Electrophoresis 2002, *23,* 3062–3070

Lev Kotler
Hui He
Arthur W. Miller
Barry L. Karger

Barnett Institute and
Department of Chemistry,
Northeastern University,
Boston, MA, USA

DNA sequencing of close to 1000 bases in 40 minutes by capillary electrophoresis using dimethyl sulfoxide and urea as denaturants in replaceable linear polyacrylamide solutions

The goal of this work was to reduce the capillary electrophoresis (CE) separation time of DNA sequencing fragments with linear polyacrylamide solutions while maintaining the previously achieved long read lengths of 1000 bases. Separation speed can be increased while maintaining long read lengths by reducing the separation matrix viscosity and/or raising the column temperature. As urea is a major contributor to the separation buffer viscosity, reducing its concentration is desirable both for increase in the separation speed and easier solution replacement from the capillary. However, at urea concentrations below 6 M, the denaturing capacity of the separation buffer is not sufficient for accurate base-calling. To restore the denaturing properties of the buffer, a small amount of an organic solvent was added to the formulation. We found that a mixture of 2 M urea with 5% v/w of dimethyl sulfoxide (DMSO) resulted in 975 bases being sequenced at 70°C in 40 min with 98.5% accuracy. To achieve this result, the software was modified to perform base-calling at a peak resolution as low as 0.24. It is also demonstrated that the products of thermal decomposition of urea had a deleterious effect on the separation performance at temperatures above 70°C. With total replacement of urea with DMSO, at a concentration of 5% v/w in the same linear polyacrylamide (LPA)-containing buffer, it was possible to increase the column temperature up to 90°C. At this temperature, up to 951 bases with 98.5% accuracy could be read in only 32 min of separation. However, with DMSO alone, some groups of *C*-terminated peaks remained compressed, and column temperature at this level cannot at present be utilized with existing commercial instrumentation.

Keywords: Capillary electrophoresis / Dimethyl sulfoxide / DNA / DNA denaturation / Elevated column temperature / Polymer solutions / Separation solutions / Sequencing EL 4984

1 Introduction

Over the past decade, capillary electrophoresis (CE) has rapidly developed [1–6] to become the method employed to sequence the Human Genome [7, 8]. In spite of close to achieving the full sequence of the Human Genome, high-throughput *de novo* sequencing will still be required to complete other important genomes, as well as for applications in genetic screening, single-nucleotide polymorphism (SNP) discovery and scoring, pharmacogenomics, *etc.*, where sequencing is viewed as the gold standard [9–11]. These demands create the need for even higher throughput from sequencing instrumentation.

Correspondence: Prof. Barry L. Karger, Barnett Institute, Northeastern University, 341 Mugar Bldg., 360 Huntington Ave., Boston, MA 02115, USA
E-mail: b.karger@neu.edu
Fax: +617-373-2855

Abbreviations: LPA, linear polyacrylamide; **SNP**, single-nucleotide polymorphism

In our laboratory, a robust separation matrix for long read DNA sequencing by CE was developed [5, 12]. With a polymer solution containing 2% w/w linear polyacrylamide (LPA) with molecular mass above 10 MDa and 0.5% w/w 270 kDa, read lengths of up to 1300 bases in less than 2 h with 98.5% accuracy of M13mp18 DNA template was achieved. For faster separation, 1000 base sequence reads could be generated in roughly 1 h with the same accuracy [12]. In this latter work, we applied an integrated approach to optimize the electric field strength, injection conditions, LPA matrix formulation, sample cleanup, capillary column dimensions, and other parameters for a maximum total read length in the short time.

One of the key factors for long read DNA sequencing by CE was found to be column temperature [13, 14]. Elevating the temperature is beneficial for separation of long ssDNA Sanger fragments, because high temperature leads to improved selectivity (*i.e.*, extent of difference in electrophoretic mobilities) for the long fragments, due to

© 2002 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
0173-0835/02/1709–3062 $17.50+.50/0

shifting of the onset of biased reptation to higher base numbers [15]. Additionally, elevated temperature can also be helpful in reducing separation time and in resolving compressions [1, 15–17]. For LPA, the optimum column temperature was found to be in the range of 60–70°C. Current-automated DNA sequencers utilizing LPA solutions are designed to maintain a temperature at this level throughout the run.

An increase in the separation speed in long read length DNA sequencing may also be achieved by reducing the low shear viscosity of the polymer solution while preserving its separation performance. Two properties of the matrix solution are major contributors to viscosity: the concentration of LPA and urea. For an optimized concentration of LPA, the viscosity can in principle be reduced by partial or complete replacement of urea with another denaturant. Besides urea, other widely used denaturants for DNA are organic solvents, such as formamide [18–20] and other amides [21]; alcohols; 1,4-dioxane [22]. However, many of these denaturants are problematical for CE.

Formamide was tested as denaturation agent in a polyacrylamide gel-filled capillary column [23]. However, it is well known that formamide is prone to thermal decomposition, and this property would preclude its use in separations performed at high temperature. Ethanol and methanol are weak denaturants, and these alcohols must be used in sufficiently high concentrations that the separation would be significantly slowed due to dielectric friction caused by interaction of charged DNA molecules and the organic solvent [24]. Similarly, the presence of 1,4-dioxane mixed with urea leads to slower separations [22].

On the other hand, properties of DMSO, another widely used denaturant, make it a good choice for use in CE. Because of its excellent compatibility with enzymatic reactions, DMSO has been used as a denaturant in PCR and DNA sequencing sample preparation [7, 21, 25–28]. In DNA separation, it was selected to correct the electrophoretic image of DNA sequencing products in the presence of dithiothreitol (DTT) [29] and was examined for use in a time-resolved fluorescence detection method [30]. The denaturing ability of a substance is quantitated by the decrease of the melting temperature (Tm) of the double helix per unit concentration of the denaturant in solution. From this standpoint, DMSO is a stronger DNA denaturant than formamide [31, 32], and it is considered a good denaturant for DNA [33] and proteins [34]. In addition, its toxicity is much lower than that of urea [35, 36], and the thermal stability of DMSO is much higher [37].

