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Capillary electrophoresis of DNA Potential utility for clinical diagnoses

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Abstract

The last few years have witnessed a tremendous shift in the use of capillary electrophoresis for clinical applications, particularly with DNA analysis. As a result of the large number of DNA-based clinical assays, there is an intense interest in making DNA analysis faster, less expensive and more automated. We describe the evaluation of CE-based single-strand conformation polymorphism (SSCP) and dideoxy fingerprinting (ddF) analysis for the detection of single-point mutations within a *Mycobacterium tuberculosis*-specific amplified DNA fragment. Both were found to be capable of detecting the mutation in the resistant isolate but ddF showed the most promise with respect to specificity and ease of implementation. In addition, initial results with a CE-based sizing method is shown to be competitive and, perhaps, superior to a Southern blot analysis for the detection of hepatitis C viral (HCV) infection.

1. Introduction

The potential application of capillary electrophoresis (CE) for both routine and esoteric clinical assays is rapidly being realized [1]. CE has been shown to be a viable alternative to many of the standard clinical techniques as a result of its ability to perform rapid, efficient, reproducible analyses in an automated format. CE analyses have been documented for a variety of analytes of biological importance; ranging from small organic ions to large macromolecules (see Ref. [1] and references therein).

The last decade has seen a tremendous shift toward the development of deoxyribonucleic acid (DNA)-based assays for use in the clinical laboratory. The analysis of polymerase chain reaction (PCR)-amplified DNA products is the basis for an ever-increasing number of diagnostic assays, in which the amplified fragments are typically sized by agarose gel electrophoresis followed by DNA–DNA hybridization analysis (i.e., Southern blot evaluation). Alternatively, post-amplification analysis can be accomplished by using DNA sequencing, with the ultimate goal being the identification of specific nucleotide base changes. Since DNA sequencing analysis is a lengthy and more technically-involved process, screening methods have developed to identify those samples possessing a mutation. Single-strand conformation polymorphism (SSCP) [2], dideoxy fingerprinting (ddF)

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[3] and heteroduplex analyses [2], assess the DNA fragment for mutations without having to sequence it. All three of these methods detect mutations based on the migration of the single or double stranded DNA under non-denaturing conditions. The presence of a single-point (or multiple-point) mutation affects the secondary structure of the molecule and, hence, its electrophoretic migration. Differences observed in comparison with the electrophoretic behavior of the wild type provide the basis for identification of the mutation.

A substantial number of reports have highlighted the utility of CE for the analysis of DNA. The speed, efficiency, sensitivity (with laser-induced fluorescence detection) and potential for automation make CE a particularly attractive analytical method for the clinical laboratory, especially in light of the fact that the number of DNA-based analyses used for diagnoses is growing rapidly. CE is proving to be a promising tool for effectively replacing standard gel electrophoretic techniques for DNA analysis, particularly for use with the aforementioned methods. Recent literature describes the use of CE to size DNA fragments generated by restriction endonuclease digestion (RFLP analysis) [4], or fragments produced by using PCR [5]. The characteristics that make CE attractive for methods such as SSCP and DNA-sizing analysis include its speed, sensitivity, efficiency, not to mention its potential for providing qualitative and quantitative results under native or denaturing conditions.

In the present study, we describe preliminary results from attempts to exploit CE as a means of analyzing DNA for clinical diagnostic purposes. CE analysis using both fixed (crosslinked) gels (CGE) and polymer networks (pnCE) are used for the detection of single-base mutations within a *Mycobacterium tuberculosis*-specific amplified DNA fragment by using two approaches: SSCP and ddF. The significance of these analyses is to rapidly identify those organisms harboring a mutation that may be associated with resistance to the drug, rifampin. In addition, DNA sizing by pnCE is exploited for the quantitative and qualitative analysis of PCR products indicative of hepatitis C viral (HCV) infection.

2. Materials and methods

2.1. CE instrumentation

HPCE separation was carried out on a Beckman P/ACE System 5510 equipped with either a laser-induced fluorescence detector or a UV detector and a sample cooling tray. The laser system was a single-wavelength (488 nm) argon ion laser (3–4 mW) with detection at 510 nm (using YO-PRO-1; Molecular Probes, Eugene, OR, USA) or 520 nm (using fluorescein). The UV detector monitored absorbance at 254 nm. An IBM 486 ValuePoint computer utilizing System Gold software (V. 8.1) was used for instrument control and data collection. All peak information (migration time, peak areas and height) was obtained through the System Gold software.

2.2. SSCP-CE

The sample was *rpo*B amplicon-derived from a clinical isolate of *M. tuberculosis* as described by Whelen et al. [6]. The amplified DNA (1–10 μg), suspended in water, was diluted in PCR buffer ($1\times$ buffer was 10 m*M* Tris pH 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$) such that the PCR buffer was at $0.25\times$ strength. The samples were boiled for 3 min and immediately put on ice. Separation was carried out using a polymer network obtained from Dionex (Sunnyvale, CA, USA), using a 50 cm (resolving length) $\times$ 50 μm capillary. The temperature of the sample holding tray was maintained at 8°C using an external chiller while the capillary was maintained at 18°C. Ethidium bromide (EtBr) was added to the buffer system at a final concentration of 10 μg/ml. The sample was applied to the capillary by electrokinetic injection (45 s $\times$ 5 kV) and separated with 150 V/cm. A DNA standard (ϕX174 Rf digested with *Hae*III; BRL-Gibco) was run as a control to ascertain performance of the pnCE system.

