Histone H1° is distributed unlike H1 in chromatin aggregation

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Non-uniform distribution of H1 histone in bovine thymus chromatin was demonstrated previously. Two classes of chromatin differ in aggregation properties and histone content. The class aggregatable by physiological saline is enriched in H1, especially H1ab, the variant known to be most powerful in condensing DNA. Now, the distribution of H1 subtypes is reported for brain chromatin, where H1ab and H1c were distributed as in thymus. In contrast, H1° preferred neither the aggregatable chromatin nor the aggregation-resistant class. It is suggested that H1° is uniformly distributed with regard to euchromatin and heterochromatin, whereas H1 is cencentrated in heterochromatin.

Chromatin structure *Euchromatin* *Heterochromatin* *Histone H1* *Histone H1°*

1. INTRODUCTION

Recently we reported that H1 histone is non-uniformly distributed in chromatin. Evidently, there are two classes of chromatin, an H1-rich class that is aggregatable by physiological concentrations of sodium chloride, and an H1-poor class that resists aggregation [1]. This uneven distribution of H1 histone did not seem to be caused by H1 exchange reactions. We also observed that the H1 variant most powerful in the condensation of superhelical DNA [2] was especially enriched in the aggregatable class of chromatin, while the H1 subtype least effective in the condensation of DNA was less enriched. With the thought that this uneven distribution of H1 subtypes might be a reflection of functional differences between the variants, we studied the distribution of histone H1°, an H1 subtype that is quite distinct from the usual H1 histones in the amino acid sequence of its 80-residue globular region [3,4], a region that is conserved in the usual H1 histones, comparing mammals to fish [5,6]. Increased levels of H1° have been associated with the cessation of cell division [7] and with the maintenance of differentiated states [8].

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2. EXPERIMENTAL

Nuclei were isolated (0–4°C) from brains by the procedure of Lin et al. [9] except that Tris was used in buffers instead of triethanolamine.

Micrococcal nuclease digestion was for 5 min at 37°C on nuclei ($A_{260} = 50$) in 300 mM sucrose, 50 mM Tris, 25 mM KCl, 5 mM $MgCl_2$, and 1 mM phenylmethylsulfonyl fluoride (pH 6.5). $CaCl_2$ was added to 1 mM immediately before digestion, and 20 units/ml micrococcal nuclease (Worthington, 15 000 units/mg) was added. Digestion was quenched by Na EDTA (pH 6.5) to 5 mM, 0°C. Nuclei were pelleted (5 min at $3000 \times g$) and lysed by resuspension in 0.2 mM Na EDTA (pH 6.5), and incubation for 1–2 h at 0°C with intermittent gentle agitation. Lysed nuclei were centrifuged for 6 min at $3500 \times g$, and the supernatant was stored at 0–4°C.

The soluble chromatin was dialyzed against 10 mM sodium phosphate, 1 mM Na EDTA (pH 6.5), overnight with two changes of buffer. The chromatin solution at $A_{260} = 6$ was brought to

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various salt concentrations by slow addition of 5 M NaCl or NaCl crystals. After incubation, ~ 20°C for 2 h, the chromatin suspension was centrifuged at $400 \times g$ (low speed) for 3 min to separate supernatant (S_1) and precipitate (P_1) fractions.

Chromatin samples were analyzed by SDS-polyacrylamide gel electrophoresis [10] on discontinuous slab gels at 30 mA, with 4.5% acrylamide stacking gels and 12.5% acrylamide separating gels. Gels were stained with Coomassie blue and scanned at 525 nm in a Kratos spectrodensitometer model SD3000 equipped with a Hewlett-Packard integrator 3380A.

Samples were deproteinized for DNA gel electrophoresis by dissolving in an aqueous phase containing 1% SDS and 1 M NaCl, and then extracting twice with equal volumes of chloroform-isoamyl alcohol (24:1, v/v). The DNA precipitated overnight in 2.5 vols ethanol at −20°C was centrifuged at $8000 \times g$ for 10 min and redissolved in electrophoresis sample buffer. DNA fragments were separated in 1.6% (w/v) agarose horizontal gels. The electrophoresis buffer was 2 mM Na EDTA, 10 mM triethanolamine-HCl (pH 7.6) [11]. The gels were stained with ethidium bromide (5 mg/l) and photographed under short wavelength UV light through a red filter.

The DNA concentration was determined by A_{260} assuming $E^{1\,cm,1\%} = 200$. Aliquots of chromatin preparations were diluted 1:50 in 2% SDS before measuring the absorbance.

3. RESULTS

The previous work [1] was done with bovine thymus chromatin, which does not contain appreciable levels of histone H1°. Therefore, here, we used chromatin from bovine brain to measure H1° distribution. Chromatin was prepared from isolated brain nuclei under conditions that produce large fragments (fig.1). The bulk of the chromatin preparation contained DNA of 5–50 kb. More extensive digestion was avoided to minimize transfer of H1 from mononucleosomes and small oligonucleosomes, to larger fragments during handling [12]. The product corresponds to the usual preparation of soluble chromatin, meaning chromatin fragments soluble at very low concentrations of sodium chloride.

