The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and chromatin structure

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Histone methylation regulates chromatin function dependent on the site and degree of the modification. In addition to creating binding sites for proteins, methylated lysine residues are likely to influence chromatin structure directly. Here we present crystal structures of nucleosomes reconstituted with methylated histones and investigate the folding behavior of resulting arrays. We demonstrate that dimethylation of histone H3 at lysine residue 79 locally alters the nucleosomal surface, whereas trimethylation of H4 at lysine residue 20 affects higher-order structure.

The methylation of lysine and arginine side chains in histones has emerged as an important class of epigenetic marks that occur on various residues within the histone tails^{1,2}. One of the best-studied methylation marks in the structured region of a histone occurs on histone H3 lysine 79 (H3K79; ref. 3), located in the solvent-exposed C-terminal end of H3 α 1 (**Fig. 1a**). Recently developed methodology to chemically introduce specific modifications into recombinant histones⁴ allows the investigation of the effect of methylation on the structure of nucleosomes and chromatin. This approach uses methylated lysine analogs, which were previously shown to behave identically to methylated lysine residues in various assays⁴. Here we study two methylation marks with opposing effects on chromatin: dimethylated H3K79 (H3K79me2), which is enriched at active promoters⁵; and trimethylated histone H4 lysine 20 (H4K20me3), which marks specific repetitive elements found in repressive chromatin⁶.

Recombinant *Xenopus laevis* histones were chemically modified to yield H_{3K_c79me2} and H_{4K_c20me3} , as described⁴. Most of the histones used here carry the chemical modification, as shown by mass spectrometry analysis (**Supplementary Fig. 1** online). Histone octamers containing either H_{3K_c79me2} , H_{4K_c20me3} or unmodified recombinant H3 and H4 (in addition to unmodified recombinant H2A and H2B) were reconstituted onto a 146-bp DNA fragment derived from human α -satellite DNA⁷ to yield nucleosome core particles (NCPs)⁸ (**Supplementary Methods** online). We determined by molecular

replacement the crystal structures of NCPs containing either $H3K_c79me2$ or $H4K_c20me3$ to a resolution of 3.2 and 2.2 Å, respectively (**Supplementary Table 1** online).

The side chains carrying either modification are clearly visible in the initial electron density and in SA-omit maps of the refined structures (Supplementary Fig. 2 online). We have analyzed data from three H3Kc79me2 crystals and from five H4Kc20me3 crystals, in both cases from two independent preparations. These crystals diffracted to different resolutions and had subtly different unit cell dimensions (as is usually the case with nucleosome crystals); however, the conformations of the modified side chains were consistent between the different data sets. For H3K_c79Me2 (Supplementary Fig. 2a,b), the side chain seems to assume two alternative conformations that, owing to the limited resolution of the structure, were not refined as such. The methyl groups for H4Kc20Me3 are not as defined at the contour level shown (Supplementary Fig. 2c,d), but do appear at lower contour levels because of the increased side chain disorder of this residue (not shown). As expected, methylation of either residue has no effect on the global structure of the nucleosomes, as shown by an r.m.s. deviation of 0.325 or 0.181 between NCPs reconstituted with either H3Kc79me2 or H3K_c20me3, respectively, and unmethylated NCP (PDB 1KX3).

Unmodified H3K79 is in a position to make a weak hydrogen bond with the main chain in the L2 loop of H4. The added bulk from the two methyl groups on the ɛ-amine of H3Kc79me2 causes the side chain to assume alternative conformations in both copies of H3K_c79me2, making them almost completely solvent accessible (Fig. 1b). Although there is no evidence that the loss of a single weak hydrogen bond has an effect on global nucleosome stability, the addition of methyl groups together with side chain rearrangement alters the local electrostatic potential as well as the molecular surface of the nucleosome. Specifically, unmodified K79 covers a small hydrophobic pocket lined by H4 residue Val70 and H3 residue Leu82 (compare Fig. 1c and Fig. 1d). The relocation of H3K_c79me2 (shown in gold in Fig. 1b) partially uncovers this region. Together, these changes result in a reshaping of the local surface near the C-terminal end of H3 a1, near superhelix location 2.5. Recent published work has demonstrated that subtle changes of the nucleosomal surface can significantly affect chromatin structure and function^{9,10}.