In this paper, we demonstrate successful DNA sequencing with a mixture of 5% v/w DMSO and 2 M urea as denaturant in the separation matrix producing close to 1000 bases read length with high accuracy for M13mp18 as template in only 40 min at a column temperature of 70°C. This denaturant mixture may be considered as an alternative to the 6–7 M urea, if a significant increase in speed in DNA sequencing with LPA solutions is desirable. In addition, urea concentration in the mixture can be increased to resolve templates with stronger compressions, if necessary.

2 Materials and methods

2.1 Instrumentation

The design of the single-capillary instrument with laser-induced fluorescence (LIF) was similar to that previously described [38]. The fluorescence emission was collected with a microscope objective (Model 13600; Oriel, Stamford, CT, USA), and the spectra of the labeled sequencing fragments were acquired in 16 channels in the range from 500 to 660 nm with a CCD camera (Model NTE/CCD-1340/400-EMB; Roper Scientific, Trenton, NJ, USA). The laser and other components are the same as reported previously [4, 38]. The CE columns were fused-silica capillaries of 75 μm ID, 365 μm OD (Polymicro Technologies, Phoenix, AZ, USA), covalently coated with polyvinyl alcohol (PVA) [39]. The capillary was placed completely horizontally in the instrument. The effective capillary length (distance from injection point to the detection window) was 30 cm, with a total length of 45 cm. The sample was injected for 10 s at a constant current of 0.7 μA, and electrophoresis was performed at 200 V/cm.

2.2 Chemicals

Acrylamide, TEMED, ammonium persulfate and urea were purchased from ICN Biomedicals (Aurora, OH, USA). Tris, TAPS and EDTA were obtained from Sigma (St. Louis, MO, USA), and DMSO was from Aldrich (Milwaukee, WI, USA). All reagents were either electrophoresis or analytical grade, and no further purification was performed. Span 80 emulsifier and petroleum special with a boiling range from 180–220°C were purchased from Fluka Chemicals (Milwaukee, WI, USA). Water was deionized with a Milli-Q purification system to 18.2-MΩ grade (Millipore, Bedford, MA, USA).

2.3 Polymer synthesis and characterization and preparation of the separation matrices

LPA with molecular mass 5.6 MDa was prepared in powder form using inverse emulsion polymerization, as described previously [40]. After polymerization, the LPA

powder was washed with acetone and vacuum dried. The molecular mass of LPA was determined by multiangle laser light scattering [41]. Polymer solutions containing 2.5% w/w LPA (5.6 MDa) and a denaturant were utilized to separate the DNA sequencing reaction products. To prepare a typical mixed solution (20 g), appropriate amounts of dry LPA polymer, denaturant, 10 × buffer concentrate (500 mM Tris:500 mM TAPS:20 mM EDTA) and water were added in a glass jar and slowly stirred with a magnetic bar. All components were added by weight, except that DMSO was measured volumetrically. The solutions were usually homogenized slowly for two days and then were ready for use. Each polymer solution was replaced from the capillary after a given run using a gas-tight syringe, and the voltage was applied for 5 min before injection to reduce the current in the matrix solution to a constant value. LPA powder and other dried polymers have almost unlimited shelf life. The working solutions of LPA could be stored in the refrigerator at 4°C for up to 3 months. DNA sequencing reactions were performed using standard cycle sequencing chemistry with Ampli-Taq-FS and BigDye (-21) M13 universal primers (Applied Biosystems, Foster City, CA, USA) on an M13mp18 single-stranded template (New England Biolabs, Beverly, MA, USA). The temperature cycling protocol for this sequencing chemistry was made on a PTC200 thermocycler (MJ Research, Watertown, MA, USA), consisting of 15 cycles of 10 s at 95°C, 5 s at 50°C and 1 min at 70°C, followed by 15 cycles of 10 s at 95°C and 1 min of 70°C. After completion of the reaction, the samples were heated for 5 min at 100°C in order to inactivate the enzymes prior to the cleanup procedure.

2.4 Purification of the reaction products

Sequencing reactions were cleaned using a method previously described [42] with minor modifications. Template DNA (M13mp18) was removed using spin columns with a polyethersulfone ultrafiltration membrane, molecular weight cutoff of 300 000 (MWCO 300K; Pall Filtron, Northborough, MA, USA), which was pretreated with a 0.005% w/w solution of LPA with a molecular mass of 700–1000 kDa. The filtrate was dried under vacuum and dissolved in 50 µL of deionized water. The reconstituted template-free sequencing samples were then desalted using prewashed Centri-Sep 96 (gel filtration) plates (Princeton Separations, Adelphia, NJ, USA). The desalting procedure was performed twice per sample, after which the sample volume was adjusted to 55 µL. A 5 µL aliquot of the purified sample was diluted with 20 µL of deionized water prior to injection. The specific injection conditions are described in the figure captions. The purified sequencing samples were stored at –20°C in deionized water.

