SIZING OF DNA FRAGMENTS BY PREPARATIVE, BENZOYLATED DEAE-CELLULOSE CHROMATOGRAPHY

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1. Introduction

Nucleic acids may be fractionated by chromatography on benzoylated DEAE-cellulose (BD-cellulose) and benzoylated–naphthoylated DEAE-cellulose (BND-cellulose) [1]. Native DNA is eluted from BND-cellulose by a 1 M salt solution, while DNA containing single-stranded regions is selectively eluted with caffeine [2–4]. In [5] fractionation of nucleic acids by BND-cellulose was suggested to be related to the proportion of single-stranded regions in each nucleic acid. Similar relationships have been reported between extent of single-stranded regions in DNA and concentration of caffeine necessary for elution from BND-cellulose. Anomalous elution characteristics were exhibited by single-stranded DNA of chainlength >2000 nucleotides [6,7].

BD-cellulose can be used to separate native DNA from DNA containing single-stranded regions using procedures similar to those employed with BND-cellulose [8–10]. This report concerns a direct relationship between length of single-stranded DNA and concentration of caffeine required for elution from BD-cellulose that is fundamentally different from that described for BND-cellulose. The relationship is continuous for DNA in the range from 10^2–5×10^4 nucleotides and hence provides a quantitative basis for fractionation of DNA by BD-cellulose chromatography.

2. Materials and methods

[^{3}H]DNA M_r-markers (bacteriophage λ, *Hin*dIII digest of bacteriophage λ, and *Hae*III digest of bacteriophage φX174; New England Nuclear, Boston MA) and [^{32}P]DNA markers (the 206 and 1670 nucleotide fragments yielded by successive cleavage of plasmid pBR-322 with *Hin*dIII and *Hae*II; generously supplied by Dr P. Nagley, Monash University VIC) were heated to 100°C for 15 min, rapidly cooled in ice-water, then chromatographed on BD-cellulose columns (Boehringer-Mannheim) prepared as in [11]. Following application of the DNA samples, columns were washed with 10 ml 1 M NaCl in 10 mM Tris–HCl–1 mM EDTA buffer followed by caffeine in a linear concentration gradient generated by a programmable pump (Dialagrad Model 382, ISCO Instruments, Nebraska). Caffeine solutions were pre-warmed, the flow rate was 1 ml/min and radioactivity in collected fractions (2 ml) was determined. The size of all DNA M_r-markers was confirmed by gel electrophoresis.

3. Results

Resolution of individual components within DNA marker mixtures by BD-cellulose chromatography was critically dependent upon the slope of the caffeine concentration gradient. An example of resolution achieved is shown in fig.1. Appropriate caffeine gradients for the various DNA markers were thus determined empirically. Three separate gradients were required for complete resolution of all DNA preparations examined, lower M_r-markers requiring a shallower caffeine gradient for resolution than higher M_r-markers. Once gradients had been defined appropriate for particular M_r ranges, single M_r-markers were chromatographed separately under the same conditions (table 1).

The relationship between DNA size and caffeine concentration required for elution was analysed sepa-

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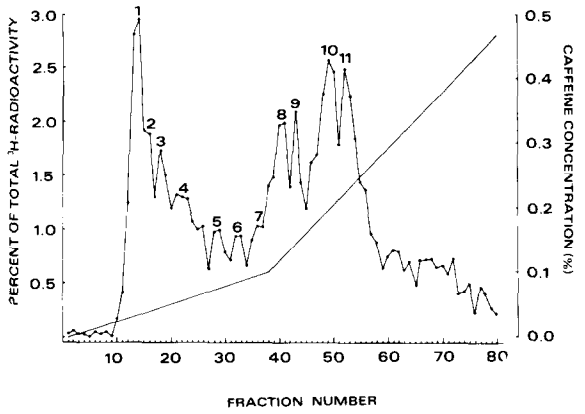

Fig.1. Resolution by BD-cellulose chromatography of the 11 restriction fragments generated by cleavage of bacteriophage ϕX174 with the restriction endonuclease *Hae*III (DNA preparations 1 and 3, table 1). The distribution of radioactivity (total cpm = 15 075) among the peaks of the elution profile parallels that obtained from a densitometer scan (supplied by New England Nuclear, Boston MA) of the electrophoretic profile (2% agarose) of these 11 fragments.

