Acetylation Increases the α-Helical Content of the Histone Tails of the Nucleosome*

The nature of the structural changes induced by histone acetylation at the different levels of chromatin organization has been very elusive. At the histone level, it has been proposed on several occasions that acetylation may induce an α-helical conformation of their acetylated N-terminal domains (tails). In an attempt to provide experimental support for this hypothesis, we have purified and characterized the tail of histone H4 in its native and mono-, di-, tri-, and tetra-acetylated form. The circular dichroism analysis of these peptides shows conclusively that acetylation does increase their α-helical content. Furthermore, the same spectroscopic analysis shows that this is also true for both the acetylated nucleosome core particle and the whole histone octamer in solution. In contrast to the native tails in which the histone acetylation; however, the precise structural role of this important post-translational modification remains elusive. Initial hypotheses proposed that this modification was responsible for weakening histone-DNA interactions, thereby producing a more “open” chromatin conformation, but the situation does not appear to be that simple.

At the chromatin fiber level, in the absence of linker histones, histone acetylation induces an extended chromatin conformation (2), which is more amenable to transcription. However, when the full complement of histones is present, the extent of folding of the fiber does not appear to be greatly affected by this post-translational modification (3, 4).

At the nucleosome level, the acetylated particle adopts a more asymmetric structure (5). This is mainly the result of the DNA ends flanking this chromatin particle binding less tightly to the histones and adopting a stretched conformation (2, 6). As ionic strength is increased, acetylated histone tails are more readily released from DNA interaction(s) (7) than their nonacetylated counterparts. This is as expected and is a consequence of the charge neutralization resulting from acetylation. However, under physiological ionic conditions, the histone tails are persistently bound (7) to the nucleosome regardless of the extent of acetylation. Thus, not surprisingly, the evidence in support of histone acetylation facilitating the binding of transcription factors to nucleosomally organized DNA has been very controversial (8–10). In fact, it has recently been shown that binding of the developmental transcription factor HNF3, which preferentially binds to nucleosomal DNA, is not affected by histone acetylation (11). A more recent hypothesis proposes that histone acetylation provides a histone code (12). However, the structural changes associated with this code remain undefined.

The tails of the core histones have been shown to adopt a helical conformation in nucleosomal DNA (13). However, it was not made clear whether the helical conformation preexisted in the histone tails or was a result of their interaction with DNA. Early studies (14) have also indicated that histone acetylation increases the overall α-helical content of these proteins in a way that was not defined. Given the relevance of both acetylation and the histone tails in the processes of chromatin folding (2, 15–17) and the regulation of gene expression (18), we decided to determine the structural effect of acetylation on these histone domains.

Finally, it has been recently postulated that the spacing of the acetylatable lysine residues of the H3-H4 histone tails is “reminiscent of that of an α-helix” (12). This is an idea first proposed 30 years ago by Sung and Dixon (19). This paper represents the first experimental evidence that such a postulate is indeed correct.

MATERIALS AND METHODS

Cell Cultures and Tissues—MSB cells (chicken erythroleukemic cells transformed by Marek's virus) were kindly provided to us by Dr. Vaughn Jackson. The cells were grown in 5% fetal calf, 5% newborn serum in 1:1 Dulbecco's modified Eagle's 1640 medium supplemented with 50 mM HEPES, 30 mM bicarbonate, and 2 mM glutamine as described previously (20). The cells were grown to a density of 1–2 × 10⁶ cells/ml and then were harvested or incubated in the presence of 5 mM sodium butyrate for 20–22 h before harvesting. After harvesting (3800 × g for 10 min at 4 °C), the cell pellets were suspended in 0.5 M Dulbecco's modified Eagle's medium, 40% glycerol at a density of approximately 2 × 10⁶ cells/ml, and the suspension was stored at −80 °C until further use. Chicken erythrocytes were used as a source of native and trypsinized nucleosomes.