2.3. ddF-CE

Samples for ddF-CE were generated using a ddG cycle sequencing reaction [7]. The template

for the cycle sequencing reaction was the same *rpo*B gene fragment generated for SSCP analysis. The primer used for gel electrophoresis ddF analysis was a ^{32}P-5′-labeled oligonucleotide, and the same primer was 5′-labeled with fluorescein for ddF-CE. The ^{32}P-labeled DNA fragments were directly analyzed by using a non-denaturing polyacrylamide gel electrophoresis system [7] and visualized by autoradiography. The fluorescein-labeled DNA fragments were precipitated with alcohol, and suspended in deionized water. The fluorescent ddF reaction products were first analyzed using an automated DNA sequencer [7] to establish the existence of the chain terminated products. The sample was then analyzed by using a CGE system (3%T–3%C polyacrylamide fixed gel capillary, Scientific Resources, Eatontown, NJ, USA) with 50 μm I.D., an effective length of 50 cm, and a TBE (0.089 *M* Tris pH 8.3, 0.089 *M* borate, 2 m*M* EDTA) buffer system. The samples were injected electrokinetically (60 s × 3.5 kV) and separated with 150 V/cm. The fragments were detected by laser-induced fluorescence of the fluorescein fluorophore (Ex 488 nm, Em 520 nm).

2.4. HCV amplicon analysis

The samples used for this analysis were amplified DNA products using an HCV-specific reverse transcription and PCR amplification [8]. The oligonucleotide primers were specific for the HCV 5′ untranslated region and generated a 308 bp product. The template for PCR were nucleic acid extracts obtained from patient serum. The amplified DNA fragments were treated with isopsoralen [9] to prevent re-amplification, which serves to eliminate false positive PCR results. The pnCE conditions used were essentially those of Butler et al. [5] and were as follows: 20 cm (effective length) × 50 μm I.D. DB-17 coated μ-Sil capillary (J&W Scientific, Folsom, CA, USA). The polymer network was TBE (same as above), 1% (w/v) hydroxyethyl cellulose (HEC), 0.06 mg/ml YO-PRO-1. The DNA fragment standard used was a *Hae*III digest of pBR322 (Boehringer Mannheim Biochemicals, Indianapolis, IN, USA). The samples were analyzed directly from the PCR mixture, such that the capillary was pre-injected with water (3 s × 3.5 kV) before the electrokinetic introduction of the sample (90 s × 3.5 kV) [10]. The separation conditions were adjusted to 260 V/cm. Southern blot results were generated by using standard agarose slab-gel electrophoresis and blotting procedures [11]. The Southern blot was probed by using a DNA fragment internal to the amplified sequence and the ECL labeling kit (Amersham, Arlington Heights, IL, USA) [8].

3. Results

3.1. SSCP-CE

Fig. 1 illustrates the comparison of standard non-denaturing polyacrylamide gel electrophoresis and pnCE as used to perform SSCP analysis on the *M. tuberculosis*-specific *rpo*B-directed PCR product. SSCP distinguishes DNA fragments by virtue of secondary structural differences generated by the single strands (ssDNA) of the DNA fragments [12]. In this example, organisms resistant to rifampin harbor a mutation within the sequence of the amplified fragment being studied. As illustrated using standard non-denaturing gel polyacrylamide electrophoresis conditions [5] (Fig. 1A), the amplified DNA derived from the genomes of rifampin sensitive and resistant organisms can be differentiated. However, only a slight migration difference was detectable between the ssDNA derived from wild type (rifampin sensitive clinical isolate) and L363 (rifampin resistant due to one base change; [7]). As a contrast, isolate L219 provided an example were the ssDNA migration differences were readily detected by routine gel electrophoresis. We chose to challenge the CE analysis with amplified DNA from these two isolates to determine if CE could better resolve the ssDNA fragments. Initial experiments were aimed at differentiating these two isolates from each other and DNA fragments derived from a wild-type isolate.

Various capillary and polymer network systems were employed to identify the system that would provide the best resolution and repro-

ducibility for the SSCP-CE analysis. The polymer network systems tested included both a 3% and 4% linear polyacrylamide system in TBE, and 0.5 to 0.75% HEC in TBE. The Dionex pnCE system yielded the highest level of resolution for the *rpo*B-derived DNA fragments.

