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Acetylation of core histones causes the unfolding of 30 nm chromatin fiber: analysis by agarose gel electrophoresis

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In the present work it was directly demonstrated that increased acetylation level of histones causes the decompactization of 30 nm chromatin fiber, as revealed by low-percentage agarose gel electrophoresis. In the light of obtained results the possible molecular mechanism of the decompactization of acetylated fiber is discussed. © 1993 Academic Press, Inc.

The chain of amino acids of histones within nuclei of eukaryotic cells may undergo a number of modifications [1, 2]. At the moment, however, it is not quite clear what role these modifications play *in vivo* in functioning of genes. Considerable evidence was obtained only for participation of histone acetylations in transcription.

The correlation between degree of acetylation of histones and transcription level was found *in vivo* in a number of studies [3-8]. However, the question of how the acetylation of histones affects the chromatin structure remains unanswered [review 2, 9]. In the present work, we directly demonstrate that the chemical acetylation of histones leads *in vitro* to unfolding of 30 nm nucleosomal fiber.

MATERIALS AND METHODS

Cell nuclei were isolated from rat liver at 0^0C as described by Bloom and Anderson [10], and treated with micrococcal nuclease (2u/10^6 nuclei) at 0^0C

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for 5 minutes [11]. The reaction was terminated by adding EDTA up to 10 mM and the nuclei were then lysed by overnight dialysis against 1 mM EDTA at $0^{o}C$. Isolated chromatin fragments containing, on the average, 15 nucleosomes were used in the following procedures directly as well as after additional purification by gel-filtration on a column TOYOPEARL HW75F.

For removal of H1 histone, the chromatin was treated with an ion exchange resin AG50W-X2 [12].

Chemical acetylation of the chromatin was conducted at $15^{0}C$ for 30 minutes according to Rampoui et al. [13]. Then the chromatin solution was portioned and dialyzed overnight against buffers containing 5 mM triethanolamine pH 8.0, 2mM EDTA and NaCl in different concentrations. Afterwards the material was fixed by glutaraldehyde at $4^{0}C$ for 24 hours [12], dialyzed against 1mM EDTA and analyzed in 0.3% agarose gel in TAE buffer (40mM Tris-base, 20 mM acetic acid, 2 mM EDTA; pH 8.0) at 0.5 V/cm for 24 hours.

Histones were extracted from the chromatin and analyzed in 12% polyacrylamide gel as described in [14].

The calculation of excessive free energy of supercoiling was performed as described in [27, 28].

RESULTS AND DISCUSSION

When suppressing the deacetylase activities *in vivo* by Na-butyrate [1-3], significant hyperacetylation of histones in nucleosomal core may be obtained. This approach is common for the preparation of chromatin with high level of acetylation of histones. At the same time, however, the treatment of cells with Na-butyrate gives rise to a number of other effects, including considerable phosphorilation of histones [3, 15] that may result in additional stabilization of chromatin structure.

Among a number of acetyl derivatives of phosphates, acetyladenylate is characterized by high efficiency of the transfer of an acetyl group as well as by the practically complete inability to denature the chromatin [13, 18]. The result of electrophoretic analysis of histones from acetyladenylate-treated chromatin is presented in Figure 1. The chemical acetylation causes a considerable increase in the share of higher acetylated forms of all four core histones.

Previously we found [19] that electrophoretic mobility of chromatin in agarose gels depends directly on the degree of chromatin condensation. Acetylated chromatin was transferred by dialysis in the solutions of different ionic strength (usually containing 5 or 120 mM NaCl) and fixed by glutaraldehyde with subsequent analysis in 0.3% agarose gel (Figure 2A). Thus we found that highly acetylated forms of histone prevent the chromatin from condensation in the solution with high ionic strength (120 mM NaCl).

The results of analogous experiments for histone H1-depleted chromatin are shown in Figure 2B. After the removal of histone H1, the chromatin loses

