Magnesium-dependent Association and Folding of Oligonucleosomes Reconstituted with Ubiquitinated H2A*

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The MgCl2-induced folding of defined 12-mer nucleosomal arrays, in which ubiquitinated histone H2A (uH2A) replaced H2A, was analyzed by quantitative agarose gel electrophoresis and analytical centrifugation. Both types of analysis showed that uH2A arrays attained a degree of compaction similar to that of control arrays in 2 mM MgCl2. These results indicate that attachment of ubiquitin to H2A has little effect on the ability of nucleosomal arrays to form higher order folded structures in the ionic conditions tested. In contrast, uH2A arrays were found to oligomerize at lower MgCl2 concentrations than control nucleosomal arrays, suggesting that histone ubiquitination may play a role in nucleosomal fiber association.

Although for many years histones were thought to be merely structural components of nucleosomes, the primary level of DNA organization required to compact the genome in the nucleus, they are now recognized as important players in the mechanisms underlying gene expression. One of the keys to chromatin’s dynamic nature is post-translational modification of the flexible histone tails. These modifications include acetylation, phosphorylation, methylation, and ubiquitination (1–3). Ubiquitin is reversibly attached to bovine H2A by means of an isopeptide bond between its terminal glycine and the ε amino group of H2A lysine 119 (8), which lies in the trypsin-accessible region of the carboxyl-terminal tail (9). Histones are among the most abundant ubiquitin-protein conjugates in higher eukaryotes, where 5–15% of the total H2A is ubiquitinated (10). The function of histone ubiquitination remains unclear. Although ubiquitin has been shown to play an important role in the degradation of many short-lived proteins (for reviews see Refs. 11, 12), two independent studies have shown that ubiquitination does not tag histones for degradation (13, 14). Nonetheless, H2A ubiquitination may play a role in nucleosomal fiber association. This paper is available on line at http://www.jbc.org

EXPRESSMENT PROCEDURES

Materials—Fresh calf thymus and whole chicken blood were obtained from the local abattoir.

Ubiquitinated histone H2A was purified from calf thymus as described previously (16, 27). All chemicals were of reagent grade.

Preparation of Template DNA—The DNA template (208-12) consisting of 12 tandem repeats of a 208-bp sequence derived from Lytechinus variegatus 5 S rDNA was amplified and purified from plasmid p58 208-12 (a kind gift from Dr. R. T. Simpson (28)). The plasmid was purified using Nucleobond (Machery-Nagel) columns followed by HhaI digestion. Template DNA thus excised was purified from the remainder of the plasmid by centrifugation through a linear 5–12% (w/v) sucrose gradient in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for 16 h at 4 °C at 30,000 rpm in a Beckman SW 40 Ti rotor. Template DNA was concentrated from selected fractions by ethanol precipitation.

Octamer Reconstitution—Reconstitution of control or uH2A octamers containing either calf H2A or uH2A instead of H2A in the chicken erythrocYTE octamer was carried out as described previously (16, 29). Nucleosomal Arrays Reconstitution—Nucleosomal arrays were reconstituted from control or uH2A octamers and template DNA by salt gradient dialysis (30) as described previously (31). A ratio of 1.3 mol of histone octamer to 1 mol of 208-bp DNA was used to generate saturated nucleosomal arrays. Reconstituted arrays were stored at 4 °C no longer than 1 week.

Quantitative Agarose Gel Electrophoresis—A nine-lane multigel system as described by Hansen and co-workers (32–34) was used to determine the electrophoretic mobilities (μ) of reconstituted nucleosomal arrays in 0.2–3.0% (w/v) agarose. Running gels were prepared in 40 mM Tris-HCl, 0.25 mM EDTA, pH 7.8) containing a final concentration of 0 or 2 mM MgCl2. Samples containing 0.6 μg of bacteriophage T3 standard and 0.5 μg of nucleosomal array were dialyzed for 4 h against running buffer prior to electrophoresis at 2.65 V.cm−1 at 20 ± 2 °C for 8 h with buffer recirculation. Control and uH2A arrays were analyzed in parallel on each multigel. Samples were visualized by UV illumination after ethidium bromide staining. The gel free migration was calculated by extrapolation of the line fitted by linear regression to a plot of migration distance versus percentage agarose concentration (≤1% (w/v) agarose) to 0% agarose. The gel-free migration was converted to the gel-free mobility (μr), which was then corrected for electro-osmosis and normalized as described previously (34) to obtain μr. The average gel pore radius (Pr) and effective radius (Re) of nucle...
FIG. 1. Reconstitution of control and uH2A nucleosomal arrays. A, SDS-polyacrylamide gel electrophoresis analysis of reconstituted control and uH2A hybrid octamers (lanes 2 and 4). A total acid extract of calf thymus nuclei is included as a standard in lanes 1 and 3. Bands were visualized by staining with Coomassie Blue G-250. B, comparison of saturated control and uH2A nucleosomal arrays. Nucleoprotein gel of native 167-bp core particles (lane 1), Avai digestion products of: naked 208-12 template DNA (lane 2), control nucleosomal arrays (lane 3), and uH2A nucleosomal arrays (lane 4). Bands were visualized under UV illumination after ethidium bromide staining.