Chromatin Preparation—The cell suspension was thawed and centrifuged at 3800 × g for 15 min at 4 °C. The pellets were resuspended in buffer A (0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 10 mM MES (pH 6.5), 5 mM MgCl₂, 1 mM CaCl₂, 0.5% Triton X-100, with or without 10 mM sodium butyrate) at a ratio of 5 ml/g of pellet using a disposable plastic transfer pipette. The suspension was then centrifuged at 3000 × g for 5 min at 4 °C. The pellets were resuspended in buffer A (0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 10 mM MES (pH 6.5), 5 mM MgCl₂, 1 mM CaCl₂, 0.5% Triton X-100, with or without 10 mM sodium butyrate) at a ratio of 5 ml/g of pellet using a disposable plastic transfer pipette. The suspension was then centrifuged at 3000 × g for 10 min at 4 °C. The supernatant was dialyzed against buffer A at 4 °C for 4 h and then centrifuged at 20000 × g for 1 h at 4 °C. The supernatant was then dialyzed again against buffer A at 4 °C for 4 h and then centrifuged at 20000 × g for 1 h at 4 °C.
The nuclear pellets were resuspended again in buffer B (10 ml) using a transfer pipet and the DNA was centrifuged under the same conditions described above. The nuclear pellets were resuspended again in buffer B (10 ml) using a transfer pipet and the DNA was centrifuged under the same conditions described above. The supernatant was stored on ice in the presence of 1:100 (v/v) (protease inhibitor mixture; see below). The supernatant that typically contains 20 mg of DNA. The compositions of the fractions that contain these two proteins. In the case of the histone H4 tail, the trypsinized histone octamer as determined from amino acid analysis. The average molar extinction coefficient of the trypsinized histone octamer as determined from amino acid sequences of the individual histones and from the data reported by Böhml and Crane-Robinson (29) on trypsinized histones as described in Ref. 22. All of the analyses were carried out in triplicate. A value of 3200 cm \(^{-1}\) mol \(^{-1}\) was used for the molar extinction coefficient of the histone tails (30) at 205 nm. Using a comparative amino acid analysis with an internal nucleosome standard, no significant variation of the extinction coefficient resulting from the addition of the acetyl groups could be detected at this wavelength. DNA concentrations were determined using \(A_{260} = 0.45\) cm \(^{-1}\) cm \(^{-1}\). The molecular weight of the 145-base pair nucleosome core particle DNA was taken to be 9.6 \(\times\) 10\(^6\) g/mol. The absorption coefficient of the native nucleosome core particles was \(A_{260} = 9.5\) cm \(^{-1}\) cm \(^{-1}\) (31), and that of the trypsinized nucleosome core particles was \(A_{260} = 10.5\) cm \(^{-1}\) cm \(^{-1}\). From the latter calculation, the absorption coefficient of the trypsinized histone octamer at 260 nm was considered to be the same as that of the native histone octamer (\(A_{260} = 0.23\) cm \(^{-1}\) cm \(^{-1}\)).

Circular Dichroism—Circular dichroism spectra were recorded at 20 °C on a Jasco J-720 spectropolarimeter as described previously (5). The nucleosomes and the histone octamers were dialyzed against the different buffers described here. The histone tails were dissolved directly in the corresponding buffers or in 90% TFE. Nucleosome and DNA samples were analyzed in 1-cm path length cells, and histone and H4 tail spectra were taken in 0.1-cm cells.

For the calculation of the mean residue molecular ellipticity \(\theta\), an average \(M\) value of 110.6 was used for the calculation of the mean molecular residue ellipticity of the native histone octamer and 110.4 for the aspartic acid endopeptidase Asp-N (EC 3.4.24.33) (Roche Molecular Biochemicals) in either 100 mM ammonium bicarbonate (pH 8.0) or 50 mM Tris-HCl (pH 6.0) at room temperature with an enzyme/substrate ratio of 1:1500 (w/w). Endoproteasine Asp-N cleaves the protein at the N-terminal site of aspartic acid with differing specificity depending on pH. Immediately after digestion, the sample was directly loaded onto a reversed-phase HPLC Vydac C\(_4\) (5-\(\mu\)m) 0.46 \times 25-cm column (Vydac, Hesperia, CA) and was eluted with a linear acetonitrile gradient in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. The fractions corresponding to the non-, mono-, di-, tri-, and tetraacetylated H4 tail were collected and lyophilized.

Amino Acid Analysis—Amino acid analyses were carried out on an ABI model 420A derivatizer analyzer system as described elsewhere (28).