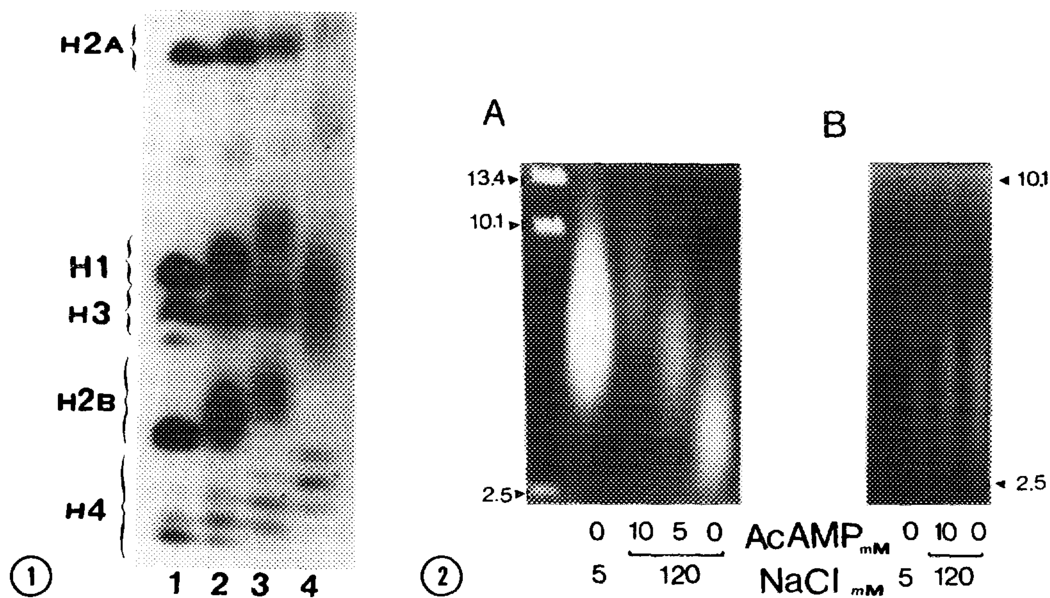

Figure 1. Histones of acetylated chromatin samples analyzed by electrophoresis in triton-acid-urea-containing gel. The concentration of acetyladenylate in reaction solution was the following: 1 - 0 mM (control); 2 - 5 mM; 3 - 10 mM; 4 - 20 mM.

Figure 2. Electrophoretogram of acetylated chromatin samples in agarose gel. The chromatin was fixed by glutaraldehyde at the different NaCl concentrations indicated in the Figure. The concentrations of acetyladenylate in reaction solution are also shown. A - the chromatin with the complete number of histones; B - H1-depleted chromatin.

its ordered organization and attains the structure of a random tangle [12]. As one would expect, the acetylation of histones has no significant effect on parameters of the tangle. Hence, most likely the decrease in electrophoretic mobility of highly acetylated chromatin fragments reflects the reorganization of supernucleosomal chromatin structure.

Our results lead to two important conclusions. Firstly, it was directly demonstrated that increase in the share of highly acetylated forms of histones causes decompactization of supernucleosomal structure. This conclusion agrees with the results of a number of other investigations (see reviews [1, 2, 9, 20, 21]), in which high degree of acetylation of histones in active chromatin, as well as incompatibility of gene activities with condensed structure of 30 nm fiber, was demonstrated. Secondly, from the present results one can draw inferences about the molecular mechanism of 30 nm fiber unfolding. During compactization the nucleosomal filament winds into solenoidal structure with 6-8 [12] (according to other data up to 12 [22]) nucleosomes per turn to form 30 nm fiber stabilized by H1 interactions. This solenoidal structure unfolds gradually with decreasing ionic strength of the medium, forming a helix with

two or less nucleosomes per turn and a diameter of 10 nm (reviews [1, 12, 22]). Histone H1-depleted nucleosomal filament has a structure of a random tangle, which parameters depend only weakly on the ionic strength.

It is apparent that unfolded 10 nm chromatin filament and the random polynucleosomal tangle are principally distinct structures despite the fact that either structures can be considered as decondensed state of 30 nm nucleosomal fiber. So far it is not quite clear which of these two structures corresponds *in vivo* to the decompactization state of nucleosomal fiber. Obviously it would be incorrect to use the mechanism of *in vitro* salt-induced decompactization of nucleosomal fiber just for description of the processes occurring *in vivo* in a cell nucleus.

In this regard the decompactization of nucleosomal fiber as a result of the acetylation of histones reflects more adequately the process that operates *in vivo*. The acetylation proceeds at "physiological" ionic strength of the medium and has quite "physiological" action on chromatin structure. The increase in share of highly acetylated forms of core histones causes the considerable decrease in electrophoretic mobility of chromatin fragments, which is proportional to the degree of acetylation of histones (Figure 2A). Since the electrophoretic mobility of the random polynucleosomal tangle is significantly higher than that of unfolded fiber (Figures 2A, 2B), therefore there are good grounds to believe that the unfolding of 30 nm fiber caused by the acetylation occurs in a similar way as salt-induced unfolding of nucleosomal solenoid rather than in the way the structure of random tangle arises (Figure 3).