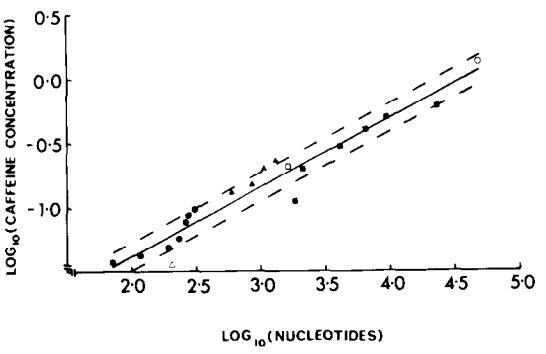

Fig.2. Linear regression of the double log transformation of the data obtained from DNA M_r-markers in the range 72–49 230 nucleotides (R = 0.969). Data from DNA preparations 1–6 as listed in table 1 are indicated by ●, △, ▲, □, ■ and ○, respectively. The solid line represents the mean regression and the broken lines indicate ±1 SD.

rately for the sets of DNA M_r-markers eluted using each of the 3 gradients. A linear regression of the double log transformation of the data was performed for each set of markers and the 3 regressions did not differ significantly in slope (F = 0.068; df = 2.15; $p > 0.05$). All data was therefore pooled and a combined regression obtained which was highly significant (fig.2). The combined relationship between DNA length in nucleotides (L) and eluting caffeine concentration (C) is given by $C = (3.410 \times 10^{-3}) \times L^{0.541}$ (R = 0.969; df = 18, $p < 0.0001$).

Table 1
Chromatographic conditions using BD-cellulose for analysis of single stranded DNA preparations of known M_r[a]

DNA markers		Caffeine gradient			Regression coefficient[b]
Preparation	Size (no. nucleotides)	Concentration range (%)	Total elution volume (ml)	Δ% Caffeine/ fraction	
1	72; 118; 194; 234; 271; 278; 310	0 –0.1	74	0.0027	0.90[c]
2	206	0 –0.1	74	0.0027	–
3	603; 872; 1078; 1353	0.1–0.8	166	0.0084	0.97[d]
4	1670	0.1–0.8	166	0.0084	–
5	1846; 2153; 4153; 6461; 9538; 23 230	0 –2.0	384	0.0104	0.94[c]
6	49 230	0 –2.0	384	0.0104	–

[a] M_r, relative molecular mass
[b] For the regression analysis of the double log transformation of the data relating DNA size to eluting caffeine concentration
[c] $p < 0.005$; [d] $p < 0.025$

4. Discussion

The relationship between DNA size and caffeine concentration required for elution from BD-cellulose evident from these present data differs markedly from those described for BND-cellulose. For low M_r DNA chromatographed on BND-cellulose, the eluting caffeine concentration is reported to be proportional to chainlength [6]. Attempts to define this relationship have been limited by quantitative differences between reports [7,12]. More importantly, as chainlength exceeds 1000–2000 nucleotides, DNA is recovered from BND-cellulose with decreasing caffeine concentrations [7]. In contrast, the linear relationship (fig.2) governing elution of DNA from BD-cellulose extends over a wide M_r-range. Confidence in the relationship encompassing available data is increased both by the high regression coefficient ($R = 0.969$) and by the lack of evidence of deviation from the relationship at high M_r-values. In the lower M_r-range, where direct comparison is possible, much lower concentrations of caffeine are required for elution of DNA from BD-cellulose than from BND-cellulose, a difference attributable to the tighter binding of nucleic acids by naphthoyl groups [1].

Lack of significant difference between slopes of the regressions of data obtained using 3 different caffeine gradients (table 1) indicates that the slope of the caffeine gradient has no effect on the relationship between DNA size and eluting caffeine concentration. For practical purposes, however, the slope of caffeine gradients is extremely important since use of inappropriate caffeine gradient results in very poor resolution or needless distribution of recovered DNA over a large number of collected fractions.

Binding of DNA to BD-cellulose may have been anticipated to depend not only on chainlength but also on base composition since in [1] purine nucleotides bound more tightly than pyrimidine nucleotides. As a parameter determining chromatographic behaviour on BD-cellulose, base composition is clearly of secondary importance to chainlength. Nonetheless, differences in base composition between DNA fragments of similar M_r may account for certain observations made here. These include the ability to differentiate between DNA fragments differing by only a few nucleotides (fig.1). In addition, during chromatography of some high M_r species, bifurcation within individual peaks was evident. This result, presumably due to separation of complementary strands is under further examination.

Gradient elution from BD-cellulose of DNA offers a means of determining M_r-value after which analysed samples may be recovered in aqueous solution. The consistent relationship between DNA chainlength and eluting caffeine concentration observed for BD-cellulose has obvious advantages over anomalous elution characteristics exhibited by BND-cellulose. Greatest advantage from BD-cellulose would appear to lie in circumstances requiring measurement and/or separation of DNA of high M_r-value, especially on a preparative scale.

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