAC-3′) and reverse (5′-GTAGCAAATTCATCACCTGTT-3′) primers were used. Amplification conditions for PCR consisted of an initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 40 s. Sequencing was performed using the dideoxy termination method with an automatic sequencer (ABI PRISM 3730XL DNA Analyzer, Applied Biosystems, CA, USA). Sequence identity searches were conducted using the National Center for Biotechnology Information (NCBI, National Institutes of Health, USA) BLAST network service [17].

2.4. Capillary electrophoresis system

The experimental lab-built CE system with a laser-induced fluorescence (LIF) detector was described previously (Fig. 1A) [5]. Briefly, a diode-pumped solid-state laser (λ_{ex} = 532 nm; Power Technology Inc., Alexander, AR, USA) was used as the light source in the CE system. The laser was coupled to a LIF detector as a photomultiplier tube (Hamamatsu Photonics K.K., Japan). A Bertan ARB 30 high-voltage power supply (Bertan High Voltage Inc., NY, USA) was used to drive electrophoresis. A bare fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 35 cm (effective length of 21 cm) and an I.D. of 75 μm was used for the separation. Running buffer was made with 1× TE buffer containing 0.5 μg/mL of EtBr. The sieving matrix was hydrodynamically injected at one end of the capillary through a syringe. After the sample was injected electrokinetically at 50 V/cm for 45 s, sample separation was performed by an electric field. The capillary was reconditioned after each run by rinsing successively in water, 0.1 M NaOH, water, and running buffer. Data was recorded as a function of time at 2 Hz. Data output and analysis were performed using Lab view (version 6.1, National Instruments, Austin, TX, USA).

2.5. Base stacking method

The BS mechanism is illustrated in Fig. 1B. First, $Tris^+$ containing background buffer (1× TE buffer) was injected onto the capillary. Then the DNA sample was introduced by applying 50 V/cm. Immediately afterward, OH^- ions were injected into the capillary by applying an electric field. A neutralization reaction occurred between OH^- ions and $Tris^+$ buffer ions forming a low-conductivity zone. As a result, concentrated DNA fragments stacked in front of the sample plug and were separated by different electric mobility.

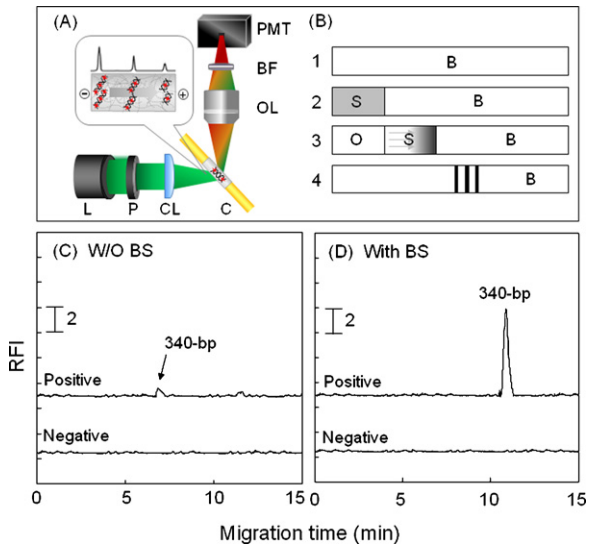

Fig. 1. Schematic diagram of the lab-built CE-LIF system (A) with BS mechanism (B) and representative CE electropherograms of the amplified PCR products of FP (C) without BS and (D) with BS using 0.1 M NaOH. *CE conditions*: sample, PCR product of FPV; running buffer, 1 × TE buffer (pH 8.0) with 0.5 ppm EtBr; sieving matrix, 2.0% PVP (M_r = 1 000 000); injection electric field, 50 V/cm for 45 s; OH^- injection electric field, 200 V/cm for 0–90 s; separation electric field, 200 V/cm; capillary total length, 35 cm (75 μm I.D.); capillary effective length, 21 cm. *Indications*: L, laser; P, pinhole; CL, convex lens; C, capillary; OL, objective lens; BF, band-pass filter; PMT, photo-multiplier tube; B, background buffer; S, DNA sample; O, hydroxide.

