of elution was also found when the nucleotides were eluted with 0.25 $M$ or even I $M$ $NH_4HCO_3$ (not shown in this paper). The results described in Fig. 3 remain to be explained.

*Department of Biological Chemistry,*                                        YEHUDA LAPIDOT
*The Hebrew University of Jerusalem,*
*Jerusalem (Israel)*

1  A. N. SCHWARTZ, A. W. G. YEE AND B. A. ZABIN, *J. Chromatogr.*, 20 (1965) 154.
2  M. UZIEL AND W. E. COHN, *Biochim. Biophys. Acta*, 103 (1965) 539.
3  M. JOHN, M. SKRAHEI AND H. SELLWEG, *FEBS Lett.*, 5 (1969) 185.
4  F. J. KULL AND M. SOODAK, *Anal. Biochem.*, 32 (1969) 10.
5  D. SHUGER, in L. GROSSMAN AND R. MOLDAVE (Editors), *Methods in Enzymology*, Vol. XII, Academic Press, New York, 1967, p. 131.
6  D. H. RAMMLER, Y. LAPIDOT AND H. G. KHORANA, *J. Amer. Chem. Soc.*, 85 (1963) 1989.
7  R. LOHRMANN AND H. G. KHORANA, *J. Amer. Chem. Soc.*, 86 (1964) 4188.

## Chromatography of DNA on wool cortical cell protein

Several proteins or polypeptide materials have been used for the liquid chromatography of DNA or RNA[1–9]. One of the most important of these has been the methylesterified albumin/kieselguhr (MAK) column described by MANDELL AND HERSHEY[1]. It allowed free passage of native, but largely retained the heat-denatured form of bacteriophage DNA. The same property was also demonstrated for bacterial DNA on a silk fibroin column[7] (non-esterified).

We recently reported the formation of thin layers of protein from wool cortical cells[10] and described examples of both inorganic[11] and organic[12] TLC on them. Cortical cells are spindle-shaped cell residues (approx. 4 × 80 $\mu$m) consisting almost entirely of insoluble protein that can be conveniently packed into columns. This note describes some observations on liquid chromatography of DNA on such columns.

*Experimental*

*Materials.* Calf thymus and salmon sperm DNA was obtained from Sigma Chemical Company. Heat-denatured DNA was prepared by heating a solution of native DNA (0.12 mg/ml) in 0.015 $M$ NaCl/0.0015 $M$ sodium citrate for 15 min at 100° and then immediately cooling it in an ice–salt bath.

*Preparation of cells.* Cortical cells were prepared by papain–bisulfite digestion (2 h) of wool fibres as previously described[11]. A short digestion time was used in this case to limit the formation of fine particles. After homogenising, the liberated cells were collected by filtration, washed thoroughly and then suspended in distilled water.

The pH was adjusted to 3 with dilute HCl and the suspension of cells heated at 60° (30 min). The cells were again collected and thoroughly washed.

*Preparation of cortical cell (CC) columns.* Cortical cells were suspended in the appropriate buffer (see Figs. 1 and 2) and the thin slurry poured into a column (1 cm diameter). The cell bed was allowed to pack to a height of about 10–12 cm and this was washed with several column volumes of buffer before use. A hydrostatic pressure head of about 150 cm provided buffer flow rates of approximately 30 ml/h.
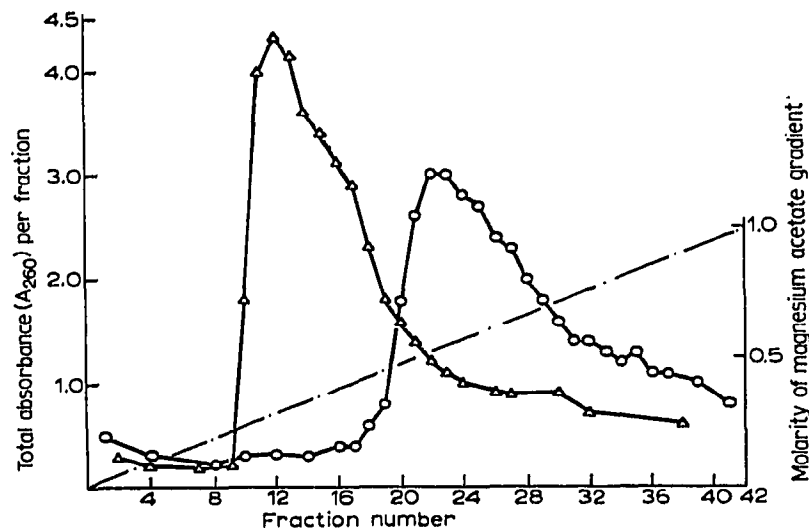