2.5 Base-calling software

An expert system developed in this laboratory was used for base-calling [12, 43]. Data processing began by determining the primer dye spectra from the relatively intense peaks in the data and performing color separation by a least-squares fit to these spectra. The electropherogram was divided into sections containing 20–40 bases, and the fifth percentile value among the amplitudes of all data points in each section was computed. This calculation established the background at the center of each section, and in other regions, the background was derived by linear interpolation. After background subtraction, the starting point of the sequence-containing region was determined by locating the primer peak and examining the time interval for the beginning of relatively uniform peak heights. The end point was designed as a migration time shortly before the position at which oriented reptation caused the elution of the remaining DNA as a single peak, which was detected by a dramatic increase in the standard deviation of the signal. If no such terminating peak was found, the end point was the completion of the electropherogram. Dye mobility shifts and average peak heights were computed throughout the sequence-containing region, and a set of empirical rules was employed to find peak boundaries and estimate the number of bases in each peak. The optimum performance on DMSO-containing matrices required minor adjustments to the parameters of some rules developed for matrices containing 7 M urea, due to higher separation speed, lower peak resolution, *etc.* Sequence reads down to a peak pair resolution of 0.24 was achieved. Sequencing data (read length, migration time, *etc.*) obtained from the base-caller were processed with Origin 6.0 software (Microcal, Northampton, MA, USA).

3 Results and discussion

In DNA sequencing by CE, the separation time may be reduced by several approaches, including the use of short columns, increase in column temperature and/or electric field, or change in the separation matrix/buffer composition, but, in these cases, often at expense of read length. In previous work from our laboratory, the separation of 1000 bases in roughly 1 h at 60–70°C was demonstrated [5, 12]. The conditions for such performance were optimized for a defined long read length in the shortest separation time. Shortening the capillary length yielded faster separations but lower read lengths, and an increase in the electric field strength had a similar effect.

One of the means for more rapid DNA sequencing by CE is to lower the low shear viscosity of the LPA solution. As mentioned previously, the separation solution viscosity

is mainly determined by the average molecular mass and concentration of LPA and the urea concentration. The LPA concentration and its molecular mass were previously optimized for long read lengths. Therefore, lowering the concentration of urea was the remaining parameter to reduce the viscosity, but this step by itself would inevitably reduce the denaturing ability of the polymer solution. In a preliminary study, we examined a number of organic solvents, which are commonly used for DNA denaturation, as a substitute for some or all of the urea (along with a column temperature of 70°C). In our experiments, ethanol was found to be an ineffective denaturant in CE of DNA at concentrations as high as 25% w/w, and, moreover, separation was slower than that with 7 M urea. Addition of 15% w/w 1,4-dioxane to the separation solution had the same effect. With DMSO, separation of DNA fragments occurred faster than that with urea, and the improvement in migration speed was combined with good quality sequence at 5% v/w concentration in the LPA solution (see below). Based on this preliminary study, we examined DMSO further as a possible complete or partial urea substitute in the separation of ssDNA Sanger fragments. We first determined the effect of lowering the urea concentration, followed by optimization of concentrations of both DMSO and urea in the polymer solution at 70°C while maintaining close to 1000 bases read length.

Before proceeding, it is important to note that the long read lengths reported in previous papers from our laboratory were obtained mainly using ssM13mp18 DNA as template. This is a *de facto* standard template used both for research in DNA separation and development of commercial sequencing instrumentation. While this template is not a production genomic template, the latter of which may possess high GC content, single- and polynucleotide repeats, *etc.*, it is nevertheless a good standard template for testing separation properties of polymer solutions, including an ability to resolve mild compressions, as well as to benchmark polymer solution performance. For these reasons, in the current work, we use DNA Sanger samples prepared with ssM13mp18 template.

3.1 Effect of urea concentration

We initially explored the possibility of decreasing the separation time by stepwise lowering the urea concentration in the LPA solution from 7 M to 3 M, while maintaining the column temperature at 65°C. Sections of the representative electropherograms are presented in Fig. 1. With 6 M urea (top panel), a read length of 930 bases with 98.5% accuracy was generated in roughly 1 h, a result similar to that with the separation matrix with 7 M urea. A further decrease in the urea concentration to 5 M resulted in a significant reduction of the read length to 750 bases at 98.5% accuracy. While separation indeed was faster at this lower urea concentration, requiring less than 48 min for the 1000 bases-long fragment to pass the detector window, several compressions were not resolved (see below for details about the compression sites), and the resolution of the T-terminated peaks in the late part of the run was substantially lower than that for other terminations (middle panel). This latter effect could possibly be a result of hydrophobic interactions between dROX, the dye on the T-terminated DNA fragments, and ssDNA [44]. Decreasing the urea concentration to 3 M resulted in the DNA fragments migrating twice as fast as with 7 M urea; however, frequent miscalls due to compression made the sequence practically unreadable (bottom panel). Thus, lowering urea concentration in the separation matrix, even at column temperature of 65°C, resulted in a decrease of migration time for the DNA fragments; however, this strategy reduced sequence read length due to insufficient denaturation of DNA. We considered two alternative approaches to improve denaturation – higher column temperature and organic solvent addition.

3.2 Thermal stability of LPA matrixes at high temperatures

It is known that at elevated column temperature, DNA fragments are denatured more effectively; we therefore explored whether further temperature increase could improve the denaturing ability and thus increase read length. Previously, we found with LPA solutions that, at column temperatures above 70°C, read length decreased rapidly [5]. In the present work, we demonstrate that urea, due to its thermal instability [45], is the principal reason causing decreased read length at temperatures above 70°C. The value of the current and its constancy during separation was chosen as a measure of solution thermal stability. We compared the current at a fixed electric field for the LPA solutions with or without 7 M urea at various column temperatures (Fig. 2). The current was measured 10 min after the start of each run, which lasted up to 30 min. The 10 min was arbitrarily chosen in order to allow sufficient time for the current to stabilize after polymer solution replacement in the column. As the column temperature was raised from 30 to 70°C, the current increased linearly with both denaturant-free and 7 M urea-containing polymer solutions. However, for the buffer containing 7 M urea, this current deviated from linearity at temperatures above 70°C, and, at 85–90°C, the current became unstable. When the LPA solution without urea was electrophoresed in the same range of temperatures, the current increased linearly with column temperature (Fig. 2), and, importantly, it was stable at least to 90°C.