The histone N-terminal tails are critically involved in the formation of higher-order chromatin structure^{11–13}. For example, one molecule of histone H4 within the nucleosome makes essential crystal contacts that are likely to be biologically relevant^{7,14}. Acetylation of histone H4 at lysine 16 (H4K16ac) impedes the ability of model chromatin fibers to condense into more compacted fibers¹³. The crystal lattice formed by modified nucleosomes is the same as that formed by unmethylated nucleosomes (**Supplementary Table 1**), but the geometry of the H4

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Figure 1 Crystal structures of nucleosomes containing $H3K_c79me2$ and $H4K_c20me3$. (a) Location of H3K79 and H4K20 (red) on the unmodified NCP structure (surface representation; PDB 1AOI). Histones H2A, H2B, H3 and H4 are shown in light yellow, red, blue and green, respectively. (b) $H3K_c79me2$ adopts an alternative side chain conformation. The structures of the NCP containing H3_c79me2 (light blue and light green) were superimposed onto unmodified NCP (dark blue and dark green). H3K_c79me2 is shown in yellow and unmodified K79 is shown in red. (c) Electrostatic potential of the same region in NCP containing H3K_c79me2 as shown in b. (d) The equivalent region for unmodified NCP. Red indicates negative surface potential and blue indicates positive surface potential, scaled from -15 to +15. Note the small hydrophobic cavity (indicated with an arrow in c) that is uncovered by the reorientation of H3K_c79me2. (e) Superposition of nucleosomes with H4K_c20me3 (light green, light blue) with unmodified NCP (dark green, dark blue). (f) Superposition of H4 tails from published nucleosome structures. Blue, human NCP (PDB 2CV5)19; yellow, X. laevis NCP (PDB 1KX5)²⁰; red, acetylated histone H4 (PDB 16EI)²¹; green, NCP with H4K_c20me3 (this work, PDB 3C1B).

exposed surface of the histone octamer as key player in mediating nucleosome-nucleosome interactions^{9,10}. To test whether H4K20me3 or H3K79me2 affect the ability of nucleosomal arrays to condense into more compact structures, we reconstituted either unmodified histone octamers or histone octamers containing H3K_c79me2 or H4K_c20me3 onto a DNA template with 12 repeats of a 207-bp '601' nucleosome-positioning sequence (601-207-12)^{16,17}. The saturation of the assemblies

tail preceding amino acid 21 is different compared to previously published nucleosome structures (**Fig. 1e,f**). Unmodified H4K20 makes hydrogen bonds with the surface of a symmetry-related nucleosome (**Supplementary Fig. 3a** online). Trimethylation affects both the orientation of this residue and that of neighboring side chains. The H4K_c20me3 side chain points at the DNA backbone (**Fig. 1e**). His18 of H4 assumes a different conformation, forming a hydrogen bond with DNA, consistent with earlier solution studies in which His18 was crosslinked to DNA at superhelix location \pm 1.5 within nuclei¹⁵. Arg19 of H4 now adopts a position where it contributes to interactions with a symmetry-related molecule (**Supplementary Fig. 3b**). Together, these results underscore the adaptability of the conformation of the histone tails in response to the subtle chemical changes introduced by posttranslational modifications (**Fig. 1f**).

Previous studies demonstrated an important role for the H4 tail, and particularly for residues near K20, in higher-order chromatin structure. In solution, H4-V21C cross-links to the long α 2 helix of H2A in moderately folded arrays where adjacent nucleosomes are in close contact, implicating this region of the H4 tail in interactions with neighboring nucleosomes¹⁴. This is further supported by the observation that H4K16ac inhibits the formation of 30-nm fibers as well as cross-fiber interactions¹³. Recent studies have implicated the was confirmed by sedimentation velocity experiments (Fig. 2a) and EcoRI digestion experiments (Supplementary Fig. 4 online)⁹.