Fig. 1. Elution profiles of native calf thymus (△—△) and native salmon sperm (○—○) DNA from separate wool cortical cell columns (10 × 1 cm) at pH 4.5. Linear salt gradients constructed from 0.1 $M$ sodium acetate (100 ml) and 1.0 $M$ magnesium acetate (100 ml) were used.
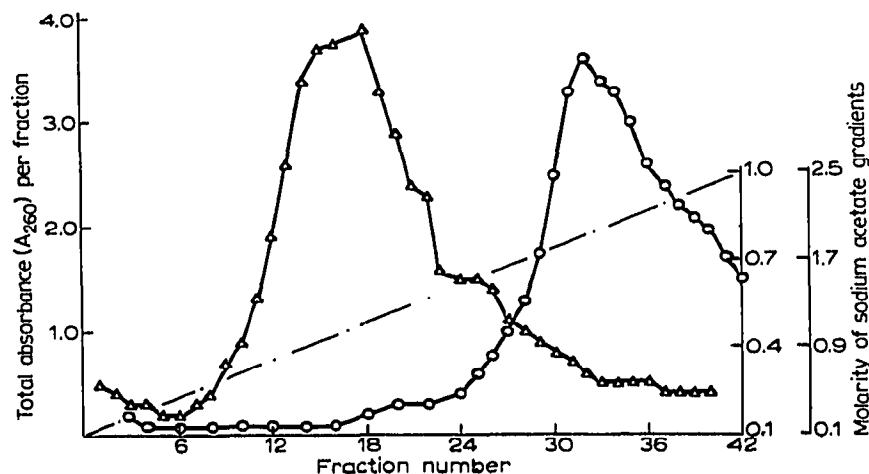


Fig. 2. Elution of calf thymus DNA from separate wool cortical cell columns (10 × 1 cm) at pH 4.5. Linear gradients constructed from 100-ml solutions of sodium acetate were used: 0.1–2.5 $M$ (△—△); 0.1–1.0 $M$ (○—○).

*Chromatographic procedure.* Buffered DNA solutions (5 ml) of either DNA containing approximately 55 $A_{260}$ units* were applied to the top of the column. The eluted buffer, collected during application of the sample, was checked for the pres-

---

* $A_{260}$ units are absorbance (optical density) units per ml as measured in a 1-cm light path cuvette at 260 nm wavelength.

ence of DNA by measuring its UV absorption. A linear salt gradient (see Figs. 1 and 2) was then applied to the column. Fractions (5 ml) were collected and their absorbance (260 nm) was measured. Some shrinkage of the column occurred when loads exceeding about 80 $A_{260}$ units were applied.

*Results and discussion*

Despite differences in the amino acid composition of wool and silk[13] it was expected that the chromatographic properties of wool cortical cell columns would resemble those of silk fibroin[7]. Because elution of DNA from the latter was successful with magnesium ions, we first evaluated the ability of magnesium ions to elute DNA from the CC column. Fig. 1 presents the elution profiles of native calf thymus and native salmon sperm DNA from separate CC columns with identical magnesium acetate gradients at pH 4.5. These two DNA's had quite different affinities for the column. Peak tubes were eluted reproducibly at buffer ionic strength of about 0.85 and 1.45, respectively. While both DNA's showed some tailing, recoveries were 90 % (calf thymus) and 70 % (salmon sperm). It seems unlikely that the differing column affinities were due to small variations in the guanine–cytosine contents of the DNA's (40 % and 44 %, respectively)[14] but differences in molecular size[2] may have been responsible.

It has been suggested[15] that the binding of native DNA to a polycarboxylic resin is due to the formation of a chelation complex involving magnesium ion. It was therefore of interest to determine whether, in the presence of salts incapable of chelation, DNA could be adsorbed and eluted from cortical cell protein. Fig. 2 shows the profile of calf thymus DNA elution at pH 4.5 with two different sodium acetate gradients. Where the final salt concentration was 1 $M$, the peak tube was eluted at an ionic strength of about 0.85. This value is identical to that found with the magnesium acetate gradient (Fig. 1). Where the final sodium acetate concentration was 2.5 $M$, the peak tube was eluted at ionic strength 1.1. These results demonstrate that, in the elution of native DNA from the CC column, magnesium ion has no special function that cannot also be performed by sodium ion.

