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Journal of Chromatography A, 853 (1999) 371–379

JOURNAL OF CHROMATOGRAPHY A

Highly alkaline electrolyte for single-stranded DNA separations by electrophoresis in bare silica capillaries ☆

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Abstract

A new, highly denaturing electrolyte system based on a solution containing 0.01 *M* NaOH, 0.0015 *M* $Na_2B_4O_5(OH)_4$ and a replaceable polymer sieving medium was designed for the separation of single-stranded DNA fragments in bare fused-silica capillaries. Extreme denaturing power, together with the optimized composition of the electrolyte, allows for a separation efficiency as high as 2 300 000 height equivalents to a theoretical plate per meter. Sample denaturation in alkaline solutions provides single-stranded DNA fragments without any intra- or intermolecular interactions at room temperature. Their electrophoretic mobilities were found to be twice those of fragments denatured by dimethylformamide or HCl. This can be interpreted in terms of an increased effective charge on the DNA molecules. The surprisingly weak electroosmosis ($6 \cdot 10^{-10}$ m^2 V^{-1} s^{-1}) of polymer solutions at pH 12 or higher is considered to be the result of the dissolution of the silica capillary wall. A highly viscous thin layer of dissolved silica probably causes a shift of the slipping plane further away from the wall to the lower value of the ζ potential. Applications of the electrolyte in clinical diagnostics demonstrate its remarkable properties.

Keywords: Background electrolyte composition; DNA

1. Introduction

Denaturing electrophoresis proved to be more selective in the separation of DNA fragments. While a difference of a single nucleotide (nt) in single-stranded (ss) DNA sequencing fragments of up to 1000 nt can be resolved under denaturing conditions [1], single base-pair (bp) resolution of double-stranded (ds) DNA fragments longer than 300 bp can hardly be attained under native conditions [2]. Therefore, denaturing electrophoresis can potentially provide fast and high-resolution separations. In some instances, highly denaturing conditions, where all intra- and intermolecular interactions are completely destroyed, need to be applied for a reliable analysis. Various organic compounds have been tested as denaturing agents towards DNA [3]. It has been shown that the effectiveness of organic denaturants is not always adequate. In particular, the melting of the guanine- and cytosine-rich regions is not always complete [4,5]. A more reliable denaturation process can be attained using acidic or alkaline conditions. Thus, at pH≤2 adenine–thymine and even guanine–cytosine pairs are completely disrupted [6–8]. However, the use of acidic buffers for DNA electrophoresis is impractical, since the dissociation of phosphate groups is reduced and, therefore, electrophoretic mobilities decrease. Because of this, effi-

☆A preliminary report was presented at the 11th International Symposium on Capillary Electroseparation Techniques, Venice, 4–7 October 1998.

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00476-8

cient alkaline denaturation is more convenient. DNA fragments can be separated in solutions at pH 12–13 [9–12] without a risk of destroying them, as verified by the comet assay technique, which is widely used in the genetic research [13–15].

The most frequently applied denaturing agent in capillaries is urea at a concentration range from 3.5 to 8.3 *M* [1,16,17]. In some instances, however, its denaturing ability is not satisfactory and electrophoresis must be performed at an elevated temperature [18]. Therefore, there is still the demand for a highly efficient denaturing electrolyte in capillary electrophoresis (CE). An alkaline electrolyte would potentially be convenient, since denaturation is complete at laboratory temperature and there is maximum dissociation of phosphate groups. On the other hand, the lower stability of covalently bonded polymer coatings and an increased electroosmotic flow (EOF) due to the increased dissociation of the silanol groups at the silica wall can cause difficulties in the application of an alkaline environment in capillaries. The mobility of the EOF is determined by the ζ potential and viscosity of an electrolyte [19]. Therefore, all known methods for EOF suppression are based on a reduction of ζ potential and/or an increase in the electrolyte viscosity in close proximity to the wall.

