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INTERACTIONS BETWEEN HISTONE AND DNA FROM CALF AND HUMAN TISSUES USING ACTIVATED SEPHAROSE COLUMN CHROMATOGRAPHY

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SUMMARY

Histone–DNA interactions have been studied using columns of histones bound to cyanogen bromide-activated Sepharose. Significant differences in the strength of ionic binding between histone and DNA in homologous systems of calf thymus and human skin tissue, and the heterologous system of calf histone and human DNA have been observed. The interaction depends on the source of histone and DNA and is highly specific.

INTRODUCTION

It is now widely accepted from polyacrylamide gel electrophoresis and other physical data that histones from different species vary only slightly in their molecular size, charge and amino acid sequence. This invariance implies that either the sequence of amino acids is important or that histones are non-specific in their interaction with DNA. We have examined the specificity of this interaction by comparing calf thymus and human skin histones and DNA with respect to the salt molarity required to dissociate the histone–DNA complex.

Following the successful development of histone–Kieselguhr columns in this laboratory[1], it was decided to immobilize the histones still further by chemically binding them to cyanogen bromide-activated Sepharose 4B. DNA can be loaded on to this column and eluted with a linear salt gradient. The eluting salt molarity differs for each histone fraction and also varies according to the species.

MATERIALS AND METHODS

DNA

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

Preparation of human skin DNA

Under sterile conditions a Davol dermatone was used to remove a thin layer of skin at dermal level from amputated legs. The skin was deep frozen with a small

amount of Hanks balanced salt solution. The dermis was removed from the epidermis by clamping the skin between two glass plates (20 cm square) and heating at 60° in a water-bath for 2 min. Separation was effected using forceps.

Saline EDTA (1 ml; 0.15 *M* NaCl + 0.1 *M* EDTA; pH 8.0) was added per gram of epidermis. Pronase (1 mg/ml) and sodium dodecyl sulphate (2% by wt.) digested the epidermis at 25° to a homogeneous suspension. Protein was then extracted three times with chloroform–isoamyl alcohol (24:1) using sodium perchlorate to dissociate the DNA–protein complex.

DNA was subsequently spooled by precipitation in two volumes of ice-cold ethanol and RNA removed by digesting with heat-treated RNAase (50 μg/ml) at 37°.

The molecular weight was determined by sedimentation analysis: $S^0_w20 = 16.4 \times$ molecular weight $= 4.6 \times 10^6$. The guanine+cytosine content by T_m measurement was 44%.

The diphenylamine estimation for DNA[2] and the Lowry assay for protein[3] were also performed.

Preparation of histones

Calf thymus histones were prepared from fresh tissue by Method No. 2 of Johns[4] and the dried histone fractions stored at −20°.

Human skin histones were prepared from epidermis separated by the above method. Using liquid nitrogen, the epidermis was ground to a fine powder in a mortar and pestle, and Method No. 2 of Johns[4] was used for histone extraction. Polyacrylamide gel electrophoresis was carried out on each fraction.

Preparation of histone–CNBr–Sepharose

Sepharose 4B was obtained from Pharmacia Fine Chemicals. 20 ml were suspended in 20 ml of distilled water, and 4 g of finely powdered CNBr were added. The suspension was stirred and maintained at 4° and pH 11.0 (adjusted by the addition of potassium hydroxide) for 15 min.

The reaction was quenched by the addition of ice and the product washed on a glass-filter with phosphate buffer 0.01 *M* pH 8.5 (250 ml).

Histone (3 mg) was dissolved in 3 ml of 0.01 *M* phosphate buffer containing 0.5 *M* NaCl, pH 8.5. The histone solution was added to the CNBr–Sepharose and gently stirred for more than 20 h. The excess protein was removed by washing with saline phosphate buffer, and any remaining active groups were blocked by treatment with 1 *M* ethanolamine for 2 h at pH 8.0. The final product was washed four times alternately with high and low pH buffer solutions containing 1 *M* NaCl. The suspension was poured into a Pyrex glass column (10 cm × 1.2 cm I.D.) with a gauze filter covering the exit bung and the column washed with 0.01 *M* phosphate buffer, pH 6.7.