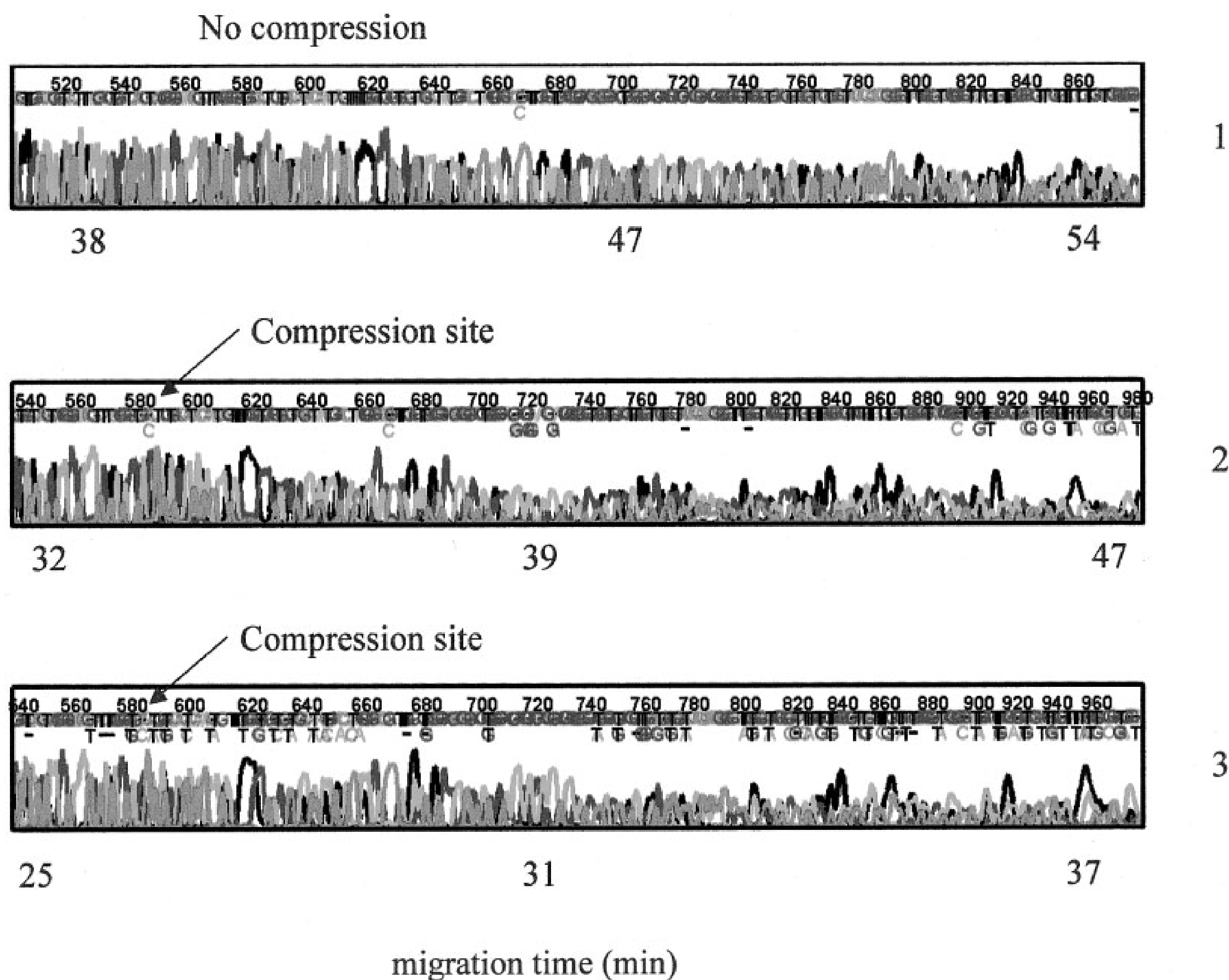

Figure 1. Sequencing of M13mp18 template using 2.5% w/w MDa LPA matrix containing (1) 6 M, (2) 5 M, and (3) 3.5 M urea at temperature 65°C. A compression site is marked by the arrow. For other conditions, see Sections 2.3 and 2.4.

Based on these results, we concluded that urea decomposition at high temperature had a concentration-dependent, deleterious effect on separation current.

In the process of thermal decomposition, urea can form various products, including ammonia, nitrogen oxides, cyanuric acid, cyanic acid, biuret, and carbon dioxide [36]. While it was concluded that significant accumulation of these decomposition products occurred at 130°C and above [45], we assumed that at column temperatures higher than 70°C, some formation of decomposition products may already occur. At these column temperatures, ammonia and other gaseous products may become less soluble in the LPA buffer and form microbubbles, thus causing instability in the separation current. At the same time, ammonia forms ions in aqueous solutions and, along with other ionic products of decomposition, may increase current in the column to higher values than predicted by Ohm's law. An increase in the current in turn would cause a nonlinear increase in heat generation inside the column, at some point, leading to insufficient heat removal and subsequent loss of efficiency. The extent of these urea decomposition effects will be dependent on the total electrophoretic run time at a given column temperature. We then tested DMSO with LPA. When we added 5% v/w DMSO to the LPA solution, this matrix showed no signs of deterioration in the entire tested range of column temperatures up to 90°C (Fig. 2). Importantly, DMSO addition did not change the excellent thermal stability of the LPA network.