Determination of Protein and DNA Concentrations—The absorption coefficient of the native core histones was \(A_{260} = 0.45\) cm \(^{-1}\) cm \(^{-1}\) (14). The molecular weight of the native histone octamer (10.8 \(\times\) 10\(^6\) g/mol) and that of the trypsinized histone octamer as determined from amino acid sequences of the individual histones and from the data reported by Böhml and Crane-Robinson (29) on trypsinized histones as described in Ref. 22. All of the analyses were carried out in triplicate. A value of 3200 cm \(^{-1}\) mol \(^{-1}\) was used for the molar extinction coefficient of the histone tails (30) at 205 nm. Using a comparative amino acid analysis with an internal nucleosome standard, no significant variation of the extinction coefficient resulting from the addition of the acetyl groups could be detected at this wavelength. DNA concentrations were determined using \(A_{260} = 20.0\) cm \(^{-1}\) cm \(^{-1}\). The molecular weight of the 145-base pair nucleosome core particle DNA was taken to be 9.6 \(\times\) 10\(^6\) g/mol. The absorption coefficient of the native nucleosome core particles was \(A_{260} = 9.5\) cm \(^{-1}\) cm \(^{-1}\) (31), and that of the trypsinized nucleosome core particles was \(A_{260} = 10.5\) cm \(^{-1}\) cm \(^{-1}\). From the latter calculation, the absorption coefficient of the trypsinized histone octamer at 260 nm was considered to be the same as that of the native histone octamer (\(A_{260} = 0.23\) cm \(^{-1}\) cm \(^{-1}\)).

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RESULTS

The Far UV Region of the Nucleosome Spectrum Can Be Reliably Used to Determine the Spectrum of the Histone Octamer—Fig. 1 summarizes the experimental approach followed in this paper. It was designed in a way that would allow analysis of the secondary structure of the histone octamer and selectively look at the conformation of its N-terminal domains (tails) in the presence or absence of interaction with the nucleosomal DNA. The electrophoretic characterization of the DNA, nucleosome, and histone components is shown in Fig. 2.

Fig. 3A shows the circular dichroism spectra of native and trypsinized chicken erythrocyte nucleosome core particles as well as that of their constitutive 145-base pair DNA. The histone octamer has a strong contribution to the far UV region of the spectrum, whereas the DNA component exerts its main contribution in the near UV region (see Fig. 3A, inset). The spectra of the trypsinized nucleosome and that of the native counterpart in the near UV region are identical to those reported earlier (22) with an ellipticity increase (~17%) at the maximum (282.5 nm) within this region of the spectrum for the trypsinized nucleosomes.

As is shown in Fig. 3B, it is possible to subtract the DNA contribution to the far UV region of the spectrum from that of the nucleosome and obtain a protein spectrum that is very similar to the spectrum of the histone octamer in a high salt concentration solution (2 M NaCl) in which the histone octamer exists as a stable entity (33, 38). The similarity of the two spectra shown in this figure is remarkable. The ellipticity of the octamer in the nucleosome form was calculated from the concentration of nucleosomes using the absorption coefficient of DNA ($A_{260} = 20.0 \text{ cm}^2 \text{ mg}^{-1}$) assuming 1 mol of histone/mol of DNA and using $M_r = 110.6$. The ellipticity of the octamer in solution was calculated directly from the concentration of the protein using its absorption coefficient (14). Furthermore, the histone octamer spectra are almost identical to those previously reported for the histone octamer in 2 M NaCl (14). From the ellipticity values at 222 nm and using Equations 1 and 2, it is possible to estimate the amount of $\alpha$-helix as 46.6 and 52.5%, respectively, for the histone octamer in the nucleosome and 43.7 and 48.7% for the histone octamer in 2 M NaCl. The values determined with Equation 2 compare very well with the value of 49.4% as determined from the crystallographic structure (34), assuming that the regions not visualized in that analysis (see Fig. 3C) did not have any $\alpha$-helical structure.

The Far UV Spectra of Native and Fully Trypsinized Nucleosome Core Particles Are Very Similar—Once it was established that the far UV region of the CD spectrum of the nucleosome core particle could be reliably used to determine the secondary structure of its constitutive histone octamer, we decided to analyze the changes in the histone octamer resulting from the removal of the histone tails by immobilized trypsin.

It is interesting to note (see Fig. 3A) that the far UV region of the spectrum of the nucleosome for both the native and the trypsinized core particles look very similar, despite the differences observed in the near UV region (see Fig. 3A, inset). However, when this region of the spectrum for the trypsinized particles is normalized for the mass lost from the histone octamer as a result of trypsinization (see thin dashed line in Fig. 3A), then there is an increase in the ellipticity at 222 nm that corresponds to an 11.6% increase in the $\alpha$-helical content of the trypsinized histone octamer (see also Fig. 3B). The $\alpha$-helical content determined in this way is in good agreement with the corresponding value (62%) estimated from the crystallographic data (34). When the value of the $\alpha$-helical content (64.4%) for the trypsinized core is combined with that estimated for the whole molecule (52.8%) and taking into consideration their relative molecular masses, it is possible to estimate the $\alpha$-helical content of the tail domains to be approximately 17%. This
value is considerably lower than the value of 30–35% previously estimated (13).

