

Structure of hyperacetylated chromatin: light scattering and flow linear dichroism study

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Cation-induced folding of 10 nm chromatin filament to 30 nm fiber was studied with hyperacetylated chromatin using light scattering at 90° and flow linear dichroism. Acetylated chromatin folded in a way indistinguishable from that of the control chromatin: both the compactness of chromatin and the orientation of nucleosomes relative to the fiber axis were identical at a given salt concentration.

Histone Acetylation Chromatin structure Light scattering Flow linear dichroism

1. INTRODUCTION

Acetylation of the N-terminal tails of core histones suggests an attractive possibility for regulation of gene activity [1,2]. The literature data, however, represent provocative correlations [2–6] rather than direct evidence for involvement of histone acetylation in gene activation and transcription. The failure to confirm the expected effect of acetylation on the stability of nucleosome core particles ([7–9] but see [10]) focussed attention on the higher order chromatin structure [11]. Two papers addressed this question, both reporting contradicting results [12,13]. On the basis of the melting profiles and circular dichroism spectra of acetylated and control chromatin it was concluded that the butyrate treatment caused a condensation of chromatin [12]. Using electric dichroism and sedimentation to follow the cation-induced folding of chromatin it was found that hyperacetylation did not prevent transition from the 10 nm filament to 30 nm fiber, although the acetylated chromatin appeared slightly less con-

densed than control chromatin [13]. This contradiction as well as the controversial results obtained with electric dichroism and flow linear dichroism (LD) concerning the optical anisotropy of the chromatin fiber [11,14–16] prompted us to investigate the higher order structure of acetylated chromatin using flow LD and light scattering. Hyperacetylation affected neither the condensation of chromatin nor the orientation of nucleosomes.

2. MATERIALS AND METHODS

HeLa cells, strain S3, were grown in Eagle's MEM with 10% calf serum. Sodium butyrate (20 mM final concentration) was added 18 h before collecting the cells. Butyrate-treated and control cells were processed in parallel. Nuclei and chromatin were isolated essentially by the procedure of McGhee et al. [13]. After treatment with boiled ribonuclease A (2 µg/ml, 5 min) chromatin was loaded on a 10–30% linear sucrose gradient containing 25 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.25 mM EDTA and 5 mM Na butyrate. Centrifugation was carried out in a Beckman SW27 rotor at 4°C for 7 h at 20 000 rpm. Fractions containing high-*M_r* chromatin were collected, extensively dialysed against 0.25 mM EDTA, 1 mM Na

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butyrate and used for LD and light scattering experiments.

Histone H1-depleted chromatin was prepared as in [18]. Histone acetylation was controlled electrophoretically [17].

Flow LD measurements were carried out as in [16]. Light scattering experiments were performed using an Aminco SPF1000 spectrofluorimeter. The variations in intensity of the scattered light were measured at 90° to the monochromatic beam ($\lambda = 350$ nm) using a fluorescence cuvette with a path length of 1 cm.

3. RESULTS AND DISCUSSION

Our preliminary experiments as well as those of McGhee et al. [13] showed that chromatin isolated from HeLa cells contained RNA in amounts which seriously affected the results of the optical measurements. All chromatin samples were treated with ribonuclease before to separate high- M_r material. Histone acetylation of this chromatin was checked electrophoretically (fig.1). The average acetylation

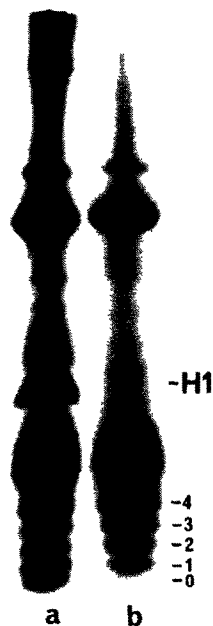


Fig.1. Triton-acetic acid-urea gels of histones extracted from chromatin (a) and H1-depleted chromatin (b) isolated from HeLa cells grown in the presence of Na butyrate. Acetylated forms of histone H4 are shown by numbers indicating the number of acetyl groups.

of histone H4, as estimated from gel scans, was approx. 2.3–2.7 acetyl groups/molecule. These values are comparable with those reported in [13] and higher than those in [12].

3.1. Light scattering

It was recently shown ([19,20]; I. Smirnov, V. Makarov and S. Dimitrov, submitted) that the increase in intensity of the scattered light at 90° of chromatin upon increasing the ionic strength reflected the condensation of 10 nm filament into 30 nm fiber. Measurements were carried out at salt concentrations no higher than 70 mM NaCl where no aggregation was observed and the results are presented in fig.2. Clearly, the variations in intensity of the scattered light with increasing ionic strength are indistinguishable for chromatin from HeLa cells grown in the presence and absence of butyrate. Therefore, hyperacetylated chromatin

