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Conformation of Nucleosome Core Particles and Chromatin in High Salt Concentration†

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ABSTRACT: The conformation of nucleosome core particles and chromatin under different ionic strength conditions has been studied by electron microscopic, hydrodynamic, and spectroscopic techniques. In the range of ionic strength used (6–600 mM), all four core histones were bound to the DNA. The sedimentation coefficient of the core particle decreases from 11.3 in 6 mM NaCl to 9.4 in 600 mM NaCl, and an alteration of the circular dichroic spectrum was observed when the ionic strength was increased. Direct evidence for the alteration of the chromatin structure in high salt was obtained by electron microscopy where a very extended conformation of the nucleosome was observed. The protein cross-linking agent dimethylsuberimidate was used to study the histone–histone proximities in the core particles; our experiments reveal that the same histones are in contact in the extended particles and in the compact native particles.

The structure of chromatin is not static but undergoes structural changes necessary for the functions in which it participates in the cell. The most obvious change is a gross conformational change of the superstructure of chromatin during mitotic condensation of the chromosomes. During interphase, the bulk of chromatin is less condensed and is organized in repeating units or nucleosomes. Electron microscopic observations, as well as nuclease digestion studies, have shown that subtle changes occur at the nucleosomal level during replication or in the transcriptionally active genes [for a review, see Felsenfeld (1978) and Chambon (1977)]. However, the precise structure assumed by chromatin during transcription or replication is not known. In vitro studies of chromatin conformation may help in understanding the properties of chromatin in vivo. Indeed, recent reports have shown that the nucleosome is able to assume different conformations depending on the salt concentration (Griffith & Christiansen, 1977; Oudet et al., 1977; Woodcock & Frado, 1977; Gordon et al., 1978; Dieterich et al., 1979); i.e., at very

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0006-2960/80/0419-4327$01.00/0

low salt (10^{-5}–10^{-2} M) or at high salt (0.6 M) it is much more open than at moderate salt concentration (10^{-2}–0.35 M). Interestingly, transcription studies of in vitro assembled chromatin have revealed that the propagation rate of RNA polymerase along the nucleoprotein template is increased at high salt concentration (Williamson & Felsenfeld, 1978; Wasylik et al., 1979; Wasylik & Chambon, 1979). This has prompted us to study in more detail the conformation of chromatin in high salt. In this paper, we have used electron microscopic, hydrodynamic, and spectroscopic techniques to study the conformation of nucleosome core particles and chromatin under different ionic strength conditions. We have been able to show that the nucleosome assumes a very extended conformation when the salt concentration is increased. On the other hand, cross-linking experiments with the protein cross-linking agents dimethylsuberimidate show that, although the nucleosome is open in high salt, the same contacts between the histones exist in the extended particles and in the compact nucleosome.

