

STUDY OF HISTONE–HISTONE INTERACTIONS BY AFFINITY CHROMATOGRAPHY

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1. Introduction

The structure and the function of the chromatin are depending upon the interactions between its major components: DNA, histones and non-histone proteins (NHP). Histones and probably NHP are known to interact with DNA at least by electrostatic binding. But little is known about protein–protein interactions.

Histone–histone interactions have been shown by studying native chromatin and in vitro reconstituted nucleohistones [1]. Further evidence for such interactions was obtained by the isolation of an equimolecular complex formed by the association of histones H₂B and H₂A [2,3]. More recently, complex formation involving all histones but H₁ has been reported [4–6].

These histone–histone complexes were studied either by chemical methods [2,6] or by physico-chemical methods such as ultracentrifugation [3,6] and measurements of fluorescence anisotropy and circular dichroism [4,5].

This paper describes the use of affinity chromatography to study the histone–histone interactions. In previous experiments, the fixation of histones on an insoluble matrix was used for fractionating nucleic acids [7] and protein kinases [8].

Abbreviations: PCA: perchloric acid; TCA: trichloroacetic acid. **Histones Nomenclature:** H₁ (formerly histone I_A or F₁); H₂B (formerly histone II_{B2} or F_{2b}); H₂A (formerly histone II_{B1} or F_{2a2}); H₃ (formerly histone III or F₃); H₄ (formerly histone IV or F_{2a1}). This nomenclature has been proposed at the recent Symposium on the structure and function of Chromatin. Ciba Foundation. London April 1974.

2. Materials and methods

Calf thymus whole histone was extracted with 0.25 N HCl from the chromatin prepared according to Johns [9]. Histone H₄ was isolated by gel-filtration chromatography [10] from the F_{2a1} fraction obtained by the selective extraction of Johns [11].

The fixation of the histone H₄ to Sepharose 4B (Pharmacia, Uppsala) was achieved by using the cyanogen bromide method of Ayad and Parker [7].

Whole histone dissolved in 0.01 M HCl was applied to a column of histone H₄ Sepharose (20 × 0.9 cm) equilibrated in 0.01 M sodium phosphate buffer pH 6.7 containing 0.02% sodium azide.

The column was eluted first by 2 M NaCl, washed with deionized water and then eluted by 0.01 N HCl.

All eluants contained 0.02% sodium azide.

Proteins were detected in the effluent by the method of Lowry et al. [12] and analyzed further after dialysis and freeze-drying, on gel electrophoresis according to Panyim and Chalkley [13].

3. Results and discussion

The elution pattern of histones fractionated by affinity chromatography is presented in fig. 1. Four fractions were obtained and submitted to gel electrophoresis (fig. 2). The histone H₁, which is the major component of the fraction A, is not retained by the histone H₄-Sepharose. In a control experiment performed in the same conditions with untreated Sepharose 4B, all histones were eluted in one single