Elution and fraction collection

Columns were eluted with a linear gradient of sodium chloride in 0.01 *M* phosphate buffer at 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 an LKB peristaltic pump. Fractions (1 ml) were collected on an LKB Ultrorack fraction collector.

The extinction of the eluate was continuously monitored using a Uvicord

recording system at 257 nm. Each fraction was analysed for DNA and NaCl by measuring the extinction at 260 nm and the refractive index, respectively. The refractive index of each fraction was converted to NaCl molarity by comparison with a standard curve.

RESULTS

Elution of calf and human DNA from columns of calf and human histone fractions F_1, F_{2b} and F_3

Columns of calf and human histone fractions F_1, F_{2b} and F_3 were prepared. Calf or human DNA (100 μg) was eluted from the column by a linear salt gradient in 0.01 *M* phosphate at pH 6.7. The results are shown in Fig. 1 (a, b, c) and Table I. The columns were reproducible and could be used again.

Control columns of Sepharose 4B, cyanogen bromide-activated Sepharose not treated with histone and/or ethanolamine and an ethanolamine-treated CNBr–Sepharose column were prepared.

Human DNA was loaded and eluted with a linear salt gradient. The DNA was eluted in the bed volume with 100% recovery in the Sepharose and ethanolamine-treated columns. The untreated CNBr–Sepharose columns retained 70% of the DNA, 30% being eluted by 0.25 *M* NaCl in two peaks.

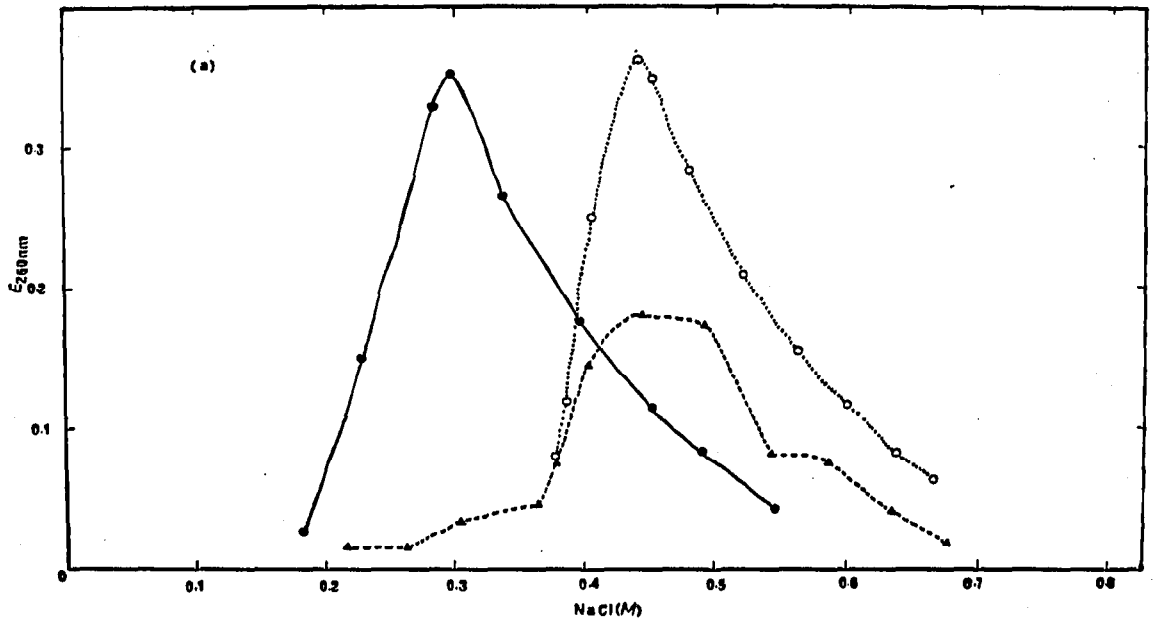
DISCUSSION

Histone-activated Sepharose columns have proved to be a model system for studying histone–DNA interactions *in vitro*. Complex precipitation is avoided and, since the columns can be re-used, only small amounts of histone are required. Non-ionic dissociators can be used since the histone is covalently bound to the Sepharose. This technique will enable histone–DNA interactions to be fully characterized with respect to dissociation of the complex.

