CHROM. 4949

Some aspects of fractionation of DNA on an IR-120 Al^{3+} column

II. Effect of the physical state of DNA on chromatographic profiles

Native DNA consists of two right-handed helical complementary polynucleotide chains of opposite polarity coiled around the common axis. Hydrogen bonds between the two bases of the complementary chains stabilise such an orderly structure. Deformation of such a structure into a disorderly state by breaking down hydrogen bonds leads to denaturation of DNA. In apurinic acid, all adenine and guanine species are quantitatively removed from a polynucleotide chain without any dislocation of pyrimidines in the macromolecule. Degradation of DNA by splitting up of the phosphodiester linkage gives rise to partially depolymerised DNA. Mononucleotides are the building blocks of DNA polymer, and it is obvious from this that differently treated DNA molecules possess different physicochemical characteristics. Thus it was worthwhile to investigate if the physical state of DNA has any effect on the chromatographic behaviour, using an IR-120 Al^{3+} column.

With the tool developed, IR-120 Al^{3+} column[1,2], studies are carried out on the chromatographic behaviour of denatured DNA, apurinic acid, partial and complete enzymic digest of DNA, and compared with the studies of native DNA on IR-120 Al^{3+} column.

Experimental

Deoxyribonucleic acid. Sodium salt of DNA, used in these experiments, was isolated from buffalo liver (Mammalia, Ruminantia) by the method of SEVAG *et al.*[3]. It was a white fibrous and fairly pure preparation[2]. Its purity and nativity were examined by the usual methods[4].

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 chromatographic studies of differently treated DNA molecules.

Treatments. DNA was deliberately denatured by controlled heat treatment (96°, 15 min) and cooled suddenly to room temperature in an ice bath. Denaturation by this method is very specific and causes no side reactions such as hydrolysis or deamination. Apurinic acid was achieved by keeping DNA at pH 4.0 with perchloric acid at 27° for 24 h (ref. 5). Perchlorate ions were subsequently removed as potassium salt. A partial digest of DNA was obtained by incubating DNA with pancreatic deoxyribonuclease at 37° for 6 h. As an activator 0.001 *M* Mg^{2+} was added. DNA was reduced to mononucleotides by the combined action of pancreatic deoxyribonuclease and spleen phosphodiesterase at 37° for 48 h. 0.001 *M* Mg^{2+} was also added. The chromatographic behaviour of these DNA's treated specifically was studied and compared with native DNA as follows.

Procedure. A known amount of the treated DNA (not treated in the case of the native one) was chromatographed on an IR-120 Al^{3+} column (size 1 × 15 cm), and

TABLE I

CHROMATOGRAPHIC PROFILES OF DNA SUBJECTED TO DIFFERENT TREATMENTS ON AN IR-120 Al^{3+} COLUMN

State of DNA	*Treatment*	*Retention (%)*	*Elution (%)*	*Profiles*
Native	None	100	100	Typical seven fractions
Denatured	96° for 15 min	95	100	Typical seven fractions
Apurinised	pH 4.0, 27°, 24 h	25	100	One fraction only, 0.5 *M* saline elutable
Partial digest	DNAase I + 0.001 *M* Mg^{2+}, 37°, 6 h	55	100	One fraction only, 0.5 *M* saline elutable
Complete hydrolysate	DNAase I + Phosphodiesterase + 0.001 *M* Mg^{2+}, 37°, 48 h	0	—	—

the adsorbed DNA was eluted using 100 ml of each of the different eluting agents in the following sequence: 0.5 *M*, 1.0 *M*, 2.0 *M* saline; 1.0%, 2.0% EDTA, 1.0 *M* ammonium acetate, glass-distilled water and finally 0.1 *M* sodium hydroxide. The flow rate during adsorption and elution was 10–15 ml/h. The fractions, each 25 ml, were