2.6. Programmed field strength gradients

PFSG changes the field strength during analysis and is used to decrease the separation time of differently sized DNA fragments [13]. PFSG separation eliminate portions of the gradient prior to the first DNA peak and following the last DNA peak or decrease the portion between the two DNA base pair fragments in the regions of interest. Different electric fields are optimization for different-sized DNA fragments. Thus, PFSG can be programmed to give the best separation and resolution (R_s > 1.5) of all DNA fragments.

2.7. Combined on-line capillary electrophoresis with BS and PFSG

For faster and more sensitive DNA molecule separation, the BS and PFSG methods were combined during one CE separation. Changing the electric field for BS-PFSG was considered to be a BS optimized condition because of the stacking procedure. While the concentration process progressed after introducing OH^-, applying a high electric field can cause electrical discontinuity through the channel. After injecting OH^- using an electrokinetic method of 50 V/cm for 90 s, the high electric field of 286 V/cm was applied to separate DNA fragments under constant electric field strength of 200 V/cm for 120 s.

3. Results and discussion

3.1. Sensitivity enhancement of DNA molecules by base stacking

The stacking procedure simultaneously introduced OH^- ions within the low-conductivity zone, in which DNA fragments moved more rapidly than in the untreated zone. Optimum conditions were determined during our previous study by varying the concentration of hydroxide and the injection times of NaOH [5]. The optimum BS conditions were determined to be a 90 s injection of 0.1 M NaOH

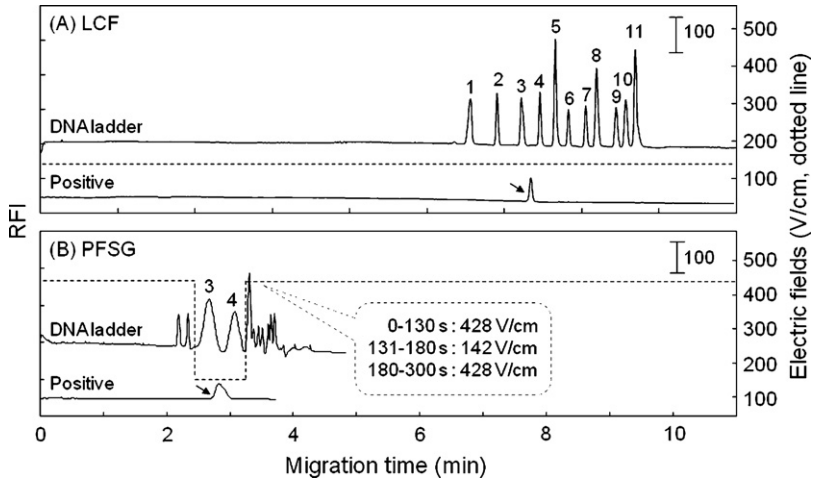

Fig. 2. Representative CE electropherograms of the PCR product of FPV obtained using (A) LCF and (B) PFSG methods. *CE conditions*: injection electric field, 50 V/cm for 45 s; applied separation electric field strength, 142 V/cm for LCF; 428 V/cm for 0–130 s, 128 V/cm for 131–180 s, 428 V/cm for 181–300 s for PFSG. Other conditions are the same as Fig. 1. *Indications*: 1, 100; 2, 200; 3, 300; 4, 400; 5, 500; 6, 600; 7, 800; 8, 1000; 9, 1500; 10, 2000; 11, 3000-bp DNA.

with a 45 s injection of DNA sample. Under these optimum conditions, the amplified PCR product of FPV was separated successfully with detection sensitivity enhanced about 10.31-fold (Fig. 1D) more than a normal CE separation (Fig. 1C).

3.2. Fast detection by PFSG

In general, an increase in electric field strength enhanced the velocity of negatively charged DNA fragments and reduced migration time. A higher applied voltage increases the separation velocities of DNA fragments [18,19] and results in fast analysis of target DNA molecules. However, higher voltages result in Joule heating due to high currents in the inner capillary [20]. The formation of heat induced the problems in separation, such as peak broadening, non-reproducible migration times, sample decomposition, and electrical discontinuity through the capillary. Therefore, the optimum electric field strength needs to be determined to decrease analysis time under non-influenced Joule heating.

