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Separation of DNA fragments by capillary electrophoresis using replaceable linear polyacrylamide matrices

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ABSTRACT

The use of low percent (1.5–6%T) replaceable linear polyacrylamide (LPA) network matrices for rapid separation of double-stranded DNA fragments was explored. Separations of fragments ranging from 20 to 23 000 base pairs were readily achieved. Typically, $4 \cdot 10^6$ theoretical plates/m were obtained in less than 30 min. Short separation times under 2 min were also possible, using the DNA intercalating dye, ethidium bromide, along with high electric fields. The high resolving power of linear polyacrylamide was demonstrated in the separation of two fragments which differ by a single base pair (123/124 base pairs) using 6%T LPA and ethidium bromide intercalation. This LPA composition allowed for the possible single base-pair resolution of dsDNA fragments up to 300 base pairs in length. Several concentrations of the linear polyacrylamide for different ranges of fragment lengths have been employed. In addition, replaceable LPA offers the advantage of a fresh separation matrix for each run, thus overcoming column stability problems and minimizing needs for sample cleanup. Electroosmotic flow was substantially reduced using stable capillary coatings, which were required for obtaining high efficiencies and good reproducibility.

INTRODUCTION

The separation of double-stranded DNA fragments is a basic requirement in recombinant DNA technology [1,2]. Improvements in resolution, speed, quantitation, and automation are among the characteristics that capillary electrophoresis offers relative to electrophoresis in the conventional slab gel format [3]. Due to the anti-convective nature of the walls of the capillary, low-viscosity sieving media which cannot normally be successfully applied to a slab may be employed for DNA separations, *e.g.* linear (*i.e.* noncrosslinked) polyacrylamide [4–6], cellulose and its derivatives [7–11], liquified low melting point agarose [12] or polyethylene glycol [13]. For a review on this topic see ref. 14.

For the most part, slab gel electrophoresis has been limited to the use of either agarose or crosslinked polyacrylamide or related polymeric matrices [15]. The mesh size of crosslinked polyacrylamide is generally too small to separate efficiently DNA fragments larger than 2000 base pairs (bp). While agarose slab gels are employed for both small (200–1000 bp) and large (up to 10 000 kbp) DNA molecule separations, resolution and speed are often insufficient. Capillary electrophoresis using crosslinked polyacrylamide gel filled capillaries provides high separation

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efficiencies for synthetic single-stranded oligonucleotides and short restriction dsDNA fragments [16–18], but any stress conditions leading to a gel failure result in a need of the whole column replacement. For these reasons we continue our efforts in DNA separations using linear (*i.e.* noncrosslinked) polyacrylamide in capillary electrophoresis with the aim of a single base-pair resolution and extension of the size range of separated DNA fragments [4,5,19].

Linear polyacrylamide (LPA) has been shown to offer many desirable analytical characteristics and to provide for high resolving power. In this work we explore the use of linear polyacrylamide concentrations between 1.5 and 6%T.[a] These compositions have a low to moderate viscosity and can easily be refilled in the column. This replaceable matrix, as well as other commonly used polymer networks, increase the flexibility of capillary electrophoresis since problems such as polyacrylamide degradation or column fouling are eliminated. Moreover, these replaceable matrixes are simple to work with and yield reproducible results of high resolution.

EXPERIMENTAL

Equipment

The basic laboratory-made CE instrumentation has been previously described [4]. A 60 kV direct current power supply (Model PS/MK 60; Glassman, Whitehouse Station, NJ, USA) was used for continuous field electrophoresis. A UV–Vis spectrophotometer (Model 100; Spectra-Physics, San Jose, CA, USA) was employed at 260 nm to detect the DNA fragments. Column temperature was controlled by means of a water jacket connected to a heating/cooling thermostatic circulation water bath. Each end of the capillary was placed in buffer reservoirs (3 ml) with platinum wire electrodes. For all experiments, placing 3%T LPA in the reservoirs was found to be beneficial in avoiding ion depletion in the matrix with subsequent changes of current and migration times [20]. Buffer reservoirs were positioned at the same level and any coiling of the capillary after filling it with LPA was avoided to prevent the distortion of the structure of the low viscosity polymer networks [21].

Electrophoresis was performed in fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 and 75 μm I.D. and 360 μm O.D., which had a 2–4 mm portion of the polyimide coating removed for UV detection. The electropherograms were acquired at 5 Hz sampling rate using Turbochrom software (version 3.2) and stored on a IBM-compatible 486 DX-2 computer by means of an A/D converter interface (Model 762 SB, PE/Nelson, Cupertino, CA, USA).

