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The behavior of DNA on basic polyamino acid Kieselguhr columns

The fractionation of DNA on columns of poly-L-lysine supported on Kieselguhr (PLK columns) has been described by AYAD AND BLAMIRE[1], and HELLEINER[2]. Columns of poly-L-arginine (PAK) and poly-L-ornithine (POK) similarly supported, as well as PLK columns have been used by JARVIS *et al.*[3] to separate different kinds of RNA. In this paper the behavior of DNA of different molecular weights and base compositions on PAK and POK columns is compared with that on PLK columns.

Poly-L-arginine hydrochloride (mol. wt. 40,000–80,000) and poly-L-α-ornithine hydrobromide (mol. wt. 100,000), and DNA from *Clostridium perfringens* were obtained from Sigma Chemical Co., St. Louis, Mo. Other kinds of DNA were prepared in the laboratory according to MARMUR[4]. Radioactive *Escherichia coli* DNA was prepared from bacteria grown to the stationary phase in a Tris salts medium[5] containing ^{32}P as KH_2PO_4. *E. coli* DNA of different molecular weights was prepared by treating DNA in a Branson Sonifier for varying periods. The sedimentation constant was determined by means of boundary measurements in the analytical ultracentrifuge, and the molecular weights were estimated according to STUDIER[6].

Columns were prepared as described by AYAD AND BLAMIRE[1], with the substitution of other polyamino acids for poly-L-lysine as indicated. In each experiment, a mixture of 500 μg of some kind of non-radioactive DNA with a tracer amount of radioactive *E. coli* DNA was applied to the column as a solution in 0.4 *M* NaCl. Columns were eluted at room temperature with linear gradients of NaCl buffered with 0.02 *M* potassium phosphate buffer, pH 7.2. For PAK columns the gradient extended from 2.0 to 4.0 *M* NaCl; for POK columns the limits were 1.0 *M* and 3.0 *M* NaCl. Fractions of 12 ml were collected and analyzed for absorbance at 260 nm and radioactivity

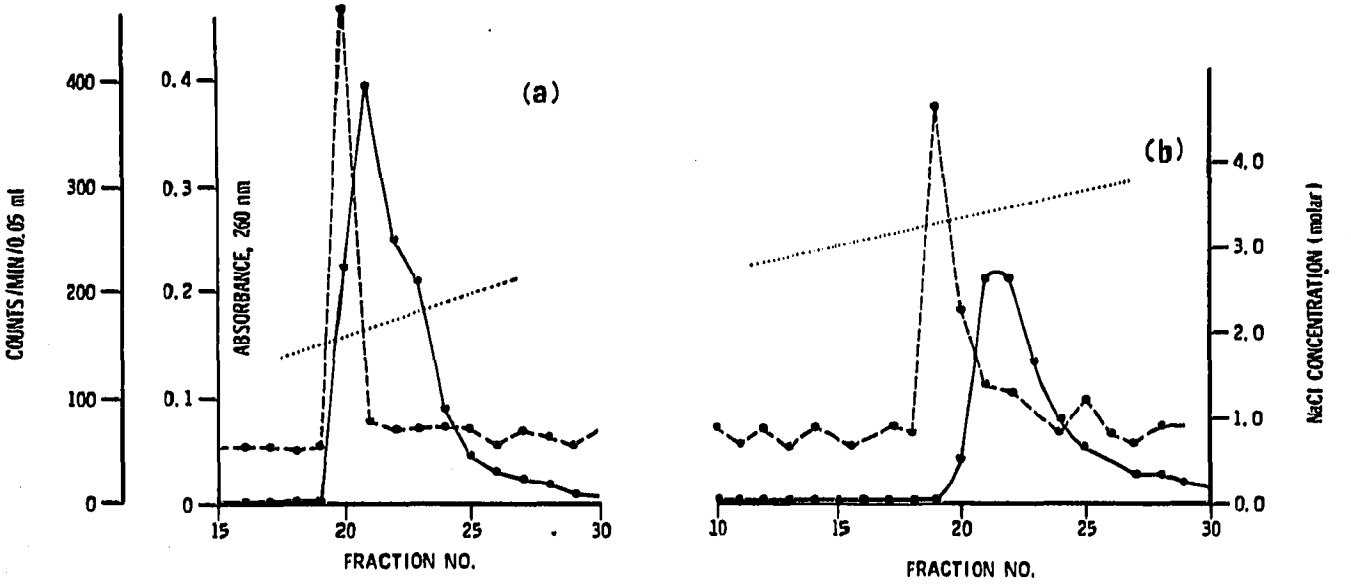

Fig. 1. Elution diagrams of bacterial DNAs from POK columns; (a) 600 μg of *Cl. perfringens* DNA + trace of radioactive *E. coli* DNA; (b) 600 μg of *M. lysodeikticus* DNA + trace of radioactive *E. coli* DNA. ———, Absorbance; — — —, radioactivity; ·····, NaCl concentration.

(by spotting samples on filter paper, drying the filters, and counting them, after addition of a PPO–POPOP scintillation fluid, in a scintillation counter).

