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Note

Separation of DNA restriction fragments by high-performance ion-exchange chromatography on a non-porous ion exchanger

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Separation of DNA restriction fragments is often necessary in the field of molecular biology and gene technology. Agarose or polyacrylamide gel electrophoresis has been mainly employed for this purpose. Although conventional and high-performance liquid chromatography was also examined, its resolution was not satisfactory in most cases, particularly for large DNA fragments[1].

Recently, we demonstrated that proteins and oligonucleotides can be separated rapidly with very high resolution by ion-exchange chromatography on a non-porous anion exchanger[2,3]. We have now tested the usefulness of ion-exchange chromatography on the same support for the separation of DNA restriction fragments.

EXPERIMENTAL

Chromatographic measurements were performed with a system consisting of a double plunger pump, Model CCPM, and a variable-wavelength UV detector, Model UV-8000, operated at 260 nm (Tosoh, Tokyo, Japan). The column was TSKgel DEAE-NPR (35 mm × 4.6 mm I.D.) (Tosoh) packed with non-porous spherical hydrophilic resin particles of 2.5 μm diameter whose surfaces are chemically bonded with diethylaminoethyl groups[2]. DNA fragments were separated by gradient elution of sodium chloride in 20 m*M* Tris–HCl buffer (pH 9.0). All eluents were filtered through a 0.22-μm membrane filter.

A pBR322 DNA-Hae III digest (Sigma, St. Louis, MO, U.S.A.) and a λ DNA-Hind III digest (Pharmacia, Uppsala, Sweden) were used as model samples of small and large DNA restriction fragments. pBR322 DNA-Hae III digest 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; the numbers in parentheses are the nucleotide compositions (A–T content). λ DNA-Hind III digest contains eight fragments of 125, 564, 2027, 2322, 4361, 6557, 9416 and 23 130 base pairs. A 1 kb DNA ladder from Bethesda Research Labs. (Gaithersburg, MD, U.S.A.) was also used

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although some components are not restriction fragments. It contains 23 fragments with a wide range of chain lengths: 75, 142, 154, 200, 220, 298, 344, 394, 506, 516, 1018, 1635, 2036, 3054, 4072, 5090, 6108, 7126, 8144, 9162, 10 180, 11 198 and 12 216 base pairs. These samples were heated at 65°C for 5 min and then cooled quickly in ice–water before injection.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of pBR322 DNA-Hae III digest. Peaks were estimated as indicated in the figure by considering that DNA fragments are mainly eluted in order of increasing chain length; fragments having high A–T contents are eluted slightly later than expected from their chain lengths and the peak areas are approximately proportional to the chain lengths[4]. (Fragments of 7 and 11 base pairs were not assigned although two of several peaks appearing before 2 min are supposed to correspond to them.) This assignment is considered to be correct by comparison between this result and one reported previously for the separation of the same sample on a porous ion exchanger, where peaks were assigned by polyacrylamide gel electrophoresis[5]. Therefore, small DNA fragments (less than 600 base pairs) can be separated almost completely when they differ in chain length by 5–10%.

Fig. 2 shows a chromatogram of λ DNA-Hind III digest. Five large peaks were collected and examined by agarose gel electrophoresis. The first peak contained two fragments of 2027 and 2322 base pairs. Another four peaks contained only single fragments of 4361, 6557, 9416 and 23 130 base pairs, respectively. This result suggests that ion-exchange chromatography on a non-porous ion exchanger is also very effective even for large DNA fragments over 1000 base pairs. It is possible to achieve almost baseline separations in a very short time, *e.g.*, 5 min, for large fragments differing in chain length by more than 50%. It is also possible to improve the separation to some extent by employing a flow-rate lower than 1.0 ml/min, as explained later, although the separation time becomes slightly longer.

