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Major Role of the Histones H3-H4 in the Folding of the Chromatin Fiber

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We have characterized the hydrodynamic behavior of nucleosome arrays in which the N- and C-terminal "tails" of the histone H2A-H2B and H3-H4 domains have been selectively removed by digestion with immobilized trypsin. The sedimentation coefficient of the polynucleosome fibers lacking the histone H2A-H2B tails exhibited a salt dependence close to that of the non-trypsinized nucleosome arrays. In contrast, the salt-dependent behavior of the H3-H4-trypsinized polynucleosome fibers was found to be closer to that observed for the nucleosome arrays on which all the histones were trypsinized. This indicates that the N- and C-terminal domains of histones H3-H4 play a major role in the folding of the chromatin fiber. Magnesium titration of the polynucleosome fibers consisting of these trypsinized histone octamer hybrids at low ionic strength indicates that the histone H3-H4 tails also play an important role in the association of the polynucleosome fibers. These findings suggest that, after linker histones (histones of the H1 family), the tails of the histone H3-H4 domains are the major players in the processes that lead to the intra-association (folding) and inter-association of the chromatin fiber.

The folding of the chromatin fiber has been extensively characterized in the past [see (1-3) for a review]. In recent years, it has again become the center of attention by several groups working on chromatin structure [reviewed in (4, 5)]. With regards to the mechanism(s) of folding and the participation of the different histone domains, it had earlier been shown that linker histones (histones of the H1 family) as well as the N- and C-terminal domains tails of the core histones (histones H2A; H2B, H3 and H4) play a critical role in the folding of the chromatin fiber (6, 7).

Recently, it has been shown that chromatin fibers depleted of linker histones can fold to a significant extent under physiological conditions (8, 9). We have shown that the tails of the core histones are mainly responsible for the intermediate state of chromatin folding (9, 10) that is observed under these conditions. This intermediate state of chromatin folding may have an important role in some of the physiologically relevant states of the chromatin fibers which involve a partial depletion of linker histones, for instance at the origin of the replication fork or during transcription (2).

In the present work, we have analyzed the individual contribution to this folding by the 'tails' of each of the two histone octamer domains: the histone H2A-H2B dimer and the histone H3-H4 tetramer (11).

MATERIALS AND METHODS

Histone octamers and DNA template. Histone octamers were prepared from chicken erythrocytes as described elsewhere (9). Removal of the C- and N-terminal domains of the core histones was carried out with immobilized trypsin (12). Native and trypsinized histone octamers (ca. 8-10 mg) were fractionated into their corresponding H2A-H2B, H3-H4 domains by hydroxylapatite chromatography (13) on a 1.5 × 15 cm column eluted with a 0.4-2.0 M NaCl gradient in 0.1 M potassium phosphate (pH 6.7) buffer containing 1mM DTT and 20 μg/ml (TLCK) at a flow rate of 15 ml/5 fractions/hour. The peaks corresponding to the H2A-H2B and the H3-H4 fractions were dialyzed against distilled water and lyophilized until further use. Histone concentrations were determined spectrophotometrically using an absorption coefficient at 260 nm of 0.23 $cm^2\ mg^{-1}$ (14).

The DNA template used was a tandemly repeated array of a 208 bp fragment of the 5*S* rRNA gene from the sea urchin *L. variegatus*, kindly provided by Dr. Robert Simpson (NIH) and was prepared and purified as described elswhere (9).

Preparation of the oligonucleosome complexes. The DNA template (in 2 M NaCl, 20 mM Tris-HCl, pH 7.5), and the histone octamers consisting of stoichiometric amounts of the different histone domains in the same buffer conditions (but containing 1 mM DTT), were mixed

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Abbreviations: DTT, dithiothreitol; PAGE: polyacrylamide gel electrophoresis; SDS, sodium dodecylsulfate; TLCK, *N*-α-tosyl-L-lysine chloromethylketone.

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together and the nucleoprotein complexes were then reconstituted by salt gradient dialysis (15) as described in (9). The following complexes were prepared $(H3\text{-}H4)_2 \cdot 2(H2A\text{-}H2B)$-DNA; $[(H3\text{-}H4)_T]_2 \cdot 2(H2A\text{-}H2B)]$-DNA; $2[(H2A\text{-}H2B)_T] \cdot (H3\text{-}H4)_2$ - DNA and $2[(H2A\text{-}H2B)_T] \cdot [(H3\text{-}H4)_T]_2$ - DNA. The subscript T refers to trypsinized histones in which the C- and N-terminal 'tails' have been removed by trypsin. All the oligonucleosome constructs used in this work contained 11.8 - 12.1 nucleosomes per molecule as determined by sedimentation equilibrium.

