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Comparison of resolution of double-stranded and single-stranded DNA in capillary electrophoresis

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Abstract

Capillary electrophoresis has become a powerful analytical tool for the analysis of DNA restriction fragments and polymerase chain reaction (PCR) products. The use of replaceable polymer solutions increases the lifetime of the capillary, improves the repeatability of the migration times and enables pressure injection. When pressure injection is used, rather than electrokinetic injection, it is assured that a representative part of the sample is introduced into the capillary. The possible lower resolution, which is a side effect of the pressure injection, can be made up for by an increase of selectivity. Using fluorescent labels, it is possible to detect DNA with a fluorescence detector under both native conditions in double-stranded (ds) form, and under denaturing conditions in single-stranded (ss) form. When DNA is separated in its ss form, as is necessary for DNA sequencing, we observe an increased selectivity compared to separation of that sample in its ds form. In this work, we exploited this increased selectivity for the analysis of denatured PCR products. It was found that DNA separated in the ss form yields superior separation; that is, a given analysis can be achieved with the same resolution in a shorter separation time compared to dsDNA. Therefore, it is advisable to separate DNA in the ss form if high resolution, size dependent separation is required. The enhanced resolution achieved with DNA migrating in the ss form enabled the separation of allelic ladders of short tandem repeats with a difference of 4 base pairs in the 200 base pair range, with separation times no longer than 6 min.

Keywords: Polymerase chain reaction; DNA

1. Introduction

Capillary electrophoresis (CE) has been recently used for the analysis of DNA restriction fragments and polymerase chain reaction (PCR) products [1–3]. Fast analysis, low sample consumption, direct quantitation ability and automation are the main advantages over conventional slab gel electrophoresis. The first publications demonstrating PCR product analysis dealt with polyacrylamide gels fixed to the capillary surface [4]. Fixed gels are susceptible to sample-induced gel damage, which results in less repeatable migration times [5]. Secondly, bubble formation reduces the lifetime of the capillary. With the advent of replaceable polymer–buffer solutions these problems were overcome and the technique became more robust. Typical polymer–buffer solutions for DNA separations consist of linear polyacrylamide [6], hydroxyethylcellulose (HEC) [7], or hydroxypropylmethylcellulose (HPMC) [8]. Besides the above-mentioned aspects, CE is also praised for its high resolution. However, "resolution" is a

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comprehensive concept and merits some additional discussion.

Suppose there are two closely eluting peaks with a certain difference in electrophoretic mobility and average mobility. The resolution can be written as the product of selectivity and efficiency:

$$R = \frac{\Delta\mu}{\mu_{av}} \frac{\sqrt{N}}{4}$$

where R is resolution, $\Delta\mu$ is difference in mobility ($m^2/V\,s$), μ_{av} is the average mobility ($m^2/V\,s$) and N the number of theoretical plates. The resolution is linear with the selectivity, which is calculated as the difference in mobility divided by the average mobility. The electrophoretic mobility of DNA can be influenced by several CE parameters like field strength [9], temperature [10] and most importantly polymer concentration [11]. The size-based electrophoretic separation of DNA fragments in gels has been described by several models including the Ogston [12] and reptation models [13–15].

However the low viscosity replaceable polymer solutions used in CE show little resemblance with the rigid gels used in slab gel electrophoresis. Slab gels are generally modeled as a rigid network of pores, a model which is not applicable to a low-viscosity polymer solution. Barron et al. have recently proposed that the mechanism behind DNA separations in uncross linked polymer solutions can be better described with a new model called "transient entanglement coupling" [16]. Experimental evidence for this new mechanism included the finding that DNA fragments can be separated in polymer solutions with polymer concentrations well below the entanglement threshold concentration [11]. It was also found that there exists a optimum polymer concentration for the separation of large DNA (>600 base pairs). As long as replaceable, low-viscosity polymer solutions are used, there seems to be no optimum polymer concentration for the separation of small DNA (<400 base pairs). A higher polymer concentration, above the entanglement threshold concentration, results in lower mobility and higher selectivity of small DNA fragments. For example dsDNA up to 300 base pairs can be separated with single base resolution using a replaceable 6% linear polyacrylamide solution [6]. However, the polymer solution is so viscous that it cannot be replaced by the rinse option of the CE instrument, but requires the use of a gas tight syringe. Furthermore, the 45-min analysis time which is required, is hardly competitive with slab gel electrophoresis. However, single-base resolution is not always necessary for PCR product analysis, hence more dilute polymer solutions can be used, shortening analysis times considerably. These low viscosity polymer–buffer solutions can be replaced by the rinse option of the CE instrument, and provide 4-base pair resolution within 10 min of electrophoresis [17]. It must be mentioned that in this case, a DNA intercalating dye was present in the running buffer. Intercalators alter the structure of dsDNA and reduce the net charge of the DNA molecule [8,18]. These two aspects result in a decrease of the mobility, and an increase in selectivity.