Reproducibility experiments under controlled temperature conditions were performed using a Beckman P/ACE 2000 system (Palo Alto, CA, USA). It was operated in both the normal- and reverse-polarity configurations, depending upon the desired column length (*i.e.* the distance from the detector window to one of the reservoirs was fixed at 6.7 cm). Data were collected and integrated using Beckman System Gold software (version 403) and transferred to Lotus 1-2-3 (Cambridge, MA, USA) for further manipulation.

Capillary coating

The capillary coating was prepared according to Hjertén [22], cross-linked maltoside surfactant coating developed in this laboratory [40], or a recently developed procedure [23], using 6%T linear polyacrylamide. In the latter case, linear polyacrylamide is grafted to a highly cross-linked polymeric sublayer. Because all coatings suppress highly electroosmotic flow, differences in resolution and efficiency were not observed. However, the latter two procedures led to the longer column lifetimes [23].

Preparation of linear polyacrylamide

In this work, both laboratory polymerized and commercially available linear polyacrylamide were used. 6%T LPA matrices were prepared as follows: 6% (w/v) acrylamide solution was dissolved in 1 × TBE buffer (100 m*M* Tris, 100 m*M* boric acid, 2 m*M* Na_2EDTA, pH 8.4) and vacuum degassed for 2 h. Polymerization was initiated by adding 0.2% (v/v) N,N,N′,N′-tetramethylethylenediamine (TEMED) (Bio-Rad, Richmond, CA, USA) and 0.04% (w/v) am-

[a] T = [g acrylamide + g N,N′-methylenebisacrylamide (Bis)]/100 ml solution.

monium persulfate (ICN, Costamesa, CA, USA), and continued at 4°C for 48 h. 3%T and 1.5%T LPA matrices were prepared by dilution of a prepolymerized stock of 6%T. 5%T LPA M_r 700 000–1 000 000 (Polysciences, Warrington, PA, USA) was diluted from 10% aqueous stock solution and running buffer was adjusted to final concentration 1 × TBE. All LPA matrices were stored in a refrigerator at 4°C and replaced in the capillary using 100-μl syringes connected to capillary by piece of PTFE tubing. Prior to storage, polymerization byproducts were removed by electrolysis.

Chemicals

Ultrapure Tris base, boric acid and Na_2EDTA were obtained from Schwartz/Mann Biotech (Cleveland, OH, USA). Acrylamide was purchased from ICN and the silane reagents from Petrarch Systems (Bristol, PA, USA). Ethidium bromide (Sigma, St. Louis, MO, USA), 10 mg/ml aqueous solution was diluted in water and added to both the separation matrix and LPA used in the electrode reservoirs. All other reagents were of the highest quality available from Sigma. Buffer and acrylamide solutions were prepared in a Millipore Alpha-Q system purified water (Millipore, Bedford, MA, USA) and filtered through a 0.2-μm pore size filter (Schleicher & Schuell, Keene, NH, USA) prior to use.

DNA Samples

Restriction digests ΦX174/*Hae* III, pBR322/Msp I and λ/*Hind* III were obtained from New England Biolabs (Beverly, MA, USA), pBR322/Hae III digest from Sigma, 123 bp ladder and 1 kbp ladder from Gibco BRL (Gaithersburg, MD, USA). All DNA samples were used at a final concentration of 100 μg/ml in water for electrokinetic or pressure injection into the capillary.

RESULTS AND DISCUSSION

DNA Restriction fragment separations

The use of low %T (≤6) linear polyacrylamide for the resolution of DNA restriction fragments was examined. A number of size-dependent separations were developed, with emphasis on analytical characteristics (*e.g.* time, efficiency and separation). While higher %T composition (6 to 14%T in ref. 4) have been useful principally for oligonucleotides, DNA sequencing reaction products, and small restriction fragment separations, the lower-viscosity systems are able to separate a wide size range of restriction fragments from tens of base pairs to twenty or greater kilobase pairs. Linear polyacrylamide compositions ≤6%T have significantly lower viscosities than those previously presented [4]. These compositions can be replaced or regenerated in the capillary column, allow pressurized sample injection, and offer versatility in sample clean-up (*e.g.* the column can be refilled, providing a fresh matrix for each separation). Four LPA concentrations are used in this work, 1.5% T, 3% T, 5% T and 6% T. The 1.5% T and 3% T matrices can be replaced with current commercial CE instrumentation. On the other hand, 5% T and 6% T matrices are more viscous and, depending on conditions, generally require *ca.* 500 p.s.i. (1 p.s.i. = 6895 Pa) for replacement. At present, this is easily done with a high-pressure syringe.

