Characterization of the Stability and Folding of H2A.Z Chromatin Particles

IMPLICATIONS FOR TRANSCRIPTIONAL ACTIVATION*

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H2A.Z and H2A.1 nucleosome core particles and oligonucleosome arrays were obtained using recombinant versions of these histones and a native histone H2B/H3/H4 complement reconstituted onto appropriate DNA templates. Analysis of the reconstituted nucleosome core particles using native polyacrylamide gel electrophoresis and DNase I footprinting showed that H2A.Z nucleosome core particles were almost structurally indistinguishable from its H2A.1 or native chicken erythrocyte counterparts. While this result is in good agreement with the recently published crystallographic structure of the H2A.Z nucleosome core particle (Suto, R. K., Clarkson, M J., Tremethick, D. J., and Luger, K. (2000) Nat. Struct. Biol. 7, 1121–1124), the ionic strength dependence of the sedimentation coefficient of these particles exhibits a substantial destabilization, which is most likely the result of the histone H2A.Z-H2B dimer binding less tightly to the nucleosome. Analytical ultracentrifuge analysis of the H2A.Z 208-12, a DNA template consisting of 12 tandem repeats of a 208-base pair sequence derived from the sea urchin Lytechinus variegatus 5 S rRNA gene, reconstituted oligonucleosome complexes in the absence of histone H1 shows that their NaCl-dependent folding ability is significantly reduced. These results support the notion that the histone H2A.Z variant may play a chromatin-stabilizing role, which may be important for transcriptional activation.

The packaging of DNA around histone octamers creates a thermodynamic obstacle to processive enzyme complexes such as RNA polymerase. To lower the energy of activation and display a template more amenable for expression, the nucleus uses several mechanisms to biochemically alter the nature of histone-DNA and histone-histone interactions. These mechanisms include post-translational modifications, nucleosome remodeling complexes, and the introduction of histone variants into the octameric core (for current reviews, see Refs. 1–3). Histone variants are nonallelic isoforms of the major H2A, H2B, H3, and H4 proteins that interact through inherent histone fold domains in nucleosomes throughout the genome (for current reviews, see Refs. 4 and 5). By inserting variant histones into the octamer noncovalent interactions between the players are altered, possibly creating particles with modified stability or functional novelty. This epigenetic feature may be utilized to silence nonessential genes in differentiated tissues or to lower the binding constants of replication, transcription, and repair machinery in active chromatin.

H2A.Z is an H2A subtype that has been identified in organisms as diverse as Saccharomyces cerevisiae (6), Tetrahymena (7), Drosophila (8), and Homo sapiens (9). The protein displays 60% homology with H2A and 90% homology between species. Mutagenic assays have demonstrated that H2A.Z is essential for development in yeast (10) and for viability in Tetrahymena (11) and Drosophila (12, 13). Initial immunochromatographic characterization of this protein uncovered that H2A.Z is exclusive to transcriptionally active domains in Tetrahymena (14–16). Recently H2A.Z has been observed to be located at yeast promoters and to display a redundant role with ATP-dependent nucleosome remodeling complexes (17) and interact directly with transcriptional machinery during gene expression (18). However, the functional dynamics of H2A.Z enrichment in active chromatin remains enigmatic as other studies describe H2A.Z deposition to have a nonspecific (19) and repressive effect on expression (20).

The characterization of the H2A.Z nucleosome crystal structure by Suto and colleagues (21) provides a snapshot of histone-DNA and histone-histone interactions within the nucleosome core particle containing this histone variant. Divergent amino acid residues in H2A.Z octamers conform to similar nucleic acid-binding sites as native octamers (22) and do not distort the superhelical path of DNA around the nucleosome perimeter (21). Despite this lack of effect on the DNA trajectory, it appears that internal protein-protein interactions are affected. Substitution of H2A Gln104 by Gly104 in H2A.Z destabilizes the 2(H2A.Z-H2B)-{H3-H4}α association and presents an opportunity for DNA activation (15, 17). Also the molecular surface of the variant nucleosome displays a novel acidic patch and a divergent cation-binding site, which may facilitate the rearrangement of higher order structure through internucleosomal electrostatic interactions or the recruitment of remodeling factors (21). However, these observations are only speculative as it is difficult to determine the direct implications of H2A.Z for chromatin from static crystallographic images.