The mobility of DNA also depends upon whether it is migrating in the native double-stranded (ds) form or the denatured single-stranded (ss) form. DNA sequencing is necessarily performed under denaturing conditions because the sequencing reactions deliver ssDNA molecules with a partly elongated complementary DNA strand attached. Denaturation is needed to give a correct, reliable, length-based separation. This also influences the selectivity of the separation. Maniatis et al. [19] have shown that the mobility of ssDNA is lower than the mobility of equally long dsDNA.

Resolution is also determined by the efficiency of the separation. The best peak efficiency is achieved with electrokinetic injection, because the DNA molecules are concentrated against the gel interface during injection [8]. This is not the case for pressure injection, where a part of the polymer solution is replaced with sample. Injection of larger sample plugs, which is often necessary for samples having low analyte concentration, will lead to peak distortion and lower efficiency [20].

Electrokinetic injection yields good results with low-ionic strength samples, such as DNA molecular mass standards. Real samples, however, such as PCR products, contain significant amounts of chloride. These chloride ions compete with the DNA molecules for entrance into the capillary during electrokinetic injection. Longer injection times must be applied in order to introduce sufficient amounts of

DNA into the capillary. Desalting of the sample in order to remove the competing small ions is one option [21]. However, this method is laborious, and one is never sure of the extent of recovery of the DNA after the desalting procedure. Dilution of the sample to create sample stacking conditions might be an option as well, but still requires a sensitive detection mode like laser induced fluorescence (LIF) [22]. In any case, if quantitation of the PCR products is required, it is necessary to use pressure injection [23]. Pressure injection is the most straightforward method of sample introduction, which furthermore ensures that a representative portion of the sample is introduced into the capillary. The loss of resolution attributable to pressure injection (due to the parabolic profile of pressure-induced flow) may be compensated by the gain in selectivity, which can be achieved when separation is carried out under denaturing conditions. This study aims to determine whether separating PCR products in their ss form results in an improvement in their CE analysis, in terms of resolution or analysis time.

2. Experimental

2.1. Capillary electrophoresis

All experiments were performed on a P/ACE 2200 from Beckman Instruments (Fullerton, CA, USA) which was equipped with an LIF detector. An Argon Ion laser (Beckman Instruments) tuned at a wavelength of 488 nm was utilized as light source. The emission light was collected through a bandpass filter of 520 nm. The sample was introduced into the capillary by pressure injection at 2 p.s.i. (1 p.s.i.= 6894.76 Pa). The voltage was applied in the negative polarity and varied between 7.4 and 20 kV. Temperature of the capillary cartridge was maintained at 20°C or 40°C.

Capillaries with a length of 37 cm (30 cm from inlet to detector, $l=30/37$ cm) were coated with polyacrylamide as described earlier [24]. Sieving buffers were prepared by polymerizing 8% acrylamide in 0.1 *M* Tris–borate buffer pH 8.3 with 0.5% N,N,N′,N′-tetramethylethylenediamine (TEMED) and 0.08% ammoniumpersulphate (APS) for 24 h at 4°C. All chemicals were purchased from Sigma, Bornem, Belgium. After polymerization the buffer was diluted with water to a final concentration of 4% linear polyacrylamide. A 4% polyacrylamide is the highest concentration that can be rinsed through the capillary on the Beckman instrument within a reasonable time of 5 min and is above the entanglement threshold concentration [25]. For separations under denaturing conditions, the 8% polyacrylamide stock solution was diluted with a 14 *M* urea solution to a final concentration of 4% polyacrylamide and 7 *M* urea. The polymer solution buffer was replaced after each run by rinsing for 5 min at 40 p.s.i..

