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# Comparison of high-performance liquid chromatography with capillary gel electrophoresis in single-base resolution of polynucleotides

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## ABSTRACT

High-resolution separations of polynucleotides were performed using capillary gel electrophoresis (cGE) and high-performance liquid chromatography (HPLC) with reversed-phase, ion-exchange and mixed-mode columns. Electropherograms showing cGE separations of single-stranded homopolynucleotides were presented and compared with HPLC separations according to the chain length of the polynucleotides. The resolving power of cGE is much higher than that of any HPLC mode. The chain length limits for complete separation within 60 min by cGE, reversed-phase, ion-exchange and mixed-mode HPLC are *ca.* 250, 30, 40 and 40 nucleotides, respectively. The plate number which is achieved for cGE of $3 \cdot 10^6$–$7 \cdot 10^6$ plates/m is about ten times larger than those of all HPLC modes of $4 \cdot 10^4$–$8 \cdot 10^5$ plates/m. The reproducibility of the migration time in cGE (2–4%) is comparable to those of retention times in HPLC in several separation modes (1–3%).

## INTRODUCTION

Analytical biotechnology [1,2] is an expanding field covering in the analytical chemistry of biopolymers such as peptides, proteins, oligonucleotides, RNA and DNA. Rapid analytical techniques with high resolution and sensitivity are required for biopolymer analysis [3]. The largest biological project, the human genome project [4,5], and RNA engineering initiated after Cech's discovery of ribozyme [6] have also led to the investigation of more rapid DNA and RNA analyses. These projects all require the achievement of single-base resolution of polynucleotides from both analytical and preparative points of views.

Chromatography [7] and electrophoresis [8] have both been recognized as the main techniques for the analysis of polynucleotides, RNA and DNA in analytical biotechnology. More recently, high-performance liquid chromatography (HPLC) [9–11] and capillary electrophoresis [12] have been developing rapidly. In HPLC separations, several novel packing materials have been developed for the separation of oligonucleotides and polynucleotides [13–31]. The packing materials developed for HPLC can be classified into four groups: ion-exchange [13–25], reversed-phase [13,26,27], mixed-mode [28–30] and hydroxyapatite [31]. Microparticulate ($< 5$ $\mu$m)

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ion exchangers based on polymers [21–25] and mixed-mode packing materials [28–30] have led to high resolving powers of polynucleotides for single-base resolution. Reversed-phase packing materials [13,26,27] have provided an excellent means of rapidly and efficiently purifying synthetic oligonucleotides from crude reaction mixtures. In capillary electrophoretic separations, Karger and co-workers [32,33] first demonstrated the ultra-high resolving power of capillary gel electrophoresis (cGE) using polyacrylamide gel-filled capillaries for the separation of polynucleotides. Several research groups have also been investigating cGE for the single-base resolution of polynucleotides [34–40] and applied it to rapid DNA sequencing [34–36].

In this paper, HPLC and cGE are compared with respect to their performance in the single-base resolution of polynucleotides. First, we examined critically the resolving power of HPLC and cGE, *i.e.*, how wide a range of chain length of polynucleotides can be separated with high resolution. For this purpose, we investigated the suitability of a laboratory-made polyacrylamide gel-filled capillary [40] for cGE and four commercially available columns for HPLC, namely (1) a TSKgel OligoDNA RP conventional silica-based ODS reversed-phase column [27], (2) a TSKgel DEAE-NPR novel microparticulate non-porous polymer-based ion-exchange column [23,24], (3) a Shim-pack WAX-1 silica-based ion-exchange column [20] and (4) a Neosorb-LC-N-7R RPC-5 type column [30]. Binary gradient elution was used for HPLC separations of polynucleotides and the gradient was optimized by using computer-assisted retention prediction and the HPLC computer simulation system reported previously [41–43]. We used commercial homooligodeoxynucleotides of specified chain length an polyadenylic acids digested enzymatically to study the limits of resolution for these systems. In addition, the reproducibility of both techniques was studied. The advantages and limitations of each technique are discussed on the basis of our own experience in the laboratory. The resolving powers of HPLC columns that have been reported previously [13–31] were also compared with that of cGE.