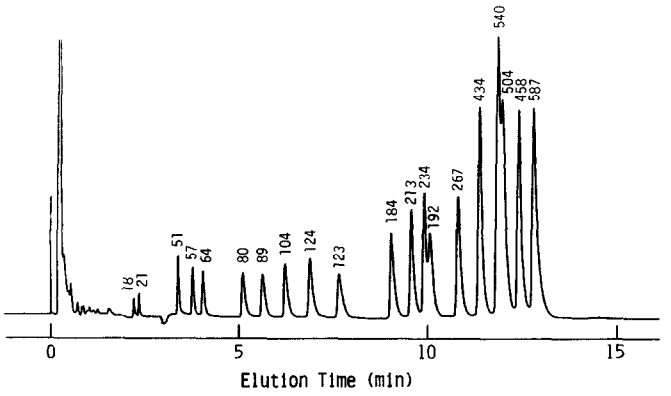

Fig. 1. Chromatogram of pBR322 DNA-Hae III digest (4.8 μg in 8 μl). The separation was performed on a TSKgel DEAE-NPR column with a 0.1-min linear gradient from 0.25 to 0.45 *M* sodium chloride followed by a 2.9-min linear gradient from 0.45 to 0.5 *M* and a 57-min linear gradient from 0.5 to 1.0 *M* in 20 m*M* Tris–HCl buffer (pH 9.0) at a flow-rate of 1.5 ml/min and 25°C. The numbers on the peaks are the estimated chain lengths of the DNA fragments in base pairs.

Hecker *et al.*[6] and Westman *et al.*[5] recently reported separations of DNA restriction fragments. They employed porous anion exchangers having large pores and obtained good separations. However, their applications were limited to rather small fragments, mostly smaller than 1000 base pairs in chain length, and took much langer times (1–8 h with some exceptions) than in Figs. 1 and 2. Stowers *et al.*[7] and Merion *et al.*[8] recently reported quite nice separations of a wide range of nucleic acids including large DNA restriction fragments by high-performance ion-exchange chromatography. The resolutions attained by them and those here seem to be equivalent, while the separation times in Figs. 1 and 2 are shorter.

The recovery of DNA restriction fragments from the column was examined for the separations in Figs. 1 and 2. The recovery was estimated from the areas of the peaks eluted. As controls, we used peak areas observed when the column was replaced with an empty 1 mm I.D. stainless-steel tube of 1 ml total inner volume. Both samples were recovered in high yield, more than 85%.

The effect of some operational variables was studied. In the separation of small fragments with constant gradient time, the resolution increased with increasing flow-rate up to around 1.0 ml/min and then decreased with further increase in the flow-rate. On the other hand, the flow-rate required to obtain the highest resolution was lower than 1.0 ml/min in the separation of large fragments, although the flow-rate dependence of the resolution was not so significant. It seemed to be 0.5–0.8 ml/min for 1000–5000 base pair fragments and 0.3–0.5 ml/min for 5000–20000 base pair fragments. Although the resolution continuously increases with decreasing gradient

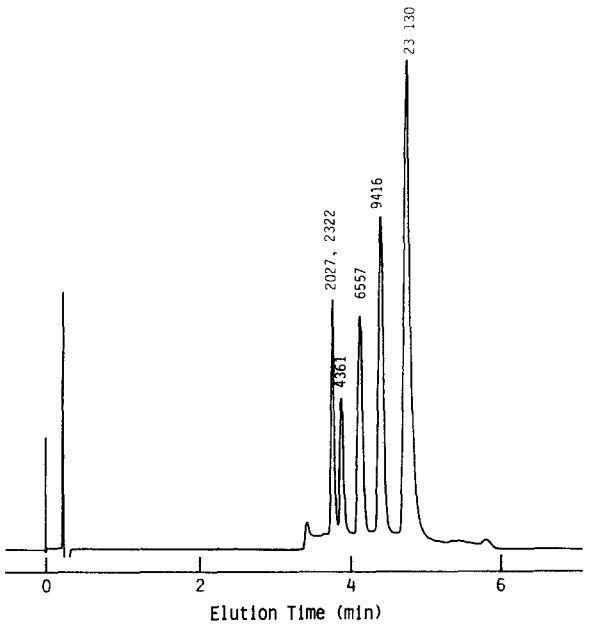

Fig. 2. Chromatogram of λ DNA-Hind III digest (2 μg in 4 μl). The separation was performed on a TSKgel DEAE-NPR column with a 10-min linear gradient from 0.5 to 1.0 *M* sodium chloride in 20 m*M* Tris–HCl buffer (pH 9.0) at a flow-rate of 1.0 ml/min and 25°C. Numbers are chain lengths of DNA fragments in base pairs identified by agarose gel electrophoresis.