In an attempt to determine if the α-helical conformation of the tails is a result of their interaction with the nucleosomal DNA as it has been routinely hypothesized (13), we looked at the ionic strength variation of the spectrum of native nucleosome core particles in the range of 25–600 mM NaCl. Nucleosome core particles retain their integrity within this range of salt concentration (31), while the histone tails are presumably released from their interaction with nucleosomal DNA as the ionic strength increases (39). Although the near UV region of the spectra showed an increase at 282.5 nm, which is characteristic of the effect of the ionic strength increase within this range (22), the far UV spectrum remained virtually unchanged, and we could not determine any significant changes in the [θ]222 at any of the salt concentrations analyzed (results not shown).

Acetylation Increases the α-Helical Content of the Histone Octamer—It has been shown that the protein environment significantly affects the amino acid preference for secondary structure (40). Thus, the ionic environment and the electrostatic interactions of the histone tails with the nucleosomal DNA may have an important impact on the structure adopted by these histone domains in the nucleosome.

If the charge neutralization of the histone tails upon interaction with DNA is responsible for their α-helical conformation (13), then it would be expected that acetylation of the lysines in the tails should favor this conformation. To test this possibility, we prepared highly hyperacetylated nucleosome core particles...
(5) and their corresponding histone octamers (see Fig. 2). To facilitate the structural comparison with the native counterparts, these particles were obtained from chicken erythroblast cells grown in the presence of butyrate (see “Materials and Methods” for more details).

Fig. 4, A and B, respectively, shows the CD spectra of hyperacetylated nucleosome core particles and histone octamers in comparison with their native counterparts. As we had reported earlier, histone acetylation increases slightly the ellipticity at the maximum at 282.5 nm in the near UV region of the nucleosome core particle spectrum (see Fig. 4A, inset) (5). The far UV region of the spectrum also exhibits a small (2–3%) but significant and very reproducible increase the negative ellipticity at 220–222 nm. Similarly the octamer also exhibits an enhanced ellipticity within this region (4–5%) when in 2 M NaCl solution. This latter value is in good agreement with the average 4.8% previously reported by Prevelige and Fasman (14) for acetylated HeLa cell octamers in 2 M NaCl under a variety of conditions. This clearly indicates that the α-helical content of the tails increases upon acetylation of their lysine amino acids. A 2–3% overall increase corresponds to a 11–17% increase in the α-helical content of the histone tails. When this value is combined with that described above (17%), the overall increase in the α-helical content of the tails as a result of acetylation increases by 64–100%.

The α-Helical Content of the Histone Tails Increases with the Number of Acetylated Lysines—In order to further analyze the effects of acetylation on the histone tails, we isolated the histone tail of histone H4 with different extents of histone acetylation. Histone H4 purified from either chromatin fraction b or c from butyrate treated cells (see “Materials and Methods”) was digested with Asp-N endopeptidase, and the resulting peptides were fractionated by reversed-phase HPLC (see Fig. 5). As seen in Fig. 5A, lanes 1 and 2, the products of the digestion vary depending on the pH of the buffer used. We found that at low pH (pH 6.0), the digestion proceeds more efficiently than at pH 8.0, and the lower pH conditions result in the production of a peptide (see arrowhead) that runs very close to the tetraacetylated form of peptide 1–23 in AU-PAGE and co-elutes with it in the HPLC. Therefore, we routinely digested H4 at pH 8.0. The elution pattern shown in Fig. 5B corresponds to acetylated histone H4 from chromatin fraction b digested under basic conditions. As can be see in Fig. 5C, this allows the complete purification and isolation of the different acetylated forms of the peptide 1–23, corresponding to the histone tail of H4. It is important to notice that each of the acetylated forms of the histone H4 tail elutes as multiple peaks (see Fig. 5B) except for the nonacetylated form. We attribute this complex elution profile to the different disposition of the multiple acetyl groups within each form and to the high resolution power of RP-HPLC. However, it is also possible that some of these bands could correspond to acetylated fractions that are additionally methylated at lysine 20.