A representative restriction fragment separation is shown in Fig. 1. Here the 11 fragments of ΦX174 DNA cut with *Hae* III are resolved on 3% T (diluted from 6% T laboratory-polymerized stock) linear polyacrylamide in less than 10 min. Similar to the higher % T compositions previously presented [4], peak efficiency for the 3% T was high. At an applied electric field of 300 V/cm, the 603 bp fragment in Fig. 1 exhibited >400 000 plates in 20 cm ($2 \cdot 10^6$ N/m), as calculated using the method of Foley and Dorsey [24] to account for non-Gaussian peaks. (At such high plate counts, the assumption of symmetrical peaks may significantly overestimate column efficiency [25]).

It can be seen in Fig. 1 that peak heights and areas increase progressively for the longer fragments. This result is expected for a restriction fragment digest since equal numbers of each fragment are present but larger fragments have a proportionally greater number of chromophores. This relationship between fragment size and peak area may be useful in the initial assignment

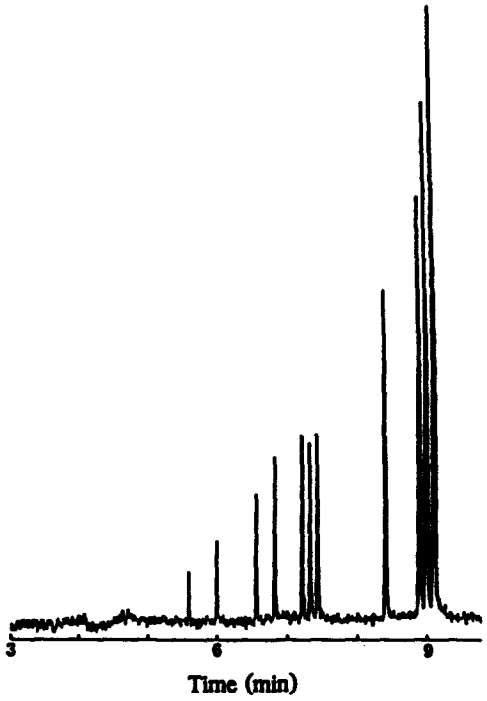

Fig. 1. Separation of ΦX174/*Hae* III using 3% T linear polyacrylamide. The eleven fragments range from 72 to 1353 bp. Conditions: l (length to detector) = 20 cm, L (column length) = 40 cm, E (voltage) = 300 V/cm, I (current) = 16 μA, capillary I.D. = 75 μm, O.D. = 360 μm, 1 × TBE buffer, electrokinetic injection at 75 V/cm for 4 s.

of migration order for this type of sample. Note also the baseline resolution of three peaks 271, 281 and 310 bp in length.

Using the ΦX174/*Hae* III restriction fragment digest as a test mixture, repetitive separations were performed using 3% T LPA at the column temperature 30°C to determine run-to-run reproducibility. Reproducibility experiments were performed using capillaries with inner walls coated with 6% T LPA [40] as described in the experimental section and the Beckman P/ACE system. Between each run, the polyacrylamide was blown out of the column and refilled. Comprehensive reproducibility data is summarized in Table I for the number of 418 separations performed. Table I demonstrates high stability of column coating and very reproducible migration times under the controlled conditions. Other coatings such as in ref. 23 work equally as well.

Another example of the separation of DNA

TABLE I

MIGRATION TIME REPRODUCIBILITY OF ΦX174/*Hae* III DIGEST ON A 3% T LINEAR POLYACRYLAMIDE COLUMN

Conditions: l = 25 cm, E = 300 V/cm, I = 23 μA, capillary I.D. = 75 μm, O.D. = 375 μm, column temperature = 30°C, running buffer 1 × TBE, pH 8.3, electrokinetic injection at 150 V/cm, 5 s. Beckman P/ACE system.

	603 bp	1353 bp
Absolute migration		
t_r average [min]	6.34	6.79
σ	$9.80 \cdot 10^{-2}$	$1.11 \cdot 10^{-1}$
n	418	418
R.S.D. (%)	1.55	1.63
Relative migration	1353/603 bp	
Δt_r [min]	1.07	
σ	$1.79 \cdot 10^{-3}$	
n	418	
R.S.D. (%)	0.17	

fragments on a laboratory-made 3% T linear polyacrylamide matrix is demonstrated in Fig. 2. This electropherogram shows separation of a 123 bp ladder (*e.g.* 123 bp fragments ligated to form 123 bp multimers). Twenty-six ladder species, of

