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Note

Separation of DNA restriction fragments by high-performance ion-exchange chromatography

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Ion-exchange chromatography on DEAE-cellulose, DEAE-Sephadex, etc., has been extensively used for the separation of biopolymers such as proteins and nucleic acids. Ion-exchange chromatography was greatly improved with respect to both speed and efficiency with the introduction of microparticulate ion exchangers of large pore size several years ago[1]. This high-performance ion-exchange chromatography has become widely accepted in protein research. However, its application to nucleic acids has not been reported so far, although the separation of oligonucleotides on high-performance ion exchangers of small pore size has been studied[2–5]. We have investigated the separation of double-stranded DNA restriction fragments on commercially available high-performance ion exchangers of large pore size and the results are presented in this paper.

EXPERIMENTAL

Double-stranded DNA fragments were prepared by cleaving plasmid pBR322 DNA with restriction endonuclease HaeIII (a gift from A. Fujiyama). The method of preparation has been described elsewhere[7]. This HaeIII-cleaved pBR322 DNA contains 22 fragments of 7 (14%), 11 (45%), 18 (50%), 21 (33%), 51 (29%), 57 (39%), 64 (33%), 80 (41%), 89 (39%), 104 (36%), 123 (44%), 124 (35%), 184 (41%), 192 (53%), 213 (39%), 234 (41%), 267 (49%), 434 (42%), 458 (57%), 504 (47%), 540 (44%) and 587 (57%) base pairs[8]; the numbers in parentheses are the nucleotide compositions (A–T content).

Ion-exchange chromatographic measurements were carried out at 25°C with a Model HLC-803C high-speed liquid chromatograph equipped with a Model GE-2 gradient generator and Model UV-8 variable-wavelength UV detector (Toyo Soda, Tokyo, Japan). The detector was operated at 260 nm. Two commercially available weak anion-exchange columns (150 × 6 mm I.D.) of TSK-GEL IEX-545 DEAE and IEX-645 DEAE (Toyo Soda) were used. IEX-645 DEAE is a silica-based support of particle diameter 10 μm and pore diameter 250 Å[9]. IEX-645 DEAE is a hydrophilic polymer-based support of particle diameter 10 μm and pore diameter 1000 Å[10].

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HaeIII-cleaved pBR322 DNA of 40 μg was commonly separated with a 180-min linear gradient of sodium chloride from 0.3 to 0.45 *M* in 0.01 *M* Tris–HCl buffer (pH 7.4) containing 0.1 m*M* EDTA at a flow-rate of 1 ml/min. In the study of the operational variables, the gradient time, pH of the eluent or flow-rate was varied.

Polyacrylamide gel electrophoresis of DNA fragments was carried out with 5% or 20% polyacrylamide gel (acrylamide: N,N′-methylenebisacrylamide = 29:1) in 50 m*M* Tris–borate buffer (pH 8.3) containing 1 m*M* EDTA. After electrophoresis, the gel was stained with 0.5 μg/ml ethidium bromide and observed under UV light around 380 nm.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of HaeIII-cleaved pBR322 DNA obtained on IEX-545 DEAE and IEX-645 DEAE. All peaks separated on IEX-545 DEAE were collected and examined by polyacrylamide gel electrophoresis. The DNA fragments identified in each peak are listed in Table I. Fragments of 11–21 base pairs were identified with 20% polyacrylamide gel and fragments larger than 51 base pairs were identified with 5% polyacrylamide gel. A fragment of 7 base pairs was not confirmed. The components in peaks k and l could not be distinguished in the electrophoresis, although they were estimated to be either 123 or 124 base-pair fragment. Therefore, they were further cleaved with restriction endonuclease Eco RV (Boehringer, Mannheim, G.F.R.), which has a cleavage site only in the 123 base-pair fragment[8], and electrophoresed again. By this method, the components of peaks k and l were assigned as in Table I. Peak n contained three fragments of 192, 213 and 234 base pairs and peak p contained five fragments of 434, 458, 504, 540 and 587 base pairs. All other peaks contained only single fragments.

