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SOME ASPECTS OF FRACTIONATION OF DNA ON AN IR-120 Al^{3+} COLUMN

III. EFFECT OF THE METHOD OF DEPROTEINISATION ON THE CHROMATOGRAPHIC PROFILES OF DNA

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SUMMARY

The effect of the method of deproteinisation on the chromatographic behaviour of DNA using an IR-120 Al^{3+} column is being studied. For this purpose, DNA's deproteinised by four different methods were chromatographed and their profiles were studied. Although the method of deproteinisation did not seem to influence significantly the DNA fractionation, retention itself was affected. DNA isolated by the chloroform–amyl alcohol method and that by the guanidine hydrochloride method were completely retainable without any treatment. DNA isolated by the phenol–fluoride method was not retainable but became significantly retainable only after Mg^{2+} equilibration under finite conditions, indicating an important role of Mg^{2+} for retention. Similar behaviour was also shown by DNA isolated by the SDS method. After retention was achieved, the elution profiles of DNA, isolated by four different methods, were similar with a little variation in percent elution in different fractions.

INTRODUCTION

It is reported[1,2] that the chromatographic, physical, or chemical properties of DNA isolated by different methods from a given tissue of a given source may not be identical. KIT[3] studied the chromatographic profiles of DNA isolated from the same source and the same tissue but by different methods and found that the profiles are quite similar. It was, therefore, thought worthwhile to study if the method of isolation and deproteinisation (methodology) has any effect on the chromatographic profiles of DNA, using an IR-120 Al^{3+} column.

EXPERIMENTAL AND RESULTS

IR-120 Al^{3+} column

10 g of dry regenerated Amberlite IR-120 (Na^+ form), polystyrene sulphonate, equilibrated with 0.2 *M* aluminium chloride solution gave the IR-120 Al^{3+} column[4,5]. Glycine–sodium hydroxide buffer (pH 8.6, 0.054 *M*) was percolated through the

column till the pH of the influent and effluent were the same. This column was then used for fractionating DNA's deproteinised by different methods[6–9].

Deoxyribonucleic acids

DNA samples were isolated from buffalo liver using the following methods:
(1) the chloroform–amyl alcohol method of SEVAG *et al.*[6];
(2) the method using 10 *M* guanidine hydrochloride of VOLKIN and CARTER[7];
(3) the 5 % sodium dodecyl sulphate (SDS) method of KAY *et al.*[8];
(4) the phenol–fluoride method of KIRBY[9].

DNA samples isolated by different deproteinising methods were native and fairly pure (90–95 %). They were associated with varying amounts (1–10 %) of residual protein and were devoid of RNA. The nativity and purity were examined as discussed earlier[4].

Procedure

DNA's, thus isolated, were dissolved in glycine–sodium hydroxide buffer (pH 8.6, 0.054 *M*) to get homogeneous solutions. The solutions were loaded on different IR-120 Al^{3+} columns, the columns were washed with 3 bed volumes of the above buffer to strip off any loosely retained DNA, and wherever adsorption occurred, DNA's from the columns were eluted with different eluting agents in the usual sequence[4]. Fractions, each 25 ml, were collected and assayed for DNA content by BURTON's reaction[10]. (The details of the experimental procedure have been discussed earlier[4].) Percent retention of DNA and percent elution of the total adsorbed DNA are given in Table I.

TABLE I

CHROMATOGRAPHIC BEHAVIOUR OF DNA'S ISOLATED BY DIFFERENT METHODS OF DEPROTEINISATION FROM BUFFALO LIVER, ON AN IR-120 Al^{3+} COLUMN

DNA isolated by	*Percent retention*	*Percent elution*	*Profiles*
Chloroform–amyl alcohol	100	100	Typical seven fractions (F_1–F_7)
10 *M* guanidine hydrochloride	90	100	Typical seven fractions (F_1–F_7)
5% SDS	Nil	—	Typical seven fractions (F_1–F_7)
Phenol–fluoride	Nil	—	Typical seven fractions (F_1–F_7)

It is clear from Table I that DNA isolated by the methods of SEVAG *et al.*[6], of VOLKIN AND CARTER[7] are 100 and 90 % retainable, respectively, and, on elution, the typical 7 fractions (F_1–F_7) were obtained, the fractionation being based upon differences in base composition of the fractions eluted[4]. DNA's isolated by the method of KAY *et al.*[8] and by KIRBY[9] are not retainable.

The series of following experiments were performed to know if the residual SDS, or phenol and/or fluoride impurities in the nonretained DNA were responsible for nonretention on an IR-120 Al^{3+} column.

DNA isolated by the SDS method

DNA isolated by the SDS method was extensively dialysed against cold 0.14 *M*

saline and chromatographed on an IR-120 Al^{3+} column and was found to be non-retainable. The effluate DNA without and with Mg^{2+} equilibration (0.001 *M* Mg^{2+} at 4° for 24 h) was chromatographed on fresh IR-120 Al^{3+} columns and the chromatographic behaviour was studied.

