Effects of Histone Acetylation on the Solubility and Folding of the Chromatin Fiber*

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The folding ability of chromatin fractions containing approximately identical nucleosome numbers and the same linker histone composition, but with different extents of core histone acetylation, were analyzed by analytical ultracentrifugation. It was found that the acetylated fractions consistently exhibited a relatively small but significantly lower extent of compaction than that of their native nonacetylated counterparts. This was regardless of the extent of the size distribution heterogeneity of the fractions analyzed. Furthermore the acetylated chromatin fibers exhibited an enhanced solubility in both NaCl and MgCl₂, which is neither the result of a differential binding affinity of the linker histones to chromatin nor of an alteration in the relative amounts of the histone H1 variants.

The possible implications of histone acetylation for eukaryotic gene transcription were recognized almost from the discovery of this post-translational histone modification more than 35 years ago (1, 2). The finding 15 years later that n-butyrate could increase the levels of histone acetylation in HeLa and Friend erythroleukemic cells (3) represented an important landmark for the structural studies designed to elucidate the structural implications of acetylation because it allowed for the production of the large amounts of material that are usually required for this kind of analyses. However, despite extensive experimental effort in the following years, no significant differences could be found either at the level of the nucleosome chromatin subunit (4, 5) or at the level of chromatin fiber folding (6, 7).

The discovery that histone acetyltransferases (8) are an integral part of the basal transcription complexes has rekindled interest in histone acetylation as an important factor in the modulation of eukaryotic gene expression (9). Histone acetylation has been linked to cancer (10–13), and histone deacetylase inhibitors are being used now for the treatment of certain cancer types (14).

Despite this, the structural implications of histone acetylation in mediating eukaryotic transcription remain to be established. Thus, although the functional implications seem clear, the mechanisms remain to be unraveled. Although the current coding hypothesis (15) would explain the localized “short range” effects, an example of a transacting factor that requires histone acetylation for its interaction with the chromatin template has not yet been identified. Furthermore the fact that acetylation can occur over long stretches (several kilobases) of DNA (16) (“long range” effect) argues against the coding hypothesis being the only structural role for histone acetylation.

In the present paper we have revisited some of the earlier structural analyses using well defined chromatin fractions that differ only in their extent of core histone acetylation. Although our results generally agree with most of the earlier data, they also underscore the structural differences and constraints that may be important for understanding the mechanisms by which histone acetylation exerts its functional effects.

MATERIALS AND METHODS

Chromatin Preparation—Chromatin was obtained from HeLa cells grown in the absence (native) or presence (acetylated) of 5 mM sodium butyrate using a protocol based on the method originally described by Perry and Chalkley (17, 18). Briefly, the cells from 4-liter cultures at ~5 x 10⁷ cells/ml were harvested at 1400 × g for 10 min at 4 °C. The pellets were suspended in 120 ml of 0.137 M NaCl, 10 mM phosphate buffer (pH 7.2, phosphate-buffered saline with or without 10 mM sodium butyrate) plus protease inhibitor mixture (“Complete” from Roche Molecular Biochemicals (19) and centrifuged at 3000 × g for 10 min at 4 °C. The pellets were resuspended in 120 ml of 0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 10 mM MES, 1 mM MgCl₂, 1 mM CaCl₂, 0.5% Triton X-100 (buffer A, with or without 10 mM sodium butyrate) plus the protease inhibitor mixture and centrifuged as before. This step was repeated twice without the protease inhibitor. The pellets thus obtained were combined and resuspended in 50 mM NaCl, 10 mM Pipes (pH 6.8), 5 mM MgCl₂, 1 mM CaCl₂, 0.5% Triton X-100 (buffer B, with or without 10 mM sodium butyrate) to a final A₂₈₀nm = 40. This absorbance was determined as described elsewhere (14). The nuclear suspension was then incubated at 37 °C for 10 min and digested at this temperature for an extra 5 min with micrococcal nuclease (Worthington) at 5 units/ml. The digestion reaction was stopped by the addition of 500 mM EDTA to a final EDTA concentration of 10 mM (on ice) and centrifuged at 10,000 × g for 10 min at 4 °C to yield a supernatant (“SI”) and a pellet. The pellet thus obtained was suspended (by repeated pipetting to lyse the nuclei) in 0.25 mM EDTA (with or without 2 mM sodium butyrate) using half the volume used for the nuclease digestion. The nuclear lysis was allowed to continue for 1 h at 4 °C with continuous stirring. Nuclear debris were removed by centrifugation at 10,000 × g for 10 min, and the chromatin thus obtained in the supernatant (“SE”) was run on a 5–20% sucrose gradient in 25 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.2 mM EDTA (with or without 3 mM sodium butyrate) to an A₂₈₀nm at 85,500 × g for 3 h at 4 °C. Fractions along the sucrose gradient were collected and dialyzed against the appropriate buffer.