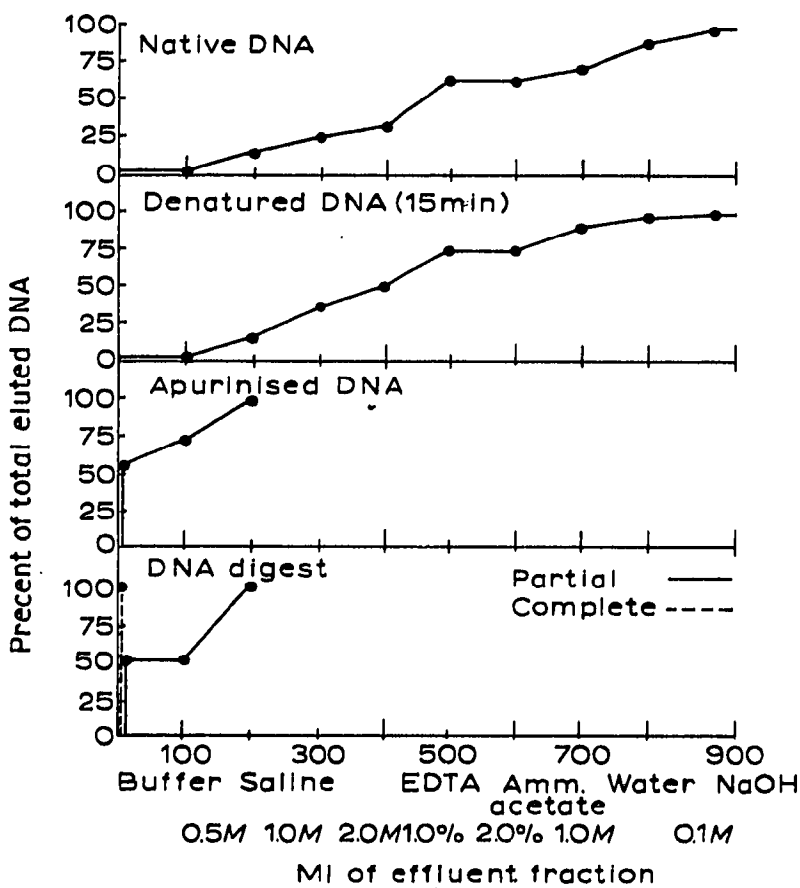

Fig. 1. Percent of total eluted buffalo liver DNA subjected to different treatments, chromatographed on an IR-120 Al^{3+} column.

analysed for their DNA content using the reaction of BURTON[6]. It was noted that DNA was fractionated on the IR-120 Al^{3+} column on the basis of its purine and pyrimidine composition[2].

The percent retention of DNA and percent elution of totally adsorbed DNA and the fractions obtained are given in Table I. The percent of total DNA eluted with different eluting agents is given in Fig. 1 and the profiles in Fig. 2.