2.2. Amplification conditions

PCR samples of the HUMTH01 and HUMFES loci were amplified according to the method described by Kimpton et al. [26]. PCR amplification was performed using 2 ng of genomic DNA in a 50 μl reaction volume. One primer of each reaction was labelled with the dye FAM (fluorescein amidite) from Applied Biosystems (Foster City, CA, USA). A part of the X-Y specific homologous amelogenin gene was amplified according to Sullivan et al. [27]. One primer was labelled with FAM.

A part of the BCR/ABL [28] fusion gene was amplified over 30 cycles for 30 s at 94°C, 30 s at 58°C and 90 s at 72°C using the following primers: B1.1 sal: 5′-TCAGTCGACCTATGAGCGTGCAGAGTGGA and anti-A2.2: 5′-GGCTTCACTCAGACCCTGAGGCTC at a concentration of 0.33 μ*M*. PCR reaction was carried out in a volume of 100 μl with 125 μ*M* of each dNTP; 50 m*M* KCl, 20 m*M* Tris–HCl pH 8.4, 2.5 m*M* $MgCl_2$ and 1.5 units of Taq polymerase (Life Technologies, Gaithersburg, MD, USA). Three PCR reactions were pooled with a total of 255 μl. The PCR products were purified using the Wizard Preps DNA purification system from Promega (Madison, WI, USA) and collected in 50 μl water. 1 μl of the purified product was re-amplified using the same PCR buffers, however the anti-A2.2 primer was added in a concentration of 0.6 μ*M* and labelled with fluorescein (primer purchased from Pharmacia Biotech, Uppsala, Sweden). ddATP was added to a final concentration of 0.32 m*M*. The dNTP concentration was 20 μ*M*. The PCR product was amplified for 25 cycles for 30 s at 94°C, 30 s at 53°C and 90 s at 72°C.

2.3. Mobility measurements

A fluorescein labelled dsDNA sizer (50–500 bp) from Pharmacia Biotech was used for mobility measurements. The sample was injected by pressure at 2 p.s.i. for 5 s into the capillary. Double-stranded DNA was diluted with equal volume of water and injected into the capillary. Single-stranded DNA was prepared by heating the dsDNA sample for 3 min at 95°C in a 50% formamide solution and consecutive snap cooling in water–ice for 3 min.

3. Results and discussion

3.1. Mobility of ds- and ssDNA

The DNA 50–500 sizer from Pharmacia consists of ten dsDNA fragments ranging from 50 to 500 base pairs in 50-base pairs steps. One strand of each DNA fragment is labelled with fluorescein on the 5′ end. DNA fragments from the Pharmacia 50–500 sizer can be detected with the LIF detector in ds and ss form (after denaturation) without the use of an intercalator. Labelling of one strand and the use of fluorescence detection also assures that, when DNA is injected in the ss form, only one strand is detected. This is not the case when UV detection is used, where even under denaturing conditions both DNA strands are separately detected. Fig. 1 shows the electropherograms of the DNA sizer in the ds and ss form, migrating at 200 V/cm and 400 V/cm, respectively. Size-based electrophoretic separations of ssDNA are usually performed under denaturing conditions. Generally, urea is added to the running buffer to a final concentration of 7 *M*, in order to keep the DNA single-stranded and avoid the influence of DNA secondary structure on the electrophoretic mobility.

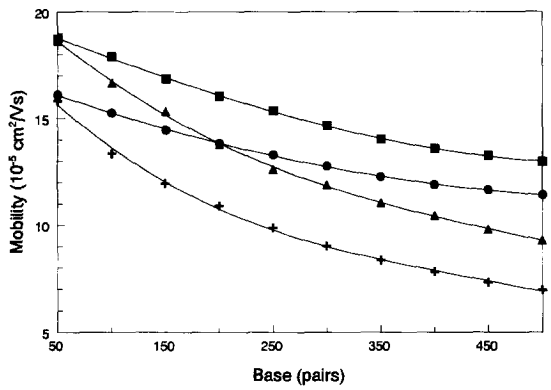

Fig. 2. Apparent mobility vs. base (pairs). Mobilities were measured in 4% linear polyacrylamide at field strength of 200 V/cm. (■) dsDNA no urea in buffer; (●) dsDNA 7 *M* urea in buffer; (▲) ssDNA no urea in buffer; (+) ssDNA 7 *M* urea in buffer.

