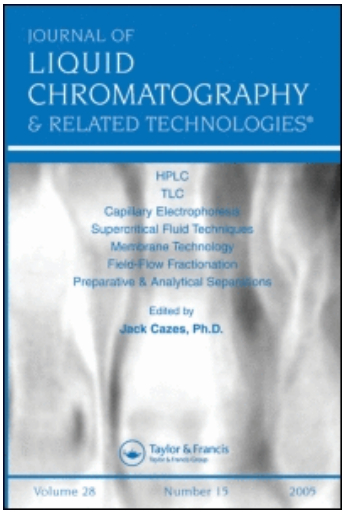

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CAPILLARY ELECTROPHORESIS OF CYTOMEGALOVIRUS DNA IN AN UNTREATED CAPILLARY

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CAPILLARY ELECTROPHORESIS OF CYTOMEGALOVIRUS DNA IN AN UNTREATED CAPILLARY

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ABSTRACT

This work demonstrates that the PCR products of the Cytomegalovirus (CMV) can be detected by capillary electrophoresis in uncoated capillaries using a zwitterionic buffer. Furthermore, dilution of the sample in phosphate buffer leads to stacking which improves the sensitivity by the ultraviolet detection without the need for sample desalting or cleanup.

INTRODUCTION

Capillary electrophoresis (CE) has a few attractive features for the analysis of double-stranded DNA (dsDNA) such as speed, small sample size, and full automation. dsDNA separations usually are performed in CE in coated capillaries to overcome the wall interactions and eliminate the effects of the electroosmotic flow (EOF). Capillaries with different coatings are used in this respect.[1-4] However, these capillaries are expensive and sometimes have a short life. As previously demonstrated a zwitterionic buffer in place of the common Tris buffer can be used to separate the digest of the DNA phage X174 Hae III in uncoated capillaries.[5] Furthermore, we have shown that the sample can be stacked by dilution in KH_2PO_4.

However, this analysis was not tried on samples obtained from the polymerase chain reaction (PCR) products where the salts and the different compounds in the reaction mixture are present and can interfere with the analysis, especially when using ultraviolet light (UV) detection.

Detection of viral infection through amplification of DNA by the (PCR) is becoming desirable and more widespread. Cytomegalovirus (CMV) is an example of viral infection which can be detected through PCR amplification. The detection of this virus is very important especially for the immuno-compromised patient such as in the transplant recipient and patients with AIDS. CMV retinitis affects 20% to 40% of patients with AIDS and is the leading cause of vision loss associated with AIDS.

CMV infection is associated with several of the lymphocyte dysfunctions, as well as bone marrow dysfunction. CMV also is the most common infectious cause of congenital anomalies of the central nervous system caused by intrauterine infection in humans.

Because of its high sensitivity laser-induced fluorescence detection (LIF) is the ideal detection system for DNA analysis by CE, but the LIF is very expensive. The majority of the CE instruments in the field are equipped only with UV detection. This type of detection for DNA analysis suffers not only from poor sensitivity but it is subject to interferences from the different reaction substrates and from the high salt content which causes band broadening. Both of these problems necessitate sample clean up.

The aim of this work is to use CMV as an example to demonstrate that the products of PCR can be detected in CE in an uncoated capillary. Furthermore, the samples can be stacked after dilution in phosphate buffer so their UV detection can be improved without desalting or sample preparation.

EXPERIMENTAL

Apparatus

A Model 2000 CE instrument (Beckman Instruments, Fullerton, CA) was set at 200 V/cm (reversed polarity) and at 280 nm. Untreated silica capillary 25 cm (effective length 17.5 cm) X 75 μm (i.d.) was washed initially for 10 min with the separation buffer.

The samples were diluted ten fold with KH_2PO_4 (5 mg/mL) and were injected for 99 s, at low pressure, filling about 25 % of the capillary volume and electrophoresed for 10 min. The capillary was rinsed between samples with the DNA separation buffer for 2 min.

Capillaries

Untreated capillary was obtained from Polymicro Technologies (Phoenix, AZ).

Chemicals

DNA standard: ds ϕX174 Hae III digest containing 11 fragments at final concentration of 100 μg/mL was obtained from Beckman Instruments. Hydroxypropylmethylcellulose (HPMC) (Viscosity of 2% solution = 3,500-5,600 centipoises) and N-2-hydroxyethyl-piperzine-N'-2-ethyanesulfonic acid sodium salt (HEPES) were obtained from, Sigma Chemicals (St. Louis, MO).

