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Note

Rapid purification of DNA fragments by high-performance size-exclusion chromatography

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The separation of large DNA fragments at a preparative scale remains a limiting step in molecular biology experiments. The most widely used method, the preparative agarose or acrylamide gel electrophoresis, is laborious and gives poor yields, especially in the preparation of large [> 2 kilo base pairs (kbp)] DNA fragments. Moreover, such preparations are contaminated by soluble agarose or acrylamide, which are known to be powerful inhibitors of DNA-processing enzymes. Therefore, further time-consuming purification steps, such as phenol extraction and/or ion-exchange chromatography, are required to remove the contaminants.

This work describes an alternative method based on gel chromatography. This technique allows fast separation (150 min) of DNA fragments (from 75 to 10 000 base pairs) with high yields of biologically active DNA after a single ethanol precipitation step.

EXPERIMENTAL

DNA fragments used in this study were produced by enzymatic cleavage of plasmids pBR322, pB1 (a derivative of pBR322 carrying a 10-kbp *Escherichia coli* chromosomal insert) and pME305 [1—3]. Plasmid DNA was purified as previously described [4]. Restriction enzymes, *Eco*RI, *Cla*I, *Bam*HI, *Ava*I, *Hin*dIII and *Sma*I, were purchased from Appligene (Strasbourg, France) and

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used as recommended by the supplier. A commercially available Spherogel-TSK 6000PW column (300 mm × 7.5 mm I.D.; particle size 10 μm), provided by Beckman-France, was used for DNA fragment separation. Gel filtration was performed at room temperature using a Beckman 112 solvent delivery pump and a Beckman Model 340 injection valve. Elution profiles were monitored by measuring absorbance at 254 nm using a Beckman 160 detector. Fractions of 2 min were collected in microtitration units (Nuclon Microwell, Nunc, Roskilde, Denmark) with a Gilson Model 201 fraction collector.

DNA eluted from the column was recovered by precipitation after addition of 2.2 vols. of ethanol; pellets were washed with ethanol and redissolved in water. Purity was estimated by electrophoresis on 1% (w/v) agarose gel.

RESULTS AND DISCUSSION

The Spherogel-TSK 6000PW column was equilibrated with 50 ml of 50 m*M* Tris—HCl buffer (pH 7.6) containing 1 m*M* sodium EDTA to suppress nuclease activity and 0.3 *M* sodium chloride to eliminate DNA—matrix interactions.

In order to determine optimal conditions, several analyses were performed using an equimolar mixture of three DNA fragments (2961, 1050 and 352 base pairs) resulting from enzymatic cleavage of 10 μg of pBR322 plasmid DNA, catalysed by *Cla*I, *Bam*HI and *Ava*I. Maximal resolution was achieved at a flow-rate of 80 μl/min. Under these conditions, the void volume was 5.0 ml, as determined by the elution volume of a 46-kbp linearized pME305 plasmid DNA. The total volume, corresponding to deoxyadenosine triphosphate (dATP) elution, was 11.7 ml. Thus, the total analysis time did not exceed 150 min.

The calibration curve shown in Fig. 1 was obtained with several DNA frag-

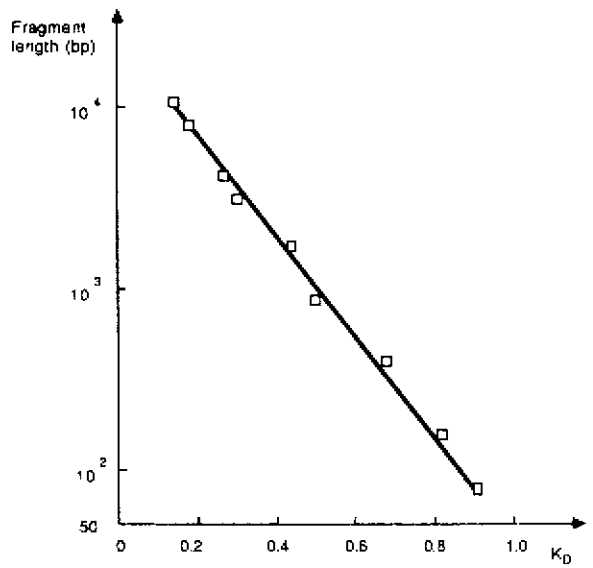

Fig. 1. Calibration curve for DNA fragments analysed using a TSK 6000PW column. Logarithm of DNA fragment size (base pairs; bp) is plotted versus the distribution coefficient (K_D).