Table I summarises the effect of buffer pH on the affinity of DNA for the CC column.

A critical pH region (approximately 4.5–5.8) was found within which native DNA could be chromatographed. Below the critical pH range, it was retained; above

TABLE I

EFFECT OF BUFFER pH ON CHROMATOGRAPHY OF DNA ON CORTICAL CELL PROTEIN COLUMNS

| Buffer pH[a] | Behaviour of DNA | |
|---|---|---|
| | Native | Denatured |
| 3.8 | Retained (CT)[b] | |
| 4.5 | Chromatographed (CT, S) | Retained (CT,S) |
| 5.1 | Chromatographed (CT) | Not held up (CT) |
| 5.8 | Chromatographed (CT) | Not held up (CT) |
| 7.0 | Not held up (CT, S) | Not held up (CT) |

[a] A magnesium acetate gradient (see Fig. 1) was used in each case.
[b] CT and S indicate observations with calf thymus and salmon sperm DNA, respectively.

the critical pH region, native DNA had no affinity for the column and was eluted almost immediately.

Apparently, denatured DNA was irreversibly bound at pH 4.5. Once applied it could not be eluted with a magnesium acetate gradient (Fig. 1 type), a sodium acetate gradient (0.1 $M$–2.5 $M$), 0.2 $M$ sodium phosphate buffer (pH 8), 0.2 $M$ EDTA (pH 8), or 0.1 $M$ sodium thioglycollate (pH 8). Above pH 5.1 denatured DNA had no affinity for the CC column and, in this respect, cortical cells differed from silk fibroin which would bind DNA at pH 5.8 if magnesium ions were available.

The important property of the CC column, requiring further study, is that at pH 4.5 native, but not denatured, DNA can be eluted with salt gradients. In this respect it resembles both the MAK and silk fibroin columns. It has distinct advantages in the ease with which it is prepared and the fact that no protein carrier is required. The capacity of these cortical cell columns (10 cm) exceeded 6 mg of DNA because this amount was chromatographed satisfactorily.

*C.S.I.R.O., Division of Textile Industry, P.O. Box 21,*          G. N. FREELAND
*Belmont, Victoria 3216 (Australia)*                              R. M. HOSKINSON

1  J. D. MANDELL AND A. D. HERSHEY, *Anal. Biochem.*, 1 (1960) 66.
2  N. SUEOKA AND T. CHENG, *J. Mol. Biol.*, 4 (1962) 161.
3  S. R. AYAD AND J. BLAMIRE, *Biochem. Biophys. Res. Commun.*, 30 (1968) 207.
4  S. R. AYAD, J. BLAMIRE AND S. J. LEIBOVICH, *J. Chromatogr.*, 37 (1968) 326.
5  S. R. AYAD AND J. BLAMIRE, *J. Chromatogr.*, 42 (1969) 248.
6  S. R. AYAD AND J. BLAMIRE, *J. Chromatogr.*, 48 (1970) 456.
7  T. Y. HUH AND C. W. HELLEINER, *Anal. Biochem.*, 19 (1967) 150.
8  G. W. RUSHIZKY, *Anal. Biochem.*, 29 (1969) 459.
9  T. I. TIKHONENKO, *Biokhimiya*, 27 (1962) 131; *C.A.*, 57 (1962) 3767.
10 P. R. BRADY, J. DELMENICO AND R. M. HOSKINSON, *J. Chromatogr.*, 38 (1968) 540.
11 P. R. BRADY AND R. M. HOSKINSON, *J. Chromatogr.*, 54 (1971) 55.
12 P. R. BRADY AND R. M. HOSKINSON, *J. Chromatogr.*, 54 (1971) 65.
13 D. HILDEBRAND, *Z. Ges. Textilind.*, 71 (1969) 274.
14 C. L. SCHILDKRAUT, J. MARMUR AND P. DOTY, *J. Mol. Biol.*, 4 (1962) 430.
15 F. R. FRANKEL AND C. F. CRAMPTON, *J. Biol. Chem.*, 237 (1962) 3200.