All coatings based on covalently bonded polymers via Si–O- [20–22] or Si–C- [23] bonds will definitely suffer from alkaline hydrolysis at pH values higher than 12. In this respect, dynamic coatings are more convenient, i.e., additives to background electrolytes (BGEs) that cover the capillary wall noncovalently. Such an addition of polymers or surfactants increases the viscosity at the wall or compensates for its negative electrostatic charge [24–31]. Another way to avoid difficulties with coatings is the application of uncoated capillaries where the EOF dominates electrophoresis. Here, DNA fragments migrate selectively against a faster bulk EOF, which transports them to the detector in order of their descending size. This counter-flow technique has also been used for the separation of DNA fragments [32,33]. In these cases, the EOF mobility is evaluated with the help of a neutral marker, which possesses an adequate extinction coefficient and/or fluorescence quantum yield. The most commonly used markers are mesityloxide [29,30,34,35], phenol [36], acetophenone [37] and pyridine [38], for absorbance detection, and coumarin [27], 4-nitroaniline, riboflavin and umbelliferone [39], for fluorescence detection.

In this paper, the results of the development of a new, highly denaturing electrolyte system based on a 0.01-*M* solution of NaOH are presented. To prove the interesting properties of the electrolyte for DNA analysis, several parameters have been investigated. The EOF mobility in an uncoated capillary was evaluated as a function of pH. The electrophoretic mobilities of ssDNA fragments denatured using dimethylformamide (DMF), 0.01 *M* HCl, and 0.01 and 0.1 *M* NaOH were compared. The composition of the alkaline BGE was optimized with respect to the separation efficiency. The utility of the methodology is demonstrated through the detection of short tandem repeat polymorphism in the endothelin 1 gene. Thus, the optimized analysis proved to be applicable in DNA diagnostics where polymerase chain reaction (PCR)-amplified fragments, which are resistant to denaturation and create heteroduplexes easily, are to be analyzed.

2. Experimental

2.1. Instrumentation

The experiments were performed using a BioFocus 3000 system (Bio-Rad, Hercules, CA, USA) with absorbance detection at 260 nm. Capillaries were installed in a user-assembled cartridge (Bio-Rad, catalog no. 148-3050) and kept at a constant temperature of 40°C for EOF measurement as well as DNA separations. Electric field strengths ranged from 58 to 289 V/cm. Fused-silica capillaries of 50 and 100 μm I.D. (367 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). The inner walls of the 100 μm I.D. capillaries were coated with linear polyacrylamide (LPAA) according to Hjertén's procedure [20] and the 50 μm I.D. capillaries were uncoated. The total length of the capillaries was 34.6 cm and the effective lengths were 4.6 and 30 cm. Before each run, the capillaries were rinsed as follows: (i) for the EOF measurements: 10 min with 0.1 *M* NaOH, 10 min with water

and 10 min with a BGE; (ii) for the EOF measurement of a 2% (w/v) solution of agarose with 0.01 *M* NaOH as the BGE: 10 min with 0.01 *M* NaOH and 10 min with the BGE; (iii) for the separation of DNA fragments in coated capillaries at pH 8.3: 3 min with water and 3 min with the BGE; (iv) for the separation of DNA fragments at pH 12: 5 min with 0.01 *M* NaOH and 3 min with the BGE. Samples were injected by electromigration in all instances.

2.2. Samples and reagents

Fragments of pBR322 DNA–*Alu*I digest (catalog no. SM0121, MBI Fermentas, Vilnius, Lithuania) were used as a model sample. Purified PCR amplification products from a short tandem repeat region of the endothelin 1 gene were obtained from the Institute of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic. The fragment sizes of samples 1 and 2 were 201 and 211, and 191 and 203 bp, respectively. Low-melting-point agarose BRE (catalog no. 1503; FMC Bioproducts, Rockland, ME, USA) was used as a sieving medium. Solutions of agarose (2%, w/v) were prepared at the boiling point with 0.1 *M* Tris–0.1 *M* *N*-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS) (both from Sigma, St. Louis, MO, USA) and with the addition of 7 *M* urea (Amresco, Solon, OH, USA) for the reference denaturing electrophoresis in coated capillaries. Three alkaline electrolytes with agarose BRE were prepared as follows: To avoid heating the agarose solution with hydroxides, a 3% stock solution of agarose BRE was mixed with 0.1 *M* solutions of NaOH, KOH and LiOH (Lachema, Brno, Czech Republic), respectively. The concentrations were adjusted to give 2% (w/v) solutions of agarose in 0.01 *M* hydroxides. Various concentrations of $Na_2B_4O_5(OH)_4$ of analytical-reagent grade (Lachema) were prepared in the 2% agarose solution with 0.01 *M* NaOH for optimization of the separation efficiency. Electrolytes with different pH values were prepared to test the EOF, by titration of 0.1 *M* NaOH with 0.5 *M* phosphoric acid to the desired pH. Mesityloxide (Fluka, Buchs, Switzerland), sulfosalicylic, phthalic and benzoic acids (all Sigma) were used as EOF mobility markers.

