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THE BEHAVIOUR OF DNA ON COLUMNS OF HISTONE KIESELGUHR

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SUMMARY

A method is described for the study of DNA histone interactions using columns of histone kieselguhr complex. This interaction depends on the type of histone used and is altered in the presence of other histones.

INTRODUCTION

Recently a method has been established in this laboratory for the fractionation of nucleic acids on columns of polylysine kieselguhr[1,2]. The mechanism of fractionation, *i.e.*, the interaction and subsequent dissociation between DNA and polylysine, suggested that a similar system might be used to study the binding of DNA to histones. Such a technique would enable detailed comparisons to be made between histones and DNA from different sources.

The feasibility of such a method has been demonstrated for several histone fractions by the work of MAZEN AND CHAMPAGNE[3]. The present work describes the preliminary results from a study of the behaviour of calf thymus DNA on columns of four calf thymus histone fractions.

PROCEDURES

DNA

Calf thymus DNA (sodium salt) was obtained from the Sigma Chemical Co. Ltd.

Preparation of calf thymus histones

Histones were prepared from fresh calf thymus (100 g) by method 2 of JOHNS[4]. The dried histone fractions were stored at −20°.

Preparation of histone kieselguhr columns

Hyflo Super Cell (kieselguhr) was obtained from Johns-Manville Co., London, and extensively washed in distilled water to remove ultraviolet absorbing material. The powder (1.5 g) was suspended in 0.01 *M* phosphate buffer pH 6.7 (10 ml) and the

suspension boiled and cooled to prevent the formation of air bubbles. Histone solution (1.5 mg/ml) in 0.01 *M* phosphate buffer pH 6.7 was prepared immediately before use and 1 ml added dropwise to the kieselguhr suspension with continuous stirring. A pyrex glass column (length 30 cm, I.D. 5 mm) was packed with: (a) 2 to 3 mm of cellulose powder, (b) the kieselguhr histone complex, (c) 2 to 3 mm of kieselguhr, using a constant air pressure of 200 g/cm^2. The packed column was washed with 0.01 *M* phosphate buffer pH 6.7 and used the same day.

Elution and fraction collection

Columns were eluted with linear gradients of sodium chloride in 0.01 *M* phosphate buffer pH 6.7 as described by AYAD *et al.*[5]. The gradients were pumped through the column at a flow rate of 5 ml/h using a LKB peristaltic pump. Fractions (1 ml) were collected on a LKB Ultrorack fraction collector.

The extinction of the eluate was continuously monitored using a Uvicord recording system at 257 nm. Each fraction in the urea of the peak was also analysed for DNA and sodium chloride by measuring the extinction at 260 nm, and the refractive index, respectively. The refractive index of each fraction was converted to sodium chloride molarity by comparison with a standard curve.

Polyacrylamide gel electrophoresis

Polyacrylamide gel containing 15% (w/v) acrylamide was prepared by the method of REISFELD *et al.*[6]. The gel was polymerized in pyrex glass tubes (length 7 cm, I.D. 5 mm) under a layer of 3 *M* urea. Samples of the histone fractions (10 µg) together with a bovine serum albumin standard (5 µg), dissolved in 0.025 ml of 10 *M* urea, were carefully layered onto the gels. Electrophoresis was carried out for 90 min at 3 to 4 mA per tube.

The gels were removed from the tubes and stained overnight in 1% (w/v) Naphthalene Black 12B (C.I. 20470) dissolved in a mixture of 40% (v/v) ethanol and 7% (v/v) acetic acid. The gels were destained by repeated washing in the ethanol–acetic acid mixture and scanned at 600 nm using the gel scanning attachment for the Unicam SP 500 spectrophotometer.

RESULTS

Recovery of DNA from a blank column of kieselguhr

In order to check that DNA did not bind to the kieselguhr a blank column was prepared, as described in METHODS, but without the addition of histone. Calf thymus DNA (79 µg) was loaded onto the column and eluted with 0.01 *M* phosphate buffer pH 6.7. 98% of the added DNA was recovered in the eluant.

Analytical polyacrylamide gel electrophoresis of calf thymus histone fractions

The results of electrophoresis are shown in Fig. 1, and indicate that histones F1, F2b, and F3 contained one main component, whereas histone F2a contained two main components. All the fractions were, however, contaminated with small quantities of other proteins.