The specificity of histone–DNA interactions has been shown by the difference in electrostatic binding between the systems. Widely differing results are obtained when the same human DNA is loaded on to columns of calf and human histones, or the same calf histone fractions are loaded with human and calf DNA.

The systems are therefore not interchangeable.

Human DNA is dissociated more easily than calf DNA from calf histones. From the difference in this dissociating salt molarity, it is possible to assess the degree of specificity of each fraction. Calf DNA is dissociated from calf histone F_1 at almost the same salt molarity as human DNA and therefore does not show any increased affinity for calf histone (see Table I). Fractions F_{2b} and F_3, however, show a wide difference in the dissociating salt molarity for the two DNAs and show a preference for their homologous DNA. The order of specificity is $F_3 > F_{2b} > F_1$. Bartley and Chalkley[6] and unpublished results in this laboratory show that the histones may be classified in terms of increasing amounts of non-electrostatic interaction with DNA, *viz.* $F_3 > F_{2b} > F_1$. This order is the same as the specificity order, and non-electrostatic interactions would appear to infer specificity. This could result in the supercoil models proposed by Pardon and Wilkins[7] from X-ray diffraction experiments and the Bram and Ris model[8].


(a)
ε_{260nm}
0
0.1
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0.6
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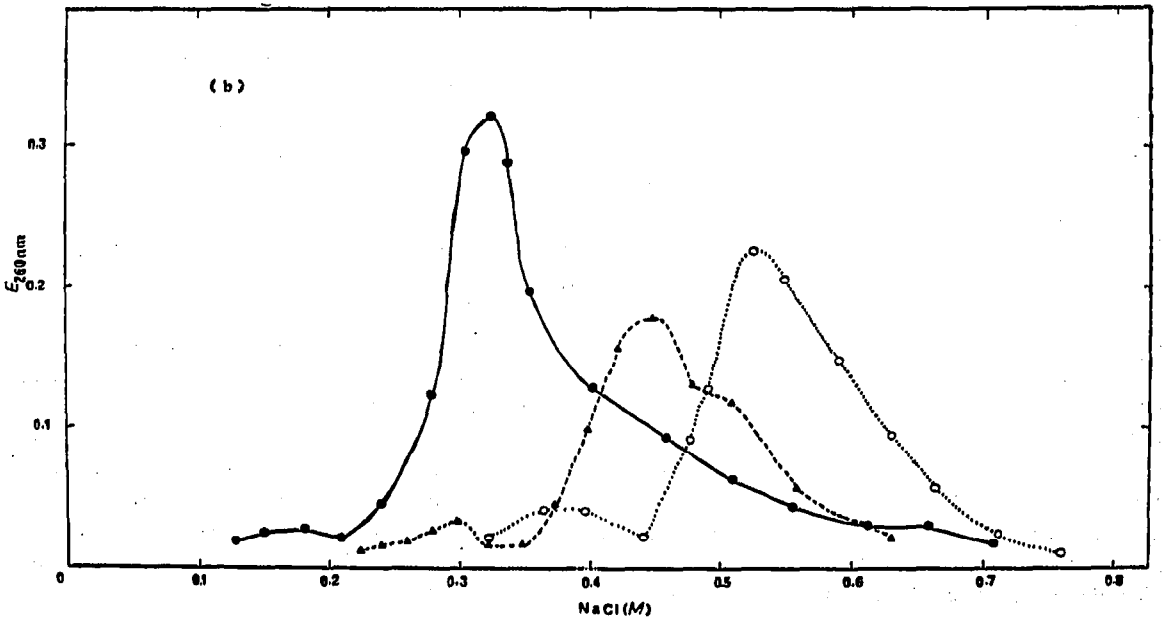
(b)
ε_{260nm}
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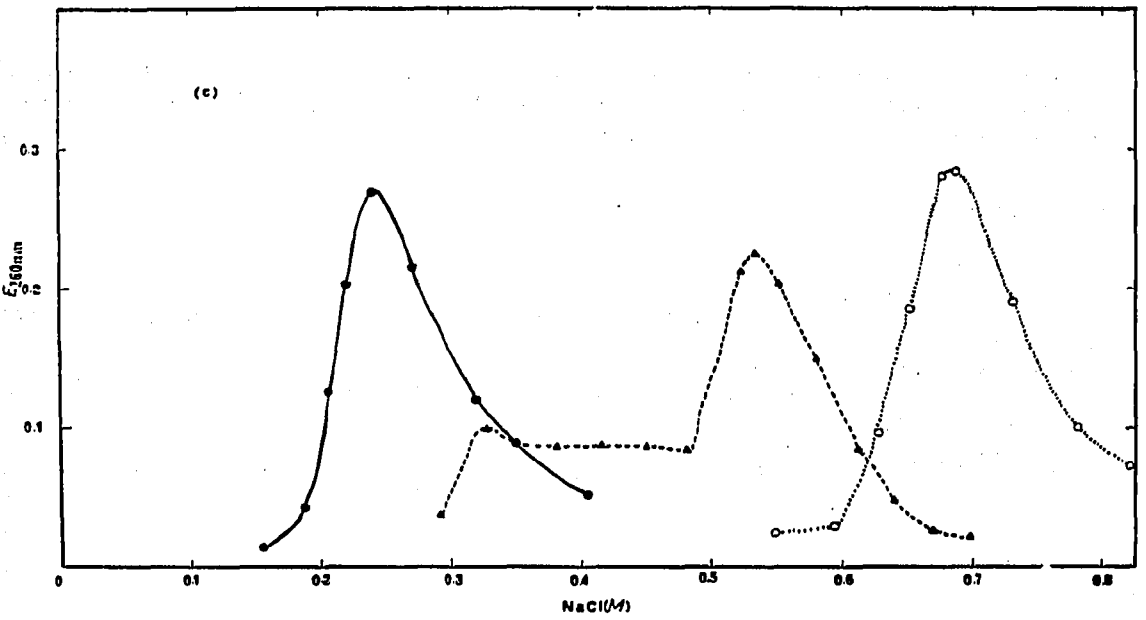

