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A Simple Method for the Preparation of Polyacrylamide Gel Filled Capillaries for High Performance Separation of Polynucleotides by Using Capillary Gel Electrophoresis

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Polyacrylamide gel filled capillaries prepared by a simple method showed ultrahigh resolution of polynucleotides. Plate number of gel filled capillary was achieved to be 5×10^6 per meter for 55mer of polyadenylic acid. Mixture of 250 kinds of polyadenylic acids were baseline resolved and analyzed within 60 min.

High resolving power of capillary gel electrophoresis(CGE)[1] has been demonstrated in the single base resolution of DNA polynucleotides by Cohen and Karger.[2-4] Recently, much attention has been focused on CGE in the Human Genome Project, because the Project requires the development of a rapid and accurate DNA sequencing technology. DNA sequencing method by using CGE[5-9] appears to be an attractive alternative to the conventional automated DNA sequencer based on slab gel electrophoresis in improving speed, resolution, and efficiency of DNA sequencing. Although several researchers[2-14] have been investigating the methods of the preparation for polyacrylamide gel filled capillaries, a simple and reliable method has not yet been established.

In preliminary experiments we tried to prepare polyacrylamide gel filled capillaries by a simple method. Resolving power, reproducibility, and stability of gel filled capillaries were examined in the separation of standard polynucleotide samples.

Capillary gel electrophoretic separations were carried out by using an Applied Biosystems Inc.(ABI) Model 270A capillary electrophoresis system. Polyimide coated fused silica capillaries(375 μm o.d., 100 μm i.d., Polymicro Technologies) of 30 cm effective length and 50 cm total length were used without pretreatment. Buffer was 0.1 M tris(hydroxymethyl)-aminomethane(Tris) and 0.25 M boric acid with 7 M urea(pH 8.3, M=mol dm^{-3}). Stock solution(50 ml) of acrylamide included 19 g of acrylamide and 1 g of *N,N'*-methylenebis-(acrylamide). Gel filled capillaries were prepared as follows. Use a low cigarette lighter flame to burn the outer polyimide coating from a 5-10 mm section of capillary located approximately 20 cm from the outlet end. Capillary was rinsed with distilled water for 10 min. Stock solution(1 ml) of acrylamide was diluted with 7 ml of buffer solution. Diluted acrylamide solution(5%T, 5%C) is carefully degassed in an ultrasonic bath. Crosslinking is initiated with the addition of both 10 μl of 10% *N,N,N',N'*-tetramethylethylenediamine(TEMED) solution and 10 μl of 10% of ammonium persulfate solution into 5 ml of diluted solution of acrylamide. The

polymerizing solution is quickly introduced into the capillary without pretreatment by using a vacuum injection system equipped with ABI Model 270A for 5 min. Polymerization in the capillary is completed within 2 h at room temperature. Pre-running with buffer at 10 kV for 20–30 min would be recommended for the reduction of baseline noise. Sample solution was introduced electrophoretically(1 s at 5 kV) into the capillary. Gel filled capillaries were run with buffer solution described above at 10 kV(200 V/cm, 9–11 μA) at 30 °C. Polynucleotides were detected at 260 nm.

Polydeoxyadenylic acid(poly(dA)) and polyadenylic acid(poly(A)) were used as standard samples. $Poly(dA)_{12-18}$, $poly(dA)_{40-60}$, and $poly(A)_{12-18}$ were purchased from Pharmacia. The concentrations of samples were 2.5 units/100 μl for $poly(dA)_{12-18}$, 5 units/100 μl for $poly(dA)_{40-60}$, and 5 units/100 μl for $poly(A)_{12-18}$, respectively. Poly(A)(50 μl, 30 mg in 1 ml of 0.3 M citrate buffer, pH 6) purchased from Yamasa was digested by nuclease P1(2 μl, 50 μg/ml) purchased from Yamasa at 40 °C for 4 min. These polynucleotide samples were stored at −18 °C until use. Other reagents were electrophoretic grade from Wako.

