The Accessibility of Histone H3 Tails in Chromatin Modulates Their Acetylation by P300/CBP-associated Factor*

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P300/CBP-associated factor (PCAF) is a transcriptional coactivator with intrinsic histone acetylase activity. Reversible acetylation of the core histone tails in chromatin has been linked to transcriptional regulation. Here we investigate the mechanism whereby PCAF acetylates its target in chromatin. We demonstrate that recombinant PACF preferentially acetylates the H3 tail in oligonucleosomes, as compared with nucleosome core particles. The rate of acetylation is directly related to the length of the oligonucleosomal substrate. Using a trypsin accessibility assay, we demonstrate that the rate of acetylation is also related to the accessibility of the H3 tail in uncondensed oligonucleosomes. We suggest that PCAF, and perhaps other histone acetyltransferases, acetylate chromatin templates more efficiently than core particle subunits and that this preference arises from an increased accessibility of the H3 tail in either condensed or uncondensed oligonucleosomes. Acetylation of the H3 tails by the native PCAF complex is not affected by the length of the oligonucleosomal substrate. Our results suggest that the accessibility of the H3 tail in chromatin is a major factor affecting their rate of acetylation and that component(s) in the native PCAF complex function to modify the organization of these tails in chromatin thereby enhancing their accessibility to PCAF.

The orderly progression of various DNA-related activities such as transcription, replication, and repair is associated with alterations in the structure of chromatin and nucleosomes. These structural changes are in part mediated by the reversible acetylation of the amino termini of the core histones. Numerous studies have demonstrated a direct correlation between the transcriptional competence of a chromatin template and the acetylation state of the core histones (1–4). The functional link between histone acetylation and transcription has been further strengthened by the demonstration that transcription factors have intrinsic histone acetylase activity (2, 5), that mutants lacking this activity fail to activate their target genes (6, 7), and that transcriptional repression correlates with histone deacetylation (1, 8, 9).

Most studies on the specificity and activity of purified histone acetyltransferases (HATs),1 or multiprotein complexes containing HAT activity, were performed using either free histone core particles, or on oligonucleosomal templates assembled in vitro on DNA sequences that position nucleosomes at specific sites. However, in the cell nucleus, the DNA has a high sequence complexity, the nucleosomes contain histone H1, and the chromatin fiber is folded in a highly condensed structure (10, 11). The molecular mechanisms whereby various HATs acetylate their targets in cellular chromatin are not fully understood. It has been demonstrated that the HAT GCN5 acetylates efficiently an oligonucleosomal template assembled on a repeated array of Xenopus 5 S RNA genes (12). We have recently reported that histone H1 specifically represses the activity of the HAT P300/CBP-associated factor (PCAF) to acetylate its target site and suggested that perturbation of the organization of this histone is a prerequisite for efficient acetylation of H3 in chromatin (13). During these studies, we noted that PCAF acetylates oligonucleosomes more efficiently than nucleosome core particles.

Here we study the molecular mechanisms responsible for the enhanced activity of PCAF on oligonucleosomal substrates. We investigate the ability of the histone acetyltransferase PCAF, either as a recombinant enzyme (rPCAF) (14) or as part of a purified multiprotein nuclear complex (cPCAF) (15), to acetylate oligonucleosome arrays isolated from chicken nuclei. We find that recombinant PCAF, but not PCAF in its native multiprotein complex, acetylates oligonucleosome substrates more efficiently than isolated nucleosome core particles. We demonstrate that this enhanced acetylation activity of rPCAF on oligonucleosomes is the result of an enhanced accessibility of the H3 tail in oligonucleosomes as compared with core particles. Furthermore, we had previously demonstrated that magnesium-induced oligonucleosome condensation significantly enhances the ability of cPCAF to acetylate the H3 histone tail (13). These results, and those showing that GCN5 activity was also stimulated by magnesium (12), indicated that these HATs preferentially acetylate a condensed oligonucleosome structure. These findings were unexpected, because it is generally accepted that condensation reduces the accessibility of chromatin targets to regulatory factors and that transcriptional activation is associated with chromatin decondensation (10, 11, 16). Here we demonstrate that magnesium-induced condensation of an oligonucleosome array does not dramatically alter accessibility of the H3 tail.