Fig. 1 and Table I indicate that the fragments were eluted in order of increasing chain length and that almost baseline separations were attained for the fragments

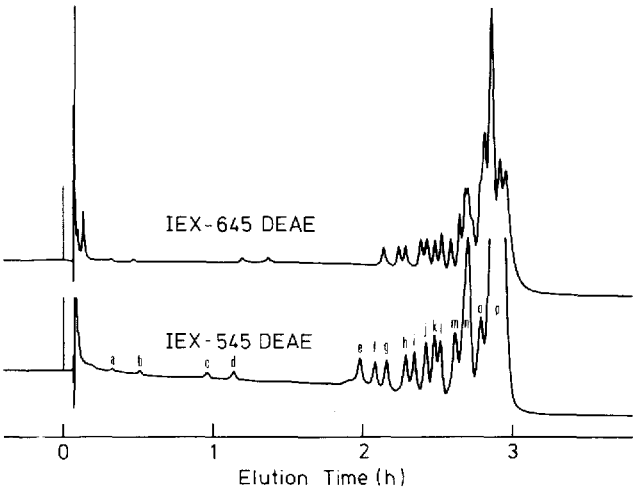

Fig. 1. Chromatograms of HaeIII-cleaved pBR322 DNA obtained on IEX-545 DEAE and IEX-645 DEAE with a 180-min linear gradient of sodium chloride from 0.3 to 0.45 *M* in 0.01 *M* Tris–HCl buffer (pH 7.4) containing 0.1 m*M* EDTA at a flow-rate of 1 ml/min.

TABLE I

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

Peak	*Chain length of component (base pairs)*	*Peak*	*Chain length of component (base pairs)*
a	–	i	89
b	11	j	104
c	18	k	124
d	21	l	123
e	51	m	184
f	57	n	192, 213, 234
g	64	o	267
h	80	p	434, 458, 504, 540, 587

smaller than *ca.* 100 base pairs when they differ in chain length by more than 10%. For larger fragments, however, inferior separation efficiency was observed. Fig. 1 also suggests that IEX-545 DEAE is superior for DNA fragments smaller than *ca.* 100 base pairs and IEX-645 DEAE is superior for DNA fragments larger than *ca.* 200 base pairs. For DNA fragments of 100–200 base pairs, IEX-545 DEAE and IEX-645 DEAE seem to be comparable. This probably resulted from the differences in pore size and base material of the two ion exchangers. Chain length is plotted against elution time in Fig. 2. The average chain length of the three fragments present was used for peak n. Fig. 2 suggests that DNA fragments were eluted mainly on the basis of chain length. Therefore, it may be possible to determine the chain length of unknown DNA fragments with the high-performance ion-exchange chromatographic system employed here.

Fig. 3 shows chromatograms of HaeIII-cleaved pBR322 DNA obtained on IEX-545 DEAE at pH 7.4 and 8.5. By increasing the pH from 7.4 to 8.5, DNA fragments were eluted earlier and the separation of DNA fragments larger than 51

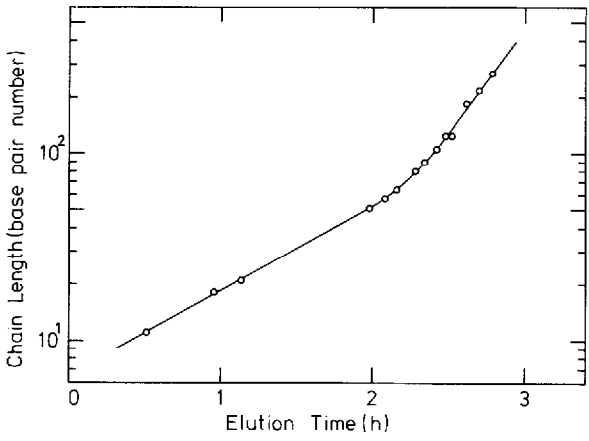

Fig. 2. Relationship between chain length and elution time in ion-exchange chromatography of double-stranded DNA fragments on IEX-545 DEAE.

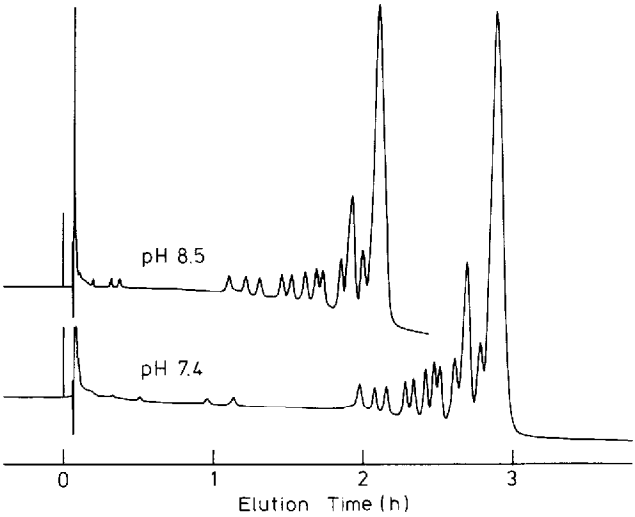

Fig. 3. Chromatograms of HaeIII-cleaved pBR322 DNA obtained on IEX-545 DEAE under the same conditions as in Fig. 1 except two buffers of pH 7.4 and 8.5 were employed.