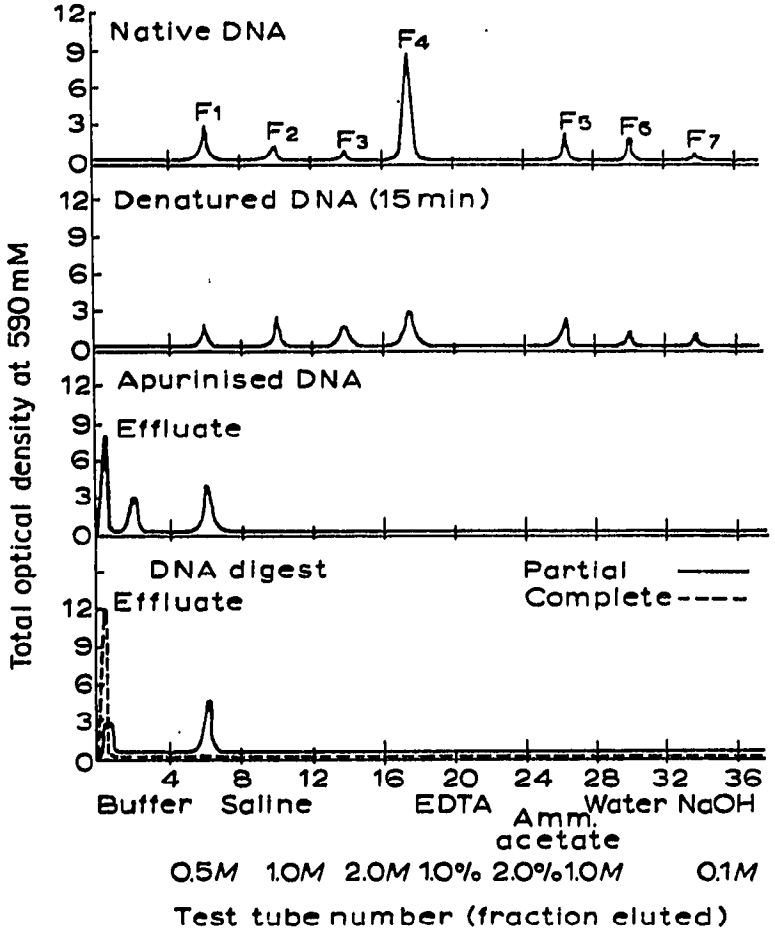

Fig. 2. Chromatographic elution profiles of buffalo liver DNA, subjected to different treatments, on an IR-120 Al^{3+} column.

Results and discussion

From Table I it is clear that the native DNA is 100% adsorbable on an IR-120 Al^{3+} column and also is completely elutable in seven distinct fractions using different eluting agents[2]. Denatured DNA was also found to be nearly 100% retainable and gave a chromatographic profile similar to that of native DNA. However, the percent distribution of DNA eluted in the fractions varies in the two cases as can be seen in Figs. 1 and 2. HERSHEY *et al.*[7] studied the effect of denaturation on the chromatographic behaviour using an MSAK column. It was observed that heat-sheared DNA has less affinity for the column, and hence it was easily elutable. Present results are comparable with those of HERSHEY *et al.*[7] as seen by more elution due to saline and less due to 1.0% EDTA. SKIDMORE *et al.*[8], however, observed that the chromatographic

profiles of heat-treated DNA are different from those of unheated DNA on ECTEOLA-cellulose. BERNARDI[9] noted that the degree of denaturation also changes the relative proportion of various fractions. Here it may be concluded that on the IR-120 Al^{3+} column, as on the protamine-coated Kieselguhr column[10], native and denatured DNA cannot be distinguished.

Apurinic acid was found to be 25 % retainable and 100 % elutable in 0.5 *M* saline fraction alone, giving only one peak. The inability to retain 75 % DNA may not be due to the breakdown of the polynucleotide chain because, under the mild conditions used in the present experiments, the chances of scission of the polynucleotide chain are minimum[11].

The partial digest of DNA is 55 % retainable on the column and is easily and completely elutable in 0.5 *M* saline alone, giving one peak. This behaviour indicates a relatively low affinity for the column. The complete digest of DNA is completely nonretainable, indicating that mononucleotides have no affinity for the column. It is known that DNAase acts on DNA giving rise to oligo-, di, tri-, and tetranucleotides[12]. If this partial digest is simultaneously treated with spleen phosphodiesterase, mononucleotides are formed. It is possible that the retainability to oligonucleotides may be due to their partial macromolecular structure. Once, however, DNA is reduced to a mononucleotide state, the macromolecular structure is completely lost and, as a result, the mononucleotides are not retained on the column. KIT[13] has also made similar observations using an ECTEOLA-cellulose column. BERNARDI[9] also found that a DNAase digest of DNA is eluted earlier than intact DNA from a hydroxyapatite column, thus indicating that the depolymerised product has poor affinity and binding on the column and, therefore, requires a polymeric structure. BROWN AND WATSON[14] have also pointed out that a finite structure of DNA is necessary for adsorption on histone coated with Kieselguhr.

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