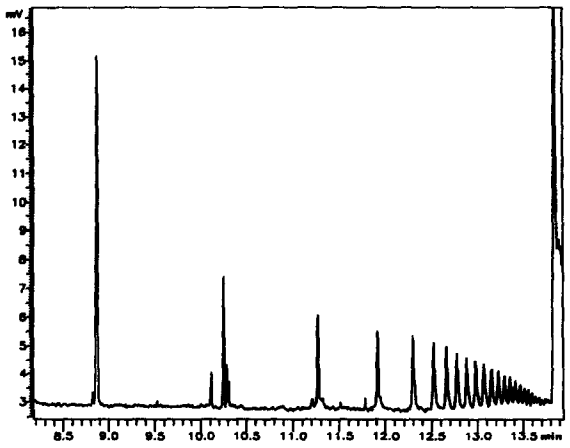

Fig. 2. Electropherogram of 123 bp DNA ladder using 3% T linear polyacrylamide. 26 fragments are separated ranging from 123 to 3198 bp. Conditions as in Fig. 1, except: l = 30 cm, electrokinetic injection at 200 V/cm for 2 s.

lengths between 123 bp and 3198 bp, are resolved in less than 15 min. High efficiencies are obtained with a maximum of $8.5 \cdot 10^6$ plates/m reached for the peak 3 (369 bp). The broad peak eluting after 13.8 min is assumed to be higher order multimers which were not resolved. The peak area (and peak height) decrease with increase in base number opposite to that of the *ΦX174/Hae* III digest in Fig. 1. Since the ladder is prepared by enzymatic ligation of the smallest unit (*e.g.* 123 bp), the smaller fragments are in the highest concentration for the specific reaction time.

We next examined the performance of the laboratory-made 3% T polymer network matrix with the separation of DNA restriction fragments up to 12 kbp. The electropherogram shown in Fig. 3 demonstrates the separation of a 1 kbp DNA ladder up to 12 kbp in less than 30 min. Peak 4 of the ladder exhibits a high efficiency of $4.4 \cdot 10^6$ plates/m. This sample consisted of 11 species in addition to the 12 species from the 1018 bp ligated monomer ladder. The additional fragments ranging from 72 to 1636 bp arise from enzymatic digestion of the pBR 322 plasmid DNA which was used in the preparation of the sample. A total of 23 fragments ranging from 72 to 12 216 bp were expected and were observed. This separation is comparable to one, shown previously using a crosslinked (3% T, 0.5% C)[a] gel filled capillary [4].

Next, separation of the sample pBR322/MspI was performed using a commercially available industrial linear polyacrylamide matrix, previously also used by other authors [26]. The sieving matrix was a 5% T LPA in 1 × TBE (Polysciences, molecular mass distribution of the polyacrylamide chains ranges from 700 000 to 1 000 000). The sample contains 26 fragments ranging in size from 9 bp to 622 bp. The first detectable peak consisted of the two fragments of 26 bp in length, the second peak consisted of the two 34 bp long fragments. Roman numeral I in Fig. 4 shows the base line separation of two 147 bp fragments. Roman numeral II shows the partial separation of the two fragments 160 bp. Similar resolutions of equivalent fragments have been shown previously using high % T nonreplaceable LPA matrix [5].

The separation of two fragments of the same length is a result of conformational differences between the two species. Similar migration phenomena have been observed by Stellwagen and

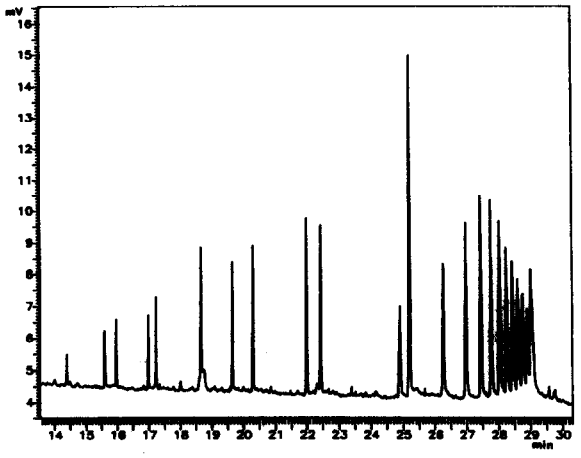

Fig. 3. Separation of the 1 kbp DNA ladder using 3% T linear polyacrylamide. Fragments range from 72 bp up to 12 216 bp. Conditions as in Fig. 1, except: $l = 40$ cm, $L = 70$ cm, electrokinetic injection at 10 kV for 0.5 s.

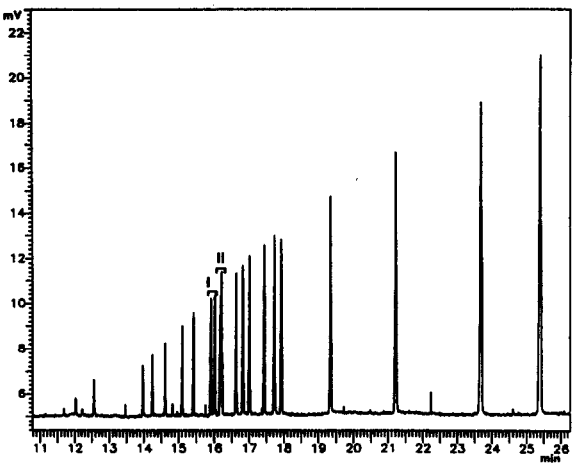

Fig. 4. Separation of the pBR322/Msp I digest using 5% T LPA Polysciences (M_r 700 000–1 000 000). Fragments range from 9 to 622 bp. The first fragment observed here is the fragment of 26 bp. Conditions as in Fig. 1, except: electrokinetic injection at 4 kV for 1 s.