Our finding that the transcription factor PCAF preferentially targets condensed oligonucleosomes suggests that the activity of this HAT is an early step in transcriptional activation, perhaps a prerequisite for subsequent chromatin decondensation. Our studies provide a molecular mechanism underlying the enhanced ability of PCAF to acetylate chromatin and clear multiprotein PCAF complex; DTT, dithiothreitol; AUT gel, triton acid urea gel; MNase, micrococcal nuclease; kb, kilobase(s); bp, base pair(s); CBP, CREB-binding protein.
suggest that chromatin condensation alters the organization of the H3 tails in nucleosomes without dramatically affecting its accessibility to factors that function to modify chromatin structure.

MATERIALS AND METHODS

Materials—Recombinant PCAF (rPCAF) (14) and a nuclear complex containing PCAF (cPCAF) (15) were prepared as described previously. [1-14C]Acetyl-CoA (55 mCi/nmol) was obtained from Amersham Pharmacia Biotech. Trypsin was obtained commercially as t-1-tosylamide-2-phenylethyl chloromethyl ketone-treated trypsin from Cooper Biochemical. Nucleosomes, core particles, and oligonucleosomes were purified from chicken erythrocyte nuclei as described previously (10). [3H]Acetylcarnitine (36 mCi/mmol) was obtained from Amersham Pharmacia Biotech. 

HAT Assay—All assays were performed in buffer A (50 mM Tris-HCl, pH 8.0, 10% glycerol (v/v), 1 mM dithiothreitol (DTT), 0.1 mM EDTA, and 10 mM butyric acid) (17). Oligonucleosome concentrations were 0.1–0.25 mg/mL and containing the [3H]Acetylcarnitine concentration was 18 μM. The assay was performed at 37 °C and initiated by addition of the enzyme to a mixture containing the substrate and acetyl-CoA in buffer A. Because the cPCAF is a more potent HAT (15) than rPCAF, the quantity of rPCAF or cPCAF added to each assay was empirically determined as the amount of preparation required to yield nearly equivalent activities on nucleosome core particles. The amount of PCAF used was empirically determined by using various amounts of the preparation to assure a linear range for the reaction (the concentration of rPCAF in all assays was approximately 10–20 ng/μL, the concentration of the complex as PCAF was approximately 1–2 ng/μL). All assays were conducted for 20 min at 37 °C. The radioactivity incorporated into the protein substrate was detected using a polyacrylamide gel assay (18). In this assay, the reactions were stopped by the addition of an equal volume of a SDS-gel sample buffer (100 mM Tris-HCl (pH 6.8), 200 mM DTT, 2% SDS, 0.1% bromphenol blue, 20% glycerol) and boiled for 5 min, and the proteins were resolved on a 15% SDS-polyacrylamide gel. The electrophoresis was performed at 15 V/cm and stopped when the bromphenol blue reached the bottom of the gel. The bands were stained with Coomassie Blue for estimation of protein quantities and vacuum-dried, and the amount of each species was quantified by software. Alternatively, the number of acetates incorporated into the individual core histones were determined using triton acid urea gels (19). In this case the acetylation assay was performed as describe above except that the reaction was stopped at the given times by addition of an equal volume AUT running buffer (20% glycerol (v/v), 8 M urea, 5% acetic acid (v/v), 0.02% Pyronin Y (w/v), 100 mM DTT, 5.0 mg/mL protamine sulfate). The samples were heated for 5 min at 50 °C and loaded onto the AUT gel. The electrophoresis was carried out for 15 h at constant voltage (200 V). The bands were visualized using Coomassie Blue, and the amount of each species was quantified by densitometry using a scanning densitometer and ImageQuant software (Molecular Dynamics).

Treatment of Oligonucleosomes—In the oligonucleosome compaction studies the nucleosome cores or oligonucleosomes were incubated in 2 mM MgCl₂ for 30 min at 4 °C, and the acetylation assays were performed as described above, except that they were performed in buffer A with 2 mM MgCl₂. Micrococcal nuclease (MNase) digestions were performed as described previously (20). For MNase digestion prior to acetylation reaction, the digests were performed as above and stopped with the addition of EDTA and EGTA to 3 and 5 mM, respectively. The digestion reactions were then diluted 2-fold into 2× buffer A (containing 36 μM [1-14C]acetetyl-CoA), and the acetylation assay was initiated by addition of either rPCAF or cPCAF. Reactions were terminated and analyzed as described above.