DNA isolated by Kirby's method

DNA isolated by KIRBY's method was chromatographed on an IR-120 Al^{3+} column after extensive dialysis against cold 0.14 *M* saline.

DNA isolated by Sevag's method and treated with phenol

DNA isolated by SEVAG's method was treated with phenol in the absence of sodium fluoride and was chromatographed on an IR-120 Al^{3+} column. Another such sample, extensively washed with cold ether till the etherial supernatant gave a negative test for phenol, indicating a complete removal of phenol, was also chromatographed on the column.

DNA isolated by Sevag's method and treated with fluoride

DNA isolated by SEVAG's method was treated with 0.3 *M* sodium fluoride in the absence of phenol. It was extensively dialysed against glycine–sodium hydroxide buffer (pH 8.6, 0.054 *M*) at 4° till the dialysed residue showed a negative test for fluoride, indicating a complete removal of fluoride ions. Dialysed DNA, thus obtained, was chromatographed on an IR-120 Al^{3+} column before and after equilibration with 0.001 *M* Mg^{2+} at 4° for 24 h.

DNA isolated by Sevag's method and simultaneously treated with phenol and fluoride

DNA isolated by SEVAG's method was simultaneously treated with phenol and 0.3 *M* sodium fluoride and freed from phenol as well as sodium fluoride by the treatments given above. The sample was then chromatographed on an IR-120 Al^{3+} column and the profiles were studied.

The percent retention of DNA and percent elution of the total adsorbed DNA (subjected to additional treatments) are given in Table II. Fig. 1 shows the percent

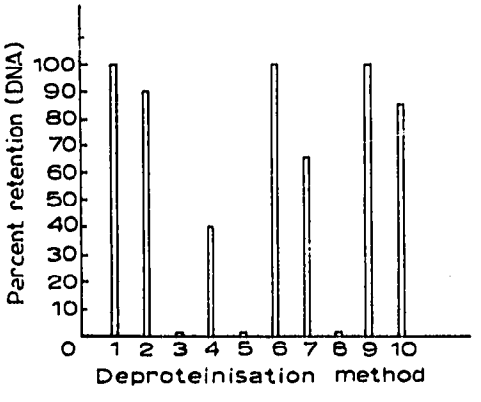

Fig. 1. Percent retention of buffalo liver DNA deproteinised by different methods on IR-120 Al^{3+} column. 1 = SEVAG's method; 2 = VOLKIN AND CARTER's method; 3 = KAY AND DOUNCE's method; 4 = KAY AND DOUNCE's method + Mg^{2+}; 5 = KIRBY's method; 6 = KIRBY's method + Mg^{2+}; 7 = SEVAG's method + phenol; 8 = SEVAG's method + NaF; 9 = SEVAG's method + NaF + Mg^{2+}; 10 = SEVAG's method + phenol + NaF.

TABLE II

RETAINABILITY OF DIFFERENTLY DEPROTEINISED BUFFALO LIVER DNA (SUBJECTED TO ADDITIONAL TI MENTS) ON AN IR-120 Al^{3+} COLUMN

Deproteinisation method	*Additional treatment*	*Percent retention*	*Percent elution*	*Profiles*
SDS	Extensive dialysis	Nil	—	—
Effluate from above after passage through IR-120 Al^{3+}	Nil	Nil	—	—
Sample as above	0.001 *M* Mg^{2+}, 4°, 24 h	40	100	0.5 *M* saline and 1% EDT elutable fractions only
KIRBY	Extensive dialysis	Nil	—	—
SEVAG	Phenol, without ether washings	70	100	0.5 *M* saline, 1% EDTA a water elutable fractions o
SEVAG	Phenol, with ether washings	66	100	0.5 *M* saline, 1% EDTA a water elutable fractions o
SEVAG	0.3 *M* NaF, extensive dialysis	Nil	—	—
SEVAG + 0.3 *M* NaF, extensive dialysis	0.001 *M* Mg^{2+}, 4°, 24 h	100	100	Typical seven fractions (F
SEVAG	Phenol–0.3 *M* NaF, extensive dialysis	85–90	100	Typical seven fractions (F

retention of DNA samples deproteinised by different methods with and without additional treatments (combined results of Tables I and II).

DISCUSSION

Method of deproteinisation and retainability

It has been noted that DNA isolated by SEVAG's method is 100% retainable, by VOLKIN AND CARTER's method 90% retainable, by KAY's and by KIRBY's methods completely nonretainable on the IR-120 Al^{3+} column. In the latter two cases, DNA is made retainable to the extent of 40 and 100%, respectively, after Mg^{2+} equilibration.