An important point is that according to this mechanism, the unfolding of 30 nm fiber leads to considerable increase in the number of nucleosomal solenoid's turns (Figure 3). Taking into account that, according to present views, the chromatin of metaphase and interphase chromosomes is separated into topologically closed independent loops or domains (reviews [23, 26]), the increase in number of solenoidal supercoils during fiber unfolding should be accompanied by considerable increase in the energy of domain supercoiling (Figure 4). Evidently this torsional tension may be then compensated by active topoisomerases. But this tension, once even short-lived, may cause significant reorganization and destabilization of nucleosomal structure [27-30].

It seems likely that the discussed mechanism may be considered as a basis of functional chromatin reorganization, for example, occurring during chromatin activation as a substrate of DNA and RNA polymerases. By the above model, the change in DNA packaging on one structural level causes the destabilization of DNA packaging on other structural levels. These changes may be fixed through additional modifications of histones and some other proteins. All this may provide not only quick and easy access of protein factors to chromatin DNA, but also an increased conformational potential of

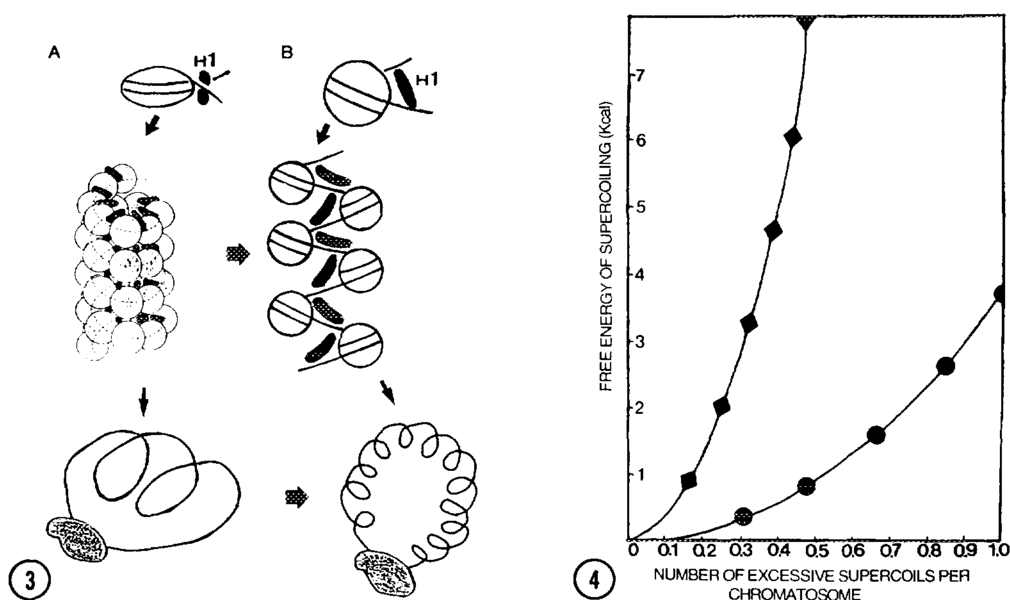

Figure 3. A schematic representation of decondensation of 30 nm fiber within chromatin topological domain. The increase in the acetylation level of core histones causes a partial unfolding of nucleosomal particles [24, 25] in a similar way, as salt-induced unfolding of nucleosomes occurs ([31] and references therein). This leads to an alteration of the spatial orientation of extranucleosomal DNA linkers [24, 25] that is accompanied by change in H1-mediated interactions. Compact 30 nm fiber containing 6-8 nucleosomes per turn (A) unfolds to the helix with 2 or less nucleosomes per turn (B); in so doing the number of nucleosomal solenoid's turns would increase more than four times.

Figure 4. The increase of free energy of supercoiling in response to the excess of superhelical turns formed by the unfolding of 30 nm chromatin fiber (see Figure 3 for details). Free energy per mole of chromatosomes [27, 28] is plotted against excessive number of supercoils per chromatosomes (200 bp DNA): ● - free energy dissipated through all the chromatosome; ◆ - free energy absorbed on linker DNA. It seems unlikely that free energy of supercoiling would be compensated by solenoid helical writhe because of the "rigidity" of nucleosomal fiber stabilized by H1 interactions. The free energy, involved here, is likely to be sufficient to weaken some histone-histone interactions [27, 28]. Clearly, excessive free energy is predicted to dissipate exclusively all the chromatosome rather than be absorbed by linker DNA that should lead to the partial release of nucleosomal supercoils with destabilization of nucleosomal structure.

DNA that is necessary for free rotation of DNA around active RNA polymerase complex (reviews [20, 21]).

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