CHROM. 5342

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

VI. The effect of pH and temperature variation on the chromatographic profiles of DNA

The shape, size and conformation of the DNA molecule as suggested by Watson and Crick's double helical model is largely dependent on a balance of opposing forces such as hydrogen bonding, Van der Waal's interionic forces, kinetic forces and varies with the environment. Thus, a change in the pH and/or temperature of DNA solution gives rise to changes in shape and rigidity *i.e.* three-dimensional structure of the molecule. DOTY AND RICE[1] have shown that lowering the pH and increasing the temperature results in structural alterations owing to breakage of hydrogen bonds. Structural transitions as a function of temperature and pH have also been observed by LUZZATI *et al.*[2] in the case of DNA and polyadenylic acid in solution. It was, therefore, thought worthwhile to investigate whether the variation in pH and temperature has any effect on the chromatographic behaviour, using an IR-120 Al^{3+} column.

DNA is quite unstable in the acidic pH range. Even at pH 6.0, it slowly starts apurinising. It is comparatively more stable in the alkaline range up to pH 10.6, because of the absence of a hydroxyl group in second position of the D-2-deoxyribose in DNA, which prevents cyclic phosphotriester formation prior to degradation, unlike that in the alkaline hydrolysis of RNA. Thus, the narrow pH range over which DNA is stable, limited the study of chromatographic behaviour at different pHs and was carried out only at pH 6.8, 8.6 and 10.0.

Experimental

IR-120 Al^{3+} column

10 g of dry regenerated Amberlite IR-120 (the Na^+ form of the cation exchanger) were equilibrated sufficiently with a 0.2 *M* aluminium chloride solution to give an IR-120 Al^{3+} column[3-8].

pH variation. Glycine–sodium hydroxide buffer at various pH, *viz.* pH 6.8, 8.6 and 10.0, was percolated through the column till the pHs of the influent and effluent were the same. The IR-120 Al^{3+} columns, thus equilibrated at the respective pHs, were then used for the fractionation studies.

Temperature variation. (1) Chromatographic studies were carried out at 4° by keeping the column in an Allwyn refrigerator. (2) The column was maintained at 27 ± 1° by the use of a jacketed column through which water from a thermostat was circulated by the peristaltic pump. The thermostat was adjusted to 27 ± 1° with the help of a mercury contact thermoregulator. A stirrer was used to avoid local variations in temperature. The relay used was from the Jumo Company. (3) The column was regulated at 42 ± 1° by means of a column condenser through which water at 42 ± 1° was circulated by a similar arrangement.

IR-120 Al^{3+} columns, equilibrated with glycine–sodium hydroxide buffer (pH 8.6, 0.054 *M*) and maintained at 4, 27 and 42°, were then used for fractionation studies.

TABLE I

EFFECT OF pH VARIATION ON THE CHROMATOGRAPHIC PROFILES OF DNA ON AN IR-120 Al^{3+} COLUMN

Fraction eluted by	*Percent elution after adsorption of DNA carried out at pH*		
	6.8	*8.6*	*10.0*
Percent adsorption	90.0	100	90.0
Effluent	9.0	Nil	7.5
Buffer	Nil	Nil	2.5
0.5 *M* Saline	23.0	10.0	16.5
1.0 *M* Saline	4.0	15.0	1.0
2.0 *M* Saline	4.4	6.7	1.5
1.0% EDTA	47.0	33.0	60.0
2.0% EDTA	Nil	Nil	Nil
1.0 *M* Ammonium acetate	4.4	8.0	4.0
Distilled water	8.0	17.5	3.0
0.1 *M* Sodium hydroxide	Nil	10.0	3.0