To separate the PCR products of FPV by increasing separation velocity, three electric field strengths were applied: low constant field (LCF), high constant field (HCF), and PFSG. A LCF of 142 V/cm (Fig. 2A) separated all standard 100-bp DNA ladder fragments within 10 min. With the HCF of 428 V/cm, all DNA fragments migrated within ~3 min with poor separation resolutions (data not shown). Because amplified PCR product has a specific DNA length (340-bp), PFSG was used to separate the 100-bp DNA ladder between 300- and 400-bp. Under PFSG, the migration times of 100-200 bp DNA fragments were reduced by applying 428 V/cm for 130 s and decreasing the electric field to 142 V/cm for 50 s (Fig. 2B). The 300–400 bp DNA fragments were then separated with adequate resolution of the regions of interest between the two DNA fragments. After the migration of the 400-bp DNA, the electric field was increased to 428 V/cm to allow rapid migration of the DNA ladder above 400-bp. As a result, the selected region of the 100-bp DNA ladder was analyzed in 3.5 min. PFSG was applied with the determined optimum conditions to rapidly detect amplified PCR products of FP infected cat blood (Fig. 2).

3.3. Clinical application of CE-BS/PFSG for rapid detection with high sensitivity

For faster and more sensitive detection of amplified PCR product of the FPV sample, BS and PFSG methods were combined during one step CE separation. The BS and PFSG methods have

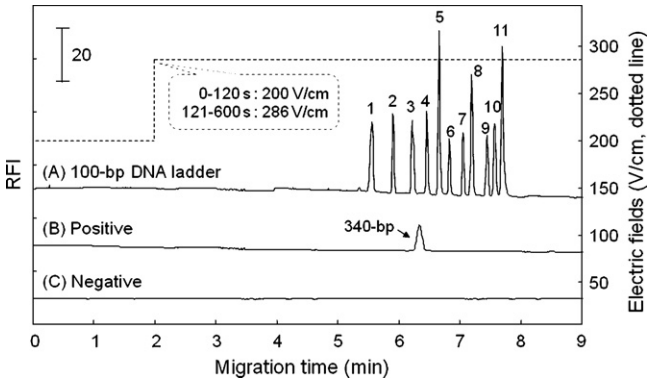

Fig. 3. Representative CE electropherograms of (A) 100-bp DNA ladder, (B) positive sample (FPV-infected cat), and (C) negative sample (normal cat) obtained using combined on-line CE-BS/PFSG method. CE conditions: separation electric field, 200 V/cm for 0–120 s, 286 V/cm for 121–600 s for PFSG. Other conditions and indications are the same as in Fig. 2.

distinct advantages that result in improved detection sensitivity and reduced analysis time, respectively. The optimum conditions of the BS-PFSG method were considered by changing the electric field under the BS optimized conditions (Fig. 3) to ensure enough stacking to prevent electrical discontinuity through the capillary channel. Accordingly, a constant field strength of 200 V/cm was applied for 120 s during the initial separation time. When the inner capillary current was held constant, a high electric field of 286 V/cm was applied for separation. The clinical sample was detected with ~10-fold higher sensitivity and ~2-fold faster analysis compared to the normal separation method.

At optimum conditions, the repeatability of the combined detection method was tested with the blood of cats infected with FPV, as shown in Table 1. The standard deviations of migration time,

Table 1
Effect of combined on-line CE-BS/PFSG method with comparison of migration time, peak height, and peak area reproducibility.

	Mean ± SD[a]	RSD (%)[b]
Migration time	6.303 ± 0.0378	0.6006
Peak height	3.960 ± 0.3109	7.8526
Peak area	89.4731 ± 9.6708	10.8086

[a] Mean ± SD = mean ± standard deviation.
[b] RSD (%) = average/SD × 100 (n = 3).

Table 2
Comparison of conventional SGE, normal CE, and combined CE-BS/PFSG at the application of clinical samples.