In order to check the performance of the polyacrylamide gel filled capillaries prepared by the present method, we tried to separate commercially available poly(dA) mixture as shown in Fig. 1. Single base resolution of poly(dA) mixture of 12–18mer and 40–60mer was achieved within 23 min. Plate number of each peak is 300,000, and with an effective capillary length of 30 cm, this value is equivalent to $1x10^6$ plates per meter. The run-to-run reproducibility of migration time was in the range of 3–5% RSD.

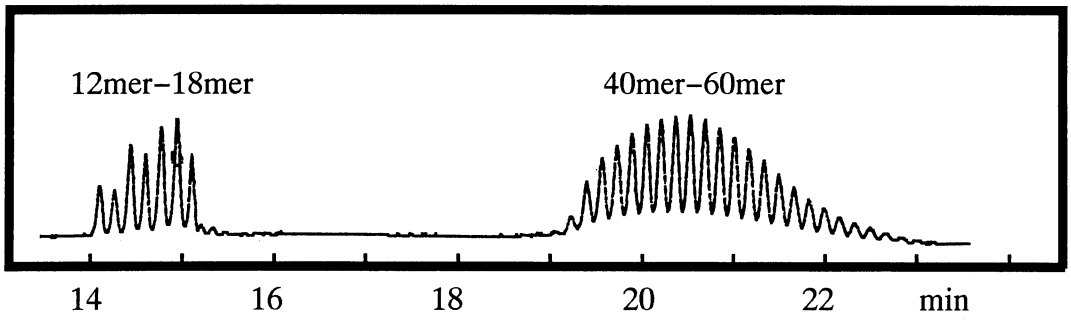

Fig. 1. CGE separation of $poly(dA)_{12-18,40-60}$. Capillary; 100 μm i.d., 375 μm o.d., length; 50 cm, effective length; 30 cm. Running buffer; 0.1 M Tris, 0.25 M boric acid, and 7 M urea, pH 8.3. Gel contained 5% T and 5% C. Field; 200 V/cm, current; 10 μA. Injection; 5 kV for 1 s. Detection; 260 nm.

Figure 2 clearly demonstrates that ultrahigh resolution of poly(A) digested by nuclease P1 was achieved by using gel filled capillary. Two hundreds and fifty bands of poly(A) were baseline resolved within only 60 min. To determine the chain length of poly(A) for each band, $poly(A)_{12-18}$ was co-injected with poly(A) mixtures. Consequently, peaks around migration time of 20 min are assigned to poly(A) from 12mer to 18mer. Large peak at 17 min would correspond to unseparated oligoadenylic acids from monomer to 5mer. If single base resolution was assumed to be achieved in all range of separation, poly(A) in the chain length range of

6mer to 255mer could be completely resolved. Chain length of such large poly(A), however, could not be determined, because of the lack of authentic sample and the difficulty in preparing large RNA samples.