DNA Buffer

The separation buffer contained the following at the final concentration per liter (pH 8.1): HEPES sodium salt 50 mmol, boric acid 65 mmol, HPMC (5 g) and ethidium bromide (1000 μg). These compounds were stirred with aid of a magnetic stirrer until dissolved completely (~ 4 hours).

Polymerase Chain Reaction

The reaction was performed using the synthetic template for CMV,[6] the polymerase and the reaction mixture in a Tris buffer 60mM, pH 8.5. The samples were cycled 30 times. The amplified samples were separated on a 2% agarose gel containing ethidium bromide for detection of the 435 nm characteristic band of the CMV.

RESULTS AND DISCUSSION

The separation of the fragments for the standard digest of the DNA phage X174 Hae III using sample loading of 25 % in the uncoated capillary is illustrated in Figure 1-A. This separation is similar to what we have described previously.[5] The zwitterionic buffer, HEPES, in place of the common buffer Tris eliminates the need for using a chemically coated capillary. Injecting a small amount (2.5 % of the capillary) of the PCR products directly without dilution produced a relatively broad peak due to the presence of salts, Figure 1-B. The UV detection poses another obvious problem which is the presence of several peaks for the different substrates used in the reaction, Figure 1-B. These peaks can interfere with the DNA peaks especially for the small molecular weight fragments (< 200bp).