To address this problem we have reconstituted nucleosome core particles and oligonucleosome arrays containing major histone H2A.1 variant and variant histone H2A.Z and have characterized the ionic strength dependence of these complexes by analytical ultracentrifugation. These experiments provide the first clues into the folding dynamics of H2A.Z mononucleosome and chromatin complexes.

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Native Histones and Native Nucleosome Core Particles—Native histone H2A-H2B dimers and H3-H4 tetramers were purified by salt gradient hydroxyapatite fractionation of chicken erythrocyte nucleosome core particles (23). H2A-H2B histones were further fractionated by gel filtration on a Bio-Gel P-60 (Bio-Rad) column according to the protocol described previously (24). Native chicken mononucleosomes were purified as described previously (25).

Recombinant Histones—Polymerase chain reaction was performed on plasmids containing the coding sequences for human H2A.1 and H2A.Z (26) maintaining the ATG codon at the 5′-end of the coding sequence and adding a HindIII site just upstream of the ATG codon and a convenient restriction site at the 3′-end so that the polymerase chain reaction products could be cloned in phase into the HindIII site of the pET17b vector (Novagen, Inc., Madison, WI). This procedure permitted the histone species to be expressed as part of fusion proteins. After constructs were checked by sequencing, duplex oligonucleotides coding for the formic acid-sensitive sequence (Asp-Pro), followed by the nickel-binding sequence His6, were inserted in phase at the HindIII site. The constructs were expressed in bacterial strain BL21(DE3)pLysS (Novagen, Inc.). When expression was maximal, the bacteria were harvested. The pellets were dissolved in 3 volumes of 98% formic acid and incubated at 37 °C overnight, leading to cleavage of the fusion protein species in the (Asp-Pro) region. The formic acid was neutralized with ammonia, and the solutions were dialyzed against 10 mM Tris-HCl (pH 7.6) overnight and passed over a nickel column in the appropriate buffer (Novagen, Inc.). The histone species with their His6 tags were eluted with an imidazole gradient. The eluted material was treated with cyanogen bromide to cleave the tagged histone species at the methionine residue of the initiation codon, lyophilized, dissolved in the appropriate buffer, and passed through a nickel column to remove His6-containing oligopeptides. The histone species were collected in the flow-through and stored at −70 °C (37).

Reconstitution of Nucleosome and Oligonucleosome Arrays—Recombinant human H2A.1 and H2A.Z proteins were mixed with chicken native H2B, H3, and H4 histones in stoichiometric amounts, and histone octamers were reconstituted onto random 146-bp fragments (28) by a 2 to 0 m stepwise salt gradient dialysis (29) in 10 mM Tris-HCl (7.5), 0.1 mM EDTA (8.0) as described elsewhere (28). The 208-12 DNA template consisting of 12 tandem repeats of a 208-bp sequence derived from the sea urchin Lytechinus variegatus 5 S rRNA gene was amplified and purified from plasmid p6S-208-12 kindly provided to us by Dr. R. T. Simpson (30). The stoichiometry of the histone octamer to the DNA in the reconstituted 208-12 oligonucleosome complexes was determined as described previously (28). The reconstituted chromatin particles thus obtained (at a concentration of ~40 mg/ml) were dialyzed against the appropriate buffers (25, 31) and used for subsequent analytical ultracentrifugation analysis (32, 33). In some instances, the reconstituted nucleosome particles were concentrated 5-fold at 4 °C using Centricron YM-10 or YM-50 (Millipore Corp., Bedford, MA).