## EXPERIMENTAL

### *Chemicals*

Polyadenylic acid [poly(A)] was obtained from Yamasa Shoyu (Chiba, Japan), polydeoxyadenylic acid [poly(dA)] from Sigma (St. Louis, MO, USA) and Pharmacia LKB (Uppsala, Sweden), polydeoxyadenylic acids of chain length from the 12mer to 18mer [poly(dA)$_{12-18}$] and from the 40mer to 60mer [poly(dA)$_{40-60}$] and polyadenylic acids of chain length from the 12mer to 18mer [poly(A)$_{12-18}$] from Pharmacia and nuclease P1 from Yamasa Shoyu. All other chemicals were of analytical-reagent or electrophoretic grade from Wako (Osaka, Japan). The concentrations of samples were 2.5 units per 100 $\mu$l for poly(dA)$_{12-18}$, 5 units per 100 $\mu$l for poly(dA)$_{40-60}$ and 5 units per 100 $\mu$l for poly(A)$_{12-18}$. These polynucleotide samples were stored at $-18$°C until used.

### *Preparation of poly(A) and poly(dA) enzymatic partial hydrolysates*

Oligoadenylate fragments from poly(A) were prepared by enzymatic hydrolysis of poly(A) with nuclease P1 [44]. An aliquot (200 $\mu$l) of an aqueous solution of poly(A) (15 mg/ml) was mixed with 200 $\mu$l of 0.3 *M* citrate buffer solution (pH 6). An aliquot (2 $\mu$l) of an aqueous solution of nuclease P1 (50 $\mu$g/ml) was added to the buffered

solution of poly(A) and the resulting solution was allowed to react at 40°C for 20 min. Oligodeoxyadenylate fragments from poly(dA) were prepared in the similar manner by using nuclease P1. A 20-$\mu$l volume of an aqueous solution of poly(dA) (5 units per 20 $\mu$l) was mixed with 20 $\mu$l of 0.3 *M* citrate buffer (pH 5.3), 3 $\mu$l of an aqueous solution of nuclease P1 (50 $\mu$g/ml) was added to the buffered solution of poly(dA) and the resulting solution was allowed to react at 40°C for 40 min. These polynucleotide samples were stored at −18°C until used.

*HPLC equipment*

A Tri-Rotar VI HPLC system (Jasco, Tokyo, Japan) and an LC-800 HPLC system (Jasco) were used for the separation of oligonucleotides. Both HPLC systems were equipped with a microcomputer-based gradient controller. All gradients were performed with a binary gradient elution technique. Sample solution (5–20 $\mu$l) was injected into the column and chromatographed at a flow-rate of 1.0 ml/min. The column temperature was kept at 40°C. Oligonucleotides were detected at 260 nm.

*Columns and eluents*

Four different types of columns were used: (1) TSKgel OligoDNA RP (TOSOH, Tokyo, Japan) [27], (2) TSKgel DEAE-NPR (TOSOH) [23,24], (3) Shim-pack WAX-1 (Shimadzu, Kyoto, Japan) [20] and (4) Neosorb-LC-N-7R (Nishio, Tokyo, Japan) [30].

The TSKgel OligoDNA RP column (150 mm × 4.6 mm I.D.) is a reversed-phase column based on a silica support, having a particle diameter of 5 $\mu$m and a pore size of 250 Å. Octadecyl groups were chemically bonded to the silica support. Eluents for this column were (A) 5% acetonitrile containing 0.1 *M* ammonium acetate and (B) 25% acetonitrile containing 0.1 *M* ammonium acetate.

The TSKgel DEAE-NPR column (35 mm × 4.6 mm I.D.) is an anion-exchange column based on a non-porous polymer support, having a particle diameter of 2.5 $\mu$m. Diethylaminoethyl groups were chemically bonded to the non-porous polymer support. Eluents for this column were (A) 0, 0.1 or 0.25 *M* sodium chloride in 20 m*M* tris(hydroxymethyl)aminomethane (Tris)–HCl buffer (pH 9.0) and (B) 1 *M* sodium chloride in 20 m*M* Tris–HCl buffer (pH 9.0).