2.3. Sample denaturation

As electrolytes for the denaturing CE, 2% solutions of agarose BRE in 0.1 *M* Tris–TAPS with 7 *M* urea and 0.01 *M* NaOH, respectively, were used. Before each series of analyses, DNA samples were denatured in solutions of: (i) pure DMF [10 μl of the sample were evaporated in vacuum, dissolved in 10 μl of DMF, heated at 98°C for 2 min in a thermocycler (MJ Research, Watertown, MA, USA) and chilled on ice]; (ii) 0.01 *M* HCl (5 μl of a sample were mixed with 5 μl of 0.02 *M* solution of HCl at room temperature); (iii) 0.01 M NaOH (9 μl of a sample were mixed with 1 μl of 0.1 *M* NaOH at room temperature); (iv) 0.1 *M* NaOH (5μl of a sample were mixed with 5 μl of 0.2 *M* NaOH at room temperature).

3. Results and discussion

3.1. EOF of alkaline solutions in a silica capillary

The main objective of this research was the development of a highly denaturing electrolyte for the separation of the DNA fragments that form heteroduplexes easily and are resistant to denaturation. Therefore, we decided to investigate highly alkaline solutions of linear polymers in the state of sol as the sieving medium. Agarose solutions containing 0.01 *M* NaOH have been used for this purpose. It is commonly accepted that electroosmosis in bare fused-silica capillaries increases with increasing pH due to the more extensive dissociation of silanol groups. Nevertheless, EOF mobilities at pH values over 12 and at a temperature of 40°C have not been reported in the literature. Therefore, the high EOF of our electrolyte was expected and counterflow electrophoresis in bare capillaries was assumed to be applicable. Surprisingly, the EOF of this system was small and, consequently, we were able to work in a direct mode of electrophoresis. To explain the extraordinarily low EOF mobility in the silica capillary with completely dissociated silanol groups ($pK \cong 2$) [40], we studied the dependence of EOF on pH in the alkaline region. The EOF was evaluated using mesityloxide, a neutral marker, and three stable organic acids, i.e., sulfosalicylic, phthalic and ben-

zoic, as reference anions. During a single injection procedure, the three acids and mesityloxide were injected at the cathodic and anodic ends of the capillary, respectively. Since the stability of mesityloxide at pH 12–13 is questionable, the differences in its migration times at the individual pH values were correlated with the migration times of the acids. Thus, the changes in EOF have been determined reliably. The results of the measurements are summarized in Fig. 1. The electrolytes of decreasing pH were prepared by the titration of a 0.1-*M* solution of NaOH with 0.5 *M* phosphoric acid. In this way, the ionic strength, *I*, was kept approximately constant in the pH range from 12 to 8. The calculated values of *I* for the particular pH are introduced in the caption to Fig. 1. A surprisingly low EOF was observed at pH 11–13, at a temperature of 40°C. This fact can be explained by the existence of a highly viscous thin layer at the silica capillary wall, which was formed by dissolved orthosilicic acid, $Si(OH)_4$, which has a strong tendency to polymerize [40, p. 178]. This layer probably does not allow for molecular transport in the vicinity of the wall, and causes a shift of the slipping plane further away from the wall to the lower value of the ζ potential. The EOF mobilities of agarose solutions are even lower. The EOF mobilities of 2% agarose solutions in 0.01 *M* NaOH, KOH and LiOH are shown in Table 1. The lowest EOF value was attained for NaOH, which varied within experimental error around a mobility of $6 \cdot 10^{-10}$ m^2 V^{-1} s^{-1}.