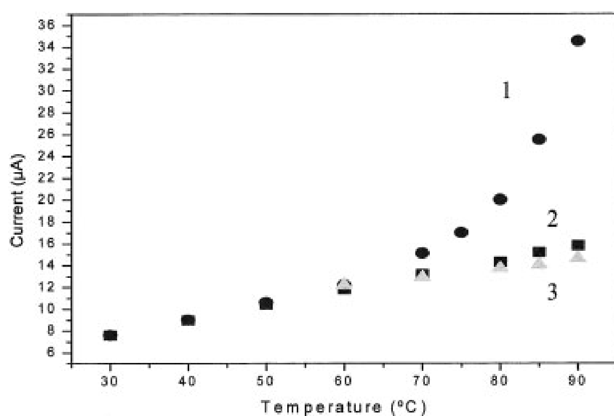

Figure 2. Plot of current in a capillary filled with separation matrix containing 2.5% w/w 5.6 MDa LPA: (1) with 7 M urea, (2) with no denaturant, and (3) with 5% v/w DMSO. The current was measured at 200 V/cm and 10 min after the start of the run. The current in (2) was adjusted to the same scale by a factor of 0.69. For each experiment at a given temperature, a fresh portion of the separation matrix was pumped into the capillary. For other conditions, see Sections 2.3 and 2.4.

3.3 DNA sequencing with DMSO-containing LPA solutions

We next examined 2.5% w/w 5.6 MDa LPA solutions, with DMSO concentrations of 5, 10, and 15% v/w (no urea present) for DNA sequencing at 70°C. The results, presented in Table 1, with a comparison to 7 M urea, indicate that DNA fragments migrated much faster in the DMSO-containing buffers, due to the decreased solution viscosity. With an increase in the DMSO concentration, the migration time of DNA fragments was longer, which was expected because of the increasing solvent viscosity and the more pronounced dielectric friction caused by the organic solvent [24]. However, even with 15% v/w DMSO in the separation matrix, DNA fragments still migrated substantially faster than with 7 M urea. In addition, DMSO-containing LPA solutions required lower pressure for replacement from the capillary. At DMSO concentrations higher than 5% v/w, shorter read lengths were obtained as a consequence of a lower resolution of G- and T- terminated peaks. Since these DNA fragments are labeled with more hydrophobic dyes than the other fragments, this effect could be attributed to an interaction between the dyes and DMSO. Based on the results in Table 1, we concluded that sufficient concentration of DMSO in the LPA matrix was 5% v/w, as even with this denaturant alone, the read length was close to 900 bases with 98.5% accuracy at 70°C.

We then performed a temperature study of DNA sequencing with 5% v/w DMSO in the 2.5% w/w 5.6.MDa LPA solution. Analyzing electropherograms generated using these LPA matrices and M13mp18 template, we identified two compression motifs located in the positions 353–355 and 583–585 bases from the first base of the primer recognition site (triplets of the *C*-terminated peaks) where the base-caller read one less base. One of these motifs is shown in Fig. 3 (top panel). Interestingly, with all the runs made with this denaturant at different column temperatures, the compression motifs (Fig. 3, top panel) were not resolved with the column temperature increase. Even at 90°C, when the read length of 952 bases with 98.5% accuracy was generated in just 32 min (roughly half the time needed to complete the run with 7 M urea), separation of these motifs was not sufficient. In addition, since commercial DNA sequencers are not designed to main-

Table 1. Migration time for specific ssDNA fragments and read length at 70°C and 200 V/cm in 2.5% w/w LPA solutions containing different denaturants[a)]

Denaturant	Migration time for base 600 (min)	Migration time for base 900 (min)	Maximum read length with 98.5% accuracy (bases)	Migration time for the highest base number read (min)
5% v/w DMSO	24.5	33.8	882	33.3
10% v/w DMSO	28.0	38.8	848	37.0
15% v/w DMSO	33.4	N/A	656	36.0
5% v/w DMSO +1 M urea	26.0	36.3	936	37.3
5%v/w DMSO +2 M urea	27.2	37.4	975	39.5
5% v/w DMSO +3 M urea	31.0	42.2	950	43.3
7 M urea	38.4	52.3	1055	58.0

a) See Section 2 for other conditions.