The Shim-pack WAX-1 column (50 mm × 4 mm I.D.) is an anion-exchange column based on a spherical silica support, having a particle diameter of 3 $\mu$m and a pore size of 100 Å. Tertiary amino groups were chemically bonded to the silica support. Eluents for this column were (A) 0.01 *M* phosphate buffer (pH 6.8) containing 20% of acetonitrile and (B) 0.3 *M* phosphate buffer (pH 6.8) containing 20% of acetonitrile.

The Neosorb-LC-N-7R column (250 mm × 4 mm I.D.) is a mixed-mode column (RPC-5 type) based on a polychlorotrifluoroethylene support, having a particle diameter of 7 $\mu$m. The support was coated with trioctylmethylammonium chloride. Eluents for this column were (A) 0.01 *M* sodium perchlorate–Tris–acetate buffer (pH 7.5)–1 m*M* EDTA and (B) 0.15 *M* sodium perchlorate–Tris–acetate buffer (pH 7.5)–1 m*M* EDTA.

*Capillary electrophoresis*

cGE separations were carried out by using an Applied Biosystems (ABI, Foster

City, CA, USA) Model 270A capillary electrophoresis system. Polyimide-coated fused-silica capillaries (375 μm O.D., 100 μm I.D.) (GL Sciences, Tokyo, Japan) of effective length 30 cm and total length 50 cm were used without pretreatment. Polyacrylamide gel-filled capillaries were prepared according to the method reported previously [40] as follows: (1) a buffered solution of acrylamide is prepared, (2) catalysts [N,N,N′,N′-tetramethylethylenediamine (TEMED) and ammonium peroxodisulphate] are added to the solution of acrylamide, (3) polymerizing solution is quickly injected into the capillary by means of a vacuum injection system and (4) acrylamide is polymerized in the capillary for 1 h at room temperature. The reproducibility and stability of gel-filled capillaries were discussed in a previous paper [40]; the gel-filled capillaries typically gave only a 10% decrease in the number of theoretical plates after 50 injections. The buffer solution was a mixture of 0.1 *M* Tris and 0.1 *M* boric acid with 7 *M* urea (pH 8.8). The sample solution was introduced into the capillary electrophoretically (1 s at 5 kV). Gel-filled capillaries were run with buffer solution at 10 kV (200 V/cm, 9–11 μA) at 30°C. Polynucleotides were detected at 260 nm.

## RESULTS AND DISCUSSION

Enzymatic digestion of poly(A) described under Experimental gave a mixture of polyadenylates containing 5′-terminal phosphate in their chain from the monomer to 300mer [40]. Poly(A) enzymatic partial hydrolysate is advantageous as a model substrate to demonstrate the resolving power of HPLC and cGE compared with poly(A) alkaline partial hydrolysate and poly(dA)$_{40-60}$, which are widely used as model substrates for HPLC and cGE. The reason is that enzymatic digestion of poly(A) produced a poly(A) mixture containing a wide chain length range in addition to single species in each chain length. In contrast, poly(dA)$_{40-60}$ has a narrow chain length range and alkaline hydrolysis of poly(A) gave double poly(A) containing 2′- or 3′-terminal phosphate in each chain length [15]. HPLC separation of poly(A) alkaline partial hydrolysate, therefore, gave a doublet peak in each chain length for small-size poly(A) and, further, high-molecular-weight poly(A) became poorer resolved [15,42]. We first examined the resolving power of cGE and HPLC with several types of columns by separating poly(A) enzymatic partial hydrolysate. In order to compare cGE and HPLC directly, identical samples were analysed.

