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Effects of ultrasonics on the resolution of DNA in gel filtration

Gel filtration on cross-linked polyacrylamide and Dextran, well established as a method for separating proteins on a molecular weight basis, has found some application in nucleic acid studies, *e.g.* for the removal of phenol and tRNA from DNA[1,2]. However, sphere-condensed agarose, with its relatively low porosity and incompressible structure, is becoming increasingly popular as a molecular sieve for DNA and deoxyribonucleoprotein[3,4].

In a previous communication[5] much of a specimen of native thymic DNA was delayed during elution on a column of Sephadex G-200 dextran, but in the presence of a trace of pancreatic DNAase eluted mainly in the exclusion volume. That this could be due to protein-mediated association of DNA molecules seemed unlikely at such low DNAase concentrations, and the effect could not be reproduced by the addition of similar amounts of bovine serum albumin.

As reported here, ultrasonication has been used to prepare a range of molecular size groups of DNA, in the absence of exogenous protein. The broad elution profile of native DNA, reproduced on columns of dextran, polyacrylamide and agarose, is shown to be sharpened by the briefest periods of sonication, and this is discussed in terms of the high viscosity of DNA solutions.

Calf thymus DNA was prepared as previously described[6]. ^{14}C-labelled DNA was prepared from *Bacillus subtilis* substantially by the phenol method of KIRBY as modified by KELLY AND PRITCHARD[7]. Sephadex G-200 was purchased from Pharmacia (G.B.) Ltd. Bio-Rad Laboratories supplied Bio-Gel P-300 polyacrylamide and Bio-Gel A-50m (100–200 mesh) 2 % agarose gels.

Ultrasonication of DNA was performed in solutions containing thymic DNA at 950 μg/ml or *B. subtilis* DNA at 250 μg/ml[6]. The method of sucrose gradient centrifugation was as previously described[6], but with the following modifications. Using the 3 × 5 ml swing-out rotor, approximately 1 μg of bacterial DNA or 150 μg of thymic DNA, contained respectively in 0.1 ml and 0.25 ml of 10 m*M* potassium phosphate buffer (pH 7.2), were layered on 4.5 ml of sucrose gradient. Centrifugation was performed at 39,500 r.p.m. ($g_{max.}$ 171,300 × g; $g_{min.}$ 92,700 × g), for 144 min and 242 min, respectively. In the case of the bacterial DNA, gradient fractions were collected on circles of Whatman No. 1 filter paper and counted for carbon-14 in a liquid scintillation counter.

Gel filtration was performed routinely with 480 μg of thymic DNA or 30 μg of *B. subtilis* DNA contained in 1.2 ml of the column buffer, 20 m*M* potassium phosphate (pH 7.2), 0.5 *M* with respect to NaCl. The dimensions of the agarose column were 2.54 × 39 cm; those of the other gels were 2.54 × 34 cm. The columns were flowed upwards at 13.6 ml/h at 4°, effluent fractions of 3.25 ml being collected. $A_{260m\mu}$ values of fractions containing thymic DNA were read in a Unicam SP 800 recording spectrophotometer. ^{14}C levels were assayed after drying portions on filter paper. Gel filtrations were often repeated, and in all cases the degree of reproducibility was excellent.

Ultrasonication of thymic DNA for as little as 5 sec was sufficient to cause virtually complete exclusion by Sephadex G-200. Even after a 1 min pre-treatment, less

than 10 per cent of the DNA was small enough to be retarded during elution. Similar results were obtained using Bio-Gel polyacrylamide. In contrast, a much smaller load of *B. subtilis* [^{14}C]DNA, 30 μg, even in the native state was almost completely excluded by Sephadex G-200, and the effect of sonication for 5 sec or longer was simply to cause a gradually increasing degree of tailing during elution. It seemed consistent with the mean $s_{20,w}$ values of the native and sonicated specimens of these two DNA specimens, as shown in Table I, to ascribe the broad elution profile of the relatively high loads of native thymic DNA to the high viscosity of these solutions.