Next, we analyzed the CD spectra of each of these acetylated forms both in the presence of TFE (a well known α-helix stabilizer) (Fig. 6A) and in aqueous solution (Fig. 6B). As seen in Fig. 6A, the histone H4 tail adopts an α-helical conformation in TFE. In contrast, in aqueous solution, these peptides display a spectrum that is clearly characteristic of a random coil (37) as it had already been reported (41). The amount of α-helix in the native nonacetylated form, as calculated from Equations 1 and 2, is approximately 17% and exponentially increases to about 24% in the tetraacetylated form (see Fig. 6C). In the aqueous solution, there is a decrease in the intensity of the negative band at 195 nm, which is most likely the result of some protein compaction resulting from the charge neutralization effects of acetylation (see Fig. 6D).

DISCUSSION

The Tails of the Histones Adopt an α-Helical Conformation upon Binding to DNA in the Nucleosome—The value of 48.7% (using Equation 2; see “Materials and Methods”) for the α-helical content of the histone octamer in solution as determined from the CD spectrum (see Fig. 2B) is in surprisingly good agreement with the value of 49.4% determined from the crystal structure (34). Such excellent agreement is most likely due to the fact that the secondary structure of the core histones consists almost exclusively of α-helix and random coil, and both Equations 1 and 2 are based on peptide models consisting of these two structures. Equation 1 (30) is based on information from polypeptide data (42). Equation 2, in principle, should provide better estimates for histones because it was empirically derived from the analysis of globular proteins and is based on α-helices of 10–20 residues, a size that corresponds to those found in histones (see “Materials and Methods”).

We have consistently found that the α-helical content of the histone octamer in the nucleosome appears to be slightly higher (∼4%). If we assume that the extinction coefficients for DNA and histones were both determined with similar accuracy, then this difference is as expected from and could be attributed to the histone tails adopting a 15–20% increase in helical content upon binding to DNA. This is consistent with the α-helical content of the tails as determined from the analysis of trypsinized nucleosomes described above (see also Fig. 2B) and suggests that the helical conformation of the tails is the result...
However, the estimate of the helical content resulting from this interaction is about half the value determined using nucleosome core particles that had been proteolyzed to different extents with clostripain (13). While the reason for such a difference is not completely clear, it may reflect experimental differences in the proteolysis experiments, both in terms of the different enzymes and the nature of the enzymes used. We have already described and discussed the importance of using immobilized proteases for such analyses (22) and have shown that the use of free trypsin can lead to overdigestion during storage of nucleosome core particles prior to their analysis.

Based on this possibility, Fig. 3C can be used to illustrate this. While the regions predicted to be in an α-helical conformation (stippled boxes) within the N-terminal domains of the histones amount to approximately 40–45%, the amount corresponding to the tails removed by trypsin is about 20–25%, which is in agreement with our experimental determinations.

It has been shown that increasing the salt from 0.2 to 0.6 M NaCl causes the tails of the histones to dissociate completely from the nucleosomal DNA (39). Therefore, if the histone-DNA interactions play a role in the α-helical conformation, it would be expected that their salt-dependent dissociation would lead to a decrease in the band at 220–222 nm of the spectrum.
Unfortunately, the salt-dependent variation of the far UV region of the CD spectra of nucleosomes did not provide the experimental support expected, since no change could be observed in this region of the spectrum as the salt was increased from 200 to 600 mM NaCl. However, as seen in Fig. 3C, the regions of the tails predicted to have helical potential are close to the histone boundaries defined by the trypsin digestion. The use of free trypsin in preparing trypsin-digested chromatin controls in earlier experiments (39) could have easily led to an overestimate of the effects due to these domains.

Despite all of this, the question still remains regarding the lack of structure found within the portions of the tails that could be visualized by crystallographic analysis. As extensively discussed in Ref. 13, this probably has to do with both the stringent conditions used in the preparation of the nucleosome crystals and the use of polyamines, which may have affected these histone domains.

**Lysine Acetylation Increases the α-Helical Content of the Tails Regardless of Its Interaction with DNA**—The results with acetylated nucleosome core particles and acetylated octamers (see Fig. 4, A–B) conclusively show that histone acetylation increases the α-helical content of the histone tails. The fact that this occurs both in solution and when bound to DNA suggests that such an increase is not dependent on the interaction of the affected regions with DNA. This supports observations from the analyses of model peptides, which have shown that the removal of the lysine side chain charges by acetylation stabilizes the helical structure (43).