[a] C = g Bis/% T.

Stellwagen [27] in crosslinked polyacrylamide slab gels. It was concluded that numerous AT-rich regions in one of the 147 bp fragments imparted a bend to the molecule, causing it to migrate more slowly than the other 147 bp fragment. Differences in tertiary structures are analogously assumed to be the reason for partial separation of the two 160 bp fragments. Thus, the high resolving power of linear polyacrylamide can potentially be used to probe structural differences between DNA fragments of the same or similar size, but of a different origin. DNA conformation dependent separations using capillary electrophoresis with linear polyacrylamide matrices is a subject of a separate paper [28].

The separation in Fig. 4 shows the possible usefulness of commercially available linear polyacrylamide solutions; however, in the present work we have found poor batch-to-batch reproducibility of the commercial product, compared to laboratory made matrixes. There are several linear polyacrylamide molecular mass standards on the market, which may yield improved reprodubility. It can be noted, nevertheless, that the current polymerization procedure is quite straightforward, and the resultant material highly reproducible [19].

We next extended the DNA size range by decreasing our laboratory-polymerized LPA concentration to 1.5% T. A separation of λ/*Hind* III digest on such a matrix is shown in Fig. 5. The λ/*Hind* III digest contains 8 fragments ranging from 125 bp to 23.1 kbp. The 125 bp fragment, the smallest of the digest, is not observed in the figure due to its relatively low absorbance (since absorbance ∝bp). The peak for this fragment could be observed by loading more sample onto the column; however, overloading of the larger fragments occurred. Peak assignment was made by peak height analysis.

An interesting feature in Fig. 5 is the broad 23.1 kbp peak. Similar separation of λ/*Hind* III on 0.5% methylcellulose matrix was previously shown and broadening of the 23.1 kbp fragment was explained by reptation with stretching model [29]. This phenomenon is, however, believed to be caused by *cos*-end annealing of the 4361 bp and 23.1 kbp fragments, resulting in a 27 kbp

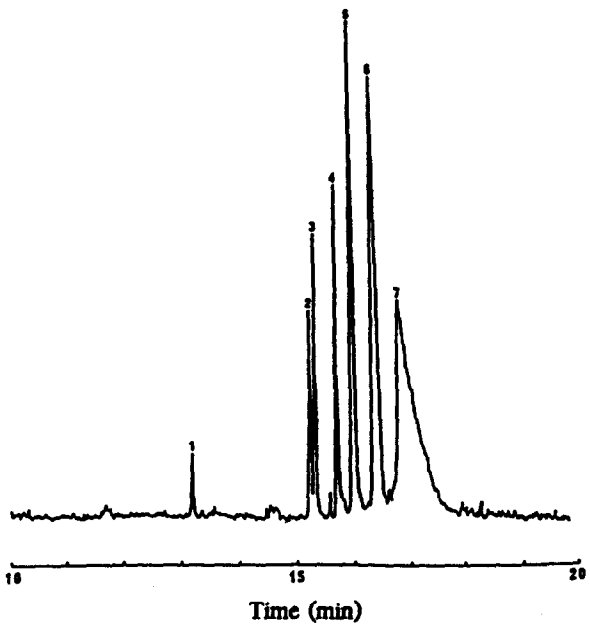

Fig. 5. Electropherogram of λ/*Hind* III DNA using 1.5% linear polyacrylamide. Presumed peak identification: 1 = 564 bp; 2 = 2.0 kbp; 3 = 2.3 kbp; 4 = 4.4 kbp; 5 = 6.6 kbp; 6 = 9.4 kbp; 7 = 23.1 kbp. Conditions as in Fig. 1, except: 1.5% T, l = 30 cm, sample heated to 65°C then chilled on ice, injection by siphoning (Δh = 10 cm, 2 s.)

species [30]. Evidence of this was obtained by loading the sample after immediate removal from −20°C storage. In this case, the 4361 bp peak was not observed and the 23.1 kbp peak was further broadened, due to strong annealing (Fig. 6A). Heating of the sample or addition of urea to the running buffer disrupted this interfragment annealing and single, sharper peaks were obtained (Fig. 6B).