### *cGE separation of poly(A) digested by nuclease P1*

Fig. 1 shows an electropherogram for mixtures of poly(A) separated by cGE with a polyacrylamide gel (5% T and 5% C)[a]-filled capillary at 200 V/cm. To determine the chain length of poly(A) for each band, poly(A)$_{12-18}$ was co-injected with poly(A) mixtures. Consequently, peaks with a migration time of *ca.* 20 min are assigned to poly(A) from the 12mer to 18mer. The large peak at 17 min would correspond to unseparated oligoadenylates from the monomer to 5mer. Fig. 1, therefore, clearly demonstrates that polynucleotides of different chain length are baseline resolved within the poly(A) series in the chain length range from the 6mer to 255mer under the conditions given, and yet the separation was completed in less than 62 min. The resolution, $R_s$, of each band pair was more than 1.5.

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

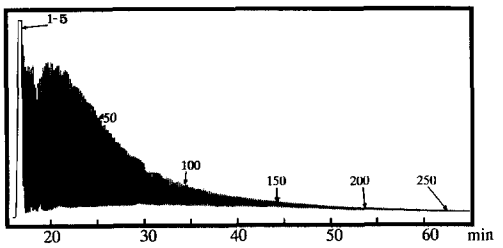


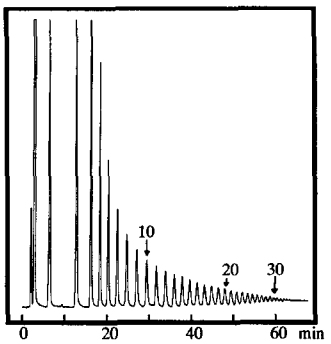

Fig. 1. cGE separation of poly(A) enzymatic partial hydrolysate. Capillary, 100 μm I.D., 375 μm O.D., length 50 cm, effective length 30 cm; running buffer, 0.1 *M* Tris–0.1 *M* boric acid–7 *M* urea (pH 8.8); gel matrix, 5% T and 5% C; field, 200 V/cm; current, 10 μA; injection, 5 kV for 1 s; detection, 260 nm.

The plate number of each peak was estimated to be $2.3 \cdot 10^6$ ($7 \cdot 10^6$/m) for peak 30, $1.5 \cdot 10^6$ ($5 \cdot 10^6$/m) for peak 50 and $9.6 \cdot 10^5$ ($3 \cdot 10^6$/m) for peak 100. The run-to-run reproducibility of the migration time was in the range 2–4% relative standard deviation (R.S.D.) ($n = 5$).

*HPLC separations of poly(A) digested by nuclease P1*

Figs. 2–5 demonstrate the separations of poly(A) enzymatic partial hydrolysate by HPLC in the reversed-phase (Fig. 2), ion-exchange (Figs. 3 and 4) and mixed modes (Fig. 5). All separations were performed by utilizing gradient elution techniques, the gradients being optimized by HPLC computer simulations [41–43]. The chain length of poly(A) was determined by co-elution of $poly(A)_{12-18}$.

Fig. 2 shows the chromatogram of mixtures of poly(A) separated by reversed-phase HPLC (TSKgel OligoDNA RP column). Poly(A) up to the 30mer were



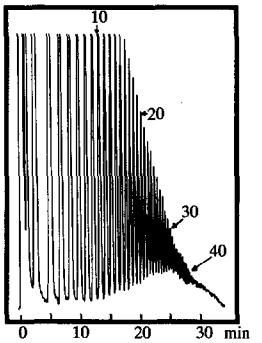
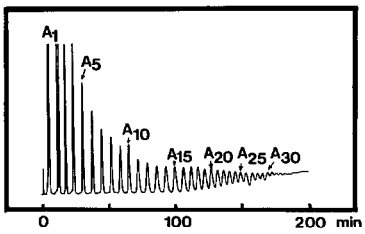




Fig. 2. Reversed-phase HPLC separation of poly(A) enzymatic partial hydrolysate. Column, TSKgel OligoDNA RP (150 mm × 4.6 mm I.D.); eluent, (A) 5% acetonitrile containing 0.1 *M* ammonium acetate and (B) 25% acetonitrile containing 0.1 *M* ammonium acetate; gradient programme, 0–10 min from 0 to 18% B, 10–30 min from 18 to 26% B, 30–100 min from 26 to 45% B at 40°C; flow-rate, 1.0 ml/min.

