Core Histone Tail Domains Mediate Oligonucleosome Folding and Nucleosomal DNA Organization through Distinct Molecular Mechanisms*

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Defined oligonucleosome model systems have been used to investigate the molecular mechanisms through which the core histone tail domains modulate chromatin structure. In low salt conditions, the tail domains function at the nucleosome level to facilitate proper organization of nucleosomal DNA, i.e. wrapping of DNA around the histone octamer. Mg²⁺ ions can substitute for the tail domains to yield a trypsinized oligonucleosome structure that is indistinguishable from that of an intact nucleosomal array in low salt. However, Mg²⁺-dependent formation of highly folded oligonucleosome structures absolutely requires the histone tail domains, and is associated with rearrangement of the tails to a non-nucleosomal location. We conclude that the tail domains mediate oligonucleosome folding and nucleosomal DNA organization through fundamentally different molecular mechanisms.

Nucleosomal arrays, which consist of core histone octamer-DNA complexes spaced at about 200-bp intervals, are the fundamental nucleoprotein assembly of chromatin fibers and higher order chromosomal domains. Nucleosomal arrays in various configurations also are the substrates for both transcription and replication (1). Consequently, it has become important to elucidate the structure-function relationships that pertain to nucleosomal arrays. At the supranucleosomal level, it has long been known that nucleosome arrays exhibit a moderate degree of salt-dependent compaction, even in the absence of linker histones (2–9). Recent studies have shown that the intrinsic folding of nucleosomal arrays is more effective at repressing transcription initiation and elongation by RNA polymerase III than the nucleosome per se (10, 11). Thus, oligonucleosome folding has potential functional importance, both for the regulation of eukaryotic gene expression, and presumably for other nuclear processes that involve nucleosomal arrays as well.

The solution-state folding of nucleosomal arrays is complex, and until recently has been poorly understood. In 10–200 mM NaCl, nucleosomal arrays appear to equilibrate between the extended beads-on-a-string conformation exclusively present in low salt, and a partially folded structure that is equivalent to a contacting zig-zag in its extent of compaction (12, 13). Furthermore, in 1–2 mM MgCl₂, regularly spaced nucleosomal arrays equilibrate between the zig-zag-like conformation and a more highly folded conformation that is equivalent to a 30-nm fiber in its extent of compaction (14, 15). While these observations demonstrate that the core histones in and of themselves can direct formation of highly folded chromatin structures, the mechanism(s) through which the core histones function have yet to be identified.

Trypsinized oligonucleosomes lacking their core histone tail domains remain unfolded in the presence of NaCl (8, 13), suggesting that these domains in some way participate in core histone-directed oligonucleosome folding. To identify the molecular mechanism(s) of tail domain-mediated functions in chromatin, we have used a combination of quantitative agarose gel electrophoresis (16, 17) and analytical ultracentrifugation to determine the hydrodynamic shape, conformational deformability, and surface charge density of trypsinized and intact nucleosomal arrays in the presence and absence of MgCl₂. Results indicate a direct role for the tail domains in mediating both oligonucleosome folding and proper wrapping of nucleosomal DNA; however, these functions are mutually exclusive and occur through fundamentally different molecular mechanisms. These observations imply that the tail domains rearrange in chromatin in conjunction with separate actions at the nucleosomal and supranucleosomal levels.

EXPERIMENTAL PROCEDURES

Materials—pPOL208-12 plasmid, 208-12 DNA template, and bacteriophage T3 were isolated as described previously (16, 17). Agarose (LE) was obtained from Research Organics. Trypsin immobilized on glass beads and soybean trypsin inhibitor were obtained from Sigma. All other chemicals were of reagent grade.

Preparation of Trypsinized Histone Octamers—Native oligonucleosomes and core histone octamers were isolated from chicken erythrocytes as described (12). To remove the N-terminal tail domains (and C-terminal tail for histone H2B), native oligonucleosomes were exposed to immobilized trypsin as described by Ausio et al. (18). After trypsinization, histones were electrophoresed on a 18% SDS-polyacrylamide gel; only those preparations consisting of the P1-P5 peptides described previously² (18, 19) were processed further. Once an appropriate preparation was identified, the trypsinized core histone octamers were separated from oligonucleosomal DNA using hydroxyapatite chromatography (20).