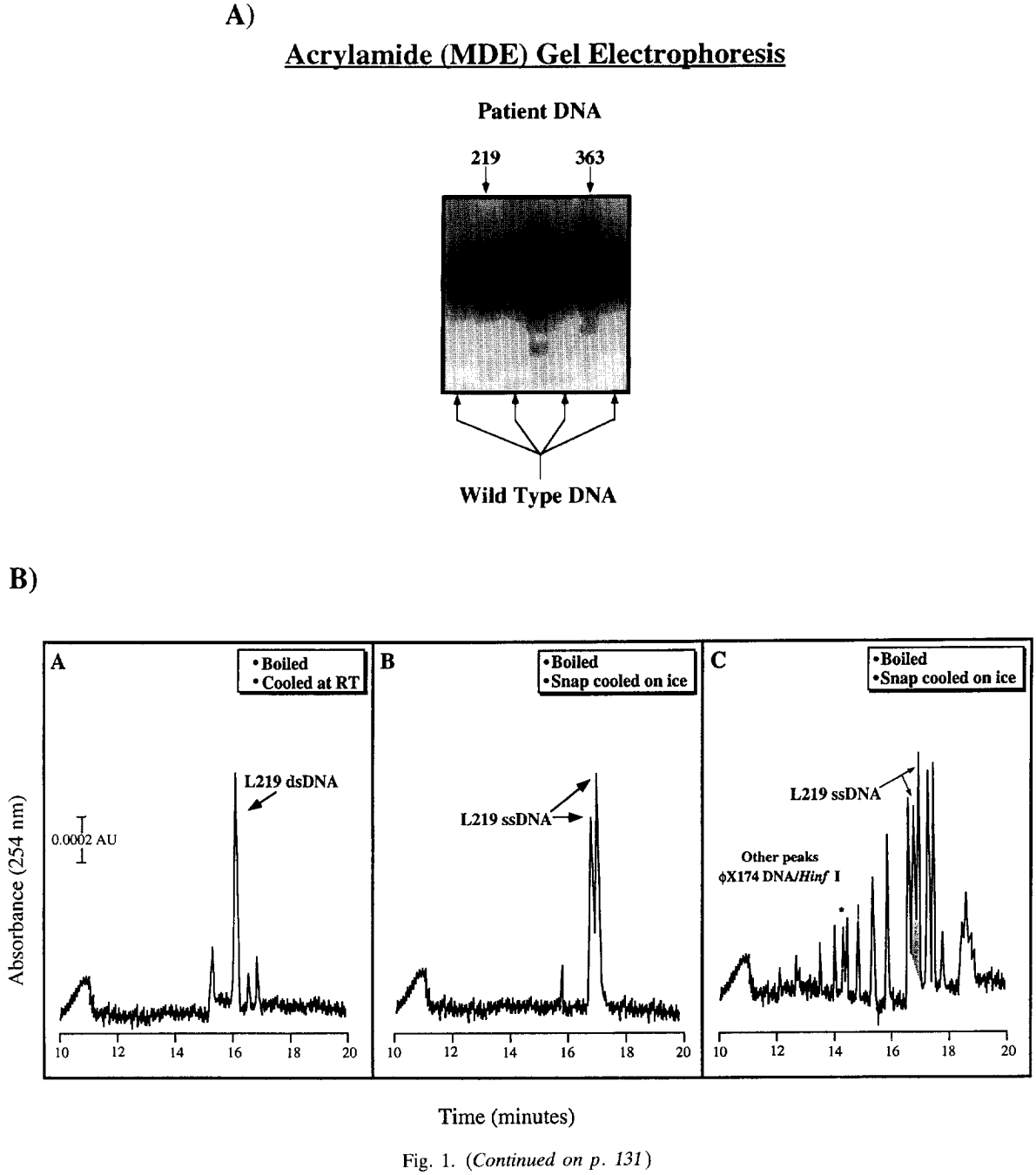

Fig. 1. (*Continued on p. 131*)

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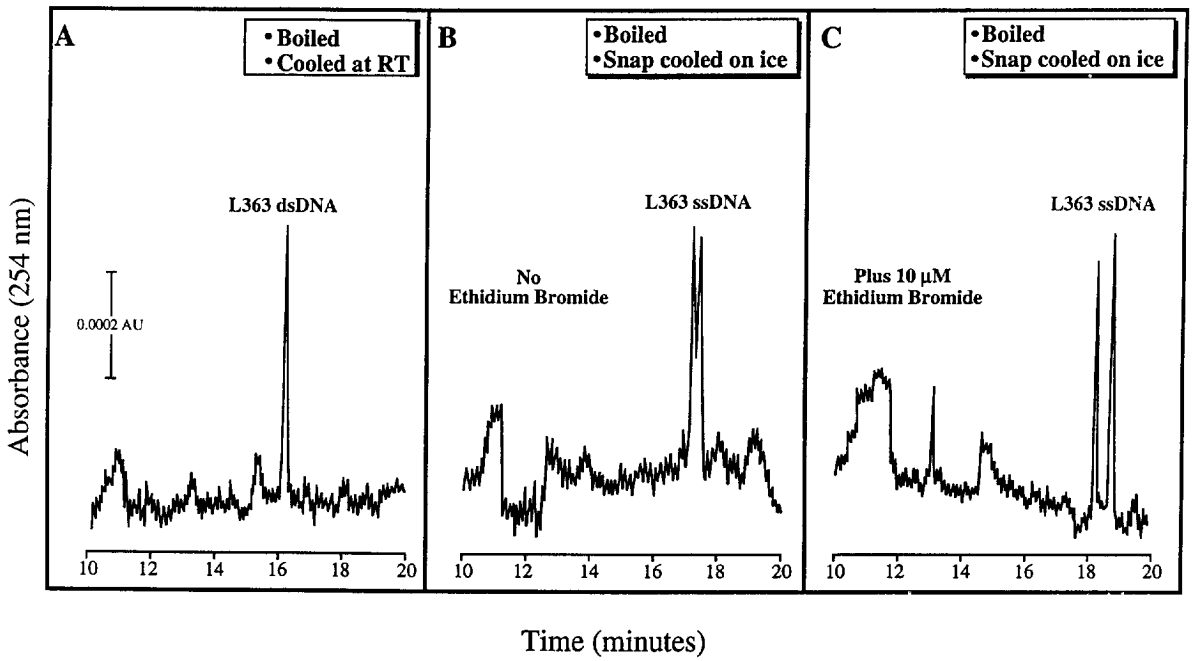