Materials and Methods

Preparation of Nuclei, Chromatin, and Core Particles. Nuclei were isolated from mature chicken erythrocytes according to the procedure of Hewish & Burgoyne (1973) with the following exceptions: (a) erythrocytes were collected in 0.024 M EDTA–0.075 M NaCl, frozen, and thawed to allow lysis of the cells; (b) nuclei were homogenized in buffer A–0.34 M sucrose–2 mM EDTA–0.5 mM EGTA (buffer A = 15 mM Tris-HCl, pH 7.4, 60 mM KCl, 15 mM β-mercaptoethanol, 0.15 mM spermidine, and 0.5 mM spermine), and the homogenate was layered on 0.5 volume of buffer A–1.22 M sucrose–1.5 mM EDTA–0.5 mM EGTA and centrifuged for 15 min at 16000*g*; (c) the nuclear pellet was resuspended and again pelleted as in step b; (d) the nuclei were washed once in buffer A–0.34 M sucrose and dispersed in the same buffer. The nucleosome core particles were prepared from nuclei according to the method of Lutter (1978) with slight modifications: nuclei were suspended in buffer A–0.34 M sucrose containing 1 mM $CaCl_2$ at a DNA concentration of ~1 mg/mL, heated at 37 °C, and digested with 60 units/mL micrococcal nuclease (Worthington) until 3–5% of the chromatin DNA was digested. The reaction was stopped by adding EDTA to a final concentration of 2 mM. Nuclei were centrifuged at 4000*g* for 5 min, and the nuclear pellet was lysed in 0.65 M NaCl which also dissociates H_1 and H_5. The chromatin and the free histones were separated by centrifugation in a zonal rotor on a sucrose gradient (5–30%) containing 0.6 M NaCl. The peak of H_1- and H_5-depleted chromatin was dialyzed against a buffer containing 10 mM Tris-HCl, pH 8.0, 0.1 mM Na_2EDTA, and 5 mM $NaHSO_3$, concentrated, and redigested with micrococcal nuclease after adding $CaCl_2$ to a final concentration of 1 mM. The reaction was stopped after 20–30% of the DNA was digested. The nucleosome core particles were then fractionated on a sucrose gradient in a zonal rotor. At the end of the preparation, the core particles were immediately dialyzed against the buffers of the desired ionic strength.

Physical Methods. Circular dichroism measurements were performed on a Roussel-Jouan DC III in 1-cm path length thermostated quartz cells. Analytical sedimentation velocity measurements were made with a Beckman Model E ultracentrifuge using the ultraviolet optics at 260 nm. The observed sedimentation coefficients were corrected to $s_{20,w}$.

Electron Microscopy. Electron microscopy was performed according to Dubochet et al. (1971). A 50-μL drop of core particles or chromatin in the desired buffer was deposited on a parafilm sheet and immediately covered with the grid. After 5-min adsorption, the specimen was positively stained with uranyl acetate at a concentration of 1 or 0.1% for 1 min. The grid was then washed in distilled water for 1 min, air-dried, rotary shadowed with platinum and paladium (80:20), and viewed in a Phillips 300 electron microscope.

FIGURE 1: $NaDodSO_4$ gel electrophoresis of histones bound to the core particle in 6 (a) and 600 mM (b) NaCl. The nucleosome core particles were sedimented on a sucrose gradient containing the appropriate amount of salt. The peak fractions were pooled, and the histone was precipitated by Cl_3AcOH, 25%, and electrophoresed on slab gels.

Results

Sedimentation. The nucleosome core particles dialyzed against buffers of increasing ionic strengths were analyzed on 5–25% sucrose gradients. The samples were sedimented 60 h at 25000 rpm by using a SW27 rotor. At all the ionic strengths studied (6–600 mM) more than 97% of the material was found in a single fast sedimenting peak. The histones bound to the DNA after the sucrose gradient were analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis (Figure 1). A very similar pattern was observed at all the ionic strengths, and equimolar quantities of chromatin core histones appeared to be bound to the DNA. Furthermore, we did not detect any free histones moving slower than the fast sedimenting peak. In agreement with our previous results (Wilhelm et al., 1978), all the histones are thus bound to the DNA, even at the higher salt concentration used in this study.

In contrast to other reports (Stein, 1979; Stacks & Schumaker, 1979), our native samples never contained more than 3% of material sedimenting slower than the fast sedimenting peak, and the amount of slow sedimenting material did not increase at higher ionic strength (up to 0.6 M NaCl). One of the reasons for this result might be that our population of core particles is very homogeneous with a very narrow range of DNA sizes (145 ● 5 bp). On the other hand, in order to minimize the amount of slow sedimenting material, the core particles were always used immediately after preparation. We also carefully avoided freezing the core particles and increasing abruptly the ionic strength by dilution with a solution of high salt concentration which could induce the dissociation of the core particles by transiently creating high local concentrations of NaCl.