steepness in general, there existed a certain gradient steepness which provided the highest resolution. This depended on the size of the fragment. It was 10–20 m*M* NaCl/min for fragments less than 1000 base pairs, 20–30 m*M* NaCl/min for 1000–5000 base pair fragments and 30–50 m*M* NaCl/min for 5000–20 000 base pair fragments. Accordingly, gradients shallower than these values should not be employed because there is no advantage. They result in not only lower resolution but also longer separation times and greater dilution of the sample. Steeper gradients should be selected of course when more rapid separations are required. The separation in Fig. 1 was carried out with a rather complicated gradient constructed with three linear portions for speed and yet satisfactory resolution. The column length had little influence on the resolution, particularly in the separation of large fragments. Slightly better separations were achieved with longer columns. Therefore, separations can be improved by using two or three columns connected in series, as exemplified in Fig. 3. The resolution was almost independent of temperature in the range of 25–65°C, while the elution of DNA fragments was slightly delayed with increasing temperature. The maximum sample load in order to obtain the highest resolution was rather low, as anticipated. In the separation of λ DNA-Hind III digest the resolution was almost constant at sample loads up to 10 μg, and then gradually decreased with further increase in the sample load.

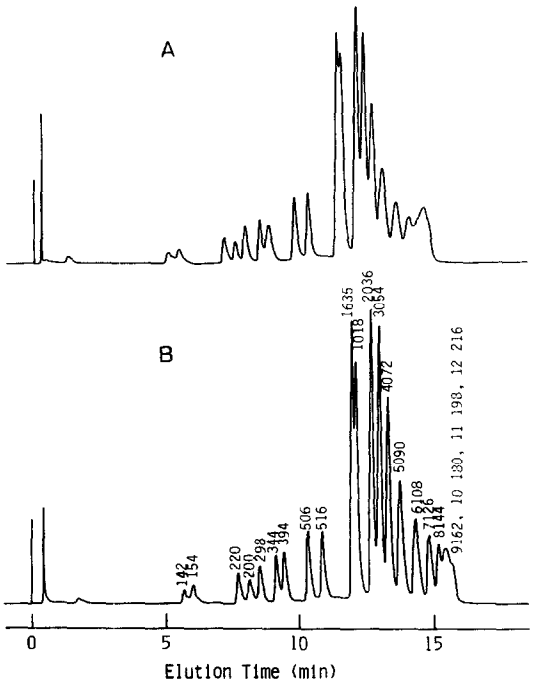

Fig. 3. Chromatograms of a 1 kb DNA ladder (6.3 μg in 6 μl). The separations were performed on one (A) and two (B) TSKgel DEAE-NPR columns with a 60-min linear gradient from 0.5 to 1.0 *M* sodium chloride in 20 m*M* Tris–HCl buffer (pH 9.0) at a flow-rate of 1.0 ml/min and 25°C. Numbers are the chain lengths of the DNA fragments in base pairs, estimated by comparing this result with one obtained by Merion *et al.*[8], who separated the same sample and identified peaks by agarose gel electrophoresis of collected fractions.

As demonstrated, ion-exchange chromatography on the non-porous anion exchanger, TSKgel DEAE-NPR, is very useful for the separation of DNA restriction fragments. A wide range of DNA fragments, from small to very large ones, can be separated in 5–15 min with high resolution. The recovery of DNA fragments was also high (>85%). Although gel electrophoresis is the most common technique used to separate DNA fragments owing to its high resolution, it has some problems in quantitative measurements of the components, scaling up, recovery of the separated components, etc. On the other hand, ion-exchange chromatography does not have such problems. Accordingly, ion-exchange chromatography on TSKgel DEAE-NPR should be a good alternative to gel electrophoresis for the analysis and purification of DNA fragments.

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