Fig. 1. SSCP analysis comparing electrophoretic separations using a standard acrylamide gel or pnCE. (A) Illustration typifying non-denaturing MDE (mutation detection enhancement) gel separation as described in Ref. [4]. The isolates used were either rifampin-sensitive wild-type DNA (lanes marked at the bottom of the autoradiogram), or rifampin-resistant isolates (lanes between the wild-type samples). Isolates L219 and L363 (designated at the top of the figure) were used to generate the SSCP-CE results that follow. (B) Panel A is the electropherogram resulting from boiling the amplified DNA from isolate L219 and allowing the sample to cool slowly, as indicated by the detection of re-annealed dsDNA. Panel B is the same sample as panel A, except the DNA was snap cooled by quickly placing the boiled sample on ice. The ssDNA species are noted in this panel. Panel C illustrates mixing the snap cooled, ssDNA from L219 with a ϕX174-*Hinf*I DNA fragment standard (Boehringer Mannheim Biochemical, Indianapolis, IN, USA). The peaks derived from L219 are filled in. (C) The first panel illustrates the re-annealed amplified DNA derived from L363. The middle panel illustrates the ssDNA migration pattern obtained from the same amplified DNA that has been snap cooled. The final panel illustrates the effects of adding ethidium bromide (10 μM final concentration) to the buffer system. The ssDNA peaks are labeled as such.

Fig. 1B illustrates the initial SSCP results using L219. The first two panels contrast cooling the heat denatured DNA slowly (first panel) or rapidly (middle panel). There was just a trace of single stranded material detected upon slowly cooling the DNA, whereas all of the DNA was in the single stranded state in the rapidly cooled sample. The ssDNA migrated at a slower rate through the matrix, which was consistent with standard non-denaturing polyacrylamide gel electrophoresis. We also mixed the snap-cooled sample with ϕX174-HaeIII DNA fragment standards to determine if the separation of ssDNA was achievable within a mixture of dsDNA fragments. The third panel of Fig. 1B illustrates the ability to include dsDNA fragments as internal markers for this analysis without affecting the resolution.

The next sample analyzed was L363, which produced an SSCP pattern that was barely distinguishable from wild type using standard non-denaturing polyacrylamide gel electrophoresis

(Fig. 1A). CE was able to separate the ssDNA of L363, as illustrated in the first two panels of Fig. 1C. The degree of separation between the ssDNA fragments was similar to that of the L219 separation; however, the migration time was different for the two isolates (compare Fig. 1B and 1C). Ethidium bromide was added to the buffer system in an attempt to increase the resolution [13,14]. An enhanced (baseline) resolution of the ssDNA fragments was achieved with sample L363 (Fig. 1C, third panel), with a corresponding increase in peak height and with only a slight increase in analysis time. Consistent with the observations of others, EtBr-ssDNA strands migrated more slowly than that observed in the absence of the intercalator. Ethidium bromide did not produce the same effect with sample L219 (no significant change in strand migration and resolution).

The wild-type sequence was analyzed along with L363 and the level of resolution was insufficient to differentiate the two samples (data not shown). This result was comparable to what was detected by non-denaturing polyacrylamide gel electrophoresis in that the DNA strands of wild type and L363 amplified products almost co-migrated.

3.2. ddF-CE

As with the SSCP analysis, evaluation of the ddF method was conducted by comparing standard non-denaturing polyacrylamide gel electrophoresis and CGE. The theory and development of the ddF technique have been described in detail elsewhere [3,15]. Briefly, the ddF analysis involved non-denaturing polyacrylamide gel electrophoretic separation of dideoxy terminated fragments generated by a typical Sanger sequencing reaction using one dideoxy chain terminating nucleotide (Fig. 2). Thus, the fragments were essentially analyzed based on the chain termination differences (similar to DNA sequencing) and secondary structural differences (similar to SSCP). Fig. 3 illustrates the ddF results from an *rpo*B-specific amplicon derived from clinical isolates of *M. tuberculosis*. Using standard non-denaturing polyacrylamide acrylamide gel electrophoresis, strand migration differences were readily discernible between the products generated from the wild-type sequence and those derived from a rifampin resistant strain harboring a single C→T base mutation (Fig. 3A). This sample typified the degree of strand migration differences expected in a ddF analysis.

For ddF-CE analysis, the same samples were analyzed by using a fluorescein-labeled oligonucleotide primer. These reactions were first analyzed using an automated DNA sequencer to assure the production of chain-terminated products. Of the systems tested, the best resolution was obtained using a chemically cross-linked acrylamide gel system (Fig. 3B). The resolution was equivalent to that obtained with the non-denaturing slab-gel system. The reproducibility of the shift was established by running the samples in triplicate. The current associated with running the fixed gel system (at 15 kV) was initially 6.0 μA and decreased with subsequent runs. Use of the capillary was discontinued when the system current was < 5.2 μA.