The analytical ultracentrifuge measurements confirmed the results of the sucrose gradient experiments. At all the ionic strengths, the nucleosomes sedimented as a single boundary and no slow moving boundary was detected. We have listed

Table I: Effect of Ionic Strength on the Sedimentation Coefficient and Ellipticity at 284 nm of Nucleosome Core Particles

NaCl concn (mM)	$s_{20,w}$	Θ_{284nm}
6	11.3	1610
400	9.8	2052
500	9.6	2359
600	9.4	2662

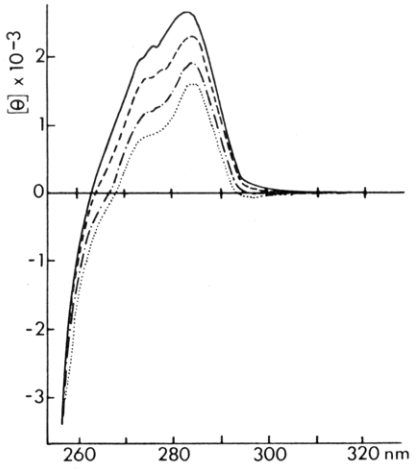

FIGURE 2: Circular dichroic spectra of the core particles at different ionic strengths: 6 (···), 400 (-·-), 500 (- - -), and 600 mM (—). The ellipticity is expressed in deg cm²/dmol of PO_4.

in Table I the $s_{20,w}$ values obtained for the core particle at the different ionic strengths: the sedimentation coefficient decreases from 11.3 in 6 mM NaCl to 9.4 in 600 mM NaCl. This observed decrease of the $s_{20,w}$ value is indicative of a conformational change of the nucleosome and, in agreement with other observations reported below, corresponds to a more extended conformation of the core particle.

Circular Dichroism. The circular dichroic spectrum of native core particles in 6 mM NaCl is shown in Figure 2. There is a maximum at 284 nm and a shoulder at 275 nm in the positive band. Between 290 and 300 nm a small negative band with a minimum at 294 nm is observed. This negative band is also characteristic of native chromatin (De Murcia et al., 1978). The maximum value of the molecular ellipticity at 284 nm for native core particles in low salt is $|\Theta|_{284} = 1610$ as compared to a value of 8300 at the maximum (situated at 280 nm) for protein-free DNA. In high salt, the shape of the circular dichroic spectrum changes; there is a shift of the maximum and of the X intercept toward lower wavelength, and the intensity of the ellipticity at the maximum increases (Table I). Proportionally, the intensity at 275 nm where the shoulder in the positive band is observed increases more rapidly than the intensity at the maximum. The negative band between 290 and 300 nm disappears completely in 0.5 and 0.6 M NaCl.

The observed changes in the circular dichroic spectrum do not correspond to the dissociation of histones in high salt, since we have shown [see above and Wilhelm et al. (1978)] that all the histones are still bound to the core particle at the ionic strengths used in our experiments. The altered circular dichroic spectra in high salt correspond most certainly to modifications of the secondary structure of the chromatin DNA but may also arise from a modification of the superstructure of the core particles as suggested by De Murcia et al. (1978). In particular, the disappearance of the negative band at 290–300 nm may be related to the opening of the core particle.

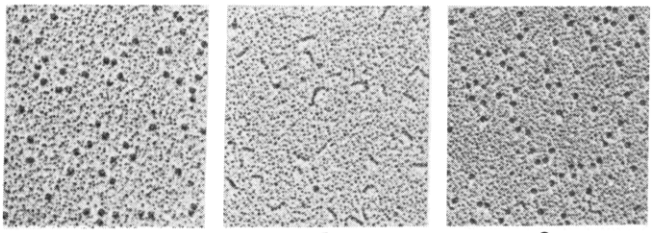

FIGURE 3: Electron micrographs of nucleosome core particles at low (6 mM) and high (600 mM) ionic strength. (A) The core particle centrifuged on a sucrose gradient containing 6 mM NaCl was dialyzed against 6 mM NaCl and adsorbed on the grids in low salt. (B) The core particle centrifuged on a sucrose gradient containing 600 mM NaCl was dialyzed against 600 mM NaCl and adsorbed on the grids in high salt. (C) The core particle centrifuged in a sucrose gradient containing 600 mM NaCl was dialyzed against 6 mM and adsorbed on the grids in low salt.