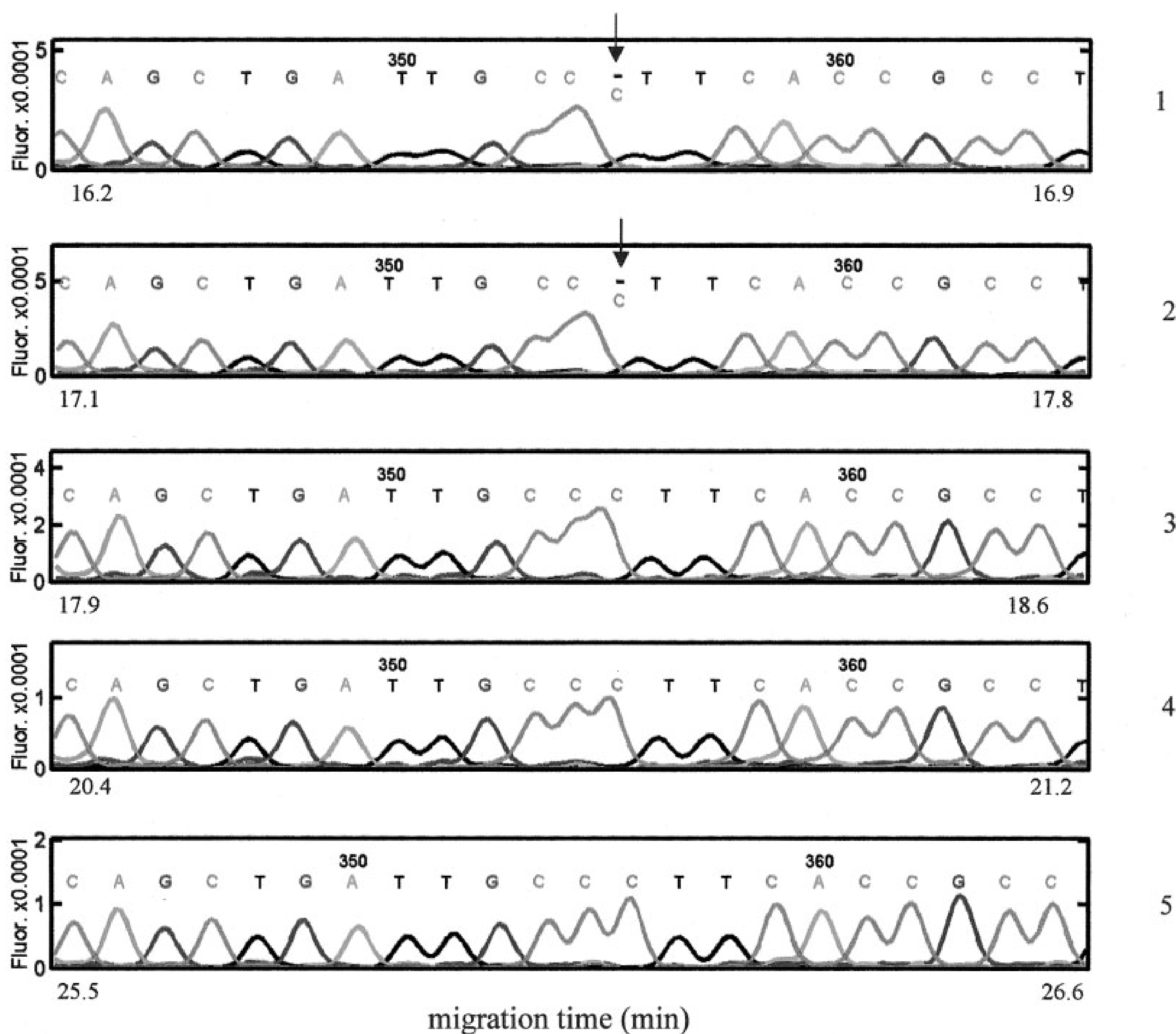

Figure 3. Sequencing through a compression motif (a triplet of *C*-terminated peaks marked with an arrow) with 2.5% w/w 5.6 MDa LPA at 70°C and 200 V/cm containing (1) 5% v/w DMSO, (2) mixtures of 5% v/w DMSO and urea in concentration 1 M, (3) 2 M, (4) 3 M, and (5) 7 M urea alone. For other conditions, see Sections 2.3 and 2.4.

tain temperature this high, and the capillary coating at this temperature may be of concern, the use of 5% v/w DMSO alone as denaturant was not studied further.

3.4 DNA sequencing with both DMSO and urea-containing LPA matrices

In order to improve resolution of compressions while maintaining rapid separations at 70°C, we decided to add small amounts of urea to the buffer containing 5% v/w DMSO. As seen in Table 1, addition of only 1 M urea led to an enhancement in the read length by more than 50 bases, while increases in run time, compared to the DMSO buffer, were small. With a LPA solution containing 5% v/w DMSO and 2 M urea, a read length of 976 bases with 98.5% accuracy was generated in less than 40 min. According to Table 1, raising the urea concentration in the LPA matrix higher than 2 M did not lead to an increase in the read length for this template. At 1 M urea (second panel), the denaturing ability of the matrix was not sufficient for resolving the compression, while increasing concentration to 2 M enhanced the resolution of the adjacent peaks sufficiently that correct base-calling was possible