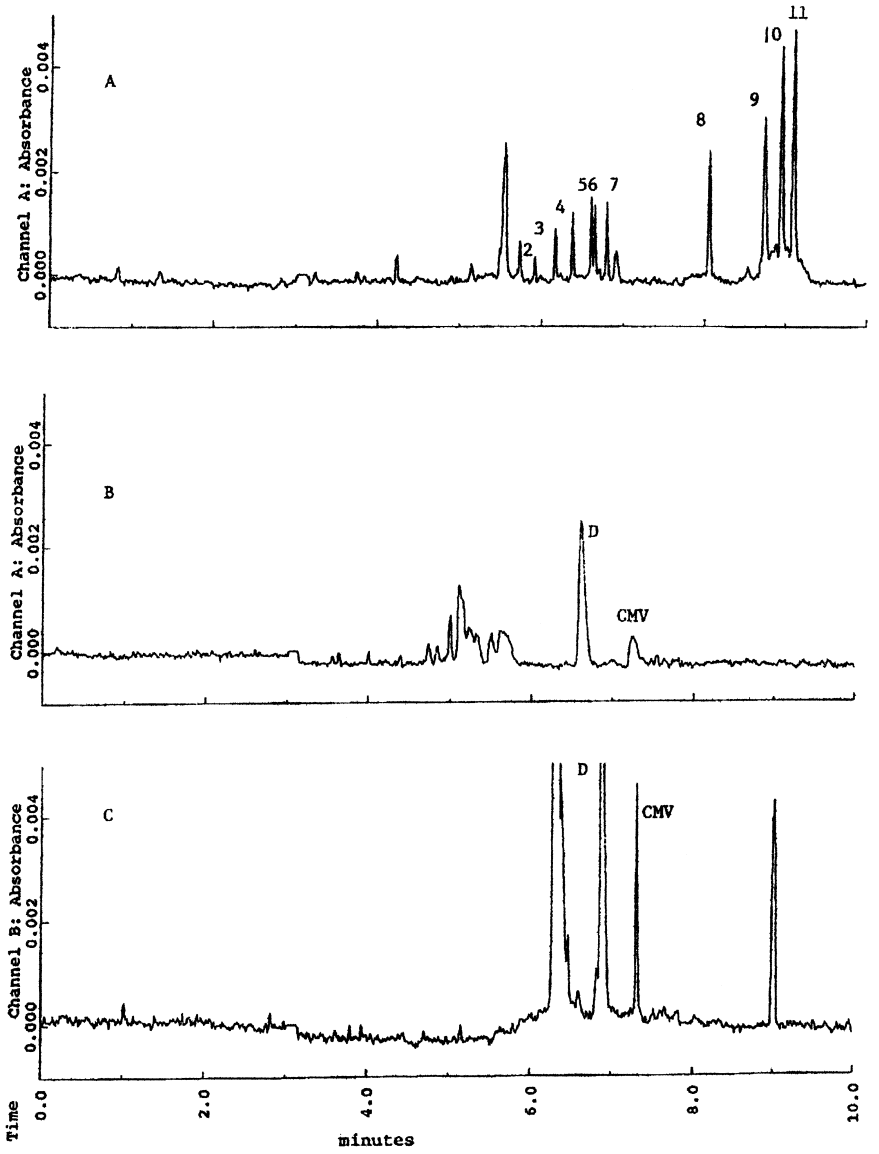

Figure 1. Separation of the DNA fragments from: A- the standard phage in the untreated capillary by pressure injection for 99 s. (Fragments: 2,118; 3,194;4, 234; 5,271; 6,281; 7,301;8,603; 9,872; 10,1078; 11,1353 bp; D, bromophenol blue dye); B- PCR of CMV(435 bp), sample injected directly for 10 s; and C- The same of B but the sample diluted ten fold with KH2PO4 and injected for 99s.

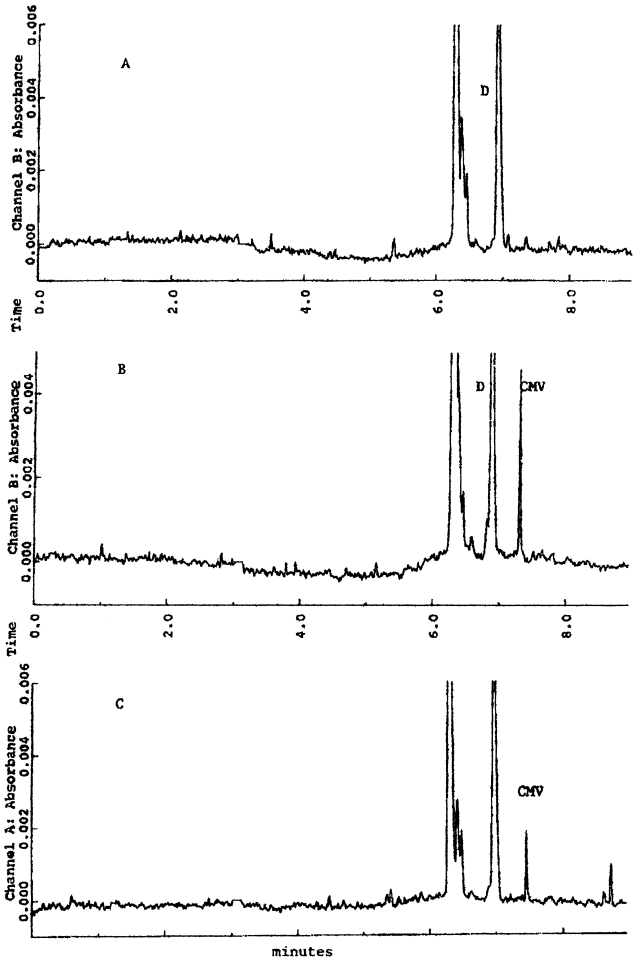

Figure 2. Separation of a sample of CMV(435 bp): A- before the amplification cycle, B- after the amplification. C- Another sample of CMV with lower intensity (on the gel electrophoresis).

On the other hand, if the PCR samples were diluted ten fold with KH_2PO_4 (5 mg/mL), as we described before,[5] the 435 bp DNA fragment of the CMV peak is very sharp and tall due to the stacking effect, Figure 1-C. This effect occurs for both the pure standard and also for the PCR products. In the case of the pure standard we were able increase the injection further to 50% of the capillary[5] but not for the CMV samples.

Figure 2-A shows a positive sample, before Figure 2-B and after the amplification step. Figure 2-C shows another sample with less viral DNA based on fluorescence on the slab gel. We have analyzed 7 samples positive for CMV based on the slab gel with fluorescence detection and 6 samples which were negative. The CE agreed in all cases with all results of the slab gel. The bromophenol blue tracking dye for the slab gel acts as a good marker or as an internal standard in CE too, Figure 1.

We have tried poly(ethyleneoxide) molecular weight 2,000,000 (Aldrich Chemicals, Milwaukee, WI) as a polymer in place of the HPMC. The separation of the standard fragments were similar to that of HPMC; however, the PCR samples showed more interfering peaks which made the detection by the UV more difficult.

The analysis of CMV DNA by this method is used as an example to demonstrate that uncoated capillaries can be used for analysis of PCR products. The UV detection allows samples with relatively high DNA concentration to be detected; however, samples with low DNA concentration require the use of LIF which enhances greatly the detection sensitivity in viral load quantification.[7]

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