Nucleosome Structural Transition during Chromatin Unfolding Is Caused by Conformational Changes in Nucleosomal DNA*

(Received for publication, August 27, 1997, and in revised form, November 13, 1997)

Igor M. Gavin‡, Sergei I. Usachenko§, and Sergei G. Bavykin¶

From the W. A. Engelhardt Institute of Molecular Biology, Academy of Sciences of Russia, Vavilova, 32, Moscow B-334, 117984, Russia

We have recently reported that certain core histone-DNA contacts are altered in nucleosomes during chromatin unfolding (Usachenko, S. I., Gavin I. M., and Bavykin, S. G. (1996) J. Biol. Chem. 271, 3831–3836). In this work, we demonstrate that these alterations are caused by a conformational change in the nucleosomal DNA. Using zero-length protein-DNA cross-linking, we have mapped histone-DNA contacts in isolated core particles at ionic conditions affecting DNA stiffness, which may change the nucleosomal DNA conformation. We found that the alterations in histone-DNA contacts induced by an increase in DNA stiffness in isolated core particles are identical to those observed in nucleosomes during chromatin unfolding. The change in the pattern of micrococcal nuclease digestion of linker histone-depleted chromatin at ionic conditions affecting chromatin unfolding also suggests that the stretching of the linker DNA may alter the nucleosomal DNA conformation, resulting in a structural transition in the nucleosome which may play a role in rendering the nucleosome competent for transcription and/or replication.

The compaction of DNA into chromatin provides many obstacles for its functioning in eukaryotic nuclei (1). The current model of transcription and replication suggests that chromatin must be remodeled in at least two distinct steps (2–5). The first step involves decondensation of chromatin fibers facilitating the access of trans-acting factors to DNA. The second step implies either a reversible dissociation of the histone octamer from DNA or a structural transition of the nucleosome allowing transcription or replication to proceed (3, 6–11). A number of reports have suggested that the nucleosome structure in active transcription or replication to proceed (3, 6–11). A number of reports have suggested that the nucleosome structure in active transcription and/or replication. The second step, which involves either remodeling or removal of the nucleosome, the distance between the points where the linker DNA enters and leaves the nucleosome core may be increased by repulsion between adjacent linker DNA segments (17, 21–24) and thereby can affect the conformation of the nucleosome core DNA. To address the question of whether or not the alterations in histone-DNA contacts during chromatin unfolding, which we observed earlier (19), are caused by the changes in the nucleosome core DNA conformation, we have analyzed the histone-DNA contacts in isolated core particles under ionic conditions affecting DNA stiffness.

Several physical and biochemical studies have demonstrated that a reduction of the monovalent ion concentration below 10 mM has a significant effect on the nucleosome core structure (reviewed in Ref. 25). It has also been well documented that the loss of counter-ions at low ionic strengths increases the electrostatic repulsion of unneutralized DNA phosphate groups, resulting in an increase in DNA stiffness (26). This increase in the stiffness may cause stretching of the nucleosomal DNA that in turn may change the conformation of the nucleosome (27) and affect histone-DNA contacts. In the present paper, we show that the alterations in histone-DNA contacts in isolated core particles induced at low ionic strength are caused by a decrease in the neutralization of negative DNA charges and are identical to those observed in nucleosomes during chromatin unfolding (19). This suggests that the conformational changes in nucleosome core particles at low ionic strength and in nucleosomes in chromatin during chromatin unfolding are due to the stretching of the nucleosome core DNA. Based on these observations, we propose a model for the reversible structural transition yielding a new nucleosome conformation in an unfolded chromatin.

**EXPERIMENTAL PROCEDURES**

Isolation of Nucleosome Core Particles and Histone-DNA Cross-linking—Nucleosome core particles were isolated from chicken erythrocyte nuclei as described previously (28, 29). Protein-DNA cross-linking under various ionic conditions, purification, and histone-labeling of cross-linked complexes with ¹²⁵I were performed as described (30, 31).

