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Note

Separation of large DNA restriction fragments by high-performance gel filtration on TSKgel DNA-PW

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Genetic engineering has been rapidly progressing in recent years. A technique to separate nucleic acids is very important in this field. Although gel electrophoresis has been mainly adopted for this purpose, high-performance liquid chromatography (HPLC) has been studied and found useful for the separation of oligonucleotides and ribonucleic acids (RNAs). However, the separation of deoxyribonucleic acid (DNA) restriction fragments has so far been limited to comparatively small molecules, and the separation of large DNA restriction fragments in the range of 1000–5000 base pairs (which are important as inserts in recombinant DNA research) has been reported only in one paper, where 1060 and 1857 base pair fragments could be separated[1]. This is probably because of the lack of suitable supports. Recently, however, a support of large pore size for use in high-performance gel filtration was developed and has become commercially available under the trade-name of TSKgel DNA-PW (Toyo Soda, Tokyo, Japan). We have evaluated its separation range and resolution. The results are presented in this paper.

EXPERIMENTAL

Double-stranded DNA fragments were prepared by cleaving plasmid pBR322 DNA with restriction endonuclease EcoRI or BstNI. The preparation method has been described elsewhere[2]. Digestion of pBR322 DNA with EcoRI produces only one fragment of 4362 base pairs[3], whereas BstNI-cleaved pBR322 DNA contains six fragments of 13, 121, 383, 928, 1060 and 1857 base pairs[3].

Gel filtration was performed at 25°C with a Model HLC-803D high-performance liquid chromatograph equipped with a Model UV-8 variable-wavelength UV detector (Toyo Soda), operated at 260 nm. DNA fragments were separated on two or four TSKgel DNA-PW columns (300 × 7.8 mm I.D.) that were connected in series and packed with hydrophilic-resin-based supports of 10 μm in particle diameter. The eluent was 0.1 *M* Tris–HCl buffer (pH 7.5), containing 0.3 *M* NaCl and 1 m*M* EDTA. The flow-rates were 0.15–0.5 ml/min.

Polyacrylamide gel electrophoresis of DNA fragments was carried out with 5

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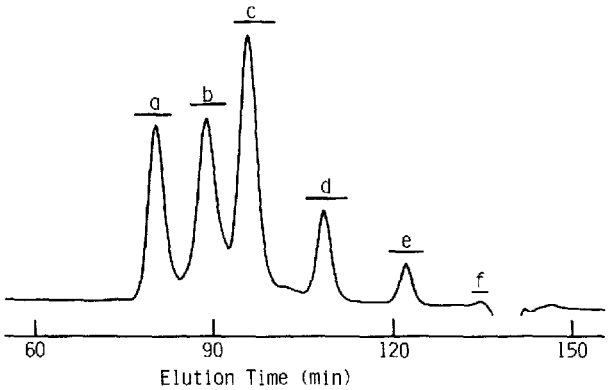

Fig. 1. Chromatogram of a mixture of EcoRI-cleaved pBR322 DNA (1.7 μg) and BstNI-cleaved pBR322 DNA (8 μg) obtained on a four DNA-PW column system. Eluent: 0.1 *M* Tris–HCl buffer (pH 7.5) containing 0.3 *M* NaCl and 1 m*M* EDTA; flow-rate, 0.3 ml/min. Injection volume, 60 μl.