3.3. dsDNA sizing analysis

As seen in Fig. 4, and as described by others [3], physical gel capillary electrophoretic separation has proven to be an excellent means of sizing amplified DNA. The PCR assay used was sensitive and specific for HCV [8], such that a fragment detected by agarose gel electrophoresis was considered to be a diagnostic marker for the presence of the HCV genome. The HCV-specific amplified DNA was easily detected with LIF-CE using a polymer network and YO-PRO-1 as a fluorescent intercalator (Fig. 4C). Routinely, Southern blot analysis is carried only to increase the sensitivity of detecting the amplified DNA fragment as shown in Fig. 4A. The signals observed with Southern blot analysis typified the range of responses from negative to strong positive. These results correlated well with the electropherograms shown in Fig. 4C. The resolution of the CGE system was assessed using a DNA fragment standard (Fig. 4B). Based on the

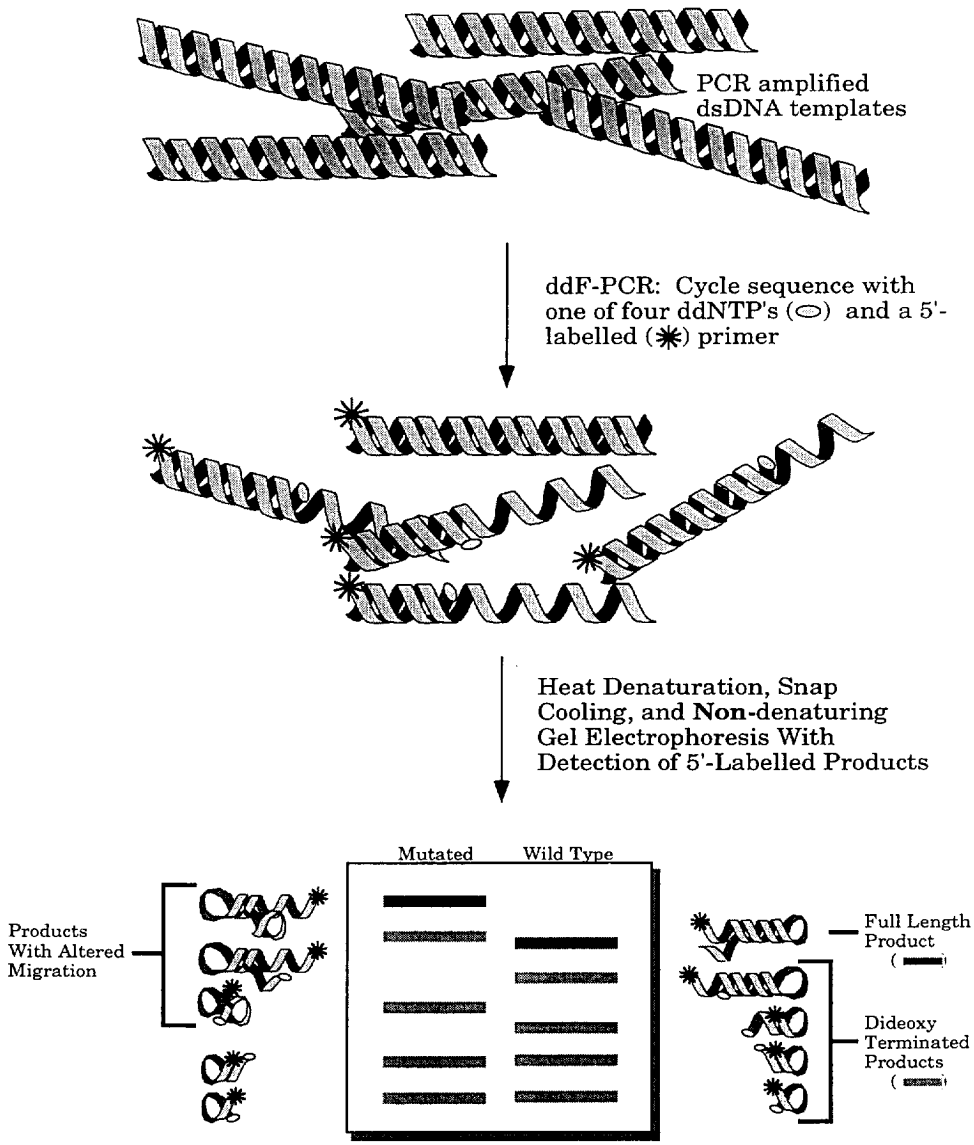

Fig. 2. The ddF theory is illustrated in this figure. The amplified DNA is used directly from the PCR mixture with no need to remove unincorporated deoxynucleotides (dNTPs) or unextended primers (top). The amplified DNA is added to a ddF reaction mixture containing a 5'-labelled oligonucleotide (32P or fluorescein) and one dideoxy nucleotide (ddNTP). The reaction is catalyzed by Taq DNA polymerase to generate a series of chain terminated products whenever the ddNTP is inserted (middle). The dNTPs added with the amplified DNA help fuel the extension of the 5'-labelled priming oligonucleotide. The chain terminated products are boiled, snap cooled and separated using non-denaturing polyacrylamide gel electrophoresis (32P 5'-labeled products) or CE (fluorescein 5'-labelled products), and the labeled ssDNA fragments are detected with autoradiagraphy or laser-induced fluorescence, respectively. Nucleotide base changes (mutations) are detected by (1) the secondary structural differences generated by the various chain terminated fragments and (2) the position of chain termination, if it has been altered by a mutation (bottom).

migration times for the standard fragments, the 308 bp amplified fragment migrated as expected between 6.9 and 7.1 min. A small shoulder was observed on the early part of the fragment peak and was the result of isopsoralen treatment [9]. CE analysis of this amplified DNA gave clear

