EXPOSURE OF HISTONE F_1 SUBFRACTIONS IN CHROMATIN

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Received May 27,1975

SUMMARY. Antisera specific to the five lysine-rich (F_1) histone subfractions, present in rat thymus, were used to study the exposure of each of the subfractions in rat thymus chromatin. The results suggest that: 1) in chromatin the five F_1 subfractions are arranged in a similar manner, and 2) the F_1 histone antigenic determinants which are exposed (are avaiable to interact with antibody) are determinants which are shared among the F_1 subfractions.

The structure of chromatin is determined by interactions between the histones and DNA (1). The histones are a group of basic proteins composed of 5 major fractions denoted as F_1, F_2b, F_2a_1, F_2a_2 and F_3 (2). The lysine rich histone fraction, F_1, is composed of several components which can be resolved by electrophoretic and chromatographic techniques (3-6). In the present study we investigate whether the various F_1 subfractions present in one tissue differ in the way they are arranged in chromatin. For this study we used rat thymus chromatin. In this tissue there are 5 chromatographic F_1 subfractions. Previously we elicited antisera specific against each of these subfractions and investigated in detail the immunological relationships between the five F_1 subfractions present in rat thymus (7). We found that each fraction contains both specific and common (i.e., cross-reacting with other F_1 histones) determinants. The techniques we use to study the arrangement of the individual F_1 subfractions in chromatin are based on our earlier findings that antisera elicited by purified histones specifically bind to chromatin and that it is possible to use serological techniques to study the state of histones in native chromatin (8,9)

MATERIALS AND METHODS

F_1 histones from rat thymus were fractionated into 5 components by chromatography on Amberlite IRC-50 (4,7). Tubes containing the various fractions were pooled, dialysed against 0.15M NaCl, 0.01M Tris HCl 0.15 mM $CaCl_2$, 0.5 mM $MgSO_4$, pH 7.4, and the volumes reduced approximately 10-fold by vacuum dialysis. The "Triton nuclear chromatin" was obtained from fresh rat thymus by the method of Tata et al. (10). Chromatin at 0.5 mg/ml in mM Tris HCl, pH 8.0 was sonicated at 4°C for 2 min at 60 kw with a Sonifier (Ultrasonics model W 185 13) equipped with a model L tip.

The antisera to purified F_1 subfractions from rat thymus used in the present study have been described before (7). Quantitative microcomplement fixation reactions were performed as described by Stollar and Ward (11). Binding of antihistone sera to chromatin was tested employing chromatin as an immunoabsorbant (8,9). To various amounts of chromatin in a total volume of 0.5 ml of 1 mM Tris HCl buffer pH 8.0 a constant amount of antiserum was added. The mixtures were incubated for 1 hr at 37°C and 4 hr at 4°C, with occasional shaking. After this time the incubation mixtures were made 0.15M in NaCl and 0.01M in sodium phosphate pH 6.8 and incubated at 4°C for 30 min. The chromatin and the antibodies bound to it were pelleted by centrifugation at 5000 x g for 20 min. The supernatant containing unadsorbed antibodies was diluted 100-fold with a solution of 0.01M Tris HCl, 0.14M NaCl, 0.15 mM $CaCl_2$, 0.5 mM $MgSO_4$, 1 mg/ml bovine serum albumin, pH 7.4, and complement. For the complement fixation assay 1.0 ml of this solution was added to 0.2 ml of antigen and incubated at 4°C for 16 hr prior to the addition of red blood cells.

RESULTS AND DISCUSSION

We have reported that sonication exposes histone determinants in chromatin and that for comparative studies it is important to use both sonicated and non-sonicated chromatin (9). The binding of the various F_1 antisera to both sonicated and non-sonicated rat thymus chromatin are presented in Fig. 1. It can be seen that each of the sera was adsorbed by chromatin. An increase in the amount of chromatin used to adsorb the antisera caused a bigger drop in the complement fixation curve indicating that more antibodies were bound to chromatin. With the exception of antiserum to peak II (Fig. 1-B, 1-G) sonication did not alter the capacity of chromatin to adsorb antibodies. In the case of anti-peak II antibodies, sonicated chromatin served as a more potent immunoadsorbant than non-sonicated chromatin. These results suggest that sonication exposed antigenic determinants in peak II so that they become more available to interact with homologous antisera. Further examination of the data presented in Fig. 1 reveals that a given amount of chromatin adsorbed the various antibodies to a different degree (for example, compare Fig. 1-C to 1-E). This effect could be due to the fact that the 5 subfractions are not present in equal amounts in chromatin. To determine the amount of each subfraction present, the chromatographic area present under each peak has been weighed. The percent of each subfraction present in F_1 is reported in Table 1. These values are taken into account when the equivalent antigenicity of each fraction was calculated.