Micrococcal Nuclease Digestion of Linker Histone-depleted Chromatin—Linker histone-depleted chromatin prepared from chicken erythrocyte nuclei as described earlier (19) was digested with micrococcal nuclease (3 µg/1 mg of DNA) in 10 mM Tris·Cl, pH 8.0, and either 0.5 mM CaCl₂ or 2 mM CaCl₂ for the time course of 20, 40, and 80 min at 37 °C. The reaction was stopped by adding EDTA to a final concentration of 4 mM.

Gel Electrophoresis Analysis—DNA from nucleosomes released by the micrococcal nuclease digestion of linker histone-depleted chromatin...
was analyzed in a 9% denaturing gel containing 7 M urea following digestion with Pronase (0.5 mg/ml) for 30 min at 37 °C (32).

Histone-DNA contacts were mapped by the “protein version” of two-dimensional gel electrophoresis of 125I-labeled cross-linked complexes as described earlier (31). For the qualitative analysis of relative intensities of certain signals, the autoradiographs of two-dimensional gels from at least three sets of experiments were scanned in a SL-504-XL Zeineh soft laser scanning densitometer (Biomed Instruments Inc., Fullerton, CA).

RESULTS

The Alterations in Histone-DNA Contacts in Isolated Core Particles Induced by Low Ionic Strength Are Identical to Those Occurring during Chromatin Unfolding—To study the histone-DNA interactions in isolated core particles under different ionic conditions, we used the method of chemically induced zero-length protein-DNA cross-linking (31, 33). Cross-linking causes a single-stranded nick of the nuclosomal DNA at the site of cross-linking such that only the 5'-end of the single-stranded core nucleosome DNA to 9Otides from the 5'-end of the nucleosomal DNA to the site of cross-linking and were determined by scanning the autoradiographs of “DNA” versions of two-dimensional gels (30). The contacts marked by circles were used as references to assess the relative intensities of altered contacts marked by stars. A signal that was not detected in the “DNA” version of two-dimensional gels is indicated with an asterisk and may appear due to the disruption of traces of histone H3 dimers in the second dimension.

The qualitative analysis of two-dimensional gels shown in Fig. 1 revealed that the position of signals corresponding to histone-DNA contacts in core particles cross-linked in the range of decreasing ionic strengths from 100 to 2 mM NaCl is the same. However, relative intensities of some signals vary. In this work, using densitometric analysis, we qualitatively assessed the variation in histone H2B and H4 signal intensities representing the extent to which a particular histone is cross-linked to a particular site of the nucleosomal DNA. The main cross-linking sites on the nucleosomal DNA for histone H2B are around nucleotides 109, 119, and 129 and for histone H4 are around nucleotides 57, 66, and 93 from the 5'-end of the nucleosomal DNA (19, 29, 30). To estimate the extent of variation in these contacts, we compared their relative intensity within the same histone line and within the same gel because, as shown previously, such a comparison prevents ambiguity caused by choosing the reference spot at different exposures of different gels (19, 30). The data represented in Fig. 1 demonstrate that by decreasing the concentration of Na+ from 100 to 2 mM, the relative intensities of the above-mentioned signals vary, showing a gradual attenuation of contacts H2B(129) and H4(66) compared with contacts H2B(109) and H4(57), respectively (numbers in parentheses indicate the distance in nucleotides from the 5'-end of one strand of nucleosome core DNA to the particular histone contact). It should be mentioned that the same alterations in the same histone-DNA contacts were ob-
served recently as a result of the stretching of a linker DNA during chromatin unfolding in linker histone-depleted chromatin (19). This indicates that an increase in the nucleosomal DNA stiffness, induced by low ionic strength, affects the analyzed histone-DNA contacts in isolated core particles much the same as the stretching of linker DNA during chromatin unfolding and suggests similar changes in the conformation of the nucleosomal DNA, which may result in the structural transition of the nucleosome (27). This suggestion is supported by the recent observation of an alteration in the shape of core particles from the oblate to the prolate form at the same low salt concentrations (36).