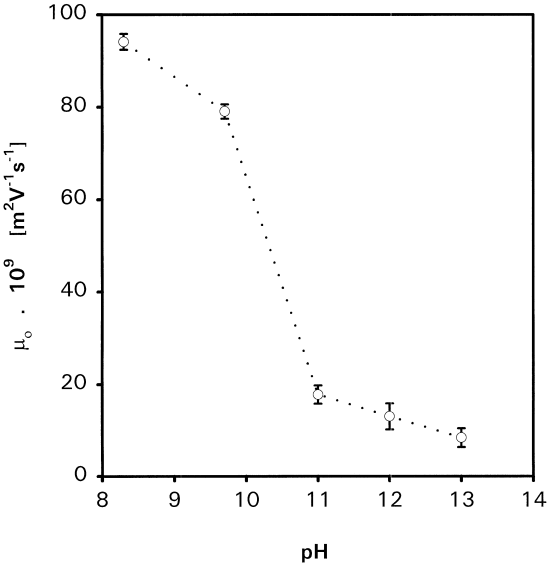

Fig. 1. Dependence of EOF in a bare fused-silica capillary on pH. Electrophoresis was performed at an electric field strength of 58 V/cm and a temperature of 40°C in a capillary with an effective length of 4.6 (34.6) cm and an I.D. of 50 μm. Error bars represent the standard deviation of five consecutive measurements in a single capillary. Ionic strengths at the individual pH values were calculated to be: pH 13–0.1, 12–0.18, 11–0.16, 9.7–0.15, 8.3–0.15 mol/l. For the pH adjustment procedure, see the text.

3.2. Separation of DNA fragments under denaturing conditions

The effects of various denaturing conditions on the separation of a model sample (pBR322 DNA–*Alu*I digest) are presented in Fig. 2. For the sake of comparison, the record of a separation under native conditions is shown in panel A. Here, the electrophoresis was performed in a 2% solution of agarose with 0.1 *M* Tris–0.1 *M* TAPS. Denaturing electrophoreses in the same electrolyte but with the addition of 7 *M* urea are shown in panels B and C. Prior to the analyses, the samples were denatured in solutions containing 0.01 (B) and 0.1 *M* (C) NaOH, to prove the reliability of the denaturation process. Electropherograms B and C show nearly identical results. Therefore, the concentration of 0.01 *M* NaOH was adopted as it was sufficient for complete denaturation at 40°C, which is in accordance with data presented in the literature [9–12]. The complete separation of all 14 fragments of the sample in a 2% solution of agarose with 0.01 *M* NaOH and 0.0015 *M* $Na_2B_4O_5(OH)_4$ is seen in panel D. The same denaturing procedure of the sample in 0.01 *M* NaOH

Table 1
Comparison of EOF mobilities of 2% agarose solutions in 0.01 *M* alkali metal hydroxides in bare fused-silica capillaries[a]

2% Agarose BRE +	$\mu_0 \cdot 10^9$ (m^2 V^{-1} s^{-1})	Standard deviation (%)
0.01 *M* KOH	0.75	1.6
0.01 *M* NaOH	0.60	1.1
0.01 *M* LiOH	0.90	1.1

[a] Other experimental conditions are the same as in Fig. 1. Experimental error is evaluated as the percentage of standard deviation from five consecutive measurements in a single capillary.