Trypsin Accessibility Assay—Trypsin digestions of the core particle or oligonucleosome substrates were performed at 37 °C in buffer A except that acetyl-CoA was omitted from the reaction. The amount of trypsin necessary for mild digestion of the substrate was empirically determined by digesting purified core particles or oligomers for 10 min with varied concentrations of trypsin. Once the appropriate concentration of trypsin was determined, the substrates were subjected to a time course of digestion. The digestions were stopped by addition of a 5×-concentrated SDS dye mix (250 mM tris-HCl (pH 6.8), 500 mM DTT, 10% SDS (w/v), 0.5% bromphenol blue (w/v), and 50% glycerol (v/v)). The samples were incubated for 5 min at 95 °C and run on a 15% SDS-polyacrylamide gel. The digestion products were visualized using Coomassie Blue, and the extent of digestion was quantified using scanning densitometry and analyzed as described above.

RESULTS AND DISCUSSION

To examine the ability of PCAF to acetylate various substrates, we purified nucleosome core particles and H1-free oligonucleosomes from chicken erythrocyte nuclei and subjected them to acetylation by either the recombinant PCAF (rPCAF) or by the nuclear multiprotein PCAF complex (cPCAF). Agarose gel electrophoresis of the oligonucleosomal DNA indicated that the starting material was on the average of 2 kilobases (kb) in length, i.e. it contained about 10 nucleosomes (Fig. 1a). Limited MNase digestion generated a typical oligonucleosomal pattern (not shown, see Ref. 13), whereas prolonged digestion resulted in protection of the canonical 147-base pair (bp) DNA fragment indicative of the nucleosome core particle (Fig. 1a). Unless noted otherwise, all studies were done under low salt conditions where the oligonucleosomal arrays are uncondensed.

Fig. 1 shows the degree of PCAF-mediated (rPCAF or cPCAF) H3 acetylation in duplicate samples of core particle and uncondensed oligonucleosomes. We find that rPCAF acetylates histone H3 in oligonucleosomes 2.7-fold more efficiently than the H3 in core particles. The specific activity of H3 isolated from the acetylated oligonucleosomes was 2.7-fold higher than that isolated from core particles (Fig. 1c). In contrast, cPCAF exhibited no preference for acetylation of either substrate, exhibiting equivalent activities toward core particles and oligonucleosomes. We note that the enzymatic activity of rPCAF cannot be compared directly to those of cPCAF, because the
former is a recombinant enzyme while cPCAF is a multiprotein complex isolated from nuclei. Therefore, the amounts of HAT used in these assays are empirically normalized to give approximately similar activities with nucleosome core. Nevertheless, the results clearly indicate that, for the free enzyme (i.e. rPCAF), the uncondensed oligonucleosomes are a better substrate than the core particles, whereas the PCAF complex acetylates both substrates with equal efficiency.

To determine if the acetylase activity of rPCAF correlates with nucleosomal length, and to eliminate the possibility that the results are due to some artifact associated with the oligonucleosomal purification procedures, we examined the acetylation efficiency for both rPCAF and cPCAF as a function of MNase digestion. MNase digestion occurs in the linker region between adjacent core particles and reduces the average size of the oligonucleosomes until most of the preparation is reduced to mononucleosomes (11, 20). Fig. 2a shows the DNA products resulting from digestion of a fragment with an average length of 10 nucleosomes. As depicted in Fig. 2b, the gradual digestion of the oligonucleosome to monomer length (estimated from the relative amounts of core particles) correlates with a gradual loss in the acetylation efficiency of rPCAF but has no significant effect on the acetylation efficiency of cPCAF. This digestion resulted in the appearance of a monomeric subunit containing approximately 176 bp of DNA (Fig. 2a). Calculation of the specific activities of H3 acetylation indicated that the oligonucleosome was acetylated approximately 8-fold better than that of the sample digested to mononucleosomes (containing linker DNA). We note that direct comparison of the rate of acetylation of purified core particles and oligonucleosomes yielded the same ratio (Fig. 1). These results indicate that the difference in acetylation efficiency observed for the oligonucleosome and the core particle does not arise simply from the presence of the linker DNA. Rather, the results indicate that the preference must arise from a feature intrinsic to an array of nucleosomes. These results are in complete agreement with those depicted in Fig. 1, indicating that rPCAF exhibits a strong preference for acetylation of the H3 tail in the oligonucleosome, whereas cPCAF acetylates all substrates with equal efficiency.