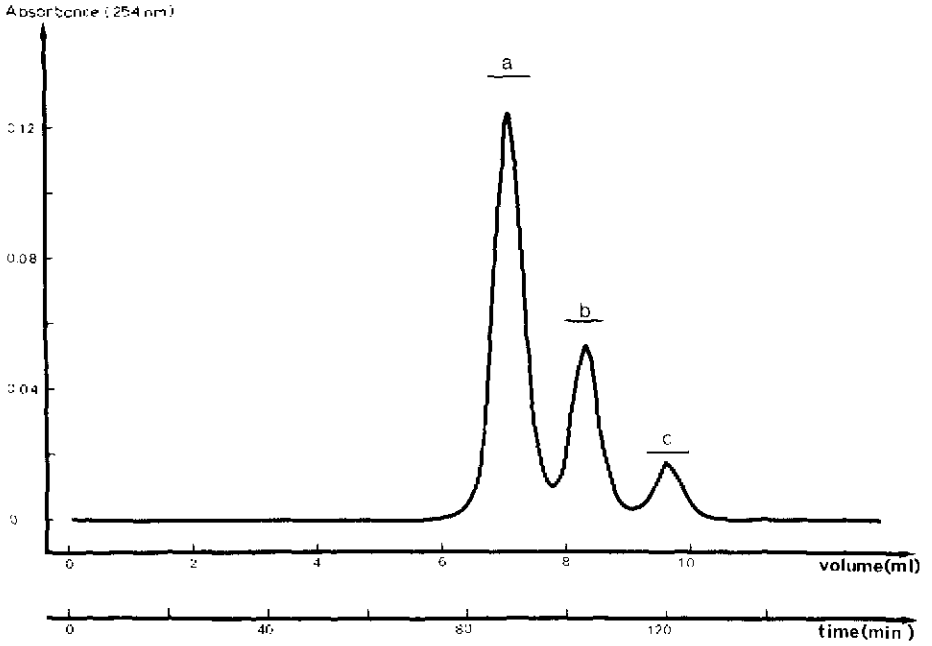

Fig. 2. Chromatogram of pBR322 fragments (2961, 1050 and 352 base pairs). Flow-rate 0.08 ml/min. Pooled fractions (a, b and c) analysed by agarose gel electrophoresis are indicated by horizontal bars.

ments. The linear portion of the calibration curve indicates that this column is suitable for the separation of DNA fragments ranging from 75 to approximately 10 000 base pairs.

The chromatogram of the three DNA fragments resulting from the enzymatic cleavage of pBR322 shows nearly baseline separation between the first two fragments (2961 and 1050 base pairs; Fig. 2). Purity of the eluted material was confirmed by the agarose gel analysis of fraction pools corresponding to the three peaks (Fig. 3).

In a manner analogous to that described for proteins [5], the resolving power of a gel chromatography column can be obtained by calculating the minimal difference in base pairs that the column can resolve. In ideal size-exclusion chromatography, the elution volume of a solute is expressed by:

$$V = V_0 + K_D V_p \tag{1}$$

where V_0 is void volume, V_p is pore volume and K_D is the distribution coefficient. The resolution R_s between two peaks is given by:

$$R_s = 2 \frac{(V_2 - V_1)}{(W_1 + W_2)} \tag{2}$$

where V_1 is the elution volume of the first eluted species, and V_2 that of the second eluted species; W_1 and W_2 are the peak widths of compounds 1 and 2, respectively. The theoretical plate count for an observed peak is given by:

$$N_i = 16 (V_i/W_i)^2 \tag{3}$$

For two closely eluting peaks having a resolution $R_s = 1$, one can assume that:

$$W_1 = W_2 = W \tag{4}$$

The difference in elution volume between the two species 1 and 2 is equal to:

$$V_2 - V_1 = V_p\ (K_{D_2} - K_{D_1}) \tag{5}$$

Combining eqns. 2 and 3 and assuming $R_s = 1$, one obtains:

$$V_2 - V_1 = 4\ \frac{V_1}{\sqrt{N_1}} \tag{6}$$

Thus:

$$K_{D_2} - K_{D_1} = 4\ \frac{V_1}{V_p\sqrt{N_1}} \tag{7}$$

In the linear portion of the size-exclusion calibration curve:

$$\ln B = a - b\ K_D \tag{8}$$

where B is the number of base pairs of the DNA species. The value of the slope b can be obtained experimentally. Thus,

$$\ln (B_1/B_2) = \ln B_{min} = b\ (K_{D_2} - K_{D_1}) \tag{9}$$

where B_{min} is the minimum ratio of fragment size that the column can resolve. The combination of eqns. 7 and 9 gives:

$$\ln B_{min} = 4\ \frac{b\ V_1}{V_p\sqrt{N_1}} \tag{10}$$

Using the TSK 6000PW column, the following values were obtained: V_p = 6.7 ml and b = 6.1; $N_1 = 1000 \pm 100$ for V_1 = 7.1 ml. Thus, B_{min} equals 2.265. As the species corresponding to V_1 is 2961 base pair long, the next fragment that can be separated with R_s = 1 should have approximately 2961/2.265 = 1307 base pairs.

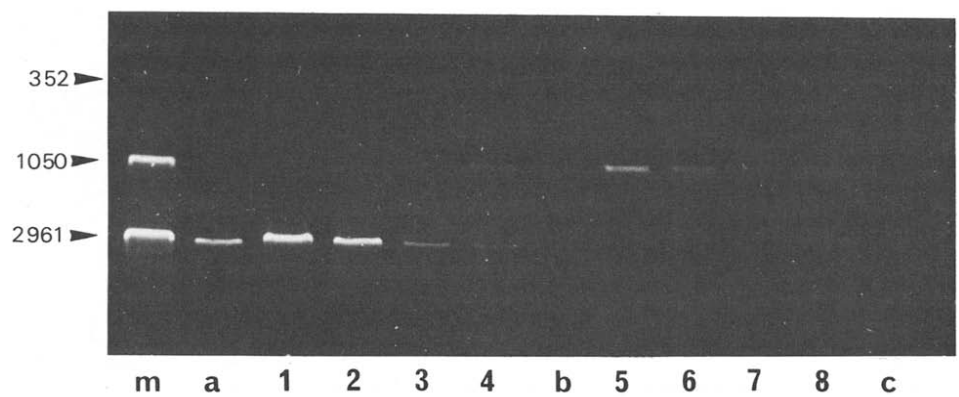

Fig. 3. Agarose gel electrophoresis pattern of the three pools and intermediate fractions obtained in the experiment described in Fig. 2. From left to right: lane m, control unfractionated mixture; lane a, pool a (one tenth); lanes 1—4, the following four fractions; lane b, pool b (one third); lanes 5—8, the following four fractions; lane c, pool c.

This evaluation of the resolving power using eqn. 10 indicates that species differing in length by a factor of 2 could be separated easily under optimal conditions by coupling two columns of this type in series. Similar results have been reported by other workers with four columns operated at high flow-rates [6].

These properties are valid for quantities of DNA that do not exceed 12 μg per fragment. Nevertheless, larger sample loads (up to 50 μg) can be analysed with little loss of resolution, which enables the preparation of large amounts of cloning vectors. For example, asymmetrical cloning in pUC vectors [7] usually requires a dephosphorylation step to avoid competition between the insertion of the small pUC fragment and that of the fragment of interest. Using this column, it is possible to prepare large amounts of the linearized vector free of the contaminating linker fragment. Since this procedure eliminates the dephosphorylation step, the overall efficiency of the cloning experiment is much higher. In addition, chromosomal DNA size-fractionation, often required for construction of genomic or c-DNA (complementary DNA synthesized from messenger RNA templates) libraries, can be achieved readily using this column. The described system has been routinely used for more than three months in our laboratory without alteration of the support.

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