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Highly sensitive analysis of nucleic acids using capillary gel electrophoresis with ultraviolet detection based on the combination of matrix field-amplified and head-column field-amplified stacking injection

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ABSTRACT

To develop a highly sensitive method for analyzing nucleic acids using capillary gel electrophoresis with ultraviolet detection (CGE-UV), we combined matrix field-amplified with head-column field-amplified stacking injection (C-FASI) to employ the advantages of two methods. Without diminishing the resolution, a limit of detection of 0.13 ng/ml (signal/noise = 3) in a 300,000-fold diluted sample was obtained, the sensitivity is 102,308 times higher than that achieved with normal pressure injection, 3077 times that with normal electrokinetic injection, 154 times that with pressure field-amplified sample stacking injection, and 31 times that with matrix field-amplified stacking injection. After establishing the method, we tested the detection of a φX174-Hae III digest DNA product without purification and with a high ionic strength. At the lowest dilution of 5000-fold, sample at a concentration of 10 ng/ml was enriched and detected. The relative standard deviations for migration time and peak area ($n = 3$) were 0.03–1.15 and 0.72–6.42, respectively. To further validate C-FASI was applicable for real sample, a 400 bp PCR product without purification was directly detected with a limit of detection at the concentration of 6000-fold dilution (signal/noise = 3), The relative standard deviations for migration time and peak area ($n = 6$) were 0.44 and 4.8, respectively. These results indicated that C-FASI had good qualitative and quantitative detection abilities and CGE-UV based on C-FASI is easy to perform, practical, highly-sensitive and robust for nucleic acid detection, which makes it a highly valuable tool for genetic diagnostics based on nucleic acid analysis.

1. Introduction

Capillary gel electrophoresis (CGE) is becoming a preferred technique for DNA analysis due to its high-resolution power, speed, and quantitative ability, especially in comparison to conventional slab gel electrophoresis [1]. However, in DNA analysis by CGE, first, the double bond formed by the base group in DNA has a low ultraviolet-visible (UV) absorption. Second, because normal UV detectors have low sensitivity, DNA analysis by CGE with UV detection (CGE-UV) is obstructed. Moreover, the intrinsically low detection sensitivity of capillary electrophoresis (CE), which are the limited light absorption dimension and the confined length resulting in small sample injection volume, also greatly restricts the application of CGE-UV to DNA analysis. This is particularly true when the sample concentration is very low, such as in the fields of clinical diagnostics, gene therapy, forensic investigations, and other biomedical studies.

Based on these facts, most researchers agree that fluorescence detection is the solution for improving sensitivity for DNA analysis by CE [2]. However, this procedure requires labeling samples with a fluorescent marker or intercalating dyes [3], which is time-consuming and expensive, and many dyes are toxic (such as ethidium bromide [EB]). Moreover, fluorescence detectors are expensive, and the data obtained in fluorescence detection electropherograms require integration with that acquired from the more widely used UV electropherograms. Consequently, fluorescence detection in CE is not yet extensively applied like traditional techniques.

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In the post-genome era following [4] the Human Genome Project [5], individual treatments for human diseases are emphasized. Consequently, future trends in medical development involve diagnosing diseases at the gene-level and prescribing preventive treatments based on the diagnoses as rapidly as possible [6–13]. Mass data analysis is inevitable for these purposes, and CE, which offers advantages of high-speed, automation, and high-throughput capabilities can undoubtedly meet the requirements for analyzing large numbers of samples quickly [14–17].

Therefore, to promote the application of CE in medical diagnostics, the critical limitation of CE sensitivity must be addressed. Toward this end, on-line sample pre-concentration techniques are considered to be the most effective strategies [18]. Among them, the on-line field-amplified stacking injection (FASI) strategy is most frequently applied to enhance the sensitivity of CE due to its simplicity, high efficiency and established mechanism.

There are several normal stacking injection modes based on field-amplification. Under field-amplified sample stacking (FASS), a sample is at least ten times less conductive than the BGE, and is injected hydrodynamically into the capillary filled with a high-conductivity BGE [19,20]. Sometimes a short low-conductivity medium (such as a water-plug) is added before injection of the sample. Under field-amplified stacking injection (FASI), two modes termed head-column field-amplified stacking injection (HC-FASI) and matrix field-amplified stacking injection (M-FASI) are frequently applied. HC-FASI injects a short low-conductivity medium (such as a water-plug) before electrokinetic injection [21–26], and M-FASI utilizes a low-conductivity medium (such as deionized water) to reduce the ion strength of the sample solution before electrokinetic injection [27–31]. However, they offer limited effects in enhancing sensitivity. For example, when FASS is applied, the length of sample-plug that can be injected into the capillary is limited by the length of capillary (5%) [32], and the extended sample matrix entering the capillary disrupts the resolution. Both effects restrain the augmentation of sensitivity. When M-FASI is applied, although the electrokinetic injection has a better sensitivity than pressure injection at the same concentration and avoids sample matrix entry into the capillary, the sensitivity is still low because at the interface of the sample solution and buffer (high and low field-strength), the sample solution and sample-plug are easily disrupted under extremely high field-strength, which causes a broad, irregular peak, bad reproducibility, limited electrokinetic injection time, etc. When HC-FASI is applied, the stacking interface is pushed into the capillary using injection of a water-plug, the interface is stabilized by the capillary and greatly extends the electrokinetic injection time [33,34]. But based on the FASI principle, more analyte is injected at a higher field-strength during electrokinetic injection. Thus, the sensitivity is still not maximized because of the low field-strength distributed in the sample solution.