Octamer concentrations were determined from the As₂₅₃ using a molar extinction coefficient of 4.3. Histone fractions were subsequently stored at 4 °C in the presence of 20 μg/ml each of aprotinin and leupeptin. Trypsinized octamers obtained by this method were used within 1 week of preparation, whereas native octamers were stable for >6 months under these conditions.

Oligonucleosome Reconstitutions—Saturated and subsaturated 208-12 nucleosomal arrays were reconstituted from either intact or trypsinized core histone octamers and DNA by salt dialysis as described (21). Moles of histone octamer/mol of 208-bp DNA (r) ranged from 0.2 to 1.2. The DNA concentration was 100 μg/ml. The final dialysis step was against 10 mM Tris-HCl, 0.25 mM EDTA, pH 7.8 (TE) buffer.

2 P1 is amino acids 27–129 of H3; P2 is amino acids 12–118 and 21–125 of H2A and H2B, respectively; P3 is amino acids 24–125 of H2B; P4 is amino acids 18–102 of H4; and P5 is amino acids 20–102 of H4.
and trypsinized nucleosomal arrays in low salt buffer are significantly different, as was first reported by Garcia-Ramirez et al. (13). In particular, the decrease in $S_{20w}$ from 32 to 25 and the increase in $R_e$ from 26 to 32 nm in E buffer confirm that the conformation of trypsinized nucleosomal arrays in low salt is significantly more elongated than that of intact arrays under the same conditions.

Effect of MgCl$_2$ on the Conformational Deformability of Trypsinized Nucleosomal Arrays—The $P_e$ dependence of the $R_e$ provides information regarding conformational deformability; as the $P_e$ approaches the $R_e$ of the molecule being electrophoresed, the $R_e$ of an undeformable macromolecule is constant, while a deformable macromolecule shows a distinctive decrease in $R_e$ (16, 23). A plot of $P_e$ versus $R_e$ for trypsinized 208-12 nucleosomal arrays in low salt E buffer $\pm$ 2.0 mM free MgCl$_2$ is shown in Fig. 1. In the absence of MgCl$_2$, the $R_e$ of a saturated trypsinized nucleosomal array decreased from 32 nm at $P_e \approx 200$ nm (Table I) to $\approx 27$ nm at $P_e = 38$ nm. Under these conditions, the $R_e$ of the naked 208-12 DNA decreased from 42 to 29 nm, while the $R_e$ of a saturated intact nucleosomal array was constant at 27 nm (14). The $R_e$ versus $P_e$ behavior of saturated trypsinized arrays is the same as that observed previously for a subsaturated intact nucleosomal array containing an average of 8-9 nucleosomes/DNA (16). These data indicate that in low salt buffer, trypsinized nucleosomal arrays are more conformationally deformable than intact nucleosomal arrays.

In 2.0 mM MgCl$_2$, however, the $R_e$ of trypsinized nucleosomal arrays was independent of $P_e$ over the range of 40–150 nm, and indistinguishable from that of intact nucleosomal arrays in low salt buffer. Together with the data in Table I, these results indicate that trypsinized nucleosomal arrays in 2.0 mM MgCl$_2$ have the same hydrodynamic shape and conformational deformability as intact nucleosomal arrays in low salt.

Effect of MgCl$_2$ on the Surface Charge Density of Trypsinized Nucleosomal Arrays—The $\mu_o$ is a measure of the average surface charge density. Small ions will contribute to the surface charge density of a large macromolecule only if they interact with the macromolecule strongly enough to be incorporated into the electrophoretic shear plane that defines the $\rho_o$ (24). For example, the $\mu_o$ of naked DNA decreases by 25% in 2.0 mM MgCl$_2$ due to the nonspecific binding of Mg$^{2+}$ ions to the DNA (17).