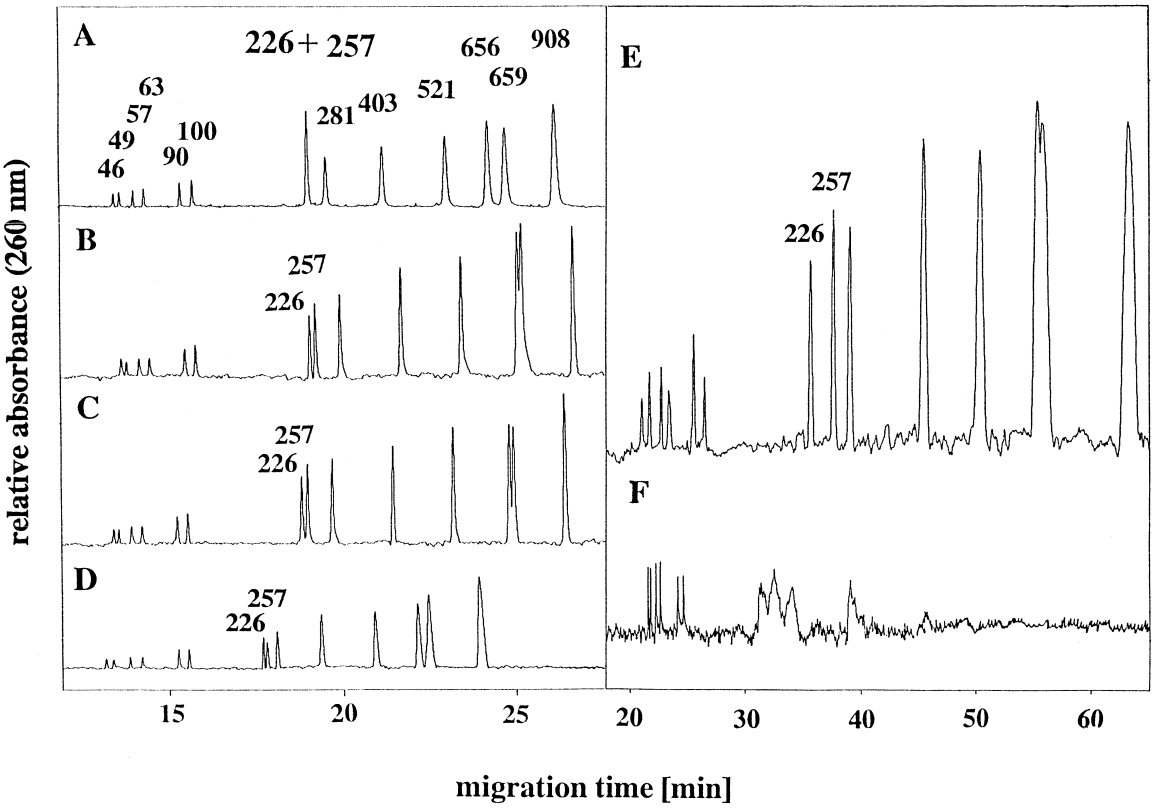

Fig. 2. Separation of standard pBR322 DNA–*Alu*I restriction fragments under various denaturing conditions. (A) Native dsDNA sample analyzed in a 2% agarose BRE solution in 0.1 *M* Tris–TAPS, pH 8.3, injected for 2 s at 58 V/cm; (B, C) samples denatured in 0.01 *M* (B) or 0.1 *M* (C) NaOH at room temperature, analyzed in 2% agarose BRE in 0.1 *M* Tris–TAPS with 7 *M* urea, pH 8.3, injected for 5 s at 58 V/cm; (D) sample denatured in 0.01 *M* NaOH at room temperature, analyzed in 2% agarose BRE in 0.01 *M* NaOH with 0.0015 *M* $Na_2B_4O_5(OH)_4$, pH 12, injected for 2 s at 58 V/cm; (E) sample evaporated, dissolved in DMF, heated for 2 min and chilled on ice, and analyzed as in (B), injected for 7 s at 145 V/cm; (F) 5 μl of sample mixed with 5 μl of 0.02 *M* HCl at room temperature, analyzed as in (B), injected for 15 s at 145 V/cm. Electrophoresis was at 145 V/cm, at a temperature of 40°C. Capillary: 30 (34.6) cm; A, B, C, E and F were LPAA-coated and had an I.D. of 100 μm I.D.; D was bare, with an I.D. of 50 μm. Fragment sizes (given as the number of nucleotides) are written over the peaks. The absorbance scales of the individual electropherograms differ.

was used here. Records B–D represent another important result of the paper, i.e., that ssDNA fragments when denatured in a solution of NaOH migrate as fast as dsDNA. Usually, the migration of individual strands is slower, since their charge-to-size ratios are half of those of dsDNA fragments. This is evident in panels E and F where the samples were denatured using DMF and 0.01 *M* HCl, respectively. A nearly twofold increase in the migration time of ssDNA fragments is seen in comparison with the migration times in panels A–D and E and F. Both separations E and F were performed in the same electrolyte with 7 *M* urea at 40°C, as in panel B. Analysis E represents a typical procedure optimized for DNA sequencing by CE. Denaturation at highly acidic pH is not suitable for DNA. Record F shows very broad zones of fragments 226, 257, 281 and 403 nt and peaks of longer fragments are absent. We can speculate that the long DNA fragments were destroyed in such a low pH. The electrophoretic mobilities of chosen DNA fragments under the conditions given for A–F are compared in Table 2.

There is no doubt that DNA fragments analyzed in the environment with 7 *M* urea at 40°C and also in 0.01 *M* NaOH at the same temperature were denatured. Therefore, the fast migration of those dena-

Table 2
Comparison of the experimentally observed mobilities of some standard DNA fragments under various denaturing conditions[a]

nt	$\mu \cdot 10^9$ (m^2 V^{-1} s^{-1})					
	A native	B NaOH–urea	C NaOH–urea	D NaOH–NaOH	E DMF–urea	F HCl–urea
46	25.72	25.30	25.82	26.30	16.44	16.05
100	21.99	21.93	22.14	22.24	12.99	14.05
281	17.65	17.32	17.55	19.14	8.80	10.18
521	15.00	14.72	14.89	16.57	6.81	
908	13.19	12.93	13.05	14.46	5.42	