Gel Electrophoresis—SDS-polyacrylamide gel electrophoresis was performed as described by Laemmli (34). Native polyacrylamide gel electrophoresis was carried out according to Yager and van Holde (35). SDS-polyacrylamide gel electrophoresis of the histones from reconstituted nucleosome core particles and 208-12 nucleosome arrays. Fig. 1, A and B, display the electrophoretic nature of the histone component of these particles.

H2A.Z octamers were reconstituted onto random sequence 146-bp DNA fragments obtained from chicken erythrocyte nucleosomes. The generated particles are shown in Fig. 2A. As it can be seen in this figure, histone octamers consisting of H2A.1 or H2A.Z are equally able to produce mononucleosome particles with identical electrophoretic mobility. The slightly lower electrophoretic mobility of these complexes when compared with native (purified) chicken erythrocyte mononucleosomes (Fig. 2A, lane 4) can be ascribed to differences in the ionic strength of the sample buffer. Indeed the fraction of free DNA that is present in lane 2 of the Fig. 2A (see white arrow) also exhibits a similar extent of mobility retardation when compared with the same DNA template used for the reconstitution of these particles (see Fig. 2A, lane 5). The structural similarity between reconstituted H2A.1 and H2A.Z nucleosome core particles and native chicken erythrocyte particles can be further depicted from the DNase I footprints which are shown in Fig. 2B.

These results clearly demonstrate that recombinant histone H2A.Z variants can be equally reconstituted into nucleosome core particles that are otherwise structurally very similar to native nucleosome core particles. This result is not surprising, and it was to be anticipated from the recently determined crystal structure of H2A.Z nucleosomes (21), which showed that the overall structure of this particle was similar to that of a nucleosome core particle that did not contain this unique histone H2A variant (22).

The apparent lack of a significant structural difference between H2A.Z and native nucleosome core particles consisting of the two major histone H2A isoforms (H2A.1 and H2A.2) is intriguing. Indeed, in contrast to H2A.1/H2A.2 variants, histone H2A.Z has been shown to be essential for survival in organisms phylogenetically as diverse as Tetrahymena (11) and Drosophila (12, 13). It has been determined by substitution experiments with H2A.1 homologous regions that the indispensable portion of histone H2A.Z maps to the carboxyl-terminal of the molecule (13). Interestingly enough, of all core histones (H2A, H2B, H3, and H4), H2A is the only histone with a prominent carboxy-terminal “tail” extending beyond the histone fold (39). The COOH domain also introduces inherent

\(^1\) The abbreviation used is: bp, base pair.
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Fig. 2. A, native (4%) polyacrylamide gel of H2A.Z-containing (lane 2) and H2A.1-containing (lane 3) reconstituted nucleosome core particles. Lane 4 is native chicken erythrocyte nucleosome core particles, and lane 5 corresponds to the 146-bp DNA obtained from these native particles and which was used in the reconstitution of the complexes shown in lanes 2 and 3. Lane 1 is a DNA marker obtained by digesting pBR 322 with HhaI. The white arrow points to the presence of a small fraction of free DNA. B, DNase I footprinting analysis of H2A.1-containing (lane 2) and H2A.Z-containing (lane 3) reconstituted nucleosome core particles in comparison to native chicken erythrocyte nucleosome core particles (lane 1).

Fig. 3. A, ionic strength-dependent variation of the sedimentation coefficient \(s_{20, w}\) of H2A.Z-containing (○) and H2A.1-containing (□) reconstituted nucleosome core particles in comparison to native chicken erythrocyte nucleosome core particles (○). The dashed line corresponds to previously published data (25, 47). B, integral distribution of the sedimentation coefficient (in Svedberg units (S)) of H2A.Z-containing (○) and H2A.1-containing (□) reconstituted nucleosome core particles in comparison to native chicken erythrocyte nucleosome core particles (○) in 0.6 M NaCl. These integral distributions were obtained after analysis of the sedimentation boundaries using the method of van Holde and Weischet (38). In these plots the ordinate represents the fraction of nucleosome arrays sedimenting with an \(s_{20, w}\) given in the abscissa. All experiments were carried out at 20 °C and 40,000 rpm. The buffer used was 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA.