Addition of ethidium bromide

The conformation of DNA, and thus its mobility, can be affected chemically. A common fluorescent dye, ethidium bromide (EtBr), alters the conformation by intercalating into the DNA helix [31,32]. EtBr is known to bind to DNA in a ratio of 1:5 and the binding constant ($1.5 \cdot 10^5$ M^{-1}) is sequence independent [32]. Intercalation of EtBr increases the contour length, but decreases the persistence length of DNA molecules, as the result of reduced DNA rigidity upon neutralization of phosphate groups after

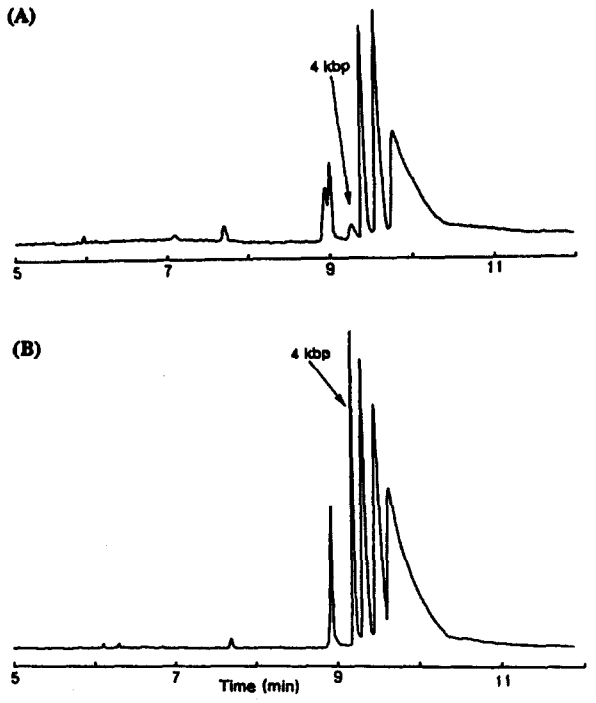

Fig. 6. Use of 1.5% T linear polyacrylamide with 0.05% agarose for λ/*Hind* III separation. See text for details. l = 20 cm, $E = 200$ V/cm, $I = 12$ μA. Injection by siphoning (Δh = 10 cm, 2 s.) (A) Sample at 4°C from −20°C; (B) sample at 25°C after heating to 65°C.

intercalation of the positively charged EtBr [33]. These properties of ethidium bromide and other DNA ligands are believed to remove sequence induced DNA curvature [34].

The separation of the sample ΦX174/*Hae* III, using 6% T LPA and electric field of 300 V/cm, exhibits changes in peak order in which DNA fragments no longer migrate according their molecular mass, *i.e.* length. The triplet of 271, 281 and 310 bp fragment in Fig. 7A is most likely not migrating according to increasing fragment length based on peak areas and relative positions. Furthermore, the peak for fragment 872 bp comigrates with peak 1078 bp (confirmed by fragment spiking experiments). For the same electrophoretic conditions, the addition of EtBr to the running buffer clearly eliminates these problems (see Fig. 7B). As another consequence of DNA charge neutralization, the migration time of DNA fragments increases, as can be seen in Fig. 7B. The relationship between changes in