The mobilities of ds- and ssDNA under native and denaturing conditions were calculated using the voltage, migration time and capillary length, and were plotted against the number of bases or base pairs (Fig. 2). It is clear that the addition of urea has

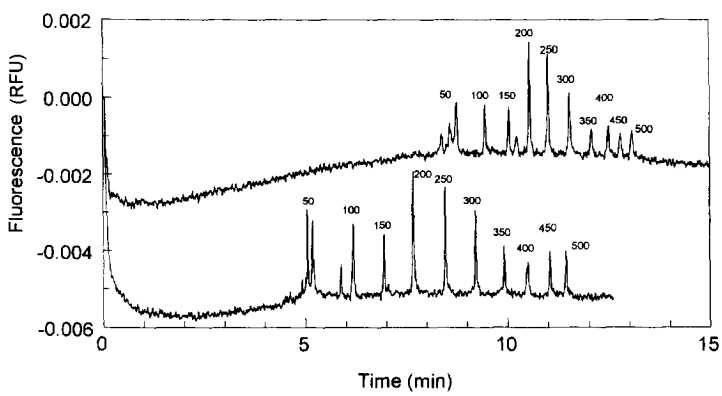

Fig. 1. Electropherogram of DNA 50–500 sizer from Pharmacia. Upper electropherogram: native conditions dsDNA, voltage = 7.4 kV. Lower electropherogram: denaturing conditions ssDNA, voltage = 14.8 kV. Capillary: I.D. = 75 μm, *l* = 30/37 cm, pressure injection of 5 s, temperature = 20°C.

an influence on the mobility of ds- and ssDNA. The denaturing, 7 *M* urea buffer has a higher viscosity, which results in lower mobilities for both ds- and ssDNA. One also observes that ssDNA longer than 50 bases has a lower mobility than dsDNA in buffers with or without urea. Single-stranded DNA has its bases available for hydrogen bond formation with water or urea molecules. This apparently results in a lower charge-to-mass ratio, and therefore, a lower electrophoretic mobility. The migration of ds- and ssDNA has also been studied in sedimentation experiments [29]. In low-ionic strength buffer, ssDNA has a lower sedimentation coefficient, which has been attributed to the fact that in its ss form the DNA adopts a more extended conformation which leaves the bases attainable for hydrogen bond formation. At high ionic strength, ssDNA adopts a random coil conformation and undergoes faster sedimentation than dsDNA. However, the DNA molecules in the Pharmacia sizer sample are too small to form true random coils; hence, the bases will be available for hydrogen bonding, regardless of the ionic strength. However, the largest fragment of the sizer appears to show some folding behavior. This folding is evidenced by the dependence of the mobility on the field strength for this particular fragment (Fig. 3). The mobilities of ds- and ssDNA were measured at different field strengths. It is well known that the mobility of large dsDNA (>1000 base pairs) strongly depends on the field strength. This effect is attributed to orientation and conformational stretching of the dsDNA molecules, induced by an electric field. It can be seen in Fig. 3 that the mobility of ssDNA is not as sensitive to the field strength as is dsDNA of the same length.

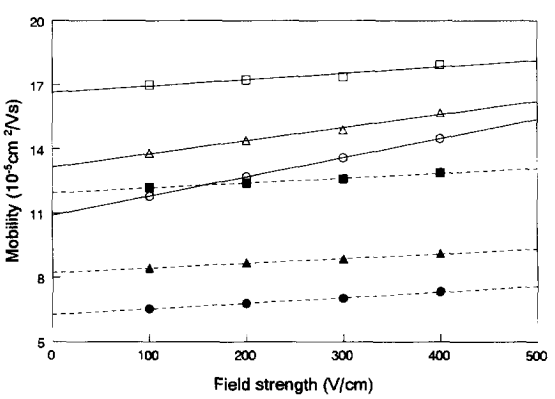

Fig. 3. Apparent mobility vs. electric field strength. dsDNA measured in buffer without urea; ssDNA measured in buffer containing 7 *M* urea; (□) dsDNA 100 base pairs; (△) dsDNA 300 base pairs; (○) dsDNA 500 base pairs; (■) ssDNA 100 bases; (▲) ssDNA 300 bases; (●) ssDNA 500 bases.