[a] Data were taken from panels A–F in Fig. 2.

tured by NaOH can be interpreted in terms of an increased effective charge. Moreover, the increased charge of the DNA molecule denatured in the NaOH solution seems to be permanent (Fig. 2 B). We have no direct proof for the explanation. This phenomenon is the subject of further investigation by us. Nevertheless, the extraordinarily fast migration of fragments denatured in solutions of NaOH is, besides having a high denaturing effectiveness and a low electroosmotic flow in a bare capillary, another positive property of using a highly alkaline BGE.

Sodium tetraborate [$Na_2B_4O_5(OH)_4$, which is frequently, but incorrectly, introduced as $Na_2B_4O_7$] is a common constituent of buffers with a maximum capacity at pH 10–12. We have found that the amount of tetraborate added to the alkaline BGE affects the separation efficiency. The dependence of the separation efficiency of ssDNA fragments on the concentration of sodium tetraborate is presented in Fig. 3. The results are based on the separation of the model sample in a 2% agarose solution with 0.01 *M* NaOH and $Na_2B_4O_5(OH)_4$. Here, the separation efficiency, expressed as the number of height equivalents to a theoretical plate (HETP), *N*, has been calculated according to the relationship: $N=5.545.(t/w_{1/2})^2$, where *t* is the migration time and $w_{1/2}$ is the peak width at half of the peak height. The maximum separation efficiency of the shortest fragments was 2 300 000 HETP/m at a concentration of 0.0015 *M* $Na_2B_4O_5(OH)_4$. However, the efficiencies decreased with the molecular mass. This fact can be explained by the larger electromigration dispersion of longer molecules. Their mobilities differ from those of co-ions more substantially and, therefore, the zones can be expected to be more dispersed. The effect of tetraborates on the separation of the model ssDNA fragments is seen in Fig. 4. Here, separations in two electrolytes are compared: 0.01 *M* NaOH (panel A) and 0.01 *M* NaOH with $Na_2B_4O_5(OH)_4$ at the optimum concentration of 0.0015 *M* (panel B). The better resolution of the zones in panel B is due to the improved separation efficiency, while the selectivity remains unaffected.

The alkaline electrolyte system was used for the

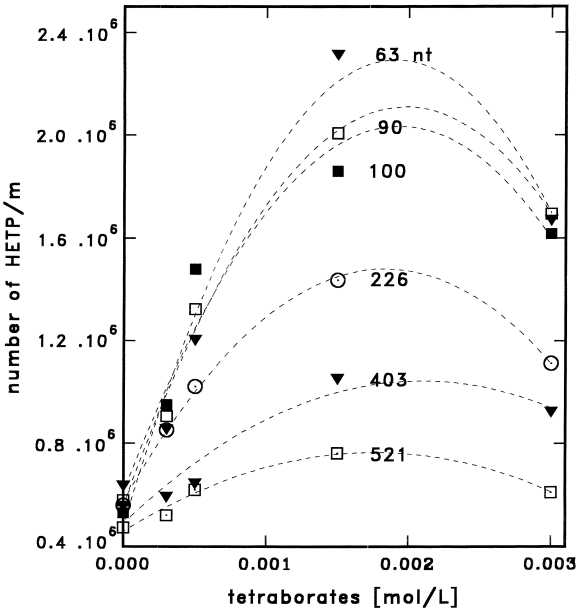

Fig. 3. Dependence of the separation efficiencies (number of HETP/m) of chosen pBR322 DNA–*Alu*I restriction fragments on the molar concentration of $Na_2B_4O_5(OH)_4$ in the BGE. Electrophoresis at 289 V/cm; temperature, 40°C; bare capillary, 30 (34.6) cm, 50 μm I.D.; BGE: 2% agarose BRE solution in 0.01 *M* NaOH; injection, 3 s at 58 V/cm. The sample was denatured in 0.01 *M* NaOH at room temperature. Dashed lines represent second order polynomial regression curves.