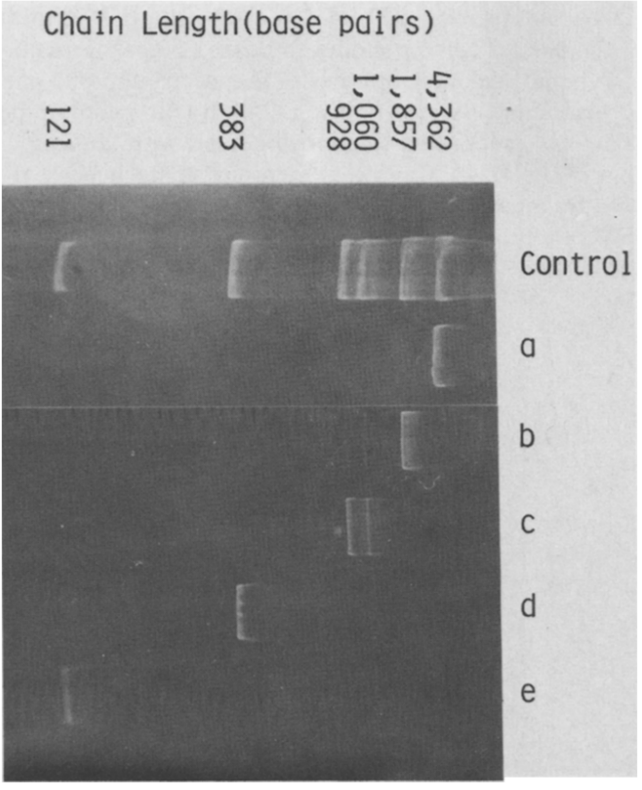

Fig. 2. 5% Polyacrylamide gel electrophoresis pattern of fractions obtained in Fig. 1. The control is the unfractionated mixture of EcoRI-cleaved pBR322 DNA and BstNI-cleaved pBR322 DNA.

TABLE I

DNA FRAGMENTS FOUND IN PEAKS a–f IN FIG. 1

Peak	*Chain length of component (base pairs)*
a	4362
b	1857
c	1060, 928
d	383
e	121
f	13

or 20% polyacrylamide gel [acrylamide–N,N'-methylenebisacrylamide (29:1)] in 50 m*M* Tris–borate buffer solution (pH 8.3) containing 1 m*M* EDTA. After electrophoresis, the gel was stained with 0.5 μg/ml of ethidium bromide and observed under UV radiation at *ca.* 380 nm.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of a mixture of EcoRI-cleaved pBR322 DNA and BstNI-cleaved pBR322 DNA obtained on a DNA-PW four-column system at a flow-rate of 0.3 ml/min. All the eluates corresponding to peaks (Fig. 1) were collected and examined by polyacrylamide gel electrophoresis. The electrophoretic pattern obtained with 5% polyacrylamide gel is shown in Fig. 2. A fraction containing a DNA fragment of 13 base pairs was submitted to electrophoresis with 20% polyacrylamide gel (data not shown). The DNA fragments identified in each peak are listed in Table I. Although two fragments of 928 and 1060 base pairs were eluted

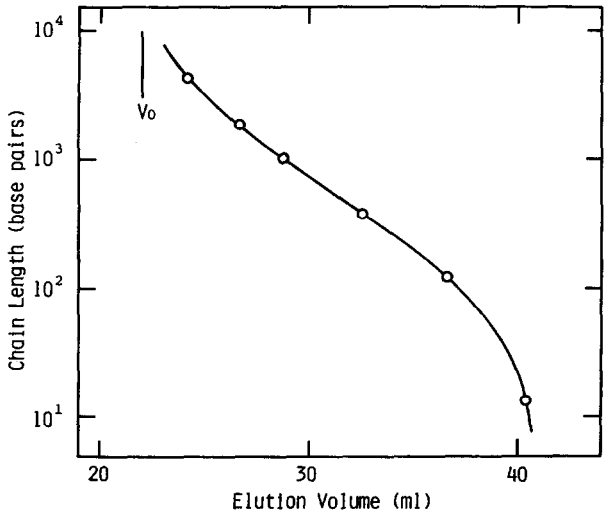

Fig. 3. Plots of chain length *vs.* elution volume for double-stranded DNA fragments obtained on a four DNA-PW column system.