Furthermore, the CD spectrum of the histone H4 tail containing different extents of acetylation in the presence of TFE (see Fig. 6) shows that the α-helical content increases exponentially with the extent of acetylation. TFE has been shown to selectively stabilize regions of peptides that have a propensity to adopt α-helical conformation in solution (44–47). From the maximum extent of this increase, the amount of α-helices in the absence of acetylation, and the predicted consensus helical region of histone H4 (see Fig. 7B), we can conclude that lysine 16 upon acetylation is the most likely residue to be responsible for such an increase (17–24%), and the region spanning amino acids 15–21 is the most likely candidate for producing the overall helical content of the histone H4 tail observed. If this is the case, the probability of this particular residue having an acetyl group would be expected to increase exponentially with the overall number of acetylated lysines present in this region, as it is indeed observed. Acetylation of lysine 16 in calf thymus histone H4 was shown to provide an altered CD spectrum for *in vitro* reconstituted histone H4-DNA complexes (48).

The fact that lysine 16 is involved in this process is very interesting, since this particular lysine is involved in the over-activation of one of the X chromosomes, which leads to dosage compensation in insects (49). Very recently, an MSL complex has been described in *Drosophila* that acetylates histone H4 at lysine 16 (50). Furthermore, the helical region highlighted in Fig. 7B corresponds to the region where amino acid insertions and deletions in yeast H4 are most detrimental to silencing (51).

**Are the Changes in the α-Helical Content Induced by Acetylation Structurally and Functionally Relevant?**—The results presented in this paper provide the first experimental evidence for acetylation increasing the α-helical content of the histone tails in chromatin. It is interesting to note that this was proposed by Sung and Dixon as early as 1970 (19), before the nucleosome structure had even been described. A similar postulate has also been recently proposed by Strahl and Allis (12). Although our results do not support an effect as extensive as those postulated by these papers, it is important to point out that acetylation may also operate in conjunction with other post-translational modifications such as phosphorylation. A quick inspection of Fig. 7D reveals that phosphorylation of serine 1 in histone H4 could neutralize arginines 3 and 19 if these two residues were part of a helix. Thus, it is possible that acetylation of all of the lysines (or most of them) in conjunction with phosphorylation of this serine could induce a much more dramatic increase in the helical content of this region than what we observed in the presence of acetylation alone. Indeed, it has been shown that during the replacement of histones by protamines during spermiogenesis in rainbow trout, histone H4 becomes extensively acetylated (52) and serine 1 is phosphorylated (19).

In the case of histone H4, the increase in α-helix due to acetylation of lysine 16 would represent a shortening of the span of interaction with DNA of approximately 4 Å (see Fig. 7C). Although this is a relatively small change, we anticipate that such an effect may be more pronounced in other histones such as histone H3, where the predicted α-helical region spans a longer amino acid range (positions 16–26; see Fig. 3C)
encompasses two of the acetylatable lysines. Similarly, the α-helical region predicted for histone H2B (amino acids 15–24) includes three of the acetylatable lysines in this protein. These relatively small decreases in the span of histone-DNA interactions may cumulatively participate in the release of the flanking DNA regions (18 base pairs) of the nucleosome that has been shown to occur upon acetylation (5, 6). It is important to point out that while acetylation has been shown to play an important role in weakening the interaction of the histone tails with the nucleosomal DNA (53, 54), which is consistent with the decrease in the positive charge of the tails, the actual decrease observed for the binding constant (53) cannot account for the release of the flanking DNA ends. In fact, such a release can be observed under physiological buffer conditions in which the acetylated tails are still bound to DNA (7).

An alternative to the possibility discussed in the previous paragraph is that the α-helical increase that occurs upon acetylation may play an important role in the modulation of interaction(s) of the core histone tails with chromatin remodeling complexes such as NURF (Drosophila nucleosome remodeling factor (55) or the yeast SWI/SNF (switch of the mating type/sucrose nonfermenting, remodeling factor from yeast) and RSC (remodel of the structure of chromatin) complex (56).

Also, it has recently been shown that a small amphiphatic α-helical region of approximately 10 amino acids is sufficient for transcriptional activation of Stat5 (57), a transcription factor involved in signal transduction and the activation of transcription. Consequently, relatively small structural changes such as those presented in this paper may have significant structural and functional implications.

Over the years, we have been looking for major structural changes in chromatin produced by histone acetylation. It is possible that the structural changes are actually as subtle as the specificity of the enzymes (histone acetyl transferases/histone deacetylases) that bring them about (58, 59).

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