The Structural Transition of Nucleosome Core Particles Induced by Low Ionic Strength Is Caused by the Change in the Nucleosomal DNA Conformation—The elevation of salt concentration causes electrostatic blocking of DNA phosphate groups, which depends on the charge of cations (26). The experimentally measured persistence length of DNA increases rapidly when the concentration of monovalent cations is decreased below 10 mM, whereas the same effect for divalent cations was observed at concentrations below 0.1 mM (37). Consistent with this observation, our data, represented in Fig. 2, demonstrate a similar change in the relative intensities of the analyzed contacts after the addition of divalent cations at 2 orders of magnitude less concentrations than monovalent cations. The weak intensity of contacts H2B(129) and H4(66) in 5 mM HEPES (Fig. 2A) is noticeably increased upon addition of 0.1 mM CaCl2 (Fig. 2B). A further increase in the concentration of divalent cations up to 2 mM causes an additional increase in the intensities of contacts H2B(129) and H4(66) (Fig. 2C). The relative intensities of the analyzed contacts at 100 and 30 mM monovalent cations (Fig. 1, A and B) resemble those at 2 and 0.1 mM divalent cations, respectively (Fig. 2, B and C) confirming previous observations that monovalent cations affect the nucleosome structure at approximately 2 orders of magnitude higher concentrations than divalent cations (36, 38, 39). We also tested various monovalent and divalent cations, such as K+, Li+, Cs+, Mg2+, Zn2+ for their ability to change histone H4/H2B contacts, and as expected, different cations that have the same charge had an identical effect on the analyzed contacts at the same concentrations. Cross-linking analysis also reveals that anions have no impact on the conformational transition induced by low ionic strength (not shown). Obtained results suggest that the conformational transition, observed as alterations in relative intensities of histone H2B/H4-DNA contacts at low ionic strength, is caused by the repulsion of adjacent unneutralized negative charges of DNA phosphate groups resulting in an increase in DNA stiffness.

The Interaction of Core Histones with DNA Regions Entering and Leaving the Nucleosome—The level of chromatin compaction induced by 1–2 mM divalent cations is similar to that observed at physiological concentrations of monovalent cations (17, 18). An increase in the salt concentration also results in folding of linker histone-depleted chromatin and bending of linker DNA (17, 40–43). To investigate how this salt-induced chromatin condensation might change the nucleosome conformation, we have analyzed the pattern of micrococcal nuclease digestion of linker histone-depleted chromatin at different concentrations of divalent cations (Fig. 3). The first time point in the course of digestion at 0.3 mM Ca2+ yields nucleosome core DNA fragments isolated from linker histone-depleted chromatin digested by micrococcal nuclease at 0.3 mM CaCl2 (A) and at 2 mM CaCl2 (B) for 20 min (lane 1), 40 min (lane 2), or 80 min (lane 3) in a denaturing 9% polyacrylamide gel (32). M, marker DNA fragments from DNase I digests of rat liver nuclei (77, 78). The size of DNA fragments in nucleotides is indicated at the side of each gel. 2N, 3N, and 5N indicate oligonucleosome DNA fragments.
particles with 146-bp\(^1\) DNA represented in the gel by a single band (Fig. 3A, lane 1). These nucleosomes are further digested to the level of subnucleosomes (Fig. 3A, lanes 2 and 3). In contrast, the first time point at 2 \(\text{mM} \text{Ca}^{2+}\) yields a set of core particles and particles with 157- and 168-bp DNA (Fig. 3B, lane 1), which then turn into particles with 146-bp DNA (Fig. 3B, lane 3). The bending of linker DNA induced by 2 \(\text{mM}\) divalent cations (17, 43) facilitates the wrapping of an additional \(-20\)-bp DNA around the histone octamer (21, 22, 24). This renders linker DNA regions less accessible to micrococcal nuclease yielding nucleosomes with 157- and 168-bp DNA (Fig. 3B). The interaction of core histones with the end regions of nucleosomal DNA in particles with 155- and 165-bp DNA (Fig. 3B) is demonstrated by protein-DNA cross-linking in isolated chromatin (44), in nuclei (45), and in H1-depleted nucleosomes (46). In contrast, at concentrations of divalent cations below 0.3 mM, linker DNA is stretched (17) such that the end regions of the nucleosomal DNA become susceptible to micrococcal nuclease. As a result, nucleosomes with 157- and 168-bp DNA are not observed under these conditions (Fig. 3A). It has been shown that micrococcal nuclease activity is only slightly reduced in 0.3 \(\text{mM} \text{Ca}^{2+}\) compared with 2 \(\text{mM} \text{Ca}^{2+}\) (47), whereas the specificity of the enzyme remains the same (48). This indicates that the observed difference in micrococcal nuclease digestion pattern reflects the change in the chromatin structure rather than the activity of the micrococcal nuclease, which is supported by early studies where a similar effect was observed at various concentrations of monovalent cations (49, 50).