Analytical ultracentrifugation analysis. Sedimentation equilibrium and sedimentation velocity runs were carried out as described previously (9) using a Beckman XL-A analytical ultracentrifuge. The partial specific volumes and the estimated molecular masses of the different complexes were determined as described in (12).

Mg^{++} titration. The solubility of the different oligonucleosome constructs was carried out as described elsewhere (16-18).

Polyacrylamide gel electrophoresis. Analysis of the histone composition of the reconstituted nucleoprotein complexes was carried out by SDS-PAGE according to Laemmli (19).

RESULTS

The major aim of this study was to determine the role played by the 'tails' of the different domains of the histone octamer (histone H2A-H2B dimer versus histone H3-H4 tetramer) in the processes of folding and association of the chromatin fiber in the absence of histone H1. To this end, homogeneous polynucleosome complexes were prepared starting from individually purified (trypsinized and/or non-trypsinized) histone domains which were reconstituted onto a sequence-defined DNA template consisting of 12 copies of a 208 bp. DNA fragments from the 5*S* rRNA gene of the sea urchin *Lytechinus variegatus* (20). The two basic histone domains of the histone octamer, the histone H2A-H2B dimer and the histone H3-H4 tetramer (11) with or without trypsinization, were fractionated by hydroxylapatite chromatography. The individual domains thus obtained were then combined to create the following histone octamer complexes: $2(H2A\text{-}H2B) \cdot (H3\text{-}H4)_2$; $2[(H2A\text{-}H2B)_T] \cdot (H3\text{-}H4)_2$; $2(H2A\text{-}H2B) \cdot [(H3\text{-}H4)_T]_2$ and $2[(H2A\text{-}H2B)_T] \cdot [(H3\text{-}H4)_T]_2$, where the subscript T stands for trypsinized. Next, salt gradient dialysis (15) was used to reconstitute these different histone octamers onto the 208-12 DNA template. Figure 1 shows the different histone composition of these complexes.

The folding dynamics of the polynucleosome fibers generated were analyzed by sedimentation velocity in the analytical ultracentrifuge as described previously (9, 10). The results of such analysis are shown in Figure 2. As can be seen in this figure, the salt-dependent sedimentation behavior of the polynucleosome fibers consisting of trypsinized histone H2A-H2B domains and intact H3-H4, shows a pattern that closely resembles that of the complexes obtained upon reconstitution with fully intact histones. In contrast, polynucleosome complexes reconstituted from trypsinized H3-H4 histones and native H2A-H2B dimers exhibit a salt dependence which is clearly intermediate between that of polynucleosomes consisting of intact and wholly trypsinized histone octamers (9). It is clear from this behavior that although the 'tails' from both histone domains (histone H2A-H2B dimer and H3-H4 tetramer) contribute to some extent to the folding of the polynucleosome fiber, the larger contribution comes from the 'tails' of the histone H3-H4 domain.

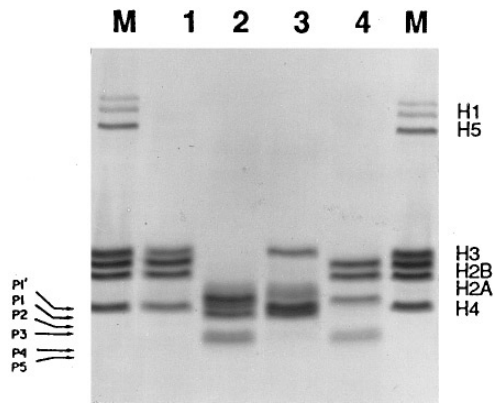

FIG. 1. SDS-PAGE analysis of the histone composition of the different polynucleosome complexes used in this paper. Lane 1: polynucleosomes reconstituted from H3-H4 and H2A-H2B native histones; Lane 2: polynucleosomes reconstituted from trypsinized H3-H4 and H2A-H2B histones, $2[(H2A\text{-}H2B)_T] \cdot [(H3\text{-}H4)_T]_2$; Lane 3: polynucleosome hybrid reconstituted from native H3-H4 and trypsinized histone H2A-H2B histones, $2[(H2A\text{-}H2B)_T] \cdot (H3\text{-}H4)_2$; Lane 4: polynucleosome hybrid consisting of trypsinized H3-H4 and native H2A-H2B histones, $2(H2A\text{-}H2B) \cdot [(H3\text{-}H4)_T]_2$; M: whole chicken erythrocyte histones used as a marker. The nomenclature used for the trypsin-resistent fragments (P1-P5) is that of Böhm and Crane-Robinson (27).