In some may have functional and/or structural implications for chromatin nucleoprotein folding dynamics

Alterations of nucleosome core particle stability in solution can be monitored by changes in their conformation resulting from variations of the ionic strength within the range of salt concentrations where histones still remain bound to DNA (≤0.6 mM NaCl) (32, 46). Changes in the ionic environment of the nucleosome under these conditions are physiologically relevant as they can mimic, to a large extent, the changes in the ionic environment resulting from interactions with other protein complexes such as RNA polymerase or chromatin-remodeling complexes (3) among others.

With this in mind, we decided to characterize the ionic strength dependence of the sedimentation coefficient of reconstituted H2A.1 and H2A.Z nucleosomes. The results of such analysis are shown in Fig. 3A. They indicate that H2A.1 nucleosomes behave in a way that is almost indistinguishable from native nucleosome core particles. In contrast, although H2A.Z reconstituted nucleosome core particles exhibit a very similar sedimentation coefficient value of 8.3 S at 600 mM NaCl, this parameter displays a characteristic declining trend to a sedimentation coefficient value of 8.3 S at 600 mM NaCl. This drop in the S value is clearly indicative of a conformational change of the nucleosome core particle. Integral distribution analysis (38) shows that at 0.6 mM NaCl, about 30–40% of the H2A.Z
nucleosome core particles sediment at 8.3 S, 30% sediment at 5.4 S, and the remaining 30% sediment with intermediate values (see Fig. 3B). This is in contrast to reconstituted H2A.1 nucleosomes, which under the same conditions exhibit a 9.4 S (70%), 5.4 S (15%), and 15% component of intermediate sedimenting particles (see Fig. 3B) in what is almost indistinguishable from native nucleosome core particles (32, 33). The 5.4 S value corresponds to free nucleosomal DNA, which is reversibly dissociating from the nucleosome core particle (32). While the conformational change in the native and H2A.1 nucleosome core particles corresponding to 9.4 S at 0.6 M NaCl is not yet clearly understood (52), the value of 8.3 S observed with H2A.Z nucleosome core particles under the same conditions could be accounted for by partial H2A-H2B depletion. We have experimentally determined the sedimentation velocity coefficient of a nucleosome core particle deficient in one H2A-H2B dimer to be 8.6 S and that of the nucleosome core particle lacking both dimers to be 6.9 S, whereas the sedimentation coefficient of free 146-bp DNA was determined to be 5.2 S.2 These values are in good agreement with similar experimental values reported by other groups (53). Thus it is possible that the decrease in sedimentation observed in the case of the H2A.Z nucleosome core particle corresponds to the progressive loss of particle integrity (H2A.Z-H2B dissociation).

H2A.Z particle lability shown in Fig. 3 was also observed elsewhere during sample manipulation. H2A.Z particles displayed an increased electrophoretic mobility compared with the unmodified patterns of its major H2A and native nucleosome counterparts following concentration (see Fig. 4). This observation corroborates the ultracentrifuge analysis and indicates that the H2A.Z nucleosome core particles have a reduced stability. The results are in very good agreement and support the crystallographic data that pointed to the existence of a "subtle destabilization of the interaction between the (H2A.Z-H2B) dimer and the (H3-H4)$_2$ tetramer" in the crystal structure (21).

We decided next to look at the effects of the H2A.Z histone variant in the modulation of internucleosomal interactions by analyzing reconstituted oligonucleosome complexes (28). The results of this analysis are shown in Fig. 5.