Folding of uH2A Nucleosomal Arrays

Quantitative Agarose Gel Electrophoresis—MgCl2-induced folding of nucleosomal arrays was analyzed by quantitative agarose gel electrophoresis (32, 33). Nucleosomal arrays were electrophoresed in multigels of concentrations ranging from 0.2 to 3% (w/v) agarose in E buffer or E buffer containing 2 mM free Mg2+. Data from multitgels were used to generate Ferguson plots. Data from multigels were used to generate Ferguson plots, which were convex in shape (32, 34) for both control and uH2A arrays (Fig. 2, A and B). The data from the linear portion of the Ferguson plots were used to calculate μ0, the gel free mobility (Table I), which is a measure of the average electrical surface charge density of a macromolecule (42). The extent of array saturation has been shown to influence μ0 values. The μ0 value obtained for control arrays in low salt buffer (Table I) lies within the range of 1.82 ± 0.04 to 1.92 ± 0.02 × 10−4 cm2/V·s previously reported for saturated arrays reconstructed on the same DNA template (31, 34, 43, 44) thereby providing further confirmation that array saturation was achieved. In the presence of 2 mM Mg2+, the gel free mobility of control arrays decreased by 45% as the arrays adopted a more compact structure (32). The gel free mobility of uH2A arrays was 10% lower than that of control arrays both in the absence and in the presence of 2 mM free Mg2+. In E buffer, this reduction in μ0 corresponds to either an increase of 25 to 30 positive charges per octamer (32) or to the shielding of an equivalent number of charges per octamer or a combination of both effects. The later explanation is more likely, because at pH 7.0, ubiquitin has 11 acidic and 11 basic residues, of which only three of the seven lysine residues are not involved in intramolecular contacts and are fully exposed on the surface of the molecule (45). The 44% reduction of μ0 values observed for uH2A arrays in E buffer + 2 mM Mg2+ was comparable to that of control arrays.

Quantitative agarose gel electrophoresis data can also be used to determine an average Rm, which can be correlated to the surface area of a rod-like nucleosomal array at low agarose concentrations (<0.6% w/v) (32, 34) as well as to the frictional coefficient derived from the average sedimentation coefficient (46). The effective radii (Rf) of control and uH2A arrays remained essentially constant at all agarose concentrations in E buffer without and with 2 mM MgCl2 (Table II), whereas naked template DNA (data not shown) was found to reptate at smaller pore sizes as reported previously (32). The Rf values obtained for control arrays at pore sizes ≥200 nm (Table II) correlate well with previous estimates of 26–28 nm in E buffer and 20.5–22 nm in the presence of 2 mM MgCl2 (32, 34, 43, 44) for equivalent arrays under the same electrophoretic conditions. No significant difference in Rf values of control and uH2A...
arrays was observed (Table II), suggesting that uH2A has little effect on the compaction of nucleosomal arrays under these experimental conditions.

**Oligomerization—**Nucleosomal arrays have been shown to oligomerize rapidly in response to increasing concentrations of divalent salts (39, 47). This association is reversible upon removal of the salt by extensive dialysis (39) and is distinct from the folding process (46). Some evidence suggests that the results obtained from *in vitro* oligomerization of relatively short chromatin fragments may be significant regarding the *in vivo* interaction of chromatin fibers during chromosomal condensation (39). Histone tails have been shown to play an important role in this process as their absence (39, 48, 49) or acetylation (43) hinder Mg\textsuperscript{2+}-induced oligomerization of nucleosomal arrays. Fig. 3 shows that uH2A arrays oligomerized at lower Mg\textsuperscript{2+} concentrations than control arrays. Control arrays were 50% oligomerized at ~4 mM MgCl\textsubscript{2} in close agreement with previous results (48), whereas uH2A arrays were almost fully oligomerized at this concentration. Because the gel free mobility of uH2A arrays was 10% lower than that of control arrays (Table I), ubiquitin may thus shield some of the DNA charge, thereby facilitating the aggregation process. Ubiquitin itself may also provide additional surfaces for inter-array contacts.