In low salt E buffer, the $\mu_o$ of saturated (n = 12) trypsinized nucleosomal arrays was only 3% lower than that of the naked 208-12 DNA (Fig. 2). In 2.0 mM MgCl$_2$, however, the $\mu_o$ of

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**Table I**

| (MgCl$_2$)$^a$ | Intact | Trypsinized |
|----------------|--------|-------------|
|                | $R_e$  | $S_{20w}$  | $R_e$  | $S_{20w}$ |
| 0 mM           | 26.7 ± 0.7 | 29 | 32.2 ± 1.1 | 25 |
| 2 mM           | 20.5 ± 1.0 | 40 | 26.2 ± 1.0 | 32 |

$^a$ n = 12 reconstitutes in TE buffer were dialyzed against E $\pm$ 2.0 mM MgCl$_2$ for 4 h at 4°C prior to electrophoresis. Running buffer was determined as described (16, 17). Each of the $R_e$ values represent the mean ± standard deviation of 10-19 determinations at $P_e = 200$–600 nm (total of 3–4 gels).

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For 208-12 DNA and nucleosomal arrays, the $R_e$ in 0.2–0.6% agarose gels ($P_e = 200–600$ nm) is constant, and has been shown previously to provide analogous structural information as the sedimentation coefficient determined in the analytical ultracentrifuge (16, 17).
saturated trypsinized nucleosomal arrays was 20% lower than that of the DNA (compare the n = 0 and n = 12 values in Fig. 3), indicating that Mg$^{2+}$ ions are binding to trypsinized nucleosomal arrays in conjunction with the Mg$^{2+}$-dependent conformational change described above (Table I; Fig. 1). Somewhat unexpectedly, the slopes of the $\mu_o$ versus n plots in 2 mM MgCl$_2$ were identical for trypsinized and intact nucleosomal arrays containing $\leq$6 nucleosomes/DNA molecule. However, although the slope of the trypsinized oligonucleosome plot in 2 mM MgCl$_2$ remained constant above n = 6 (Fig. 3), the slope of the intact nucleosomal array plot changed markedly in this region. We have shown previously that the additional decreases in $\mu_o$ observed for n > 6 intact nucleosomal arrays in 2 mM MgCl$_2$ reflect the increased extents of oligonucleosome folding that occur in proportion to the increased extent of nucleosome occupancy of adjacent 5 S repeats (17).

**DISCUSSION**

Our interpretation of the complex solution-state behavior of trypsinized and intact nucleosomal arrays is schematically illustrated in Fig. 4. Intact 208-12 nucleosomal arrays by numerous criteria are best modeled as a fully extended structure in which two complete turns of DNA are wrapped around each histone octamer (12, 13, 25). Sedimentation analyses have demonstrated unequivocally that structural heterogeneity related to partially unwrapped nucleosomal DNA (26) is not present in the solution state in low salt conditions (12–14). The $\mu_o$ of an intact nucleosomal array in low salt is 20% lower than that of the naked DNA, which is equivalent to 80–100 surface exposed positive charges added by each histone octamer (16).

Finally, a saturated 208-12 nucleosomal array in low salt is characterized less deformable than either naked 208-12 DNA or a sub saturated 208-12 nucleosomal array containing $\geq$1–2 nucleosome-free 5 S repeats (16).

In low salt buffer, trypsinized nucleosomal arrays have markedly different structural properties than intact nucleosomal arrays. A comparison of the $\mu_o$ of trypsinized and intact nucleosomal arrays in E buffer (Fig. 2) indicates that $\sim$85% of the surface positive charges of each histone octamer are contributed by the lysine and arginine residues in the tail domains. This is consistent with previous estimates derived from both chemical modification (27) and thermodynamic (9) studies. Removal of the tails also leads to a decreased $S_{20, w}$, an increased $R_e$, and increased conformational deformability in low salt. Each of these observations suggests that more linker DNA is present in a trypsinized nucleosomal array (and therefore less DNA is bound to the trypsinized octamer) in low salt. Taken together, our data strongly support the previous conclusion by Ausio and colleagues (13) that only the central $\sim$100 bp of DNA is bound to each trypsinized octamer within an array in the absence of salt. Importantly, these data indicate clearly that under low salt conditions the tails within a nucleosomal array function at the nucleosome level to keep DNA at the nucleosome periphery wrapped around the histone octamer.