base pairs was slightly improved. In order to separate DNA fragments smaller than 21 base pairs at pH 8.5, however, the ionic strength of initial buffer must be decreased.

Fig. 4 shows the results of separation at a flow-rate of 0.5 ml/min. By decreasing the flow-rate from 1 to 0.5 ml/min, the elution of DNA fragments was delayed and even smaller fragments (except very small fragments) were delayed more. As a result, DNA fragments larger than 21 base pairs were eluted more closely and the resolution became inferior. However, DNA fragments smaller than 18 base pairs were eluted with better separation.

Fig. 5 shows the results of separation obtained with a gradient time of 90 min.

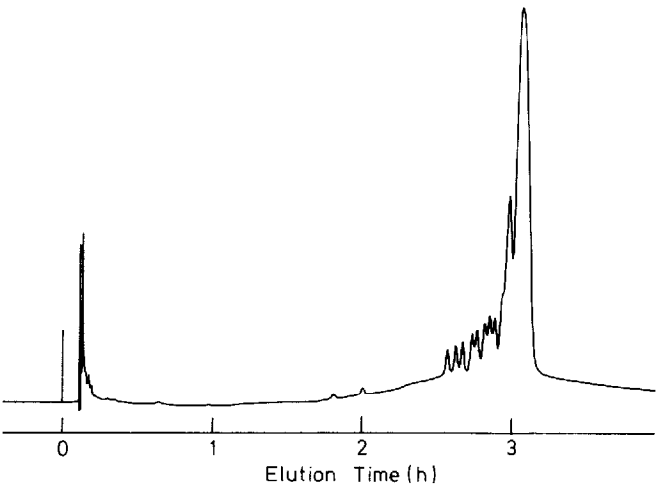

Fig. 4. Chromatogram of HaeIII-cleaved pBR322 DNA obtained on IEX-545 DEAE under the same conditions as in Fig. 1 except the flow-rate was 0.5 ml/min.

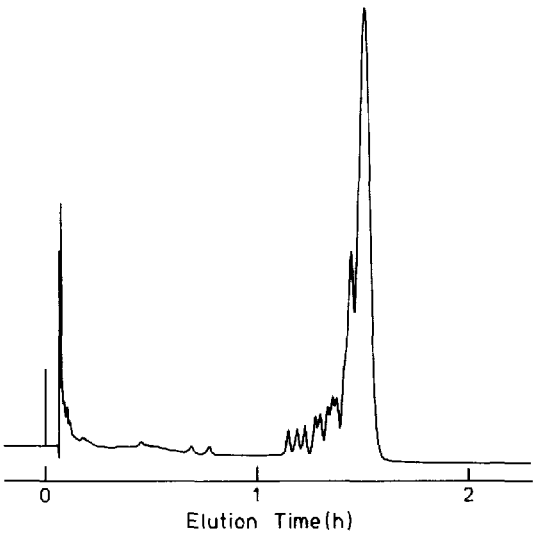

Fig. 5. Chromatogram of HaeIII-cleaved pBR322 DNA obtained on IEX-545 DEAE under the same conditions as in Fig. 1 except the gradient time was 90 min.

On decreasing the gradient time, DNA fragments were eluted earlier and the resolution decreased. The effect of gradient time was more pronounced for larger fragments.

As demonstrated above, high-performance ion-exchange chromatography can be employed as an efficient method for separating DNA restriction fragments according to chain length. However, some other factors such as nucleotide composition and sequence seemed slightly to affect the elution of DNA fragments. For example, fragments of 123, 192 and 267 base pairs, which comparatively high A–T contents, seem to be eluted slightly later than their chain lengths would indicate, as can be seen from Fig. 1. Further, it is necessary to investigate the chromatographic conditions in more detail in order to improve the separations, especially for DNA fragments larger than 100 base pairs.

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