In order to have further insight into the processes involved in this differential folding behavior we analyzed the magnesium-mediated association of all the reconstituted complexes. The results of such analysis are shown in Figure 3 in comparison to the magnesium solubility of native chicken erythrocyte chromatin containing a full complement of linked histones (H1+H5) (18). This figure shows that whereas the polynucleosome hybrid consisting of trypsinized H2A-H2B and intact H3-H4 exhibits a solubility pattern between that of the wholly non-trypsinized and wholly trypsinized complexes, the solubility of the trypsinized H3-H4 polynucleosome hybrid is identical to that of the polynucleosome complex consisting of wholly trypsinized histones. These later complexes remain soluble up to concentrations of magnesium greater than 50 mM (results not shown).

DISCUSSION

Figure 4 shows a schematic representation of the folding behavior of the different polynucleosome con-

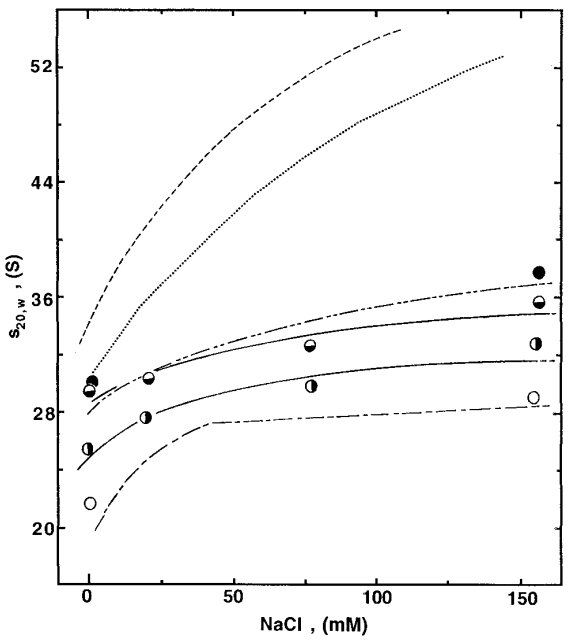

FIG. 2. Salt-dependent sedimentation behaviour of the different 208–12 polynucleosome complexes. (●) 208–12 polynucleosome reconstituted from non-trypsinized histones; (○) 208–12 consisting of fully trypsinized histones; (◒) 208–12 polynucleosome reconstituted from $2(H2A\text{-}H2B)_T \cdot (H3\text{-}H4)_2$ histone octamers; (◑) 208–12 reconstituted from $2(H2A\text{-}H2B) \cdot [(H3\text{-}H4)_2]_T$ histone octamers. Also shown is the salt-dependent sedimentation behaviour of the 208–12 polynucleosomes reconstituted from native histones (– -- –) and wholly trypsinized histone octamers (– - –) previously determined (9) as well as the salt dependence exhibited by a fragment of native chicken erythrocyte chromatin of a DNA size distribution corresponding to approximately 12–13 nucleosomes [from (28 and 29) (---) (· · ·)].

structs characterized in this paper. This analysis of the folding as assessed from the sedimentation behavior of the reconstituted complexes is based on the Kirkwood modelling theory (21) that we have previously described in detail (9, 10). It is clear from Figure 2 that as the salt concentration increases to near physiological values (≈150 mM NaCl) the polynucleosome hybrid consisting of trypsinized histone H2A-H2B dimers (Figure 4B) can fold to a similar extent to that of the polynucleosome complex consisting of nontrypsinized histones (Figure 4A). In contrast, polynucleosome hybrids consisting of histone H3-H4 tetramers in which their tails are removed by trypsin, exhibit a folding behaviour (Figure 4C) which is intermediate between that of polynucleosome complexes consisting of non-trypsinized (Figure 4A) and wholly trypsinized histones (Figure 4D). From the results shown in Figures 2 and 3, it appears that the 'tails' of the histones play a very important role in the inter- (folding) and intra-association of the polynucleosome fiber.

It has been shown that monovalent cations affect the folding of the fiber by primarily screening the residual charge of DNA whereas divalent cations bind to DNA, reducing its residual charge (22). It is possible that the histone 'tails' bind to the nucleosomal DNA in a similar fashion as histone H1 does to the linker DNA. That is, by reducing the electrostatic free energy which is achieved upon displacement of the bound cations and reduction of the residual DNA charge (22). This screening of the repulsive negative charge would allow for adjacent nucleosomes to come together during the folding of the fiber and would also allow for reversible association behaviour (23) of these particles in the presence of magnesium.