The results in Fig. 2 show that, as the oligonucleosome is digested to shorter arrays, there is a concomitant decrease in the efficiency of PCAF-mediated acetylation. These results suggested that the enzyme exhibits a size dependence in substrate preference. To examine the relationship between oligonucleosomal length and the acetylation activity of PCAF, we purified oligonucleosomes of different average lengths and examined the ability of both cPCAF and rPCAF to acetylate these substrates. The average length of the oligonucleosomal substrates were estimated from the midpoint of the DNA bands isolated from these oligonucleosomes and analyzed on agarose gels (Fig. 3a). The data revealed a direct correlation between the acetylation activity of rPCAF and the oligonucleosomal length of the substrate (Fig. 3b). A preparation with an average length of tetranucleosomes was acetylated 4.5 times more efficiently than an equal amount of nucleosome core particles, whereas a preparation with an average length of six nucleosomes was acetylated 6 times more efficiently than a core particle. In contrast, the oligonucleosomal length (Fig. 2b) did not affect the activity of cPCAF.

What is the molecular mechanism underlying the preferential rPCAF-mediated acetylation of the H3 tail in oligonucleosomes, as compared with core particles? A significant difference between the core particle and an oligonucleosomal chain is the presence of the linker DNA between adjacent core particles. Our finding that the acetylation activity is directly proportional to the oligonucleosomal length argues against the linker DNA as the major factor in determining acetylation levels. Indeed, we found that rPCAF acetylates mononucleosomes, which contain part of the linker DNA, as efficiently as core particles, which lack linker DNA (Fig. 2). We therefore suggest that the main effect is due to the interaction of the histone tail with neighboring nucleosomes (not necessarily contiguous), even in the uncondensed state, a situation consistent with the known role of H3 in the condensation of the chromatin fiber (21–28). Conceivably, by interacting with the DNA on neighboring nucleosomes, rather than with the DNA on its own core particle, the nucleosomal organization of the H3 tail is altered and the acetylation sites are sterically more accessible to rPCAF. This explanation is also consistent with the length dependence (Fig. 3). If we assume that the interactions can occur between non-contiguous nucleosomes within the array, then the increased length and greater flexibility of the fiber would facilitate these interactions. Thus, steric factors may be affecting the rPCAF-mediated acetylation of the histone H3 tail.

To test this hypothesis, we examined the ability of trypsin to digest the histone tails in core particles and in oligonucleosomes (Fig. 3). It has been previously demonstrated that the H3 tail is especially sensitive to trypsin digestion in chromatin (29). Fig. 4, a and b, shows representative digestion patterns as a function of increasing times of trypsin digestion for the core particle (Fig. 4a) and an oligonucleosome fragment containing 12 nucleosomes (Fig. 4b). Quantitative analyses of the rate of H3 tail digestion, either as a function of trypsin concentration or of the time of digestion, are shown in Fig. 4, c and d. The rate of digestion of the H3 tail increased with oligonucleosomal length, with the H3 tail in the nucleosome core particle being the most resistant, and the 12-mer being the most sensitive to...
digestion. Fig. 4d also indicates that all of the substrates can be digested to completion if sufficient trypsin is added to the reaction, indicating that there is not a particular conformer of the substrate in the reaction that is undigestable by trypsin. These results suggest that the organization of the histone H3 tails in oligonucleosomes is distinct from that in core particles. Trypsin cleaves adjacent to Lys and Arg residues without any known specific conformational requirements. These results suggest that the H3 tail in oligonucleosomes does not adopt a unique conformation that is specifically and uniquely recognized by rPCAF. We conclude, therefore, that the H3 acetylation site is sterically more accessible to rPCAF in oligonucleosomes than in core particles. Interestingly, the difference in digestion rate was specific for the H3 tail. Although the histone tails for all of the core histones are digestible by trypsin, the H3 tail adopts a distinct conformation in oligonucleosomes as compared with that in core particles.