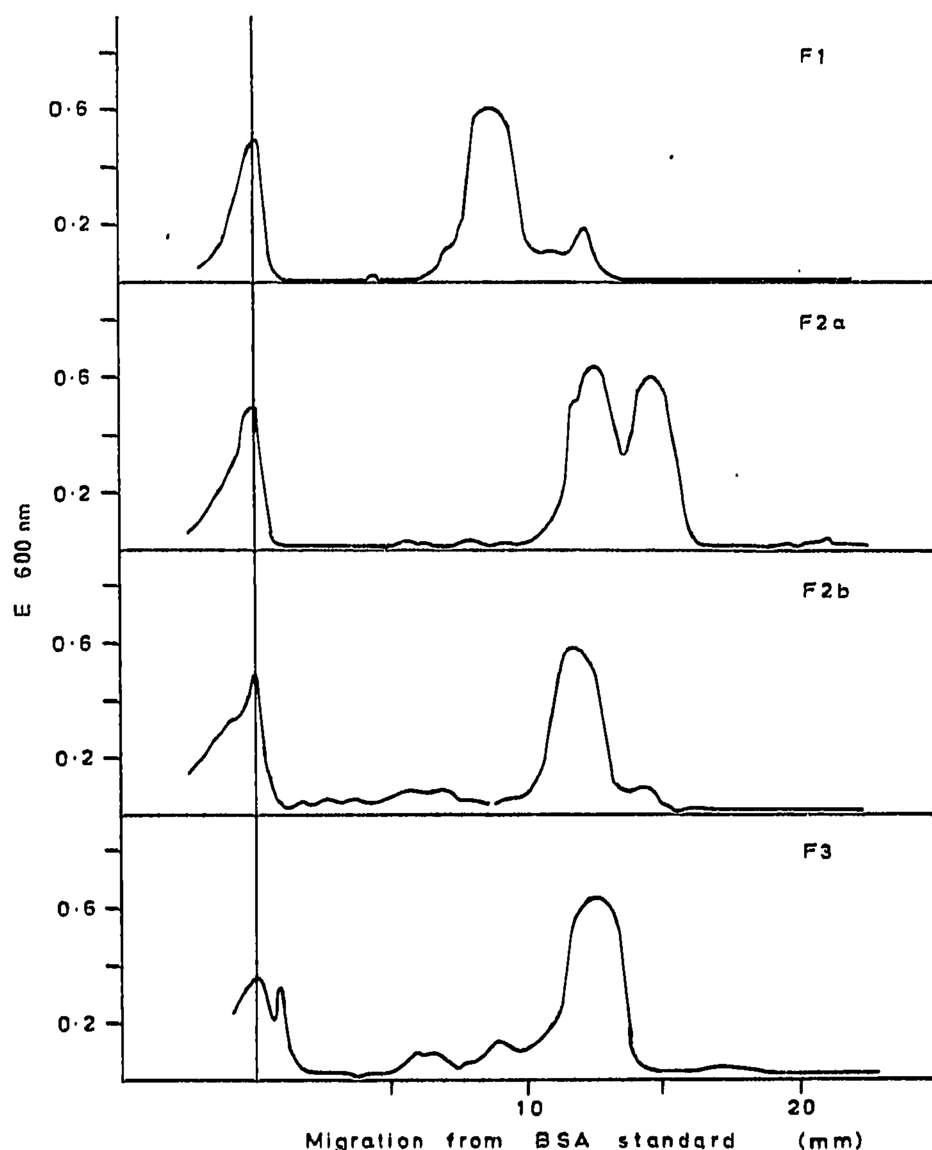

Fig. 1. Polyacrylamide gel electrophoresis of calf thymus histone fractions (see text). The first peak is the bovine serum albumin standard.

Elution of DNA from columns of calf thymus histone

Columns were prepared using calf thymus histones F1, F2a, F2b and F3 and loaded with 75 μg of calf thymus DNA. The DNA was eluted with a linear gradient of NaCl in 0.01 *M* phosphate buffer pH 6.7. The results are shown in Fig. 2 and Table I.

TABLE I

NaCl MOLARITY AT WHICH DNA ELUTES FROM COLUMNS

Histone fraction	*No. of experiments*	*NaCl molarity at peak*	
		Mean	*Range*
F1	4	0.63	0.62–0.65
F2a	7	0.66	0.56–0.85
F2b	3	0.45	0.43–0.46
F3	4	0.70	0.68–0.71

