CHROM. 5179

Some aspects of fractionation of DNA on IR-120 Al^{3+} columns
IV. Effect of protein association on the chromatographic profiles of DNA

With an IR-120 Al^{3+} column[1,2], DNA is extensively and reproducibly fractionated into seven distinct fractions that differ in base composition. While studying the effect of the method of deproteinisation[3,4] on the chromatographic profiles of DNA using IR-120 Al^{3+} column, it was found out that DNA isolated by different methods showed varying degrees of adsorption on the column. In these studies, in addition to the differences in the method of isolation, DNA's also differed in their protein association. It was therefore thought worthwhile to study if the protein association with DNA shows any effect on the chromatographic profiles.

Experimental

Deoxyribonucleic acids. DNA samples containing different amounts of associated protein were isolated from buffalo liver by the procedure of SEVAG *et al.*[5]. The amount of protein associated with different samples of DNA depended on the number of deproteinisation treatments given to deoxyribonucleoprotein (DNP) solution with chloroform–amyl alcohol (3:1). The treatments ranged from no deproteinisation to extensive deproteinisation, finally involving the use of chymotrypsin digestion to remove the last traces of protein residue. Different amounts of associated protein were thus deliberately retained by regulating the number of deproteinisation treatments. The purity of different samples, thus isolated, was examined by common methods[6]. All the samples were found to be pure and devoid of RNA contamination. They differed in their protein association, ranging from 40 to 0.1 %, as judged by the reaction of LOWRY *et al.*[7].

IR-120 Al^{3+} column. 10 g of a dry, regenerated Amberlite IR-120 Na^+ form of cation exchanger, sufficiently equilibrated with a 0.2 *M* aluminium chloride solution, gave an IR-120 Al^{3+} column. After percolating glycine–sodium hydroxide buffer (pH 8.6, 0.054 *M*) through the column until the pH of the influent and effluent were the same, it was used for the chromatography of DNA samples that differed only in the degree of protein association, the source and method of isolation being identical.

Procedure. A known amount of homogeneous DNA solution obtained in the glycine–sodium hydroxide buffer was allowed to adsorb on IR-120 Al^{3+} column and was eluted with 100 ml of different eluting agents in the usual given sequence[2]. The flow rate during adsorption and elution was 10–15 ml per h. The fractions collected, each 25 ml, were assayed for their DNA content by BURTON's diphenylamine reaction[8]. An identical procedure was followed for the other samples.

The amounts retained and eluted of the totally adsorbed DNA having different degrees of protein association are given in Table I. Amounts of DNA eluted with different eluting agents are given in Fig. 1, and also the profiles where total optical densities at 590 nm are plotted against the test-tube number or fractions eluted. DNA associated with 1.0, 6.0 and 13.0 % protein gave closely overlapping profiles which are represented by one curve only.

TABLE I

CHROMATOGRAPHIC PROFILES OF BUFFALO LIVER DNA ASSOCIATED WITH VARYING AMOUNTS OF PROTEIN, ON IR-120 Al^{3+} COLUMN

Protein association with respect to DNA (%)	*Retention* (%)	*Elution* (%)
40 (DNP, no deproteinisation)	100	95
26	100	102
13	100	100
6	100	100
1	100	85
0.1	100	90
(Extensive deproteinisation + chymotrypsin digestion, 37°, pH 8.6, 96 h)		

Results and discussion

It is clear from Table I that DNA samples, isolated from buffalo liver by Sevag's procedure, containing varying amounts of protein, ranging from 40 to 0.1 %, were found to be 100 % retainable on the IR-120 Al^{3+} column. They were quantitatively eluted into seven distinct fractions (Fig. 1), and the fractionation of DNA is on the basis of differences in base composition of the fractions eluted[1].