TABLE I

EFFECT OF SONICATION ON DNA SEDIMENTATION COEFFICIENTS

DNA	*Time of sonication*	*Mean $s_{20,w}$*	*90% of DNA recovered between*
Thymic	—	25.9 ± 0.38 S	4.5–52 S
	5 sec	14.5 ± 0.21 S	1.9–37.4 S
	15 sec	11.1 ± 0.14 S	2.0–23 S
	1 min	9.8 ± 0.13 S	1.5–20 S
	10 min	6.2 ± 0.10 S	0.4–13.2 S
B. subtilis	—	47.4 ± 0.29 S	6.3–62 S
	5 sec	13.5 ± 0.09 S	1.0–23.5 S
	15 sec	12.6 ± 0.09 S	1.0–21 S

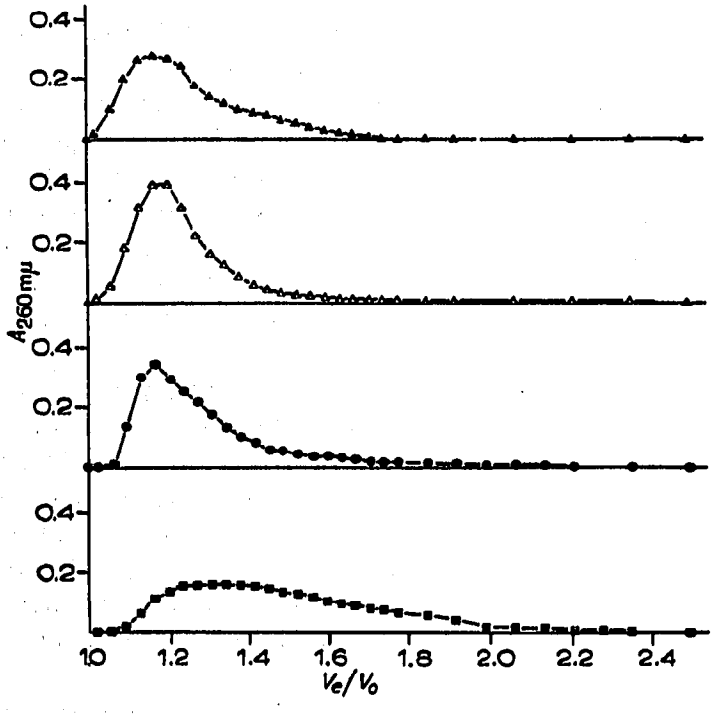

Fig. 1. Effect of sonication on elution of thymic DNA on Agarose A-50m. ▲—▲, native DNA; △—△, 5 sec sonicated DNA; ●—●, 15 sec sonicated DNA; ■—■, 1 min sonicated DNA.