In 2 mM MgCl$_2$, a trypsinized nucleosomal array has both the same hydrodynamic shape and characteristic conformational deformability as an intact nucleosomal array in low salt (Table I, Fig. 1). This demonstrates that inorganic cations can mechanically substitute for the tail domains to organize the DNA at the periphery of the nucleosome. This conclusion is supported by the previous findings that micrococcal nuclease digests of trypsinized nucleosomal arrays are indistinguishable from those of intact arrays in high salt (1 mM CaCl$_2$) but produce mainly $\sim$100-bp products in low salt (0.05 mM CaCl$_2$) (13), and that trypsinization does not influence the linking number of closed circular nucleosomal arrays in 170 mM NaCl (28). Our observation that the $\mu_o$ of saturated trypsinized nucleosomal arrays in 2.0 mM MgCl$_2$ is 20% lower than that of the naked 208-12 DNA molecule under the same conditions (Fig. 3) indicates that $\sim$40–50 Mg$^{2+}$ ions, an amount equivalent to the total positive charges in the tails, are taken up from the bulk solution concomitant with the Mg$^{2+}$-dependent wrapping of DNA around the trypsinized histone octamer. Importantly, despite having a structure that is indistinguishable from that of an intact array in low salt, a trypsinized nucleosomal array in 2.0 mM MgCl$_2$ is incapable of folding (Table I, Fig. 1; Ref. 14).

We therefore conclude that the core histone tails mediate oligonucleosome folding through a mechanism that is distinctly different than the coulombic-based DNA charge neutralization...
mechanism (29) involved in tail-mediated wrapping of nucleosomal DNA.

Do the tails remain bound to nucleosomal DNA in a folded nucleosomal array? The answer to this question lies in the $\mu_o$ values determined in 2.0 mM MgCl$_2$. Studies to date have identified four potential contributions to the $\mu_o$ of an intact 208-12 nucleosomal array in 2 mM MgCl$_2$: the surface negative charges of the naked DNA molecule, the positive charges on the surface of the histone octamer (16), nonspecific binding of Mg$^{2+}$ to naked DNA in 2.0 mM MgCl$_2$ (17), and Mg$^{2+}$ that is specifically taken up to organize nucleosomal DNA in the absence of bound tails (Fig. 3). Nonspecific Mg$^{2+}$ binding to 208-12 DNA lowers the $\mu_o$ by 25%, while the surface positive charges in the histone octamer and Mg$^{2+}$ uptake during nucleosomal DNA wrapping each lower the $\mu_o$ by 20%. Thus, any mechanism that leads to release of the tails from nucleosomal DNA in 2 mM MgCl$_2$ will result in a $\mu_o$ value that is the sum of all four potential contributions, and hence 65% lower than the $\mu_o$ of DNA in E buffer. By contrast, the $\mu_o$ of both trypsinized and intact nucleosomal arrays will be the same at any given n if the tails remain bound to nucleosomal DNA in 2 mM MgCl$_2$ (since no additional Mg$^{2+}$ ions will be taken up by the intact arrays without tail release). The data in Fig. 3 indicate that the $\mu_o$ is identical for both intact and trypsinized nucleosomal arrays in 2 mM MgCl$_2$, provided that n is ≥6. Thus, the tails remain bound to nucleosomal DNA in 2 mM MgCl$_2$ if the array is highly subsaturated and consequently unfolded (17). However, the $\mu_o$ of a folded n = 12 intact nucleosomal array in 2 mM MgCl$_2$ ($-0.80 \times 10^8$ cm$^2$/V-s) is 67% lower than the $\mu_o$ of naked DNA in E buffer ($-2.42 \times 10^4$ cm$^2$/V-s), indicating that the tails are released from their nucleosomal location in 2.0 mM MgCl$_2$ concomitant with formation of folded oligonucleosome structures. Both folding and release of the tail domains require nucleosome occupancy of adjacent 5 S repeats (17). It is important to note that the tails do not appear to be freely dissociated under salt conditions that promote oligonucleosome folding (9). Rather, the tails presumably mediate oligonucleosome folding by interacting with oligonucleosomal constituent(s) other than nucleosomal DNA. Potential candidates include linker DNA (1, 30, 31) and/or neighboring core histone components. An important implication of these results is that the tail domains rear-

range in chromatin in conjunction with their roles in mediating structural transitions at both the nucleosomal and supranucleosomal levels.

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