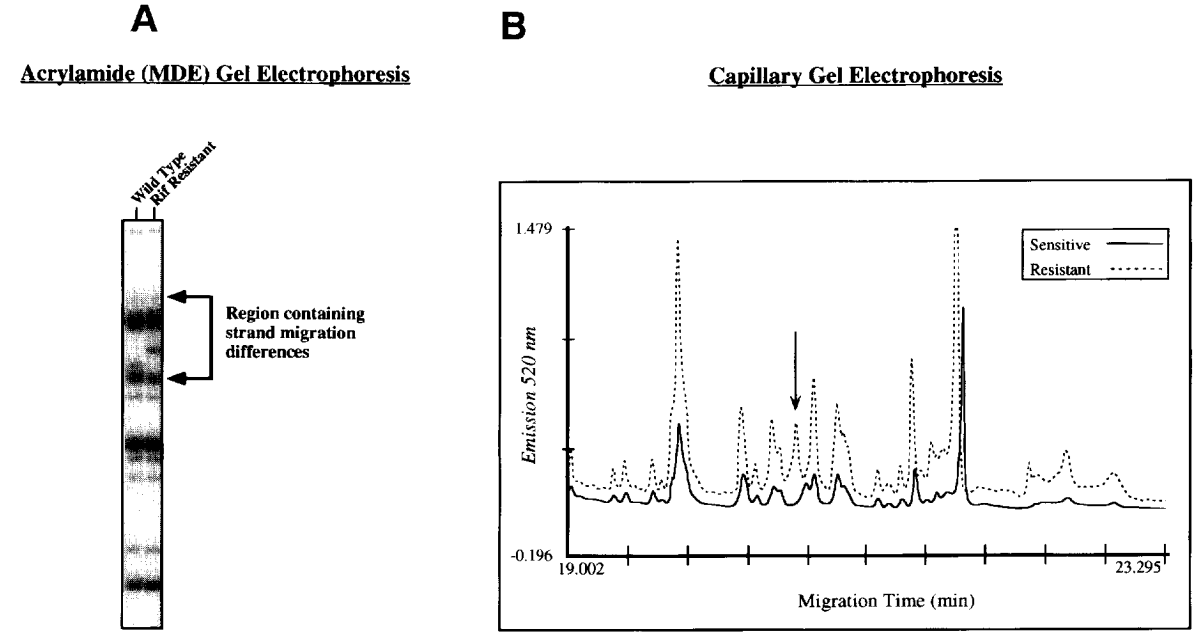

Fig. 3. ddF analysis comparing electrophoretic separations using an acrylamide gel method or CGE. (A) Illustration typifying non-denaturing gel separation as described in Ref. [4]. The top of the autoradiogram is labeled to indicate rifampin susceptibility of the isolate used to generate the amplified fragment. The rifampin resistant isolate harbored a single C to T mutation. The ddF reaction included a ^{32}P-5′-labeled oligonucleotide primer, and ddGTP as the chain terminating nucleotide. The region where strand migration differences can be detected is marked to the right of the autoradiogram. The arrow indicates the beginning of peak migration differences between the rifampin resistant and sensitive isolates. (B) The amplified DNA from the same isolates used in (A) was analyzed using a fluorescein-labeled oligonucleotide and ddGTP as the chain terminating nucleotide. The CGE separation of the fluorescein labeled fragments is illustrated in this figure. The arrow in B represents the detected mutation.

results for 39 clinical samples with a 100% correlation with Southern blot analysis [16].

4. Discussion

A recent trend in the development of clinical diagnostic procedures is to exploit molecular methods of analysis for the rapid detection of pathogens directly from patient specimens. It is clear that the most prominent molecular technique currently used is PCR, which can be developed to detect a pathogen with a high degree of specificity and sensitivity. Post-amplification analysis of DNA fragments produced by PCR is routinely carried out by agarose gel electrophoresis. Enhanced resolution can be obtained with the use of an acrylamide gel matrix, however, these gels can be more difficult to cast and require a longer separation time. When information regarding the DNA sequence is required, the use of the acrylamide format is essential. Subsequently, all of the disadvantages associated with the use of these cumbersome systems compound the labor-intensity of the clinical diagnostic procedure. It is for this reason that CE in fixed gels and polymer networks has been embraced with intense interest and that, as in this study, critical comparisons between agarose/acrylamide (standard) gel electrophoresis systems and various CE systems are being carried out. In the present study, our ultimate goal was to establish the feasibility of using CE in the clinical molecular diagnostic laboratory setting. The advantages of CE are many, but the potential for rapid analysis and the ability to automate the CE system are among the most appealing to the clinical laboratory.