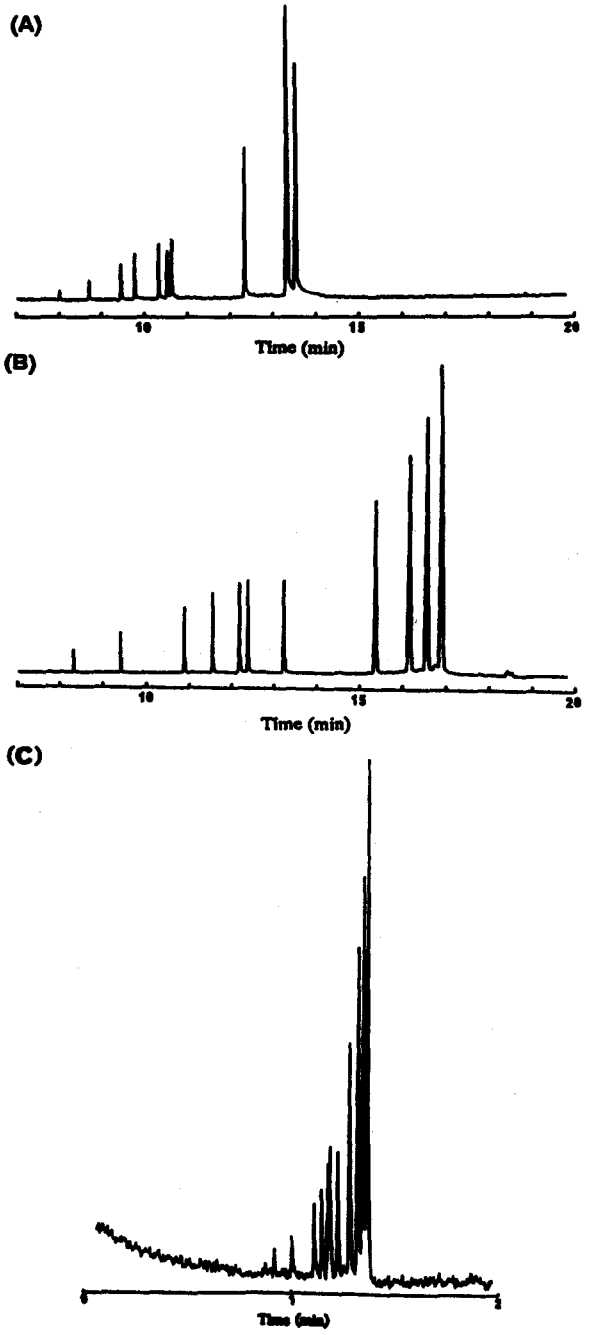

Fig. 7. Effect of ethidium bromide in migration behavior of ΦX174/*Hae* III DNA. (A) 6% T linear polyacrylamide without ethidium bromide; (B) 6% T linear polyacrylamide with 0.5 μg/ml ethidium bromide added to the running buffer. Conditions: $l = 20$ cm, $E = 300$ V/cm, other conditions as in Fig. 1. (C) Rapid separation of ΦX174/*Hae* III using 3% T linear polyacrylamide. Conditions: $E = 700$ V/cm, $I = 19$ μA, capillary I.D. = 25 μm, 0.5 μg/ml EtBr.

DNA fragment conformation and the correct migration pattern (increasing UV absorbance) is not fully understood. Nevertheless, the use of intercalating agents as well as other DNA-binding chemicals to modify DNA tertiary structures seems to be promising [28,34,35]. Other parameters such as temperature, field strength, buffer ionic strength, and matrix composition have also been studied in order to recover normal migration patterns for anomalous DNA fragments [27]. The role of these parameters, and others, on DNA separations using CE will be discussed separately [28].

The combined advantage of both ethidium bromide addition into the running buffer and the high plate counts on linear polyacrylamide allow separation under high electric fields. Migration times can be on the order of 1 min, as shown in Fig. 7C. Here, the analysis time for the ΦX174/*Hae* III digest was decreased to 1.5 min by increasing the field to 700 V/cm. Since the low viscosity of the 3% T polyacrylamide solution yields current generation similar to that observed in the open-tube mode (in contrast to the low currents found for the high %T gels), the column I.D. was reduced from 75 to 25 μm, limiting the power generation to <0.6 W/m. At this level, significant Joule heating is not expected [36,37].

Comparing the differences in the electropherograms of Fig. 7B and C, it can be seen that separation of the 271/281 bp doublet was worse in the high field experiment. This trade-off between speed and separation power, however, may be acceptable for many applications, such as in on-line polymerase chain reaction (PCR) product analysis [38], monitoring an enzymatic digest, or any type of time-dependent reaction. In fact, depending on the separation required, sub-minute analysis times can be readily realized.

In order to examine further the separation abilities of linear polyacrylamide, we have next chosen the plasmid pBR 322/*Hae* III restriction digest. This sample contained 22 fragments ranging from 8 to 587 bp, including the doublet 123/124 bp. The first detectable peak was for fragment 18 bp long and 19 peaks were detected using 6% T LPA, as shown in Fig. 8A. The seventh major peak represents comigrating fragments 123 and 124 base pairs, which were not separated on 6% T LPA, under the described conditions. Fig. 8A further shows positions of the last five peaks (434 to 587 bp) which do not correspond to the fragment lengths, based on the expected peak area increase with migration position for the restriction digest. Fragments 458 bp and 587 bp of this digest contain regions with tertiary structures which can cause retardation in polyacrylamide gels [27]. Fig. 8B shows the effect of ethidium bromide addition upon the