When the brain chromatin preparation was adjusted to various concentrations of sodium chloride, aggregation was induced in amounts dependent on salt concentration. The amount of chromatin that resisted aggregation is shown in fig.2, and as in the case of bovine thymus chromatin, minimum solubility was observed at

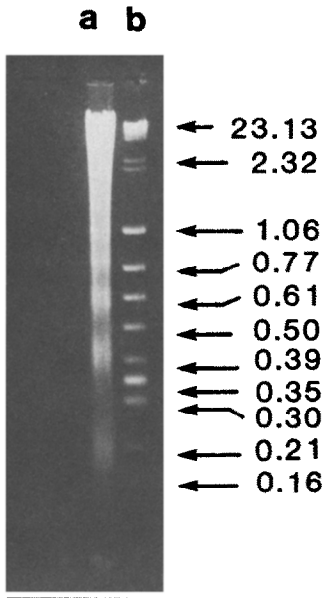

Fig.1. Size distribution of DNA from chromatin preparation. Gel electrophoresis of: (a) DNA from nuclei treated with micrococcal nuclease; (b) size markers noted in kb pairs.

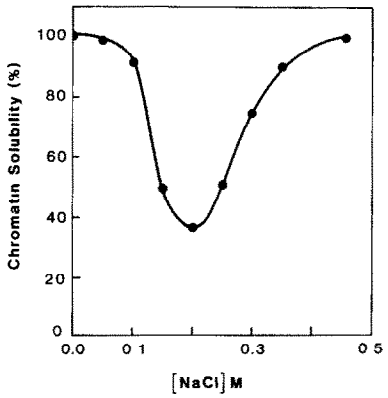

Fig.2. Salt-induced aggregation of chromatin. Chromatin soluble in the absence of NaCl was adjusted to various salt concentrations. After removing aggregated chromatin by centrifugation, the DNA contents of supernatants were measured by A_{260}.

0.2 M NaCl. The amount of brain chromatin aggregated at 0.2 M NaCl was 65%, which may be compared to 75% for the case of thymus chromatin [1]. As in the case of thymus chromatin, the aggregation-resistant chromatin had a lower H1/core histone ratio than the original chromatin preparation (fig.3). More to the point of this of study, however, when the aggregation-resistant fraction at 0.2 M NaCl was compared to the original chromatin (i.e., at 0 M NaCl) the difference in H1/core histone ratio depended strikingly on which H1 subtype was measured. As shown in fig.3 and table 1, the difference for histone H1ab was 33%, while that for H1c was 12% and for H1° was a mere 1%.

4. DISCUSSION

The deficits of H1 histones in aggregation-resistant fractions of brain chromatin can be compared with those of thymus H1 histones in table 1. The relationship of H1ab to H1c is comparable in the brain and thymus systems, although the absolute numbers differ. The difference in absolute percentages probably reflects the fact that the H1/core histone ratio (stained gels) of whole brain chromatin is different from that of thymus chromatin (0.8 compared to 1.0). Such differences in H1 stoichiometry among tissues were established more rigorously by Bates and Thomas [13]. For the purpose of the present study, the important thing to note is that H1ab is heavily favored in the aggregated chromatin from both brain and thymus, and in both systems H1c is moderately favored. The striking fact, then, is that histone H1° is not favored in either the aggregate or the aggregation-resistant chromatin. Since the aggregation-resistant fraction appears to be enriched in active genes [1], we suppose it is enriched in euchromatin, and therefore that the aggregatable fraction is enriched in heterochromatin. If so, then H1° seems not to be preferentially deposited in either of these functionally distinguishable classes.

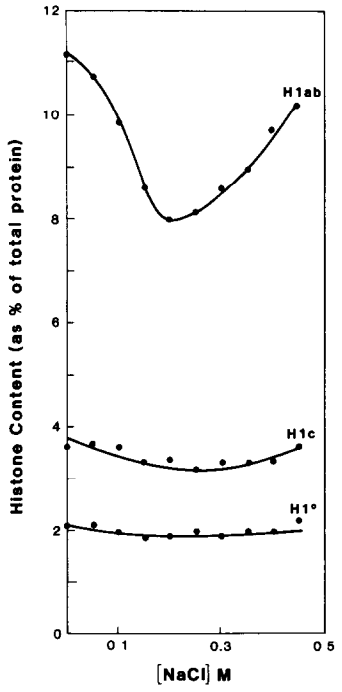

Fig.3. Content of H1 variants in aggregation-resistant fractions. The supernatants described in fig.2 were submitted to SDS gel electrophoresis. The gels were then stained and scanned and each H1 band was measured as % of the total stain. Each point is the average of 4–6 determinations. Upper, H1ab; intermediate, H1c; lower, H1°.

Table 1

Deficiency of H1 variants in chromatin soluble at 0.2 M NaCl

Tissue source	Variant	H1 content at 0.2 M / H1 content at 0 M	Deficit (%)
Bovine thymus	H1ab	0.55	45
	H1c	0.72	28
Bovine brain	H1ab	0.67	33
	H1c	0.88	12
	H1°	0.99	1

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