Apparently, the more flexible ssDNA is already more extended than the dsDNA, adopting a more rod-like conformation. This is perhaps to be expected, as the ionic strength of the CE buffer is rather low (30 m*M*). A similar finding has been published by Luckey and Smith [30]. In their model for the mobility of ssDNA, they predict a weaker dependence of ssDNA mobility on field strength than that exhibited by dsDNA.

It is well known that lower field strength will increase the selectivity of a dsDNA separation. This has been shown by McGregor and Young [9] using buffers containing 0.5% methylcellulose. The selectivity between 100 an 150 base pairs increases from 0.0604 at 200 V/cm to 0.0625 at 100 V/cm (a rise of 3.5%). The selectivity between 300 and 350 base pairs increases from 0.0416 to 0.0547 (a rise of 31%). However, a lower field strength also increases the time of analysis, which makes this option for improving the separation not so attractive.

In any case, the selectivity for ssDNA under denaturing conditions is higher than for dsDNA under native conditions. This can be seen in the electropherogram given in Fig. 1. Although the applied field strength during the run under denaturing conditions was two times higher, the greater selectivity for the ssDNA separation still results in a wider migration window (the difference in migration time between first and last eluting peak), yet the time of analysis is shorter.

The improvement in resolution which is achieved when DNA is separated in ss form can also be observed by comparing the CE separation of two allelic ladders of the HUMTH01 and HUMFES loci under native and denaturing conditions (Fig. 4). HUMTH01 and HUMFES are loci which contain short tandem repeats (STR) [31]. STRs are tri-, tetra- or pentanucleotides that are repeated sequentially on several places on the genome. They are used for testing for paternity, for personal identification and for diagnostic purposes [32,33]. They are easily amplified with the polymerase chain reaction. An allelic ladder consists of all repeats that might be