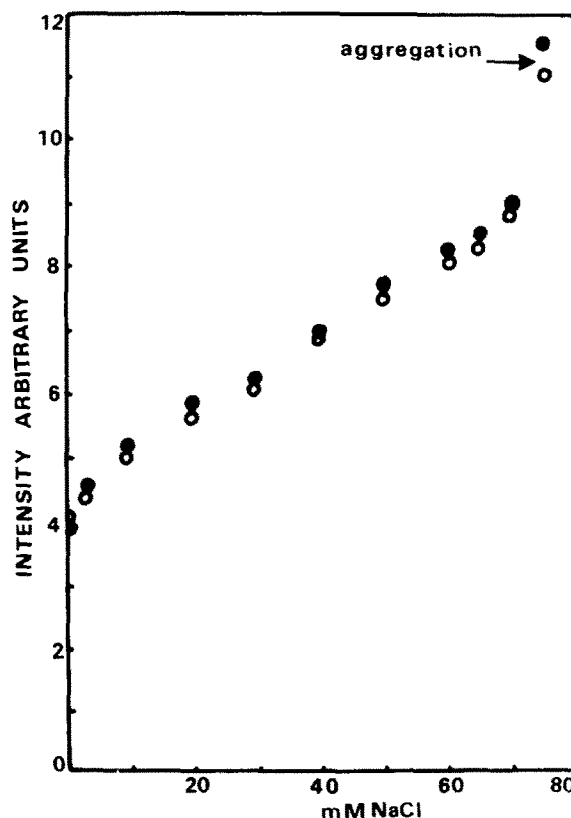


Fig.2. Dependence of the intensity of the scattered light at 90° on the ionic strength for hyperacetylated (●) and control (○) chromatin in 0.25 mM EDTA, 1 mM Na butyrate.

forms a 30 nm fiber in a way similar to that of the control unacetylated chromatin.

3.2. Flow LD

Fig.3 plots the values of the reduced LD ($\Delta A/A$) vs ionic strength for butyrate treated- and control chromatin. Both samples exhibited a positive dichroism [15,16] showing typical changes in its value upon increase in ionic strength [16]. The identical behaviour of butyrate-treated and control chromatin means that hyperacetylation does not affect the orientation of nucleosomes which changed negligibly upon increasing salt concentration up to 80 mM [16]. As shown in fig.3B, hyperacetylation has no effect on the structure of H1-depleted chromatin: both butyrate-treated and control chromatin exhibited a negative anisotropy

and behaved identically upon increasing the ionic strength.

It is curious that the expected 'loosening' of the chromatin structure as a result of histone acetylation was not proved by some more or less direct evidence ([12,13]; this paper). Reczek et al. [12] even reported a more compact structure for acetylated chromatin as judged by melting experiments and CD spectra [12]. In contrast, McGhee et al. [13] using electric dichroism and sedimentation found the acetylated chromatin to be slightly less condensed than control chromatin but indistinguishable from the latter with respect to the cation-induced transition from 10 nm filament to 30 nm fiber. Our results do not agree with those in [12] and support the conclusion in [13] concerning the lack of effect of acetylation on the orientation of nucleosomes along the chromatin fiber as well as on the cation-induced filament-solenoid transition. The slightly less compact appearance of acetylated chromatin, revealed by the sedimentation analysis in [13], was not confirmed by our light scattering data.

The lack of effect of acetylation on the orientation of nucleosomes relative to the fiber axis seems reasonable in the light of our recent data on the role of N-terminal tails of core histones in maintaining nucleosome orientation [21]. The positive anisotropy of chromatin was preserved upon very strong digestion with trypsin and changed to negative when all core histones were cleaved. On the basis of these and other data [16,22] we conclude that the orientation of nucleosomes is preserved upon folding and unfolding of chromatin.

There must be some perturbations in gene structure during transcription, but it is not yet clear to what extent the solenoid structure of an average gene is disrupted, if at all. With this in mind, the ability of acetylated chromatin to fold into 30 nm fiber as does unacetylated chromatin does not impugn the numerous suggestions for a role of histone acetylation in transcription. Moreover, if the solenoid has to be disrupted, this does not mean that acetylation should act alone. An additional complication is the multiple effect of butyrate on the cell [23]. Yet, the condensation effect of acetylation on chromatin seems surprising although it might be accommodated in some views concerning the structural changes needed for transcription [24].

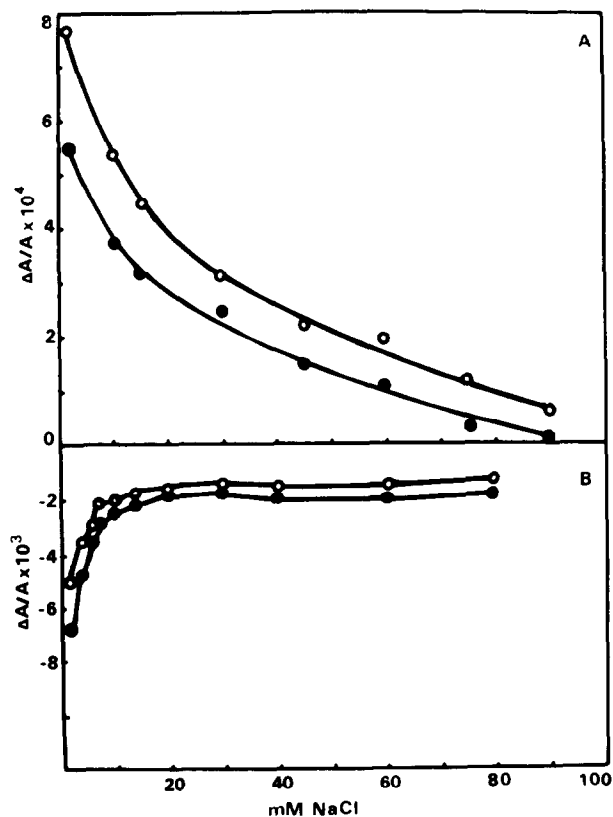


Fig.3. Reduced LD at 258 nm vs ionic strength for hyperacetylated (●—○) and control (●—○) chromatin in 0.25 mM EDTA, 5 mM Na butyrate, 1 mM Tris-HCl (pH 7.2). (A) Total chromatin, (B) H1-depleted chromatin.

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