Fig. 1. Elution profiles of DNA from activated Sepharose–histone columns. (a) Histone fraction F_1. (b) Histone fraction F_{2b}. (c) Histone fraction F_3. ○···○, Calf thymus DNA eluted from calf thymus histone column; ●—●, human skin DNA eluted from human skin histone column; ▲--- ▲, human skin DNA eluted from calf thymus histone column.

TABLE I

VALUES OF SALT MOLARITY AT PEAK OF DISSOCIATION

DNA isolated from	*Histone fraction isolated from*					
	Calf thymus			*Human skin*		
	F_1	F_{2b}	F_3	F_1	F_{2b}	F_3
Calf thymus	0.46	0.53	0.675			
Human skin	0.45	0.45	0.53	0.295	0.325	0.25

The theory is further strengthened from data on amino acid analysis of histones. The lysine-rich histones have a higher ratio of basic to acidic residues and the expected ease of dissociation should be $F_3 > F_{2b} > F_1$. The human homologous system shows much greater ease of dissociation, *viz.* $F_3 > F_1 > F_{2b}$, and emphasizes the difference between the calf and human systems.

The remarkable differences in the strength of the electrostatic binding show the specificity of the interaction. Even though polyacrylamide gel electrophoresis showed the fractions from the two species to be similar physically, the binding studies prove the importance of the non-electrostatic interaction.

The results show that histone is specific for DNA from the same species.

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REFERENCES

1 S. R. Ayad and A. Wilkinson, *J. Chromatogr.*, 66 (1972) 277.
2 K. Burton, *Biochem. J.*, 62 (1956) 315.
3 O. H. Lowry, N. J. Roubrough, A. Lewis, A. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
4 E. W. Johns, *Biochem. J.*, 92 (1964) 55.
5 S. R. Ayad, R. W. Bonsall and S. Hunt, *Anal. Biochem.*, 22 (1968) 533.
6 L. Bartley and R. Chalkley, *J. Biol. Chem.*, 247 (1971) 11.
7 J. Pardon and M. Wilkins, *J. Mol. Biol.*, 68 (1972) 115.
8 S. Bram and H. Ris, *J. Mol. Biol.*, 55 (1971) 325.