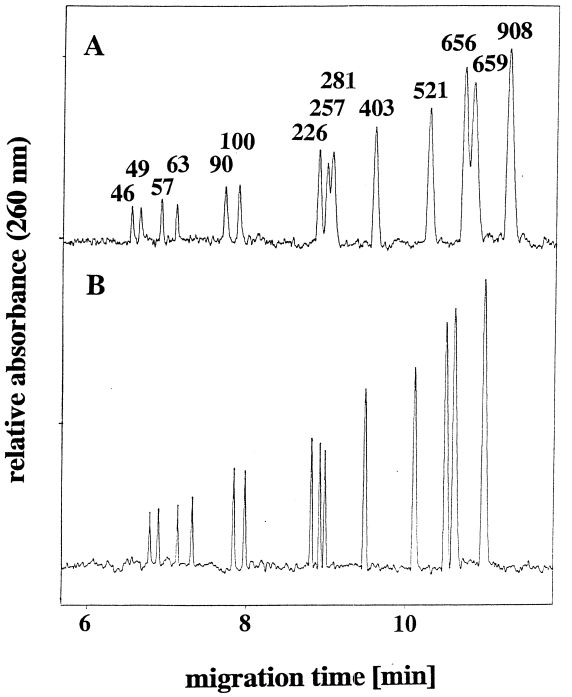

Fig. 4. Effect of the addition of the optimum amount of tetraborate to the BGE on the separation of the standard fragments. (A) 2% Agarose BRE in 0.01 *M* NaOH, (B) 2% agarose BRE in 0.01 *M* NaOH with 0.0015 *M* $Na_2B_4O_5(OH)_4$. Conditions of electrophoresis are as in Fig. 3.

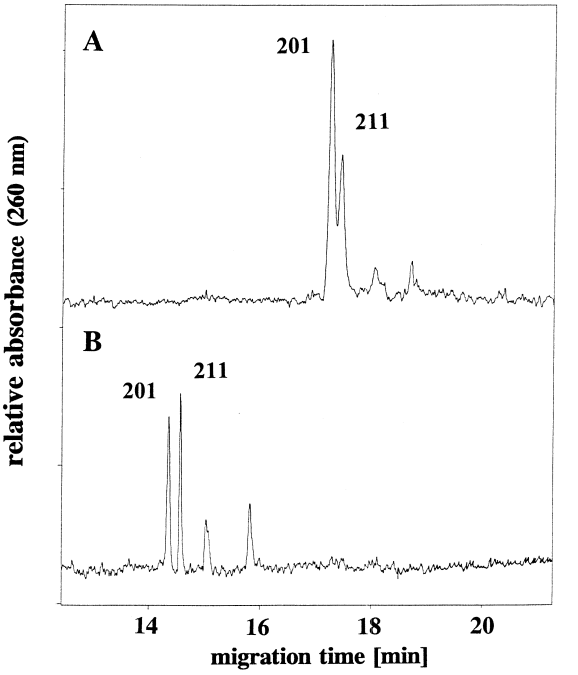

Fig. 5. Comparison of the native and denaturing CE of the PCR products from the endothelin 1 gene (sample 1). (A) Native CE: injection for 5 s at 145 V/cm, with other conditions as in Fig. 2A. (B) Denaturing CE: injection for 5 s at 145 V/cm, with other conditions as in Fig. 2D.

detection of short tandem repeat polymorphism in the endothelin 1 gene. The polymorphism, from three adjacent regions CT, CA and GC, seems to play a role in the regulation of transcription and is assumed to be associated with the onset of hypertension. The fragments in samples 1 and 2 were amplified from the endothelin 1 gene of heterozygous individuals. In Figs. 5 and 6, the separation of native dsDNA (panels A) and denatured ssDNA fragments (panels B) of both samples are compared. The GC region of repeats gives the fragments a resistance to complete denaturation and a tendency to create heteroduplexes. The molecular masses of the PCR products were determined by the calibration method of standard addition. Fragments with known sequences amplified from the same region of the gene served as standards [41]. The records in Fig. 5 were monitored in capillaries of the same length. Again, the ssDNA fragments that were denatured and analyzed in a 0.01 *M* NaOH solution were resolved faster and better than their ds analogues. Two small peaks behind those of 201 and 211 nt are PCR-amplified nonspecific fragments. Record B in Fig. 6 demonstrates the feasibility of fast DNA diagnostics based on the detection of short tandem repeat polymorphism by denaturing electrophoresis in short capillaries.