Fig. 3. Anion-exchange HPLC separation of poly(A) enzymatic partial hydrolysate. Column, TSKgel DEAE-NPR (35 mm × 4.6 mm I.D.). Eluent, (A) 0.25 *M* sodium chloride in 20 m*M* Tris–HCl buffer (pH 9.0) and (B) 1 *M* sodium chloride in 20 m*M* Tris–HCl buffer (pH 9.0); linear gradient from 0 to 100% B in 60 min at 40°C, flow-rate, 1.0 ml/min.



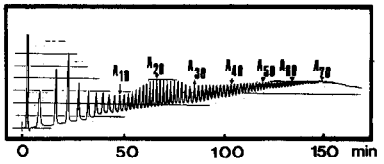




Fig. 4. Anion-exchange HPLC separation of poly(A) enzymatic partial hydrolysate. Column, Shim-pack WAX-1 (50 mm × 4 mm I.D.); eluent, (A) 0.01 *M* phosphate buffer (H 6.8) containing 20% of acetonitrile and (B) 0.3 *M* phosphate buffer (pH 6.8) containing 20% of acetonitrile; the convex gradient described previously [42] was used, from 0 to 100% B in 240 min; flow-rate, 1.0 ml/min.

Fig. 5. Mixed-mode HPLC separation of poly(A) enzymatic partial hydrolysate. Column, Neosorb-LC-N-7R (250 mm × 4 mm I.D.); eluent, (A) 0.01 *M* sodium perchlorate–Tris–acetate buffer (pH 7.5)–1 m*M* EDTA and (B) 0.15 *M* sodium perchlorate–Tris–acetate buffer (pH 7.5)–1 m*M* EDTA; linear gradient from 0 to 100% B in 150 min; flow-rate, 1.0 ml/min.

successfully separated in less than 60 min. The resolution was in the range $R_s$ = 1.0–1.5. Such HPLC separations exhibited plate numbers of 80 000 which, with a column length of 15 cm, corresponds to $5 \cdot 10^5$ plates/m. The reproducibility of the retention time was in the range 1–2% R.S.D. ($n$ = 5).

Figs. 3 and 4 show the separations of a mixture of poly(A) oligonucleotides by ion-exchange chromatography. The non-porous polymer-based ion exchanger TSKgel DEAE-NPR was used in Fig. 3 and the porous silica-based ion exchanger Shim-pack WAX-1 in Fig. 4. Baseline resolutions were obtained for up to the 20mer, and peaks appeared for up to about the 40mer (Fig. 3) and 35mer (Fig. 4). The chromatographic pattern in Fig. 4 is similar to that in Fig. 3, although the separation time in Fig. 4 was much longer than that in Fig. 3.

The plate numbers were 28 000 ($8 \cdot 10^5$ plates/m) with the TSKgel DEAE-NPR column and 13 000 ($3 \cdot 10^5$ plates/m) with the Shim-pack WAX-1 column. The resolutions for both columns were calculated to be in the range $R_s$ = 0.8–1.5. The reproducibility of the retention time for each oligonucleotide was 1–3% R.S.D. ($n$ = 5) for both columns.

The chromatogram shown in Fig. 5 was obtained with a mixed-mode column in which the matrix contains sites for both ionic and hydrophobic interactions [28–30]. The resolution possible using this mixed-mode matrix is illustrated by the resolution of a poly(A) enzymatic hydrolysate, which could be separated up to at least the 70mer in less than 150 min. The plate number estimated for each peak was 10 000 ($4 \cdot 10^4$ plates/m) and the resolution for each band pair was in the range $R_s$ = 0.6–1.5. The reproducibility of the retention time was 1–2% R.S.D. ($n$ = 5).

The performances of cGE and HPLC in the various separation modes as described above are summarized in Table I. Comparison of the resolving powers and analysis time of the four HPLC columns confirms that within a series of poly(A) the TSKgel DEAE-NPR column is the most suitable for the complete and rapid separation of polynucleotides according to chain length.