We have shown previously (9) that the folding behaviour of the 208-12 polynucleosome complexes reconstituted with native histones is identical to that of histone H1 depleted native chromatin fragments of a similar size (9). Therefore, it should be possible to compare the hydrodynamic behaviour of our complexes to that of native chromatin fragments of a similar DNA length with a full complement of linker histones as in Figure 2. Indeed, the sedimentation coefficient of these native chromatin fragments at near physiological strength50 ± 2 *S* (see Figure 2) is very close to that of 51.5 *S* predicted for a 208-12 complex folded into a higher order conformation (8). From the comparison of the folding and association behaviour of the different polynucleosome complexes to that of native chromatin in Figures 2 and 3, it is clear that the participation of the linker histones and the histone 'tails' on these processes follow a clearly defined hierarchy in which histone H1⩾H3-H4 'tails' >H2A-H2B 'tails'.

This result provides further experimental support to earlier reports (24, 25). It also provides support to our recent *in situ* (within the nucleus) trypsinization studies which showed that the 'tails' of histone H3-H4 play a critical role in chromatin folding (26).

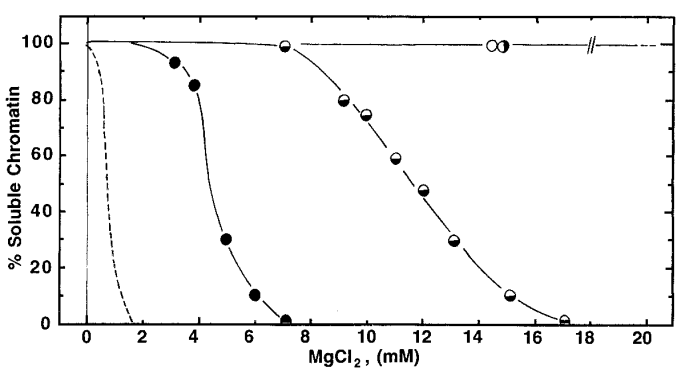

FIG. 3. Solubility of the polynucleosome complexes as a function of $MgCl_2$ concentration in 10 mM Tris-HCl (pH 7.5). The symbols for the different complexes are the same as in Figure 2. Also shown is the solubility behaviour of native chicken erythrocyte chromatin (---) (18).

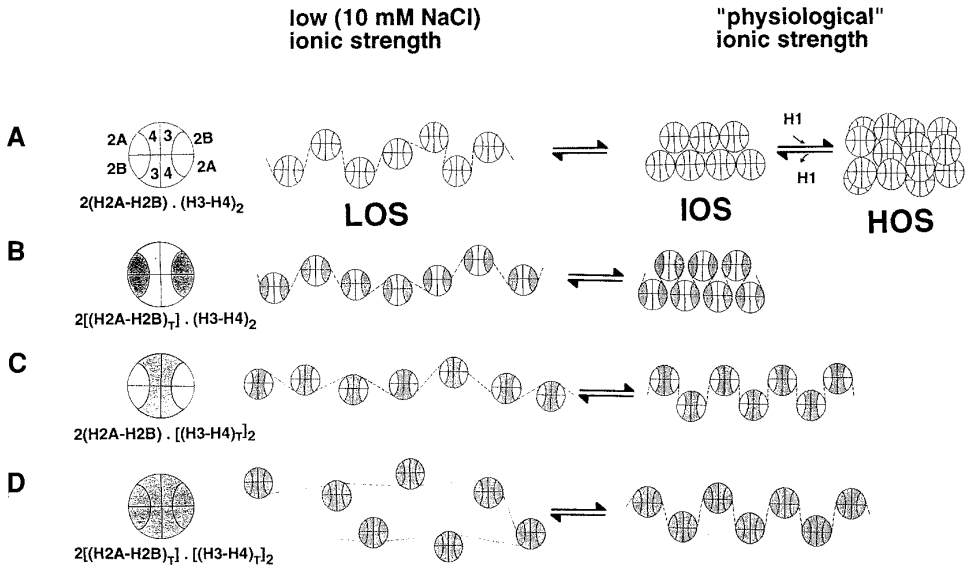

FIG. 4. Schematic representation of the folding behaviour of the different polynucleosome complexes analyzed in this paper. LOS (low order structure); IOS [intermediate order structure (10)] and HOS (higher order structure). The shaded domains of the histone octamers shown on the left-hand side of the figure correspond to those lacking the histone tails. Also shown is the folding pattern of native chromatin (upper row).

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