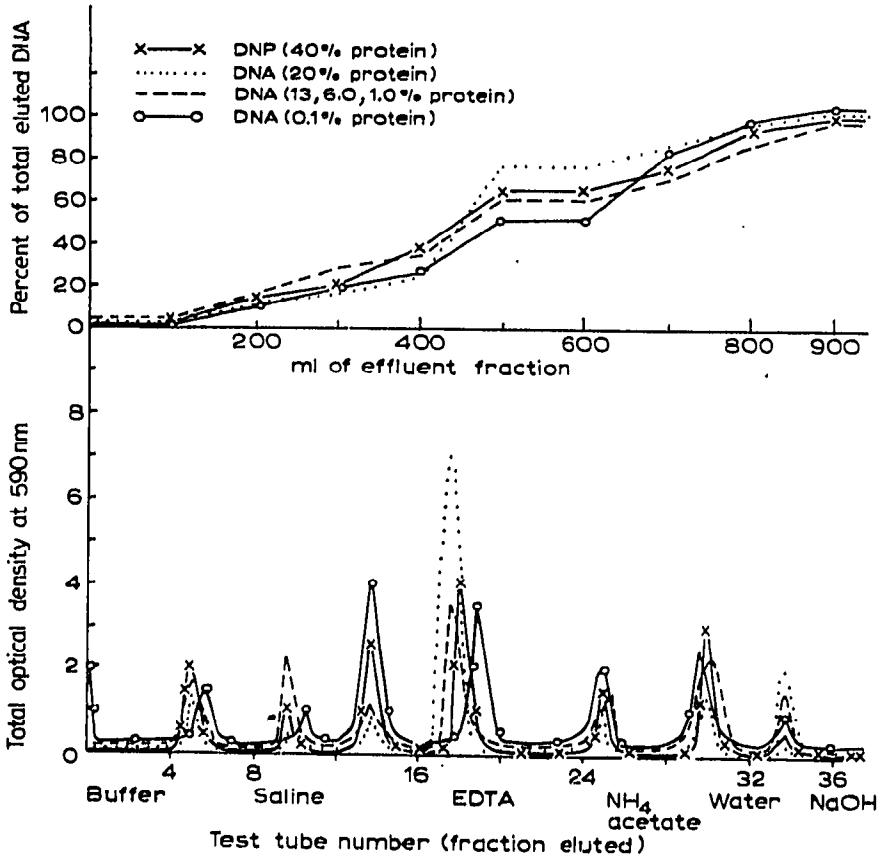

Fig. 1. Chromatographic elution profiles of buffalo liver DNA with varying degrees of protein association on a IR-Al^{3+} column.

Complete retention of DNA in spite of the large variation in protein association in different samples indicates that the retentivity of DNA on the IR-120 Al^{3+} column is independent of the amount of its protein associate. Almost complete elution was achieved in each case, giving typical comparable profiles. In each case, the diphenylamine-positive material was eluted at the same location with minor variations in the distribution produced by different eluting agents. This also indicated that protein association has no significance in the fractionation of DNA on the IR-120 Al^{3+} column.

Each diphenylamine-positive fraction, when analysed for its protein content by the reaction of LOWRY *et al.*[7], showed complete absence of protein in all the fractions. This observation further substantiated that protein plays no role in the fractionation of DNA. With the eluting agents used, the IR-120 Al^{3+} column seemed to act as a deproteinising tool in addition to its ability to give well-defined fractionation.

Protein association and properties of DNA. It is known that a small amount of protein association markedly alters the physical properties of DNA, such as increasing the sedimentation coefficient[9] or giving erroneous results for molecular weights[10]. However, it was pointed out by KIT[11] that protein association in the range of 3–4% to less than 1.0% (30% in one case) has no marked effect on the chromatographic profiles of DNA on ECTEOLA-cellulose. The present results with the IR-120 Al^{3+} column are similar to those of KIT[11]. According to KIT[11], chromatographic profiles of DNA should theoretically be altered by even traces of protein, if the protein functioned to crosslink DNA molecules to give aggregates (particularly if the retention is markedly dependent on molecular weight). However, it has been already shown[2,4] that retention on the IR-120 Al^{3+} column is a function of finite macromolecular conformation rather than molecular weight.

In conclusion, therefore, the chromatographic behaviour of DNA on the IR-120 Al^{3+} column is independent of its protein association.

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