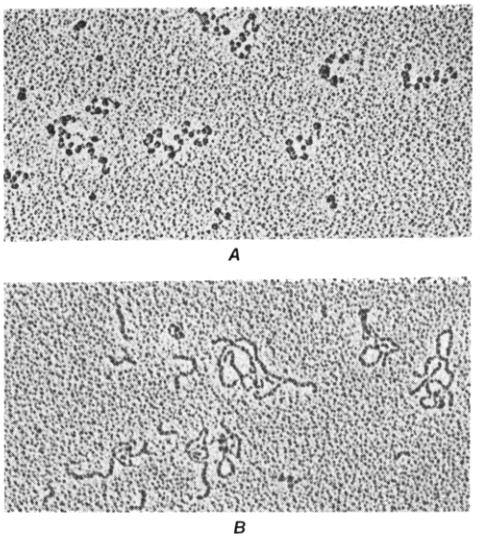

FIGURE 4: Electron micrographs of oligomers of H_1- and H_5-depleted chromatin adsorbed on the grids in low salt (A) or in high salt (B).

Electron Microscopy. The electron microscopic picture of the core particles at the two extreme ionic strengths (6 mM and 600 mM) used in this study is shown in Figure 3. The core particles were first sedimented in a sucrose gradient adjusted to the desired ionic strength as described above. After the centrifugation, the peak fractions were pooled and the core particles were dialyzed to remove the sucrose prior to adsorption on the grids.

The core particles centrifuged and dialyzed against 6 mM have the characteristic beaded appearance, as shown in Figure 3A.

The core particles sedimented in high salt were either dialyzed against a buffer of the same ionic strength or dialyzed against a buffer of low ionic strength. The result of such an experiment is shown in parts B and C of Figure 3. When the core particles are absorbed on the grids in high salt, they appear as extended particles having the shape of a small rod (Figure 3B). However, if the core particles are dialyzed against a buffer of low ionic strength after treatment at high salt, they regain a beaded appearance (Figure 3C). This observation also confirms that all the histones are still bound to the DNA in high salt and shows that the core particles can reversibly assume an extended or compact conformation.

Not only was the salt-dependent conformational change observed with nucleosomes but also the same result was obtained with long oligomers of H_1- and H_5-depleted chromatin. We show in Figure 4 that the chromatin is completely extended

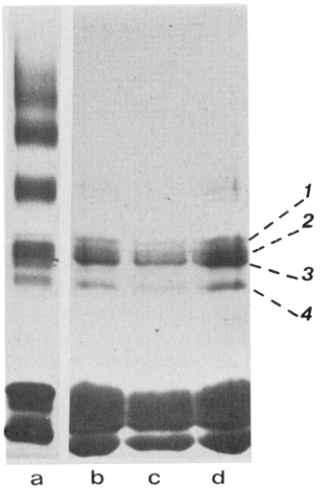

FIGURE 5: $NaDodSO_4$–polyacrylamide gel patterns of histones cross-linked with dimethylsuberimidate at different ionic strengths. Control core particle in 6 mM NaCl (a); core particle in 400 (b), 500 (c), and 600 mM NaCl (d). All the samples were buffered with 10 mM borate, pH 9, and the experimental conditions are as described under Materials and Methods. The bands numbered 1–4 correspond to $(H_3)_2$, $H_{2a}H_{2b}$, H_3H_4, and H_4H_{2b} or H_4H_{2a}, respectively.

in 600 mM NaCl whereas it has a typical beads on a string appearance in 6 mM NaCl.