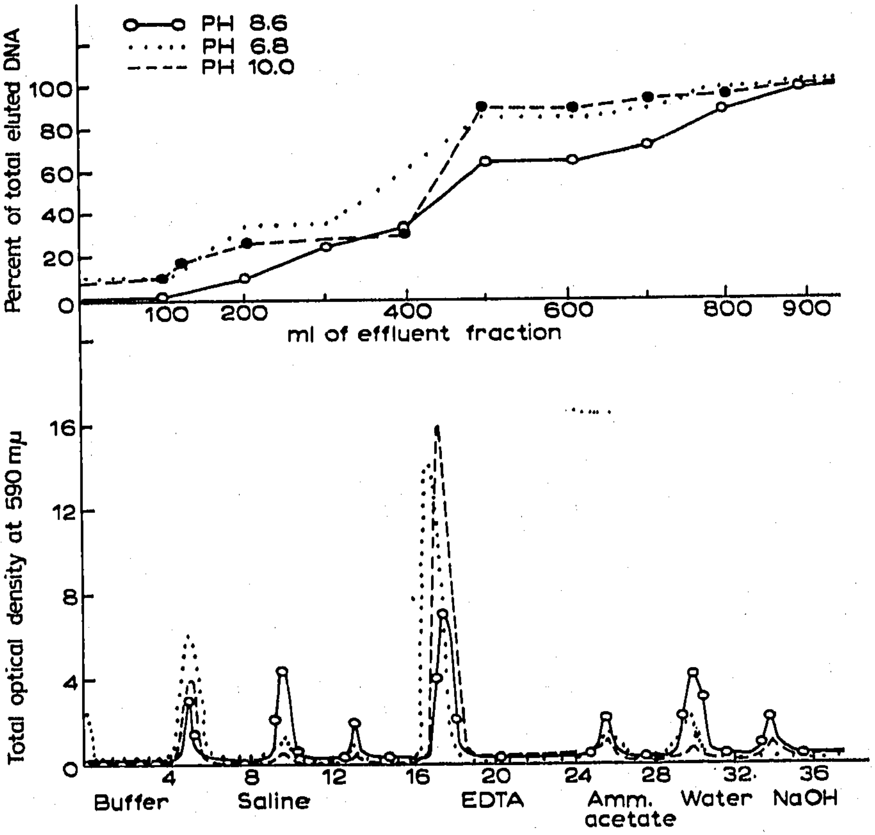

Fig. 1. Effect of pH variation on the chromatographic profiles of DNA on an IR-120 Al^{3+} column.

Deoxyribonucleic acid

The sodium salt of DNA, used in the present studies, was isolated from buffalo liver by the method of SEVAG *et al.*[9]. It was a white, fibrous and fairly pure preparation[4]. Its purity and nativity were examined by the usual methods[10]. It was devoid of any RNA contamination.

Procedure

A known amount of homogeneous DNA solution in glycine–sodium hydroxide buffers having various pHs, *viz.* pH 6.8, 8.6 and 10.0, was loaded on three separate IR-120 Al^{3+} columns, maintained at pH 6.8, 8.6 and 10.0, respectively. The adsorbed DNA was eluted with 100 ml of different eluting agents in the usual given sequence[4]. 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[11].

TABLE II

EFFECT OF TEMPERATURE VARIATION ON THE CHROMATOGRAPHIC PROFILES OF DNA ON AN IR-120 Al^{3+} COLUMN

Fraction eluted by	*Percent elution after adsorption and elution carried out at*		
	4°	*27°*	*42°*
Percent adsorption	100	100	80.0
Effluent	Nil	Nil	2.0
Buffer	Nil	Nil	18.0
0.5 *M* Saline	18.0	10.0	20.0
1.0 *M* Saline	5.5	15.0	4.0
2.0 *M* Saline	15.0	7.0	1.6
1.0% EDTA	40.0	33.0	36.0
2.0% EDTA	Nil	Nil	Nil
1.0 *M* Ammonium acetate	7.0	8.0	6.5
Distilled water	12.0	17.0	6.0
0.1 *M* Sodium hydroxide	3.0	10.0	3.0

The effect of pH variation on adsorption and elution is given in Table I. Fig. 1 gives a graph of the percentage of total DNA eluted against different fractions obtained with 100 ml of different eluting agents. It also gives the elution profiles obtained, wherein the total optical density at 590 nm is plotted against the test-tube number or the fractions eluted.

A known amount of homogeneous DNA solution in glycine–sodium hydroxide buffer (pH 8.6, 0.054 *M*) was chromatographed on three separate IR-120 Al^{3+} columns, previously equilibrated by the above buffer and maintained at three different temperatures, *viz.* 4, 27 and 42°.

The effect of temperature variation on adsorption and elution is given in Table II. Fig. 2 gives a graph of the percentage of total DNA eluted against different fractions obtained with 100 ml of different eluting agents. It also gives the elution profiles.