**DISCUSSION**

The change in histone H2B/H4-DNA contacts induced by low ionic strength occurs in the sharply bent regions of nucleosomal DNA. In the present work, we have demonstrated that a structural transition in isolated core particles induced by low concentrations of monovalent cations is caused by the change in the nucleosomal DNA conformation that alters the analyzed histone H2B/H4-DNA contacts. These altered contacts are located at sites \(\pm 5.5\) and \(\pm 1\) (Fig. 4), which are very close to the sharply bent regions of the nucleosomal DNA (51–55). Histone H4 cross-links to the sites around \(\pm 1\) mainly through the single highly basic domain (30, 56, 57), which may alter DNA structure in this region (57, 58). On the other hand, the change in nucleosomal DNA conformation induced by low ionic strength in isolated core particles or by the stretching of linker DNA during chromatin unfolding can in turn affect histone interactions with these DNA sites. Since the analyzed contacts are located close to the sharply bent DNA regions, they would be expected to be very sensitive to conformational changes in the nucleosomal DNA.

Identification of nucleotide-tagged histone H4 peptides (56, 57) and selective proteolysis of cross-linked core particles by trypsin and clostripain (30) have demonstrated that histone H4 cross-links via His-18 to three major sites, at nucleotides 57, 66, and 93, which are extended over 1.2 helical turns of the nucleosomal DNA (Fig. 4). The cross-linking domain of histone H4 has divergent structure within the nucleosome core (51), suggesting that these contacts may be formed by different histone H4 molecules, which may cross-link to two discrete sites at a given time: to nucleotide 66 around site \(\pm 1\) and to nucleotides 57 and/or 93 due to the oscillation of His-18 through the minor groove of the nucleosomal DNA around site \(\pm 2\) (Fig. 4). This is supported by our observations that the relative intensities of contacts H4(57) and H4(93) are not changed at low ionic strength (Fig. 1–2) and during chromatin unfolding (19). The change in the nucleosome conformation induced by low ionic strength or chromatin unfolding may affect the local interaction of the DNA-binding domain of histone H4 with nucleotide 66 and would result in a less intensive cross-linking of its His-18 to this DNA site.

The highly basic N-terminal domain of histone H2B also cross-links to nucleosomal DNA (59); however, it is still unknown whether the observed contacts are formed by the same domain or by different regions of histone H2B. The crystal structure of the nucleosome core particle recently resolved at 2.8 \(\text{Å}\) resolution suggests that loop 2 of histone H2B contacts DNA around sites \(\pm 3.5\) while loop 1 makes contact around sites \(\pm 4.5\) (51). Since the methodology used in this study detects protein-DNA cross-linking mainly through histidine residues (33), it is possible that histidine 79 at the C terminus of \(\alpha\)-helix 2 cross-links to the DNA site at nucleotide 109 while