We investigated two different, yet related, methods of detecting mutations using an am-

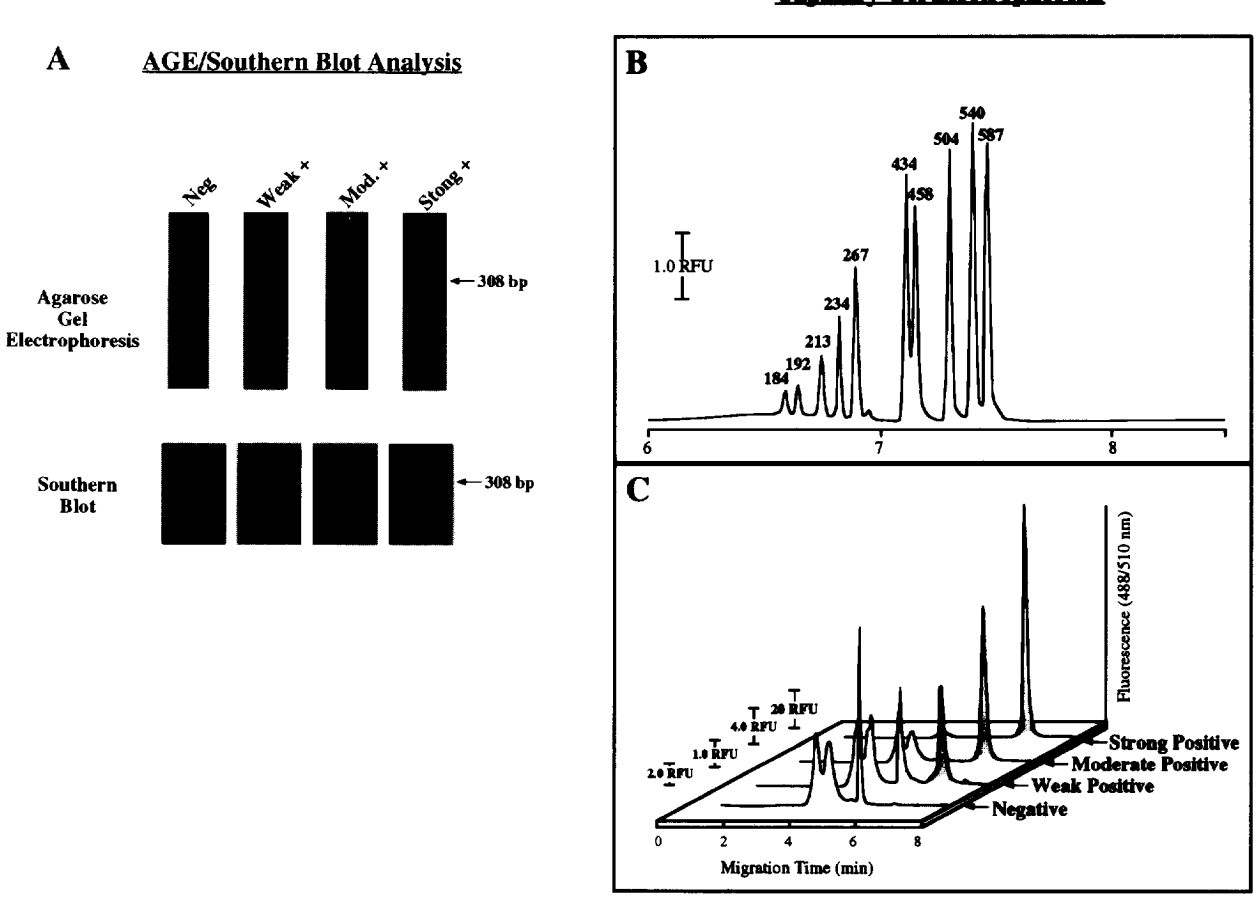

Fig. 4. DNA fragment sizing comparing electrophoretic separation and detection using standard agarose gel and Southern blotting methods, or LIF-pnCE. (A) Illustration of a typical agarose gel and Southern blot results. (B) The degree of resolution obtained with the pnCE system used is illustrated by the separation of a DNA fragment standards (2.5 ng/μl). The size of the DNA fragements is indicated at the top of each peak as the number of base pairs. (C) A stacked figure of electropherograms is illustrated, with the magnitude of the ordinate axis (emission detected at 510 nm) indicated to the left of the figure. The shaded peak represents the amplified DNA product and the range of detected signal for the various samples is indicated to the right of the figure.

plified DNA fragment: SSCP and ddF. Both methods rely on analyzing the ssDNA of a fragment, where the wild type and mutated strands can be differentiated by secondary structural differences formed during non-denaturing polyacrylamide gel electrophoresis. The standard means of analysis requires ^{32}P-labeled material and a non-denaturing polyacrylamide gel separation time of at least 3 h followed by autoradiography (typically overnight exposures). In light of this, the rapid nature of CGE becomes attractive. CE in polymer networks has previously been shown to be amenable to SSCP analysis [17]. The present results confirm these original findings since CGE was able to separate the ssDNA species of all the DNA fragments tested. However, use of the conditions defined in the present study allowed for separation in a shorter period of time. Furthermore, the addition of ethidium bromide was found to enhance the separation allowing for baseline resolution of some single strands. We can only speculate that this results from intercalator-induced secondary structural changes in the partially duplexed

ssDNA [18]. However, it is unclear why the same effect was not observed with the other sample tested. It is possible that the magnitude of the EtBr-induced structural changes is influenced by the nature of the mutation. Although this pnCE system performed best among those tested, it did not provide the level of resolution needed to differentiate various mutated DNA fragments from the wild-type sequence. This was particularly true for the L363 sample; unlike previous pnCE analyses using SSCP [17]. While there appears to be potential for SSCP-CE analysis as described by others [17], the CGE system described here is of limited application for the *rpo*B system. Clearly, the resolution achievable with CGE may be improved by altering the parameters affecting the secondary structure formation, namely the ionic strength conditions, temperature and fragment size [19] as well as the inclusion of various intercalating agents. Additionally, resolution may be increased to a functional level through altering the concentration and/or type of polymer utilized as a sieving matrix for the *rpo*B-derived amplicon.