TABLE I

PERFORMANCE OF cGE AND HPLC IN THE SEPARATION OF POLYNUCLEOTIDES

| Method | Plate number ($N$) | Separated poly(A) | Analysis time (min) | Resolution, $R_s$ | R.S.D. (%) |
|---|---|---|---|---|---|
| *cGE* | | | | | |
| 100 μm I.D.; 5% T and 5% C[a] | 2 300 000 ($7 \cdot 10^6$/m) | 6–255mer | 62 | 1.5–1.7 | 2–4 |
| *HPLC* | | | | | |
| TSKgel OligoDNA RP[b] | 80 000 ($5 \cdot 10^5$/m) | 1–30mer | 60 | 1.0–1.5 | 1–2 |
| TSKgel DEAE-NPR[c] | 28 000 ($8 \cdot 10^5$/m) | 1–40mer | 27 | 0.8–1.5 | 1–3 |
| Shim-pack WAX-1[d] | 13 000 ($3 \cdot 10^5$/m) | 1–30mer | 160 | 0.8–1.5 | 1–3 |
| Neosorb-LC-N-7R[e] | 10 000 ($4 \cdot 10^4$/m) | 1–70mer | 150 | 0.6–1.5 | 1–2 |

[a] Gel-filled capillary as in Fig. 1.
[b] Reversed-phase column as in Fig. 2.
[c] Ion-exchange colomn as in Fig. 3.
[d] Ion-exchange column as in Fig. 4.
[e] Mixed-mode column as in Fig. 5.

### *cGE and HPLC separations of poly(dA) standards and poly(dA) digested by nuclease P1*

We next examined the resolving power of cGE and HPLC in the separation of polydeoxyadenylates, poly(dA). The model solutes used were commercially available homooligodeoxyadenylates of specified chain length and poly(dA) enzymatic partial hydrolysate obtained by the method described under Experimental. The electrophoretic conditions were almost the same as described in the previous section. For the HPLC separation of poly(dA), a TSKgel DEAE-NPR column was selected because of its superior resolving power and speed compared with the other columns described. Poly(dA) was separated by gradient-elution chromatography, the gradients being optimized by HPLC computer simulations [41–43].

Fig. 6 shows separations of a mixture of poly(dA) oligonucleotides which

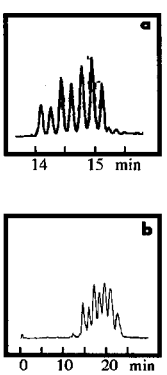



Fig. 6. (a) cGE and (b) anion-exchange HPLC separations of poly(dA)$_{12-18}$. Gel electrophoretic conditions as in Fig. 1. Chromatographic conditions as in Fig. 3 except for eluent and gradient programme. Eluent, (A) 0.1 $M$ sodium chloride in 20 m$M$ Tris–HCl buffer (pH 9.0) and (B) as in Fig. 3; gradient programme, 0–3 min from 0 to 8% B, 3–15 min from 8 to 18% B, 15–50 min from 18 to 35% B at 40°C.

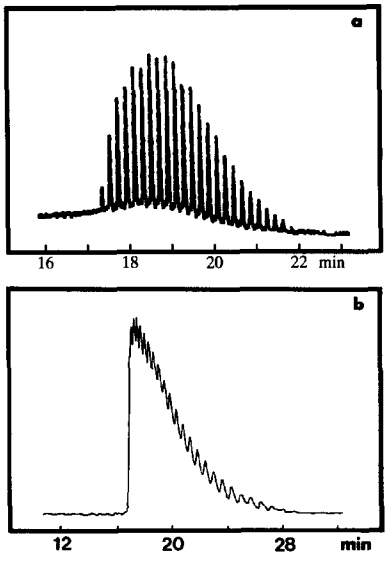



Fig. 7. (a) cGE and (b) anion-exchange HPLC separations of poly(dA)$_{40-60}$. Gel electrophoretic conditions as in Fig. 1 except for gel matrix (7% T and 5% C). Chromatographic conditions as in Fig. 3 except for eluent and gradient programme. Eluent, (A) 0.1 *M* sodium chloride in 20 m*M* Tris–HCl buffer (pH 9.0) and (B) as in Fig. 3; gradient programme, 0–4 min from 0 to 20% B, 4–12 min from 20 to 40% B, 12–55 min from 40 to 55% B at 40°C.