Fig. 1 shows the elution of *Cl. perfringens* and *Micrococcus lysodeikticus* DNA from a POK column. The *M. lysodeikticus* DNA (about 71 mole per cent guanine + cytosine) precedes the *E. coli* DNA (about 50 mole per cent guanine + cytosine), while the *Cl. perfringens* DNA (about 30 mole per cent guanine + cytosine) follows the *E. coli* DNA. This behavior resembles that on PLK columns. The effect of molecular weight on the position of elution was similar to that seen on PLK columns[2], and also to that on PAK columns (Fig. 3).

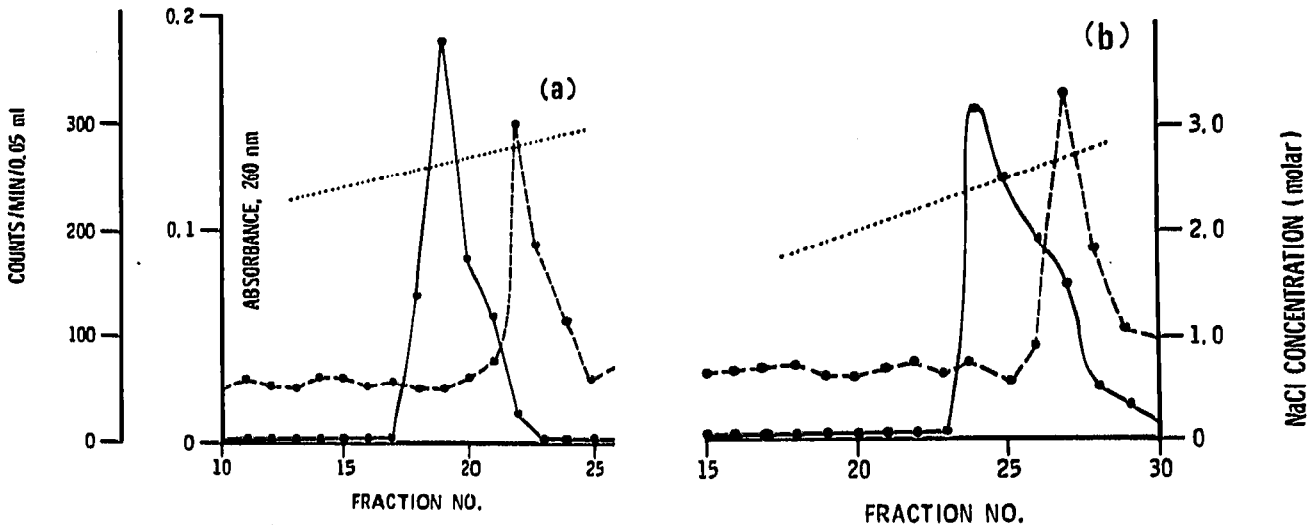

Fig. 2. Elution diagrams of bacterial DNAs from PAK columns; (a) 600 μg of *Cl. perfringens* DNA + trace of radioactive *E. coli* DNA; (b) 600 μg of *M. lysodeikticus* DNA + trace of radioactive *E. coli* DNA. ———, Absorbance; — — —, radioactivity; ·····, NaCl concentration.

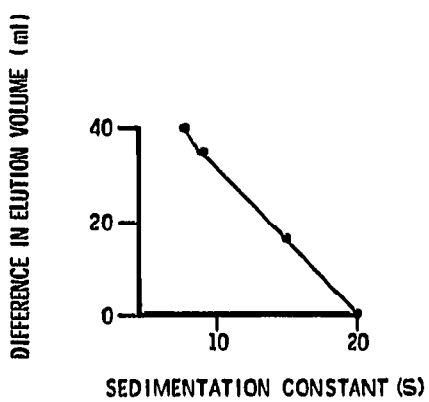

Fig. 3. Effect of sedimentation constant of DNA on elution volume. The difference in elution volume between sonically disrupted *E. coli* DNA of various molecular weights and undegraded *E. coli* DNA is plotted against the sedimentation constant.

Fig. 2 shows the elution of the same kinds of DNA from a PAK column. Here the order of elution is reversed: the DNA with the lowest guanine + cytosine content is eluted first, and that with highest guanine + cytosine content is eluted last. The effect of sedimentation constant on the position of elution of *E. coli* DNA is summa-

rized in Fig. 3. As might be expected, the smaller the molecular weight, the sooner the DNA appears in the elution.

When a preparation of calf thymus DNA was chromatographed on a PAK column, the fractions eluted earliest had the lowest buoyant density in caesium chloride solutions, and successive fractions had higher buoyant densities. This is to be expected from the relationship between buoyant density and base composition[7].

LENG AND FELSENFELD[8] measured the solubility of complexes of DNA and polylysine and polyarginine as a function of salt concentration and base composition, and concluded that while polylysine interacts preferentially with DNA rich in adenine + thymine, polyarginine shows a slight preference for DNA rich in guanine + cytosine. OLINS *et al.*[9] concluded from the hyperchromic dispersion of complexes of DNA and basic polyamino acids that polylysine preferentially stabilizes adenine + thymine-rich regions of DNA, while polyarginine is less discriminating. These results indicate that the binding of DNA to basic polyamino acids is not entirely electrostatic. While they do not explain the behavior of DNA of different base compositions on columns of the type used in this work, they are in accord with our observations.

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Department of Biochemistry,
Dalhousie University,
Halifax, Nova Scotia (Canada)

C. W. HELLEINER

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