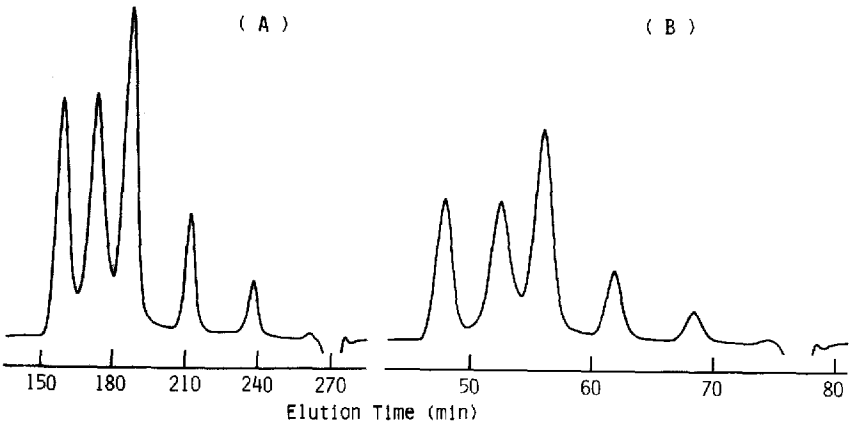

Fig. 4. Chromatograms of a mixture of EcoRI-cleaved pBR322 DNA and BstNI-cleaved pBR322 DNA. Conditions as in Fig. 1 except that the flow-rate was 0.15 ml/min (A) or 0.5 ml/min (B).

together as one peak, all the other fragments were well separated from each other. The separation of 1060 and 1857 base pair fragments and of 1857 and 4362 base pair fragments were also almost complete. This means that even fragments of greater than 1000 base pairs can be separated with little cross-contamination provided that the chain length of one is more than twice that of the other.

The chain length of DNA fragments is plotted against elution volume in Fig. 3. The average chain length of the two fragments present was used for peak b. The void volume (V_0) was determined with λ-DNA. According to this molecular weight calibration curve, the exclusion limit of DNA-PW for double-stranded DNA fragments is *ca.* 7000 base pairs. Therefore, DNA-PW can separate a significantly extended size range of DNA fragments as compared with previously evaluated gel-filtration columns[1,2].

Fig. 4 shows chromatograms of a mixture of EcoRI-cleaved pBR322 DNA

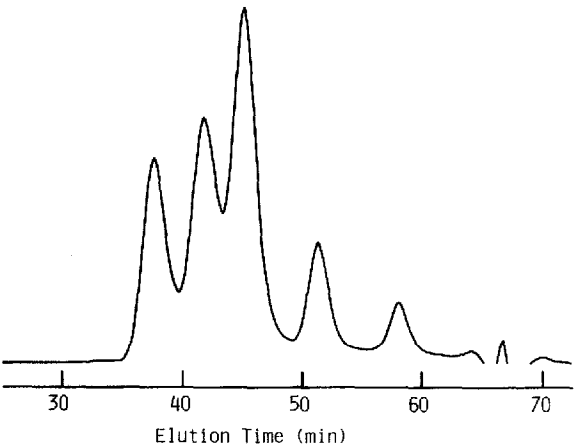

Fig. 5. Chromatogram of a mixture of EcoRI-cleaved pBR322 DNA and BstNI-cleaved pBR322 DNA. Conditions as in Fig. 1 except a two DNA-PW column system was employed.

and BstNI-cleaved pBR322 DNA obtained under the same conditions as in Fig. 1 except that the flow-rate was 0.15 or 0.5 ml/min. Lower flow-rates afforded better separation of most fragments, as expected. However, the effect of increase in flow-rate was not very significant; in particular, fragments of 1857 and 4362 base pairs were slightly better separated at higher flow-rates in opposition to the general trend. The reason for this is not known.

Fig. 5 shows a chromatogram of a mixture of EcoRI-cleaved pBR322 DNA and BstNI-cleaved pBR322 DNA obtained under the same conditions as in Fig. 1 except that a two DNA-PW column system was employed. Separation became inferior with decreasing column length. It seems that a four-column system is necessary in order to achieve base-line separation for large fragments with chain lengths differing by a factor of two.

As demonstrated above, DNA-PW had a wide separation range and could be used to separate large DNA fragments of up to *ca.* 7000 base pairs. Complete base-line separation was almost achieved for DNA fragments differing in chain length by a factor greater than two. Therefore, DNA-PW columns should be very useful in the field of genetic engineering and should be a good alternative to electrophoresis.

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