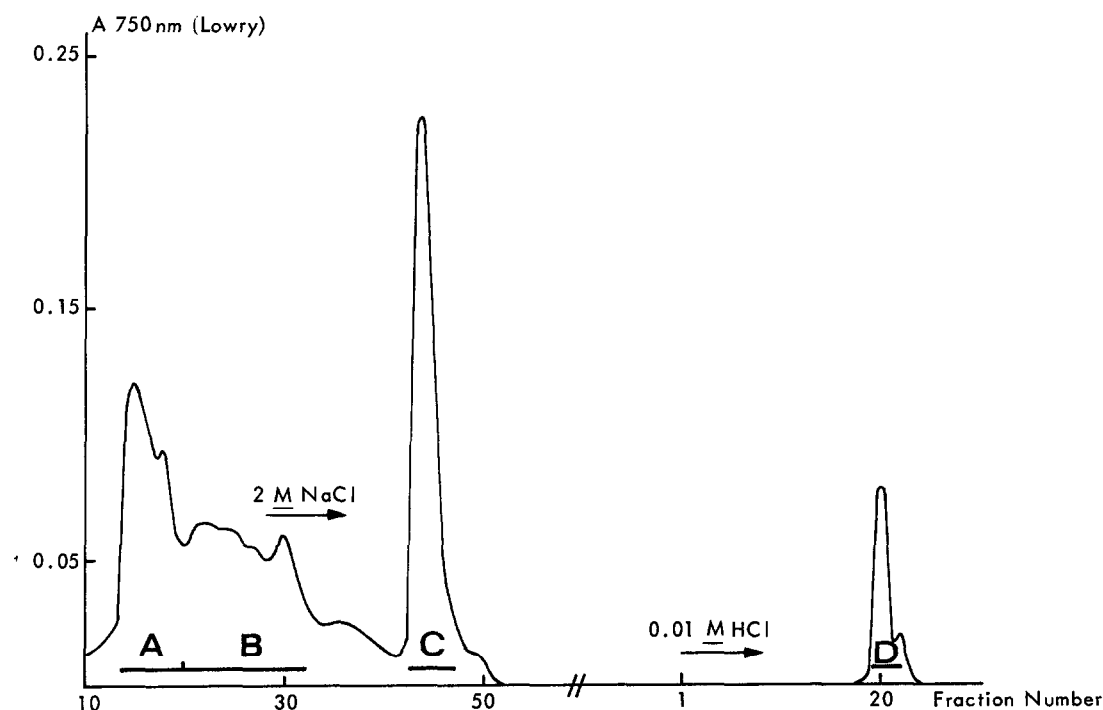


Fig. 1. Affinity chromatography of calf thymus histones on histone H_4 -Sepharose column. Whole histones (10 mg) were dissolved in 0.3 ml 0.01 M HCl and kept at 4°C for 48 hr before application on the top of the column. (20 × 0.9 cm). After washing with 0.01 M Na phosphate pH 6.7, column was eluted first with 0.01 M Na phosphate pH 6.7 containing 2 M NaCl and then with 0.01 M HCl, as indicated by the arrows. Flow rate was 4.8 ml/hr and fractions of 1.2 ml were collected.

peak, the elution volume of which being identical to that of fraction A.

The histones H_{2A} and H_{2B} are found in the fraction C, eluted with 2 M NaCl.

The fraction B corresponds to a mixture of histones H_1 , H_{2A} and H_{2B} .

The histone H_4 is the major component of the fraction D eluted with HCl 0.01 N. A minute amount of histone H_3 was also characterized in that fraction. The low recovery of histone H_3 may be due to a dimerization process or to the peculiar sensitivity of that histone to proteolysis [14].

From these preliminary results we can infer that privileged interactions are developing specifically

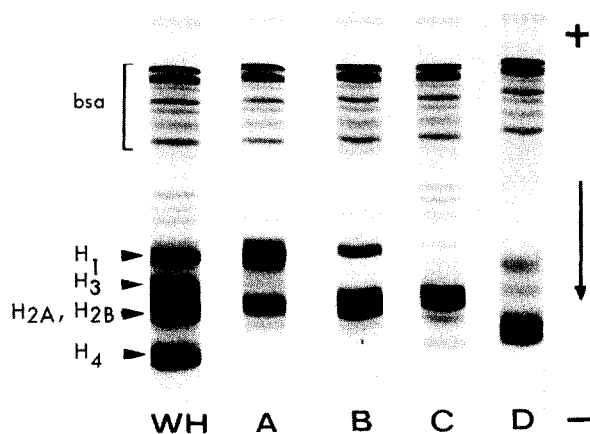


Fig. 2. Polyacrylamide gel electrophoresis of histone fractions obtained by affinity chromatography. WH: calf thymus whole histone. Gels A, B, C and D correspond to chromatographic fractions A, B, C and D, respectively. bsa: bovine serum albumine used as marker. Gels (6 × 0.9 cm) according to Panyim and Chalkley [13] were loaded with 20 µg of histone and were run at 1.5 mA/gel for 4 hr. Staining was achieved with 1% Amidoblack in ethanol-water 7:3 (v/v). Gels were destained electrophoretically in 7% acetic acid.

between some histones. Thus, with the histone H₄-Sephrose as support, among all the histones, the histone H₄ appears to be the most strongly attached. Our results are consistent with the conclusions of Bradbury [15] who considers that the self-interactions of histones are the strongest.

On the other hand the histones H_{2A} and H_{2B} are eluted simultaneously with milder conditions than those used for histone H₄. At the present time, we can only conclude that these two histones present together or separately some affinity for the histone H₄.

Moreover, these findings can be related to the cross interaction between the histones H_{2A} and H_{2B}, already evidenced by several groups [2-6].

The early elution of the histone H₁ shows the lack of affinity of that histone for the other histones. The easy extraction of this very lysine-rich histone from the chromatin either by 0,5 M NaCl or 5% PCA or TCA may be due to this lack of cross interactions with the other histones [6].

Although our results were obtained with histones prepared by acid extraction, there are in agreement with those of Kornberg and Thomas [6] obtained with histones extracted from chromatin in milder conditions.

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