Dideoxy fingerprinting-CE has not been previously reported in the literature and, based on the preliminary data described in this study, has potential for application in clinical analysis of amplified DNA fragments. The CE conditions described clearly allow for the detection of a single nucleotide base change. As with SSCP-CE, the use of ddF-CE analysis may prove to be advantageous for clinical screening of amplified DNA for mutations. A clear benefit to the use of the ddF analysis is that it is less influenced by the same factors affecting SSCP; therefore, ddF-CE may be the better choice for the post-amplification analysis of some DNA fragments. From the perspective of analysis time, ddF-CE analysis can be accomplished in ≈25 min per sample. As a result, 30 samples could be analyzed in 15 h in comparison with 36 h by acrylamide gel electrophoresis. The development of a clinical ddF-CE approach could include (1) the co-injection of internal markers, which could be used to normalize migration times and (2) the stream-lining of sample preparation techniques to minimize sample handling. With the ddF-CE system described here, this may be accomplished by using a formamide- or urea-based sample buffer which would allow for the ddF reaction products to be mixed directly with sample buffer, boiled, snap-cooled, and analyzed by CGE.

Finally, post-amplification sizing of DNA fragments is a ready-made application for CE and this is supported by the numerous reports in the literature (e.g., Refs. [5,14]). PCR assays that specifically amplify one DNA species, and require a sensitive post-amplification analysis are especially suited to pnCE analysis. The results of the present study illustrate that CE provides qualitative and quantitative information about the amplicon. CE not only affords a rapid analysis time but the inclusion of an intercalator in the buffer can increase the sensitivity dramatically over Southern blot analysis. We are currently conducting a prospective analysis to further compare the routine agarose gel and Southern blot analysis to the CE system described in this study. There is little doubt that the automated CE system will soon replace the currently used agarose and blot system for those amplified products that are specific for a template DNA. Simply altering the format of the CE sample loading tray to accept the 96 well format currently used with some commercial thermocyclers, will allow for tubes to be removed from the thermocycler and placed directly on to the CE for amplicon detection.

In summary, we envision many applications for CE in the clinical setting, especially those that involve the analysis of DNA. The attributes of CE, namely speed and automation, only encourage further investigations into clinical applications. DNA is a ready made analyte, whose potential as an agent in diagnostic evaluation of a patient is being exploited daily. It is clear that routine CE analysis of DNA in the clinical setting is rapidly becoming a reality.

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References

[1] J.P. Landers, Clin. Chem., 41 (1995) 495–509.
[2] E.P. Lessa and G. Applebaum, Molec. Ecol., 2 (1993) 119–129.
[3] G. Sarkar, H. Yoon and S.S. Sommer, Genomics, 13 (1992) 441–443.
[4] E. Avaniss-Aghajani, K. Jones, D. Chapman and C. Brunk, BioTechniques, 17 (1994) 144–149.
[5] J.M. Butler, B. McCord, J. Jung, M. Wilson, B. Budowle and R. Allen, J. Chromatogr. B. , 658 (1994) 271–280.
[6] A.C. Whelen, T.A. Felmlee, J. Hunt, D. Williams, G.D. Roberts, L. Stockman and D.H. Persing, J. Clin. Microbiol., 33 (1995) 556–561.
[7] T.A. Felmlee, Q. Lui, A.C. Whelen, D. Williams, S.S. Sommer and D.H. Persing, J. Clin. Microbiol., 33 (1995) 1617–1623.
[8] T.A. Cha, J. Kolberg, B. Irvine, M. Stempien, E. Beall, M. Yano, Q. Choo, M. Houghton, G. Kuo, J. Han and M. Urdea, J. Clin. Microbiol., 29 (1991) 2528–2534.
[9] P.N. Rys and D.H. Persing, J. Clin. Microbiol., 31 (1993) 2356–2360.
[10] A. Guttman, K.U. Ulfelder and H.E. Schwartz, Presented at the 7th International Symposium on High Performance Capillary Electrophoresis, Würzburg, Germany, 1995, Poster £ P428.
[11] J. Sambrook, E.F. Fritsch and T. Maniatis, in Molecular Cloning: A laboratory manual, 1989, Cold Spring Harbor.
[12] A. Guttman, R.J. Nelson and N. Cooke, J. Chromatogr., 593 (1992) 297–303.
[13] A. Guttman and N. Cooke, Anal. Chem., 63 (1991) 2038–2042.
[14] H.E. Schwartz, K. Ulfelder, F.J. Sunzeri, M.P. Busch and R.G. Brownlee, J. Chromatogr., 559 (1991) 267–83.
[15] Q. Liu and S.S. Sommer, PCR Methods and Applications, 4 (1994) 97–108.
[16] T. Felmlee, P.S. Mitchell, K.J. Ulfelder, D.H. Persing and J.P. Landers, J. Cap. Elec., 2 (1995) 125–130.
[17] A.W. Kuypers, P. Williams, M. vander Schans, P. Linssen, H. Wessels, C. de Bruijn, F. Everaerts and D. Mensink, J. Chromatogr., 621 (1993) 149–156.
[18] P.R. Selvin, D.N. Cook, N.G. Pon, W.R. Baurer, M.P. Klein and J.E. Hearst, Science, 255 (1992) 82–85.
[19] K. Hayashi and D. Yandell, Human Mutation, 2 (1993) 338–346.