Equivalent antigenicity of F_1 subfractions. To compare quantitatively the availability of the antigenic determinants of the histone F_1 subfractions when complexes in chromatin, the equivalent antigenicity of each fraction has been computed. The value defined as equivalent antigenicity compares the ability of chromatin-bound histones to that of non-bound histones to adsorb

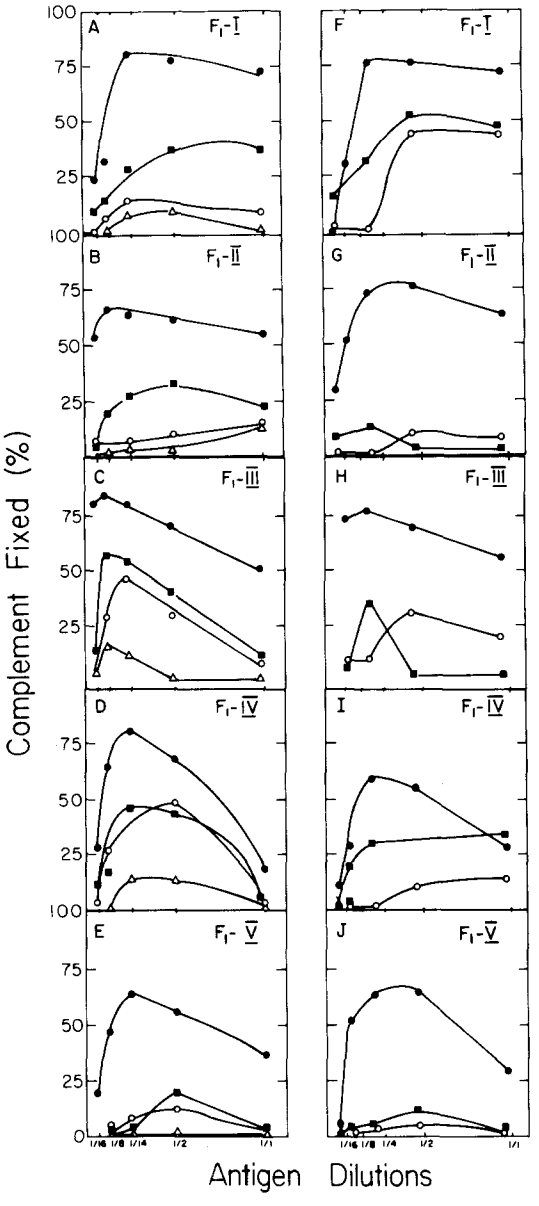

Fig. 1: Adsorbtion of antisera against different F_1 subfractions purified from rat thymus, by rat thymus chromatin. A-E, non-sonicated chromatin; F-J, sonicated chromatin. Sera dilutions: anti F_1-I 1:9600; anti-F_1-II, 1:9600; anti-F_1-III, 1:4800; anti-F_1-IV, 1:4800; anti-F_1-V, 1:3600. The amount of antigen at 1:1 dilutions were: F_1-I, 2 µg; F_1-II, 1 µg; F_1-III, 1.8 µg; F_1-IV, 1.2 µg; F_1-V, 1.8 µg. The amount of chromatin-DNA used for adsorbtion was: ●, 0 µg; ■, 10 µg; O, 20 µg; Δ, 50 µg.

Table 1. Equivalent antigenicity of F_1 histone subfractions in rat thymus chromatin*

F_1 subfraction	Relative amount of total F_1 %	Equivalent Antigenicity			
		Non-sonicated chromatin		Sonicated chromatin	
		A^+	B^+	A^+	B^+
I	11	86.6	9.6	93.8	10.3
II	14	40.4	5.6	95.7	13.4
III	28	29.0	8.1	27.0	7.5
IV	27	22.1	6.0	34.9	9.4
V	20	117.0	23.4	130.0	26.0
Average		59.1	10.5	76.0	13.3

*10 µg chromatin used for adsorbtion

+A, calculated assuming each antiserum binds exclusively to homologous subfraction. B, Calculated assuming each antiserum binds to all F_1 subfractions.

(or react with) antihistone sera (9). It is a relative value which in a defined set of conditions can be used for comparative purposes (9). The formula used to calculate this term is as follows:

$$\text{equivalent antigenicity} = \frac{A}{B} \times \frac{C-D}{C} \times 100$$

where A is the amount of antigen which gives maximal complement fixation at a given sera dilution (i.e. 0.5 µg in Fig. 1-A), B is the amount of a given histone complexed in the chromatin used as immunoadsorbant (the amount of chromatin bound histone can be calculated because it is known that in rat thymus the weight ratio of F_1 histone to DNA is approximately 0.16 (2) and the percentage of each subfraction is known from the elution profile of F_1). C refers to the maximum complement fixed by unadsorbed sera (i.e. 80% in Fig. 1-A), D is the maximum complement fixation obtained with chromatin-adsorbed antisera (i.e. 40% in Fig. 1-A).