Therefore, in the present study, we attempted to combine M-FASI with HC-FASI for the purpose of utilizing the advantages of both techniques. The combined field-amplified stacking injection approach (C-FASI) not only utilizes deionized water to reduce the ion strength of sample solutions but also involves injection of a short water-plug on the head-column of the capillary before electrokinetic injection. Compared to the normal modes, C-FASI possesses the advantages of preventing sample matrix from entering the capillary, prolonging the electrokinetic injection time, allowing more analyte to enter the capillary and stack at the interface of high and low electric field-strength, in addition to better reproducibility, secondary enrichment, and so on. Consequently, the use of C-FASI achieved the highest sensitivity.

Overall, the goal of this research was to develop a feasible method to address the intrinsic limitations of CE in practice and permit sensitive detection of nucleic acids using CGE-UV.

2. Materials and methods

2.1. Apparatus and instruments

All experiments were conducted using a Beckman P/ACE MDQ capillary electrophoresis system fitted with a UV detector. The temperature of the capillary chamber was set at 20 °C. All separations were performed in a coated fused-silica capillary of 65 cm × 100 μm inner diameter (54.8 cm effective length) purchased from Beckman Inc. with product code 477477. Pipettes, volumetric flasks, glass stirring rod, beakers, and 2 ml and 200 μl vials were purchased from Beckman Inc. Data analysis was carried out using 32 Karat 7.0 software (Beckman Inc.).

2.2. Reagents and sample preparation

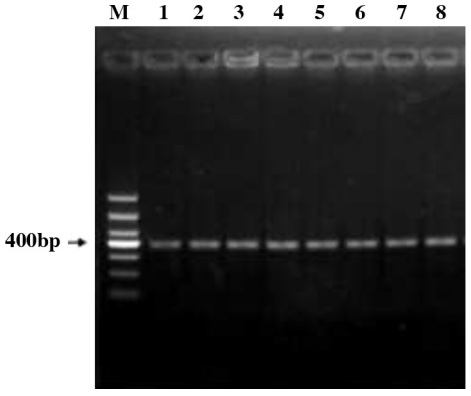
2.2.1. Reagents

A 100 bp DNA ladder composed of 10 DNA fragments of 100–1000 bp was purchased from Fermentas, Inc. The total concentration was 500 μg/ml (individual DNA fragments were 40, 40, 40, 40, 115, 45, 45, 45, 45, and 45 μg/ml for 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 bp, respectively), which was originally stored in TE solution containing 10 mM tris-HCl and 1 mM EDTA. The φX174-Hae III digest DNA product, in loading buffer, was purchased from TaKaRa Bio, Inc. The samples consisted of 11 restriction fragments from 72–1353 bp at a total concentration of 50 μg/ml. A polyacrylamide sieving gel and Tris-borate-EDTA (TBE) buffer for CE were purchased from Beckman, Inc. with a product code 477628. Acetonitrile and methanol of analytical grade were purchased from Aladdin, Inc. Deionized water was used to prepare all solutions, which were filtered through 0.45 μm Millipore filters.

2.2.2. PCR sample preparation

CTAB approach was used to extract the DNA of *Fallopia multiflora*, and then the ITS2 sequence of genomic DNA was amplified by PCR. The PCR conditions were as follows: primers were 5′-ATG CGA TAC TTG GTG TGA AT-3′ and 5′-GTT CGC TCG CCG TTA CTA G-3′; the total volume of PCR reaction system was 50 μL, containing 100 ng genomic DNA, 20 mM primers (each was 10 mM), 4 μL dNTP (each was 2.5 mM), 1.25 U Prime STAR HS DNA Polymerase and 10 μL 5 × Prime STAR Buffer (Mg^{2+} plus). Initial denaturation for 2 min at 98 °C, denaturation for 10 s at 98 °C, annealing for 15 s at 59 °C, extension for 45 s at 72 °C, 30 cycle and final, extension for 8 min at 72 °C.

The PCR product was 400 bp and the electropherogram of agarose gel electrophoresis was as follow:



2.3. Electrophoretic conditions and methods

New capillaries were washed with gel buffer for 10 min and subjected to electrophoresis for 20 min to establish a balanced status.