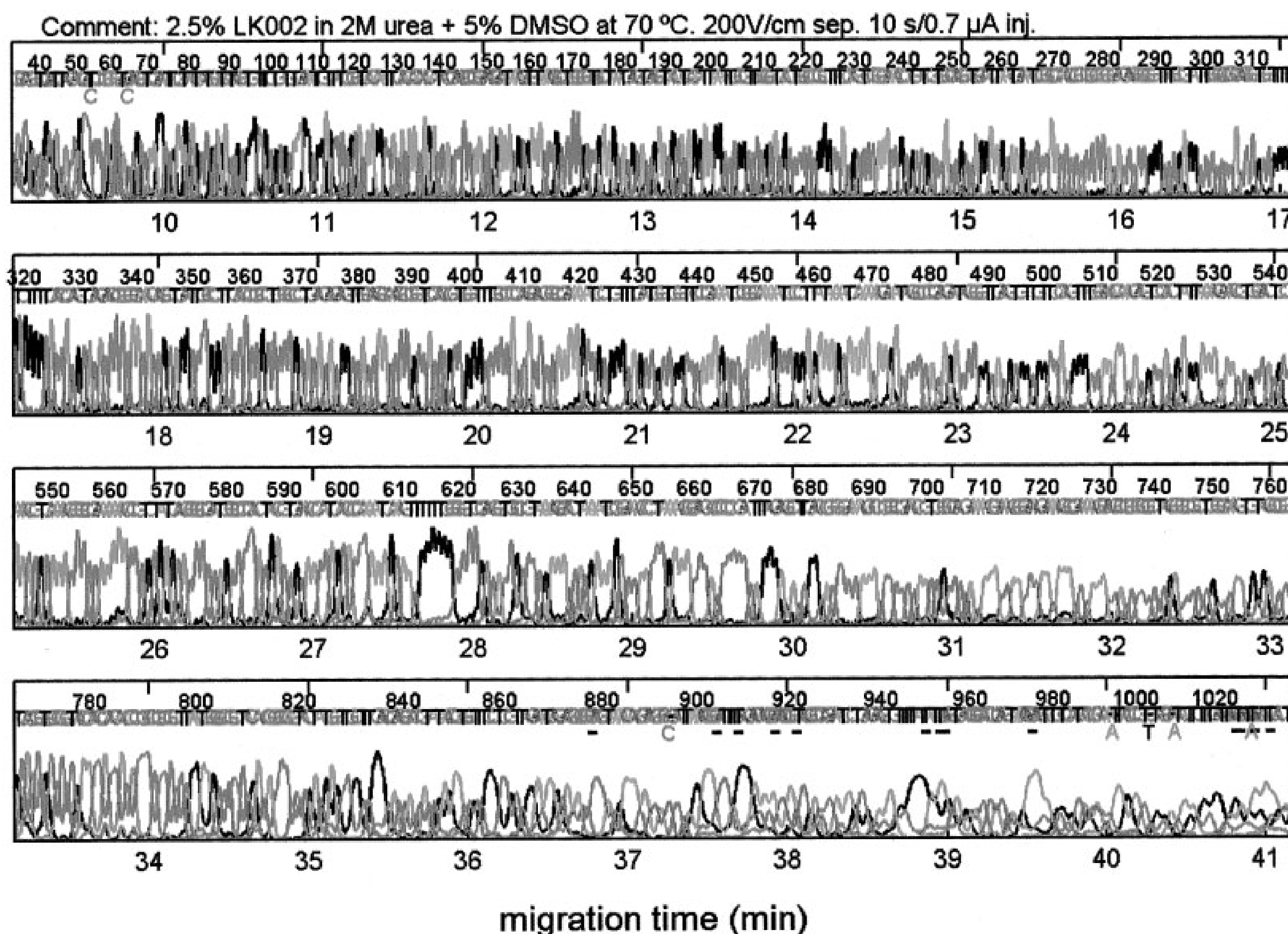

Figure 4. Sequencing of 976 bases with 98.5% accuracy in less than 40 min with 2.5% w/w 5.6. MDa LPA solution containing 5% v/w DMSO and 2 M urea at 70°C and 200 V/cm. For other conditions, see Sections 2.3 and 2.4.

(third panel). With further increase in the urea concentration to 3 M (fourth panel), resolution of all three *C*-terminated peaks was comparable to that of the original LPA buffer with 7 M urea (bottom panel). This is an important result demonstrating that in sequencing of templates with stronger compressions, higher urea concentrations in the matrix may be required. Even in such a case, the resulting sequence still may be generated faster than with an LPA matrix with a high concentration of urea. A sequencing run using LPA matrix containing 5% v/w DMSO and 2 M urea with ssM13mp18 template is shown in Fig. 4, in which close to 1000 bases were read in roughly 40 min.

The overall throughput of the sequencing process (the total number of bases generated in 1 h per capillary) with the separation buffer in Fig. 4 was over 30% higher than that with the buffer containing 7 M urea. This new denaturant can be used in both full-length capillary systems and integrated microchip devices as well. While with microchips filled with conventional matrices, the separations of 500 bases in 30 min per channel has been achieved [46], the LPA solution containing 5% v/w DMSO and 2 M urea almost doubles this read length at a price of only 10 min longer separation. Moreover, the urea concentration in the separation solution can be increased for DNA templates with more difficult compressions. Indeed, the use of 5% v/w DMSO, 7 M urea and 70°C could be a quite strong denaturant to handle some of the most problematic compressions.

4 Concluding remarks

In this work, we developed a new formulation of the LPA separation matrix containing 5% v/w DMSO and 2 M urea at column temperatures of 70°C that can be used for rapid sequencing, genotyping and SNP profiling and scoring by capillary array and microchip electrophoresis with

sequence read lengths close to 1000 bases. The polymer solution combines the high resolving power of LPA solutions previously optimized for long read length but with increased separation speed. The total throughput of DNA sequencing with this separation buffer can be over 30% higher than that with a similar LPA solution containing 7 M urea alone, published previously [12]. Moreover, compared to current commercial instrumentation, the throughput may be even higher due to much slower separation speeds than presented [12]. This new buffer has an optimum temperature of 70°C, which is commercially available in current DNA sequencers. It is interesting to note that the optimum temperature for solutions of other polymers, *e.g.*, poly(*N*,*N*-dimethylacrylamide) (PDMA), did not exceed 50°C [44]. The lower viscosity of the 5% v/w DMSO and 2 M urea containing LPA solution also facilitates rapid replacement of the solution from the capillary between runs. For sequencing DNA templates with stronger compressions, the denaturing ability of this mixture may be increased by raising the urea concentration to 3–4 M or even higher levels.

The authors gratefully acknowledge the support of this work by DOE under Human Genome Project Grant DE-FG02–90ER-60985. Support by DOE does not constitute an endorsement of views expressed in this paper. The authors also thank Dr. Haihong Zhou for experimental work in DNA sequencing with various urea concentrations and Prof. A. E. Barron (Northwestern University) for the LPA molecular mass determination. This is publication No. 797 from the Barnett Institute.

Received January 10, 2002

5 References

[1] Dovichi, N. J., *Electrophoresis* 1997, *18*, 2393–2399.

[2] Dolník, V., *J. Biochem. Biophys. Methods* 1999, *41*, 103–119.

[3] Heller, C., *Electrophoresis* 2001, *22*, 629–643.

[4] Ruiz-Martinez, M. C., Berka, J., Belenkii, A., Foret, F., Miller, A. W., Karger, B. L., *Anal. Chem.* 1993, *65*, 2851–2858.

[5] Zhou, H., Miller, A. W., Sosic, Z., Buchholz, B., Barron, A. E., Kotler, L., Karger, B. L., *Anal. Chem.* 2000, *72*, 1045–1052.