Nonretainability of DNA isolated by the SDS method

DNA isolated by the SDS method was nonretainable as such and it remained so even after extensive dialysis to remove residual SDS. The nonretainability of DNA may be explained as follows.

During the isolation of DNA, complex formation between DNA and SDS may have taken place wherein SDS occupied the same "loci" of DNA necessary for its retention on the IR-120 Al^{3+} column, and such "loci" of DNA being "capped" by SDS, no retention was possible.

It might also be that SDS may have formed a complex with resin-Al^{3+} and DNA free of SDS impurity may have come out in the effluate. Therefore, the effluate DNA was again loaded on a fresh IR-120 Al^{3+} column, but DNA was still nonretainable. The possibility that SDS may have "poisoned" the column also does not hold true because DNA isolated by SEVAG's method was 100% retainable on the same column.

It is known that SDS deproteinises deoxyribonucleoprotein (DNP) forming DNA and a denatured protein from one end[8]. If the deproteinisation is incomplete, part of the protein may still be associated with DNA in a denatured state. Retention of β-amylase and some studies[11] on phosphate metabolising enzyme on an IR-120 Al^{3+} column have shown that denatured proteins are not retained on the column. The residual denatured protein impurity along with DNA may exist as a "coating" on the DNA fibre as exists with certain viral RNA's and result in nonretainability of DNA.

Retainability of DNA isolated by the SDS method in the presence of Mg^{2+}

The effluate DNA, isolated by the SDS method, when equilibrated with Mg^{2+} and chromatographed, was found to be 40 % retainable. Mg^{2+} equilibration was tried without any specific reason except that Mg^{2+} is known to maintain a finite three dimensional conformation in the case of RNA and is a cation commonly found in biological systems. Demineralisation due to the SDS method may result in the nonretainability of DNA which may be rendered partially retainable again by Mg^{2+} equilibration.

Behaviour of DNA isolated by Kirby's method

As DNA isolated by KIRBY's method[9] employing phenol and 0.3 *M* fluoride was found nonretainable, it was thought that residual phenol and/or fluoride impurities may be inhibitory to the adsorption of DNA on the IR-120 Al^{3+} column. Sodium fluoride was however found to aid in the adsorption of DNA isolated from normal somatic tissue, on a Dowex-50 Hg^{2+} column[5]. As the results looked contradictory, care was taken to remove phenol by extensive ether washings and fluoride by dialysis. Still DNA was nonretainable on the IR-120 Al^{3+} column.

Behaviour of DNA isolated by Sevag's method and treated with phenol

It was found that DNA isolated by SEVAG's method and treated with phenol in the absence of fluoride was 66 % retainable after removal of traces of phenol and 70 % retainable in the presence of traces of phenol, indicating that nonretainability of part of the DNA isolated by KIRBY's method was apparently not due to the residual phenol impurity.

Behaviour of DNA isolated by Sevag's method and treated with fluoride

It was found that DNA isolated by SEVAG's method which is retainable on the IR-120 Al^{3+} column becomes nonretainable after fluoride treatment in the absence of phenol, even though last traces of fluoride were removed by extensive dialysis. It is unlikely that fluoride causes any damage, *viz.* depolymerisation or denaturation to the DNA rendering it nonretainable. This nonretainable DNA is rendered 100 % retainable by Mg^{2+} equilibration, indicating that the nonretainability on the IR-120 Al^{3+} column is due to some effect by fluoride which is nullified by Mg^{2+}. It is unlikely that Mg^{2+} undertakes "repairs" of DNA resulting from depolymerisation or denaturation making it retainable. Earlier studies[12,13] showed that the denatured DNA is completely retainable on the IR-120 Al^{3+} column.

Use of sodium fluoride in KIRBY's method may be removing Mg^{2+} by chelation. Mg^{2+} is also known to favour certain conformations of DNA and to aggregate nucleic

acids. Removal of Mg^{2+} as a complex possibly results in the deformation of the DNA structure, ultimately making it nonretainable.

Disaggregation due to fluoride treatment and aggregation due to Mg^{2+} equilibration may also be the probable phenomenon. Aggregation and disaggregation may be involving structural alterations, incidentally resulting in retainability and nonretainability of DNA, respectively.

It is difficult to explain why DNA, isolated primarily by SEVAG's method and then treated with KIRBY's phenol–fluoride deproteinising phase simultaneously, is completely retainable on the IR-120 Al^{3+} column, while DNA isolated directly by KIRBY's method is not retainable under an identical set of experimental conditions.

As use of different methods leaves different amounts of residual proteins, the effect of protein association on the chromatographic profiles of DNA on the IR-120 Al^{3+} column was studied and it was noted[14] that protein association has no major effect on the chromatographic behaviour of DNA.

These results, on the whole, indicate that the method of deproteinisation has a role in the retention of DNA on the IR-120 Al^{3+} column, although it does not affect the profiles, once adsorbed. The role of Mg^{2+} is also thought provoking.

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