Cross-Linking with Dimethylsuberimidate. It was of interest to determine next whether the same histone–histone proximities were found in the compact and in the extended particles. Indeed, it has been shown by Dieterich et al. (1979) that the two sulfhydryls of the two H_3 molecules which are close together in low salt move apart by more than 70 Å in 0.6 M NaCl. This result raises the question of whether the two H_3 molecules are still in close contact in the extended particle. In order to answer this question, cross-linking experiments with the protein cross-linking reagent dimethylsuberimidate have been made. Conditions have been adjusted such that the histones in the core particles are predominantly cross-linked into dimers. Figure 5 shows the result of such an experiment. The same bands are observed in the dimer region at all the ionic strengths. According to Thomas & Kornberg (1978), the dimer bands seen in Figure 4 can be assigned to the following pairs of histones in order of decreasing electrophoretic mobilities: H_4H_{2b} or H_4H_{2a} (M_r 25 100 or 25 300, respectively), H_3H_4 (M_r 26 600), $H_{2a}H_{2b}$ (M_r 27 800), and $(H_3)_2$ (M_r 30 600). For the core particles cross-linked in high salt, the band corresponding to H_3H_4 is the most intense whereas the intensities of the bands corresponding to H_3H_4 and $H_{2a}H_{2b}$ are identical in the control native core particle. The presence of a band corresponding to $(H_3)_2$ in high salt indicates that some regions of the two H_3 molecules are still close enough to allow the cross-linking. Since it has been shown (Dieterich et al., 1979) that in high salt the C-terminal regions move apart, we can speculate that the cross-linking must take place in the central region of H_3 or at the NH_2-terminal ends. However, an analysis of the cross-linked peptides will be necessary to verify this hypothesis.

Discussion

In the present paper, we present evidence that the structure of chromatin and of the nucleosome core particles is altered in high salt. The most direct evidence for this alteration is obtained by electron microscopy where the nucleosomes are no longer observed as beads but take a very extended conformation. The results described here agree with observations made by other techniques on native nucleosomes, chromatin, or reconstituted nucleosomes (Strätling, 1979; Bottger et al., 1979; Dieterich et al., 1979; Stacks & Schumaker, 1979). The precise arrangement of histones in the core particle is not known, but several models of the nucleosome structure have been proposed (Ohlenbusch, 1979; Trifonov, 1978; Weintraub et al., 1976; Finch & Klug, 1977; Mirzabekov et al., 1978). In two models, it has been suggested that the nucleosome could take an extended conformation by dissociation of the histone octamer into heterotypic tetramers $H_{2a}H_{2b}H_3H_4$ (Weintraub et al., 1976; Mirzabekov et al., 1978). Our work reveals that, if these models apply for the structures observed in high salt, the two tetramers must be very close to each other, since the two H_3 molecules are still able to be cross-linked in the extended particle.

It has been shown previously (Oudet et al., 1977) that the nucleosome could also take an extended conformation under conditions of very low ionic strength and that it was split into two half nucleosomes visualized as small beads under the electron microscope. In high salt, the nucleosomes appear rather like rods. The arrangement of histones and DNA is, therefore, different in the extended particle found in high salt and in the half nucleosomes observed in low salt. On the other hand, the rodlike shape of the nucleosome shows that in high salt the $(H_3H_4)_2$ tetramer has a loose conformation and is not sufficient to fold the nucleosomes, although it is able to form a tight nucleosome-like structure in low salt. The unfolding of the nucleosome could also explain the mechanism by which the passage of RNA polymerase is facilitated on chromatin templates in high salt (Williamson & Felsenfeld, 1978; Wasylik et al., 1979; Wasylik & Chambon, 1979). There is now some evidence that the active genes are covered by histones but that the chromatin in these regions has an altered less compact conformation as compared to inactive chromatin. We could speculate, though with caution, that the interaction of some acidic nonhistone proteins and/or the acetylation of histone could play a role similar to the ionic strength by decreasing the number of positive charges of the histones available for the interaction with the phosphate groups of DNA, thus loosening the interaction of histone with the DNA and allowing the unfolding of the nucleosome.