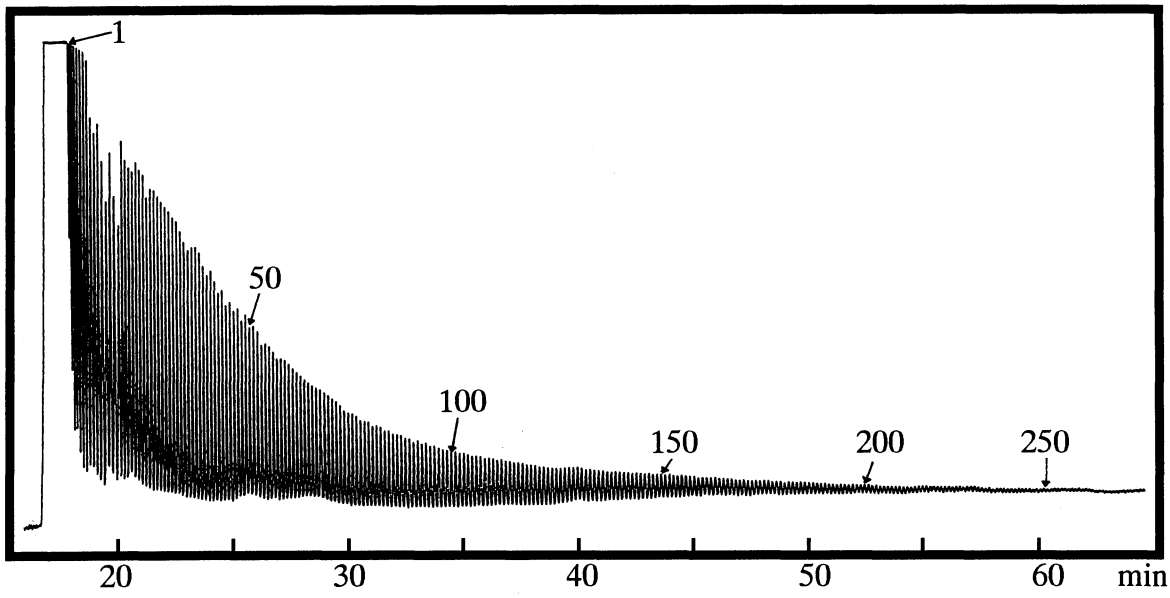

Fig. 2. CGE separation of poly(A) digested by nucelease P1. Separation conditions as in Fig. 1.

Plate number of each peak was estimated to be 1.5×10^6(5×10^6 per meter) for peak number 50(may be 55mer), 9.6×10^5(3×10^6 per meter) for peak number 100(may be 105mer), and 3.8×10^5(1×10^6 per meter) for peak number 150(may be 155mer), respectively. This high efficiency, which is obtained by using CGE without optimization of capillary size, gel matrix, and applied field, compares favorably with the reported values of plate number in the range of $3-30 \times 10^6$ per meter,[2,4,6,9] which are achieved under optimum conditions.

Reproducibility and stability of gel filled capillaries were examined by use of poly(A) mixture. The run-to-run reproducibility of migration time was in the range of 3-5% RSD (n=3), the day-to-day one was 5-7% RSD(n=3), and the capillary-to-capillary one was 5-10% RSD(n=5). Preliminary experiments showed that gel filled capillaries could be used for over 50 measurements without appreciable decrease in performance. In addition, gel filled capillaries were all free from bubbles. Reproducibility and stability are sufficient for analytical purposes in the separation of DNA and RNA samples, but more accuracy of migration time is required for use in DNA sequencing. Gel filled capillary was usually prepared with pretreatment of capillary inner surface by use of a bifunctional reagent such as 3-methacryloxypropyl-trimethoxysilane to increase the stability of gel.[2-5,7,9,10,12-14] The results presented here demonstrate that stable gel filled capillaries are obtained even by the simple method without surface pretreatment.

Several researchers[5,6,13] pointed out that the formation of bubbles during gel

polymerization remains a problem. In our opinion, the formation of bubbles would be avoidable, when gel was polymerized under the moderate conditions and polymerizing acrylamide solution was quickly introduced into capillaries, as similar reason was described by Schomburg.[11,13] In the present method, to polymerize gel under the mild conditions, we used 0.02%(W/V) ammonium persulfate and 0.02%(V/V) TEMED. Such concentrations are lower than normally used for gels. Additionally, vacuum injection of acrylamide solution into capillaries is advantageous for the production of bubble free gel filled capillaries. Capillaries prepared by the siphoning introduction of acrylamide solution tended to form gas bubbles during polymerization as described in the literature.[6]

Stable gel filled capillaries free from bubbles are obtained by the simple method. The results in Figs. 1 and 2 clearly illustrate that gel filled capillaries show an ultrahigh resolution of complex polynucleotide mixtures. Gel filled capillaries possess the possibility of using CGE as a tool for DNA sequencing.

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