**Analytical Ultracentrifugation—**Sedimentation velocity experiments were next used to monitor the effect of H2A ubiquitination on nucleosomal array folding in response to increasing MgCl\textsubscript{2} concentrations (Fig. 4). The 208-12 oligonucleosome complexes used in these experiments consisted of 11–11.5 nucleosomes per DNA template determined as described elsewhere.

**DISCUSSION**

Histone modifications such as acetylation and phosphorylation mediate changes in chromatin largely through alteration of the charge of amino acid residues in the amino-terminal...
histone tails. Ubiquitination is, by comparison, a bulky modification that has led researchers to postulate its function to lie in hindering chromatin folding (e.g. Refs. 6, 17, 18). This postulate has been difficult to confirm in vivo, because the enzymes involved in conjugating ubiquitin to histones are also required for the ubiquitination of many other proteins that may directly or indirectly affect chromatin folding. We have therefore used an in vitro model system to assay the impact of histone H2A ubiquitination on the Mg$^{2+}$-induced folding and oligomerization of nucleosomal arrays. Moreover, the extent of H2A ubiquitination used in this study was far greater than that in vivo where it is more common for only one H2A molecule to be ubiquitinated per nucleosome (51). In the absence of linker histones, nucleosomal arrays equilibrate between moderately folded and extensively folded structures in buffers containing 2 mM MgCl$_2$ (31, 37, 47). The data obtained from quantitative agarose gel electrophoresis (Tables I and II) and analytical ultracentrifugation (Fig. 4) show that uH2A and control arrays attained a similar extent of compaction in 2 mM MgCl$_2$ relative to low salt conditions. This indicates that uH2A does not affect this degree of nucleosomal array folding. Thus, although the tail domains of histones are crucial for the salt-induced folding of nucleosomal arrays (37, 48, 49, 52), the carboxyl-terminal tails of H2A can be ubiquitinated without much impact on the folding process. Furthermore, the results shown in Fig. 4A in 2 mM MgCl$_2$ are almost identical to those previously reported for unmodified arrays (see Fig. 5A of Ref. 47). Therefore, it is possible to conclude that histone H2A ubiquitination neither affects the 28–40 S folding transition, which is characteristic of the histone H1-depleted chromatin in either the presence of monovalent (31, 37) or low concentrations of divalent ions (47), nor the maximum folding (40–55 S transition), which occurs at higher levels of histone saturation in the presence of MgCl$_2$ (43, 44, 47, 49).

Although support for uH2A playing a role in hindering the final stages of chromatin compaction has been provided by reports of the loss of the uH2A ubiquitin moiety at metaphase (25, 26), not all compact chromatin structures are devoid of ubiquitin. In mice spermatocytes, uH2A has been associated with the inactive sex body that contains the heterochromatic X and Y chromosomes (53), and in Drosophila ubiquitin has been shown to be mainly associated with the band domains of polytene chromosomes (54). Further investigations are required to determine if uH2A affects the higher degree of folding attained by nucleosomal arrays containing linker histones in response to elevated salt concentrations (44). It also remains to be investigated if ubiquitination of H2A could affect the binding of other proteins involved in the formation of mitotic chromosomes. Finally, it has been suggested that histone ubiquitination could label specific chromatin regions (26, 55) and as such could be part of the “histone code” (56). This ubiquitin tag could direct as yet unidentified or known cellular machinery such as chromatin remodeling complexes (57) to uH2A-enriched chromatin regions such as the 5’-end of the mouse dihydrofolate reductase gene (17) or the copia and hsp 70 genes in Drosophila (18).

Acknowledgments—We are very grateful to Dr. R. T. Simpson for the pSS20-12 plasmid construct and Dr. J. C. Hansen for providing details of the quantitative agarose gel electrophoresis apparatus.

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J. Biol. Chem. 2001, 276:14597-14601.
doi: 10.1074/jbc.M011153200 originally published online February 2, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M011153200

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