It would be of interest now to study the structure of chromatin assembled in vitro with acetylated histones, together with nonhistone protein, such as the HMG 14 and HMG 17 which have been shown to interact specifically with active genes (Weisbrod et al., 1980) and to see whether it is possible to unfold the nucleosome under more physiological conditions.

Acknowledgments

We thank Professor J. Bonner, who has made this work possible. The early part of this work was done at the Institut de Biologie Moleculaire et Cellulaire (Strasbourg). We thank Denise Buhr for excellent technical assistance and Pat Koen for his help with the electron microscope.

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Amino Acid Analysis and Cell Cycle Dependent Phosphorylation of an H1-like, Butyrate-Enhanced Protein (BEP; H1°; IP_{25}) from Chinese Hamster Cells†

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ABSTRACT: A fraction enriched in the butyrate-enhanced protein (BEP) has been isolated from Chinese hamster (line CHO) cells by perchloric acid extraction and Bio-Rex 70 chromatography. Amino acid analyses indicate that the composition of BEP resembles that of CHO H1; however, BEP contains 11% less alanine than H1, and, in contrast to H1, BEP contains methionine. Treatment of BEP with cyanogen bromide results in the cleavage of a small fragment of ~20 amino acids so that the large fragment seen in sodium dodecyl sulfate–acrylamide gels has a molecular weight of ~20 000. Radiolabeling and electrophoresis indicate that BEP is phosphorylated in a cell cycle dependent fashion. In G_1-arrested cells, little or no phosphate is incorporated into BEP. As cells progress through interphase, BEP becomes phosphorylated so that 12–35% of the BEP molecules are phosphorylated at one to two sites by late interphase. During mitosis, all BEP molecules become phosphorylated at approximately four sites per molecule (BEP_M). Electrophoresis and the analysis of cell populations by electron microscopy indicate that the appearance of BEP_M is temporally correlated with the mitotic phosphorylation of histone H1 ($H1_M$) and with chromosomal condensation during prophase, metaphase, and anaphase. During exit from mitosis, BEP_M undergoes dephosphorylation. The dephosphorylation of BEP_M is temporally correlated with dephosphorylation of $H1_M$ and with the unraveling of fully condensed chromosomes near the anaphase–telophase transition. These data suggest that (1) BEP is a specialized histone of the H1 class and (2) BEP is the species equivalent of calf lung histone H1° [Panyim, S., & Chalkley, R. (1969) *Biochem. Biophys. Res. Commun. 37*, 1042], rat H1° [Medvedev, Zh. A., Medvedeva, M. N., & Huschtscha, L. I. (1977) *Gerontology (Basel) 23*, 334], and IP_{25}, a protein enhanced in differentiated Friend erythroleukemia cells [Keppel, F., Allet, B., & Eisen, H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 653]. The data also indicate that putative HMG1 and HMG2 proteins do not undergo the extensive cell cycle dependent phosphorylations measured for histone H1 and BEP.

Recently, this laboratory has observed an enhancement in the cellular content of an H1-like protein in Chinese hamster (line CHO) cells grown in the presence of sodium butyrate (D'Anna et al., 1980). The relative amount of that butyrate-enhanced protein (BEP) increases from 6–8% of H1 in control cultures to 32% of H1 when cultures are grown in the presence of 10–15 mM butyrate for 24 h. BEP can be extracted by 5% $HClO_4$ and by 0.20 M H_2SO_4 from the chromatin of blended whole cells and from the chromatin of isolated nuclei. BEP migrates slightly in front of H1 in both the urea–acrylamide–acetic acid electrophoresis gel system of Panyim & Chalkley (1969a) and in the sodium dodecyl sulfate ($NaDodSO_4$)[1]–acrylamide–Tris–glycine gel electrophoresis system of Laemmli (1970).

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[1] Abbreviations used: BEP, butyrate-enhanced protein; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/80/0419-4331$01.00/0