The equivalent antigenicity of the F_1 subfractions present in non-sonicated and sonicated rat thymus chromatin is presented in Table 1. We have previously found that the equivalent antigenicity of the whole unfractionated F_1 histone fraction in calf thymus is approximately 10.0 (9). The values

obtained here with individual subfractions are significantly higher. These values could be obtained if each antisera, rather than specifically binding to one specific F_1 subfraction, reacted with more than one subfraction, perhaps even with all the five F_1 histones. Therefore, we have recalculated the equivalent antigenicity assuming that the amount of chromatin-bound F_1 histone seen by the antisera equals the total amount of F_1 histone chromatin (i.e. for 10 µg chromatin-DNA, 1.6 µg F_1 histone). The results of these calculations are presented in Table 1. The values obtained are much lower. The average of these values is close to 10.0 in good agreement with that obtained when antiserum to unfractionated F_1 histones has been used. The high equivalent antigenicity values obtained suggest that determinants shared by the five F_1 subfractions are exposed and available to interact with antibodies.

Rescue of the activity of adsorbed serum by heterologous serum. Antisera specific to the various histones (not to the F_1 subfractions) do not interfere with each other in their adsorption to chromatin (8). For example, the binding of anti-F_2b to chromatin which has been previously saturated with anti-F_1 and anti-F_3 is not different from the binding to non-saturated chromatin (8). In the case of F_1 subfractions, our results indicate that antiserum against any one of the subfractions binds to other F_1 subfractions found in chromatin. Therefore, it could be expected that the binding to chromatin previously saturated with antibody would be different from the binding to non-saturated chromatin. An experiment designed to test such a situation is presented in Fig. 2. From panel 2A it can be seen that antiserum to F_1-I reacted with F_1-I did not react with F_1-V and was adsorbed by chromatin. Panel 2B shows that anti-F_1-V reacted with F_1-V, did not cross-react with F_1-I and was adsorbed by chromatin. Panel 2C shows a situation where anti-F_1-V was added to chromatin which was first saturated with anti-F_1-I (as in panel 2A). It can be seen that the loss of immunological activity of anti-I due to absorbance was restored by the addition of anti-V. Similar results were obtained when first anti-F_1-V and then anti-F_1-I were added to chromatin. Also in this case the second, heterologous, antisera rescued the immunological activity of the first antisera. Panel 2D shows that in the absence of chromatin, the reaction between F_1-I and its antiserum is not affected by the presence of the heterologous anti-F_1-V serum and that the anti-F_1-V-F_1-V system is not affected by anti-F_1-I.

The simplest interpretation of the results presented above is as follows: antisera to F_1-I contain subfraction specific antibodies (type I) and cross-reacting antibodies (type C) directed against determinants present on all F_1 subfractions. At the dilutions tested neither type I or type C antibodies by themselves can fix complement when bound to F_1-I. The binding of both

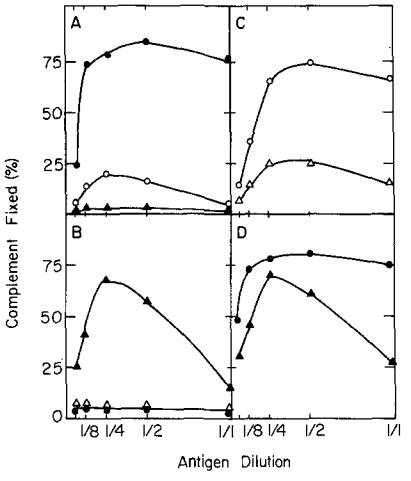

Fig. 2: Restoration of immunological activity lost, by addition of heterologous antisera. Sera dilutions and antigen concentrations as in Fig. 1. O, ●, reaction with F_1-I; △, ▲, reactions with F_1-V. Filled symbols, reactions with unadsorbed sera; open symbols, reactions with sera adsorbed on 25 µg chromatin. A, anti-F_1-I sera; B, anti-F_1-V sera; C, first addition of anti-F_1-I followed by addition of anti-F_1-V; D, a mixture of anti-F_1-I and anti-F_1-V.

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are necessary for immunological reaction. Type C antibodies are adsorbed to chromatin. The type I antibodies (and some unadsorbed type C) remain unadsorbed. Addition of the second antiserum (in this case anti-F_1-V which contains subfraction specific antibodies, type V, and cross-reacting antibodies, type C) restores the level of type C antibodies to that required for complement fixation at the dilutions tested. The rescue of immunological activity of the adsorbed serum (in our example F_1-I) by the cross-reacting antibodies (type C) present in the heterologous antiserum (anti-F_1-V) support our previous conclusion that in chromatin, determinants shared among the F_1 subfractions are exposed and available to interact with the antisera.

The relative unavailability of subfraction-specific determinants to interact with antibodies could be due to their binding to other chromosomal components. Pertinent to this point are the findings of Gottesfeld et al. (12) which suggest that the F_1 histones may exhibit some species specific regulation activity only when complexed with some non-F_1 histone chromosomal component.

Acknowledgment. We are grateful to Mrs. H. Kupfer for expert assistance. Supported by the Israeli Commission for Basic Research.

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