Gel Electrophoresis—SDS-polyacrylamide gel electrophoresis (PAGE) was carried out according to Laemmli (20). Acid-urea PAGE was carried out as described elsewhere (4). In this later case the chromatin samples to be analyzed were dissolved in 4 M urea, 5% acetic acid, and 0.5% (w/v) protamine sulfate (to displace the histones from DNA) and incubated at 65 °C for 15 min before loading them onto the gel (21). Reversed-phase HPLC—Reversed-phase HPLC analysis was carried out as described elsewhere (4). In this later case the chromatin samples to be analyzed were dissolved in 4 M urea, 5% acetic acid, and 0.5% (w/v) protamine sulfate (to displace the histones from DNA) and incubated at 65 °C for 15 min before loading them onto the gel (21).

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1 The abbreviations used are: MES, 4-morpholineethanesulfonic acid; Pipes, 1,4-piperazinediethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography.
out using a 5-μm Vydac C18 column (25 × 0.46 cm) as described elsewhere (22) and an acetonitrile gradient in the presence of 0.1% trifluoroacetic acid according to Ref. 23.

**Analytical Ultracentrifuge Analysis**—Analytical ultracentrifuge analyses were carried out on a Beckman analytical XL-A ultracentrifuge using an An-55 aluminum rotor. Analyses of the sedimentation velocity runs were carried out as described elsewhere (24, 25).

**Histone H1 Solubility**—Long native or acetylated chromatin at an \( A_{260} \) nm = 8–9 in 10 mM Tris-HCl, 2 mM sodium butyrate (pH 7.5) was brought to different NaCl concentrations by addition of a 5 M NaCl solution while vortexing (26), and in some instances (as indicated in the figure legends) the final salt concentration was reached by mixing chromatin in the 10 mM Tris-HCl buffer with an equal volume of a 2× NaCl solution prepared in the same buffer. After a 30-min incubation at room temperature, the samples were then centrifuged in a Beckman Optima TLX ultracentrifuge with a TLA 100.3 rotor at 200,000 × g for 2.5 h at 4 °C. Aliquots of the supernatants were run on SDS-PAGE, and after staining with Coomassie Blue the gels were scanned using an Alpha Innotech Chemi Imager 4000 (Alpha Innotech Corp., San Leandro, CA).

**Magnesium and Sodium Chloride Solubility**—Chromatin solubility analysis in the presence of MgCl2 was carried out in 1 mM Tris-HCl (pH 7.5) as described previously (22). The chromatin samples had an \( A_{260} \) nm = 0.8. For the NaCl studies the chromatin samples had an \( A_{260} \) nm = 6–8 in 10 mM Tris-HCl, 2 mM sodium butyrate (pH 7.5). The different NaCl concentrations were achieved by mixing equal volumes of the chromatin samples in the 10 mM Tris-HCl buffer with an equal volume of a 2× NaCl in the same buffer while vortexing.