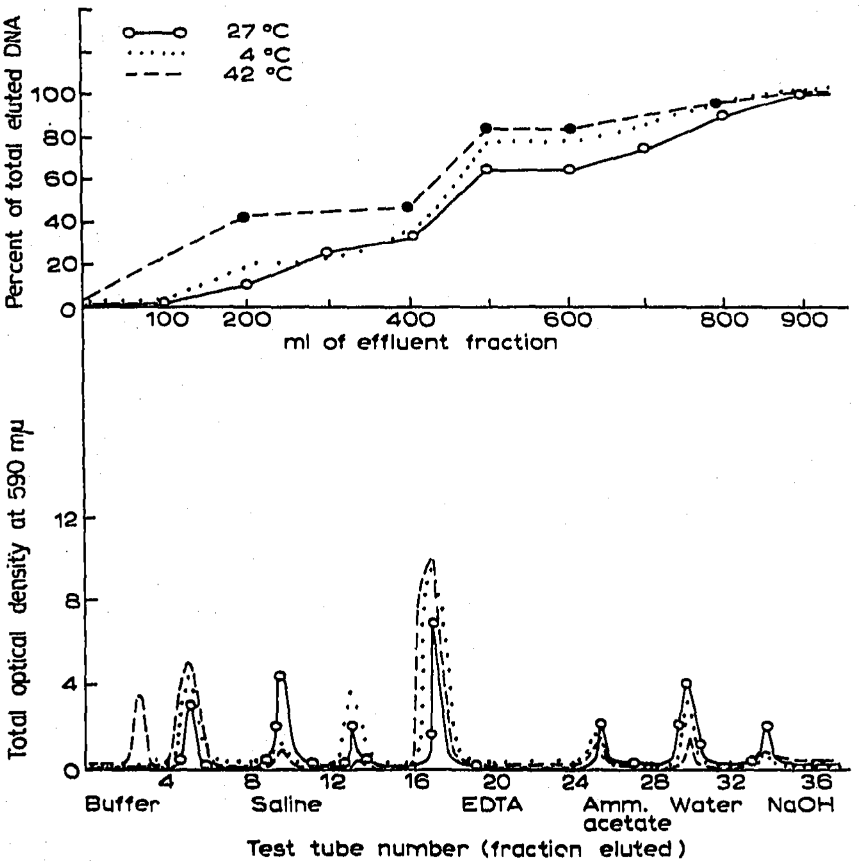

Fig. 2. Effect of temperature variation on the chromatographic profiles of DNA on an IR-120 Al^{3+} column.

Results and discussion

pH variation and profiles of DNA

Table I shows that there is 100% retention only at pH 8.6, and somewhat less retention at pH 6.8 and 10.0. The adsorbed DNA is nearly 100% eluted in each case and the nature of the elution profiles remains more or less the same. The percentage distribution of the DNA in the different fractions is, however, altered to some extent (Fig. 1).

A change in pH is known to bring about conformational changes in DNA[1,2]. MATHIESON AND MATTY[12] have noted the variation in intrinsic viscosity and streaming birefringence by changing the pH of the sodium salt of calf thymus DNA. In the present studies, however, no dramatic conformational changes in DNA can have taken place over the narrow pH range used, or else these would have been reflected in the chromatographic behaviour of DNA, as a definite conformation of DNA is necessary for its adsorption on an IR-120 Al^{3+} column[5,6,13].

Temperature variation and profiles of DNA

Table II shows that DNA is 100% retainable and 100% elutable at 4° and 27°. Only 80% of DNA is adsorbed at 42°, the remaining amount coming off in the effluent and buffer washing. The elution in this case is 95%. The nature of the elution profiles obtained at different temperatures is broadly the same. The percent distribution of eluted DNA in the different fractions obtained shows minor modifications with variation in temperature (Fig. 2).

The present observations regarding the adsorption and elution of DNA at higher temperature, using an IR-120 Al^{3+} column, are comparable to those of MILLER[14] who showed that adsorption of native DNA at the water–mercury interphase is temperature dependent. The small variations observed in percentage distribution in profiles on the IR-120 Al^{3+} column may be due to easy dissociation of a complex between DNA and IR-120 Al^{3+}. MAY[15] has also shown that temperature influences the dissociation and formation of aggregates of thermally denatured DNA. Though elution at higher temperature may be quicker than at lower temperature, chromatography at a lower temperature is desirable as it reduces the risk of cleavage of labile bonds, and of bacterial or enzymatic degradation.

Structural transitions and retainability of DNA. The partial loss of retainability of DNA at 42° may be closely related to a partial change in the three-dimensional structure of the molecule. TIKCHONENKO *et al.*[16,17] have also shown that thermal denaturation of DNA proceeds through a state characterised by a number of properties intermediate between the rigid double-stranded and flexible single-stranded structures. This has been subsequently confirmed by BRAHMS AND MOMMAERTS[18] and is also supported by electron microscopic and viscometric studies[19] of the alterations introduced by the increased temperature in the secondary structure of DNA. The temperature of 42° is, however, much below the T_m of DNA studied, and therefore, the contribution of such a transition to the overall phenomenon is likely to be only marginal.

Effect of Mg^{2+} on the chromatographic behaviour of DNA at 42°. DNA effluent from an IR-120 Al^{3+} column at 42°, containing 20% nonretainable DNA, was also nonretainable at 27°. It was equilibrated with 0.001 M Mg^{2+} at 4° for 24 h and was again chromatographed on an IR-120 Al^{3+} column regulated at 42 and 27°. It was still found to be nonretainable at 42°, but retained at 27°.

It may be possible that at 42°, DNA is "demagnesised" resulting in an alteration of structure and subsequent loss in retainability. Mg^{2+} may be undertaking a "repair" of the "injury" caused by the higher temperature, attributing a finite three-dimensional structure acceptable to the IR-120 Al^{3+} column, hence causing retention at 27°. At 42°, however, DNA may once again undergo "demagnesisation" (dissociation) resulting in its nonretainability. It may be recalled here that the suggestion made by PEACOCKE[20] that the use of heat, acid and alkali be avoided, not only during isolation of DNA, but also in the subsequent physico-chemical studies, still holds to day.

Variation in the pH over which DNA is stable had no significant effect on the profiles. DNA was 100% retainable at 4 and 27°, but only 80% was retainable at 42°, the temperature considerably below the T_m. The elution profiles were quite comparable also at the different temperatures studied. Minor deviations observed in percent distribution in different fractions, as a result of pH and temperature variation, may be due to a partial alteration, if any, in the structure of DNA.

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