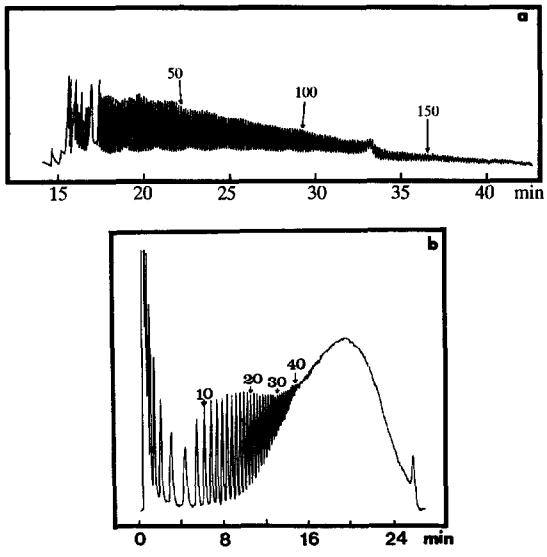



Fig. 8. (a) cGE and (b) anion-exchange HPLC separations of poly(dA) enzymatic partial hydrolysate. Gel electrophoretic conditions as in Fig. 1. Chromatographic conditions as in Fig. 3.

contains 12–18 bases [poly(dA)$_{12-18}$] according to the supplier. All components were completely separated in the cGE separation (Fig. 6a) and HPLC separation (Fig. 6b) illustrated that poly(dA)$_{12-18}$ was well resolved. The analysis time of cGE was shorter than that of HPLC.

Fig. 7 shows separations of poly(dA)$_{40-60}$ by cGE (Fig. 7a) and HPLC (Fig. 7b). In the cGE separation, all poly(dA) polynucleotides were baseline resolved within only 22 min. On the other hand, HPLC failed to resolve each component of poly(dA). We tried to improve the resolution of poly(dA)$_{40-60}$ by changing the HPLC separation conditions, but dit not achieve a better resolution. These results clearly illustrate that the resolving power of cGE is much hihger than that of HPLC in the separation of relatively high-molecular-weight poly(dA), whereas the difference in the resolving powers of the two techniques is not obviously distinguishable in the separation of relatively shorter poly(dA), as shown in Fig. 6.

Separations of poly(dA) enzymatic partial hydrolysate were performed by use of cGE (Fig. 8a) and HPLC (Fig. 8b). Fig. 8a clearly demonstrates that ultra-high resolution of poly(dA) is achieved by using cGE; 180 bands of poly(dA) were completely resolved within only 42 min, whereas baseline resolution was obtained for up to only the 20mer by HPLC as shown in Fig. 8b and peaks appeared for up to about the 40mer. Comparison of the results shows that cGE gave a superior resolution to HPLC. The number of poly(dA) bands that appeared in the cGE separation (Fig. 8a) is smaller than that of poly(A) bands in Fig. 1, because the poly(dA) sample prior to digestion has an approximate average chain length of 290 according to the supplier but the chain length of poly(A) prior to digestion is much larger than that of poly(dA).

*Comparison of resolving power and reproducibility of the two techniques*

We compared with the utility of cGE and HPLC with four different columns for the separation of homopolynucleotides according to their chain length. The results show that within a series of homopolynucleotides all systems lead to the reliable separation of small-sized poly(A) according to chain length but only the cGE system makes it possible to separate completely higher polynucleotides such as the 250mer.