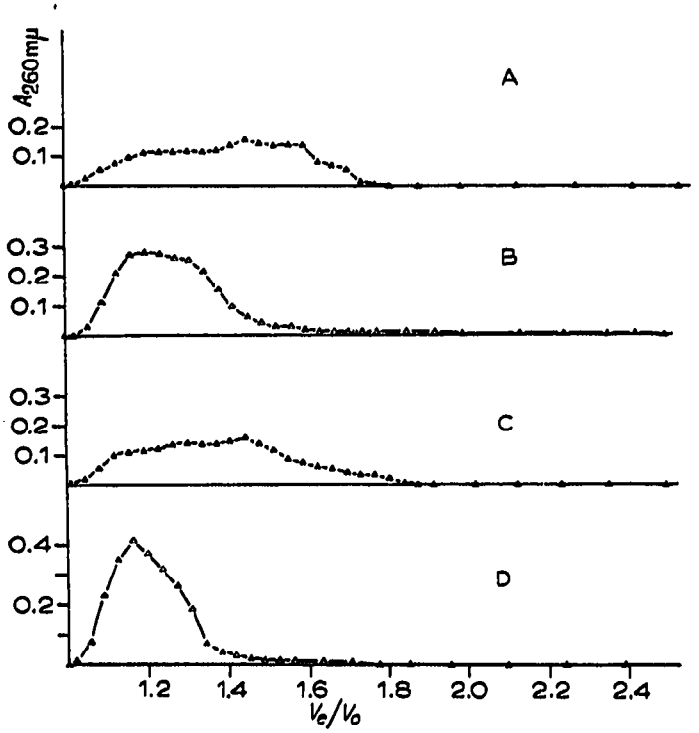

Fig. 2. Elution of thymic DNA on Agarose A-50m at extremes of ionic strength. A and B: elution in 20 m*M* potassium phosphate buffer (pH 7.2), 1 *M* with respect to NaCl. C and D: elution in 20 m*M* phosphate buffer alone. Symbols as in Fig. 1.

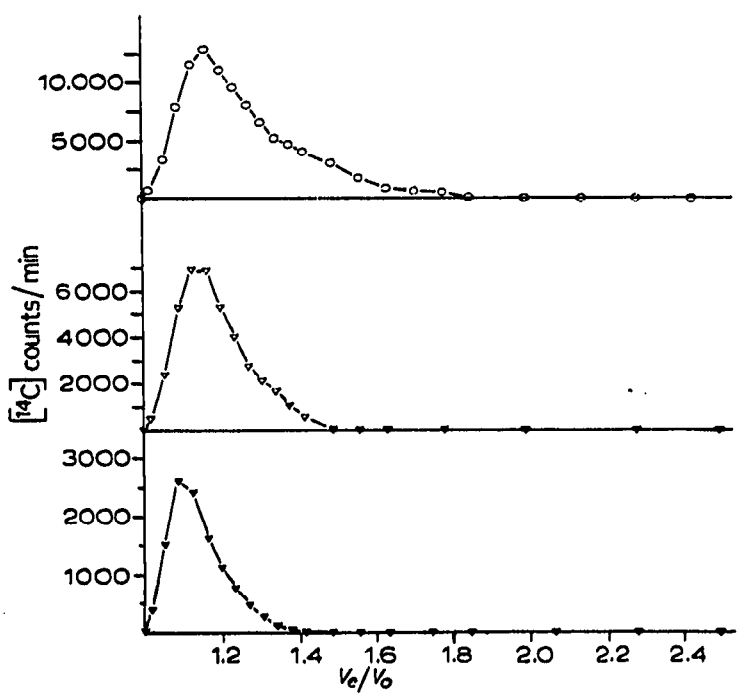

Fig. 3. Concentration dependence of elution of *B. subtilis* DNA on Agarose A-50m. DNA loads were: ○—○, 250 μg; ▽—▽, 100 μg; ▼—▼, 30 μg.

In extending the study to agarose, 480 μg of native and sonicated thymic DNA were run on an agarose column equilibrated in various buffers: the standard saline buffer; 20 m*M* potassium phosphate buffer (pH 7.2), 1 *M* with respect to NaCl; and saline-free 20 m*M* phosphate buffer. As may be seen in Figs. 1 and 2, at the two extremes of ionic strength the elution profile of the native specimen was greatly sharpened by limited sonication, yet in the standard saline buffer the profile of native DNA was little broader than that of the least sonicated specimen. The effect of ionic strength should find application in optimising the resolution of DNA gel filtration.

Because of the inferred effect of DNA load on the elution on Sephadex, several concentrations of bacterial DNA were run on the agarose column equilibrated in the standard buffer. As shown in Fig. 3, the proportion of DNA delayed by the gel increased with the DNA load. Even at 100 μg of [^{14}C]DNA, no peak sharpening was achieved by sonication.

These results may largely be explained by the high viscosity of solutions rich in native DNA. *Per se*, the concentration dependence of the elution of native *B. subtilis* DNA on agarose could implicate the involvement of DNA aggregates too large to pass through even the intersphere spaces, dissociating slowly as they are diluted. However, this hypothesis is difficult to reconcile with the sedimentation data, since the thymic DNA, when centrifuged at an overall concentration of the same order as that of the thymic DNA solutions routinely applied to the columns, sedimented rather more slowly than the 150 × lower concentration of bacterial DNA.

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