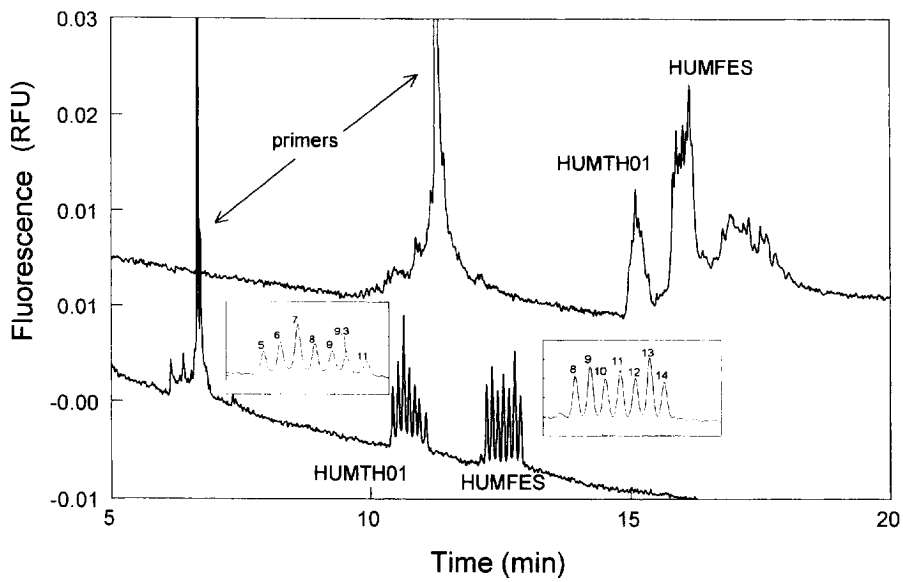

Fig. 4. Electropherogram of STR allelic ladders HUMTH01 and HUMFES loci. HUMTH01: 5 = 154 base pairs (bp), 6 = 158 bp, 7 = 162 bp, 8 = 166 bp, 9 = 170 bp, 9.3 = 173 bp, 11 = 178 bp. HUMFES: 8 = 211 bp, 9 = 215 bp, 10 = 219 bp, 11 = 223 bp, 12 = 227 bp, 13 = 231 bp, 14 = 235 bp. Upper electropherogram: native conditions dsDNA, voltage 7.4 kV. Lower electropherogram: denaturing conditions ssDNA, voltage 14.8 kV. Capillary I.D. = 75 μm, l = 30/37 cm, pressure injection of 10 s, temperature = 20°C.

present at that typical locus. Using the primer set described by Kimpton et al. [26], the HUMTH01 and HUMFES allelic ladders each consist of 7 DNA fragments ranging from 154 to 178 base pairs and from 211 to 235 base pairs, with a step size of 4 base pairs in both cases [34,35]. The HUMTH01 locus has one exception for the 9.3 repeat, which has 173 base pairs rather than of 174. Sufficient resolution of all fourteen peaks is obtained when DNA is separated in the ss form, while no resolution is visible when DNA is separated in the ds form. Baseline resolution of the HUMTH01 fragments have also been published by Butler et al. [17] with the use of an intercalator. Intercalators which are present in the running buffer form a complex with DNA molecules reducing their net charge and hence their electrophoretic mobility [8,18]. This results in better selectivity, and therefore better resolution; however, the effect is less dramatic than that observed for ssDNA. Separation of the allelic ladder HUMTH01 using labelled primers was published recently by Wang et al. [36]. However, the time of analysis can be much shorter when DNA is separated in its ss form. The applied field strength in the denaturing system was two times higher, which resulted in a faster analysis time for the denaturing system, as well as superior resolution.

The migration times of the various fragments in the DNA sizer can be fit to a second order polynomial as function of their length. Using the peak width and the plot of DNA migration time vs. fragment length, the peak width can be expressed in base pairs, reflecting the number of adjacent base pairs which are occupied by one peak. This number provides direct information about which DNA molecules can be distinguished from each other. The results are listed in Table 1. It is clear that the separation under denaturing conditions delivers better resolution. In the native system, a 5-base pair difference can be resolved in the 100 base pair range, while in the denaturing system a 2-base pair difference can be distinguished. The lower mobility of the ssDNA does require a longer analysis time. The increased resolution is of diminished value if it requires a longer separation time. However, if one employs a greater field strength for the denaturing separation (in this study, twice as high), the separation time is much reduced. Furthermore, Table 1 shows that under this higher field strength resolution is only slightly decreased.

Table 1
Resolution of DNA separation expressed in base pairs as function of DNA length

Form	ds	ss	ss	ss	ss
Temperature (°C)	20	20	20	40	40
Field strength (V/cm)	200	200	400	200	400
DNA length (bases)					
100	4.6	1.1	1.5	1.2	1.4
150	4.5	1.4	1.8	1.3	1.4
200	5.5	1.5	2.2	1.4	2.2
250	5.8	1.5	2.5	1.6	3.5
300	8.0	4.6	4.0	4.2	4.4
350	7.5	4.1	4.2	4.0	4.6
400	10	4.2	5.0	5.9	7.1
450	11	4.2	4.8	4.5	5.8
500	11	4.5	5.9	4.5	7.2

The influence of conformation (ds or ss), temperature and field strength on the resolution is listed. Capillary: I.D. = 50 μm, *l* 30/37 cm.

The numbers are only a rough indication and offer the reader the opportunity to compare the effects of field strength, temperature and the DNAs ds or ss form on the resolution. It is better not to pay too much attention to the absolute values. Resolution can always be improved if separation time is increased, or if one employs sample stacking.

The peak width for some DNA fragments (especially 400 base pairs) is aberrant. This may be caused by different folding of the ssDNA molecules, e.g., the ssDNA molecule may adopt an ensemble of conformations. Secondly, the peak widths of the DNA molecules are influenced by the amount of sample, and the amounts of the various DNA molecules are not the same. The relative standard deviation for the given values was approximately 10%.