4. Conclusions

We have developed a new, highly denaturing electrolyte system based on a 0.01 *M* solution of NaOH. The composition of the electrolyte was optimized with respect to the denaturing ability and separation efficiency. As a result, a 2% (w/v) solution of low-melting-point agarose in 0.01 *M* NaOH and 0.0015 *M* $Na_2B_4O_5(OH)_4$ proved to be an excellent denaturing electrolyte for the separation of DNA fragments products of PCR amplification, which create heteroduplexes easily and are resistant to denaturation. Thus, the electrolyte is convenient

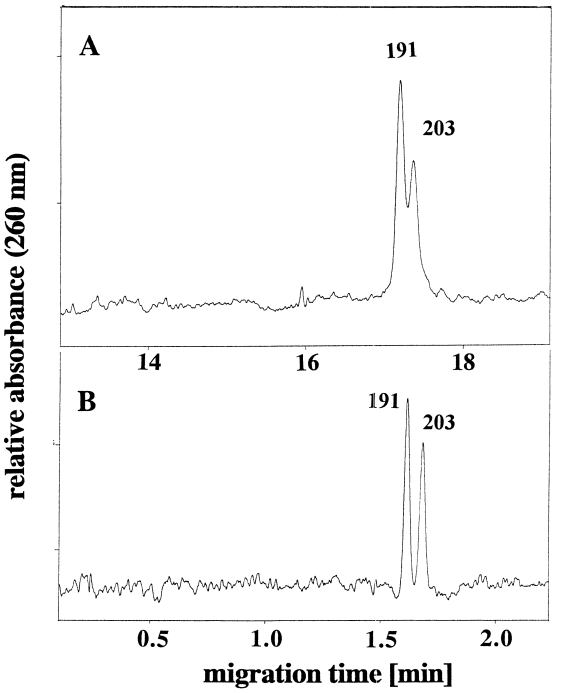

Fig. 6. Comparison of the native and denaturing CE of the PCR products from the endothelin 1 gene (sample 2). (A) Native CE: injection for 5 s at 145 V/cm, with other conditions as in Fig. 2A. (B) Denaturing CE: injection for 5 s at 145 V/cm; capillary 4.6 (34.6) cm; other conditions as in Fig. 2D.

for implementation in clinical DNA diagnostics. Several remarkable separation properties of the electrolyte have been discovered:

1. NaOH is a very strong denaturing agent and, therefore, even the DNA fragments with GC-rich regions, which are resistant to denaturation in urea, dissociate completely in a 0.01-*M* solution of NaOH. Very well resolved and sharp zones of model fragments (Fig. 2D) suggest the absence of any inter- or intramolecular aggregations in the alkaline electrolyte. Even the peaks of fragments amplified from GC regions of the endothelin 1 gene are sharp (Fig. 5B). This is a prerequisite of high throughput genetic analysis in short capillaries, where the analysis time can be reduced by a factor of ten (Fig. 6B).
2. The addition of tetraborates to the alkaline background electrolyte improves the separation efficiency. At the optimum concentration of 0.0015 *M* sodium tetraborate, $Na_2B_4O_5(OH)_4$, in the BGE, separation efficiencies as high as 2 300 000 HETP/m were reached for short DNA fragments (Fig. 3). The positive effect of tetraborates on the separation efficiency is difficult to explain due to their very complex equilibria in solutions.
3. Surprisingly, ssDNA fragments denatured in NaOH solutions (Fig. 2B–D) migrated at the same velocity or faster than dsDNA (Fig. 2A). Their fast migration can be interpreted in terms of an increased effective charge of the DNA molecule denatured by NaOH, which seems to be permanent. A direct proof for this explanation has not been achieved yet.
4. It is commonly accepted that the EOF mobility of electrolytes in silica capillaries increases with increasing pH. The results submitted show that the opposite is true at pH values higher than ten (Fig. 1). The extraordinarily weak EOF of highly alkaline solutions in fused-silica capillaries could be explained by the existence of a thin layer of orthosilicic acid, $Si(OH)_4$, at the capillary wall. A highly viscous thin layer of dissolved silica probably causes a shift of the slipping plane further away from the wall to a lower value of the ζ potential. As the EOF mobility of a 2% solution of agarose at pH 12 and at a temperature of 40°C is low ($6 \cdot 10^{-10}$ m^2 V^{-1} s^{-1}), uncoated capillaries can be used for the separation of ssDNA fragments.

Acknowledgements

This research has been supported by Grants 203/97/0347 and 301/97/1192 of the Grant Agency of the Czech Republic. The authors acknowledge Mgr. Michaela Blažková and the staff members of the Institute of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, for the preparation of genetic samples.

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