**Fig. 5.** Schematic representation of the reversible formation of a “stretched” nucleosome. A, interaction of the histone octamer with 168-bp DNA in a compact form of a nucleosome in linker histone-depleted chromatin at 2 \(\text{mM}\) divalent cations. B, formation of a “stretched” nucleosome at concentrations of divalent cations below 0.3 \(\text{mM}\). The change in the nucleosomal DNA conformation due to its stretching causes a release from the histone octamer of approximately 10 bp of DNA at each terminus. Arrows with numbers indicate the sites of preferential micrococcal nuclease cleavage yielding nucleosome particles with 146-, 157- (a and b), and 168-bp DNA. The nucleosomal DNA terminal fragments in the compact form of the nucleosome turn into a linker DNA (represented by a dashed line) in the stretched nucleosome. Arrows on the right side show the direction of the stretching resulting in a prolate shape of the nucleosome (36).

\(^1\) The abbreviations used are: bp, base pair(s).
histidine 46 located in the loop 1 cross-links to the site around the 129th nucleotide. Straightening of the nucleosomal DNA at low ionic strength or during chromatin unfolding may affect the interaction of the end fragments of the nucleosomal DNA with loop 1 of histone H2B, resulting in the loss of histone H2B cross-linking to nucleotide 129.

Models for the Conformational Transition of Nucleosomes—Two models for the conformational transition of a nucleosome core are suggested by the straightening of the nucleosomal DNA have been proposed (27). In the model for a radial transition, the nucleosomal DNA is uncoiled and the nucleosome deforms into a spherical form. In the case of the axial transition, DNA straightens along the superhelical axis, bending the nucleosome into the prolate form. Recent observations of the altered core particle shape to a prolate form (36) that have a decreased (36, 60) or unchanged (61) radius of gyration at concentrations of monovalent cations below 30 mM provide strong support for the second model. This model is also in agreement with the expected repulsion of adjacent superhelical turns of the nucleosome DNA induced by low salt concentrations, which also explains the increased repulsion of adjacent superhelical turns of the nucleosome DNA induced by low salt concentrations, which also expected repulsion of adjacent superhelical turns of the nucleosome DNA. The axial straightening of DNA may also cause destabilization of the nucleosome core, resulting in the change in the linking number of DNA in minichromosomes (21, 22) and in the altered nucleosome morphology in linker histone-depleted chromatin (21–24).

Biological Relevance of the Conformational Transition of Nucleosomes during Chromatin Decondensation—The association of the alterations in the analyzed histone H2B/H4-DNA contacts with the level of chromatin compaction and activity demonstrates the importance of these changes in chromatin structural/functional relationships (19). The alterations in the contacts formed by the highly basic histone domains may result in destabilization of the nucleosomes in unfolded chromatin facilitating nucleosome remodeling and/or removal during transcription and replication. This is strongly supported by the observation that a deletion of the histone H2B cross-linking domain changes the minichromosome topology in vivo, suggesting that this domain is required for the proper folding of DNA in the nucleosome (63). Deletion or substitution of a single amino acid in the cross-linking domain of histone H4 alters chromatin structure in vitro (63–66), decreases the ability of yeast to mate, increases the duration of S phase (67–70), and affects telomeric repression (71, 72) as well as expression of a large number of yeast genes (64, 65, 73–75). It should be mentioned also that the cross-linking domain of histone H4 contains sites for post-translational acetylation and phosphorylation (76) that may also be involved in the regulation of the interaction of this domain with DNA at different levels of chromatin activity and condensation rendering nucleosomes competent for transcription and/or replication.

Acknowledgments—We thank Andrei Mirzabekov for the initial comments and Jodie Usachenko for help in the preparation of this manuscript.

REFERENCES
Nucleosome Structural Transition during Chromatin Unfolding Is Caused by Conformational Changes in Nucleosomal DNA
Igor M. Gavin, Sergei I. Usachenko and Sergei G. Bavykin

doi: 10.1074/jbc.273.4.2429

Access the most updated version of this article at http://www.jbc.org/content/273/4/2429

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 77 references, 22 of which can be accessed free at http://www.jbc.org/content/273/4/2429.full.html#ref-list-1