3.2. Influence of DNA secondary structure

When one is working with ssDNA, it is important to be aware of its propensity to adopt secondary structure. Different conformations can give rise to band compression, or more generally, to anomalous migration [37]. The anomalous migration behavior of ssDNA is the principle upon which single stranded conformation polymorphism (SSCP) analysis is based. Different folding of ssDNA molecules gives rise to differences in electrophoretic mobility [38,39]. These differences are exploited for the detection of point mutations. However, if sequence differs, the separation of ssDNA is not purely size dependent even under denaturing conditions. Sequence-dependent migration can make accurate base pair assignment difficult. Some sequence dependence has also been reported for small dsDNA fragments [40,41], but the effect is much more dramatic for ssDNA. It is for this reason that ssDNA should be separated at elevated temperature (e.g., 40°C) [37]. At such elevated temperatures, DNA secondary structure is melted out and electrophoresis becomes more purely size-dependent. This is shown in Fig. 5. A part of the amelogenin gene was amplified and then sized using the Pharmacia DNA 50–500 sizer. PCR amplification of this region should yield two

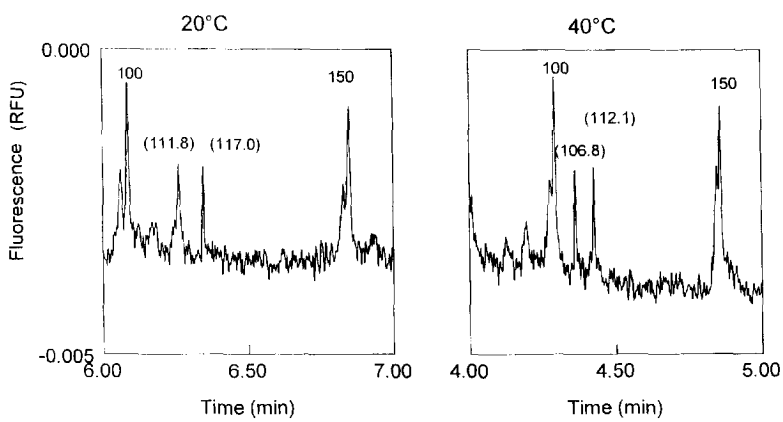

Fig. 5. Accurate sizing at elevated temperature. Double injection of DNA 50–500 sizer and amelogenin PCR product at 20°C (left) and 40°C (right). Capillary I.D. = 50 μm, *l* = 20/27 cm, voltage = 10.8 kV.

different PCR products of 106 and 112 base pairs, since the amelogenin gene is polymorphic (located on both the X and Y chromosomes), and the sample contained DNA from both of these chromosomes [27]. The calculated number of base pairs is given in parentheses in Fig. 5. At 40°C, the correct values are obtained.

A higher temperature also results in an increase in electrophoretic mobility. The average mobility rise of all DNA fragments was 44% (standard deviation was 1%; mobility rise was 2.2%/°C). Although the average selectivity for all of the DNA fragments did not change, the standard deviation in the selectivity rise was 11%. Electrophoretic separation of the DNA sizer at elevated temperature resulted in only slight differences in resolution (see Table 1). The decrease in buffer viscosity which is a consequence of running at higher temperatures results in both decreased separation times and faster replacement of the separation matrix.

3.3. Analysis of real samples

The resolution achieved by this method enables sizing of the STRs of the HUMTH01 and HUMFES loci. The difference in migration time between the peaks of the repeats is only a few seconds. This puts great demands on the accuracy of the system. If only single wavelength detection is available, the accurate sizing which is required can only be obtained if one utilizes an internal standard, as previously discussed by Butler et al. [22]. A PCR product from the amelogenin gene was used as internal standard, allowing us to size the other PCR products. This internal standard was pressure-injected just before the STR sample. The electropherograms were overlaid with matched peaks of the internal standard (Fig. 6). The repeat number could be obtained by comparison of the allelic ladder and the unknown sample. The fragment lengths of the sample were also calculated using the allelic ladder as a marker. An accuracy of 0.2 base pairs was achieved. By this method, 50 samples of forensic interest were sized, and resulted in a 100% correlation with the results achieved on a automated slab gel sequencer (Applied Biosystems 373A). These CE measurements were performed using a 20/27 cm long capillary with 50 μm I.D.. Note that sufficient resolution for accurate