In solution, 12-mer nucleosomal arrays exist in equilibrium between unfolded (29S), moderately folded (40S), maximally folded (55S) and oligometric (> 55S) structures¹⁸. At 1 mM MgCl₂ all three arrays formed folded structures, as indicated by sedimentation coefficients that exceeded $\sim 29S$ (Fig. 2b). Nucleosomal arrays reconstituted with H3Kc79me2 condensed similarly to unmodified nucleosomal arrays, with a maximum sedimentation coefficient of 40S under these conditions. In contrast, a substantial percentage of arrays reconstituted with H4Kc20me3 formed the more compact \sim 55S structures under these conditions. These differences remained pronounced at 1.5 mM MgCl₂ (Fig. 2c), where H4_c20me3-containing nucleosomal arrays started to form small 60-120 S oligomers, whereas H3K_c79me2 again behaved similarly to the wild type (note that a fraction of both the H3Kc79me2 and wild-type arrays sedimented at 55S at this salt concentration). The increased folding ability of H4K-20me3 nucleosomal arrays is not an artifact caused by the introduction of the thioether, because arrays reconstituted with the thioether but no methylation (H4K_c20) behaved similarly to the wild type nucleosomal arrays in these assays (Supplementary Fig. 5 online). Under the same conditions, H4Kc20me2 and H4Kc20me3



Figure 2 Sedimentation velocity analysis of unmodified, $H3K_c79Me2$ and $H4K_c20Me3$ nucleosomal arrays. (a–c) Analysis was carried out in TEN buffer (a), in buffer contain 1 mM MgCl₂ (b) and 1.5 mM MgCl₂ (c). (d) Self-association of unmodified, $H3K_c79me2$ and $H4K_c20Me3$ nucleosomal arrays. Symbols in a–d: •, $H3K_c79me2$ nucleosomal array; \Box , unmodified nucleosomal array; \blacktriangle , $H4K_c20Me3$ nucleosomal array. The experiments were repeated three times with identical results (see also **Supplementary Fig. 3**); one representative experiment is shown. Error bars are s.d.

nucleosomal arrays folded similarly, suggesting that H4K20me2 could also enhance chromatin condensation (**Supplementary Fig. 5**).

Notably, when assayed by differential centrifugation to pellet large oligomeric chromatin assemblies, all five arrays behaved similarly (**Fig. 2d** and **Supplementary Fig. 5d**). This suggests that the primary effect of H4K_c20me3 (and H4K_c20me2) is to shift the intrinsic equilibrium toward more locally condensed chromatin structures, in accordance with the enrichment of this modification within pericentric heterochromatin¹.

Although methyllysine analogs behave similarly to their natural counterparts in binding assays, western blots and functional assays including enzymatic assays⁴, the possibility remains that the substitution of the side chain methylene with a thioether, as found in MLA histones, may cause some perturbation in some contexts. The increased folding ability of $H4K_c20$ me nucleosome arrays, but not unmethylated $H4K_c20$, indicates that this effect is not an artifact of the MLA, but rather is regulated by methylation at this side chain.

Together, our results document the power of chemical approaches to prepare methylated nucleosomes and nucleosome arrays⁴, and demonstrate that MLA nucleosomes can be produced with sufficient purity and quantity for crystallographic analysis. The first high-resolution structures of nucleosomes bearing analogs of biologically relevant post-translational modifications demonstrate that H3K79 dimethylation is likely to exert its effect through modulating the nucleosome surface, with the likely outcome of changing interactions of macromolecules with the nucleosome. Our structures underscore the flexible and adaptable character of histone tails. Our finding that arrays reconstituted with H4K20me3, but not H3K79me2, require less Mg²⁺ to form condensed chromatin is consistent with a role for H4K20me3 in regulating the biological activity of the chromatin fiber by altering the locally condensed state of the genome.

Accession codes. Protein Data Bank: Coordinates for the structures of H3K_c79me2 and H4_c20me3 have been deposited with accession code 3C1C and 3C1B, respectively.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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AUTHOR CONTRIBUTIONS

X.L. carried out the crystallographic and array work; M.D.S. made the methylated histone analogues; J.V.C. helped with refinement and figure preparation; J.C.H., K.M.S. and K.L. supervised the work and wrote the manuscript.

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