To evaluate the potential of cGE for single-base resolution of polynucleotides, the performance of cGE was compared with that of HPLC as indicated in Table I. Table I shows a ten times higher plate number for cGE of $7 \cdot 10^6$ plates/m than those for all modes of HPLC of $4 \cdot 10^4$–$8 \cdot 10^5$ plates/m. The chain length limit by cGE, which is 255 nucleotides, is 4–8 times larger than those given by reversed-phase, non-porous polymer-based ion-exchange, porous silica-based ion-exchange and mixed mode HPLC, which are 30, 40, 30 and 70 nucleotides, respectively. cGE separated 40 bands of poly(A) in 10 min, whereas HPLC separated only fifteen bands (TSKgel DEAE-NPR) or two bands (Shim-pack WAX-1) in 10 min. Additionally, each component was resolved completely in cGE but later eluted components in HPLC showed poorer resolution. The resolution values obtained for cGE ranged from $R_s$ = 1.5–1.7, which compare favourably with those for HPLC of $R_s$ = 0.6–1.5. The reproducibility (2–4% R.S.D.) of the migration time in cGE is comparable to those of the retention times in HPLC in the various modes (1–3% R.S.D.). These results show that the cGE technique gave a superior performance to the HPLC techniques.

To examine the performance of cGE in more detail, we selected high-resolution HPLC and cGE separations of polynucleotides reported in the literature, as listed in

TABLE II

PERFORMANCE OF HPLC AND cGE REPORTED IN THE LITERATURE

| Method | Separated polynucleotides | Analysis time (min) |
|---|---|---|
| *Ion-exchange HPLC* | | |
| Partisil SAX [13] | 1–30mer | 50 |
| Laboratory-made column [15] | 4–35mer | 300 |
| Nucleogen-DMA-60 [18] | 1–37mer | 110 |
| MonoQ [19] | 1–27mer | 15 |
| Gen-Pak FAX [22] | 40–60mer | 39 |
| TSKgel DEAE-NPR [23] | 20–70mer | 17 |
| Laboratory-made column [25] | 1–18mer | 4 |
| *Reversed-phase HPLC* | | |
| Zorbax ODS [13] | 2–10mer | 25 |
| μBondapak $C_{18}$ [26] | 1–19mer | 60 |
| *Mixed-mode HPLC* | | |
| Laboratory-made column [28,29] | 4–90mer | 1140 |
| Neosorb-LC-N-7R [30] | 1–75mer | 95 |
| *cGE* | | |
| cGE [33] | 20–160mer | 25 |
| cGE [34] | 19–330mer | 70 |
| cGE [35] | 19–300mer | 115 |
| cGE [36] | 19–340mer | 70 |
| cGE [38] | 1–430mer | 130 |

Table II. The results obtained by HPLC given in Table II show that mixed-mode HPLC [28–30] can separate polynucleotide mixtures containing a wide range of chain length and HPLC with a non-porous polymer-based ion-exchange column [23,25] achieved a high-speed analysis of polynucleotides. However, the resolving power and separation speed of cGE as presented here is much better than those of the HPLC techniques listed in Table II. For example, Kato *et al.* [23] reported a rapid separation in less than 20 min of oligodeoxyadenylic acids from the 17mer to 70mer by use of a non-porous polymer-based ion-exchange column. Later eluted bands, however, represented a poor resolution, in contrast to the achievement of baseline separations for up to the 32mer as shown in Fig. 3. Other groups [28–30] have demonstrated high resolutions of polynucleotides with relative wide ranges of chain length by use of mixed-mode separation columns, but their separations required long analysis times.

Table II also shows the cGE separations of polynucleotides reported in the literature. These results demonstrate the ultra-high efficiency of cGE in the single-base resolution of polynucleotides and in DNA sequencing. The cGE separation presented in Fig. 1 gave almost the same efficiency with the respect to resolution and speed as those listed in Table II.

In conclusion, our results suggest that cGE and HPLC are equally effective for the separation of small-sized polynucleotides, whereas for longer polynucleotides cGE is superior to HPLC with respect to resolving power, speed and efficiency. The only disadvantage is that cGE is not suitable for large-scale preparative separations of

polynucleotides. Therefore, for preparative work with small-sized polynucleotides, HPLC is the only choice. Micropreparative analysis of large polynucleotides, however, is realized only by cGE [33]. For high-speed separations of larger polynucleotides there is as yet no alternative to cGE.

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