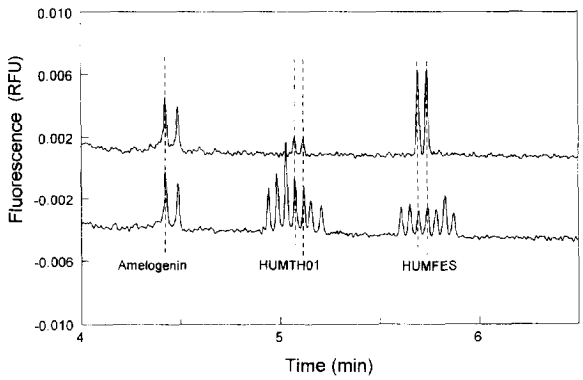

Fig. 6. Accurate sizing of STR. Upper: double injection of PCR products amelogenin and STR sample. Lower: double injection of PCR products amelogenin and allelic ladders of HUMTH01 and HUMFES. Capillary I.D. 50 μm, l=20/27 cm, voltage=10.8 kV, temperature=40°C, pressure injection of 20 s.

sizing is achieved with a separation time of only 6 min.

The resolution achieved in the separation of small DNA fragments reaches the values which are required for DNA sequencing. Fig. 7 shows the separation of a sequencing digest of a PCR product. Chain extension was terminated by the addition of ddATP to the PCR mix. This means that the sample contains large amounts of chloride, so that in order to introduce a sufficient amount of DNA into the capillary, a pressure injection is required. It is clear that the resolution is sufficient for sequencing of DNA up to 250 base pairs in length. Furthermore,

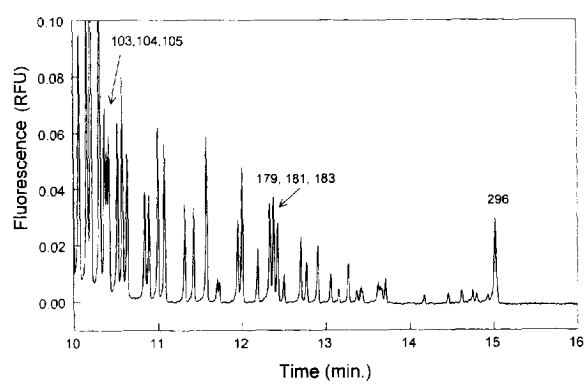

Fig. 7. Electropherogram of ddATP terminated sequencing digest of fragment of BCR/ABL fusion gene. Capillary I.D. 50 μm, l=30/37 cm, pressure injection of 40 s, voltage=10.8 kV, temperature=40°C.

the total analysis time is not more than 15 min. This type of rapid separation could serve to confirm the identity of a PCR product, an application which can be very important in the search for point mutations. The same purpose has been served by dideoxy fingerprinting (ddF) as discussed by Felmlee et al. using a fixed gel [42]. DNA sequencing using replaceable gels has also been published recently [43,44]. However, in those studies, electrokinetic injection was used. The present work demonstrates that one can achieve the resolution necessary to sequence PCR products up to 250 bases in length, even if one employs pressure injection.

4. Conclusion

Capillary electrophoresis is a powerful tool for the analysis of PCR products. The use of fluorescein-labelled primers enables electrophoretic separation under denaturing conditions. When DNA is separated in its ss form, the selectivity of the separation is greatly increased, which results in significantly higher resolution. Even when separation is performed at high temperature to aid in DNA denaturation, and using a high field strength to ensure rapid separation, better resolution is obtained under denaturing conditions than under native conditions. We have demonstrated the separation of PCR products, separated in the ss form, up to 200 bases in length in under 6 min, with base-line resolution of fragments differing by 4 bases. The use of a replaceable separation matrix assures good reproducibility, as well as an accuracy of better than 0.2 base pairs in the assignment of short tandem repeats. The resolution provided by linear polyacrylamide under denaturing conditions is sufficient for sequencing of short PCR products (<250 base pairs), with analysis times under 15 min.

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