[6] Tan, H., Yeung, E. S., *Electrophoresis* 1997, *18*, 2893–2900.

[7] Venter, J. C., *et al.*, *Science* 2001, *291*, 1304–1351.

[8] Lander, E. S., *et al.*, *Nature* 2001, *409*, 860–921.

[9] Weitzman, J., *Trends Mol. Med.* 2001, *7*, 287.

[10] Schmitz, G., Aslanidis, C., Lackner, K. J., *Clin. Chim. Acta* 2001, *308*, 43–53.

[11] Heath, K. E., Humphries, S. E., Middleton-Price, H., Boxer, M., *Eur. J. Hum. Genet.* 2001, *9*, 244–252.

[12] Salas-Solano, O., Carrilho, E., Kotler, L., Miller, A. W., Goetzinger, W., Sosic, Z., Karger, B. L., *Anal. Chem.* 1998, *70*, 3996–4003.

[13] Lu, H., Arriaga, E., Chen, D.-Y., Figeys, D., Dovichi, N. J., *J. Chromatogr. A* 1994, *680*, 503–510.

[14] Kleparnik, K., Foret, F., Berka, J., Goetzinger, W., Miller, A. W., Karger, B. L., *Electrophoresis* 1996, *17*, 1860–1866.

[15] Fang, Y., Zhang, J. Z., Hou, J. Y., Lu, H., Dovichi, N. J., *Electrophoresis* 1996, *17*, 1436–1442.

[16] Chang, H. T., Yeung, E. S., *Electrophoresis* 1995, *16*, 2069–2073.

[17] Zhang, J., Fang, Y., Hou, J. Y., Ren, H. J., Jiang, R., Roos, P., Dovichi, N. J., *Anal. Chem.* 1995, *67*, 4589–4593.

[18] Rocheleau, M. J., Grey, R. J., Chen, D.-Y., Harke, H. R., Dovichi, N.J., *Electrophoresis* 1992, *13*, 484–486.

[19] Zhang, W., Deisseroth, A. B., *Methods Mol. Biol.* 1996, *65*, 137–147.

[20] Flanagan, J. H., Jr., Owens, C. V., Romero, S. E., Waddell, E., Kahn, S. H., Hammer, R. P., Soper, S. A., *Anal. Chem.* 1998, *70*, 2676–2684.

[21] Chakrabarti, R., Schutt, C. E., *Nucleic Acids Res.* 2001, *29*, 2377–2381.

[22] Hui-Shieh, C., Hedayati, A., *US Patent 5, 961, 801,* 1999.

[23] Konrad, K. D., Pentoney, S. L., Jr., *Electrophoresis* 1993, *14*, 502–508.

[24] Roy, K. I., Lucy, C. A., *HPCE* 2001, Boston 2001, 65.

[25] Seto, D., Seto, J., Deshpande, P., Hood, L., *DNA Seq.* 1995, *5*, 131–140.

[26] Sun, Y., Hegamyer, G., Colburn, N. H., *BioTechniques* 1993, *15*, 372–374.

[27] Choi, J. S., Kim, J. S., Joe, C. O., Kim, S., Ha, K. S., Park, Y. M., *Exp. Mol. Med.* 1999, *31*, 20–24.

[28] Jung, M., Muche, J. M., Lukowsky, A., Jung, K., Loening, S. A., *Anal. Biochem.* 2001, *289*, 292–295.

[29] Yoshihiro, A., *Japan Patent JP9145673A2,* 1997.

[30] Li, L., McGown, L. B., *Fresenius' J. Anal. Chem.* 2001, *369*, 267–272.

[31] Escara, J. F., Hutton, J. R., *Biopolymers* 1980, *19*, 1315–1327.

[32] Hutton, J. R., *Nucleic Acids Res.* 1977, *4*, 3537–3555.

[33] Zimmer, C., Luck, G., *Biochim. Biophys. Acta* 1972, *287*, 376–385.

[34] Khmelnitsky, Y. L., Mozhaev, V. V., Belova, A. B., Sergeeva, M. V., Martinek, K., *Eur. J. Biochem.* 1991, *198*, 31–41.

[35] http://www.jtbaker.com/msds/D7120.htm

[36] http://www.jtbaker.com/msds/U4725.htm

[37] Head, D. L., McCarty, C. G., *Tetrahedron Lett.* 1973, *16*, 1405–1408.

[38] Ruiz-Martinez, M. C., Carrilho, E., Berka, J., Kieleczawa, J., Miller, A. W., Foret, F., Carson, S., Karger, B. L., *BioTechniques* 1996, *20*, 1058–1064, 1066–1069.

[39] Karger, B., Goetzinger, W., *US Patent 5, 840, 388,* 1998.

[40] Goetzinger, W., Kotler, L., Carrilho, E., Ruiz-Martinez, M. C., Salas-Solano, O., Karger, B. L., *Electrophoresis* 1998, *19*, 242–248.

[41] Wyatt, P. J., *Anal. Chim. Acta* 1993, *272*, 1–40.

[42] Ruiz-Martinez, M. C., Salas-Solano, O., Carrilho, E., Kotler, L., Karger, B. L., *Anal. Chem.* 1998, *70*, 1516–1527.

[43] Miller, A. W., Karger, B. L., *US Patent 6, 236, 944,* 2001.

[44] He, H., Buchholz, B., Kotler, L., Miller, A. W., Barron, A. E., Karger, B. L., *Electrophoresis* 2002, *23*, 1421–1428.

[45] Nachbaur, E., Baumgartner, E., Schober, J., *Proc. Eur. Symp. Therm. Anal.* 1981, 417–421.

[46] Medintz, I. L., Paegel, B. M., Mathies, R. A., *J. Chromatogr. A* 2001, *924*, 265–270.