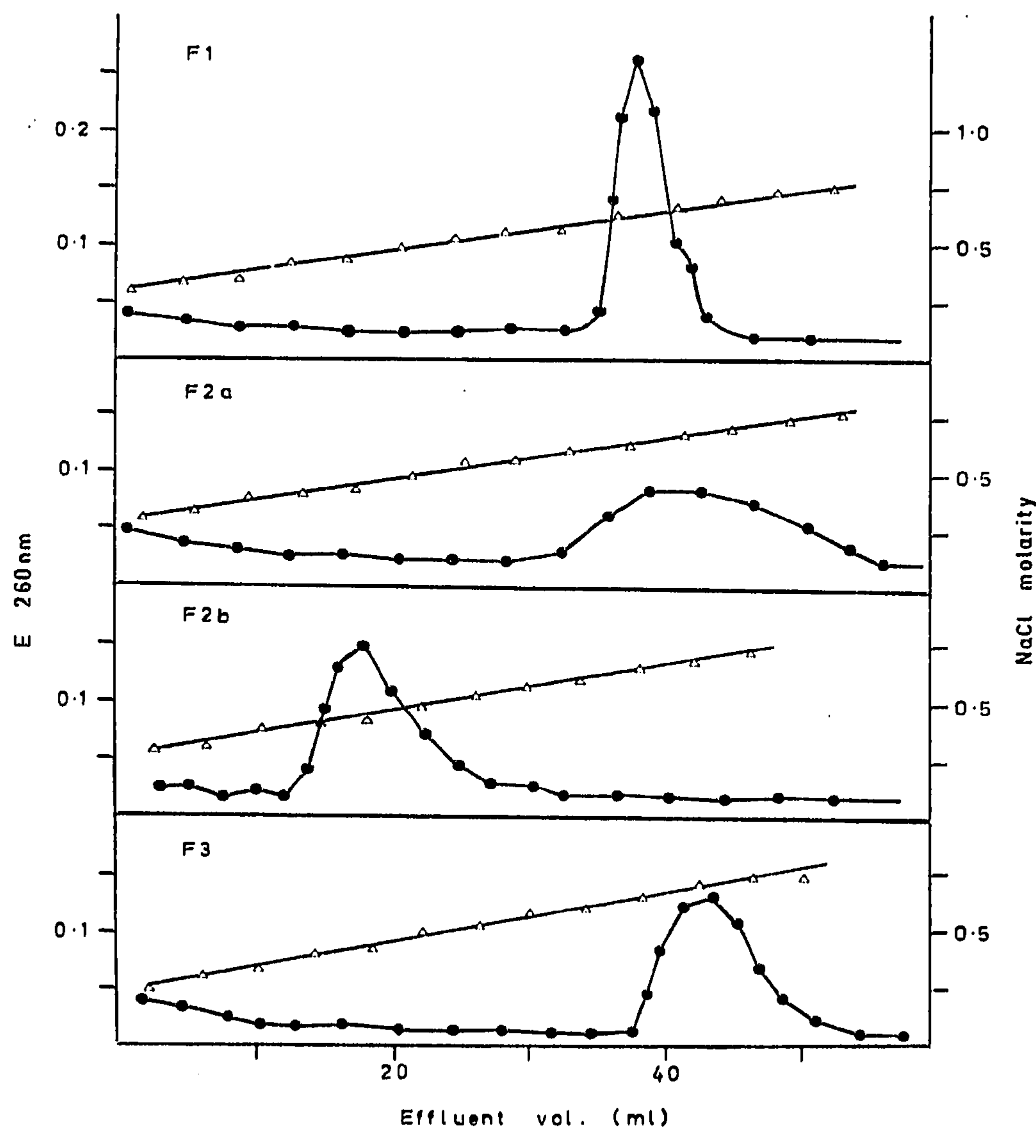

Fig. 2. Elution profiles of calf thymus DNA (75 μg) from columns of calf thymus histones. ●—●, E 260 nm; △—△, NaCl molarity.

Reproducibility of the elution pattern was good except for columns prepared with histone F2a. The variability of these results could be related to the fact that the histone contained two main components as judged by polyacrylamide gel electrophoresis. The mean recovery of loaded DNA was 68%.

Elution of DNA from a column of mixed histone F2b and F3

In order to demonstrate the effect of the presence of one histone on the dissociation of DNA from another, a column was prepared containing 1.5 mg of both histones F2b and F3. DNA was eluted from this column by a linear gradient of NaCl in 0.01 *M* phosphate buffer pH 6.7. The results are shown in Table II.

Dissociation of DNA from histone F2b would be expected to occur at 0.45 *M* NaCl (see Tables I and II). However, at this molarity no DNA elution was detected,

TABLE II

THE EFFECT OF MIXING F3 AND F2b ON THE ELUTION OF DNA FROM COLUMNS

Histone fraction	*No. of experiments*	*Mean NaCl molarity at peak*
F3	4	0.70
F2b	3	0.45
F3 + F2b	2	0.62

the dissociated DNA remaining bound to histone F3. Dissociation from histone F3, however, occurred at 0.62 *M* NaCl compared with 0.70 *M* for a column of F3 alone.

DISCUSSION

The results show that all four fractions of calf thymus histone bind to kieselguhr, and that DNA can subsequently bind to the histone. Bound DNA can be eluted with a gradient of increasing sodium chloride molarity. The molarity at which DNA was eluted from the column varied with the histone fraction used but was reproducible for those histone fractions containing one main component.

DNA added to a column of mixed histone F2b and F3 eluted at a molarity lower than was obtained on a column of F3 alone. It is suggested that this effect is due to a reduction in the number of binding sites on the DNA available to histone F3 due to competitive binding with F2b at low ionic strength. The subsequent restriction of mobility of the DNA would prevent further binding to F3 at ionic strengths high enough to dissociate the DNA–F2b complex. This model would suggest that the elution molarity depends primarily on the number of binding sites rather than the nature of the binding sites.

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