Sample	SGE	Normal CE	CE-BS/PFSG
1	+[a]	+[a]	+[a]
2	+[a]	+[a]	+[a]
3	ND[b]	+[a]	+[a]
4	+[a]	+[a]	+[a]
5	ND[b]	ND[b]	ND[b]
6	+[a]	+[a]	+[a]
7	ND[b]	ND[b]	+[a]

[a] Detected the amplified PCR product of FPV-infected clinical sample (340-bp DNA).
[b] ND: not detected.

peak height and peak area demonstrated the high reproducibility. Moreover, clinical samples from five cats with suspected FP and two normal cats were analyzed to compare the CE-BS/PFSG method with conventional SGE and normal CE (Table 2). The target DNA molecule related to FPV (340-bp DNA) from sample 3 was not detectable using slab gel electrophoresis. In the FPV-infected clinical cat sample (sample 7), the target DNA was not detected by either SGE or normal CE methods. However, the combined on-line CE-BS/PFSG method could detect FP disease-related DNA fragments within 6.5 min in clinical samples with high detection sensitivity.

4. Conclusions

A novel combined on-line CE/BS-PFSG method was developed for accurate and rapid FP diagnosis. BS and PFSG have been used for improved detection sensitivity and reduced analysis time. The PCR products of FPV were analyzed within 6.5 min with detection sensitivity enhanced by ~10-fold at the optimum combined on-line CE/BS-PFSG condition. There was no significant loss of baseline separation (R_s > 1.5). In clinical applications, the combined method showed higher accuracy than conventional SGE and CE methods. The combined on-line CE/BS-PFSG method was proved to be an effectively rapid analysis technique for the highly sensitive detection of disease-related specific DNA molecules.

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References

[1] U. Truyen, D. Addie, S. Belák, C.B. Baralon, H. Egberink, T. Frymus, T.G. Jones, K. Hartmann, M.J. Hosie, A. Lloret, H. Lutz, F. Marsilio, M.G. Pennisi, A.D. Radford, E. Thiry, M.C. Horzinek, J. Feline Med. Surg. 11 (2009) 538.
[2] N. Decaro, C. Desario, M.S. Lucente, F. Amorisco, M. Campolo, G. Elia, A. Cavalli, V. Martella, C. Buonavoglia, J. Virol. Methods 147 (2008) 67.
[3] F.F. Neuerer, K. Horlacher, U. Truyen, K. Hartmann, J. Feline Med. Surg. 10 (2008) 247.
[4] J. Esfandiari, B. Klingeborn, J. Vet. Med. B 47 (2000) 145.
[5] A. Shin, S.H. Kang, Bull. Korean Chem. Soc. 32 (2011) 4316.
[6] M. Lee, K. Cho, D. Yoon, D.J. Yoo, S.H. Kang, Electrophoresis 31 (2010) 2787.
[7] H. Go, M. Lee, D. Oh, K.-S. Kim, K. Cho, D.J. Yoo, S.H. Kang, Bull. Korean Chem. Soc. 30 (2009) 2141.
[8] S. Jeon, M.-J. Lee, J. Park, S.H. Kang, J. Chromatogr. B 854 (2007) 268.
[9] D.-K. Kim, S.H. Kang, J. Chromatogr. A 1064 (2005) 121.
[10] M.E. Hoque, S.D. Arnett, C.E. Lunte, J. Chromatogr. B 827 (2005) 51.
[11] Y. Xiong, S.-R. Park, H. Swerdlow, Anal. Chem. 70 (1998) 3605.
[12] S.H. Kang, S. Jang, S.-K Park, Bull. Korean Chem. Soc. 27 (2006) 1346.
[13] S.H. Kang, M. Park, K. Cho, Electrophoresis 26 (2005) 3179.
[14] Y.-J. Kim, J.-S. Chae, J.K. Chang, S.H. Kang, J. Chromatogr. A 1083 (2005) 179.
[15] K.S. Kumar, S.H. Kang, Electrophoresis 28 (2007) 4247.
[16] K.S. Kumar, H.G. Lee, D.J. Yoo, S.H. Kang, Bull. Korean Chem. Soc. 29 (2008) 153.
[17] http://blast.ncbi.nlm.nih.gov/
[18] A. Guttman, B. Wanders, N. Cooke, Anal. Chem. 64 (1992) 2348.
[19] D.R. Baker, Capillary Electrophoresis, John Wiley & Sons, New York, 1995.
[20] C. Heller, Electrophoresis 21 (2000) 593.