Saturated H2A.Z 208-12 oligonucleosome complexes (30) sedimented in low salt (10 mM Tris- HCl (pH 7.5), 0.1 mM EDTA buffer) as a very homogeneous population with a sedimentation coefficient of 29.5 S (see Fig. 5A), similar to its H2A.1 208-12 counterpart (results not shown). However, as the salt was titrated to 150 mM NaCl, the increase in the sedimentation coefficient of the 208-12 H2A.Z reconstituted complexes was consistently lower than that of the 208-12 complexes reconstituted with either H2A.1 octamers or native histone octamers (28, 31). As in H2A.1 or in native histone 208-12 complexes, a plateau was reached at 100–150 mM NaCl but at a (10%) lower average sedimentation coefficient value (34 S) (see Fig. 5B). The increase in sedimentation coefficient of the 208-12 reconstituted complexes under these conditions reflects an increase in the folding of the complexes (31, 33, 54). The very similar values of the sedimentation coefficients of H2A.1 and H2A.Z reconstituted 208-12 complexes at low salt was to be expected from the similarity of the sedimentation coefficient values of the nucleosome core particles under the same conditions (see Fig. 3A), which suggest that both species of nucleosome core particles sediment at 8.3 S, 30% sediment at 5.4 S, and the remaining 30% sediment with intermediate values (see Fig. 3B). This is in contrast to reconstituted H2A.1 nucleosomes, which under the same conditions exhibit a 9.4 S (70%), 5.4 S (15%), and 15% component of intermediate sedimenting particles (see Fig. 3B) in what is almost indistinguishable from native nucleosome core particles (32, 33). The 5.4 S value corresponds to free nucleosomal DNA, which is reversibly dissociating from the nucleosome core particle (32). While the conformational change in the native and H2A.1 nucleosome core particles corresponding to 9.4 S at 0.6 M NaCl is not yet clearly understood (52), the value of 8.3 S observed with H2A.Z nucleosome core particles under the same conditions could be accounted for by partial H2A-H2B depletion. We have experimentally determined the sedimentation velocity coefficient of a nucleosome core particle deficient in one H2A-H2B dimer to be 8.6 S and that of the nucleosome core particle lacking both dimers to be 6.9 S, whereas the sedimentation coefficient of free 146-bp DNA was determined to be 5.2 S.2 These values are in good agreement with similar experimental values reported by other groups (53). Thus it is possible that the decrease in sedimentation observed in the case of the H2A.Z nucleosome core particle corresponds to the progressive loss of particle integrity (H2A.Z-H2B dissociation).

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particles have a very similar conformation at this low salt. The reason for the inability of the 208-12 H2A.Z nucleosome arrays to fold to the same extent as the H2A.1 counterpart (as indicated by the lower sedimentation coefficient values observed in this later instance) is not clear. However, it could possibly be attributed to novel internucleosomal electrostatic interactions resulting from the H2A.Z-H2B dimer acidic patch, which was observed in the crystal structure of the H2A.Z nucleosome core particles (21). It is important to note, however, that this does not preclude the formation of the 40 S higher folding structures (see Fig. 5C), which are observed for 208-12mers constituted with native core histones under physiological ionic strength conditions of 100–150 mM NaCl and which have also been well characterized in the presence of divalent ions such as magnesium (55).

When the results described above are considered together, the data suggest that histone variant H2A.Z has a destabilizing effect on both intranucleosomal histone-histone interactions and at the internucleosomal level. Such destabilization is consistent with the physiological roles attributed to this variant, especially its participation in the regulation of transcription through its enrichment at promoter sites and redundancy with nucleosome remodeling complexes (17) and in recruitment of RNA polymerase complex through the nucleosomal DNA during transcriptional elongation (56).

In the future, it will be interesting to determine the structural effects that covariant modification have on the folding of H2A.Z fibers. In this regard, it has recently been shown that H2A.Z particles retain a nonspecific charge neutralization requirement for viability (57), which may facilitate cooperative regulation of DNA activation.

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