Because our results indicate that the H3 tail in oligonucleosomes is more accessible than that in core particles, our enhancement in acetylation could result from the acetylation of additional lysines in the H3 tail. We therefore examined the number of acetylated species in core particles versus oligonucleosomes using triton acid urea (AUT) gels. Core particles or oligonucleosomes were acetylated with rPCAF for 20 or 40 min, and the resultant reaction products were analyzed on AUT gels (Fig. 5). Fig. 5a shows the Coomassie Blue-stained AUT gel from the acetylation reaction. Fig. 5b and c, shows the densitometric scan of the H3 region of the gel. Acetylation of both core particles and oligonucleosomes results in an increase of the mono-acetylated H3 species. The increase in the relative levels of the mono-acetylated species in oligomers was significantly higher than that in core particles (Fig. 5a, arrow at H3). Analysis of the [14C]acetate incorporation indicated that all counts were incorporated into the mono-acetylated species (not shown). The results show that acetylation of the oligonucleosomes result in greater accumulation of the mono-acetylated species and are not caused by additional acetylated forms arising from the reaction. Therefore, we conclude that the preferential acetylation of the oligonucleosome over the core particle arises from an enhanced rate of acetylation at a specific site and not from the appearance of additional acetylation sites.

PCAF also acetylates the histone H4 tail, albeit with a much-reduced efficiency compared with the H3 tail (14, 15, 30). Quantitative analysis of the acetylation pattern for H4 in the AUT gels (Fig. 5) indicated that there was an accumulation of mono- and di-acetylated forms of H4 as a function of time of acetylation with rPCAF for both the core particle and the oligonucleosome (Fig. 5a, arrow at H4). However, in contrast to H3, the rate of appearance of the acetylated H4 species for core particles was very similar to that of the oligonucleosome. These results are in full agreement with our trypsin digestion studies that indicated that the sensitivity of H4 to trypsin in core particles is the same as that in oligonucleosomes (Fig. 4). Taken together, these results indicate that the enhanced accessibility, as measured by acetylation or trypsin digestion, is specific for the H3 tail. Furthermore, we conclude that the H3 tail adopts a distinct conformation in oligonucleosomes as compared with core particles.

Magnesium ions are known to condense oligonucleosomes (22, 31, 32). Previous studies have demonstrated that oligonucleosome condensation by magnesium resulted in a slight enhancement of the rate of H3 acetylation by GCN5 (12) and by both rPCAF and cPCAF (13). We reasoned that, if the accessibility of the H3 tail affects the acetylation activity of these HATs, then the magnesium-induced condensation of the oligonucleosome might also enhance the sensitivity of the H3 tail to trypsin digestion. Control experiments with the linker histone
The mono-acetylated peak increased. acetylated (1), and di-acetylated (1) states. Note that only the amount of some (3) oligonucleosomes incubated with rPCAF for 40 min and decreased at 10 mM Mg2+. Conditions known to condense oligonucleosomes (22, 31, 32), matin regions, containing genes that may be either transcrip-
tively active or poised to be active, exist in an altered confor-
mation and are accessible to various nucleases (10, 11). Therefore, our present studies with uncondensed oligonucleo-
somes taken together with our previous studies on H1 and magnesium-condensed oligonucleosomes (13) may be relevant to the in vivo situation.

All of these data are consistent with the conclusion that the steric accessibility of the amino terminus of H3 in chromatin is the major factor determining the rate of its acetylation by PCAF. In addition, both our previous results (13) and the data presented here, suggest a difference in the regulation of the activity of the free enzyme (rPCAF) and the enzyme when in a complex. cPCAF can overcome the inhibitory effects of H1 more efficiently than rPCAF (13), and it can overcome the repressive conformation of the H3 tail in the core particles (Figs. 1 and 2). The differences between rPCAF and cPCAF suggest that components present in the multiprotein PCAF complex modulate the organization of H1 and that of the H3 tails in chromatin and affect the steric accessibility of the H3 tails to acetylation.

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