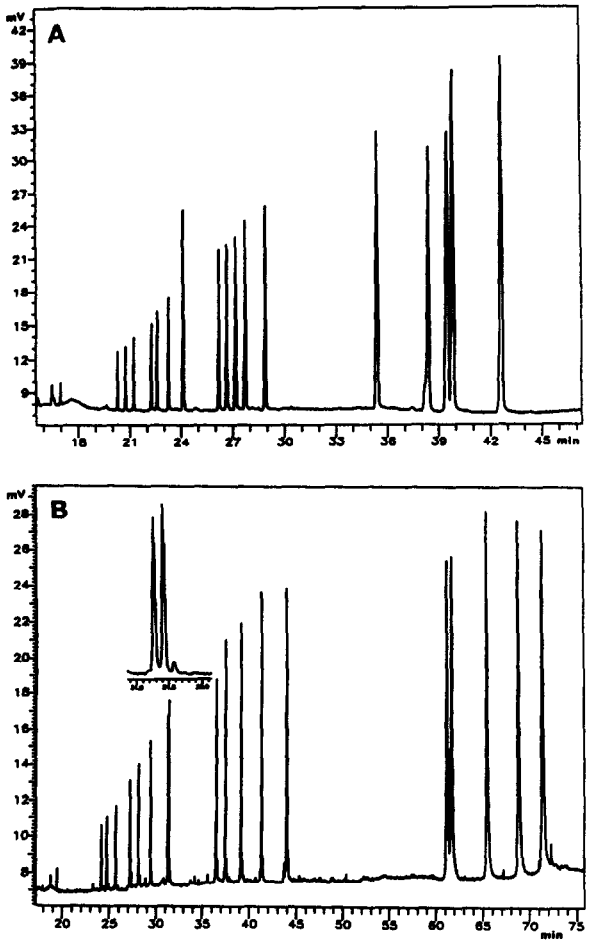

Fig. 8. Electropherogram of the pBR 322/*Hae* III sample separation from peak 18 bp to 587 bp using 6% T LPA without (A) or with (B) 1 μg/ml ethidium bromide. Conditions: 1 × TBE running buffer, $E = 150$ V/cm, $L = 45$ cm, $l = 30$ cm, column temperature 30°C, electrokinetic sample injection at 10 kV/2.5 s.

separation of pBR 322/*Hae* III. Note baseline resolution of peaks for fragments 123 and 124 bp (insert at Fig. 8B) and correct migration order of peaks for fragments 434–587 bp (based on peak areas). On the other hand, overall migration time increased to 72 min.

Fig. 9 shows a plot of migration time (mobility^{-1}) *vs.* bp number, N for the electropherogram in Fig. 8B. It can be seen that a close to linear relationship exists at least to 267 bp. The number of peaks that can be resolved in this region (51–267 bp) can be determined from

$$N = \frac{(t_{r267} - t_{r51})}{W} \quad (1)$$

where W = average peak width. For the given time window (*ca.* 20 min) and peaks widths ($4 \cdot 10^6$ plates/m), it is determined that single base pair resolution is possible in this region, assuming the DNA molecules do not anomalously migrate.

In Fig. 9, it can be seen that the slope of the last five peaks is lower than for the shorter fragments, probably as a consequence of the early stages of DNA stretching [39]. In addition, relative peak widths tend to be wider. As a consequence, the bp resolution changes to 2–3 bp in this region. Further optimization may decrease this number. However, what is important is that whereas in Fig. 8A the peaks do not appear to migrate in terms of increasing bp size (as determined from peak areas), the order is correct when ethidium bromide is the agent.

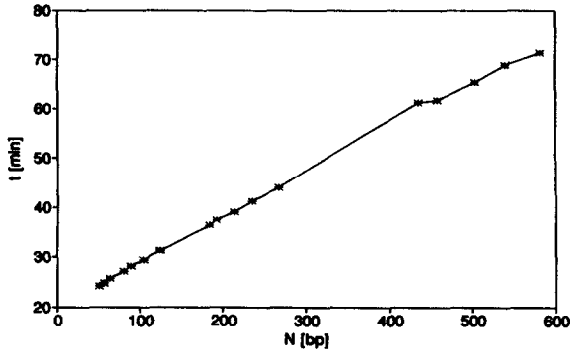

Fig. 9. Plot of migration time *vs.* base number for pBR 322/*Hae* III for electropherogram in Fig. 8B.

However, as seen in Fig. 9, there appears still to be a small amount of anomalous migration. These points will be discussed separately [28].

CONCLUSIONS

This paper presents results on the separation of double-stranded DNA molecular mass standards using replaceable linear polyacrylamide. Close to 1-min size-dependent separations were achieved using high electric field and ethidium bromide as an additive in the running buffer. Single base pair resolution of dsDNA fragments of 123 and 124 bp in length was attained with ethidium bromide in the running buffer. Possible single base pair resolving power of the 6% T linear polyacrylamide matrix was calculated in the range up to 267 bp. We have also demonstrated the sensitivity of linear polyacrylamide matrices to sequence induced DNA curvature of double-stranded DNA fragments.

With the perspective of an automated multicapillary instrument, equipped with the fluorescence detection, replaceable sieving matrices will allow the use of various compositions of separation media during consecutive runs without replacing and aligning new capillaries. This includes various pore-size matrixes optimized for specific size ranges of dsDNA fragments, native or denaturing compositions for mapping and detection of mutations, or finally, high resolution denaturing linear polyacrylamide for DNA sequencing [19]. Such a design would be cost effective and accessible to many research, clinical and forensic laboratories.

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