**RESULTS AND DISCUSSION**

Fig. 1, A and B, shows the ionic strength dependence of the sedimentation coefficient of native and acetylated chromatin fractions with different average nucleosome content (\( N_{w} \)) and size distribution (see Fig. 1C). To facilitate the comparison between the native and acetylated counterparts as well as to account for differences in \( N_{w} \), the sedimentation coefficients at any given ionic strength (\( s_{20,w} \)) for a given chromatin sample were normalized by dividing them by the sedimentation coefficient of the sample in plain buffer (control) at the same ionic strength (\( s_{20,w}^{0} \))

![Fig. 1](https://example.com/fig1.jpg)

**Fig. 1. Dependence of the sedimentation coefficient (\( s_{20,w} \)) of chromatin on the NaCl concentration.** A, two fractions with a narrow size distribution (\( N_{w} = 31 \) nucleosomes (acetylated) and \( N_{w} = 35 \) nucleosomes (native)) are compared. B, two fractions with a broad size distribution (\( N_{w} = 16 \) (acetylated) and \( N_{w} = 12 \) (native)) are compared. The average number of nucleosomes (\( N_{w} \)) was determined as described in Ref. 43 and using an average nucleosome repeat length of 192 base pairs for HeLa cell chromatin (44). C, 1% agarose electrophoresis of DNA from chromatin fractions with a narrow (lanes 1 and 3) or broad size distribution (lanes 2 and 4). Acetylated fractions in lanes 1 and 2 and lanes 3 and 4 correspond to the native counterparts. Lane M is a BstEII digest of λ DNA.
to prepare chromatin fragments yields two chromatin fractions (SI and SE) (see “Materials and Methods”). In the case of the cells grown in the absence of butyrate (native), SI consists mainly of mononucleosomes that are almost completely depleted of histone H1. In contrast fraction SI from the butyrate-treated cells (acetylated) consists of an oligonucleosome (mainly 1–6 nucleosomes) fraction that (i) is histone H1-deficient (results not shown), (ii) contains highly hyperacetylated core histones (4), and (iii) is enriched in transcriptionally active sequences (30). Only the SE fraction that had been size fractionated using sucrose gradients was used in our experiments.

The results of the MgCl₂ and NaCl solubility are shown in Fig. 2 (A and B, respectively). Fig. 2C shows the difference in the extent of core histone acetylation of the samples being compared that otherwise exhibit an undistinguishable linker histone composition. Although the solubility data for the native
fractions are very similar to those reported earlier (24, 31, 32), acetylated chromatin exhibited an enhanced solubility either in the presence of NaCl or MgCl₂. The increased solubility of the acetylated fraction could be because of the acetylated histone tails adopting an α-helical conformation that would reduce the span of their interaction with nucleosomal DNA (19) resulting in the presence of more free DNA.

To assess whether the folding and solubility differences could be the result of differences in the binding affinity of linker histones, an analysis of the salt-dependent dissociation of these histones from native and acetylated chromatin was carried out (see Fig. 3). These results conclusively show that the binding affinity of histone H1 to chromatin is independent of the extent of the core histone acetylation. This is regardless of the more dynamic nature of the association between histone H1 and acetylated chromatin (33). As shown in Fig. 3B the relative stoichiometry of histone H1 to core histones in the native and acetylated chromatin fractions used for these analyses was the same.

It was argued several years ago that the reason why only relatively small changes were observed for the folding of the acetylated chromatin fiber could be the result of an increased amount of histone H1c (34, 35). The results shown in Fig. 4 show that under the experimental conditions used by us the histone H1 variant composition of the native and acetylated chromatin samples analyzed in this work was identical. Hence none of the structural differences described above are the result of an altered linker histone composition.

The results shown in Fig. 3 clearly indicate that core histone acetylation does not affect the binding affinity of linker histones to chromatin. However, it has been shown recently that histone H1 phosphorylation regulates gene expression in vivo by creating a charge patch (36). This possibly occurs by weakening the binding affinity of phosphorylated H1 by DNA in chromatin, thus mimicking loss of H1 (37). We have shown also that in the absence of linker histones core histone acetylation results in a major unfolding of the chromatin fiber (38). This opens the possibility that under physiological (not-butyrate induced) conditions core histone acetylation may operate in conjunction with linker histone phosphorylation and lead to the formation of long stretches of unfolded fibers. This could account for the long range or so-called global effects of this modification (6, 18, 39) including the observed DNase I hypersensitivity usually associated with these chromatin domains (13).

The fact that the overall amount of acetylation in yeast is very high (~13 acetylated lysines per nucleosome (40)) implies that in addition to operating as a histone code (15, 42), histone acetylation must have an important structural role at the level of the chromatin fiber.

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